

THE DEVELOPMENT OF A HEPATITIS C VIRUS PROPHYLACTIC VACCINE STRATEGY

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DECLARATION

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text. It has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

In accordance with the Nuffield Department of Clinical Medicine guidelines, this thesis is does not exceed 50,000 words, and it contains less than 150 figures.

Signed:_____

Timothy Donnison

Date:_____

THE DEVELOPMENT OF A HEPATITIS C VIRUS PROPHYLACTIC VACCINE STRATEGY

The success of directly-acting antivirals (DAA) has revolutionised hepatitis C virus (HCV) treatment, yet a prophylactic HCV vaccine remains an important clinical asset to achieve HCV elimination by 2030. To date, the development of a prophylactic HCV vaccine has been hindered by viral genomic variability. In human trials, a viral vectored genotype (gt)-1b immunogen induced genotype-1b specific T cell responses targeting highly variable dominant epitopes that displayed reduced cross-recognition of epitope variants at the population level. Therefore, a simian adenoviral vectored (ChAd) HCV T cell vaccine encoding conserved sequences across HCV genotypes was developed, containing known T cell epitopes.

In this thesis, I show that conserved sequence vaccines (ChAd-gt1/3, -gt1-6) induce broad, high magnitude, and functional T cell responses that target subdominant highly conserved HCV epitopes in mice, that can be increased by encoding the novel genetic adjuvant—truncated shark invariant chain ($sIi_{47\text{-}72}$) at the 5' end of the transgene. In addition, I demonstrate that conserved HCV epitopes were not targeted by a ChAd-gt-1b prime and an MVA-gt-3a boost vaccination, unlike a ChAd-gt1-6 prime-boost vaccination, highlighting the importance of priming naïve T cells specific for conserved epitopes. Furthermore, I show that a mixed modality HCV vaccination strategy—combining ChAd-gt1-6 and the recombinant $E2\Delta123_{HMW}$ protein does not compromise either the T cell or antibody response compared to either vaccine alone. For the prospects of a single viral vector vaccine that encodes both the gt1-6 T cell immunogen and the E2Δ123 protein sequence (a 'bivalent' vaccine), I demonstrate that a monovalent ChAd-E2Δ123 prime vaccine induces higher antibody titres targeting E2 and CD81-binding epitopes that neutralise HCVpp/cc, when compared to recombinant $E2\Delta123_{HMW}$ protein-induced antibody responses. This body of work represents a significant step forward in the development of a universal prophylactic HCV vaccine and the next step is the assessment of HCV bivalent vaccine induced cross-reactive T cells and broadly neutralising antibodies.

A universal HCV vaccine that will contribute to the global elimination strategy will need to target multiple HCV genotypes that circulate in a population and conserved epitopes that can resist viral escape. The work described in this thesis is the next step towards the generation of a universal prophylactic HCV vaccine.

THESIS CONTRIBUTIONS BY OTHERS

Experiments and analyses presented in this thesis that were performed by those other than me are as follows:

All vaccine GMP production was undertaken at the Viral Vector Core Facility by Ali Turner and colleagues. Claire Hutchings was my mentor and trainer for all animal work in the Biomedical Science animal facility.

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List of Abbreviations and Acronyms

aa: amino acid.

AAV: adeno-associated virus.

Ad: adenovirus.

Ag: antigen.

APC: allophycocyanin. ; antigen presenting cell.

BCIP/NBT: 5-bromo-4-chloro'-indolyphosphate p-toluidine salt / nitro-blue tetrazolium chloride.

Bcl-2: B cell lymphoma 2.

bNAb: broadly neutralising antibody.

BOC: boceprevir.

bp: base pair.

BSA: bovine serum albumin.

CD: cluster of differentiation.

CD81-LEL: long elongated loop of CD81.

ChAd: chimpanzee adenovirus.

CHC: chronic hepatitis C.

CLDN: claudin.

CMV: cytomegalovirus.

conA: concavalin A.

CTLA: cytotoxic T lymphocyte associated antigen.

DAA: directly acting anti-viral.

DC: dendritic cell.

DMSO: dimethyl sulfoxide.

EDTA: ethylenediaminetetraacetic acid.

eGFP: enhanced green fluorescent protein.

EGFR: epidermal growth factor receptor.

ELISA: enzyme linked immunoassay.

ELISpot: enzyme-linked immunoSpot assay.

FITC: fluorescein isothiocyanate.

FMO: fluorescence minus one.

GAG: glycosaminoglycans.

GC: germinal centre.

gp: glycoprotein.

gt: genotype.

GWAS: genome wide association study.

H-2: murine H antigen.

HAV: hepatitis A virus.

HBV: hepatitis B virus.

HCC: hepatocellular carcinoma.

HCIG: hepatitis C antibody enriched immune globulin product.

HCV: hepatitis C virus.

HCVcc: cell cultured HCV.

HCVpp: hepatitis C virus pseudoparticle.

hIi: human invariant chain.

HIV: human immunodeficiency virus.

HLA: human leukocyte antigen.

HRP: horse radish peroxidase.

HVR: hypervariable region.

ICS: intracellular cytokine staining.

IFN: interferon.

Ig: immunoglobulin.

Ii: invariant chain.

IL: interleukin.

IRES: internal ribosomal entry site.

ISG: IFN stimulated gene.

IVDU: intravenous drug user.

JAK-STAT: janus kinase signal transducer and activator of transcription proteins.

KIR: killer cell immunoglobulin like receptor.

KLRG1: killer cell lectin-like receptor subfamily G member 1.

LDL: low density lipoproteins.

LILR: leukocyte immunoglobulin-like receptor.

LMIC: low to middle income country.

LVP: lipoviroparticle.

mAb: monoclonal antibody.

MAVS: mitochondrial antiviral signalling protein.

MERS: middle east respiratory syndrome.

MHC: major histocompatibility complex.

miR: micro RNA.

mRNA: messenger RNA.

MSM: men who have sex with men.

MV: measles virus.

MVA: modified vaccinia Ankara.

nAb: neutralising antibody.

NK: natural killer.

NPC1L1: Niemann-Pick C1-like cholesterol update receptor.

NrHV: Norway rat hepacivirus.

NS: non structural. ; non-structural protein.

OCLN: occludin.

ORF: open reading frame.

PBS: phosphate-buffered saline.

PBST: PBS tween (0.05% of Tween20).

PCR: polymerase chain reaction.

PD-1: programme death receptor 1.

PE: phyceoerythrin.

PKR: protein kinase R.

PMA: phorbol 12-myristate 13-acetate.

PPR: pattern recognition receptor.

RAV: resistance associated variants.

RBD: receptor binding domain.

RBV: ribovirin.

RIG-I: retinoic acid inducible gene I.

RNA: ribonucleic acid.

SFU: spot forming units.

SFV: semliki forest virus.

sIi: shark invariant chain.

SNP: single nucleotide polymorphism.

SOF: sofosbuvir.

SR: spontaneous resolver/resolved.

SR-BI: scavenger receptor class B type I.

SVR: sustained virological response.

TCF: T cell factor.

TCR: T cell receptor.

Tfh: T follicular helper cell.

Th: t helper cell.

Tim-3: T cell immunoglobulin and mucin-domain containing-3.

TIR: Toll/Il-1 receptor.

TLR: toll-like receptor.

TNFα: tumour necrosis factor alpha.

TPA-LS: tissue plasminogen activator - leader signal sequence.

TRIF: TIR domain containing adapter inducing IFN-beta.

T_{RM}: resident memory T cell.

TVR: telaprevir.

UTR: untranslated region.

VLDL: very low density lipoproteins.

VLP: virus like particles.

VV: vaccinia virus.

WHO: world health organisation.

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1 INTRODUCTION

1.1 Hepatitis C virus

1.1.1 A global health problem

The hepatitis C virus (HCV) is an important human pathogen and since its discovery thirty years ago has remained a focus of intense immunological and epidemiological research (Choo et al. 1989; Kuo et al. 1989). HCV is a hepatotropic RNA virus that infects an estimated 71 million people worldwide with approximately 1.75 million new acute infections each year (WHO, 2017). As HCV infection is often asymptomatic for the first twenty years of infection, these statistics likely underestimate true global HCV prevalence (Hanafiah et al. 2013).

Although some HCV-infected patients present with acute infection, 60-80% of acute infections lead to persistent chronic infection. Chronic HCV (CHC) exhibits varying levels of severity which are correlated with progression of liver inflammation and consequently a spectrum of liver disease ranging from mild inflammation to extensive liver fibrosis and cirrhosis. Chronically-infected individuals are at risk of developing hepatocellular carcinoma (HCC) and end stage liver disease (ESLD) resulting in 400,000 annual deaths and significant morbidity for patients with CHC (But et al., 2008; El-Serag 2012; Thrift et

al., 2017). The progression of CHC remains a significant clinical problem as HCV infection is often asymptomatic and is therefore diagnosed after the onset of intrahepatic pathophysiology.

The recent advent and success of Directly Acting Antivirals (DAAs) has revolutionised treatment of HCV infection. Nevertheless, global HCV prevalence has not yet decreased as outlined by the World Health Organisation's 2017 global hepatitis report (World Health Organisation, 2017). The global implementation of DAA treatment programmes remains a significant obstacle to eliminating HCV, particularly in third world countries and in at-risk populations, where many infected individuals have limited access to DAA treatment (Iyengar et al., 2016; Midgard et al., 2016; Vermehren et al., 2018). The emergence of DAA drug resistant HCV mutants may yet hinder the efficacy of DAA treatments in achieving elimination. Whilst DAA treatment can cure HCV infection, therapy does not prevent re-infection which may occur in high-risk populations such as intravenous drug users (IVDUs) and men who have sex with men (MSM; Midgard et al. 2016), nor does it abrogate the risk of developing HCC in cirrhotic patients after treatment (Conti et al., 2016). Therefore, the prevention of HCV infection through the implementation of a prophylactic vaccination programme will be an important tool to combat the HCV epidemic, in particular re-infection of cured high-risk populations (Scott et al. 2015; Stone et al. 2016; Major et al. 2018). If viral hepatitis elimination is to be achieved by 2030 (WHO, 2017), HCV vaccine development will need to be a focal point of HCV research efforts and a priority for funding bodies (Bartenschlager et al., 2018).

1.1.2 Prevalence and incidence of HCV infection

HCV exhibits extreme genetic variability and exists as seven major genotypes with a global distribution and genotype 1 (1a and 1b) is the dominant worldwide genotype (Messina et

al. 2014; **figure 1-1**). It is estimated that 1% of the world's population are HCV positive and recent World Health Organisation (WHO) modelling showed that there were 1.75 million new infections in 2015 alone, with mean global incidence rates of 23.7/100,000 people (World Health Organisation, 2017). HCV prevalence differs between developed and developing countries with an estimated HCV prevalence of 1-2% and 5-10%, respectively (World Health Organisation, 2017). Despite established healthcare infrastructures in developed countries that can facilitate screening, diagnosis, and treatment of HCV patients, these countries are witnessing an increase in genotype 1 and 3 HCV infections in high-risk populations such as intravenous drug users (IVDUs) and men who have sex with men (MSM). This is a recent trend observed in North America's opioid epidemic (Bruneau et al., 2012; Platt et al., 2018; Zibbell et al., 2018) with a doubling of the incidence between 2010 and 2014 (National Academies of Sciences, 2016). Similarly, Europe and the Eastern Mediterranean region had the highest reported incidence rate (61.8 and 625. per 100,000 people, respectively; World Health Organisation, 2017). Other regions of high HCV prevalence include Africa and South-East Asia which are predominantly genotype-2 and -6 and have less-developed healthcare systems and sub-optimal medical practices which lead to poor HCV surveillance and screening programmes, and high transmission rates. HCV genotype-4 is prevalent in North Africa and the Middle East, and South Africa harbours the majority of global genotype-5 infections (Messina et al., 2014).

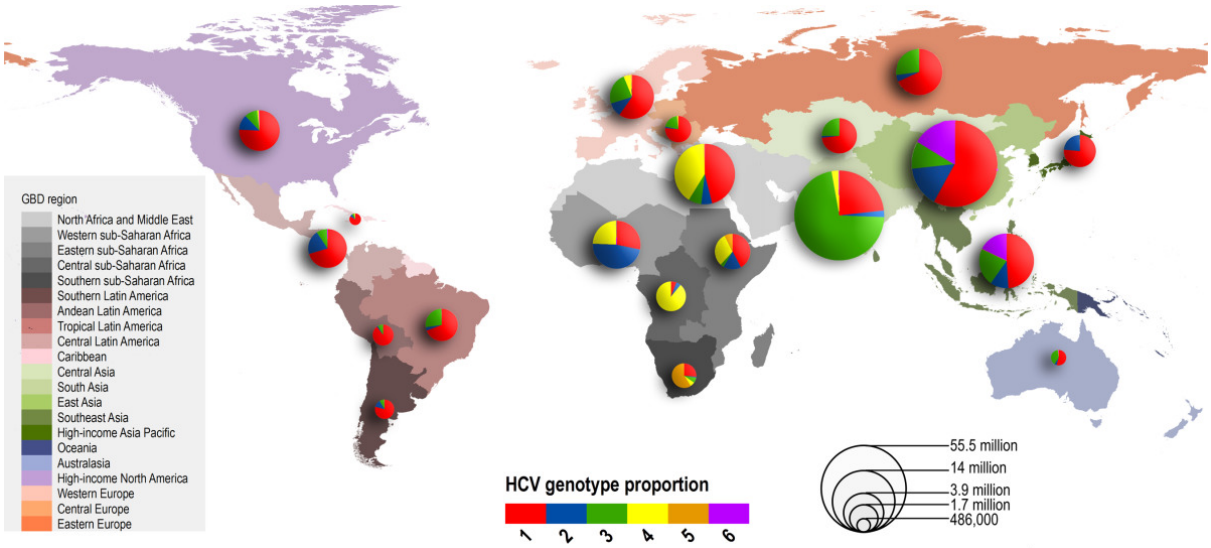


Figure 1-1. Global prevalence of HCV genotypes: The worldwide distribution of HCV genotypes is displayed as pie charts by GBD region (global burden of disease). Pie slice colour indicates HCV genotype (1-6) and size of slice reflects proportion of genotype prevalence within total prevalence of all genotypes in each GBD region. The size of pie charts is proportional to the number of seroprevalent cases determined by Hanafiah et al. 2013. Genotype 7 is not shown due to unknown global prevalence (Figure courtesy of Messina et al. 2014).

1.1.3 HCVs genetic diversity

HCVs error-prone polymerase generates its huge genetic diversity and based on its sequence diversity, HCV is separated into phylogenetic clades, designated as seven genotypes and over sixty-seven distinct subtypes (Smith et al., 2014; International Committee for the Taxonomy of Viruses, ICTV; Figure 1-2). At least 15% sequence difference across the complete coding region sequence and sequence information from at least two other isolates in Core/envelope 1 (E1) and non-structural protein 5b (NS5b) sequences is the criteria to separate subtypes (Simmonds et al., 2005). Yet HCV exhibits greater than 30% variability between genotypes and 20-25% between subtypes in genes that encode HCVs surface envelope proteins (Smith et al. 2014). Combined with the production of a large virus population due a high replication rate (10^{-5}–10^{-4} nucleotides per

replication cycle, (Behrens et al., 1996), a genetically related population of mutant viruses—termed 'quasispecies'—is harboured within an infected individual. The HCV population has the inherent capacity to escape immune and treatment-driven selection pressure (Martell et al. 1992; Bowen and Walker 2005).

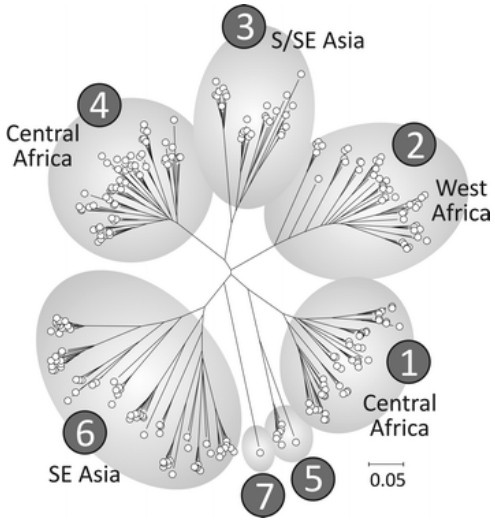


Figure 1-2. Evolutionary tree of HCV genotypes: The evolution of HCV genotypes 1-7 are displayed as determined by their respective NS5b sequences (position 8276-8615 in the H77 reference sequence). Recently discovered viruses including additional genotype-7 and -8 subtypes are not shown. The tree was constructed by neighbour-joining maximum composite likelihood distances as used by the MEGA 4 programme (Tamura et al., 2007). The scale bar represents an evolutionary distance of 0.05. Figure from Simmonds, 2013.

1.1.4 Modes of HCV transmission

Blood or blood-related products are the most common mode of HCV transmission. It was observed that blood donations resulted in post-transfusion hepatic disease that was identified as neither hepatitis A (HAV) or hepatitis B virus (HBV). This observation started the search for additional hepatitis viruses leading to the discovery of HCV (Choo et al. 1989). Since the introduction of pre-transfusion blood screening, HCV infection from

contaminated blood is now rare in developed countries (Pépin et al., 2014). The WHO reported that of the majority of the 1.75 million new infections in 2015 were from unsafe healthcare procedures and injection drug use (World Health Organisation, 2017). In the U.S.A. and Europe shared needle use in IVDU populations (Mitruka et al., 2015), particularly in young persons (Van Handel et al., 2016), may account for the doubling of HCV incidence between 2010 and 2014 (National Academies of Sciences, 2016). Transmission of HCV has also been reported in MSM (men who have sex with men) populations in Europe, Australia, and the U.S.A. (Chan et al., 2016; A. J. Khan et al., 2000; Mohsen et al., 2015). Whereas, in developing countries, unscreened blood transfusions continue to be a major source of new infections (Prati, 2006). Non-sterile needle acquisition of HCV from inadequate equipment sterilisation and insufficient numbers of trained professionals in remote rural areas ensures that blood transfusions remain a high-risk scenario for infection of HCV and other infectious blood-borne agents. Other barriers to achieve adequate safety in blood transfusions in developing countries include the inadequate development and implementation of national policies for transfusion, donor recruitment and mobilisation, and poverty (Tagny et al., 2008). Rarer modes of HCV infection include vertical mother-to-infant transmission, and activities that increase the risk of percutaneous exposure of HCV-infected blood such as tattooing, body piercing, and cosmetic procedures (Lanini et al., 2016; Poulin et al., 2018).

1.2 HCV infection

1.2.1 The virus

HCV is a positive-sense, single-stranded, enveloped RNA virus belonging to the flaviviridae family. The 9400 base-pair (bp) HCV genome encodes three structural proteins: a nucleocapsid that forms the core and two glycoproteins that form the

heterodimer envelope (E1 and E2); and seven non-structural proteins: p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b (Scheel & Rice, 2013).

1.2.2 Establishment of HCV infection

HCV infects and replicates in hepatocytes. Initial binding between HCV and the hepatocyte is facilitated by LDL receptor and glycosaminoglycans (GAG; Scheel and Rice 2013) which leads to CD81 and scavenger receptor class B type I binding (SR-BI; Pileri et al. 1998; Scarselli et al. 2002). Tight junction proteins claudin-1 (CLDN; Evans et al. 2007) and occludin (OCLN; Ploss et al. 2009) facilitate the late stages of HCV entry into the hepatocyte. Additional receptors Niemann-Pick C1-like 1 cholesterol update receptor (NPC1L1; Sainz et al. 2012), epidermal growth factor receptor (EGFR), and ephrin receptor type A2 are also required for HCV entry (Lupberger et al., 2011). Following successful HCV binding to hepatocyte receptors, the viral envelope fuses with the host's cellular membrane by clathrin-mediated endocytosis, followed by fusion with the endosomal membrane release of the viral genome into the cytosol (Dubuisson et al., 2008). HCV genome and polyprotein processed is outlined in **Figure 1-3**. Host miR-122 molecules stabilise the HCV RNA by binding to two 5' untranslated regions (UTR) seed sequences (Jopling et al. 2005; Jangra et al., 2010), allowing for the internal ribosomal entry site (IRES) to recruit the host 40S ribosomal subunit and initiate translation of the ORF (open reading frame) into a full-length HCV polyprotein (mRNA; Pestova et al., 1998). HCV does not use the cap-dependent translation of mRNA, typical of eukaryotic cells. Following translation, the HCV polyprotein that is subsequently post-translationally processed into viral proteins. The structural proteins in the HCV polyprotein are initially processed by host signal peptidases derived from the endoplasmic reticulum. The HCV non-structural proteins are subsequently processed by the NS2/3 autoprotease and the

NS3/4a serine protease (Bartenschlager et al. 1994; Moradpour et al., 2007). HCV NS5b RNA-dependent RNA polymerase mediated replication takes place within an ER derived structure called the 'membranous web'. Newly synthesised RNA genomes are used for translation (production of new viral proteins), RNA replication, or formation of new infectious virions. Genetically diverse HCV progeny are assembled within lipid-raft like structures by which the HCV nucleocapsid is built from units of the core protein with viral RNA, surrounded by membrane derived from the hepatocyte with embedded gpE1E2 heterodimers (Scheel & Rice, 2013). The new virions associate with low- and very-low-density lipoproteins (LDL and VLDL) forming lipoviroparticles (LVPs) for release from the cell (Merz et al., 2011).

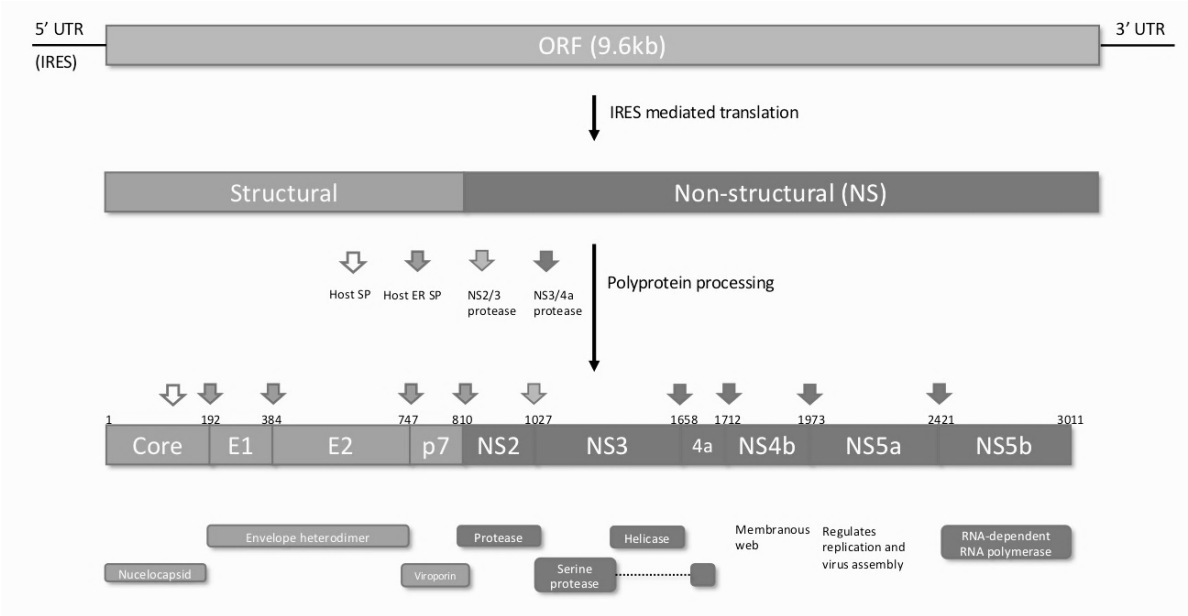


Figure 1-3. The HCV genome and polyprotein processing: The HCV RNA genome contains one ORF (grey; top) flanked at both ends by a 5' and 3' untranslated region (UTR). Host miR-122 sequences stabilises the HCV genome by binding to the 5' UTR. IRES-mediated translation of the ORF leads to the production of a single polyprotein (middle). Endoplasmic reticulum (ER) Signal peptide peptidases ('*Host ER SP'* arrows) cleave the structural proteins: core, E1, and E2, and p7. The white arrow indicates further C-terminal processing of HCV core polyprotein by a host signal peptide peptidase. The NS2/3 protease cleaves itself via an autocleavage mechanism ('*NS2/3*

protease' arrow). The NS3 protease associates with its membrane-bound co-factor, NS4a, and cleaves the non-structural proteins: NS3, NS4a, NS4b, NS5a, and NS5b. The function of each HCV protein is described.

1.2.3 HCV subversion of the innate response

The innate immune response serves to recognise HCV as foreign and recruit local innate antiviral defences to clear infection. In hepatocytes, pattern recognition receptors (PRRs) including TLR3, protein-kinase R (PKR) and retinoic-acid-inducible gene I (RIG-I) are the main responders to HCV entry and replication, recognising HCV RNA. However, the majority of individuals infected with HCV fail to mount a protective immune response. Underlining this problem is the fact that HCV has evolved numerous strategies to regulate and evade the innate immune response. For example, NS3/4a serine protease activity cleaves proteins such as mitochondrial antiviral signalling protein (MAVS) and TIR domain-containing adapter inducing IFN-β (TRIF) which are downstream of PKR/RIG-I and TLR3, respectively. HCV core, E2, and NS5a regulate the JAK-STAT signalling pathway that is activated by IFN type I and III, further inhibiting the generation of an anti-viral state within the infected cell (reviewed by Horner and Gale 2013).

The innate response can function to recruit and modulate the proliferation, differentiation and functionality of the immune cells that constitute an adaptive immune response. Therefore, upon infection, HCV can immediately disrupt both the innate and adaptive immune response to maximise its chances of survival within the liver (Rehermann, 2009).

1.2.4 The clinical course of HCV infection

Acute HCV infection is underdiagnosed because it is often asymptomatic. At 2-weeks post infection, HCV RNA is detectable and is followed by a delayed seroconversion and emergence of HCV-specific T cell response approximately 8 weeks later, suggesting that

the virus outpaces the immune system initially and impairs its response (Netski et al., 2005; Thimme et al., 2001). Nevertheless, intrahepatic interferon stimulated genes (ISGs) are detected early and some innate responses, such as NK cells, are activated regardless of clinical outcome (Thimme et al. 2002; Alter et al. 2011).

Screening for HCV constitutes assessing patient serum/plasma for anti-HCV antibodies using an enzyme-linked immunoassay (ELISA) and recombinant HCV proteins. Positive ELISA results are confirmed by PCR to determine viral tire and infecting genotype. Of the 1.75 million new worldwide HCV infections reported annually, approximately 25% of primary infections will spontaneous resolve, while 75% will advance to chronic HCV infection (WHO, 2017). Most chronic infections cause a degree of fibrosis and 10-20% of chronic infections lead to cirrhosis within 20 years and this is accelerated by certain risk factors: alcohol use (>20-30 dL/day), male sex, age (>40 years), comorbid diabetes, obesity, fatty liver, and co-infection with HIV-1 or hepatitis B virus (HBV; WHO, 2017). Cirrhosis and hepatocellular carcinoma are the leading causes of HCV-related deaths with 400,000 reported deaths each year (WHO, 2017). Chronic HCV can manifest into other extrahepatic conditions including cryoglobulinemia, membranoproliferative glomerulonephritis, porphyria cutaneous tarda, lichen planus, visceral vasculitis, vitiligo, and B cell non-Hodgkin lymphoma (Negro et al., 2015).

1.2.5 Immune-mediated liver injury

HCV persistence, rather than initial infection, in the liver is the driving force behind its associated liver injury, which is a result of immune-mediated mechanisms (Guidotti & Chisari, 2006; Shin, Sung, & Park, 2016). Liver injury is manifested by increased serum levels of alanine aminotransferase (ALT). Although HCV interferes with the induction of IFNs to an extent, type I and III IFNs and consequently ISGs are produced by hepatocytes

during HCV infection (Sheahan et al., 2014; S. Wieland et al., 2014) with many ISGs upregulated in CHC (Su et al., 2002). IFNγ is also produced by HCV-specific $CD8^+$ T cells during HCV infection and correlates with the increase in ALT levels (Guidotti & Chisari, 2006; S.-H. Park & Rehermann, 2014). These virus-specific T cells facilitates virus elimination without severe liver injury due to its non-cytolytic effector function (Jo et al., 2009). $CD4^+$ T cells may contribute to liver injury through the generation of T cell subset Th17 which produces IL-22 (Lee et al., 2012). In chronic HCV infection, $CD8^+$ T cells become exhausted (Radziewicz et al., 2007), which indicates that non-HCV specific immune cells may drive immune-mediated liver injury during chronic infection. One such example may be immunoregulatory cells (e.g. myeloid derived suppressor cells; MDSC) which suppress T cells through the production of reactive oxygen species (Tacke et al., 2012).

The contribution of the anti-HCV immune response to the extent of liver disease seen in chronic HCV infection is not fully understood and remains an important focus of research.

1.3 Correlates of protective immunity during acute HCV infection

The acute phase of hepatitis C infection is defined as the first 6-months following infection. Approximately 25% of acute infections are self-limiting with individuals clearing viral infection within 6-months without therapeutic intervention (WHO, 2017). Conversely, 75% of acute HCV infections progress beyond 6-months and this is deemed the onset of chronic HCV (CHC) infection (WHO, 2017). The exact correlates of immune protection are not fully understood, yet there is convincing evidence for the role of host genetics and the adaptive immune response (reviewed by Abdel-Hakeem & Shoukry, 2014). For the prospects of a prophylactic HCV vaccine, the vaccine-induced immune response should

align with protective immune correlates identified in acute HCV infection and successful clearance of secondary HCV infection.

1.3.1 Role of interferons in spontaneous HCV clearance

To elucidate the role, if any, of host genetic determinants in spontaneous HCV clearance, genome-wide association studies (GWAS) were undertaken. The single nucleotide polymorphism (SNP) rs12979860—located 3kb upstream of the *IL28B* gene which encodes the type III interferon, IFNλ3—was identified as possessing a strong association with increased spontaneous HCV clearance and HCV drug treatment success when the CC genotype was present (Ge et al., 2009; Rauch et al., 2010; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009). In 2013, a new SNP—ss469415590 (TT/ΔG)—was discovered which displayed high linkage disequilibrium with rs12979860 (Prokunina-Olsson et al., 2013). ss469415590-ΔG is a frame-shift variant that leads to expression of the IFNλ4 protein which upregulates hepatic ISGs yet is associated with viral persistence (Terczyńska-Dyla et al., 2014). In addition, high ISG expression has been shown to render the host less amenable to exogenous IFNα and IFNγ treatment, and is associated with more infected cells in the liver (Sheahan et al., 2014). It was reported that the IFNλ4 ΔG genotype and subsequent IFNλ4 and ISG expression is associated with viral adaptations (for example, NS5a serine2414) leading to low viral load (Ansari et al., 2017), yet IFNλ4 protein expression correlates with poor treatment response rates (Bibert et al., 2013; Terczyńska-Dyla et al., 2014). It may be that an increased immune pressure selects for the 'best survivors' to an antiviral IFN/ISG response, resulting in HCV progeny that can survive the hostile environment that anti-HCV treatment creates. The association of favourable and non-favourable IFNλ4 genotypes with subsequent viral adaptations to the host immune response underscores the importance of understanding virus-host interactions

and the innate anti-viral response to predict clinical outcome and inform the treatment of HCV infection.

1.3.2 Protective T cell responses during acute infection

The HCV-specific $CD4^+$ and $CD8^+$ T cell response appears late in acute infection, between 6-8 weeks, despite high HCV replication levels (Bowen et al. 2005). The tolerogenic nature of the liver (Crispe, 2009), the time required for viral antigens to be presented by dendritic cells (DCs; Bowen et al. 2005), or evasion of the innate immunity may explain the late appearance of HCV-specific T cells (Horner, 2014). The appearance of HCV-specific IFNγ secreting $CD4^+$ and $CD8^+$ T cells in the blood and liver is kinetically linked with the decline of intrahepatic HCV viral loads and HCV resolution (Su et al., 2002; Thimme et al., 2002; Thimme et al., 2001) whereas loss of T cells leads to HCV reoccurrence (Gerlach et al., 1999; Lechner et al., 2000). In addition to a delayed T cell response, HCV infection confers an initial 'stunned' T cell phenotype by which the first appearance of T cells coincides with limited proliferative capacity and effector function, regardless of clinical outcome (Lechner et al., 2000; Thimme et al., 2001; Urbani et al., 2006).

The HCV-specific T cell response in resolved HCV infection is characterised by its high magnitude, breadth, longevity, and polyfunctionality (reviewed by Abdel-Hakeem et al., 2014). The magnitude and breadth of the $CD4^+$ and $CD8^+$ T cell response correlates with spontaneous clearance in acute infection (Cooper et al., 1999; Lechner et al., 2000; Missale et al., 1996). The magnitude of the T cell response is measured by the frequency of antigen-specific T cells or by the number of T cell exerting a specific effector function, such as IFNγ production. While no definitive threshold of a protective $IFN\gamma^+$ T cell response exists for HCV, individuals who clear infection typically display a HCV-specific T cell response of approximately a few hundred $IFN\gamma^+$ cells per million PBMCs (Folgori et al., 2006;

Kaplan et al., 2007; Georg M Lauer et al., 2004; Franziska Lechner, Wong, et al., 2000). The efficacious HCV-specific T cell response targets many HCV antigens in contrast to individuals who develop CHC whose T cell response is a lower magnitude and targets a narrow range of epitopes (Cox et al., 2005; Day et al., 2002; Lechner et al., 2000). Specifically, the T cell response to non-structural (NS) proteins are immunodominant, correlating with HCV clearance (Diepolder et al., 1995; Schulze zur Wiesch et al., 2005; Smyk-Pearson et al., 2006). These T cell responses are characterised by their polyfunctionality with the capacity to secret multiple cytokines including IFNγ, IL-2, and CD107a (Badr et al., 2008). Patients who go on to develop CHC display progressive loss of T cell function (especially $CD4^+$ T cells) with reduced polyfunctional and proliferative capacity, effector dysfunction, and increased expression of T cell exhaustion markers (PD-1, 2B4, CD160, KLRG1, Tim-3, CTLA-4), suggesting that the failure to sustain a T cell response, rather than to prime one, is critical to HCV control (Badr et al., 2008; Bengsch et al., 2010; Gruener et al., 2001; Semmo et al., 2005; Wedemeyer et al., 2002). Furthermore, whilst the presence of T cells in acute infection does not predict or correlate clinical outcome, the early expression of IL-7α receptor (CD127) on HCV-specific T cells has been demonstrated to be a predictor of spontaneous resolution in acute infection, while a delayed appearance of $CD127^+$ T cells is associated with HCV persistence (Golden-Mason et al., 2006; Shin et al., 2013). Additionally, in SRs, the HCV-specific T cells develop a memory phenotype of $CD127^{hi}$ $Bcl\text{-}2^{hi}$ (Badr et al., 2008). Whereas in CHC patients, peripheral T cell frequencies decrease yet highly activated and exhausted intrahepatic T cells ($PD\text{-}1^{hi}$ $CD127^{lo}$) can be readily detected (He et al., 1999; Radziewicz et al., 2007).

The importance of the T cell response to control and clear HCV infection has been demonstrated in chimpanzee challenge studies. Two chimpanzees who had spontaneously

resolved HCV infection were re-infected with HCV resulting in low serum RNA levels and a shorter course of viraemia of four weeks compared to ten weeks of viraemia in the naïve animal (Major et al. 2002). This coincided with intrahepatic cytokine levels which rose quicker and reached a 30-fold higher peak by week four compared to week twelve in the naïve animal. Viraemia in a subsequent secondary HCV infection was undetectable after fourteen days compared to initial HCV infection which took four months to reduce viraemia to undetectable levels. A lack of detectable anti-HCV envelope antibodies and the rapid onset of intrahepatic IFNγ and tumour necrosis factor alpha (TNFα) after re-infection of HCV suggests immune memory T cell responses were recalled, conferring protective immunity (Major et al. 2002). Follow-on studies demonstrated that intrahepatic $CD8^+$ T cells rapidly acquired cytolytic activity specific to HCV which coincided with the expansion of $CD4^+$ and $CD8^+$ memory T cells in the blood (Shoukry et al., 2003). Subsequently, the antibody-mediated depletion of $CD8^+$ memory T cells prolonged viral replication despite the presence of fully functional $CD4^+$ T cells (Shoukry et al. 2003). Viral control was regained with the re-appearance of liver resident HCV-specific $CD8^+$ T cells (Shoukry et al., 2003). In a complementary experiment, the antibody-mediated depletion of $CD4^+$ memory T cells in chimpanzees who had previously cleared HCV failed to control HCV infection upon re-challenge despite the presence of HCV-specific $CD8^+$ T cells (Arash Grakoui et al., 2003). The redundant $CD4^+$ T cells led to the emergence of MHC class I-restricted variants with escape mutations (Grakoui et al., 2003). A sustained $CD4^+$ T cell response appears to be necessary for maintaining viral control in chimpanzees, reflecting the reoccurrence of viraemia in humans which exhibits a waning $CD4^+$ T cell response (Gerlach et al., 1999; Semmo et al., 2005). These animal studies highlight the need for a sustained functional complementary $CD4^+$ and $CD8^+$ T cell response to clear HCV infection.

Natural history studies of single source outbreaks and HCV patient cohorts have also highlighted the importance of the HCV-specific T cell response by identifying a clear relationship between human leukocyte antigen (HLA) alleles and clinical outcome. MHC class I molecules—HLA-B27, -B57, and -A3—have been associated with an increased rate of spontaneous HCV clearance (Dazert et al., 2009; Fitzmaurice et al., 2011; Kim et al., 2011; Neumann-Haefelin et al., 2006). Mechanisms through which HLA alleles confer a protective advantage may be through preferential presentation of highly constrained T cell epitopes that are less likely to mutate due to associated fitness costs (Cox et al., 2005; Dazert et al., 2009; Neumann-Haefelin et al., 2006, 2011). Other mechanisms could include preferential thymic selection, large naïve T cell populations, greater concordance with the antigen presentation pathways, greater flexibility to bind in the MHC binding groove, lower susceptibility to inhibition by regulatory T cells, and interactions with other receptors aside from TCR, such as killer-cell immunoglobulin like receptors (KIRs) and leukocyte immunoglobulin-like receptors (LILRs) on natural killer cells and DCs, respectively (reveiwed by Goulder & Walker, 2012). Nevertheless, the association of HLA alleles with favourable clinical outcome supports the role of T cells in protective immunity and HCV clearance, giving merit to a vaccine approach that aims to elicit an HCV-specific T cell response.

1.3.3 Protective antibody response

Soon after the discovery of HCV in 1989, the screening of sera from patients who had previously resolved HCV infection identified circulating anti-HCV antibodies (Gretch et al. 1992; Sheu et al. 1992; Zhang et al. 1992). However, the importance of antibodies in HCV clearance remains unclear to date. In some clinical settings antibodies are not required to clear HCV, for example, hypoglobulinaemia patients are able to clear HCV

infection despite lacking immunoglobulins (Semmo et al. 2006) and the administration of human hepatitis C antibody enriched immune globulin product (HCIG) does not protect against HCV infection (Davis et al. 2005). Furthermore, induction of antibodies is delayed until week-8 post-infection and are of low titre (Logvinoff et al., 2004; Netski et al., 2005). One study showed that broadly neutralising antibodies (bNAbs) appeared after establishment of chronic infection, selected for escape mutants, and did not clear HCV infection (von Hahn et al., 2007). Yet, the correlation of an acute anti-HCV antibody response with HCV clearance is supported by a number of natural history studies (Lavillette et al., 2005; Osburn et al., 2010; Pestka et al., 2007). In a cohort of HCV positive acute-phase patients, all individuals seroconverted but only those whose serum neutralised infectious HCV pseudoparticles (HCVpp) resolved acute HCV infection (Lavillette et al., 2005). A single-source HCV outbreak in a homogenous cohort of women demonstrated the association between rapid induction of neutralising virus-specific antibodies and HCV clearance (Dowd et al., 2009; Pestka et al., 2007). A delayed induction of neutralising antibodies in late acute infection resulted in HCV persistence suggests that an HCV-specific humoral response can only clear HCV if the immune system responds early enough in acute HCV infection (Dowd et al., 2009; Pestka et al., 2007). A recent study by Osburn et al. supports the notion that a rapid antibody response mediates HCV clearance (Osburn et al. 2014). Using an extensive HCVpp library covering the natural variability of HCV genotype-1a E1E2 sequences, a bNAb response was detected earlier in patients who controlled viraemia compared to a delayed antibody response in patients who developed CHC that progressively broadened as HCV persisted (Osburn et al. 2014).

The association of HCV clearance with an early onset bNAb response may reflect HCV clearance before HCV can establish intrahepatic infection and produce a quasispecies of diverse virions that can survive anti-HCV immune pressure. The HCV quasispecies

harbours vast sequence diversity in its viral surface envelope glycoproteins (Kato et al., 1992; Martell et al., 1992; Okuda et al., 1999). The main target of the anti-HCV antibody response is the envelope glycoprotein E2 with sequence variation primarily occurring in E2s hypervariable regions ($HVR1_{384-410}$, $HVR2_{461-481}$, $HVR3_{434-450}$, $igVR_{570-580}$; Drummer & Poumbourios, 2004; Drummer, 2015; Kato et al., 1992; Weiner et al., 1991). Antibodies targeting the HVR1 have been reported to be immunodominant in infection (Kato et al. 1993, 1994; Zibert et al. 1995), with detectable titres observed in SR within 6 months of infection (A Zibert et al., 1997) and in CHC (Dowd et al., 2009; von Hahn et al., 2007). As the E2 HVR sequences are highly variable in the HCV quasispecies and that immune pressure to this region drives viral evolution, it may be that anti-E2 nAbs must be produced early enough in infection to mediate HCV clearance. A delayed anti-E2 antibody response may be the result of an already established diverse virus population that can evade antibody neutralisation and persist in the infected host (Dowd et al., 2009; von Hahn et al., 2007).

Strikingly, high sequence variability in E2 was exhibited in both defined B cell and T cell epitopes suggesting sequence evolution may be critical to avoid virus clearance from a coordinated host humoral and cellular adaptive response (von Hahn et al., 2007). Studies of patients who spontaneously cleared chronic HCV infection indicate that a functional antibody and T cell response is required to eliminate HCV, associated with the production of neutralising antibodies and the reversal of intrahepatic T cell exhaustion (Raghuraman et al., 2012). Furthermore, direct cell-to-cell transmission of HCV in the liver could evade circulating bNAbs, thus underpinning the need for a rapid bNAb response and a complementary intrahepatic T cell response (Timpe et al. 2008; Witteveldt et al. 2009; Brimacombe et al. 2011).

A number of animal experiments have investigated the passive transfer of neutralising antibodies to deduce the role antibodies play in control of HCV infection. In naïve chimpanzees, pre-incubation of HCV with immune serum resulted in reduced HCV infectivity upon serum/HCV mix infection (Farci et al., 1994, 1996). In chimpanzees chronically infected with HCV, the administering of hepatitis C immunoglobulin (HCIG) prolonged the acute HCV incubation period (Krawczynski et al., 1996), and resulted in only a minor reduction in viraemia (Morin et al., 2012). While upon secondary HCV infection in resolved chimpanzees, viral clearance correlated with a dramatic increase in anti-HCV antibodies and a T cell response (Bassett et al., 2001). Furthermore, chimpanzees that were challenged with HCV genotype 1a (H77) after a single dose of human monoclonal antibody, HCV1, displayed undetectable levels of viraemia (Morin et al., 2012). Yet, in one animal viral load rebounded after 14 days in concordance with an E2 mutation conferring resistance to HCV1 neutralisation (Morin et al., 2012). In genetically humanised mouse models, passive immunisation with broadly neutralising antibodies prevented HCV infection after a heterologous virus challenge (Dorner et al., 2011; Law et al., 2008). Furthermore, the administration of CHC patient sera decreased viraemia in human liver-chimeric mice, with 5/8 animals exhibiting sterilising immunity to the ancestral strain derived from the CHC patient (Vanwolleghem et al., 2008).

Aligning with natural history studies of HCV infection in humans, animal challenge models of HCV reiterate the importance of a rapid antibody response that is capable of neutralising HCV envelope proteins. The interplay between the host antibody response and mutability of the HCV E2 antibody epitopes may be critical in determining the outcome of HCV infection. Finally, identification of bNAbs from animal and human immune sera remains an important area of HCV research in order to assess the candidacy of antibodies

and antibody producing vaccines for prophylactic and therapeutic intervention (Bailey et al., 2017; Giang et al., 2012).

1.4 Protective immunity upon HCV re-infection

Spontaneous clearance of HCV infection generates long-lived HCV-specific memory T cells that may serve to protect individuals from re-exposure to HCV (Shoukry et al., 2003; Takaki et al., 2000). A cohort of German women, who had previously cleared HCV infection, retained a detectable $CD4^+$ and $CD8^+$ memory T cell population 20 years after infection (Takaki et al., 2000). Strikingly, in contrast to women who developed CHC over 20 years, the spontaneous resolvers had low titres of anti-E1 and -E2 antibodies after 10 years that waned to undetectable levels after 20 years suggesting that T cells, not antibodies, may provide durable protective immunity (Takaki et al., 2000). Furthermore, in IVDU cohorts, HCV resolved individuals were more likely to resolve subsequent infection compared to primary infection in naïve individuals, with reduced magnitude and duration of viraemia (Mehta et al., 2002; Osburn et al., 2010; Sacks-Davis et al., 2015). HCV resolution in secondary infection correlated with rapid expansion of T cells with high avidity and polyfunctionality (Abdel-Hakeem et al., 2017), and the generation of cross-reactive antibodies (Osburn et al., 2010). Reduced viraemia and detection of a durable T cell response after HCV re-infection is supported by challenge studies in chimpanzees (Bassett et al., 2001; Grakoui et al., 2003; Major et al., 2002; Shata et al., 2002; Shoukry et al., 2003). Importantly, whilst rates of spontaneous clearance increased after secondary infection compared to primary infection, protective immunity was not absolute as some chimpanzees and humans could still develop CHC in the presence of a strong adaptive immune response (Abdel-Hakeem, Bédard, Murphy, Bruneau, & Shoukry, 2014; Bukh et al., 2008). These data indicate the importance of generating a broad memory T cell and

antibody response capable of recognising multiple antigens across multiple genotypes and diverse HCV variants to protect against HCV infection and persistence.

In summary, chimpanzee challenge models and natural history studies of primary and secondary infection in humans indicate that a high magnitude, broad, sustained, and coordinated HCV-specific $CD4^+$ and $CD8^+$ T cell response is necessary to clear HCV infection in the context of preventing persistent HCV infection. Furthermore, the generation of a nAb response early in acute HCV infection is associated with viral clearance. Whilst both arms of the adaptive immune response have the capacity to clear HCV, both rely on a quick and sustained immune response to counter HCVs inherent ability to quickly establish a quasispecies in the liver that is capable of epitope mutation, immune escape, and exploiting the tolerogenic nature of the liver to generate an exhaustive dysfunctional immune response incapable of clearing HCV infection. These observations suggest that HCV vaccination is a feasible endeavour. Finally, understudied aspects of the host immune system are probably involved in mounting an efficacious immune response against HCV—such as distinct T cell subsets and antibody isotypes—emphasising the importance of basic science research to inform translational science research.

1.5 Treatment of HCV infection

1.5.1 Interferon therapy

Since the discovery of HCV in 1989, HCV treatment and management has undergone considerable progress (Gentile et al., 2016). For several years there was no treatment programme, until reports of the benefits interferon-alpha (IFNα) treatment initiated the introduction of a 24- or 48-week course of IFNα to treat CHC (Carithers & Emerson, 1997). Patients required three weekly injections of IFNα but despite the intense treatment

course only 10% of patients cleared HCV (Carithers & Emerson, 1997). In 2001, the standard treatment for HCV infection and its liver-associated disease improved from tri-weekly doses of IFNα to a 24- or 48-week course of pegylated interferon-alpha and ribavirin (peg-IFNα + RBV; Ghany et al. 2009), administered weekly. Sustained virologic responses (SVRs; defined as HCV PCR negative 12-24 weeks after the end of treatment) reached 50% in HCV genotype 1 infected patients, 80% in genotype-2 and 65% in genotype-3 (von Wagner et al., 2005). Not every HCV patient had access to the treatment as patients diagnosed with contraindications related to IFNα or RBV, such as depression or psychosis, were excluded from HCV treatment enrolment (Ghany et al., 2009). HCV-infected patients with severe liver failure were excluded from receiving treatment despite being most at-need of anti-HCV treatment.

1.5.2 Emergence of directly acting antiviral drugs (DAAs)

The low response rates of patients on treatment courses of peg-IFNα drove the search for more effective therapy options. In 2011, novel revolutionary drugs called 'directly acting antivirals' (DAAs) emerged as a standalone treatment option and a combinatory therapy with peg-IFN (Thompson et al., 2009). The first HCV DAAs developed, telaprevir (TVR) and boceprevir (BOC), were protease inhibitors targeting HCV proteins NS3/4a and preventing HCVs serine protease from cleaving the HCV polyprotein into its constituent parts. The co-administration of peg-IFNγ+RBV and BOC/TVR to individuals infected with HCV genotype 1 infection improved SVR24 to 70% (sustained virologic response at week-24 post-treatment; Dusheiko et al., 2008; Nelson, 2011). A drawback to the peg-IFN+RBV, TVR, BOC triple therapy was that CHC genotype-1 was treated exclusively and treatment programmes recorded low patient tolerance due to the pill burden (taken

with a fatty meal every eight hours) and adverse side effects such as anaemia, fatigue, and rash (Jacobson, 2011).

The second wave of licensed DAAs was introduced in 2013-14 and included second-generation NS3/4a inhibitors, for example simeprevir (QUEST-1, -2, and RESTORE phase III clinical trials; Jacobson et al. 2014; Manns et al. 2014; Moreno et al. 2015), grazoprevir (C-SPIRIT trial; Summa et al. 2012; Gane et al. 2016), and paritaprevir. In simeprevir trials, SVR rose to 80-90% in the majority of patient groups, with the DAAs demonstrating antiviral activity towards HCV genotypes-2, -4, -5, and -6 (Moreno et al., 2012).

Among the second wave of licensed DAAs were inhibitors that targeted other HCV non-structural proteins. NS5a inhibitors were introduced, such as Daclatasvir among others (ledipasvir, elbasvir, ombitasvir, velpatasvir, odalasvir), which inhibit the NS5a protease and therefore prevent a viral mechanism of subverting the anti-viral interferon response (Blight et al., 1998). Daclatasvir proved effective as part of a triple therapy (with peg-IFN+RBV) in phase II clinical trials (Hézode et al., 2015; Pol et al., 2012), and is currently being combined with other DAAs. The targeted inhibition of the NS5b RNA-dependent RNA polymerase using NS5b inhibitors, sofosbuvir (others include nucleoside and non-nucleoside: dasabuvir and beclabuvir), was undertaken in human clinical trials (NEUTRINO trial; Lawitz et al. 2013). Pan-genotype SVR was achieved in 80-100% of patients in a SOF-based triple therapy regimen (Lawitz et al., 2013; Lawitz et al., 2015).

The introduction of anti-HCV DAAs revolutionised treatment of HCV. Treatment programmes offer straight-forward dosing schedules, shorter enrolment periods, and reduction in unpleasant side effects. The standard treatment combinations now include DAAs exclusively without administration of interferon, with patient retention and enhanced DAA efficacy with SVR reaching 100% in some patient groups (Gentile et al.,

2014; Gentile et al., 2013). For those who have access to DAA therapy, HCV is a curable disease.

1.6 Barriers to HCV elimination

Despite the success of oral, IFN-free treatments of DAA combination therapies which achieve greater than 90% cure rates, a number of barriers exist that could limit the impact DAA treatment have on HCV elimination.

1.6.1 HCV infection remains undiagnosed

A significant drawback for the HCV treatment programmes is that HCV positive individuals are unaware they harbour HCV until the onset of advanced liver disease when symptoms are noticed by patients or the disease is diagnosed by practitioners. Approximately 20% of HCV infections are diagnosed with only 7% of diagnosed HCV positive patients enrolled on DAA treatment programmes (WHO, 2017). While DAA therapy is effective, it can only be utilised if the patient seeks medical help, therefore lack of diagnosis remains a significant problem. In addition, HCV screening programmes require national hepatitis-specific action plans, yet only 18 counties have implemented such plans according to the WHO. Nation-wide implementation of screening programmes require laboratories, trained staff, and specialised equipment, a challenging hurdle for lower to middle income countries (LMICs) that have limited resources—a significant problem considering that LMICs harbour the majority of HCV infections (Messina et al., 2014). Even if HCV screening is accessible, the associated cost of screening is relatively higher for the patient from the LMIC, due to upfront costs, time, and travel, when compared to the ease of accessibility to healthcare systems in developed countries (Thursz & Lacombe, 2016). Furthermore, surveillance programmes are difficult to implement in

large at-risk populations who do not present with symptoms of HCV infection (Hagan et al., 2013)

1.6.2 DAA treatment is expensive

A major obstacle for HCV treatment is the prohibitive costs of DAAs. The high price of DAAs represents a major barrier to patient enrolment on DAA treatment plans which range from $37,000 in Japan to $64,000 in U.S.A. for a single 12-week course of sofosbuvir (Iyengar et al., 2016). With limited national healthcare resources, varying DAA costs between countries, and limited space on treatment programmes, DAA therapy will not be available to all patients (Andrieux-Meyer et al. 2015; Hagan et al., 2013). To address the limit spaces on DAA enrolment programmes, patients are prioritised based on liver disease progression and the potential benefit attained from DAA triple therapy (Martin et al., 2016). A tiered selection process for treatment enrolment raises ethical concerns, particularly when DAA treatment is prioritised to patients with advanced fibrosis or cirrhosis. Consequently, recently infected individuals are less likely to be treated despite pro-actively seeking medical help, and who would otherwise have a chance of avoiding onset of pathophysiology and HCV-associated morbidity.

As a consequence of DAA treatment availability and cost, generic licensing offers have emerged. Generic drugs—equivalent to ‘brand drugs’—are manufactured by external pharmaceutical companies under license to the originator company, enabling the upscaling of product (Jensen et al., 2016). Generic drugs are much cheaper than their original counterparts and have become an attractive alternative for LMICs to provide access to DAA therapy (Jensen et al., 2016). Despite this, without low DAA prices—implemented by a fairer pricing framework to consider affordability—countries are unlikely to increase investment in HCV treatments to reduce HCV burden.

1.6.3 Antiviral resistance

With the widespread use of DAAs, HCV resistant-associated variants (RAVs) have emerged (Coppola et al., 2016; Perales, 2018; Sorbo et al., 2018). DAA failure is associated with a selection of viral variants that have become resistant to DAA action, which include mutations in NS5a, a target of all approved antiviral combination regimens. The frequency of HCV mutation is dependent on the HCV genotype, the DAA combination, previous patient treatment regimens, and geographical location (Li et al., 2017; Ramirez et al., 2016), indicating that careful surveillance is required to detect and characterise DAA resistance. While RAVs are rare, patients who are unresponsive to DAA treatment still represent a significant number of patients due to the high prevalence of HCV infection. As HCV diagnosis, surveillance, and treatment enrolment improve so will the likelihood of generating HCV RAVs to DAAs.

1.6.4 HCV reinfection after treatment

While high SVR is attainable through DAA therapy, cured individuals still remain at risk of re-infection of HCV after treatment ends (Midgard et al., 2016; Sarrazin et al., 2017). Considering the asymptomatic nature of acute HCV infection and the high-risk behaviour of susceptible populations, lack of post-treatment protective immunity is a major cause for concern in managing HCV incidence (Mehta et al., 2002). To further complicate secondary infection treatment, the newly infecting HCV strain may be genetically distinct from the previous infecting strain (Sarrazin et al., 2017), an important consideration for use of DAAs with limited pan-genotype application, particularly in LMICs where diagnosis may not be possible and DAA options limited.

1.7 HCV vaccination

1.7.1 The need for an HCV vaccine

With an estimated 95% of undiagnosed HCV cases, a trend towards higher transmission rates and risk of re-infection, and coupled with diverse virus heterogeneity, the impact DAAs will have on HCV elimination is uncertain (Bartenschlager et al., 2018; Cox, 2015). Furthermore, liver disease can still progress, and hepatocellular carcinoma can develop despite cure of HCV infection in cirrhotic patients (Bailey et al., 2018). Therefore, prevention of infection offers significant advantages over treatment. The shortcomings of current HCV treatments indicate that there is an unmet need for a prophylactic vaccine in the management of HCV infection. Mathematical models have suggested that even the use of a low efficacious vaccine of 30% could confer significant benefit to reduce HCV prevalence and transmission compared to treatment alone to reach prevalence reduction targets (Major et al., 2018; Scott et al., 2015; Stone et al., 2016). Global research efforts to develop an HCV vaccine will need to be supported by major funding bodies and policy makers if HCV is to be eliminated by 2030.

1.7.2 HCV vaccine development

There is convincing evidence that spontaneously resolved HCV infection protects against persistence of secondary HCV infection suggesting a role for immunological memory in HCV clearance (see section 1.3). A meta-analysis of chimpanzee vaccination studies demonstrated that re-challenging vaccinated animals confers reduced viraemia duration and peak magnitude, comparable to that observed in naïve animals who cleared virus spontaneously (Dahari et al., 2010). A parallel result in humans showed that only 20% of secondary HCV infections in IVDUs advanced to chronic disease compared with 80% of primary HCV infections (Grebely et al., 2012), indicating that protective immunity does

not need to be sterilising. Therefore, the clinical objective of vaccination is to prevent HCV persistence, rather than initial infection, as it is the establishment of long-term HCV infection that leads to disease. A true 'cure' is possible given that HCV does not have a cellular reservoir in humans and latency is not observed in natural infection (Lauer & Walker 2001).

To date, all licensed vaccines primarily elicit an antibody response. The historical approach to vaccine design is to use either a live strain with attenuated pathogenicity or to use an inactivated/killed strain. Both approaches have been used for licensed vaccines, such as the smallpox and yellow fever vaccines, respectively, and confer long-lasting cellular and humoral immunity in those vaccinated (Pulendran, 2009; Smith, 2011). While live-attenuated or inactivation vaccine production is straight-forward, this approach is not feasible for all pathogens, such as those that cause serious disease, e.g. HIV and HCV (Minor, 2015).

Rationale vaccine design should, ideally, be informed by the correlates of protection with vaccine-induced immune responses aligning with the dominant form of protection in natural infection. For example, the HBV vaccine is a recombinant version of the HBV surface antigen (HbV sAg) that elicits anti-sAg antibodies in the majority of vaccines at titres that protect against infection (Meireles et al., 2015). Understanding the correlates of protective immunity against pathogen infection is typically a prerequisite to vaccine design, but this is not possible for HCV due to the small number of cohorts identified during acute infection and the lack of an animal model permissive to CHC. Consequently, the correlates of protection in HCV are not well defined, and although there is a large body of literature to suggest that both innate and adaptive immune responses play key roles, the exact mechanisms of virus clearance are not clear and likely not due to a single correlate

of protection. Consequently, the HCV field has contrasting opinions on the importance of antibody and T cell responses that should be generated by a prophylactic vaccine. As such, research groups have attempted to design and assess HCV vaccine candidates with the primary aim of eliciting either an HCV-specific antibody or T cell response (Shoukry, 2018; Walker & Grakoui, 2015). A spectrum of approaches ranging from recombinant proteins and virus-like-particles (VLPs) to synthetic peptides and recombinant viral vectors have been attempted to date, and these are outlined in **Table 1**.

Table 1. Animal and human studies describing prophylactic HCV vaccine candidates

Table 1. Animal and human studies describing prophylactic HCV vaccine candidates						
Type of vaccine	Investigator	Vaccine	Stage	Immunity	adjuvant	Reference
Recombinant protein	Innogenetics	rE1 (T2S-918/InnoVac-C; gt-1b)	Phase 1 human	Antibody	Alum	Leroux-Roels et al., 2004
	Chiron/Novartis	rE1E2 (gt-1a) rE1E2 (gt-1a) rE1E2 (ΔHVR1, gt-1a)	Chimpanzee Phase 1 human Mouse, guinea pig	Antibody	MF59	Choo et al., 1994; Meunier et al., 2011 Frey et al., 2010; Law et al., 2013 Law et al., 2018
	CSL Ltd	rCore (gt-1a)	Phase 1 human	Antibody, T cell	ISCOMATRIX	Drane et al., 2009

	BPRC	rE1 or rE2 (gt-1b)	Chimpanzee	Antibody, T cell	Alum	Verstrepen et al., 2011
	Burnet Institute	rE2Δ123 (gt-1a)	Guinea pigs	Antibody	AddaVax	Vietheer et al., 2016
	NIHR, Nottingham, U.K.	E2 (ΔHVR1, consensus gt-1)	Guinea pigs	Antibody	CFA, IFA	Tarr et al., 2018
	University of Gdansk, University of Glasgow	E1E2 (HVR1 replaced with Flag tag; gt-1a, -1b, or -2a)	Mouse	Antibody	IFA	Krapchev et al., 2018
	Institut Pasteur of Shanghai	Trivalent rE2 (gt-1a, -1b, -3a)	Mouse, Rhesus macaques	Antibody	Alum	Wang et al., 2019
Virus-like particle	NIH	VLP	Chimpanzee	Antibody, T cell	AS01B	Elmowalid et al., 2007
	Monash University	Quadrivalet gt-1a/1b/2a/3a VLP	Mouse	Antibody, T cell	-	Christiansen et al., 2018

	Indian Institute of Science	VLP (gt-3a)	Mouse	Antibody, T cell	Alhydrogel	Kumar et al., 2016
	INSERM/Epixis	VLP, MV, Ad (E1 and/or E2, gt-1a)	Mouse and rhesus macaque	Antibody, T cell	-	Garrone et al., 2011
	INSERM	VLP (E1E2, gt-1a) VLP (E1 or E2, gt -1a)	Rabbit Rabbit	Antibody	AddaVax	Beaumont et al., 2013 Beaumont et al., 2016
Peptide	Intercell AG	7 HLA-A2 restricted peptides (IC41)	Phase 1 human	T cell	Poly-l-arginine	Firbas et al., 2010, 2006
Viral/plasmid vectored	Transgene Co.	DNA/MVA (core, E1, E2, NS3) DNA, SFV, Ad5, MVA (core, E1, E2, NS3)	Chimpanzee Rhesus macaque	Antibody, T cell Antibody, T cell	- -	Rollier et al., 2007 Rollier et al., 2016

	Okairos Co.	Ad6/Ad24 + DNA/Ad6/ChAd32 (NS-gt-1b)	Chimpanzee Rhesus macaque	T cell T cell	- -	Folgori et al., 2006 Fattori et al., 2006
	University of Oxford/Okairos Co.	Ad6/ChAd3 (NS-gt-1b) ChAd3/MVA (NS-gt-1b)	Phase 1 human Phase 1 human	T cell T cell	- -	Barnes et al., 2012 Swadling et al., 2014
	University of Oxford	ChAdOx1 (conserved sequence, gt1/3 and gt1-6)	Mouse	T cell	-	von Delft et al., 2018
	NIH/Okairos Co.	Ad/DNA (NS-gt-1b)	Chimpanzee	T cell	-	Park et al., 2012
	NYC Blood Center	rVV (C-NS3, gt-1b)	Chimpanzee	T cell	-	Youn et al., 2008
	Jining Medical University	rAAV (E2, gt-1b)	Mouse	Antibody	-	Zhu et al., 2018

Ad: adenovirus, ChAd: simian adenovirus (numbers refer to serotype), MVA: modified vaccinia Ankara virus, VV: vaccinia virus, AAV: adeno-associated virus, SFV: Semliki forest virus, MV: measles virurs, r: recombinant protein, gt: genotype, HLA-A2: human leukocyte A2, CFA: complete Freund's adjuvant, IFA: incomplete Freund's adjuvant, VLP: virus like particle, HVR: hyper variable region in HCVs E2 protein

1.7.3 HCV vaccines that have progressed to human trials

Despite almost three decades of HCV research, two main prophylactic vaccine strategies are moving forward in human clinical trials: (1) a recombinant E1 and E2 HCV glycoprotein vaccine that induces production of nAbs in a phase I trial (Novartis, formerly Chiron; (Frey et al., 2010; Law et al., 2013), and (2) a T cell vaccine comprised of a non-structural HCV 1b sequence encoded within viral vectors as a prime-boost strategy in phase I and II trials (Okairos & the University of Oxford; Barnes et al. 2012; Swadling et al. 2014).

1.7.3.1 The E1E2 protein vaccine

The E1E2 protein vaccine, pioneered by Novartis (formerly Chirion), is an antibody-based vaccine that has advanced to phase I human clinical trials.

In pre-clinical studies, vaccination of seven chimpanzees with envelope glycoproteins (gpE1E2 with MF59 oil-in-water emulsion adjuvant), displayed high titre antibody responsess with complete protection against homologous HCV genotype-1a challenge in the five highest responders (Choo et al., 1994). Further chimpanzee studies showed that none of the re-infected chimpanzees with genotype-1a had sterilising immunity from prior HCV exposure, but still did not develop chronic HCV infection unlike the unimmunised controls. Chimpanzees immunised with recombinant gpE1/E2 also demonstrated protection from HCV genotype-1a challenge and exhibited antibodies capable of neutralising HCVpp from genotypes-1a, -4a, -5a and -6a with limited protection to -2a and -3a (Meunier et al. 2011). In another study, chimpanzees were vaccinated with gpE1E2 that lacked the hyper variable region 1 (HVR1) and displaying reduced nAb titres compared to wildtype gpE1E2 (Law et al., 2018).

A phase I clinical trial was undertaken to assess the immunogenicity and safety of the HCV gpE1/E2 vaccine with an MF59 adjuvant at three dosages in humans (Frey et al. 2010). The vaccine was well-tolerated in all subjects at all dosages. All volunteers elicited high antibody titres capable of HCV neutralisation and modest magnitudes of vaccine-specific T helper cells (Frey et al. 2010). Characterisation of recombinant gpE1E2 vaccine-induced antibodies in the phase I clinical trial was further investigated (Ray et al. 2010). Serum samples from gpE1E2 vaccinated volunteers showed an increased avidity for HCV gpE1E2 over the course of vaccination indicating the selection of high-avidity memory B cells. Specific HCV epitopes: $E1_{313-327}$, $E2_{384-411}$ (within HVR1), $E2_{412-419}$ and $E2_{434-446}$, were detected in all volunteers with varying neutralisation of HCV (Ray et al. 2010). The breadth of the neutralising Abs (nAbs) elicited in gpE1/E2 vaccination was further elucidated in two follow-up studies using HCVpp containing gpE1E2 from different HCV genotypes (Law et al., 2013; Stamataki et al., 2011). Stamataki et al. demonstrated that E1/E2 vaccine-induced nAbs neutralised gpE1E2 from genotypes 1a H77, 1b (CON1 and OH8) and 2a (strain J6; Stamataki et al. 2011). Law et al. further showed greater neutralisation coverage to include a HCVpp panel expressing E1E2 from 1a (H77), 1b (J4), 2a (J6), 4a (ED43), 5a (SA13) and 6a (HK6a) and to a lesser extent E1E2 from genotypes 2b (J8), 3a (S52) and 7a (QC69), albeit in only 3 of 41 individuals (Law et al. 2013).

1.7.3.2 The genotype-1b NSmut viral vector vaccine

The second vaccine that is progressing through human clinical trials is a HCV genotype-1b non-structural sequence encoded in viral vector vaccines administered as a prime-boost vaccination (Barnes et al., 2012; Swadling et al., 2014). The immunogen contained many $CD4^+$ and $CD8^+$ T cell epitopes that are described in natural infection with the intention of inducing a high magnitude T cell response to multiple T cell epitopes, reflecting the broad T cell response seen in spontaneous HCV resolvers (reviewed by Abdel-Hakeem & Shoukry, 2014). The proof-of-concept for an HCV T cell vaccine was demonstrated in

rhesus macaque and chimpanzee studies (Fattori et al., 2006; Folgori et al., 2006). Vaccine-induced T cell responses were of a high magnitude that could recognise multiple HCV epitopes and subsequently protected chimpanzees from heterologous HCV challenge (Folgori et al. 2006).

Due to the likely presence of pre-existing immunity to human adenovirus in humans, a prime boost regimen using rare serotypes of human adenovirus (Ad6) and Chimpanzee adenovirus (ChAd3) was assessed in humans (Barnes et al. 2012). Flow cytometry analysis revealed vaccine-induced high magnitude, broad $CD4^+$ and $CD8^+$ T-cells subsets that secreted IL-2, IFNγ and TNFα and could recognise, to a limited degree, HCV genotype-3a (Barnes et al. 2012). In this study, the induction of cross-reactive anti-adenovirus antibodies limited the boosting effect of ChAd3. To further counter pre-existing host immunity to adenovirus, an additional prime-boost strategy was conducted with a ChAd3 priming vector and a modified vaccinia virus Ankara (MVA) boosting vector (Swadling et al., 2014). The ChAd3-MVA heterologous regimen demonstrated optimal prime and boosting with the production of broad $CD4^+$ and $CD8^+$ T cell responses of high frequency and polyfunctionality compared to previous human trials and analogous with $CD4^+$ and $CD8^+$ responses observed in spontaneous HCV clearance (Swadling et al. 2014). Functional T cell analysis showed that a ChAd3-MVA prime-boost vaccination regimen produced sustained levels of memory and effector T cell subsets with improved proliferative and functional capacity following MVA boost vaccination (Swadling et al. 2014). Current phase II trials in high-risk IVDU populations in Baltimore, MD, USA. San Francisco, CA, USA, and Albuquerque, NM, USA may provide proof of efficacy that a T cell vaccine prime-boost regimen can induce the cellular arm of the immune system to prevent persistent HCV infection in a susceptible population (ClinicalTrials.gov NCT01436357).

1.8 Obstacles to HCV vaccine development

HCV vaccine development has been hindered by a number of obstacles which need to be addressed if an HCV vaccine is to be effective at targeting diverse HCV genotypes and utilised as part of a broader strategy to eliminate HCV by 2030. These include:

1.8.1 Immunological challenges

While natural history studies and animal challenge studies have revealed, to some degree, the immune correlates of protection against HCV, protective immunity is not fully understood. Specifically, what constitutes a protective anti-HCV immune response such that 25% of those with acute infection clear the virus? It is clear that a sustained $CD4^+$ T cell response is critical to clearing HCV as the absence of $CD4^+$ T cells impairs $CD8^+$ T cell responses, rendering them dysfunctional and facilitating selection of class I T cell epitope escape variants (Arash Grakoui et al., 2003). Yet, it is not clear how $CD4^+$ T cell responses are rendered exhausted and dysfunctional in CHC. In parallel, the mechanisms of $CD8^+$ T cell exhaustion are not fully understood. Furthermore, while both antibody and T cell responses are associated with HCV clearance, it is not known how these two arms of the adaptive immune response coordinate in viral clearance. Recently, it was revealed at the annual HCV2018 international conference that T follicular helper cells (Tfh) are highly active early in acute infection of individuals who go onto clear HCV but not in CHC individuals (Shoukry group), in line with previous observations (Raziorrouh et al., 2016). Considering Tfhs role in somatic mutation and affinity maturation of the B cell response in the germinal centres (Crotty, 2015), it is plausible that anti-HCV bNAbs are generated through a robust Tfh response, signifying the importance of a coordinate T cell and antibody immune response in HCV clearance and therefore in a vaccine-induced immune response.

While HCV populations in the infected host are extremely diverse, an efficacious immune response may be compounded by the heterogeneity of the human population. For the prospects of a T cell vaccine, it is important to include epitopes that can be presented by a plethora of MHC alleles to ensure most of the target population is accounted for. A prophylactic HCV vaccine would also need to overcome host factors associated with high-risk HCV populations such as ethnicity, age, liver-disease status, opioid usage, HIV co-infection, and previous HCV treatment that may alter the immune response and therefore require tailoring of vaccination regimens to be effective. In the example of cured high-risk populations, such as IVDUs, some studies have demonstrated partial restoration of the HCV-specific immune response (Knapp et al., 2010; Martin et al., 2014; Wieland et al., 2017). Specifically, in CHC individuals, $CD8^+$ T cells during and after treatment expressed both memory and exhaustion markers—$TCF^+CD127^+PD1^+$—and produced lower levels of IFNγ and TNFα upon antigen stimulation compared to conventional $CD8^+$ T cells (D. Wieland et al., 2017). Whether partial HCV-specific immunity will protect from subsequent HCV infection is not known. One study in chimpanzees with DAA-cured CHC showed that an HCV-specific T cell response could be induced but was narrow-focussed and did not prevent HCV persistence (Callendret et al., 2014).

Use of prime boost vaccination strategies should be considered to expand optimal HCV-specific immune cell populations such as highly proliferative and polyfunctional $CD4^+$ helper T cell and $CD8^+$ cytolytic T cell populations in addition to affinity matured B cell populations that can produce bNAbs. Novel prime-boost strategies that aim to prime T cells in the periphery and then relocate them to the liver should also be considered to promote high levels of intrahepatic T cell populations, including resident memory T cells (T_{RM}). For example, a prime-target vaccination strategy uses an intramuscular prime vaccination to generate high numbers of T cells and an intravenous boost vaccine to express the immunogen in the liver to target T cells to the liver (Gola et al. 2018).

1.8.2 Lack of pre-clinical small animal models

Humans and chimpanzees are the only two species that are permissive to HCV infection. Proof of immunogenicity of the two most clinically advanced HCV vaccines were first demonstrated in chimpanzees (Choo et al. 1994; Folgori et al. 2006), before being assessed in humans (Frey et al. 2010; Barnes et al. 2012; Swadling et al. 2014). However, since 2011, use of chimpanzees as experiment animal models is no longer permitted (Committee on the Use of Chimpanzees in Biomedical and Behavioral Research (U.S.) 2011). Animal models such as rodents are the preferred experimental animal model due to their small size, ease of handling, genetic manipulation, and scalability. While mice have been used to assess the HCV life cycle, they are immunodeficient rendering them insufficient for assessment of vaccines (Burm et al., 2018). Immunocompetent rodents that are susceptible to hepacivirus infection have been recently described such as the Norway rat hepacivirus (NrHV). NrHV displays chronic infection in rats, exhibiting viral intrahepatic persistence, and a disease profile analogous to human CHC (Trivedi et al., 2017). In mice, NrHV is a self-limiting infection that can induce chronic disease through depletion of $CD4^+$ T cells (Billerbeck et al., 2017). The NrHV model of infection in rats and mice warrants consideration for future assessment of immunogenicity and protective efficacy of novel prophylactic HCV vaccine candidates.

1.8.3 Cohorts for clinical trials

As HCV infection is asymptomatic upon acute infection, the majority of acute infections remain undiagnosed (WHO, 2017). However, high-risk populations such as IVDUs and men who have sex with men (MSM) provide obvious cohorts to assess vaccine efficacy. Yet, a number of challenges exist that limit enrolment of individuals into trials and make working with these cohorts challenging (Maher et al., 2010). For example, IVDU populations typically suffer from multiple social, psychological, and marginalisation issues

that need to be considered when undertaking human clinical trial phase II efficacy studies. Another cohort that could be leveraged for HCV vaccine efficacy trials, is healthy volunteers that are challenged with HCV. The rationale is that the effective DAA triple therapy can treat volunteers should the vaccine fail and viraemia ensue. This approach has been successfully implemented in the malaria field (Rampling et al., 2016, 2018). Nevertheless, ethical implications in trial design and execution remain complex.

1.8.4 HCV sequence variability

HCV exhibits high genetic diversity and is classified into seven major genotypes and over sixty-four subtypes, differing by more than 30% sequence diversity (Smith et al. 2014). An HCV-infected individual harbours a viral population of closely genetically related virus particles—a 'quasispecies'—that has high sequence variation, a result of HCVs error prone RNA polymerase, NS5b, that possesses a high mutation rate of 10^{-5}–10^{-4} nucleotides per replication cycle (Behrens et al., 1996). Therefore, HCV has an inherent mutability in the protein sequences (epitopes) that are targeted by host T cells, B cells, and antibodies. The emergence of viruses with variant sequences that are no longer effectively presented to or recognised by immune cells is termed 'immune escape', and this is a common mechanism employed by HCV to rapidly escape immune selection pressure (Bowen and Walker 2005). For example, the HCV envelope proteins, gpE1E2, are highly diverse, especially in immunodominant epitopes such as those found in HVR1, to facilitate subversion of the neutralising antibody response (Mondelli et al., 2001). In the context of the T cell response, variant epitope sequences can lead to loss of recognition of HCV-infected cells as sequence mutation may alter peptide processing, MHC peptide loading, recognition of MHC:peptide comples, and shift epitope hierarchies (Bowen and Walker 2005; Timm and Walker 2015).

HCVs vast genetic diversity makes vaccine design challenging. Lack of cross-reactivity in vaccine-induced immune responses has been observed in animal and human studies

assessing vaccine candidates that aim to elicit antibodies and T cells (Houghton, 2011). In phase I clinical trials evaluating the recombinant E1E2 glycoprotein vaccine candidate, high antibody titres were detected in healthy volunteers but only a fraction had the capacity to neutralise diverse heterologous HCV genotypes (Ray et al., 2010). In human trials assessing the HCV gt-1b T cell vaccine candidate, NSmut, IFNγ responses were markedly lower when assessing the vaccine-induced T cell responses against non-gt-1b genotypes and epitope variants (Kelly et al., 2015; Swadling et al., 2014). In natural HCV infection, anti-HCV T cell responses are genotype-specific with immunodominant T cell epitopes exhibiting low homology, if any, between genotypes (Harcourt et al., 2003; Humphreys et al., 2012; Lunburger et al., 2018; von Delft et al., 2015).

An ideal HCV vaccine should induce robust cross-reactive immune responses against multiple genotypes and recognise epitope variants to ensure the vaccine is efficacious in any setting regardless of the prevalent infecting genotype and to minimise successful immune escape that leads to HCV persistence. Therefore, HCV sequence variability remains a critical aspect of the design and development of a 'universal' HCV vaccine.

1.9 Vaccine strategies to overcoming HCV sequence variability

An important criterion of a prophylactic HCV vaccine is the induction of a T cell response against multiple HCV genotypes and epitope variants. An intergenotypic immune response—a response that targets multiple genotypes—would be critical in endemic regions where multiple HCV genotypes circulate, in addition to recognising diverse epitopes in the virus quasispecies in the infected host that are poised for immune escape. Therefore, vaccination strategies that aim to induce cross-reactive antibodies in addition to T cell responses to conserved epitopes may be effective against multiple HCV genotypes and epitope variants within genotypes. The premise of targeting conserved viral sequences in vaccine design is based on the hypothesis that mutation of conserved sequences comes

at a detrimental fitness cost to the virus due to the functionally importance of conserved sequences (Lindenbach & Rice, 2005). Vaccines containing T cell epitopes may be easier to engineer due to the linear and discontinuous nature of peptides presented as part of the MHC:peptide complex in cellular immunity. Conversely, targeting conserved B cell epitopes may prove more challenging than T cell epitopes due to the conformational shape that B cell epitopes take as part of their respective protein's tertiary structure, for example envelope glycoproteins, gpE1E2. Methods to select conserved viral sequence include identifying conserved sequences between variants ('conserved'), selecting the most frequently occurring amino acids at each position in a sequence within a population ('consensus'), selecting an ancestral sequence with shared homology to all circulating strains ('ancestral'), and algorithmic calculations to maximise epitope coverage within a sequence ('mosaic'). Each approach has advantages and disadvantages as outlined in **table 2**. For example, a protein sequence that is the consensus of all genotypes or subtypes may contain dominant epitopes which include immunodominant decoy epitopes and escape variants that may be genotype-specific, non-neutralising, and have the capacity to rapidly mutate. Conversely, while conserved HCV sequences can exclude immunodominant variable epitopes, it is possible that conserved epitopes are not sufficiently processed in the cytosol, have limited utility in the host MHC presentation pathway, display insufficient HLA avidity, target a low frequency cognate TCR population, and consequently, be immunologically inert. Nonetheless, a number of vaccine approaches have aimed to overcome HCV sequence variability through the use of consensus, ancestral, or mosaic sequences to target conserved viral sequences. Alternative approaches that do not select for conserved or consensus sequences include immunisation with multiple genotype-specific antigens and use of genetic adjuvants to enhance suboptimal genotype-specific immune responses.

Table 2. Vaccine strategies to overcome viral variability

Vaccine design	Strategy	Possible Benefits	Possible Limitations	Vaccine	Reference
Consensus sequence	Minimise sequence divergence (distance) between antigen and circulating virus	Maximise similarity to many genotypes	Artificial sequence (circulating virus sequence not used or represented) Lack of tertiary conformational structure Immunodominant variant epitopes targeted Escape variants may be in the consensus sequence	HCV HIV MERS Influenza	Wijesundara et al., 2018 Tarr et al., 2018 De Groot et al., 2004 Muthumani et al., 2015 Weaver et al., 2011
Ancestral sequence	Minimise sequence divergence (distance) between antigen and circulating virus	Maximise similarity to many genotypes	Artificial sequence (circulating virus sequence not used or represented) Lack of tertiary conformational structure Immunodominant variant epitopes targeted Escape variants may be in the consensus sequence	HCV HIV Influenza	Burke et al., 2012 Kothe et al., 2006 Ducatez et al., 2011

Mosaic sequence	Improved T cell epitope coverage	Increased breadth (multiple Ags) and depth (epitope variants)	Artificial sequence (circulating virus sequence not used or represented) Immunodominant variant epitopes targeted including escape variants	HCV HIV	Yusim et al., 2010 Yusim et al., 2013 Barouch et al., 2010 Abdul-Jawad et al., 2015
Conserved sequence	Selection of most conserved sequences within proteome across circulating genotypes	Conserved epitopes under fitness constraint Increased epitope valency (subdominant epitopes) Vaccine sequence match circulating genotypes	Potentially immunologically inert or less immunogenic Low frequency of TCR populations Limited applicability to full-length protein Ag Inclusion/exclusion of epitopes regardless of associated protection or escape	HCV Influenza	von Delft et al., 2018 Drane et al., 2009 Klade et al., 2009 Firbas et al., 2006 Antrobus et al., 2014 Coughlan et al., 2018
Multiple Ag (different gt)	Increase immune responses to multiple gt's	Increased gt-specific immunogenicity	Immunodominant variant epitopes targeted Variable regions included Non-neutralising epitopes included Escape epitopes	HCV	Youn et al., 2005 Wijesundara et al., 2018 Christiansen et al., 2018 Wang et al., 2019

		Vaccine sequence match multiple circulating genotypes			
Adjuvanted Ag	Increase immune responses to all gt's and epitope variants	Increased immunogenicity	Immunodominant variant epitopes targeted Response may still be skewed to one gt	HCV HIV	Capone et al., 2014 Ondondo et al., 2016
Gt: genotype, Ag: antigen, TCR: T cell receptor, HLA: human leukocyte antigen					

Table 2. Vaccine strategies to overcome viral variability

1.10 Background work to thesis

This thesis is concerned with the development of a prophylactic vaccine strategy to induce an HCV-specific T cell response that targets conserved HCV sequences. The rationale to this work stems from the first-generation HCV T cell vaccine—a genotype-1b non-structural sequence encoded in viral vectors—which displayed reduced immunogenicity to non-genotype 1b antigens compared to genotype-1b antigens, and abrogated T cell responses targeting sequence variants at immunodominant epitopes in human trials (Barnes et al., 2012; Kelly et al., 2015; Swadling et al., 2014). The HCV T cell vaccines used in this thesis constitute the second generation of our viral vectored T cell vaccines and they encode gene segments derived from conserved HCV sequences (from the Los Alamos database) across all subtypes within (1) genotypes 1 and 3 (72 sequences used) and (2) genotypes 1 to 6 (216 sequences used; von Delft & Donnison et al. 2018). To reduce sampling bias, only full-length HCV sequences were used. This ensured equal coverage of conserved and variable viral regions, since variable viral regions are difficult to sequence and possibly underrepresented in proteome databases. Furthermore, sequences from different research groups and countries were used for each strain and manually checked for human origin. It was hypothesised that immune-driven amino acid mutation in conserved sequences results in a detrimental fitness cost to the virus rendering immune escape redundant. Furthermore, conserved HCV sequences display homology between genotypes and subtypes suggesting that vaccines will be immunogenic to multiple HCV genotypes. However, as conserved HCV sequences may have retained sequence homology between genotypes because of immunological inertia, this thesis investigates the capacity of vaccines that encode conserved HCV sequences to elicit T cell responses *in vivo.* Prior background work to thesis identified known B cell and T cell epitopes in the conserved HCV vaccine immunogens and demonstrated *in silico* epitope prediction (von Delft & Donnison et al. 2018).

Secondly, this thesis utilises the ChAd prime and MVA boost vaccination regimen to investigate whether primed T cell epitopes that are conserved between two different HCV genotype-specific immunogens (non-structural protein sequences: NS) can be preferentially boosted instead of genotype-specific T cell epitopes that are not conserved between the two immunogen sequences.

Lastly, this thesis proposes the generation of a third-generation viral vector HCV vaccine that incorporates a conserved sequence T cell immunogen and an antibody component that utilises the conserved ectodomain of HCVs envelope protein, E2 (McCaffrey et al., 2016; Vietheer et al., 2016).

1.11 Hypotheses tested in this thesis

1.11.1 Data chapter 1 hypothesis

Simian adenovirus vector vaccines encoding conserved HCV proteomic regions across viral subtypes are immunogenic *in vivo.*

1.11.2 Data chapter 2 hypothesis

A prime-boost HCV vaccination strategy that employs different HCV genotype-specific immunogens encoded in a ChAd prime, MVA boost viral vector strategy can generate T cells that target conserved epitopes between the two genotypes, therefore eliciting a T cell response against both HCV genotypes.

1.11.3 Data chapter 3 hypothesis

A conserved sequence HCV T cell adenoviral vectored vaccine and the recombinant E2Δ123 protein vaccine can be combined in a mixed modality prime-boost vaccination strategy to generate HCV-specific intergenotypic T cell responses and broadly neutralising antibody responses.

2 MATERIALS AND METHODS

Chapter 2 describes the materials and methods that are used throughout this thesis. Chapter specific methods such as vaccination regimens are described in each chapter.

2.1 Vaccine viral constructs & immunogens

2.1.1 Vaccine production

The HCV vaccine immunogens were encoded in a transgene immunogen downstream of an ATG start sequence, Kozak sequence, and CMV promoter. The HCV immunogen was inserted into the donor vector via In-Fusion™ cloning to generate the shuttle vector encoding the HCV immunogen. The immunogen encoding shuttle vector was cloned into the ChAdOx1 destination vector using ThermoFisher's LR gateway cloning method and linearised using *PmeI* restriction site and transfected into T-REx™-293 cells (Thermo Fisher Scientific) for generation of viral vector vaccines. ChAdOx1 HCV vaccines were manufactured by the Viral Vector Core Facility (Jenner Institute, University of Oxford, Oxford, U.K.).

2.1.2 Viral vectors

In all the experiments described in this thesis, the ChAdOx1 viral vector was used to encode the HCV vaccine immunogens as previously described (von Delft et al., 2018). The

design of ChAdOx1 has been previously described (Dicks et al., 2012). The modified vaccinia Ankara (MVA) vector was used for boost vaccinations.

2.1.3 Vaccine nomenclature

The following ChAdOx1 vaccines were used throughout this thesis. Chapter specific vaccines are stated in that chapter's materials and methods.

2.1.3.1 Conserved sequence long and short HCV immunogens

The ChAdOx1 conserved sequence HCV T cell vaccines encode the conserved HCV sequences of (1) genotype-1 and -3 subtypes and (2) all subtypes in genotype-1 to -6 as previously described (von Delft et al., 2018). They are referred to in this thesis as '*ChAd-gt1/3*' and '*ChAd-gt1-6'*. Both vaccines have long and short immunogen versions, i.e. the shorter gt1/3 immunogen of 1000 amino acids is referred to as '*ChAd-gt1/3S*'. The longer gt1/3 immunogen of 1500 amino acids is referred to as '*ChAd-gt1/3L*'.

2.1.3.2 Subtype-1b HCV T cell immunogen nomenclature

The ChAdOx1 HCV subtype-1b T cell vaccine encodes the subtype-1b HCV NS3-5 sequence and is referred to as '*ChAd-gt-1b-NS*'. The HCV subtype-1b ChAdOx1 T cell vaccine does not contain an NS5b mutation to render the NS5b polymerase dysfunctional, as was previously described in human clinical trials (Barnes et al., 2012; Swadling et al., 2014).

2.1.4 Western blot

HEK293 cells were either infected with ChAdOx1-HCV conserved sequence vaccines, at a MOI of 100, or transfected using lipofectamine 2000 transfection reagent with 1mg of plasmids expressing HCV immunogens. 24 hours post infection or transfection, cells were lysed in 100ml of SDS-PAGE sample buffer, heated at 95^{o}C for 5 minutes and loaded on to 4-15% SDS-PAGE gels and the separated proteins were transferred onto nitrocellulose

membrane. The blots were then incubated overnight at 40ºC with 5% skim milk powder diluted in PBST (PBS with 0.1% Tween-20). Subsequently, the blots were incubated with primary antibody and secondary antibody, diluted in 5% skim milk powder diluted in PBST, at room temperature for 1 hour and washed 4x with PBST. Finally, the protein bands were visualized by incubating the blots with chemiluminescent substrate and imaging in a photographic image collector. The anti-HCV core 1b antibody (C7-50, AB2740, Abcam) was used to detect AA sequence 21–40 (DVKFPGVGQIVGGVYLLPR) as it is contained within all HCV immunogens.

2.2 Vaccine study design

2.2.1 Animals and ethics

All mouse studies were performed at the Biomedical Services Building, Oxford, according to U.K. Home Office Regulations (project license numbers 30/2744 and 30/3293) and approved by the local ethical review board at the University of Oxford. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedure) Act, 1986. Groups of age-matched female mice were used throughout this thesis.

2.2.2 Vaccination route

After a 1-week adaptation period after arrival at the BMS animal facility in Oxford, mice were vaccinated intramuscularly (I.M.) in the left quadricep with 40μL of viral vector vaccine solution (vaccine in sterile PBS). For intravenous (I.V.) vaccinations, mice were pre-warmed in a hot box, one at a time, to dilate the tail vein and subsequently restrained in a cylindrical mouse constrainer. Using a 29G needle, 100μL of vaccine solution was injected into the lateral caudal vein.

2.3 Organ harvest & processing

Mice were culled by schedule 1 (cervical dislocation confirmation) and organs were harvested immediately.

2.3.1 Spleen harvesting

Mouse spleens were extracted immediately after schedule 1 and left on ice until mechanical processing within two hours. Using a 40µn cell strainer, mouse splenocytes were mechanically processed by pushing the spleen into the membrane of the cell strainer using a sterile plunger. Cell suspensions were centrifuged at 1500rpm for five minutes and the pellets resuspended in 1mL of ACK lysis buffer (Gibco, NY, USA). The reaction was stopped at 60 seconds by addition of excess PBS (to 40mL total volume). Cells suspensions were centrifuged at 1500rpm for five minutes and pellets resuspended in 10mL of R10 media for cell counting. Cell yields were calculated using a Guava Personal Cell analysis system (Merck Millipore 0100-14230) and the Muse® Cell Analyser (Merck Millipore). The machine was calibrated prior to cell counting using Guava check beads (16-0040).

2.3.2 Blood collection

Mouse blood was collected using a tail bleed and terminal cardiac bleed.

For tail bleeds, mice were pre-warmed, one at a time, in a hot box to dilate the tail veins and then restrained in a cylindrical mouse constrainer. Using a sterile 29G needle, the needle tip was inserted into the paired lateral caudal veins in the tail for venous blood collection. Blood was collected, dropwise in sterile 300µL Microvette® tubes without EDTA (CB300 K2E, Sarstedt).

For terminal cardiac bleeds, mice underwent schedule 1 one at a time, and immediately received a cardiac puncture after cessation of the heartbeat. Blood was extracted using a 18G needle via capillary action and collected in a 1.5mL sterile Eppendorf tube.

2.4 Peptides and antigens

HCV genotype-1a (H77), -1b (J4), and -3a (K3a650) peptides of 15-18mer amino acids in length overlapping by 11 amino acids spanning the whole HCV proteome (optimal for both $CD4^+$ and $CD8^+$ T cell activation) were obtained from the National Institute of Health (NIH, MA, USA) and used in T cell assays. Peptides were initially dissolved in dimethyl sulfoxide (DMSO) at 40mg/mL and subsequently pooled into 10 minipools at 300µg/mL labelled A (core), B (E1), C (E2), D (NS2), F (NS3p, protease), G (NS3h, helicase), H (NS4), I (NS5a), L, (NS5bI, amino acids 2421-2718), and M (NS5bII, amino acids 2719-3011). Each pool was split into minipools (H1, H2, H3, etc.) and individual peptides within each minipool (H1-1, H1-2, H1-3, etc.). See **Appendix 2: HCV vaccines and peptide sequence comparison**.

2.5 Sequence analysis

HCV amino acid sequences were aligned and analysed in Aliview (version 1.18). HCV subtype amino acid sequences – as identified by the International Committee on the Taxonomy of Viruses, June 2017 – were obtained from UniProt.org. Basic Local Alignment Search Tool (BLAST) analysis was done using the protein BLAST tool on the NCBI website (National Centre for Biotechnology Information).

2.6 Enzyme-linked immunospot assay (ELISpot)

2.6.1 ELISpot assay

Multiscreen®$_{HTS}$ IP filter plates (PVDF; Merk Millipore) were pre-wetted with 20µL of 35% ethanol per well for no longer than 60 seconds. After three PBS washes, plates were pre-coated with anti-mouse anti-IFNγ mAb (AN18, 0.5µg/well, 1:200 dilution, Mabtech, Sweden) overnight. Plates were subsequently washed three times with PBS and blocked with R10 for two hours at 37°C. After blocking, cells were plated at 1-2x10^5 cells per well

in 50µL R10 media and stimulated for 20-24 hours at 37°C with 50µL HCV genotype-1a, -1b, -3a peptide pools, minipools, or peptides (3µg/mL final peptide concentration in 100µL total R10 media; NIH, MD, USA), a DMSO negative control and a concanavalin positive control (conA, 10µg, Sigma). After incubation, cells were washed seven times with PBS and incubated at room temperature with 50µL anti-mouse IFNγ mAb R4-6A2 biotinylated (1:2000 dilution, Mabtech, Sweden). After three hours, cells were washed four times with PBS and incubated at room temperature with 50µL anti-biotin alkaline phosphatase for two hours (1:750, Vector Laboratories, Burlingame, CA, USA). Filtered room temperature BCIP/NBT phosphatase substrate (Thermo Scientific, IL, USA) was added to the wells (50uL) and allowed to develop the plate for fifteen minutes before being washed thoroughly with DI water and read on an ELISpot plate reader.

2.6.2 Defining the cut-off for the ELISpot assay

Positive ELISpot wells were determined as: 1) having a spot forming unit (SFU) count of greater than the DMSO negative control mean SFU plus three standard deviations, and 2) a greater SFU count than the mean SFU plus three standard deviations from a naïve unvaccinated control group stimulated with HCV peptide pools.

2.6.3 Measuring the intergenotypic response

Intergenotypic T cell responses were measured by determining the ratio of the genotype-specific T cell responses: a ratio to measure how comparable the genotype-specific T cell response is. The intergenotype ratio is calculated with the following formula:

$$\frac{\text{lowest magnitude T cell response}}{\text{highest magnitude T cell response}} = \text{Intergenotype ratio}$$

For example, for an ELISpot response measured in SFU/10^6 splenocytes:

$$\frac{300\ (\text{gt3a T cell total magnitude})}{1500\ (\text{gt1b T cell total magnitude})} = 0.2\ \text{intergenotype ratio}$$

The intergenotype ratio ranges between 0 and 1, with 1 being highly intergenotypic due to the generation of comparable genotype-1b and -3a specific T cell responses. Importantly, the intergenotype ratio is not reflective of a high or low total magnitude T cell response. For a comparison of the genotype-1b and -3a sequences, see **Appendix 4: Genotype-1b and -3a vaccine sequences.**

2.7 Fluorescence activated cell sorting (FACS)

2.7.1 Fluorescence minus one (FMO) controls

When antibodies did not display a clear bimodal staining profile, a fluorescence minus one control was performed. Cells of interest were stained with the full ICS antibody panel minus one antibody and a comparison between stains with and without this antibody was made to determine an appropriate gating strategy to define cells that were positive for that antibody.

2.7.2 Intracellular cytokine staining (ICS)

ICS was performed as described previously on fresh mouse cells at $1x10^6$ cells/100µL (von Delft et al., 2018). Mouse splenocytes were stimulated using HCV genotype-1a, -1b, and -3a peptides in pool combinations (A+B+C = Core, E1, E2; F+G+M = NS3-4, I+L+M = NS5a-b, 1.5µg/mL, 15-18mers overlapping by 11 amino acids). An unstimulated negative control (DMSO) and PMA (phorbol 12-myristate 13-acetate)/ionomycin positive control (50 and 500ng/mL, respectively) were used. Cells were stimulated for 6 hours with peptide pools and after 2 hours 4µg/mL GolgiPlug™ (BD) was added. After the 6-hour incubation, the plates were stored overnight at 4°C. The following day, cells were centrifuged at 1500rpm for 3 minutes with the supernatant discarded and the pellet resuspended in FACS

buffer (PBS, 2% FCS, 0.1% NaN3, 5mM EDTA), and subsequently centrifuged again at 1500rpm for 3 minutes. The supernatant was discarded, and the pellet resuspended with a staining solution containing fixable Near-IR live/dead dye (Life Technologies, Carlsbad, CA, USA) and surface markers: CD3-efluor450, CD4-AlexaFluor700, and CD8-peridinin chlorophyll protein (PerCP) Cy5.5 (see **table 3**).

Table 3: Staining antibody panel

Marker	Stain	Clone	Dilution	Supplier	Ref. No.
live dead	Surface	-	1:1000	Invitrogen	L34976
CD4	Surface	GK1.5	1:50	eBioscience	56-0041-82
IL-2	Intracellular	JES6-5H4	1:25	Biolegend	503810
CD3	Surface	17A2	1:50	eBioscience	18-0032-82
CD8a	Surface	53-6.7	1:100	eBioscience	45-0081-82
IFNγ	Intracellular	XMG1.2	1:100	BD	12-73-11-82
TNFα	Intracellular	MP6-XT22	1:100	eBioscience	11-7321-41

Table 3. Staining antibody panel

After a 30-minute stain at 4°C in the dark and a subsequent wash with FACS buffer and centrifugation, pellets were resuspended with either 1) fixation/permeabilization solution (BD Biosciences, San Jose, CA, USA) for immediate intracellular staining or 2) 1% formaldehyde for 30 mins followed by resuspension in PBS for overnight storage at 4°C in PBS for ICS the following day. After permeabilization (with BD Perm/Wash™ buffer 10x for cells left overnight after 1% formaldehyde fixation), cells were washed with 150µL perm/wash buffer (1:10 dilution with milliQ water; BD Biosciences, San Jose, CA, USA), centrifuged at 1500rpm for 3 minutes and stained with intracellular staining solution (IFNγ-phyceoerythrin (PE), TNFα-fluoroscein isothiocyanate (FITC), and IL-2-

allophycocyanin (APC), see **table 3**) in perm/wash buffer for 30 minutes at 4°C in the dark. Following the stain and a subsequent 1500rpm 3-minute centrifugation, the pellets were washed twice with perm/wash buffer and resuspended in 200μL FACS buffer to be run on the LSRII flow cytometer.

All ICS data are corrected for background by subtracting the cytokine production as a percentage of $CD4^+$ or $CD8^+$ T cell subsets in a matched unstimulated DMSO negative control sample.

2.7.3 FACS analysis

Photomultiplier tube (PMT) detector setup of flow cytometer, BD LSRII (Peter Medawar Building for Pathogen Research, Oxford, United Kingdom), is shown in

Table 4. Gating and analysis were performed in FlowJo (TreeStar, v10.5, Ashland, OR, USA). Cytokine co-expression within T cell subsets was determined using Boolean gating in FlowJo and graphs produced in Pestle (v1.8), and SPICE (NIAID, NIH, v5.35).

Table 4: flow cytometer detector setup (LSRII)				
Excitation (nm)/laser	Detector	Bandpass filter	Longpass filter	Fluorochrome
633 /red	A780	780/60	755	APC-C7, APC-Alex760
	B730	730/45	710	Alexa Flour 700
	C670	670/14	-	APC, Alexa 647
405 /violet	A655	655/8	630	Qdot 655
	B605	605/12	595	Qdot 605
	C585	585/15	575	Qdot 585
	D560	560/20	545	Qdot 565, Pacific Orange, BV510, BV605, V500
	E525	525/50	475	Qdot 525, AmCyan
	F450	450/50	-	Pacific Blue, eFluor 450, BV421, BV450
488 /blue	A780	780/60	755	PE-Cy7
	B695	695/40	685	PerCP-Cy5.5
	C670	670/14	655	PE-Cy5, PerCP
	D610	610/20	600	PE-Texas Red, ECD
	E575	575/26	550	PE
	F530	530/30	505	FITC, Alexa Fluor 488
	G488	488/10	-	SSC

Table 4. Flow cytometer detector set up

2.8 Antibody assays

2.8.1 ELISA

Nunc Maxisorb flat bottom 96 well plates were incubated overnight at 4°C with 50µL/well of 2µg/mL monomeric HCV (H77c) E2 (RBD or Δ123) or H77c peptides specific for HCV E2 epitopes I, II, or III in 50mM carbonate bicarbonate buffer at pH9.6. The next day, plates were washed twice with PBST (PBS + 0.05% Tween20) and then blocked with 100µL/well of BSA_{10}PBS for 1 hour at room temperature. After a further four washes with PBST, mouse serum at a dilution of 1:20 in BSA_5PBST was added to the first row with half logarithmic serial dilutions of the mouse serum, titrating down the plate so that every second row was diluted by 10-fold (e.g. 1:20, 63.2, 200, 632, 2000, 6320, 20000, etc.). Positive controls used were HCV1, mAb24, mAb44, AR3C anti-HCV antibodies at 1µg/mL in BSA_5PBST. After a one-hour incubation at room temperature, the plate was washed four times with PBST. Polyclonal rabbit anti-mouse horseradish peroxidase (HRP)-conjugated antibody (Dako) was added at 1:1000 dilution of 1mg/mL per well, followed by a one-hour room temperature incubation and four subsequent washes with PBST. A reaction mix of 1 tablet of 3',3',5',5 tetramethylbenzidine dihydrochloride (TMB; Sigma-Aldrich) in 10mL of phosphate citrate buffer was prepared. Just before use, 2µL of hydrogen peroxide was added to the TMB substrate reaction mixture. To each well, 100µL of TMB reaction mix was added and left to develop for four minutes after which 50µL of HCL was added to stop the reaction. Plates were read on microplate reader (CLARIOstar) at absorbance 450-630nm.

2.8.2 CD81-LEL inhibition assay

Nunc Maxisorb flat bottom 96 well plates were incubated overnight at 4°C with 50µL/well of 5µg/mL MBP-CD81-LEL prepared in 50mM carbonate bicarbonate buffer at pH9.6. In

the top row of a U-bottom 96 well starstead plate, 58.5µL of BSA_5PBST (PBS + 0.05% Tween20) and 14.6µL of serum (1:5 dilution) was added with half logarithmic serial dilutions of the mouse serum, titrating down the plate. For a positive control, 73.1µL of control antibodies, mAb24 and HC84.27 anti-HCV antibodies at 5ug/mL were used. For a negative control, BSA with no serum and E2 only was used and the BSA result was result subtracted from all other conditions. To all wells in the starstead plate, 50µL of E2RBD at 1µg/mL was added, mixed, and incubated for one hour at room temperature. The MBP-CD81-LEL coated plate was washed twice with PBST and blocked with BSA10PBS for one hour at room temperature. From the starstead plate, 80µL of the sera/E2RBD mix was transferred from the dilution plate to the CD81 plate and incubated for two hours at room temperature. After the incubation phase, the CD81/sera plate was washed four times with PBST and 50µL of mouse anti-His tag antibody (Rockland) was added at a 1:500 dilution and incubated for one hour. Following four washes with PBST, 50µL of polyclonal rabbit anti-mouse horseradish peroxidase (HRP)-conjugated antibody (Dako) was added at 1mg/mL (1:1000 dilution) to all wells. Following a one-hour incubation and PBST wash, a reaction mix of 1 tablet off 3',3',5',5 tetramethylbenzidine dihydrochloride (TMB; Sigma-Aldrich) in 10mL of phosphate citrate buffer (2µL of hydrogen peroxide added just before use) was prepared and 100µL added to all wells and allowed to develop for four minutes. The reaction was stopped after the addition of 50µL of HCL and the plate was read on the microplate reader (CLARIOstar) at 450nm absorbance.

2.8.3 HCV pseudoparticle and cell culture neutralisation assay

Serial dilutions mouse serum was added to HCVpseudoparticles (HCVpp, gt-1a) and incubated for one hour at 37°C before added to Huh7.5 cells that were seeded twenty-four hours earlier at 30,000 cells/well in 48 well plates. The inoculum was removed after a four-hour incubation at 37°C and replaced with DMF10 media containing non-essential amino

acids (NEAA) for seventy-two hours. Cells were washed with PBS before lysis in Cell Lysis Buffer (Promega). Firefly luciferase activity in lysates was measured using luciferase substrate (Promega) and a CLARIOstar microplate reader set on luminescence detection (gain: 4095; time: 5 seconds; injection volume: 10µL/well of 1x luciferase substrate).

Neutralisation assays using HCV cell culture (HCVcc gt-2a, -3a, -5a) were performed by mixing the HCVcc virus with serially diluted mouse sera (1:1 ratio). The virus/serum mixture was incubated for one hour at 37oC before added to Huh7.5 cells seeded twenty-four hours earlier at 30,000 cells/well in 48 well plates for four hours. Cells were washed four times with PBS and replenished with DMF10 media (NEAA) and incubated for an additional seventy-two hours. Cells were washed with PBS before lysis with Renilla Cell Lysis Buffer (Promega). Renilla luciferase activity in lysates was measured using luciferase substrate (Promega) and a CLARIOstar microplate reader set on luminescence detection (gain: 4095; time: 5 seconds; injection volume: 10µL/well of 1x luciferase substrate).

The neutralisation titre was calculated from a dilution curve as the reciprocal dilution of mouse serum to reduce luciferase activity by 50% (IC50) or 80% (IC80).

2.8.4 Antibody isotype assay

Antibody isotype assay was performed solely by Joey McGregor. Nunc Maxisorb flat bottom 96 well plates were incubated overnight at 4ºC with 50µL/well of 2µg/mL monomeric HCV (H77c) E2 (RBD) in 50mM carbonate bicarbonate buffer at pH9.6. The next day, plates were washed twice with PBST (PBS + 0.05% Tween20) and then blocked with 100µL/well of BSA_{10}PBS for 1 hour at room temperature. After a further four washes with PBST, mouse serum at a dilution of 1:20 in BSA_5PBST was added to the first row with half logarithmic serial dilutions of the mouse serum, titrating down the plate. Positive controls used were mAb44 (IgG1 control), mAb25 (IgG2a control), mAb24 (IgG2b

control), and mAb64 (IgG3) anti-HCV antibodies at 1μg/mL in BSA_5PBST, whilst 5μg/mL BSA was used as a negative control. After a one-hour incubation at room temperature, the plate was washed four times with PBST (PBS + 0.05% Tween20). Goat anti-sera to mouse IgG1, G2a, G2b, G3, A, and M was added at 1:1000 dilution in BSA_5PBST of 1mg/mL per well (Mouse Monoclonal Antibody Isotyping Reagent Kit, Sigma-Aldrich), followed by a one-hour room temperature incubation and four subsequent washes with PBST. To each well, 50μL of donkey anti-goat horse radish peroxidase (HRP) antibody was added at a dilution of 1:1000 in BSA_5PBST. A reaction mix of 1 tablet of 3',3',5',5 tetramethylbenzidine dihydrochloride (TMB; Sigma-Aldrich) in 10mL of phosphate citrate buffer was prepared. Just before use, 2μL of hydrogen peroxide was added to the TMB reaction mixture. To each well, 100μL of TMB reaction mix was added and left to develop for four minutes after which 50μL of HCL was added to stop the reaction. Plates were read on microplate reader (CLARIOstar) at absorbance 450-630nm.

2.9 Reagents

R10 medium was used for all experiments. R10 is RPMI medium (Gibco) plus 10% heat inactivated foetal calf serum (FCS; heat inactivated by 1 hour at 64^{o}C in water bath; 50ml FCS in 500ml of RPMI), 1% by volume 200mM L-glutamine (Sigma) and 1% by volume 100x penicillin and streptomycin solution (Sigma).

2.10 Statistical analysis

Data were analysed using Graphpad prism (version 8.0.1). The D'Agostino and Pearson test was used to determine the normality of the data distribution. Non-parametric tests were used throughout (unless stated) paired for within-individual comparisons (Wilcoxon) and unpaired for group comparisons (Mann Whitney) to determine significant difference between two group medians at 95% confidence intervals. For multiple comparisons a RM

two-way ANOVA with Tukey's multiple comparisons was used. Group averages are presented as medians unless otherwise stated. Two-tailed *p* values were used unless stated and a *p* value less than 0.05 indicate a significant difference: $p < 0.05$ = *, <0.01 = **, <0.001 = ***, and <0.0001 = ****.

3 A SECOND-GENERATION HCV T CELL VACCINE CONTAINING CONSERVED VIRAL SEQUENCE ACROSS MULTIPLE HCV SUBTYPES

3.1 Primary hypothesis

Simian adenovirus vector vaccines encoding conserved HCV proteomic regions across viral subtypes are immunogenic *in vivo.*

3.2 Introduction

Despite the advent of successful anti-viral drug treatment programmes to cure HCV-infected individuals, incidence and prevalence of HCV remain high (WHO, 2017). Therefore, the World Health Assembly endorsed the *Global Health Sector Strategy* (GHSS) which aims to eliminate viral hepatitis, including HCV infection, as a public health threat by 2030, reducing new HCV infections by 90% and HCV-associated mortality by 60%. The development of a prophylactic HCV vaccine will be a valuable clinical asset to achieve HCV elimination by 2030. A prophylactic vaccine against HCV is feasible as 25% of acute infections are naturally resolved with convincing evidence to support the role of

T cells in viral control and subsequent viral clearance (Bowen & Walker, 2005; Holz & Rehermann, 2015; Klenerman & Thimme, 2011; Lauer & Walker, 2001).

The proof of concept for a prophylactic HCV T cell vaccine was demonstrated in chimpanzee challenge studies and human clinical trials using rare and non-human serotypes of adenoviruses as viral vectors (Barnes et al., 2012; Folgori et al., 2006). A modified viral vector regimen utilising an adenovirus vector prime encoding a subtype-1b immunogen followed by an MVA boost vaccine induced the highest recorded T cell response to date in a human clinical trial, generating high magnitude T cells in healthy volunteers, targeting multiple HCV proteins, and specific for dominant T cell epitopes found in natural HCV infection (Swadling et al., 2014). Despite the success of this prime-boost T cell HCV vaccine regimen, reduced immunogenicity to other common non-1b genotypes in these studies suggested that a vaccine induced genotype-1b specific T cell response may have reduced cross-reactivity with non-1b subtypes and would be insufficient to provide pan-immunity to HCVs seven genotypes and many subtypes. Furthermore, epitope mapping analysis revealed that subtype-1b immunogen-targeted T cell epitopes resided in proteomic regions of high sequence variability at the population level, and subtype epitope variants displayed significantly reduced immunogenicity (Kelly et al., 2015). In addition, a comprehensive assessment of HCV genotype-1 and -3 antigenic targets in acute, chronic, and spontaneously resolved HCV infection revealed that T cell specificity is distinct between genotypes with limited T cell cross-reactivity at immunodominant epitopes, regardless of clearance or chronicity (von Delft et al. 2015). Data from vaccine trials and in natural HCV infection strongly suggest that the HCV-specific T cell response is genotype-specific and would be insufficient to provide T cell immunity to multiple HCV genotypes – i.e. an ‘intergenotypic T cell response’.

In order to overcome the lack of intergenotypic T cell responses induced by viral vector vaccines, an *in silico* approach was undertaken to identify HCV proteomic sequences that are conserved across HCV subtypes to inform the design of second generation T cell vaccines (described in detail in 1.10 Background work to the thesis). *In silico* analysis predicted that genetically conserved gene segments across (1) genotype-1 and -3 subtypes (gt1/3), and (2) genotype1-6 subtypes (gt1-6), all contain multiple CD4 and CD8 T cell epitopes described in natural HCV infection (von Delft et al., 2018). Conserved sequence immunogens were encoded in an simian adenovirus vector (ChAd; Dicks et al. 2012). The aim of these conserved sequence T cell vaccines is to elicit pan-genotypic immunity against multiple HCV genotypes and subtype virus variants through the induction of a robust, broad, intergenotypic T cell response. To date, only the design and *in silico* analysis of conserved sequence HCV vaccines that are described by von Delft and colleagues has been undertaken prior to the work in this thesis.

In this body of work, I undertake an in-depth pre-clinical assessment of the second-generation conserved sequence HCV T cell vaccines, ChAd-gt1/3 and ChAd-gt1-6, (described by von Deflt et al.) to determine if conserved HCV sequences are immunogenic in mice. Vaccine-induced T cell responses are measured in inbred, outbred, and human transgenic mice to determine overall magnitude, breadth, and functionality after a single prime immunisation. Importantly, conserved sequence T cell vaccines are assessed head-to-head with the subtype-1b HCV non-structural protein sequence immunogen encoded in an adenovirus vector (ChAd-gt-1b-NS), analogous to the vaccine in human clinical trials. In addition, the effect of novel genetic adjuvants located upstream (5') of the T cell immunogen is investigated to determine if vaccine-induced T cell responses can be increased.

3.3 Key questions

I address the following key questions:

1. Are conserved sequence HCV T cell vaccines immunogenic in inbred mice?
2. What effect, if any, does the presence of linker sequences between conserved gene segments have on vaccine-induced immunogenicity?
3. Do conserved sequence HCV T cell vaccines induced a genotype-1b specific T cell response comparable to the subtype-1b HCV T cell vaccine, ChAd-gt-1b-NS?
4. Does the conserved sequence vaccine-induced T cell response target epitopes in multiple HCV proteins?
5. Do conserved sequence T cell vaccines induce an intergenotypic T cell response? If so, is it of a higher magnitude compared to the intergenotypic response of ChAd-gt-1b-NS?
6. What capacity do conserved sequence vaccine-induced T cells have for producing anti-viral and immunomodulatory cytokines?
7. Are HCV T cell vaccines immunogenic in human HLA-A*0201 transgenic mice and outbred CD-1 mice?
8. How conserved are vaccine targeted T cell epitopes across HCV genotypes?
9. Can novel genetic adjuvants increase the vaccine-induced T cell response?

Some of this work is published in the following manuscripts:

Von Delft & Donnison, *et al*. The generation of simian adenoviral vectored HCV vaccine encoding genetically conserved gene segments to target multiple HCV genotypes. ***Vaccine* 36 (2). Elsevier: 313–21. doi:10.1016/J.VACCINE.2017.10.079.**

An additional manuscript is in preparation:

Donnison & von Delft, *et al.* A viral vector hepatitis C virus T cell vaccine strategy elicits a broad, high magnitude T cell response targeting conserved sub-dominant viral epitopes. **Manuscript in progress.**

3.4 Chapter specific material and methods

3.4.1 Vaccine design and nomenclature

In this chapter, I used the following simian adenovirus vector vaccines. The immunogens of the vaccines are shown **(Figure 3-1A-C**) and described in detail in **Vaccine viral constructs & immunogens**.

3.4.2 Genetic adjuvants

Genetic adjuvants used in this study are the tissue plasminogen activation leader sequence (TPA-LS) and the truncated sequence (residues 47-72 of the transmembrane domain) of the shark invariant chain (sIi; **Figure 3-1C**). Genetic adjuvants were encoded at the 5' end of the HCV immunogen sequence downstream of the ATG start codon. Where a genetic adjuvant is used, the abbreviated adjuvant name is added to the vaccine name. For example, when ChAd-gt1-6L is adjuvanted with a tissue plasminogen activation leader sequence (TPA-LS), the vaccine name becomes ChAd-gt1-6L-TPA-LS.

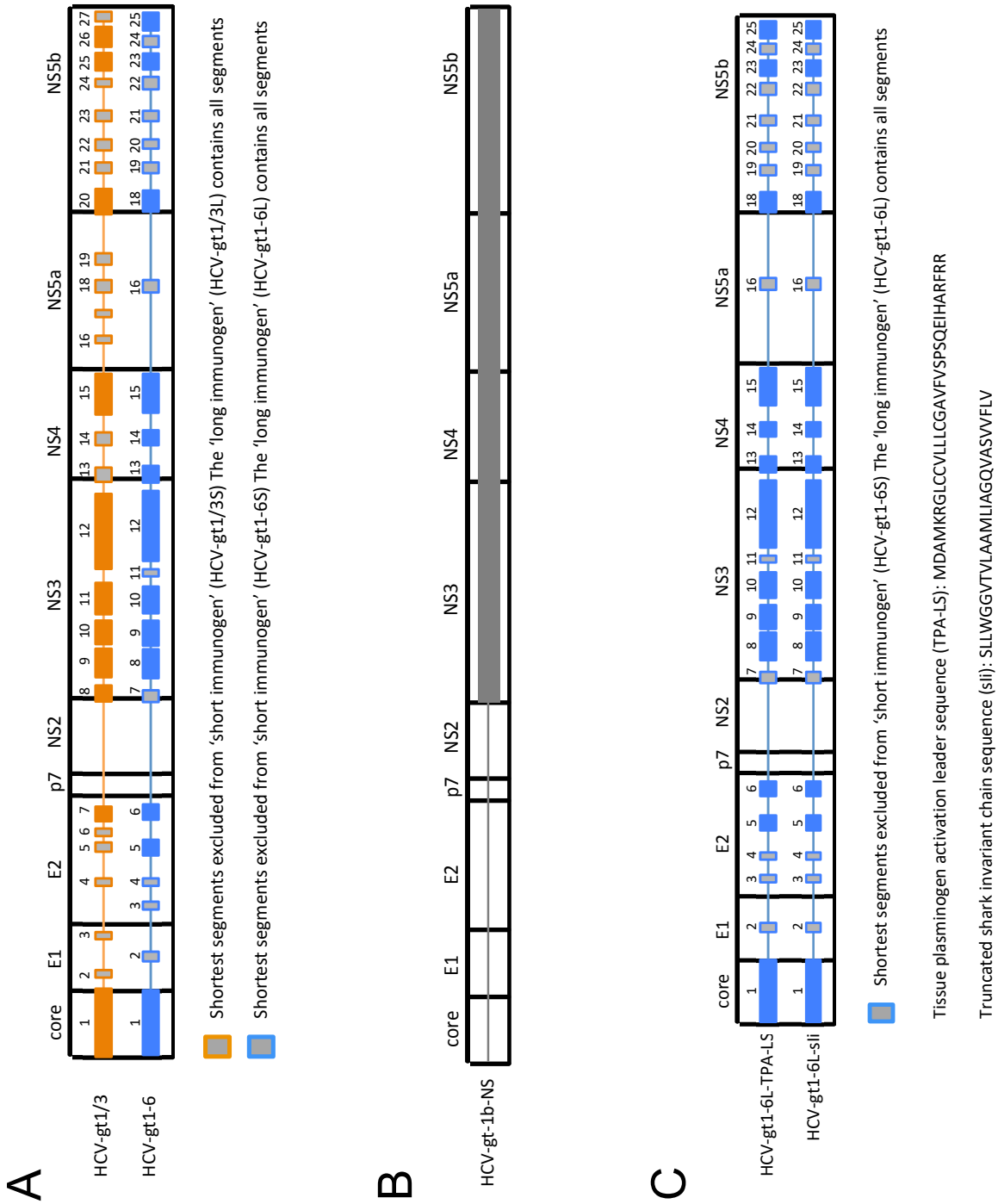

A
core
E1
E2
p7
NS2
NS3
NS4
NS5a
NS5b
HCV-gt1/3
HCV-gt1-6
Shortest segments excluded from 'short immunogen' (HCV-gt1/3S) The 'long immunogen' (HCV-gt1/3L) contains all segments
Shortest segments excluded from 'short immunogen' (HCV-gt1-6S) The 'long immunogen' (HCV-gt1-6L) contains all segments
B
HCV-gt-1b-NS
C
HCV-gt1-6L-TPA-LS
HCV-gt1-6L-sli
Shortest segments excluded from 'short immunogen' (HCV-gt1-6S) The 'long immunogen' (HCV-gt1-6L) contains all segments
Tissue plasminogen activation leader sequence (TPA-LS): MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR
Truncated shark invariant chain sequence (sli): SLLWGGVTVLAAMLIAGQVASVVFLV

Figure 3-1. HCV immunogen design: (**A**) Conserved sequence HCV T cell immunogens that contain gene segments that correspond to conserved viral sequences across subtypes 1 and 3, and 1 to 6. Segments are numbered. Grey segments correspond to shorter length gene segments that are excluded from the short immunogens and are included in the long immunogens. (**B**) the subtype-1b immunogen corresponding to the wildtype subtype-1b (J4) non-structural HCV protein sequence. (**C**) The gt1-6 conserved sequence HCV T cell immunogen with genetic adjuvants at the 5' end of the immunogen sequence.

3.4.3 Animals & immunisations

All mouse studies were performed at the Biomedical Services Building, Oxford, according to U.K. Home Office Regulations (project license numbers 30/2744 and 30/3293) and approved by the local ethical review board at the University of Oxford. Groups of age-matched female BALB/c, C57BL/6, CD-1, or HLA-A*0201 transgenic mice were vaccinated intramuscularly in the left quadricep at 8-10 weeks of age with 10^7 or 10^8 infectious units (I.U.) of non-replicating simian adenovirus ChAdOx1 containing the T cell immunogen. Mice were culled at 2- or 3-weeks post-immunisation and organs were harvested and processed for immunoassays as described in full in **chapter 2**.

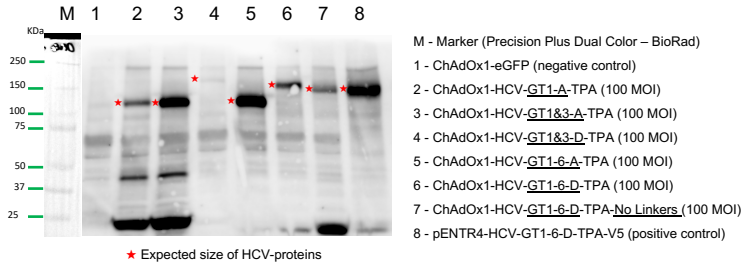
3.5 Results

3.5.1 Second-generation conserved sequence HCV T cell vaccine are immunogenic in inbred mice

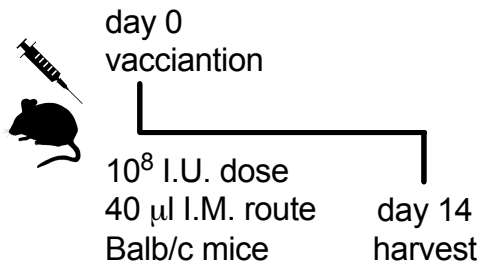
First, I showed the expression of all conserved sequence HCV immunogens by targeting the HCV $core_{21\text{-}40}$ amino acid sequence in a Western blot using an anti-HCV core 1b antibody (C7-50, AB2740, Abcam). The ChAd-eGFP was used as a negative control and the pENTR4-Gt1-6L-TPA plasmid as a positive control (Figure 3-2**A**). I then compared the ChAd vaccines containing the long and short HCV immunogens for both gt1/3 and gt1-6 immunogens in a single prime vaccination with a 2-week vaccination to harvest time

interval (Figure 3-2**B**). ChAd-gt1-6L and ChAd-gt1/3L had significantly higher T cell responses (2418 and 2486 median SFU/10^6 splenocytes respectively) compared to their respective short immunogen vaccines (450 and 988 median SFU10^6 splenocytes respectively; Figure 3-2**C;** p = 0.0286). When accounting for immunogen length by normalising the IFNγ^+ SFU ELISpot response using immunogen length (number of amino acids), no significant difference was observed between vaccine (**Figure 3-2D).**

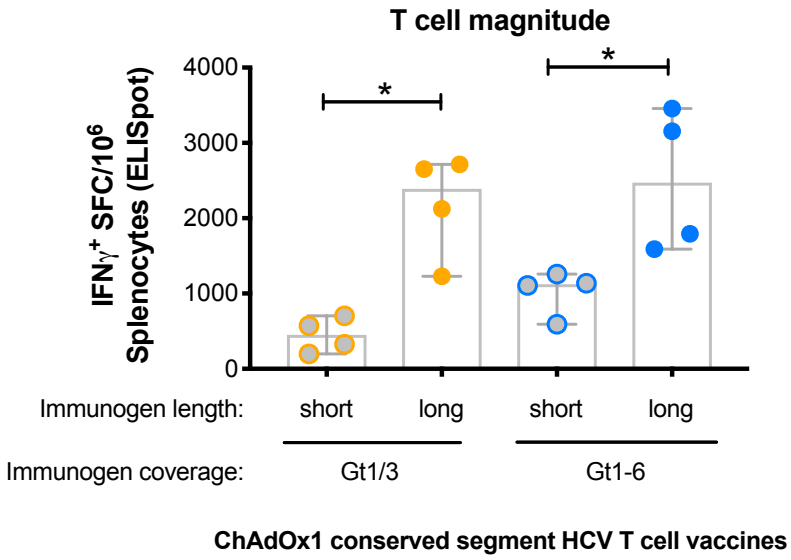
A


M 1 2 3 4 5 6 7 8
KDa
250
150
100
75
50
37
25
★ Expected size of HCV-proteins
M - Marker (Precision Plus Dual Color – BioRad)
1 - ChAdOx1-eGFP (negative control)
2 - ChAdOx1-HCV-GT1-A-TPA (100 MOI)
3 - ChAdOx1-HCV-GT1&3-A-TPA (100 MOI)
4 - ChAdOx1-HCV-GT1&3-D-TPA (100 MOI)
5 - ChAdOx1-HCV-GT1-6-A-TPA (100 MOI)
6 - ChAdOx1-HCV-GT1-6-D-TPA (100 MOI)
7 - ChAdOx1-HCV-GT1-6-D-TPA-No Linkers (100 MOI)
8 - pENTR4-HCV-GT1-6-D-TPA-V5 (positive control)

B


day 0
vacciantion
10^8 I.U. dose
40 µl I.M. route
Balb/c mice
day 14
harvest

C


T cell magnitude
IFNγ+ SFC/10^6 Splenocytes (ELISpot)
4000
3000
2000
1000
0
*
*
Immunogen length:
short
long
short
long
Immunogen coverage:
Gt1/3
Gt1-6
ChAdOx1 conserved segment HCV T cell vaccines

D

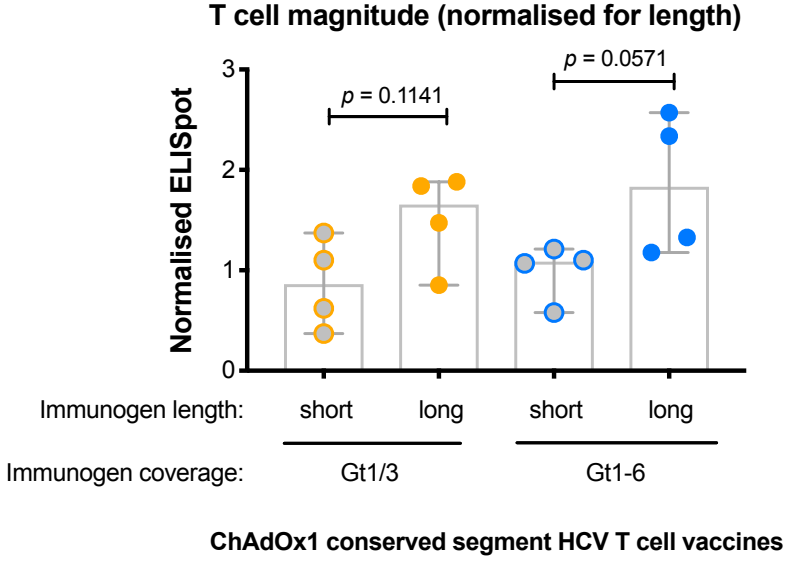

T cell magnitude (normalised for length)
Normalised ELISpot
3
2
1
0
p = 0.1141
p = 0.0571
Immunogen length:
short
long
short
long
Immunogen coverage:
Gt1/3
Gt1-6
ChAdOx1 conserved segment HCV T cell vaccines

Figure 3-2. Immunogen expression in vitro and vaccine immunogenicity of conserved segment HCV T cell vaccines: (**A**) HCV immunogen expression was evaluated in HEK 293A cells by Western blot using anti-HCV core 1b antibody (C7-50, AB2740, Abcam) that detects HCV $core_{21-40}$ sequence which is present in all HCV immunogens. (**B**) Conserved sequence HCV T cell vaccines were evaluated for immunogenicity in 8-week old female BALB/c inbred mice (4/group) that were immunised with a single 10^8 infectious units (I.U.) intramuscular immunisation in the left quadricep and harvested two weeks post-vaccination. (**C**) Harvested splenocytes ($2x10^5$ cells/well) were stimulated with HCV genotype-1b (J4) peptide pools (final concentration of 3μg/ml) that cover the full length of the HCV proteomic sequence (15-18mers overlapping by 11aa) and assessed in *ex vivo* IFNγ ELISpot assay. (**D**) ELISpot data was normalized using immunogen length. Data presented includes 'short' or 'long' versions of the vaccines 'gt1/3' and 'gt1-6'. T cell magnitude is the total of the positive individual peptide pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. Bars represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.2 Conserved sequence HCV vaccines induce broad T cell response in inbred mice

The number of antigenic targets induced in BALB/c inbred mice was measured to assess the breadth of the T cell response to vaccination. The breadth of the vaccine-induced T cell response was measured using 15-18mer peptides that overlap by 11 amino acids in 10 pools that cover the full length of the HCV proteome. The breadth of the response was defined as the number of IFNγ positive pools. The ChAd-gt1-6S and ChAd-gt1-6L vaccines induced high numbers of IFNγ positive peptide pools compared to ChAd-gt1/3S and ChAd-gt1/3L vaccine induced T cell responses, and ChAd-gt1-6L displayed a significantly greater breadth compared to ChAd-gt1/3L (Figure 3-3**A**, $p = 0.0286$). The magnitude of the vaccine induced T cell response was measured by ELISpot assay using genotype-1b peptide pools that correspond to specific HCV protein sequences. The T cell

response of all vaccines targeted preferentially non-structural proteins, including NS3h, NS4 and NS5b (Figure 3-3**B**). ChAd-gt1-6L and ChAd-gt1/3L induced significantly higher median IFNγ$^+$ spot forming units (SFUs) compared to ChAd-gt1-6S and ChAd-gt1/3S. This was reflective in the total magnitude of the response and also in the response to the individual peptide pools.

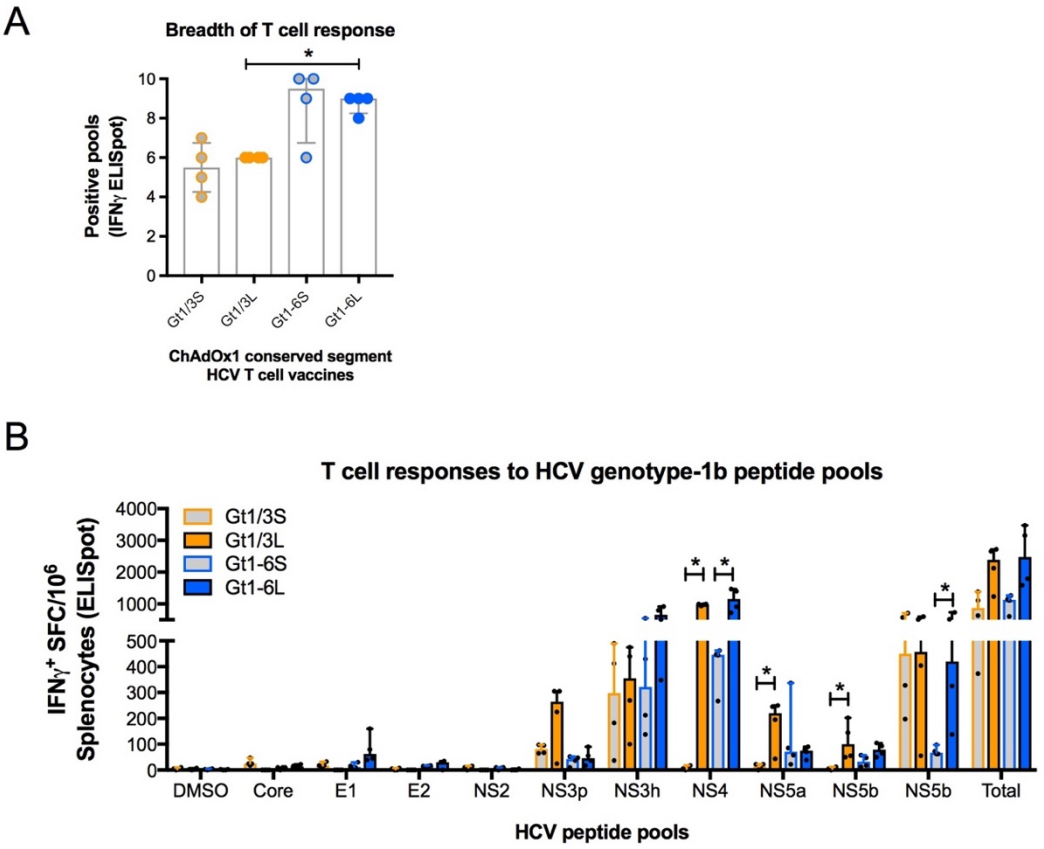
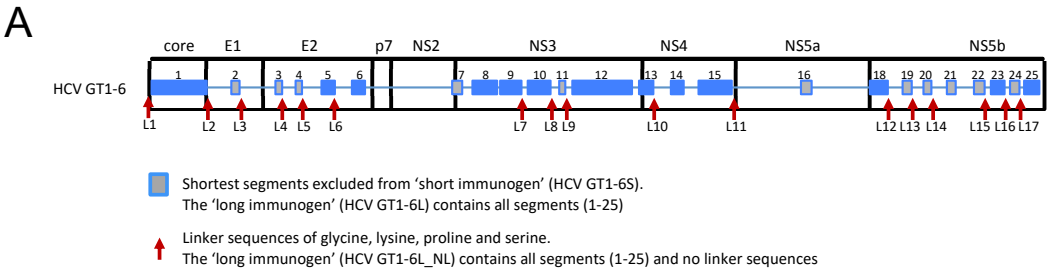


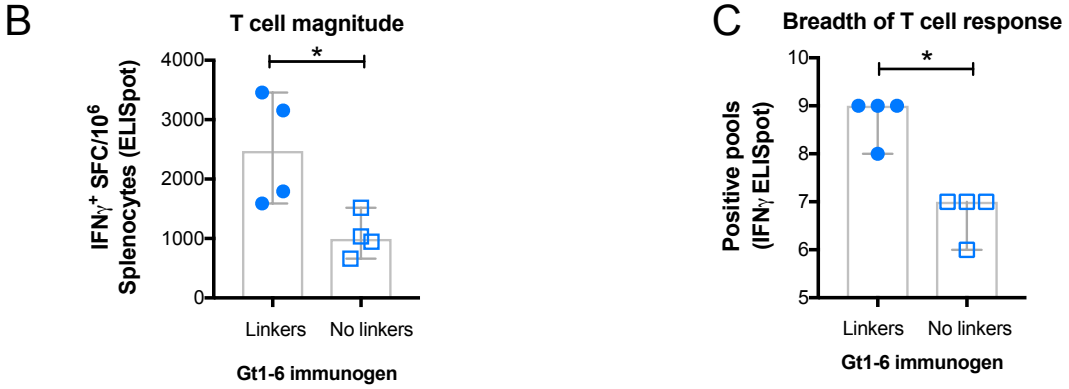
Figure 3-3. The breadth of the T cell response induced by conserved sequence HCV T cell vaccines: The breadth of the vaccine-induced T cell response in IFNγ ELISpot assays by number of positive peptide pools (**A**) and by spot forming units (SFUs) to individual HCV peptide pools that cover the full length of the genotype-1b HCV proteome (15-18mers overlapping by 11aa; **B**). Pools are considered positive when greater than the mean SFU of the DMSO negative control plus three standard deviations. Four female age-matched BALB/c mice were vaccinated per group, each mouse receiving 10^8 IU of vaccine in a 40μL intramuscular injection and harvested 14 days post-vaccination. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests

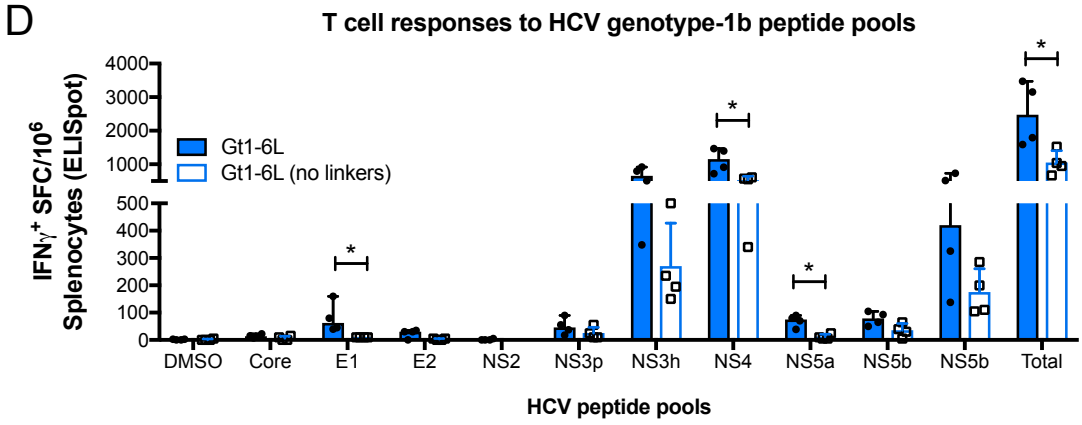
were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.3 The presence of linker sequences between gene segments increases vaccine immunogenicity

As the HCV immunogens were mosaic constructs joining together gene segments, artificial epitopes between gene segments that are not representative of epitopes found in natural infection may be present. *In silico* analysis revealed that some of these artificial epitopes are predicted to bind strongly to HLA class I (von Delft et al., 2018). Therefore, the insertion of short linker sequences (combinations of glycine, serine, proline, and lysine residues) were inserted between gene segments that had *in silico* predicted strong binding artificial epitopes (Figure 3-4**A**). The effect of the presence of these linker sequences on vaccine immunogenicity were assessed in mice. IFNγ ELISpot responses demonstrated that vaccine induced HCV-specific T cell responses were significantly increased in the presence of linker sequences in the immunogen (Figure 3-4**B**; $p = 0.0286$). The breadth of the vaccine-induced T cell response was significantly increased by the presence of linker sequences in the immunogen **(**Figure 3-4**C-D**; $p = 0.0286$). The peptide pools, NS3p, NS4, and NS5b displayed the highest IFNγ SFU magnitude in both the linker version and non-linker version of ChAd-gt1-6L. Peptides corresponding to linker sequences that span junction regions between HCV gene segments were generated for assessment of T cell responses specific for these linker sequences. Vaccine-induced T cell responses targeting linker sequences between HCV gene segments was low, below the negative control (DMSO) cut off of 22 median SFU/10^6 splenocytes, with the exception of linker 6 (40 SFU/10^6 splenocytes).


A
core
E1
E2
p7
NS2
NS3
NS4
NS5a
NS5b
HCV GT1-6
L1
L2
L3
L4
L5
L6
L7
L8
L9
L10
L11
L12
L13
L14
L15
L16
L17
Shortest segments excluded from 'short immunogen' (HCV GT1-6S).
The 'long immunogen' (HCV GT1-6L) contains all segments (1-25)
Linker sequences of glycine, lysine, proline and serine.
The 'long immunogen' (HCV GT1-6L_NL) contains all segments (1-25) and no linker sequences


B
T cell magnitude
IFNγ+ SFC/10^6 Splenocytes (ELISpot)
Linkers
No linkers
Gt1-6 immunogen
C
Breadth of T cell response
Positive pools (IFNγ ELISpot)
Linkers
No linkers
Gt1-6 immunogen


D
T cell responses to HCV genotype-1b peptide pools
IFNγ+ SFC/10^6 Splenocytes (ELISpot)
Gt1-6L
Gt1-6L (no linkers)
DMSO
Core
E1
E2
NS2
NS3p
NS3h
NS4
NS5a
NS5b
NS5b
Total
HCV peptide pools

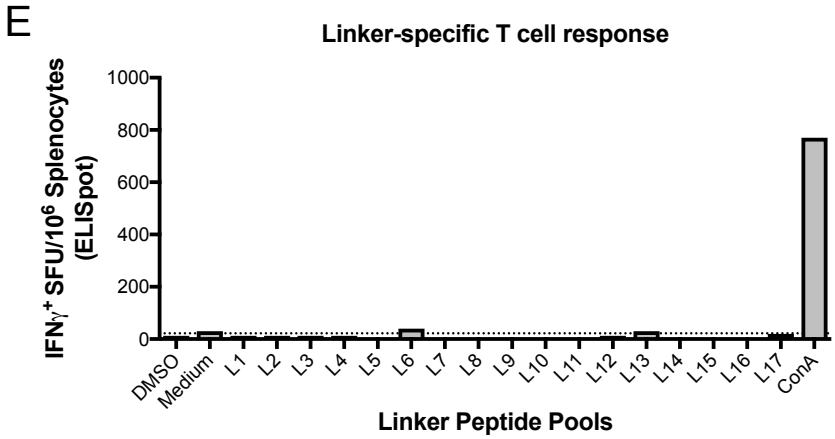

E
Linker-specific T cell response
IFNγ+ SFU/10^6 Splenocytes (ELISpot)
DMSO
Medium
L1
L2
L3
L4
L5
L6
L7
L8
L9
L10
L11
L12
L13
L14
L15
L16
L17
ConA
Linker Peptide Pools

Figure 3-4. The effect of linker sequences between gt1-6 gene segments on vaccine-induced immunogenicity: The conserved sequence vaccine, ChAd-gt1-6, with linker sequences. Red arrows indicate the location of all 17 linkers (L1-17) that were inserted between conserved gt1-6 gene segments (**A**). The *ex vivo* IFNγ ELISpot assay for vaccine-induced T cell magnitude (**B**), breadth indicated by number of positive peptide pools (**C**), T cell magnitude of individual peptide pools (**D**), and vaccine-induced immunogenicity to linker sequences in junction regions between HCV gene segments (**E**). DMSO, medium (R10), and Concanavalin A (ConA) were used as two negative controls and a positive control, respectively. T cell magnitude is the total of the positive individual peptide pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. Four female age-matched BALB/c mice were vaccinated per group, each mouse receiving 10^8 IU of ChAd-gt1-6L in a 40μL intramuscular injection and harvested 14 days post-vaccination. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.4 Conserved sequence HCV T cell vaccines have comparable immunogenicity to the HCV T cell vaccine, ChAd-gt1b-NS

The HCV vaccine currently in phase II human clinical trials is a viral vectored T cell vaccine that encoded the subtype-1b non-structural HCV proteins (gt-1b-NS). In order to assess the conserved sequence HCV T cell vaccines as potential vaccine candidates, their immunogenicity was assessed head-to-head with the HCV subtype-1b NS vaccine. An HCV subtype-1b vaccine identical to that in current human trials was generated for comparative assessment with the conserved sequence HCV T cell vaccines.

The conserved sequence HCV T cell vaccines were assessed head to head with the ChAd-gt-1b-NS vaccine and a ChAd vector containing the sequence of the green fluorescent protein (ChAd-eGFP) as a control vaccine. Total vaccine induced HCV-specific IFNγ ELISpot responses demonstrated that conserved sequence HCV T cell vaccines had

superior immunogenicity compared to gt-1b-NS and eGFP vaccines. Gt1/3L and gt1-6L vaccines had significantly higher total ELISpot responses of medians 2390 and 2473 IFNγ SFU/10^6 splenocytes respectively, whilst gt-1b-NS and eGFP had medians of 650 and 16.25 SFU/10^6 splenocytes respectively (Figure 3-5**B**; $p = 0.0286$). Next, I determined the breadth of the vaccine-induced T cell response and revealed that significantly more peptide pools were IFNγ positive for gt1-6L compared to gt1/3 and gt-1b-NS, whilst gt1/3 had significantly higher number of IFNγ^+ peptide pools than gt-1b-NS (Figure 3-5**C**; $p = 0.0286$). Evaluation of the vaccine induced T cell response to individual peptide pools showed that gt1-6L and gt1/3L vaccines displayed significantly greater T cell responses compared to the gt-1b-NS vaccine to NS3p, NS3h, NS4, and NS5b (Figure 3-5**D**; $p = 0.0286$).

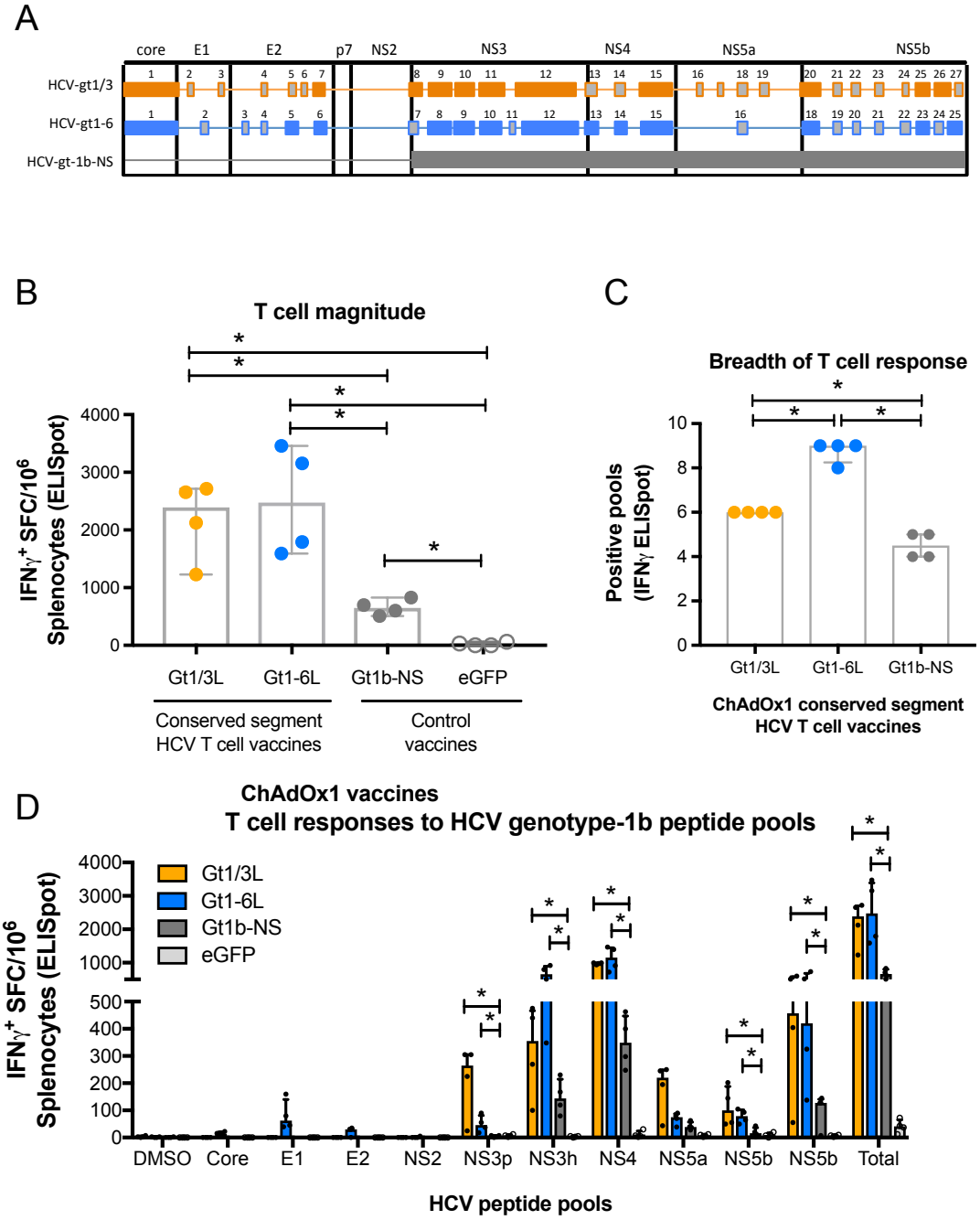


Figure 3-5. Comparative assessment of vaccine-induced T cell responses between conserved sequence HCV T cell vaccines and subtype-1b HCV T cell vaccine: HCV T cell vaccine immunogen design (**A**). The ex vivo IFNγ ELISpot assay response for vaccine-induced T cell magnitude (**B**), breadth by number of positive peptide pools (**C**), and T cell magnitude to individual peptide pools across the full length of the HCV proteome (15-18mers overlapping by 11aa; **D**). T cell magnitude is the total of the positive individual peptide pools. Pools are considered

positive when greater than the mean of the DMSO negative control plus three standard deviations. Four female age-matched BALB/c mice were vaccinated per group, each mouse receiving 10^8 IU of vaccine in a 40μL intramuscular injection and harvested 14 days post-vaccination. Bars represent the median SFU/106 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. P values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.5 Comparative analysis of intergenotypic T cell responses between conserved sequence HCV T cell vaccines and ChAd-gt-1b-NS vaccine

An ideal prophylactic HCV vaccine should elicit cross-reactive intergenotypic T cell responses to protect against multiple infecting HCV genotypes. A limitation of the subtype-1b immunogen viral vector vaccine is reduced immunogenicity to non-subtype-1b sequences (Swadling et al. 2014). Conserved sequence HCV T cell vaccines encode viral sequences with high sequence homology across all HCV genotypes (von Delft et al., 2018), with the potential to protect against multiple HCV genotypes. Therefore, I determined the capacity of conserved segment HCV T cell vaccines to elicit intergenotypic T cell responses. Total HCV-specific IFNγ ELISpot responses and the number of IFNγ positive HCV peptide pools for ChAd-gt1-6L and ChAd-gt1/3L were comparable to ChAd-gt-1b-NS when stimulating splenocytes with genotype-1a peptides, but $IFN\gamma^+$ ELISpot responses and number of $IFN\gamma^+$ positive pools were significantly higher when stimulating with genotypes-1b and -3a compared to ChAd-gt-1b-NS **(**Figure 3-6**A-B**). Differences between genotype-1a, -1b, -3a, and the vaccine sequences are reported in **Appendix 2: HCV vaccines** and peptide.

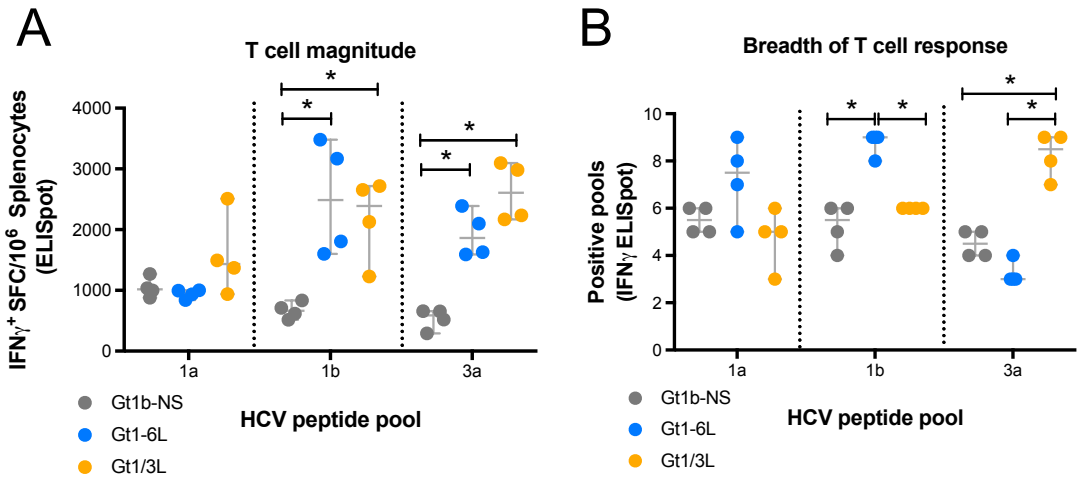


Figure 3-6. Intergenotypic T cell responses induced by HCV T cell vaccines: Splenocytes from age-matched female BALB/c mice (4/goup) that received a single 10^8 I.U. vaccine dose in a 40μL intramuscular immunisation with three different HCV T cell ChAd vaccines (gt-1b-NS, gt1-6L, gt1/3) were harvested 14 days post-vaccination and stimulated with genotype-1a (H77), -1b (J4), and -3a (k3a650) peptides in 10 pools and IFNγ producing cells were detected by *ex vivo* IFNγ ELISpot for comparison of T cell magnitude (**A**) and number of positive peptide pools (**B**). T cell magnitude is the total of the positive individual peptide pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

All three vaccines—ChAd-gt1-6L, ChAd-gt1/3L, and ChAd-gt-1b-NS—predominantly generated T cell responses that targeted the non-structural HCV proteins when assessed using HCV genotype-1a, -1b, and -3a antigens (Figure 3-7**A-C**). ChAd-gt1-6L also induced low magnitude immune responses to HCVs structural proteins. Significantly higher IFNγ$^+$ T cell responses to NS3h, NS4, and NS5b peptide pools were observed for conserved sequence HCV T cell vaccines compared to ChAd-gt-1b-NS for all HCV genotypes (Figure 3-7**A-C**). Strikingly, the ChAd-gt1-6L and -gt1/3L vaccines induced no T cell responses targeting core epitopes despite the vaccines expressing the full length of

core and its epitopes that have been linked to spontaneous clearance (Day et al., 2002; Lauer et al., 2004; Schulze zur Wiesch et al., 2005). Interestingly, ChAd-gt1/3L generated the highest genotype-3a specific T cell response out of all the vaccines, targeting at least four highly immunogenic epitopes (**Figure 3-7C**). This result is reflective of the greater amount of genotype-3a sequence present in the ChAd-gt1/3L vaccine compared to the ChAd-gt1-6L and ChAd-gt-1b-NS vaccines (**Appendix 2: HCV vaccines** and peptide).

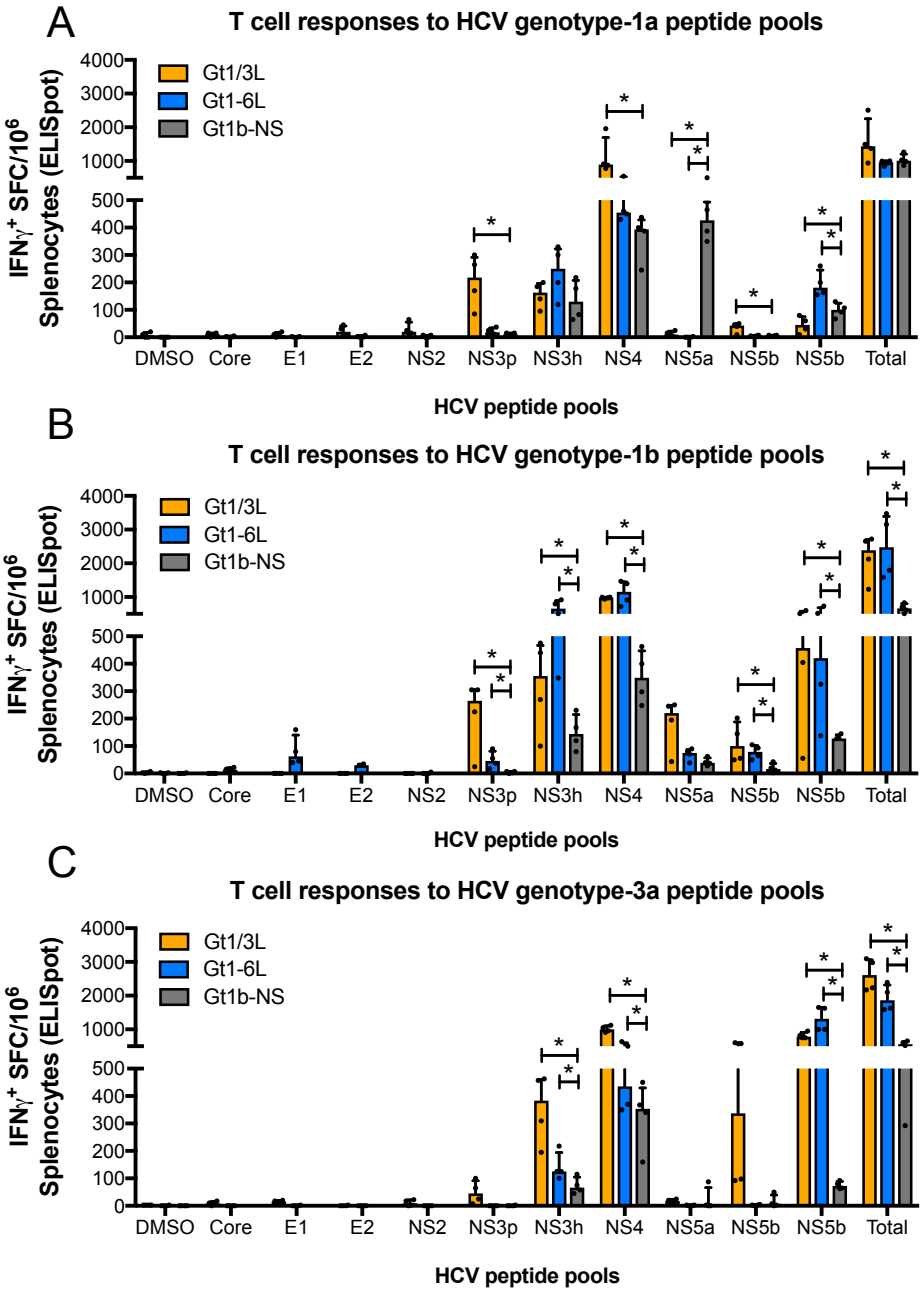


Figure 3-7. Vaccine-induced intergenotypic T cell responses to HCV proteins: The vaccine-induced T cell response from splenocytes of age-matched female BALB/c mice (4/group) that received a single 10^8 I.U. vaccine dose in a 40μL intramuscular immunisation with three different HCV T cell ChAd vaccines (gt-1b-NS, gt1-6L, gt1/3L) and were stimulated with genotype-1a (**A**), -1b (**B**), and -3a (**C**) peptides in 10 pools that cover the full length of the HCV proteome. Peptides are 15-18mers in length and overlap by 11 amino acids within peptide pools. T cell magnitude is the total of the positive individual peptide pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.6 Conserved sequence HCV T cell vaccine, ChAd-gt1-6, induces functional $CD4^+$ and $CD8^+$ T cells against common HCV genotypes

Intracellular cytokine staining (ICS) and flow cytometry was used to assess the production of proinflammatory and immunomodulatory cytokines by vaccine-induced T cells, and to assess the magnitude of the T cell subsets, CD4 and CD8, primed by adenovirus vaccination (example plots in **Figure 3-8A-B**).

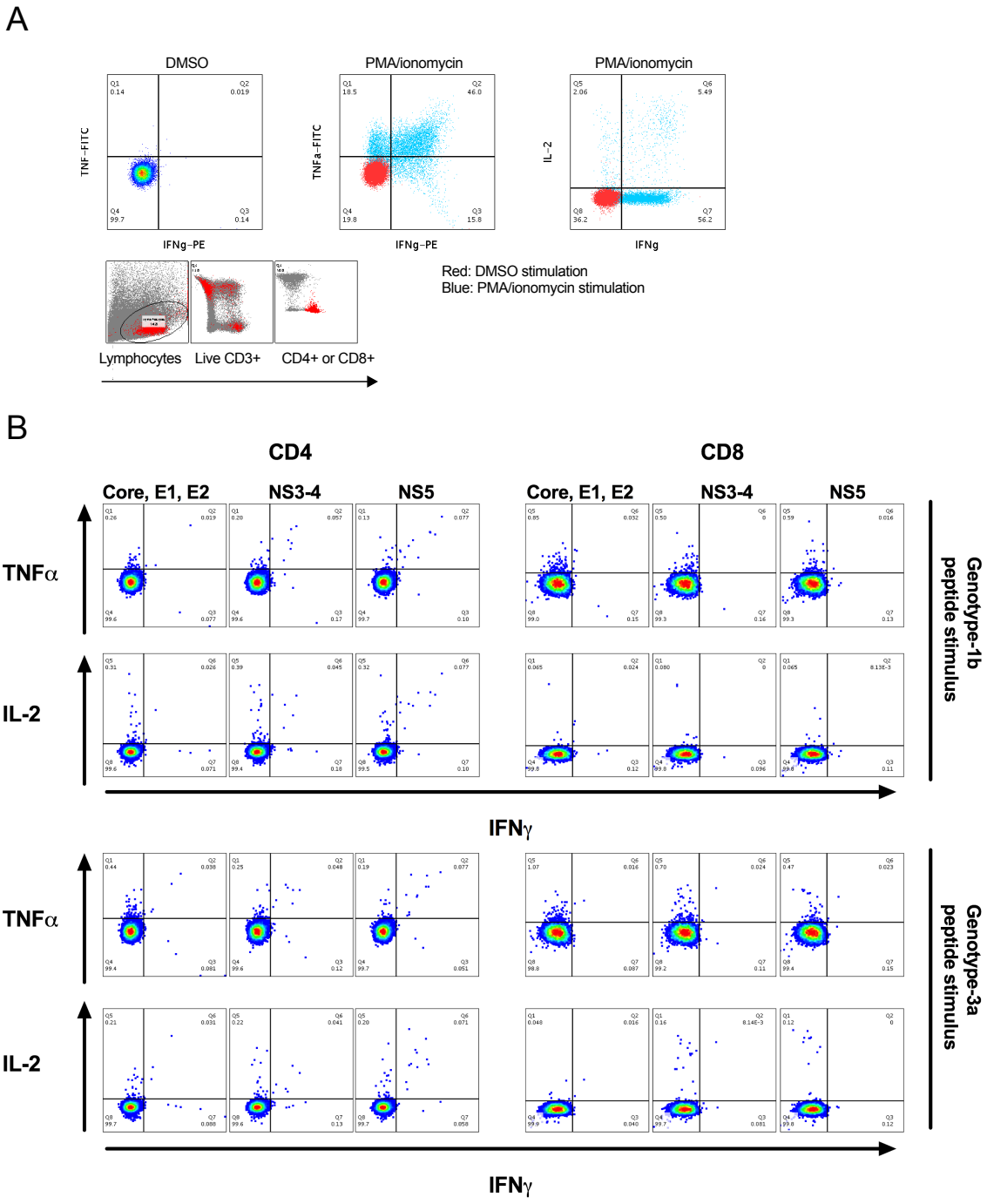


Figure 3-8. Flow cytometry plots of intracellularly stained murine splenocytes: (**A**) Gating strategy to identity $CD4^+$ and $CD8^+$ T cell subsets cytokine production using DMSO and PMA/ionomycin controls. (**B**) Example plots of intracellular staining of IFNγ, TNFα, and IL-2 produced by splenic $CD4^+$ and $CD8^+$ T cells from age-matched female C57BL/6 mice immunised with 10^8 IU I.M. prime ChAd-gt1-6L vaccination and harvested 14 days post-vaccination. Cells were stimulated with either genotype-1b or -3a specific HCV peptides in 3 pools that cover the full HCV

protein sequence (i. HCV core-E1-E2, ii. NS3-4, and iii. NS5; peptides are 15-18mers overlapping by 11aa).

Many vaccine studies have demonstrated that adenovirus vector vaccines predominantly generate a high magnitude $CD8^+$ T cell response (Barnes et al., 2012a; K. J. Ewer et al., 2016; Tatsis et al., 2007). $CD8^+$ T cells that produced IFNγ, TNFα, and IL-2 were readily detected in murine splenocytes two-weeks post vaccination with IFNγ the predominant cytokine produced after both a genotype-1b and -3a peptide stimuli (Figure 3-9**A**). In this analysis, it is unclear if multi-cytokine production is from the same specific T cell or an effect of multiple activated T cells acting in an endocrine manner.

Vaccine-induced $CD4^+$ T cells were also detected but at a lower frequency. These produced predominantly TNFα and IL-2 after stimulation with genotype-1b and -3a peptides with a trend for lower IFNγ production after genotype-3a stimulation that did not reach statistical significance (Figure 3-9**B**).

There was a trend for the mice that had high responding genotype-1b specific cytokine-producing $CD8^+$ T cell populations to have a lower genotype-3a $CD8^+$ T cell response, and vice versa (Figure 3-9**C**). In contrast, mice that produced genotype-1b specific TNFα and IL-2 producing $CD4^+$ T cell populations also responded to genotype-3a antigen stimulation (Figure 3-9**D**).

To further characterise the $CD4^+$ and $CD8^+$ T cell's capacity to generate a intergenotypic T cell response, i.e. a T cell response targeting multiple HCV genotypes, the intergenotype ratio was determined (see **methods**). When the individual intergenotype ratios of IFNγ, TNFα, and IL-2 cytokine producing cells were combined, the $CD4^+$ T cell response displayed a significantly higher intergenotype ratio compared to the vaccine-induced $CD8^+$ T cell response, particularly regarding IL-2 production (Figure 3-9**E**).

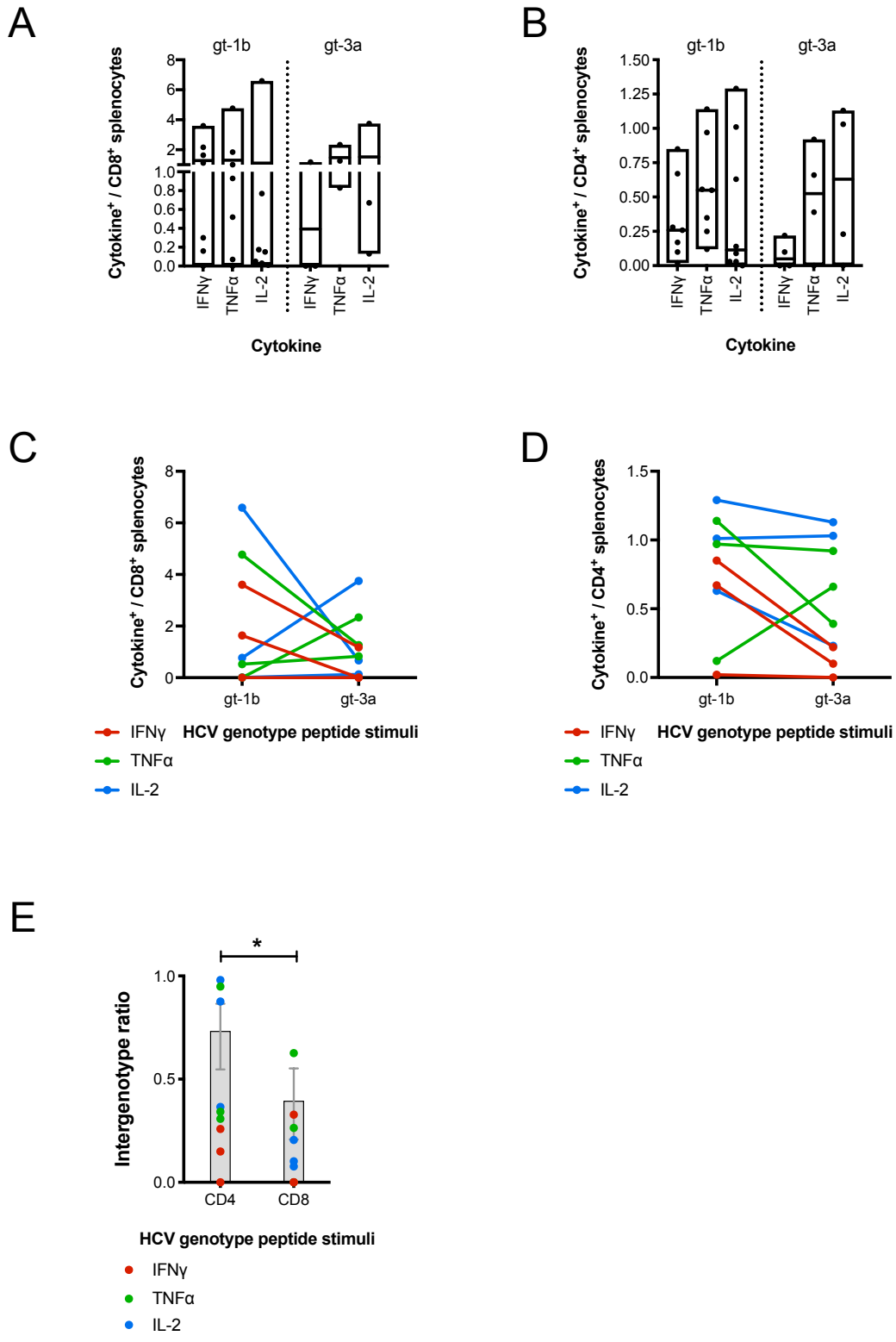


Figure 3-9. Functionality of genotype-1b and -3a vaccine-induced T cell responses: Intracellular staining of IFNγ, TNFα, and IL-2 produced by splenic $CD4^+$ and $CD8^+$ T cells from age-matched female C57BL/6 mice immunised with 10^8 IU I.M. prime ChAd-gt1-6L vaccination and harvested 14 days post-vaccination (n=4-8; data presented are from two experiments). Cells were

stimulated with either genotype-1b or -3a specific HCV peptides in 3 pools that cover the full HCV protein sequence (i. HCV core-E1-E2, ii. NS3-4, and iii. NS5; peptides are 15-18mers overlapping by 11aa). The experiment was performed twice. Cytokine production of vaccine-induced $CD8^+$ (**A**) and $CD4^+$ (**B**) T cells is shown and displayed as the sum response of all three peptide pools in the left column as floating box plots with medians shown). (**C-D**) The level of cytokine production in genotype-1b and -3a-specific T cell responses are compared in each mouse. (**E**) The intergenotype ratio of vaccine-induced $CD4^+$ and $CD8^+$ T cells. The intergenotype ratio is defined as the ratio of the lowest genotype-specific (gt-1b or -3a) T cell response to the highest genotype-specific T cell response (gt-1b or -3a). For example, if the gt-1b and -3a responses were the same, then the intergenotype ratio would be 1, and if no T cell response was shown for any genotype-specific T cell response, the intergenotype ratio is 0. Individual data points correspond to the secretion of a particular cytokine and the grey bars indicate the median and range of the total cytokine producing T cell response. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.7 Conserved sequence HCV vaccine, ChAd-gt1-6, produces a polyfunctional $CD4^+$ and $CD8^+$ T cell response

To assess the capacity of individual T cells to secrete multiple cytokines, I used Boolean gating, Pestle software, and Spice analysis on peptide stimulated T cell populations. Vaccine-induced $CD4^+$ and $CD8^+$ T cells were predominantly mono-functional after genotype-1b and -3a peptide stimulation. Yet, some polyfunctionality was observed: genotype-1b and -3a specific $CD4^+$ T cells and genotype-1b specific $CD8^+$ T cells (Figure 3-10).

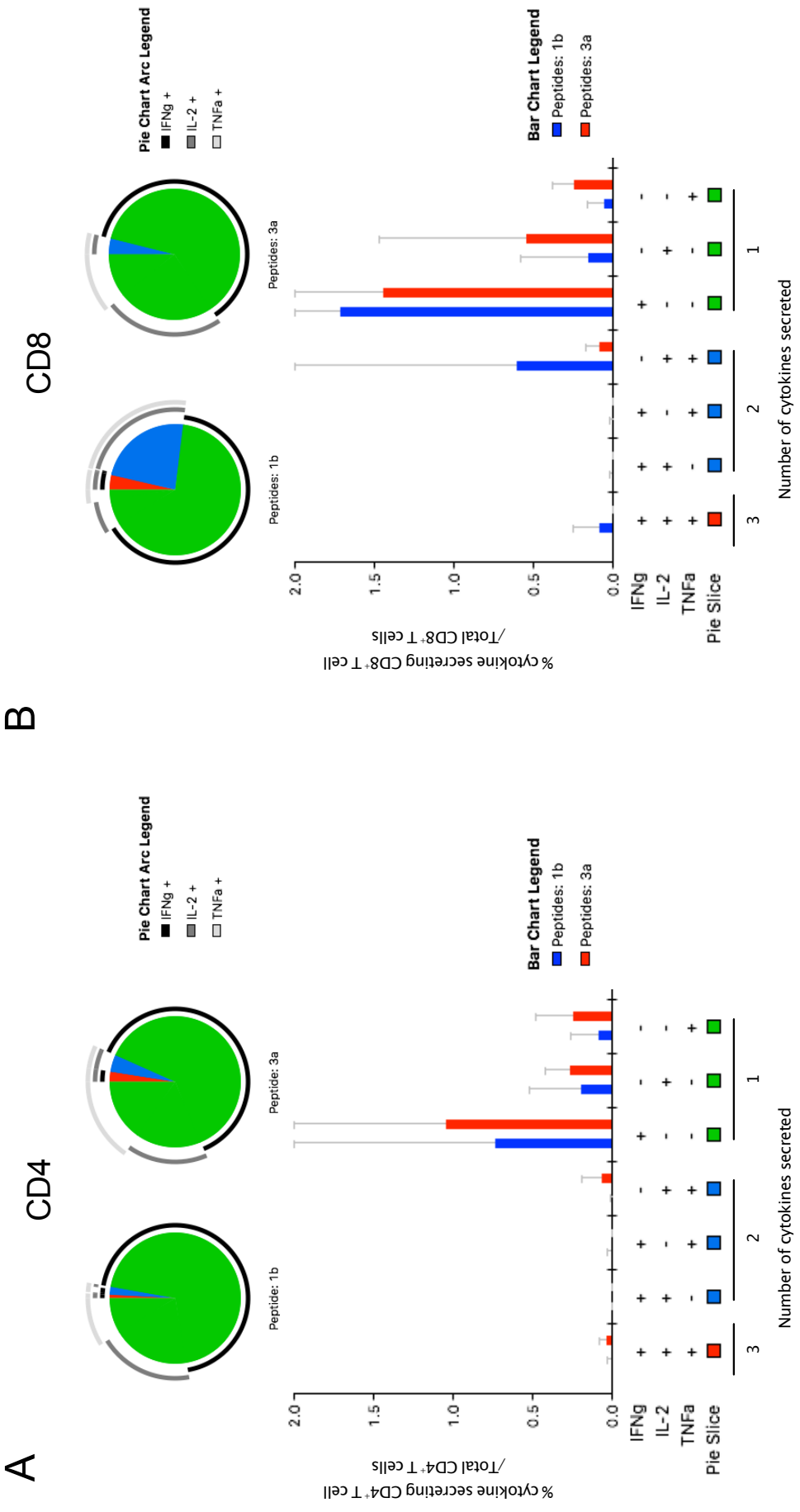

A
CD4
B
CD8
Pie Chart Arc Legend
IFNg +
IL-2 +
TNFa +
Peptide: 1b
Peptide: 3a
Peptides: 3a
Peptides: 1b
Bar Chart Legend
Peptides: 1b
Peptides: 3a
% cytokine secreting CD4+ T cell /Total CD4+ T cells
% cytokine secreting CD8+ T cell /Total CD8+ T cells
2.0
1.5
1.0
0.5
0.0
IFNg
IL-2
TNFa
Pie Slice
3
2
1
Number of cytokines secreted

Figure 3-10. Polyfunctionality of HCV-specific CD4+ and CD8+ T cells in mice receiving ChAd-gt1-6L-TPA vaccination: Cytokine combinations produced by individual HCV-specific splenic $CD4^+$ (**A**) and $CD8^+$ (**B**) T cells from age-matched female C57BL/6 mice immunised with 10^8IU of ChAd-gt1-6L and harvested two-weeks post-vaccination (n=4-8; data presented are from two experiments). Cells were stimulated in an intracellular cytokine staining assay with genotype-1b or -3a specific peptide pools that correspond to HCV protein sequences and cytokine secreting $CD4^+$ and $CD8^+$ T cell subsets were analysed for polyfunctionality using Boolean gating, Pestle software and SPICE analysis. Peptides are 15-18mers overlapping by 11aa. The experiment was performed twice. Pie charts and graphs represent the proportion of cytokine-secreting T cells that produce one (green), two (blue), or three (red) cytokines of IFNγ, TNFα, and IL-2. Pie arcs (in greyscale) show the proportion of cytokine-producing cells that make a given cytokine, where overlap of arcs indicate polyfunctionality. Pie bases and bars are displayed as means of all samples.

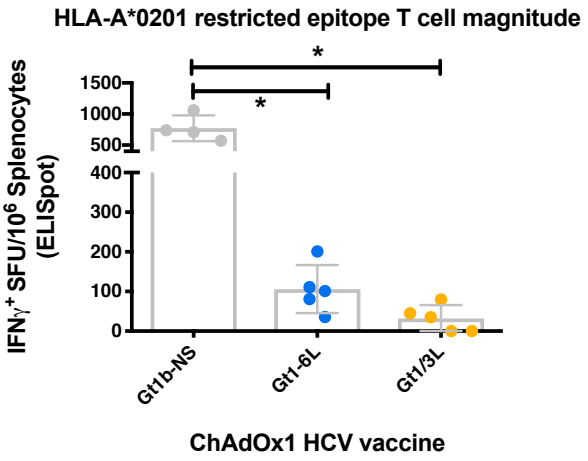
3.5.8 Conserved sequence HCV T cell vaccines are immunogenic in the humanised HLA-A*0201 transgenic mice

Humanised transgenic mice are surrogate models of human immunogenicity as the background C57BL/6 inbred mouse strain carry the HLA-A*0201 transgene and therefore express the human class I MHC antigen HLA-A*0201 on the cells of the spleen, bone marrow, and thymus. Therefore, vaccinated HLA-A*0201.1 transgenic mice may express HLA-A*0201.1 restricted HCV epitopes in the MHC class I complex.

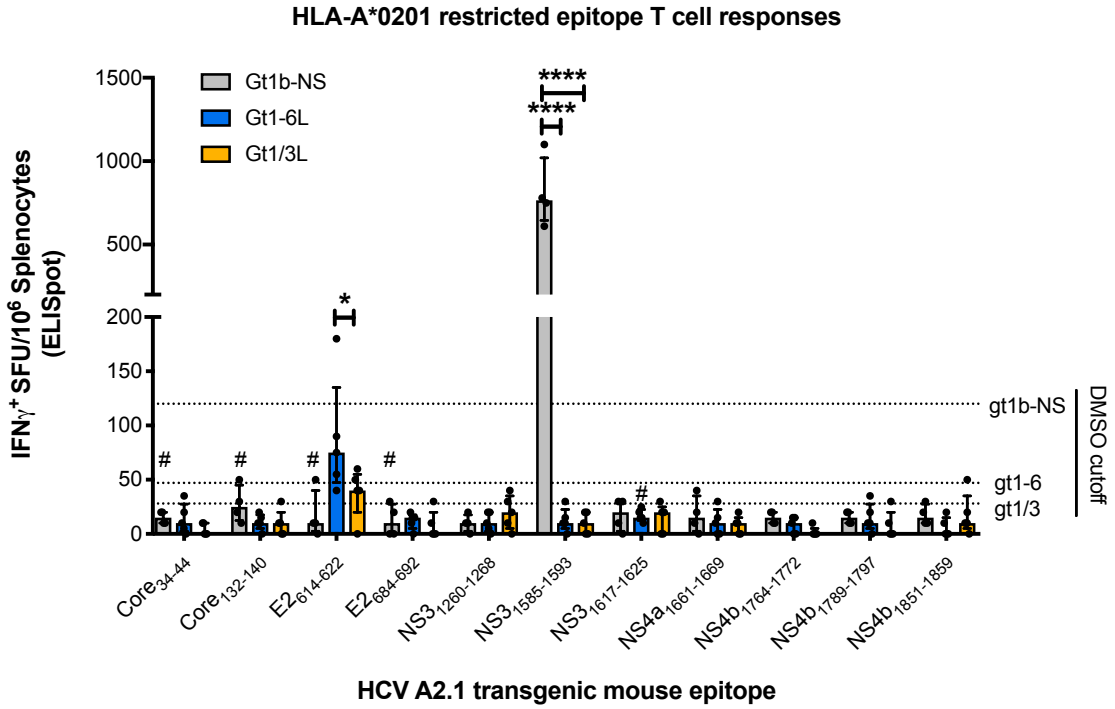
I assessed the HCV vaccines—gt1-6L, gt1/3L, and gt-1b-NS—in HLA-A*0201 mice for their immunogenicity to HLA-A*0201.1 restricted epitopes previously via IFNγ ELISpot assays using HCV peptides that contain known transgenic mouse HLA-A*0201.1 restricted epitopes. The total IFNγ ELISpot response to HLA-A*0201 epitopes in conserved HCV regions was significantly higher for gt-1b-NS than the conserved sequence vaccines (Figure 3-11**A**). Epitope mapping revealed that the gt-1b-NS HLA-A*0201 T cell response was restricted to epitope $NS3_{1585\text{-}1593}$ while the conserved sequence HCV T cell

vaccine HLA-A*0201 restricted T cell response was to epitope $E2_{614-622}$ (Figure 3-11**B**). Sequence mismatch between conserved sequence vaccine and peptide stimulus was observed for $E2_{614-622}$ and $NS3_{1585-1593}$ HLA-A2.1 epitopes (Figure 3-11**C**).

A



B



C

	Sequence	
	$E2_{614-622}$	$NS3_{1585-1593}$
Peptide	RLWHYPCTI	YLVAYQATV
Gt1-6L	- - - - - - - - V	- - - - - - - - -
Gt1/3L	- - - - - - - - x	- - T - - - - - -
Gt1b-NS	N/A	- - - - - - - - -

Figure 3-11. Vaccine immunogenicity in HLA-A*0201 transgenic mice: (**A**) The total IFNγ ELISpot response of HLA-A*0201 transgenic mouse splenocytes (5 mice/group received 10^8 IU I.M. single immunisation and were harvested 14 days post-vaccination) stimulated with genotype-1b peptides (15-18mer) containing HLA-A*0201 identified epitopes that cover the full length of the HCV protein sequence (15-18mer peptides that overlap by 11a). (**B**) Total IFNγ ELISpot responses to individual peptides that correspond to HLA-A*0201 epitopes. The experiment was performed once. T cell magnitude is the total of the positive individual peptide pools. Pools are defined as positive when greater than the mean of the DMSO negative control plus three standard deviations. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****. Hashes indicate epitopes that are not present in the vaccine. (**C**) The peptide and vaccine amino acid sequence of $E2_{614-622}$ and $NS3_{1585-1593}$ HLA-A2.1 restricted epitopes.

3.5.9 ChAdOx1-gt1-6L induces a broad intergenotypic T cell response in outbred mice

The use of inbred mice strains are standard for pre-clinical vaccine studies due to the limited number of H-2 antigens restricted to a single allele and therefore its ease of producing reproducible results with minimal variation within experiment groups. One limitation of inbred mice is that the limited variety of antigen presenting molecules may restrict the diversity of the peptides that are processed and presented on the surface of murine cells, and therefore potentially limit the immunogenic responses detected in inbred mice or fail to show the heterogeneity of T cell responses that may be seen in human studies. An outbred strain of mice better represents vaccination in an outbred population such as humans due to the increased diversity of the murine H-antigen. To further assess the breadth of conserved sequence HCV T cell vaccine-induced immunogenicity, peptides pools corresponding to each conserved gt1-6L segment were created and used in immunoassays (S1-15).

I immunised CD-1 (outbred) and C57BL/6 mice (inbred) with the conserved segment HCV T cell vaccine, gt1-6L, to assess the breadth of the vaccine-induced T cell response. The total IFNγ ELISpot response to genotype-1a, -1b, and -3a peptides in CD-1 mice were all of a total magnitude of 500-600 SFU/10^6 splenocytes (Figure 3-12**A**). CD-1 mice induced comparable numbers of IFNγ^+ ELISpot positive pools to C57BL/6 mice (Figure 3-12**B**). After mapping T cell responses to individual conserved segments, the majority of the vaccine-induced immunogenicity in C57BL/6 mice was attributed to conserved segment 14 ('S14'; NS4b), and to a lesser extent segments 5 (E2), 9 (NS3p), 18 (NS5a), and 23 (NS5b). CD-1 mice displayed a diverse breadth of T cell responses, targeting segments 5 (E2), 8 (NS3p), 13 (NS3h/NS4a), 14 (NS4b), 15 (NS4b), 16 (NS5a), 19 (NS5a), 22 (NS5b), 25 (NS5b), albeit at a lower magnitude compared to the S14-targeted magnitude in C57BL/6 mice (Figure 3-12**C**).

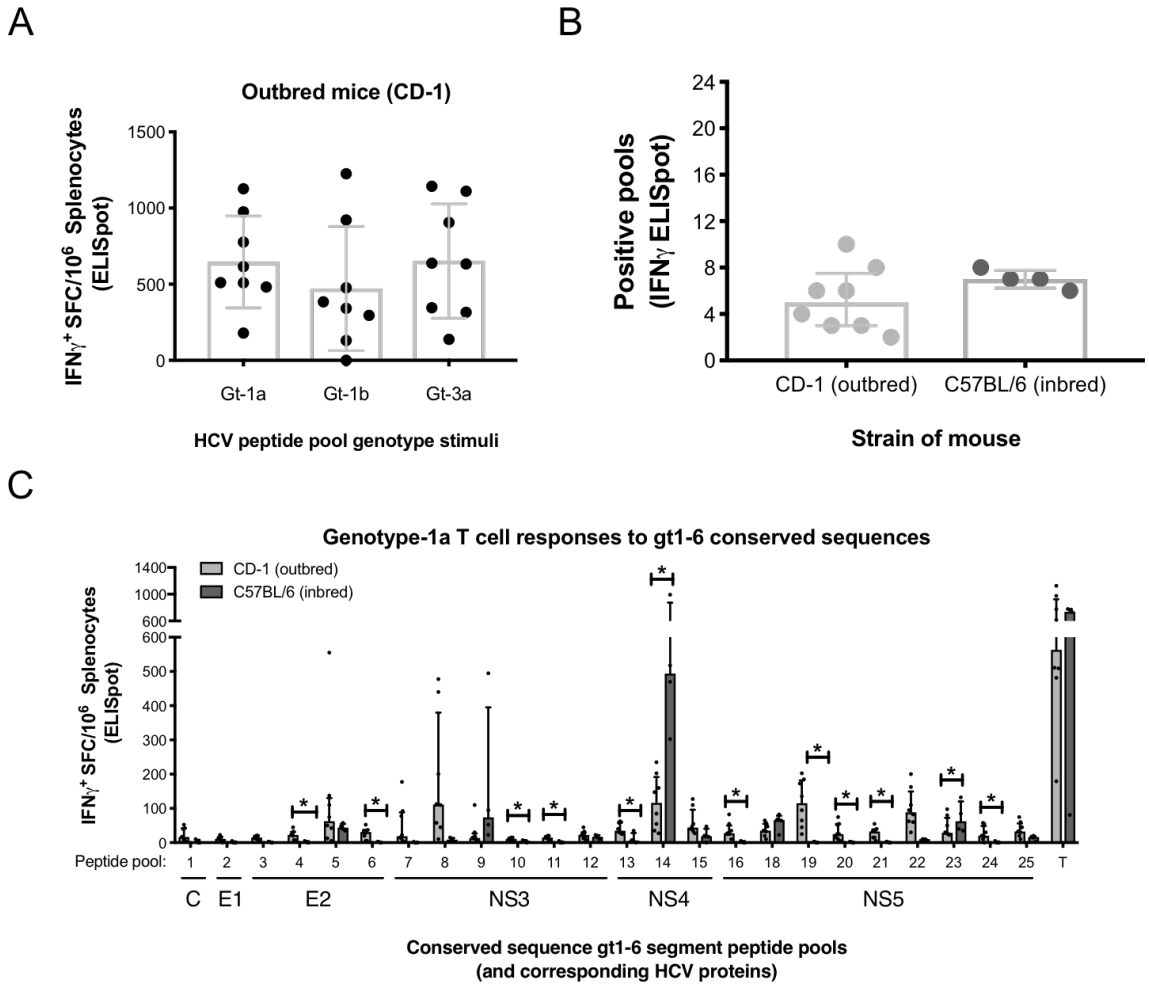


Figure 3-12. Gt1-6L vaccine immunogenicity in CD-1 outbred and C57BL/6 inbred mice: Groups of 8 age-matched female CD-1 mice were vaccinated with 10^8 IU of vaccine in a 40uL intramuscular injection and harvested 3-weeks post vaccination. The experiment was performed twice. (**A**) Total genotype-specific IFNγ ELISpot responses of ChAd-gt1-6L in CD-1 outbred mice splenocytes. T cell magnitude is the total of the positive individual peptide pools. (**B**) The number of IFNγ positive ELISpot pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. (**C**) The breadth of the T cell response to conserved gt1-6 sequence peptide pools (S1-25) from ChAd-gt1-6L vaccinated CD-1 outbred mice and C57BL/6 inbred mice. Peptides are 15-18mers, overlap by 11aa, and cover the subtype-1b specific conserved sequence of the Gt1-6L immunogen. Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant

difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.10 Conserved sequence HCV T cell vaccines induce $CD4^+$ and $CD8^+$ T cells that target conserved subdominant T cell epitopes

I next assessed the specificity of the ChAd-gt1-6L and ChAd-gt-1b-NS induced T cell response for conserved HCV sequences using peptides pools and corresponding to conserved segments in the ChAd-gt1-6L immunogen. Intracellular staining (ICS) was used to determine the vaccine-induced T cell subsets generated by each vaccine.

The total IFNγ ELISpot response was similar between gt1-6L and gt-1b-NS vaccines but gt1-6L displayed a broader ELISpot response (Figure 3-13**A-B**). Gt1-6L vaccine-induced T cell responses targeted S5 (E2), S9 (NS3p), S14 (NS4), S18 (NS5a), and S23 (NS5b), whilst S13 (NS3h/NS4a) was the only responding peptide pool in mice vaccinated with gt-1b-NS (Figure 3-13**C**).

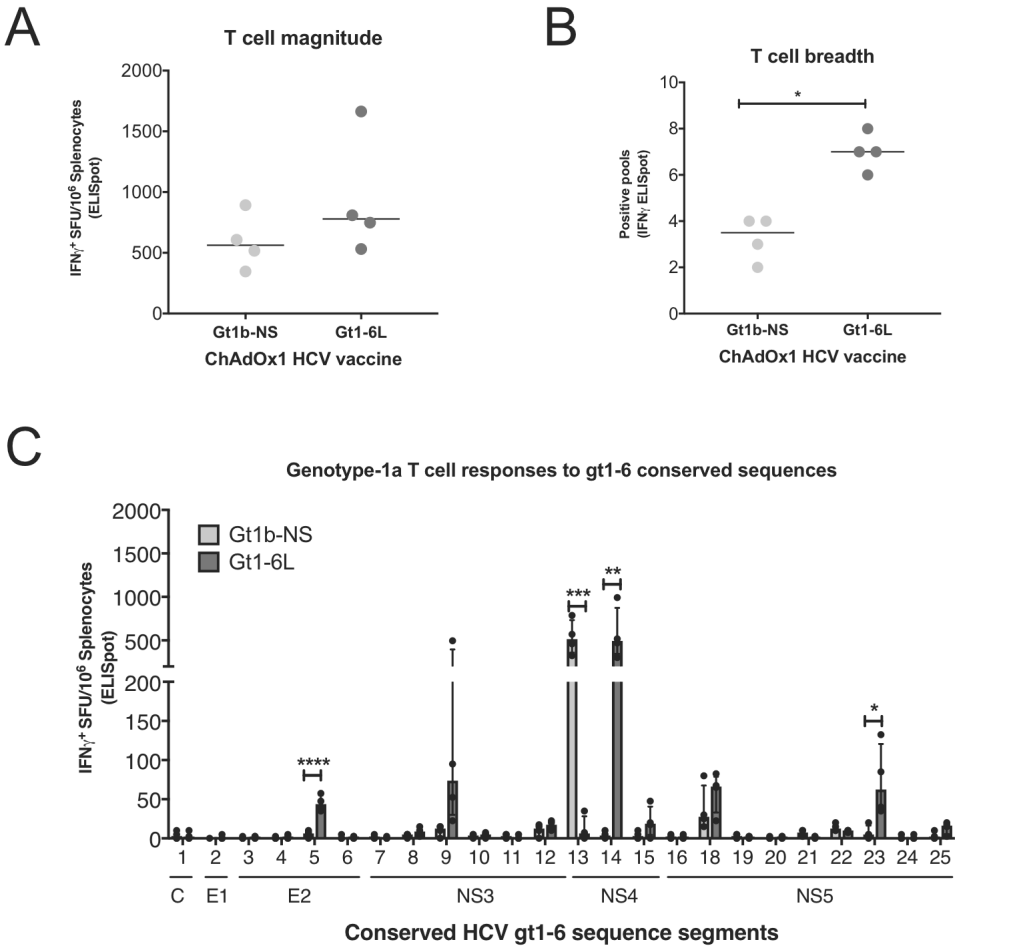


Figure 3-13. Comparative analysis of ChAd-gt-1b-NS and ChAd-gt1-6 vaccine-induced immunogenicity to conserved HCV sequence: Age-matched female C57BL/6 mice were vaccinated with 10^8 IU of either ChAd-gt-1b-NS or ChAd-gt1-6L in a 40uL intramuscular injection and harvested 3-weeks post vaccination (n=4 for each vaccine, respectively, and the experiment was performed once). (**A**) Total genotype-specific IFNγ ELISpot responses of ChAd-gt1-6L in mouse splenocytes. T cell magnitude is the total of the positive individual peptide pools. (**B**) The number of IFNγ positive ELISpot pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. (**C**) The breadth of the vaccine-induced T cell response to conserved gt1-6 sequence peptide pools (S1-25) that cover the subtype-1b specific conserved sequence of the Gt1-6L immunogen (15-18mers overlapping by 11aa). Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

Next, I characterised the gt1-6L-induced T cell response to conserved HCV sequence segment pools: S5, S9, S14, and S23 using ICS (example plots shown; Figure 3-14**A**). Both $CD8^+$ and $CD4^+$ T cell subsets were detected, but the $CD8^+$ T cell responses were of a higher magnitude than the $CD4^+$ T cell response. Both S9 and S14-specific $CD8^+$ T cells secreted IFNγ and TNFα and to a less extent IL-2, while S23-specific $CD8^+$ T cells secreted markedly less cytokines reflecting the IFNγ ELISpot response, and no cytokine-producing $CD8^+$ T cells were detected for S5 (Figure 3-14**B**). S9-specific $CD4^+$ T cells secreted IFNγ and IL-2 whilst S14- and S23-specific $CD4^+$ T cells displayed lower magnitudes of cytokine secretion. A low but detectable $CD4^+$ T cell response was detected to S5, the conserved segment lying in the HCV E2 glycoprotein (Figure 3-14**C**).

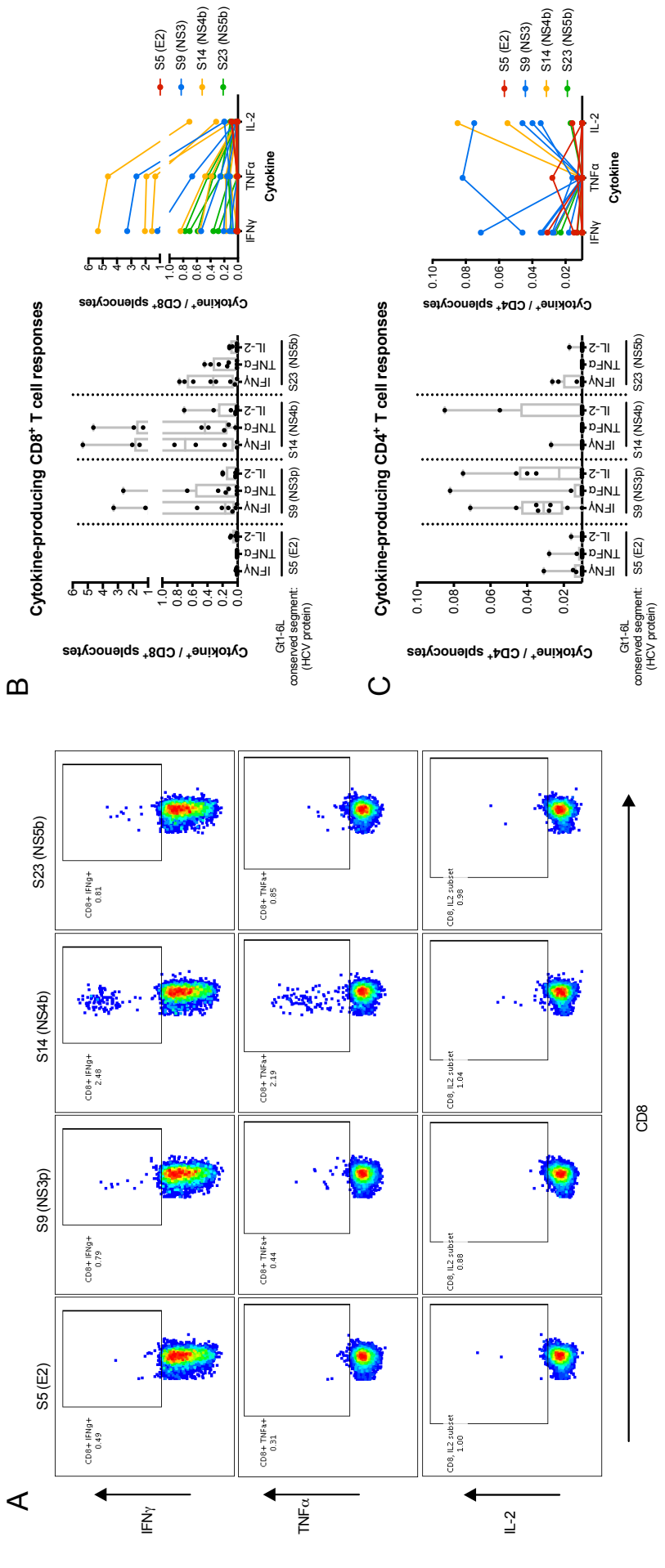

A
S5 (E2)
S9 (NS3p)
S14 (NS4b)
S23 (NS5b)
IFNγ
TNFα
IL-2
CD8
B
Cytokine-producing CD8+ T cell responses
Cytokine+ / CD8+ splenocytes
Cytokine
IFNγ
TNFα
IL-2
S5 (E2)
S9 (NS3)
S14 (NS4b)
S23 (NS5b)
Gt1-6L conserved segment: (HCV protein)
C
Cytokine-producing CD4+ T cell responses
Cytokine+ / CD4+ splenocytes

Figure 3-14. Functionality of gt1-6L vaccine-induced CD8⁺ and CD4⁺ T cell responses to conserved HCV sequences: Age-matched female C57BL/6 mice were vaccinated with 10^8 IU of ChAd-gt1-6L in a 40uL intramuscular injection and harvested 2-weeks post vaccination (n=8, and the experiment was performed once). (**A**) Example plots of cytokine-producing CD8⁺ T cells. Intracellular staining analysis of vaccine-induced CD8⁺ (**B**) and CD4⁺ (**C**) T cells stimulated with conserved sequence peptides in four pool: S5 (E2), S9 (NS3p), S14 (NS4b), and S23 (NS5b) for the detection of T cell-secreted IFNγ, TNFα, and IL-2 cytokine. Peptides were 15-18mers and overlapped by 11aa. Graphs are box and whisker plots with medians and ranges displayed.

In order to assess the quality of the conserved HCV sequence specific T cell response, a re-analysis of cytokine-producing T cell responses primed by ChAd-gt1-6L and ChAd-gt-1b-NS vaccines, was undertaken using each vaccine's most immunogenic peptide pool (S13 and S14, respectively; see Figure 3-13**C**). The ChAd-gt-1b-NS induced T cell response to S13 was a CD8⁺ subset whereas the ChAd-gt1-6L generated a CD4⁺ and CD8⁺ T cell response to S14 (Figure 3-15).

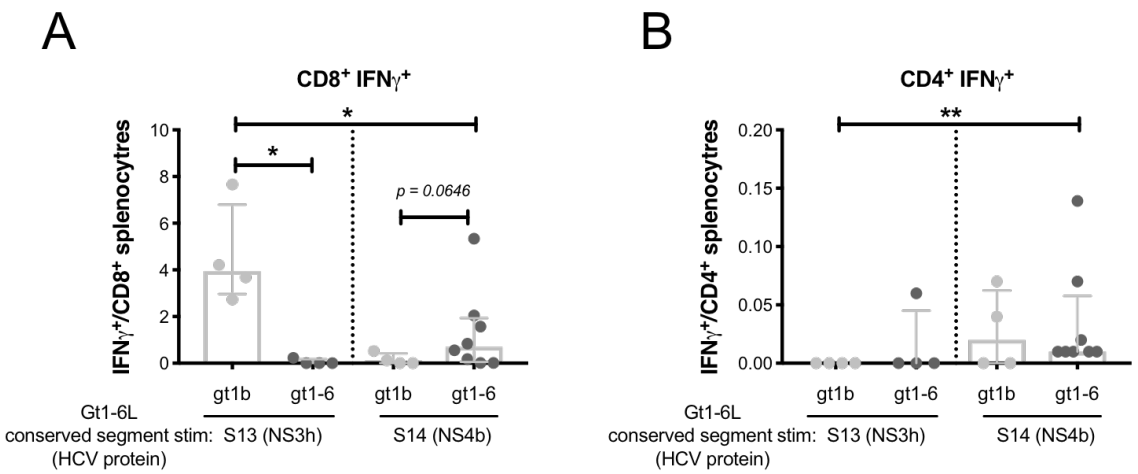
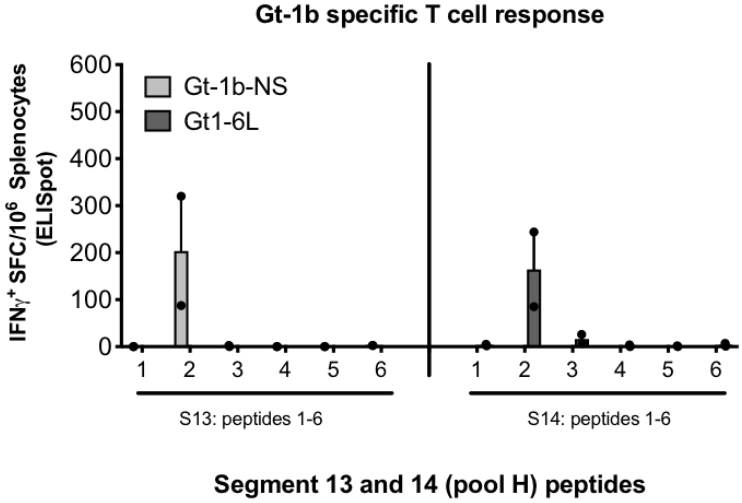


Figure 3-15. Functionality of ChAd-gt-1b-NS and ChAd-gt1-6L vaccine-induced T cells targeting their most immunogenic respective epitope: C57BL/6 mice were vaccinated with 10^8 IU of ChAd-gt-1b-NS or ChAd-gt1-6L in a 40uL intramuscular injection and harvested 2-weeks post vaccination (n=4-8; data presented is from two experiments). (A) Total vaccine-induced S13 and

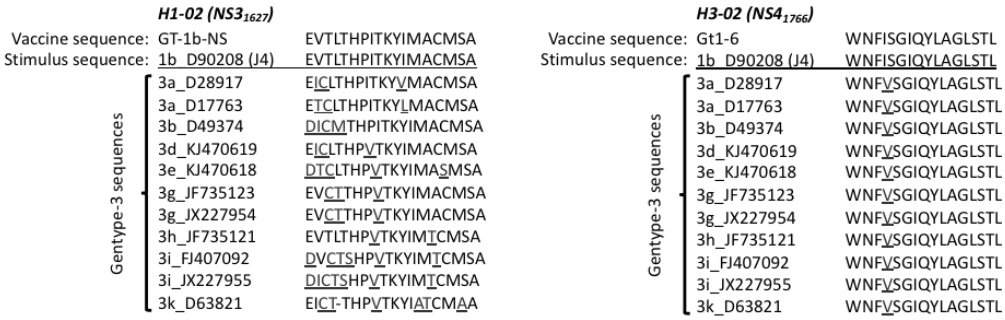
S14-specific IFNγ^+ CD8$^+$ T cell response in mouse splenocytes. (B) Total vaccine-induced S13 and S14-specific IFNγ^+ CD4$^+$ T cell response in mouse splenocytes. The S13 and S14 peptide pools cover the subtype-1b specific conserved sequence of the NS3h and NS4b HCV protein sequence (15-18mers overlapping by 11aa). Bars represent medians and interquartile ranges are displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

Next, using the IFNγ ELISpot assay, I mapped the T cell responses to the S13 and S14 segment peptide pools to single epitopes. I identified one immunogenic T cell epitope in each of the S13 and S14 peptide pools: $NS3_{1634}$ and $NS4_{1766}$ respectively (Figure 3-16**A**). A major limitation of the ChAd-gt-1b-NS vaccine is its reduced immunogenicity to HCV genotype-3, therefore I compared the vaccine-targeted T cell epitopes for their conservation across the subtypes of HCV genotype-3. The ChAd-gt1-6L targeted epitope in NS4b ($NS4_{1766}$) displayed one amino acid mismatch between the vaccine sequence of that T cell epitope and all gt-3 subtypes, whilst the ChAd-gt-1b-NS targeted epitope in NS3 ($NS3_{1634}$) revealed between 2 and 6 amino acid mismatches with the vaccine sequence of that T cell epitope and all gt-3 subtypes (Figure 3-16**B**). Using all the confirmed HCV subtypes (listed by the International Committee for the Taxonomy of Viruses on www.talk.ictvonline.org), a comparison of the $NS3_{1634}$ and $NS4_{1766}$ T cell epitopes across all HCV subtypes revealed significant differences in percentage of sequence conservation between the epitopes (Figure 3-16**C**). As expected, the gt1-6L targeted epitope, $NS4_{1766}$, was highly conserved across HCV subtypes compared to the gt-1b-NS targeted T cell epitope, $NS3_{1627}$, which displayed reduced conservation in genotype-1 (90.91% for $NS3_{1627}$ vs 99.15% in $NS4_{1766}$), -2 (76.52% vs 98.86%), -3 (83.26% vs 93.75%), and -6a (83.16% vs 94.23%; means displayed, $p < 0.0001$****).

A


Gt-1b specific T cell response
IFNγ+ SFC/10^6 Splenocytes (ELISpot)
Gt-1b-NS
Gt1-6L
600
500
400
300
200
100
0
1 2 3 4 5 6
1 2 3 4 5 6
S13: peptides 1-6
S14: peptides 1-6
Segment 13 and 14 (pool H) peptides

B


H1-02 (NS3 1627)
Vaccine sequence: GT-1b-NS EVTLTHPITKYIMACMSA
Stimulus sequence: 1b_D90208 (J4) EVTLTHPITKYIMACMSA
Gentype-3 sequences
3a_D28917 EICLTHPITKYVMACMSA
3a_D17763 ETCLTHPITKYLMACMSA
3b_D49374 DICMTHPITKYIMACMSA
3d_KJ470619 EICLTHPVTKYIMACMSA
3e_KJ470618 DTCLTHPVTKYIMASMSA
3g_JF735123 EVCTTHPVTKYIMACMSA
3g_JX227954 EVCTTHPVTKYIMACMSA
3h_JF735121 EVTLTHPVTKYIMTCMSA
3i_FJ407092 DVCTSHPVTKYIMTCMSA
3i_JX227955 DICTSHPVTKYIMTCMSA
3k_D63821 EICT-THPVTKYIATCMAA
H3-02 (NS4 1766)
Vaccine sequence: Gt1-6 WNFISGIQYLAGLSTL
Stimulus sequence: 1b_D90208 (J4) WNFISGIQYLAGLSTL
Gentype-3 sequences
3a_D28917 WNFVSGIQYLAGLSTL
3a_D17763 WNFVSGIQYLAGLSTL
3b_D49374 WNFVSGIQYLAGLSTL
3d_KJ470619 WNFVSGIQYLAGLSTL
3e_KJ470618 WNFVSGIQYLAGLSTL
3g_JF735123 WNFVSGIQYLAGLSTL
3g_JX227954 WNFVSGIQYLAGLSTL
3h_JF735121 WNFVSGIQYLAGLSTL
3i_FJ407092 WNFVSGIQYLAGLSTL
3i_JX227955 WNFVSGIQYLAGLSTL
3k_D63821 WNFVSGIQYLAGLSTL

C

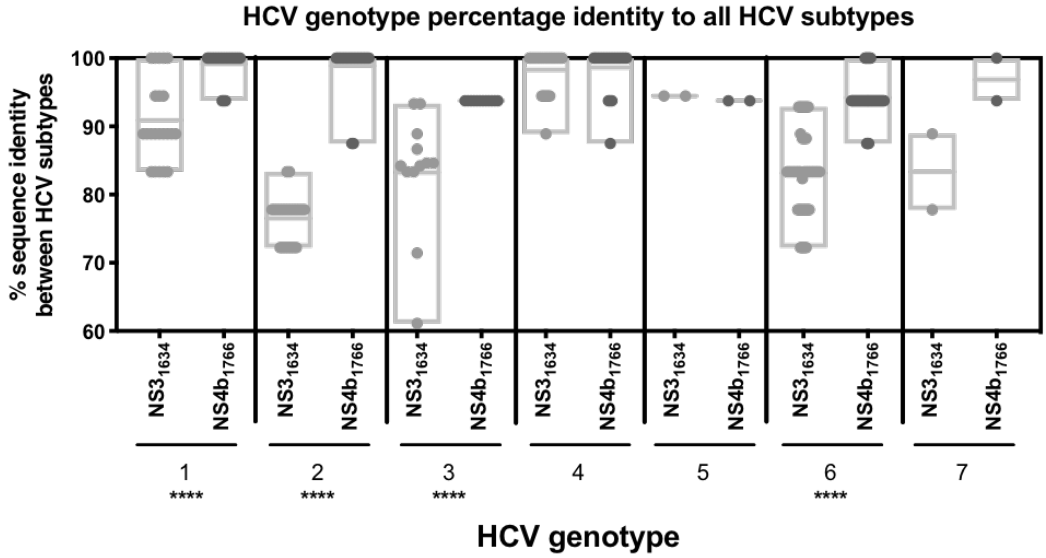

HCV genotype percentage identity to all HCV subtypes
% sequence identity between HCV subtypes
100
90
80
70
60
NS3 1634
NS4b 1766
1 2 3 4 5 6 7
**** **** **** ****
HCV genotype

Figure 3-16. Conservation of vaccine-induced T cell targeted epitopes: C57BL/6 mice were vaccinated with 10^8 IU of ChAd-gt-1b-NS or ChAd-gt1-6L in a 40uL intramuscular injection and harvested 2-weeks post vaccination (n=2 due to limited splenocyte yield). The data displayed is a combination of two vaccination experiments performed at different times. (**A**) The vaccine-induced IFNγ^+ T cell response in mouse splenocytes. The S13 and S14 peptide pools cover the subtype-1b specific conserved sequence of the NS3h-4a and NS4b HCV protein sequence (15-18mers overlapping by 11aa). Bars represent medians and interquartile ranges are displayed. (**B**) The S13-2 ($NS3_{1634}$) and S14-03 ($NS4_{1766}$) amino acid sequences in the vaccine immunogen, peptide stimulus, and genotype-3 subtypes are shown. Amino acid mutations are in red and underlined. Accession numbers are shown (International Committee for the Taxonomy of Viruses). (**C**) The percentage sequence identity of S13-2 ($NS3_{1634}$) and S14-03 ($NS4_{1766}$) sequences across all HCV subtypes (each HCV subtype is a data point) with means and ranges displayed (subtypes listed and defined by the International Committee for the Taxonomy of Viruses). Mann Whitney tests were performed to determine a significant difference between two group means at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

3.5.11 A novel genetic adjuvant – the transmembrane region of the shark invariant chain (sIi) – increases vaccine-induced T cell magnitude, breadth, and cross-reactivity

Genetic adjuvants—sequences that are encoded within a DNA or virally vectored vaccine to enhance vaccine immunogenicity—have been a complementary avenue of investigation in vaccine research. A number of studies support the use of the MHC class II invariant chain (Ii) as a vaccine genetic adjuvant, with increased transgene-specific T cell responses when Ii is tethered directly to the transgene (Capone et al., 2014; P J Holst et al., 2008; Spencer et al., 2014; Wälchli et al., 2014). The Ii sequence domains responsible for the adjuvant effect have been investigated and the minimal sequence required for the adjuvant effect is the Ii transmembrane domain, amino acid residues 47-72 (Ii_{tr} or $Ii_{47\text{-}72}$; Halbroth et al. 2018).

The use of Ii as a genetic adjuvant in vaccine trials is theoretically associated with the risk of generating autoimmunity (Baerlecken et al., 2014; Baraliakos et al., 2014), as Ii is a self-antigen: a constituent component of the MHC class II presentation pathway in human cells (reviewed by Blum et al. 2013). In short, the MHC class II invariant chain functions as a chaperone by which it stabilises the interaction of the α and β chains of MHC as they are formed in the endoplasmic reticulum (ER). Ii then occupies the peptide-binding groove of MHC class II molecules, inhibiting peptide loading in the ER and targeting MHC class II to the endosomal pathway (Sandoval & Bakke, 1994). In the late endosome, Ii is cleaved by cathespins so that class II associated invariant chain peptide (CLIP) remains in the MHC peptide-binding groove until replaced by exogenous peptides for presentation to $CD4^+$ T cells.

To limit generation of autoimmunity, non-human Ii—such as chicken, trout, frog, and shark invariant chains—with limited homology to human invariant chain has been explored as an alternative strategy in order to decrease the risk of inciting autoimmunity (Halbroth et al., 2018).

For this study I chose the truncated sequence of shark invariant chain (sIi_{tr}) as it displays 24.6% human homology to hIi, the lowest sequence homology of all species investigated (Figure 3-17**A**; Halbroth et al. 2018). Furthermore, sIi adjuvanted viral vectored HBV vaccine induced high magnitude transgene-specific T cell responses in mice (personal communications with Senthil Chinnakannan in the Barnes Laboratory at the University of Oxford) and enhanced the protective capacity of viral vectored malaria vaccines (Halbroth et al. 2018).

I therefore investigated the immunogenicity of sIi_{tr} adjuvanted conserved sequence HCV T cell vaccines and determined that vaccine-induced immunogenicity was enhanced when the sIi_{tr} was tethered to the conserved HCV sequence transgene in the ChAd viral vector

(**Figure 3-17A**). The total HCV specific IFNγ ELISpot response of vaccine-induced HCV-specific immunogenicity in C57BL6 inbred mice was compared between genetic adjuvants sIi_{tr}, a control adjuvant tissue plasminogen activator leading sequence which is a proven vaccine genetic adjuvant as it is targeted to the secretory pathway by its N-terminus (TPA-LS; Parmer et al. 1997; de Cassan and Draper 2013), and an immunogen with no adjuvant ('No GA'). Only sIi_{tr} demonstrated a significant increase in T cell magnitude compared to the non-adjuvanted vaccine (Figure 3-17**B**). The vaccine-induced HCV-specific T cell response was significant broader in sIi_{tr} adjuvanted vaccines than TPA-LS or the non-adjuvanted vaccine, targeting up to 19 of the 24 conserved sequence segments contained in the gt1-6L immunogen (Figure 3-17**C-D**).

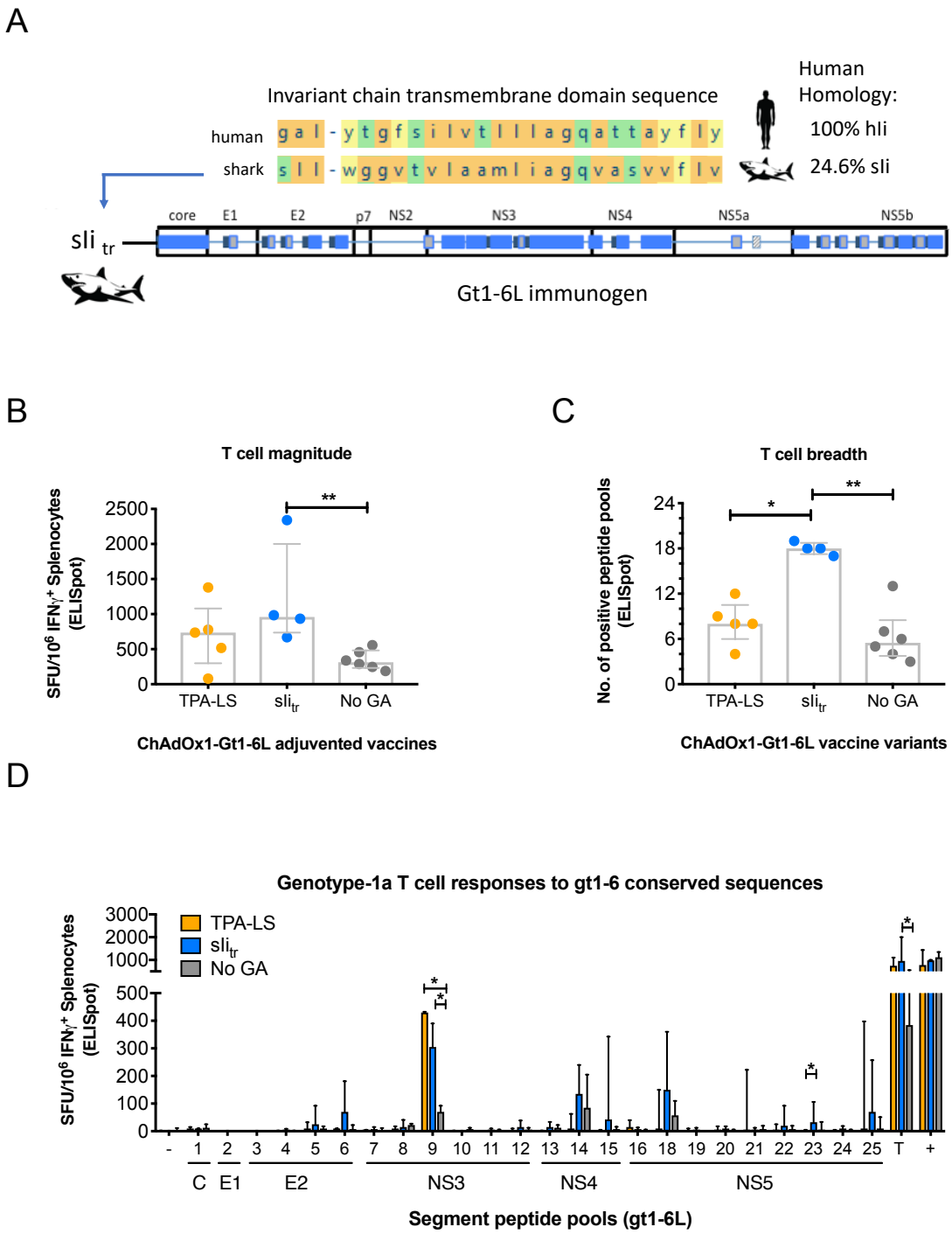


Figure 3-17. The design of HCV viral vector vaccines with genetic adjuvant truncated shark invariant chain (slitr) and vaccine immunogenicity in C57BL/6 mice: (**A**) A schematic of the truncated shark invariant chain (sli_{tr}) sequence and truncated human invariant chain sequence alignment (hli_{tr}). Sli_{tr} is encoded at the 5' end of the gt1-6L HCV conserved immunogen sequence. C57BL/6 mice (4-6/group) were vaccinated with 10^8 IU of ChAd-gt1-6L-TPA-NS ('*TPA-LS*'), ChAd-gt1-6L-sli ('*sli_{tr}*'), or ChAd-gt1-6L (no genetic adjuvant, '*No GA*') in a 40uL intramuscular injection

and harvested 2-weeks post vaccination. (**B**) T cell magnitude is the total of the positive individual peptide pools. (**C**) The number of IFNγ positive ELISpot pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations. (**D**) The breadth of the vaccine-induced T cell response to conserved gt1-6 sequence peptide pools (S1-25) that cover the subtype-1b specific conserved sequence of the Gt1-6L immunogen (15-18mers overlapping by 11aa). Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.0005***, <0.0001****.

Next, I assessed the cross-reactivity of vaccine-induced HCV-specific immunogenicity to common HCV genotypes in CD-1 outbred mice to determine TPA-LS and sIi_{tr} adjuvanted vaccine-induced immunogenicity. The sIi_{tr} adjuvanted gt1-6L vaccine increased the HCV genotype-1a, -1b, and -3a specific total IFNγ ELISpot response compared to the TPA-LS adjuvanted gt1-6L vaccine (Figure 3-18**A**). The total IFNγ ELISpot response of gt1-6L-sIi was compared against the total ELISpot response of gt-1b-NS-TPA-LS and revealed similar HCV genotype-1b specific immunogenicity between the vaccines, and a significant increase in HCV genotype-3a specific gt1-6L-sIi-induced immunogenicity (Figure 3-18**B**).

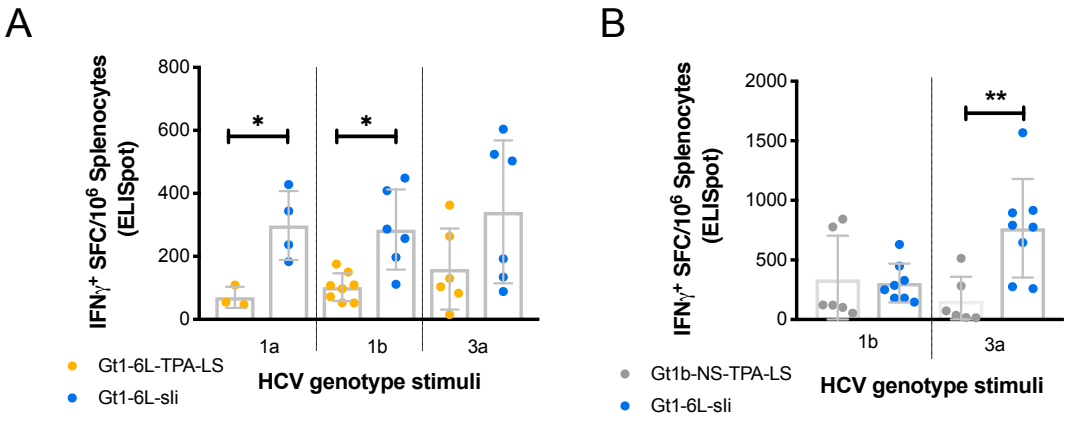


Figure 3-18. Immunogenicity of genetically adjuvanted HCV viral vector vaccines in CD-1 outbred mice: (**A**) Outbred CD-1 8-week old age-matched female mice (n=3-8/group due to limited cell yield during harvest) were vaccinated with 10^7 IU of ChAd-gt1-6L-TPA-NS or ChAd-gt1-6L-sIi in a 40uL intramuscular injection and harvested 2-weeks post vaccination. The experiment was repeated once. Total vaccine induced genotype-specific IFNγ ELISpot responses in mouse splenocytes are displayed. (**B**) Outbred CD-1 8-week old age-matched female mice (6-8/group) were vaccinated with 10^8 IU of ChAd-gt1b-NS-TPA-LS or ChAd-gt1-6L-sIi in a 40uL intramuscular injection and harvested 2-weeks post vaccination. Total vaccine induced genotype-1b and -3a IFNγ ELISpot responses in mouse splenocytes are displayed. T cell magnitude is the total of the positive individual peptide pools. Pools are considered positive when greater than the mean of the DMSO negative control plus three standard deviations and when greater than the unvaccinated control. HCV peptides were pooled into 10 pools that cover the full length of the subtype-1b or -3a specific HCV protein sequence (15-18mers overlapping by 11aa). Bars represent the median SFU/10^6 splenocytes, with interquartile range displayed. Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001 ***, <0.0001****.

3.6 Discussion

The primary hypothesis of the work described in this chapter was that simian adenovirus vector vaccines encoding conserved proteomic regions across HCV subtypes are immunogenic *in vivo.*

The experimental data presented in this chapter confirms the primary hypothesis. Simian adenovirus vector HCV T cell vaccines that encode conserved gene segments across HCV subtypes induce high magnitude, broad, functional $CD4^+$ and $CD8^+$ T cell responses. Specifically, conserved sequence HCV T cells induce significantly higher genotype-3a specific T cell responses than the subtype-1b vaccine (ChAd-gt-1b-NS) targeting subdominant T cell epitopes that are highly conserved across all HCV subtypes, unlike ChAd-gt-1b-NS. Using the truncated shark invariant chain (sIi_{tr}), I show that transgene-

specific immunogenicity can be increased when tethering the sIi_{tr} to the transgene in the viral vectored vaccine.

3.6.1 HCV immunogen length and linker sequence between gene segments affects vaccine immunogenicity

It is not known if conserved HCV viral regions are immunogenic or immunologically inert *in vivo.* In this study, I showed that conserved sequences HCV T cell vaccines—ChAd-gt-1/3 (short and long immunogens) and -gt1-6 (short and long immunogens)—are immunogenic in mice. Vaccines that contained longer HCV conserved sequence immunogens displayed higher magnitude and broader T cell responses than vaccines containing shorter HCV conserved sequence immunogens, likely due to the reduced number of T cell epitopes present in the vaccine. This is concurrent with *in silico* analysis of conserved sequence vaccines as it was previously shown that they contain both known CD4 and CD8 T cell epitopes described in natural infection (von Delft et al., 2018). The compromise of shortening the immunogen, and therefore reducing the number of T cell epitopes contained in the immunogen, was to increase manufacturing feasibility yet this came at the cost of reduced immunogenicity *in vivo.*

As conserved sequence vaccines are an assembly of gene segments that do not appear in nature, artificial epitopes are generated between adjoining gene segments in the transgene. Artificial epitopes may be immunogenic, potentially diverting the T cell response away from the intended HCV-specific immune response. Therefore, the addition of linker sequences between gene segments was used to abrogate *in silico* predicted strong binding epitopes found in adjoining regions of conserved HCV gene segments. Importantly, linkers displayed no immunogenicity *in vivo* as was predicted *in silico* (von Delft et al., 2018) and their presence in the immunogen enhanced HCV-specific immunogenicity. The increased HCV-specific T cell response could be because strong-binding artificial epitopes may also

be immunodominant within the immunopeptidome of the vaccine infected cell and therefore artificial epitope abrogation may enable HCV epitopes to dominate naïve T cell induction.

3.6.2 Conserved sequence HCV T cell vaccines induce broad, high magnitude T cell responses to multiple HCV genotypes

While protective immune correlates against HCV are not fully understood, there is a strong case for the role of cellular mediated immunity in virus control and resolution in natural and in chimpanzee challenge studies (reviewed by Abdel-Hakeem & Shoukry, 2014). Therefore, a vaccine-induced T cell response should be high magnitude, target multiple antigens and HCV subtypes, and be polyfunctional (Gerlach et al., 1999; Osburn et al., 2010; Takaki et al., 2000). To this end, the viral vectored HCV subtype-1b T cell vaccine meets the majority of this criteria and is the most clinically advanced HCV vaccine, currently in phase II human clinical trials (NCT01436357; Folgori et al. 2006; Barnes et al. 2012; Swadling et al. 2014; Kelly et al. 2015). Yet, the subtype-1b vaccine-induced T cell response displayed reduced immunogenicity to non-subtype-1b antigens and sequence variants at immunodominant epitope (Swadling et al. 2014; Kelly et al. 2015).

In this study, I demonstrated that conserved sequence HCV T cell vaccines, ChAd-gt1-6L and -gt1/3, induce high magnitude T cell responses of a comparable magnitude to ChAd-gt-1b-NS in inbred, outbred, and HLA-A2.1 transgenic mice. A high magnitude adenovirus-induced T cell response is in accordance with a number of other studies that target other pathogens using adenoviruses (Ewer et al. 2016), in contrast to other vaccine platforms used for HCV development such as DNA vaccines and recombinant proteins (Houghton, 2011).

T cell breadth is associated with clinical outcome of HCV infection. Broad T cell response is readily detectable in spontaneous resolver of HCV infection, whereas, T cell responses

in the chronic phase of HCV infection target a narrow repertoire of T cell epitopes that undergo viral escape from the host immune system (Day et al., 2002; Lauer et al., 2004; Neumann-Haefelin & Thimme, 2013; Osburn et al., 2010; Schulze zur Wiesch et al., 2005). Therefore, increasing the breadth of the vaccine-induced HCV-specific T cell response may increase the likelihood of T cell epitope recognition and effectively mount a robust population of T cells to control and resolve acute HCV infection before epitope mutation occurs and HCV persists. The conserved sequence HCV T cell vaccines induce a broad T cell response targeting epitopes in multiple HCV proteins, comparable to the ChAd-gt-1b-NS vaccine, in mice. Strikingly, the vaccine-induced T cell response was skewed towards the HCV non-structural proteins with limited detection of core-specific T cell responses. T cells targeting core epitopes have been previously associated with spontaneous HCV clearance (Day et al., 2002; Lauer et al., 2004; Schulze zur Wiesch et al., 2005). Their absence in pre-clinical studies of conserved sequence HCV T cell vaccines warrants investigation through core epitope specific tetramers and a ChAd vector expressing only core sequence to demonstrate to abrogate any epitope hierarchal dominance exerted by conserved epitopes in non-structural sequence.

An important criterion of a prophylactic HCV vaccine is the induction of a T cell response against multiple HCV genotypes and epitope variants. An intergenotypic T cell response would be critical in endemic regions where multiple HCV genotypes circulate in addition to recognising diverse epitopes in the virus quasispecies in the infected host that are prone to immune escape. However, HCV exhibits approximately 30% sequence diversity between genotypes and 15-30% within subtypes (Smith et al., 2014), with immunodominant T cell epitopes predominantly residing in variable proteomic regions and conferring genotype-specific T cell responses (Humphreys et al. 2012; von Delft et al. 2015; Lunburger et al. 2018). Consequently, HCV vaccines encoding a specific HCV

genotype sequence display limited immunogenicity to non-vaccinal genotype antigens in humans (Kelly et al., 2015; Swadling et al., 2014), rhesus macaques (Fattori et al., 2006), and confer lack of protection against non-vaccinal HCV genotypes in the surrogate HCV infection model, HCV-rVV, in mice (Eisenbach et al., 2006). Therefore, HCV sequence variability represents a major obstacle to vaccine development. In this study, I demonstrate that conserved sequence HCV vaccines elicit robust T cell responses against multiple epitopes contained in HCV structural and non-structural proteins from common circulating subtypes: 1a, 1b, and 3a. Importantly, conserved sequence vaccine induced genotype-3a T cell response was significantly higher than the ChAd-gt-1b-NS induced genotype-3a specific T cell response. Furthermore, targeting conserved HCV epitopes by vaccination may prevent T cell immune escape as mutations in cognate epitopes will come at detrimental fitness cost to the virus.

While genotype-1a, -1b, and -3a peptides used in this study display conservation across peptides that cover HCV sequences that represent diverse subtypes (such as gt-4a and gt-6a), this study did not assess vaccine-induced T cell responses to complete peptide pools sets based on diverse HCV subtypes due to financial reasons. For future studies, utilising single peptide pools or peptides that cover immunogenic conserved HCV sequence that represent diverse subtypes may be useful to further assess the extent of the vaccine-induced intergenotypic T cell response.

3.6.3 Conserved sequence vaccines induce T cell subsets that are important for protective immunity to HCV

The central role of the $CD4^+$ T cell subset in generation and maintenance of the $CD8^+$ T cell response in HCV viral control is evident in natural infection (Gerlach, et al., 1999; Thimme et al., 2001) and proven in chimpanzee challenge studies (Grakoui et al., 2003). Conserved sequence HCV T cell vaccines induce a polyfunctional and mono-functional

$CD4^+$ T cell response consisting of IFNγ, TNFα, and IL-2 secretion. Synergistic action of TNFα and IFNγ enhances mechanisms of cytotoxicity in cell-mediated immunity (Bogdan et al., 1990; Chan et al., 1992; Liew et al., 1990) indicating that mono-functional $IFN\gamma^+$ or $TNF\alpha^+$ T cells may constitute a protective T cell response in some settings. A vaccine-induced polyfunctionality T cell response targeting multiple antigens is concurrent with protective immune responses in HCV (Lauer et al., 2004; Lechner et al., 2000), and other chronic viral infections. In HIV infection, $CD4^+$ T cell polyfunctionality correlated with low viral load and resolution of viral infection in individuals who resolved infection (Betts et al., 2006; Kannanganat et al., 2007; Kannanganat et al., 2007). The lack of a vaccine-induced $CD4^+$ T cell response may compromise $CD8^+$ T cell functionality and permit the emergence of viral escape mutants and lead to chronic viral infection (Gerlach et al., 1999; Grakoui et al., 2003).

A broad polyfunctional $CD8^+$ T cell response is a key feature of a successful HCV-specific T cell response in individuals who spontaneous resolve acute HCV infection (Cooper et al., 1999; Lechner et al., 2000). Simian adenovirus vectored HCV vaccines are efficient at priming a high magnitude HCV-specific $CD8^+$ T cell response in chimpanzees and humans (Barnes et al., 2012; Folgori et al., 2006). In mice, conserved sequence HCV T cell vaccines elicited polyfunctional $IFN\gamma^+/TNF\alpha^+/IL\text{-}2^+$ $CD8^+$ T cell responses targeting multiple conserved HCV sequences after a single prime vaccination. The ChAd-induced T cell response shown in this study aligns with other ChAd vaccine studies as it is the intrinsic property of adenovirus vectors, not the transgenes, to induce functional T cell phenotypes (Ewer et al., 2017).

3.6.4 Conserved sequence HCV vaccines target highly conserved subdominant T cell epitopes

Immunodominant $CD8^+$ and $CD4^+$ T cell epitopes in variable viral regions display limited cross reactivity between HCV genotypes (Sugimoto et al. 2003; Harcourt et al. 2003; Humphreys et al. 2012; von Delft et al. 2015; Luxenburger et al. 2018). The exclusion of variable HCV sequences containing immunodominant epitopes from HCV vaccine immunogen design may increase the valency of subdominant epitopes to naïve T cells and therefore increase immunogenicity of subdominant T cell epitopes after vaccination (von Delft et al., 2018) as has been demonstrated in the HIV field (Im et al., 2011). In this study, conserved sequence HCV vaccines induced T cells targeted highly conserved $CD4^+$ and $CD8^+$ T cell epitopes. This is in line with reports of conserved T cell epitopes described in acute HCV infection resolution (Lamonaca et al. 1999; Giugliano et al. 2009) including a $CD8^+$ T cell epitope, $NS4_{1766}$ (Lauer et al. 2004). Strikingly, the vaccine-induced $CD4^+$ T cell subset generated a significantly higher intergenotype T cell response to HCV genotype-1b and -3a antigens than the $CD8^+$ T cell response. A cross-reactive $CD4^+$ T cell response to multiple genotypes is in accordance with *in silico* epitope prediction analysis that identified a greater proportion of all identified CD4 epitopes, compared to CD8 epitopes, are found in conserved HCV sequence regions (von Delft et al., 2018). Furthermore, the ChAd-gt1-6L vaccine T cell response targeted conserved T cell epitopes that displayed, on average, greater than 93% amino acid homology across all seven HCV genotypes and 100% amino acid homology in many HCV subtypes in genotypes-1, -2, -4, and -6. Whereas, ChAd-gt-1b-NS vaccine-induced T cells targeted different, more variable T cell epitopes, reflecting the dynamics of an antigen hierarchy that can be leveraged by excluding immunodominant epitopes.

Numerous studies in the HIV field that support the utility of conserved subdominant T cell epitopes in vaccine design to elicit robust T cell responses (Létourneau et al. 2007; Im et

al. 2011; Ondondo et al. 2014; Borthwick et al. 2014; Hancock et al. 2015; Ahmed et al. 2016; Steffensen et al. 2016). In HIV positive individuals, preferential targeting of subdominant T cell epitopes, rather than T cell magnitude or breadth, correlated with low viral load (Hancock et al., 2015; Masemola et al., 2004). Subsequent studies demonstrated that conserved HIV epitopes are targeted by T cells isolated from peripheral blood of HIV-1 positive individuals and correlate with a high $CD4^+$ T cell count and low viral load (Ondondo et al., 2016). Furthermore, T cells targeting conserved HIV sequences suppressed *in vitro* HIV-1 replication from HIV-1 patient isolates (Murakoshi et al., 2018). In human clinical trials, the conserved mosaic HIV T cell vaccine—HIVcons—elicited robust $CD8^+$ T cell responses (Borthwick et al. 2014), that recognise conserved *pol* and *gag* protein sequences including novel subdominant epitopes capable of mediating HIV inhibition *in vitro* (Ahmed et al., 2016; Borthwick et al., 2017). Follow up of this trial demonstrated long term vaccine efficacy as T cell memory persisted for at least two year post-vaccination with a polyfunctional effector T cell phenotype (Moyo et al., 2017).

For the prospects of an HCV vaccine, immunodominant T cell epitopes targeted in chronic HCV infection steer the T cell response away from protective T cell epitopes that are targeted in acute infection of those who clear infection (Harcourt et al. 2003; Smyk-Pearson et al. 2006; Schulze Zur Wiesch et al. 2007; Humphreys et al. 2012). Therefore, targeting conserved epitopes—that are also typically subdominant—via vaccination may ensure protective T cell epitopes are sufficiently primed before the virus can manipulate the immune system to target its preferred immunodominant variable T cell epitopes upon infection. The generation of high magnitude HCV-specific $CD4^+$ and $CD8^+$ T cell responses to conserved, subdominant epitopes in multiple HCV genotypes is a criterion that conserved sequence HCV vaccines fulfil, warranting progression of conserved sequences HCV T cell vaccines to human clinical trials.

3.6.5 Shark invariant chain increases vaccine-induced T cell responses

A limitation of a vaccines that utilise sub-dominant T cell epitopes may be the low frequency of TCR populations specific for sub-dominant conserved epitopes in uninfected individuals. Therefore, increasing the magnitude of the T cell response to these subdominant epitopes may be necessary to achieve protective efficacy. Genetic adjuvants are sequences encoded at the N terminus of the transgene in DNA and viral vector vaccines to increase transgene-specific immune responses. One of the most promising genetic vaccine adjuvants is the MHC class II invariant chain (Ii), and this has been shown to enhance the transgene-specific T cell response in vaccines (Holst et al. 2008; Spencer et al. 2014; Capone et al. 2014; Mikkelsen et al. 2011; Holst et al. 2011). However, as Ii is a self-antigen and therefore carries the risk of autoimmunity when used in a vaccine, the use of non-human species specific Ii may be necessary (Halbroth et al., 2018). Tethering the truncated shark invariant chain to the conserved HCV sequence transgene increased vaccine-induced T cell response in mice, in line with other preclinical studies (personal communication with Senthil Chinnakannan; Halbroth et al. 2018).

3.7 Conclusion

The exact correlates of a protective T cell response to present HCV disease are not fully understood. Studies from natural infection and chimpanzee studies indicate that a vaccine should generate $CD4^+$ and $CD8^+$ T cell subsets (Lechner et al. 2000; Grakoui et al. 2003; Shoukry et al. 2003; Urbani et al. 2006; Semmo et al. 2006; Gerlach et al. 1999; Thimme et al. 2001), that target multiple HCV antigens (Lauer et al. 2004), and are polyfunctional (Cooper et al. 1999; Lechner et al. 2000). Conserved sequence HCV T cell vaccines encode the most conserved HCV sequences across HCV subtypes in a simian adenovirus vector and generate high magnitude, broad $CD4^+$ and $CD8^+$ T cell responses targeting multiple HCV genotypes compared to the HCV ChAd-gt-1b-NS T cell vaccine.

The diversity of the HCV genome represents the greatest obstacle to the development of a universal HCV vaccine, as immunodominant T cell epitopes are found in variable HCV regions and are genotype specific (Sugimoto et al. 2003; Lauer et al. 2004; von Delft et al. 2015; Lunburger et al. 2018). The association of immunodominant T cell epitopes with chronic infection indicates that their inclusion in a universal prophylactic HCV vaccine may be ineffective to produce protective immunity (Gerlach et al., 1999; Humphreys et al., 2012). Human clinical trials have revealed that the inclusion of full length non-structural genotype-1b proteomic sequence induced broad, high magnitude genotype-1b-specific T cell responses but conferred limited immunogenicity to other common genotypes and to immunodominant epitope variants (Barnes et al. 2012; Swadling et al. 2014; Kelly, Swadling, Brown, et al. 2015). Importantly, conserved sequence HCV T cell vaccines displayed superior intergenotypic T cell responses targeting genotype-1a, -1b, and 3a, compared to the HCV subtype-1b vaccine, in pre-clinical studies. The conserved sequence vaccine-induced T cell response constitutes $CD4^+$ and $CD8^+$ T cell subsets that are polyfunctional and target subdominant T cell epitopes that are conserved across the majority of HCV subtypes. These vaccine-induced T cell responses can be increased through the use of a novel genetic adjuvant, sIi, tethered to the HCV immunogen.

3.8 Summary of results

- All *in silico* designed conserved sequence HCV T cell immunogens encoded in a simian adenovirus vector vaccines are immunogenic in inbred mice, specifically:
- Vaccine-induced immunogenicity correlated with immunogen length and was enhanced by the presence of linker sequences between gene segments.
- ChAd-gt1-6L and ChAd-gt1/3L vaccines induced a high magnitude, broad genotype-1b specific T cell response comparable to ChAd-gt-1b-NS.

- ChAd-gt1-6L and ChAd-gt1/3L vaccines induced a T cell resposne that targets multiple conserved HCV antigens in inbred and outbred mice and are preferentially located in non-structural HCV regions.
- ChAd-gt1-6L and ChAd-gt1/3L vaccines induce a significantly higher intergenotypic T cell response targeting genotype-1a, -1b, and -3a, compared to ChAd-gt-1b-NS-induced T cell response.
- ChAd-gt1-6L and ChAd-gt1/3L vaccines induce both robust genotype-1b and -3a specific $CD4^+$ and $CD8^+$ T cells that are monofunctional and polyfunctional secreting combinations of IFNγ, TNFα, and IL-2.
- ChAd-gt1-6L and ChAd-gt1/3L vaccines are immunogenic in HLA-A*0201 transgenic and outbred CD-1 mice.
- ChAd-gt1-6L and ChAd-gt1/3L targeted T cell epitopes are subdominant and display significantly greater sequence homology (90-100%) across all HCV subtypes compared to ChAd-gt-1b-NS targeted T cell epitopes.
- The transmembrane domain sequence of the shark invariant chain (sIi_{tr}: aa47-72) increase vaccine immunogenicity when encoded at the 5’ end of the transgene.

4 VACCINATION STRATEGIES TO BOOST HCV-SPECIFIC T CELL IMMUNE RESPONSES AGAINST MULTIPLE HCV GENOTYPES

4.1 Primary hypothesis

A prime-boost HCV vaccination strategy that employs different HCV genotype-specific immunogens encoded in a ChAd prime, MVA boost viral vector strategy can generate T cells that target conserved epitopes between the two genotypes, therefore eliciting a T cell response against both HCV genotypes.

4.2 Introduction

A prophylactic HCV vaccine will be a valuable asset to reduce the incidence of HCV infection globally. There is evidence that HCV may be amenable to a T cell based vaccination strategy (Barnes et al., 2012; Folgori et al., 2006; Swadling et al., 2014). Viral vector vaccines induce potent antigen-specific T cell responses and have become key components of vaccine prime-boost strategies against many pathogens including malaria (Ewer et al., 2013; Ogwang et al., 2015), influenza virus (Antrobus et al., 2014), and Ebola virus (Henao-Restrepo et al. 2015; Ewer et al. 2016). Many prime-boost T cell vaccine

strategies use an simian adenovirus (ChAd) prime followed by a modified Ankara vaccinia (MVA) virus boost vaccination to induce high magnitude T cell responses against the viral vector transgene (reviewed in Ewer et al. 2016).

The current prophylactic HCV vaccination strategy in phase II clinical trials employs an immunogen encoding HCVs genotype-1b non-structural proteins 3-5 (NS) sequence in a chimpanzee adenovirus prime immunisation and a subsequent MVA boost immunisation. Vaccine-induced T cell responses high in magnitude and breadth targeting the vaccine genotype (1b) in healthy volunteers but displayed reduced immunogenicity against other common HCV subtypes – genotype-1a, -3a and -4a (Swadling et al., 2014). Analysis revealed that T cells targeted genotype-specific epitopes that display reduced immunogenicity to genotype epitope variants (Kelly et al., 2015). A parallel study revealed that dominant HCV T cell epitopes in natural infection are genotype-specific suggesting that a T cell vaccine strategy that uses a single genotype immunogen may only elicit T cell responses to that genotype (von Delft et al., 2015). As HCV is a highly variable pathogen with its genotypes following a geographical distribution (e.g. genotype-1 and -3 co-circulate in the U.K.) and with many subtypes within each genotype, an ideal prophylactic HCV vaccine strategy should be effective against multiple infecting HCV genotypes.

In this body of work, I aimed to develop an HCV prophylactic T cell vaccination strategy that uses two different vaccine genotypes (Gt-1b and -3a) to prime and boost conserved HCV-specific T cells against these two HCV genotypes, i.e. a T cell response that is 'intergenotypic' (for genotype-1b and -3a vaccine sequence comparison, see **Appendix 4: Genotype-1b and -3a vaccine sequences**). The genotype-1b NS immunogen was encoded in the ChAd prime viral vector vaccine and a genotype-3a NS immunogen encoded in the MVA boost viral vector vaccine. This vaccine strategy will utilise the priming capacity of the ChAd with an MVA boost viral vector. ChAd is a potent inducer of naïve T cells

whereas MVA is not. The rationale is that the ChAd vaccine will prime a high magnitude T cell response to genotype-1b epitopes and following an MVA-gt-3a-NS vaccination, ChAd-primed T cells that target conserved sequence HCV epitopes between genotype-1b (prime genotype) and -3a (boost genotype) will be targeted resulting in a high magnitude T cell response to conserved genotype-1b and -3a epitopes. By using two distinct HCV subtypes separately in a prime vaccine and a boost vaccine, a prime-boost T cell response targeting conserved HCV epitopes may confer high magnitude T cell responses against both vaccine genotypes. If successful, this approach would address a limitation of current HCV T cell vaccine strategies: reduced immunogenicity to multiple HCV genotypes.

I performed pre-clinical immunogenicity studies in mice to assess the proof-of-principle of a two-genotype prime-boost approach to induce intergenotypic HCV-specific T cell responses (Figure 4-1). In the following work, the vaccine-induced genotype-1b and genotype-3a specific T cell response is characterised, focusing on the overall magnitude, breadth, functionality, and epitope specificity and hierarchy of the T cell response.

4.3 Key questions

I address the following key questions:

1. Is the HCV MVA-gt-3a-NS vaccine immunogenic?
2. Does the MVA-gt-3a-NS vaccine boost a ChAd-gt-1b-NS primed HCV specific T cell response?
3. Does the MVA-gt-3a-NS vaccine increase the magnitude of the ChAd-gt-1b-NS-primed genotype-3a specific T cell response compared to a homologous genotype-1b ChAd prime MVA boost regimen?

4. Does an MVA-gt-3a-NS boost vaccine increase the **intergenotypic** T cell response to HCV compared to MVA-gt-1b-NS boost vaccination, after a ChAd-gt-1b-NS prime vaccination?
5. Does the MVA-gt-3a-NS vaccine increase the **breadth** of the ChAd-NS-1b primed T cell response compared to MVA-gt-1b-NS?
6. Does the MVA-gt-3a-NS vaccine change the **dominance** of the prime-boosted T cell response to specific T cell epitopes compared to a homologous genotype-1b vaccine prime-boost regimen?
7. Do MVA-NS-3a boosted T cells target epitopes that display conserved amino acid sequence between HCV genotype-1b and -3a?
8. Do the ChAd prime, MVA boost vaccination regimens enhance the $CD4^+$ and $CD8^+$ T cell response compared to the ChAd-gt-1b-NS prime vaccine?

For the following chapter, prime-boost regimens are written as '*ChAd prime/MVA boost*'. For example, a vaccination group that has received a day 0 ChAd-gt-1b-NS prime vaccine and a day 63 MVA-gt-3a-NS boost vaccine will be denoted as '*1b/3a*'. Where no day 63 MVA boost vaccination was administered, the regimen is denoted as '*1b/-*'. Where no prime vaccination was administered at day 0, the regimen is denoted as '*-/3a*'. In this regimen the MVA-gt-3a-NS vaccine was administered at day 63 as a prime vaccination.

4.4 Chapter specific material & methods

4.4.1 Vaccine design and production

Viral vector vaccine design and production is described in full in **chapter 2**.

4.4.2 Animals & immunisations

All mouse studies were performed at the Biomedical Services Building, Oxford, according to U.K. Home Office Regulations (project license numbers 30/2744 and 30/3293) and approved by the local ethical review board at the University of Oxford. Groups of age-matched female C57BL/6 mice were vaccinated intramuscularly at 8-10 weeks of age with 10^8 infectious units (IU) of non-replicating simian adenovirus ChAdOx1 incorporating the subtype-1b sequence of HCVs non-structural proteins, NS3, NS4, and NS5. Mice were vaccinated intramuscularly nine-weeks post-prime vaccination with $5x10^7$ plaque forming units (PFU) of modified vaccinia Ankara (MVA) vector vaccines that encode either the subtype-1b or subtype-3a HCV NS3-5 sequence. Mice were culled at one-week post-boost vaccination and splenocytes were harvested and processed for immunoassays to assess T cell reactivity to HCV genotype-1b and -3a peptides (Figure 4-1).

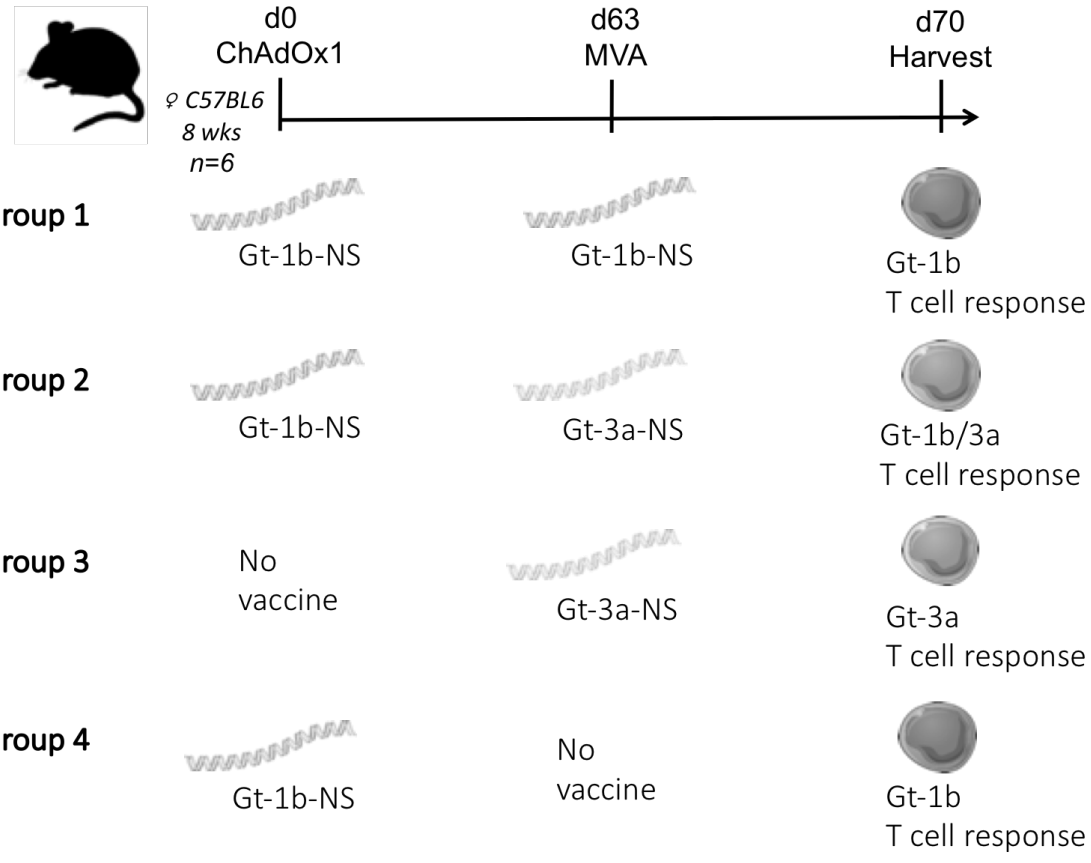


Figure 4-1. Vaccination schedule of a heterologous genotype ChAd prime MVA boost regimen: Age-matched 8-week old female C57BL/6 mice were immunised intramuscularly with 10^8 infectious units (IU) of ChAd-gt-1b-NS vaccine. Mice were intramuscularly administered a $5x10^7$ PFU MVA-gt-1b-NS, MVA-gt-3a-NS boost, or nothing, at 63-days (9 weeks) post-prime vaccination. A control group of an MVA-gt-3a-NS prime was used at day 63 to account for MVA primed T cell responses. Mice were sacrificed by schedule 1 at 7-days post-boost vaccination (70 days since start of study) and spleens harvested, and immunogenicity assessed; see methods).

4.5 Results

4.5.1 The design of MVA vaccines

Modified vaccinia Ankara (MVA) is an attenuated poxvirus and is an efficient viral vector vaccine to boost transgene-specific T cell responses that are primed from a prior vaccination (Ewer et al., 2016; Gómez et al., 2013). In order to assess an HCV vaccination regimen to elicit intergenotypic T cell responses using MVA as a boosting vaccine, two MVA vaccines were generated encoding (1) the genotype-1b HCV non-structural proteomic sequence (MVA-gt-1b-NS), or (2) the genotype-3a HCV non-structural proteomic sequence (MVA-gt-3a-NS; Figure 4-2**A**). MVA vaccine immunogens encoded the truncated shark invariant chain (sIi_{tr}) and utilise the F11L cytomegalovirus (CMV) promotor which enhances *in vivo* transgene expression and immunogenicity (Alharbi et al., 2016). MVA vaccine immunogens were encoded within shuttle vectors, generated using In-Fusion^{TM} cloning and restriction enzyme based cloning (Zhu et al., 2007), and submitted to Oxfords Viral Vector Core Facility at the Jenner Institute for MVA vaccine production (Figure 4-2**B**).

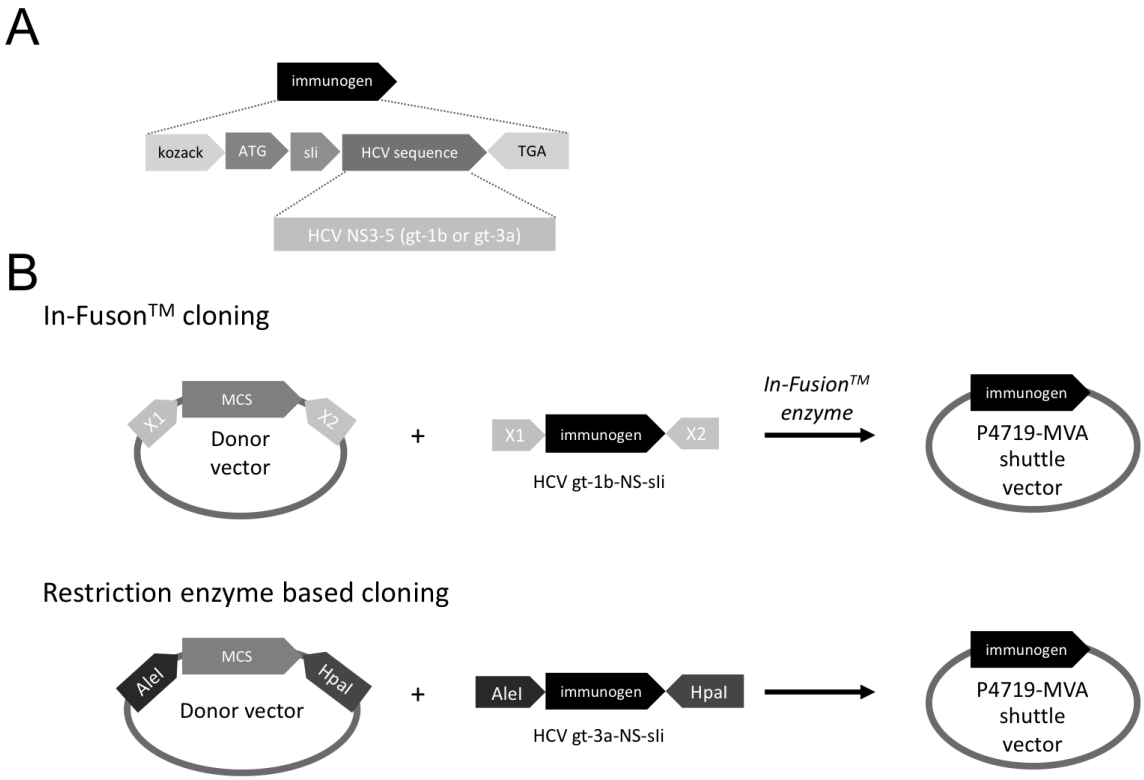


Figure 4-2. Production of MVA vectored HCV T cell vaccines: (**A**) The HCV immunogen design contains a kozak sequence, ATG start codon, the sequence of the truncated shark invariant chain (sIi) genetic adjuvant, the HCV sequence (the non-structural, NS3-5 sequence of HCV subtype-1b or -3a), and a TGA stop codon. (**B**) human codon optimised HCV immunogens were inserted into the donor vector via Clontech's In-Fusion™ cloning method or by restriction enzyme-based cloning using restriction enzyme sites *AleI* and *HpaI*, to generate the p4179-MVA shuttle vector for submission to the Viral Vector Core Facility in Oxford.

4.5.2 MVA-gt-3a-NS vaccine primes a gt-3a specific T cell response

MVA vector vaccines are considered poor prime vaccines compared to other viral vector vaccines such as recombinant adenoviruses in humans (Ewer et al. 2016). In order to assess the MVA-gt-3a-NS boosted T cell response in mice, I first addressed the MVA's capacity to induce immunogenicity as a prime vaccine prior to its use as a boost vaccine to a ChAd-gt-1b-NS prime vaccination (Figure 4-3**A**). MVA-gt-3a-NS elicited low magnitude genotype-1b and -3a IFNγ^+ ELISpot responses between 10 and 100 IFNγ^+ spot forming

units (SFU/10^6 splenocytes) at the low dose of $1x10^6$ PFU (plaque forming units). At the $5x10^7$ PFU dosage, the T cell response was three times higher with genotype-3a producing a total IFNγ^+ response reaching 300 SFU/10^6 splenocytes (Figure 4-3**B**). The MVA-gt-3a-NS prime vaccination induced a broader T cell response with a greater number of positive peptide pools for gt-3a compared to gt-1b. Breadth was significantly higher at the $5x10^7$ PFU dosage **(**Figure 4-3**C**).

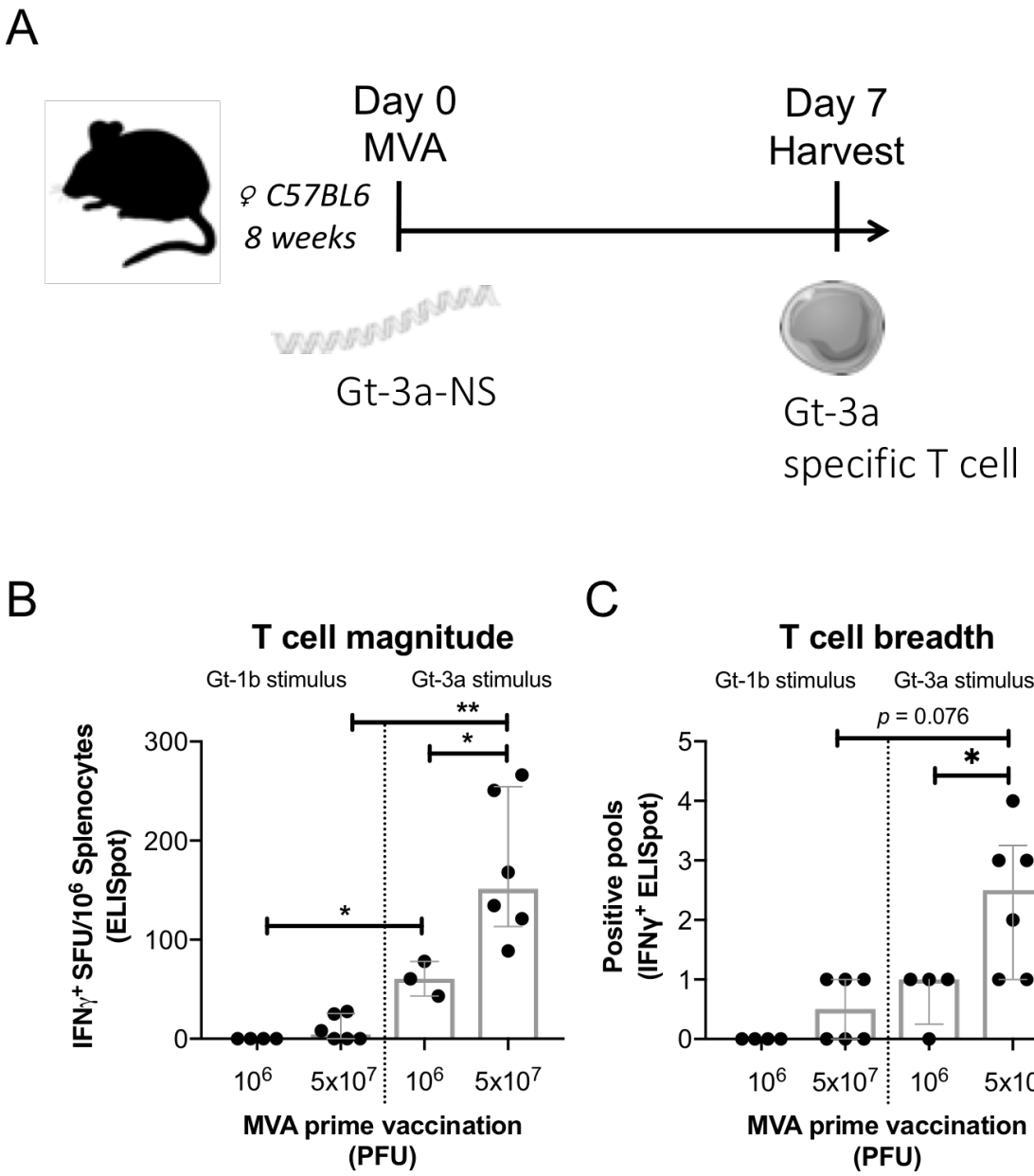


Figure 4-3. Assessment of MVA-gt-3a-NS primed T cell response: (**A**) Age-matched C57BL/6 mice were immunised intramuscularly with 10^6 or $5x10^7$ plaque forming units (PFU) of MVA-gt-3a-

NS and harvested 7 days post-vaccination. (**B**) The total IFNγ ELISpot response of MVA-gt-3a-NS immunised mice to HCV genotype-1b and -3a specific peptide pools (15-18mers overlapping by 11aa) covering the non-structural HCV proteomic sequence. (**C**) The total breadth of the IFNγ ELISpot response of MVA-gt-3a-NS immunised mice to genotype-1b and -3a specific peptide pools that cover the amino acid sequences of HCV proteins, NS3p, NS4, and NS5b. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. Bars represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

4.5.3 An MVA-gt-3a-NS boost vaccine does not increase the genotype-3a specific T cell response compared to an MVA-gt-1b-NS boost vaccination

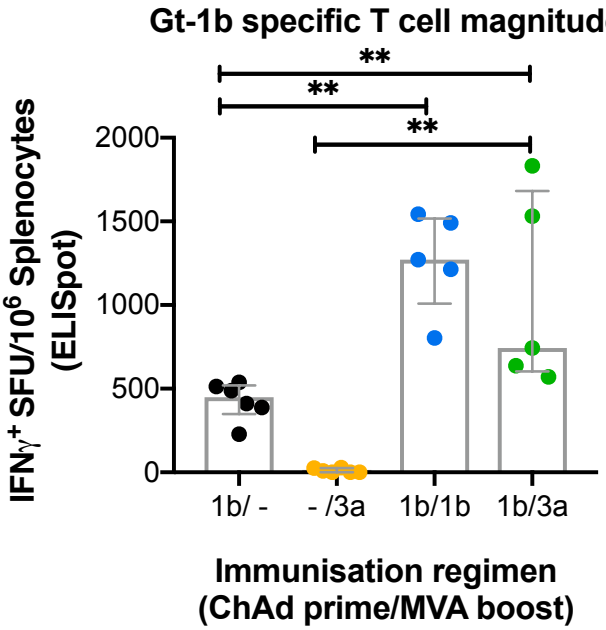
I determined the capacity of an HCV vaccination strategy—that utilises a genotype-1b specific ChAd prime vaccination and genotype-3a MVA boost vaccination (regimen denoted as '*1b/3a*') to induce a genotype-1b specific and genotype-3a specific T cell response compared to a homologous genotype-1b ChAd prime, MVA genotype-1b boost regimen (regimen denoted as '*1b/1b'*), and prime only regimens: a ChAd-gt-1b-NS prime (1b/-) and a MVA-gt-3a-NS prime (-/3a).

A ChAd-gt-1b-NS prime and MVA-gt-3a-NS boost (1b/3a) regimen induced an HCV genotype-1b specific T cell response of 743 median SFU/10^6 splenocytes at day 70 post-prime (7-days post-boost), which was lower than a 1b/1b ChAd prime MVA boost regimen induced genotype-1b specific response of 1271 median SFU/10^6 splenocytes (Figure 4-4**A**), though this did not reach statistical significance. Both prime-boost regimens were significantly higher than the ChAd-gt-1b-NS prime T cell response (449 median SFU/10^6

splenocytes; Figure 4-4**A**). The 1b/1b, 1b/3a, and 1b/- vaccination regimens all induced higher magnitude T cell responses compared to the MVA-gt-3a-NS vaccination group (denoted: '*-/3a*') which displayed a median of 28 SFU/10^6 splenocytes (Figure 4-4**A**).

There was no difference in the genotype-3a specific T cell response between the 1b/1b and 1b/3a vaccination regimens (580 and 608 SFU/10^6 splenocytes, respectively; Figure 4-4**B**). Both prime-boost regimens generated significantly higher genotype-3a specific T cell responses compared to the ChAd-gt-1b-NS and MVA-gt-3a-NS prime vaccinations (Figure 4-4**B**). No difference in the genotype-3a specific T cell response was observed between the prime vaccinations (Figure 4-4**B**).

A



B

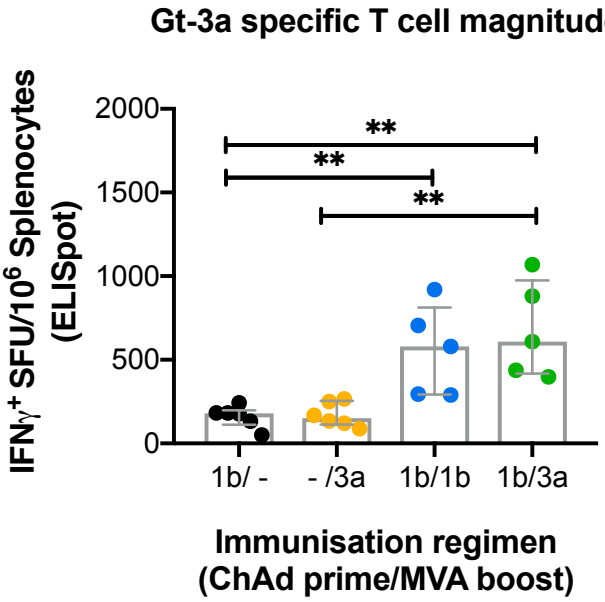


Figure 4-4. Genotype-1b and genotype-3a specific immunogenicity of prime-boost and prime only vaccination regimens: The total magnitude genotype-1b specific (**A**) and total genotype-3a specific (**B**) IFNγ ELISpot response of harvested splenocytes from age-matched female C57BL/6 mice (n=5-6) vaccinated with a 10^8 IU ChAd prime at day 0 and/or a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or

4) a ChAd-gt-1b-NS prime vaccination only (1b/-), stimulated with HCV genotype specific peptide pools (15-18mers, overlapping by 11aa) covering the HCV non-structural proteomic sequence. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. Bars represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

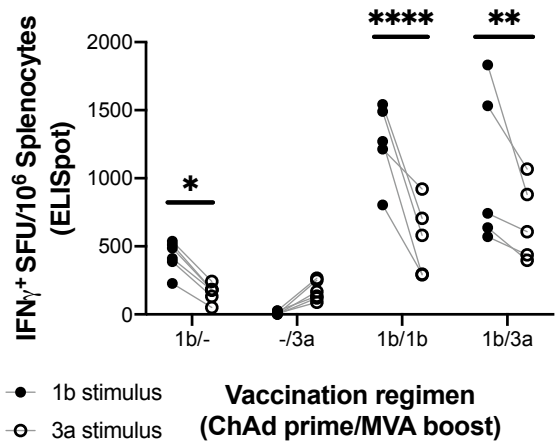
4.5.4 MVA-gt-3a-NS increases the intergenotype T cell response compared to a ChAd-gt-1b-NS prime and homologous genotype-1b ChAd MVA prime-boost regimen

Having demonstrated that the overall genotype-3a specific T cell response is not increased by an MVA-gt-3a-NS boost compared to an MVA-gt-1b-NS boost following a ChAd-gt-1b-NS prime, I next determined the capacity of individual mice within each vaccination regimen to generate genotype-1b and -3a T cell responses, i.e. intergenotypic T cell responses. All regimens that received a ChAd-gt-1b-NS prime vaccination displayed T cell responses that were skewed towards genotype-1b with regimen 1b/1b displaying the highest statistical difference between its genotype-1b and -3a specific T cell response ($P < 0.0001$; Figure 4-5**A**). The 1b/3a and 1b/- regimens also displayed significant differences between the genotype-1b and -3a specific T cell responses but to a less significant extent than the 1b/1b regimen ($P = 0.0039$ and 0.0312, respectively; Figure 4-5**A**).

Where a comparable total T cell magnitude is elicited by an individual mouse, the T cell response is deemed 'intergenotypic', i.e. displaying a greater capacity of responding to both genotype-1b and -3a stimuli (see Error! Reference source not found. in materials and m

ethods for calculation). The 1b/3a vaccination regimen displayed a significantly higher intergenotype ratio of 0.7 compared to all other vaccination regimens. The 1b/1b and 1b/- vaccination regimens displayed a low intergenotype ratio of <0.4 (Figure 4-5**B**).

A



B

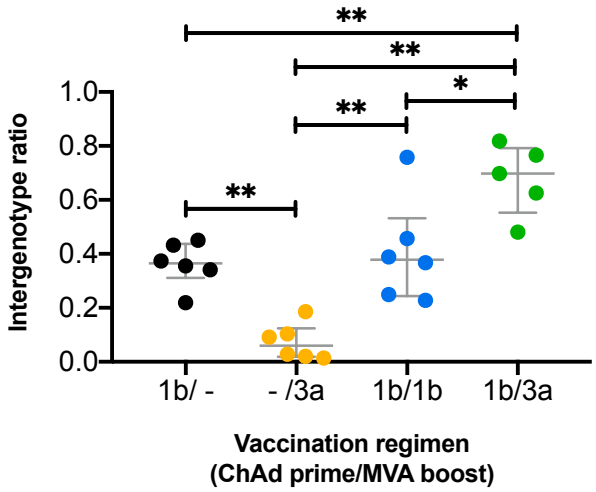


Figure 4-5. The intergenotype T cell response of all vaccination regimens: (**A**) The total genotype-1b specific and total genotype-3a specific IFNγ ELISpot response of harvested splenocytes from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-

gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-), stimulated with HCV genotype specific peptide pools (15-18mers, overlapping by 11aa) covering the HCV non-structural proteomic sequence. T cell responses are displayed as linked data points for each individual mouse. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. (**B**) The intergenotype ratio of all vaccination groups, where the intergenotype ratio is calculated as the lowest genotype specific T cell response divided by the highest genotype specific T cell response. Ratio values of 0 indicate a genotype-specific T cell response and ratios closer to 1 indicate T cell responses to both genotype-1b and -3a, i.e. intergenotypic. Data points are individual mice and bars represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

4.5.5 MVA-gt-3a-NS boost vaccination does not change the immunodominance of the primed T cell response, but increases the breadth of the prime-boost T cell response

I have demonstrated that the vaccine-induced genotype-3a T cell response is similar between prime-boost vaccination regimens and that the genotype-1b T cell response is lower after an MVA-gt-3a-NS boost compared to an MVA-gt-1b-NS boost. Next, I assessed whether the T cell response to HCV peptide minipools is influenced by the MVA boost vaccination between 1b/1b and 1b/3a regimens.

The breadth of the genotype-1b specific T cell response (number of positive ELISpot peptide pools) to individual HCV proteins was significantly higher in the 1b/3a regimen compared to the prime vaccine regimens, and whilst not significant, did display a higher median of positive peptide pools compared to the 1b/1b regimen (Figure 4-6**A**). The 1b/3a regimen significantly increased the breadth of the genotype-3a specific T cell response

compared to the 1b/1b and 1b/- regimen, but not the -/3a vaccination regimen (Figure 4-6**B**).

To investigate the breadth of the T cell response further, I assessed the T cell response magnitude for HCV peptide minipools: NS3p (pools F1-5), NS4 (pools H1-6), and NS5b (pools M1-9). The F4 and H1 minipools induced the highest T cell responses in all prime-boost vaccination regimens irrespective of the MVA boost vaccine used, that was significantly higher compared to the prime vaccination only. The 1b/1b regimen displayed a significantly higher genotype-1b T cell magnitude to minipool H1 and F4 compared to the other vaccination regimens **(**Figure 4-6**C**). The minipool H1 T cell response, but not the F4 T cell response was observed after genotype-3a stimulation (Figure 4-6**D**). An increase in the genotype-3a T cell response targeting the M2 minipool (NS5b) was seen in the 1b/3a and -/3a vaccination regimens, but not the 1b/1b and 1b/- regimens (Figure 4-6**D**).

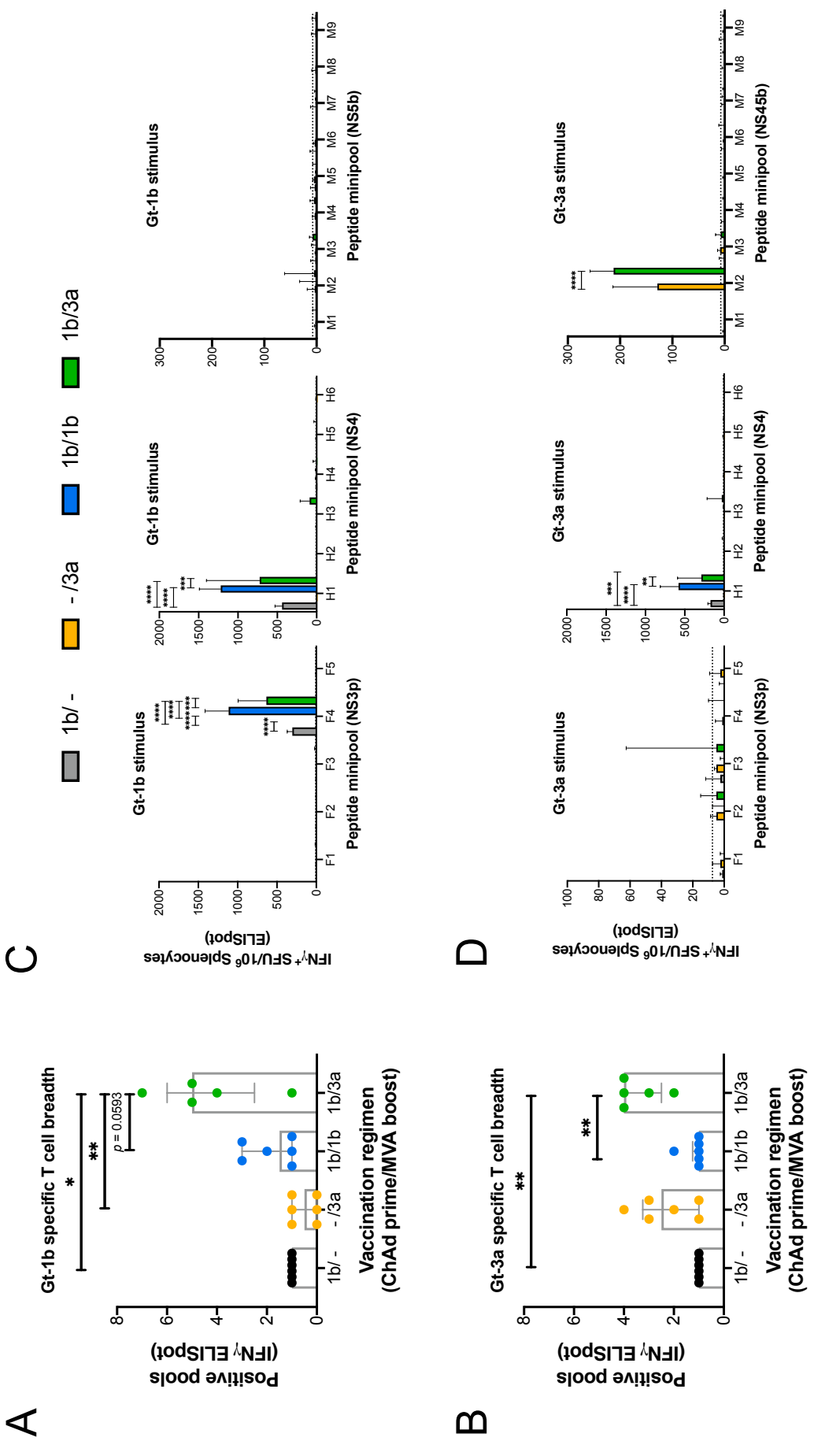

A
Gt-1b specific T cell breadth
Positive pools (IFNγ ELISpot)
Vaccination regimen (ChAd prime/MVA boost)
1b/ -
- /3a
1b/1b
1b/3a
p = 0.0593
B
Gt-3a specific T cell breadth
C
1b/ -
- /3a
1b/1b
1b/3a
Gt-1b stimulus
Peptide minipool (NS3p)
Peptide minipool (NS4)
Peptide minipool (NS5b)
IFNγ+ SFU/10^6 Splenocytes (ELISpot)
D
Gt-3a stimulus
Peptide minipool (NS45b)

Figure 4-6. Vaccine-induced immunogenicity to HCV minipools and corresponding T cell breadth: HCV genotype-specific minipools of 15-18mer peptides corresponding to HCV proteins, NS3p, NS4b, and N5b, were used to assess the specificity of the vaccine-induced T cell responses from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-). The breadth of the T cell response—defined by the number of positive peptide pools—is displayed for genotype-1b (**A**) and -3a (**B**). A positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. The IFNγ ELISpot response to minipools corresponding to the NS3p, NS4, and NS5b for genotype-1b (**C**) and -3a (**D**) amino acid sequence is shown. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians (for T cell breadth) at a 95% confidence interval. For statistical comparisons between vaccination regimens within minipools, a RM two-way ANOVA with Tukey's multiple comparisons test was used. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

4.5.6 The MVA-gt-1b-NS and MVA-gt-3a-NS boosted T cell response targets genotype-specific epitopes with mismatched sequence between genotypes

Having demonstrated that the MVA boosted T cell response targeted a limited number of HCV peptide minipools, I sought to identify the epitopes targeted by 1b/1b and 1b/3a regimen-induced T cells and determine the epitope sequence homology between genotype-1b and -3a.

Prime-boost vaccine-induced T cell responses targeted four HCV peptides: one in NS3p (peptide F4-3), two in NS4 (peptides H1-2 and H1-3) and one in NS5b (peptide M2-1). The vaccine-induced T cell response targeting F4-3 and H1-2 peptides was genotype-1b

specific (Figure 4-7**A**), with prime-boosted T cell responses significantly higher than prime only T cell responses. No difference was observed between 1b/1b and 1b/3a regimens despite a trend for a lower T cell magnitude in the latter regimen (Figure 4-7**A**). The vaccine induced T cell response targeting H1-3 and M2-1 was genotype-3a specific (Figure 4-7**B**). No difference in the T cell response was observed between the 1b/- prime vaccine regimen and the 1b/1b and 1b/3a prime-boost regimens to H1-3 (Figure 4-7**B**). Only 1b/3a and -/3a regimens generated T cell responses to M2-1 which were not statistically different from each other (Figure 4-7**B**). The intergenotype ratios of all T cells was low with no difference between prime-boost vaccination regimens (Figure 4-7**C**).

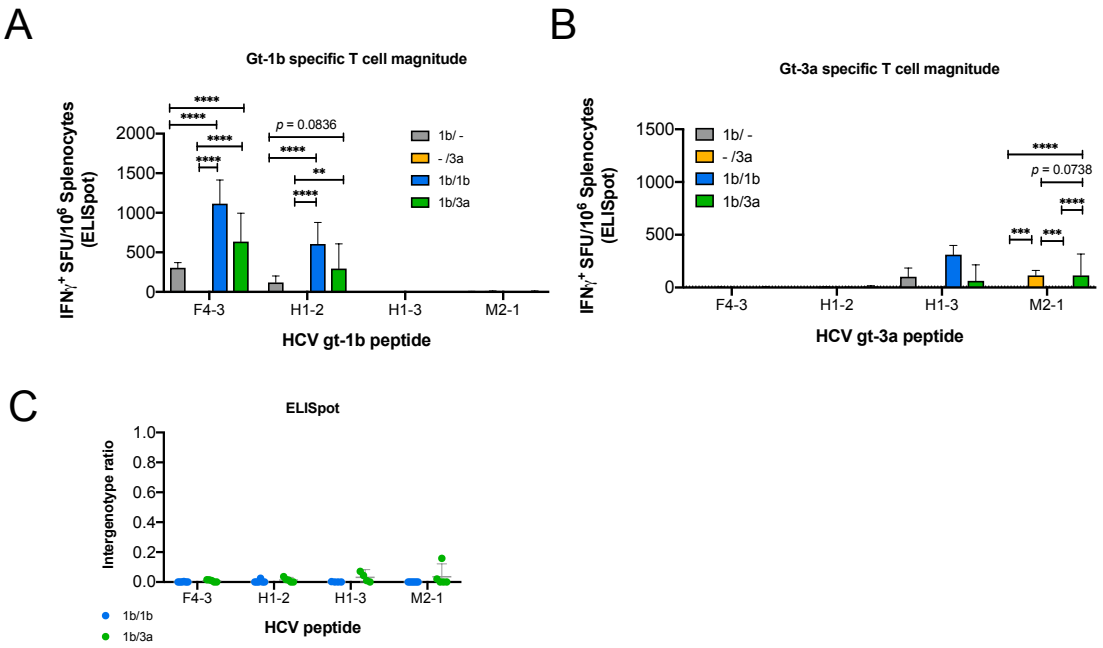


Figure 4-7. Assessment of vaccine-induced T cell immunogenicity to T cell epitopes: The IFNγ ELISpot response to HCV genotype-1b (**A**) and -3a (**B**) specific peptides F4-3 ($NS3_{1215-1230}$), H1-2 ($NS4_{1634-1651}$), H1-3 ($NS4_{1641-1657}$), and M2-1 ($NS5b_{2730-2747}$) with corresponding intergenotype ratio (**C**) of harvested splenocytes from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-). For statistical comparisons between vaccination regimens, a RM two-way ANOVA with Tukey's

multiple comparisons test was used. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

Comparison of the amino acid sequence of the responsive T cell epitopes was undertaken to determine if boosted T cells targeted conserved epitopes (Figure 4-8). Epitope sequence comparison revealed that the F4-3 peptide ($NS3_{1215-1230}$) that was boosted in a genotype-1b manner in both the 1b/1b and 1b/3a vaccination regimens displayed sequence mismatch between genotype-1b and -3a sequences, with five amino acid substitutions and a maximum conserved sequence length of six amino acids. The H1-2 peptide ($NS4_{1634-1651}$) displayed three amino acid substitutions between genotype-1b and -3a and was only boosted after a genotype-1b stimulus. The H1-3 peptide ($NS3_{1641-1657}$) displayed two amino acid substitutions between genotype-1b and -3a and was only boosted after a genotype-3a stimulus. The amino acid overlap between H1-2 and H1-3 was eleven amino acids and contained one amino acid substitution ($NS3_{1641-1651}$; I1638V). The immunogenicity of the overlapping amino sequence was not assessed as a single peptide in this study, yet no single peptide containing the $NS4_{1641-1651}$ epitope stimulated IFNγ production in T cells that was positive for both genotype-1b and -3a stimuli. The M2-1 peptide ($NS5b_{2730-2747}$) displayed sequence mismatch between genotype-1b and -3a and contained eight amino acid substitutions. The vaccine-induced T cell response to M2-1 was genotype-3a specific and present only in vaccination regimens with MVA-gt-3a-NS.

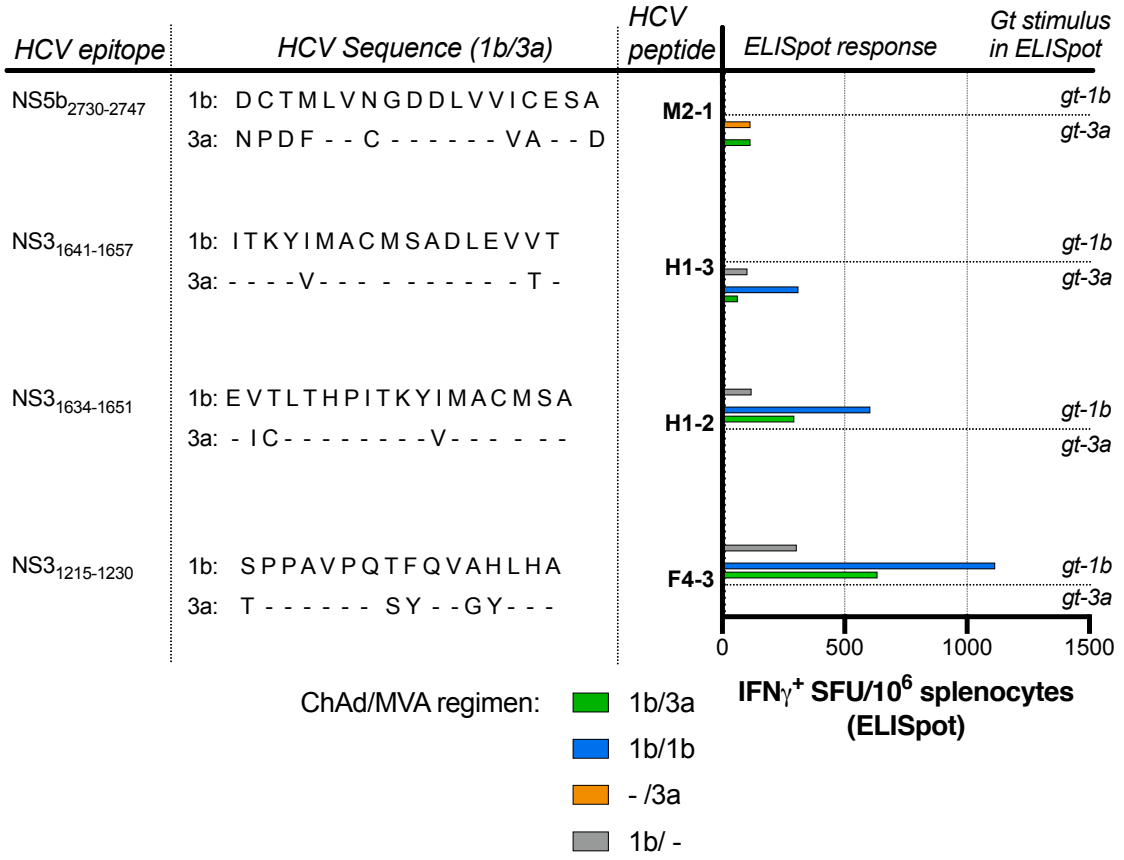


Figure 4-8. Comparison of vaccine-induced T cell epitope sequences between genotype-1b and -3a with corresponding IFNγ ELISpot response: The HCV amino acid sequence for HCV genotype-1b (J4) and -3a (K3a650) subtypes for vaccine-induced T cells targeting epitopes F4-3 (NS3p$_{1215-1230}$), H1-2 (NS4$_{1634-1651}$), H1-3 (NS4$_{1641-1657}$), and M2-1 (NS5b$_{2730-2747}$). Dashes in the epitope sequence represent sequence homology and one letter amino acids codes represent amino acid substitution mutations between the genotype-1b and -3a sequence. T cells were derived from splenocytes harvested from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-). Corresponding IFNγ ELISpot responses to HCV peptide minipools for all vaccination regimens to both genotype-1b and -3a peptide stimuli are shown with bars representing SFU/10^6 splenocyte medians. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus

three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group.

4.5.7 MVA-gt-3a-NS boost vaccination increases the genotype-3a specific $CD8^+$ T cell response

Having shown that the total magnitude of the genotype-3a specific T cell response was similar between regimens 1b/1b and 1b/3a, I next assessed the genotype-specific $IFN\gamma^+$ $CD8^+$ T cell response between vaccination regimens.

The genotype-1b specific $IFN\gamma^+$ $CD8^+$ T cell response was significantly higher in the 1b/1b regimen compared to prime vaccination regimens (Figure 4-9**A**). While not statistically significant, a higher genotype-1b specific $IFN\gamma^+$ $CD8^+$ T cell response was observed in 1b/1b when compared to the 1b/3a regimen. Conversely, the 1b/3a regimen induced a significantly higher genotype-3a specific $IFN\gamma^+$ $CD8^+$ T cell responses compared to all other vaccination regimens (Figure 4-9**A**), a trend that was reflected in individual mouse $IFN\gamma^+$ $CD8^+$ T cell responses (Figure 4-9**B**). The intergenotype ratio of $IFN\gamma^+$ $CD8^+$ T cell responses was significantly higher for the 1b/3a vaccination regimen (0.145) compared to the 1b/1b and 1b/- regimens, albeit still low (0.004 and 0, respectively; Figure 4-9**C**).

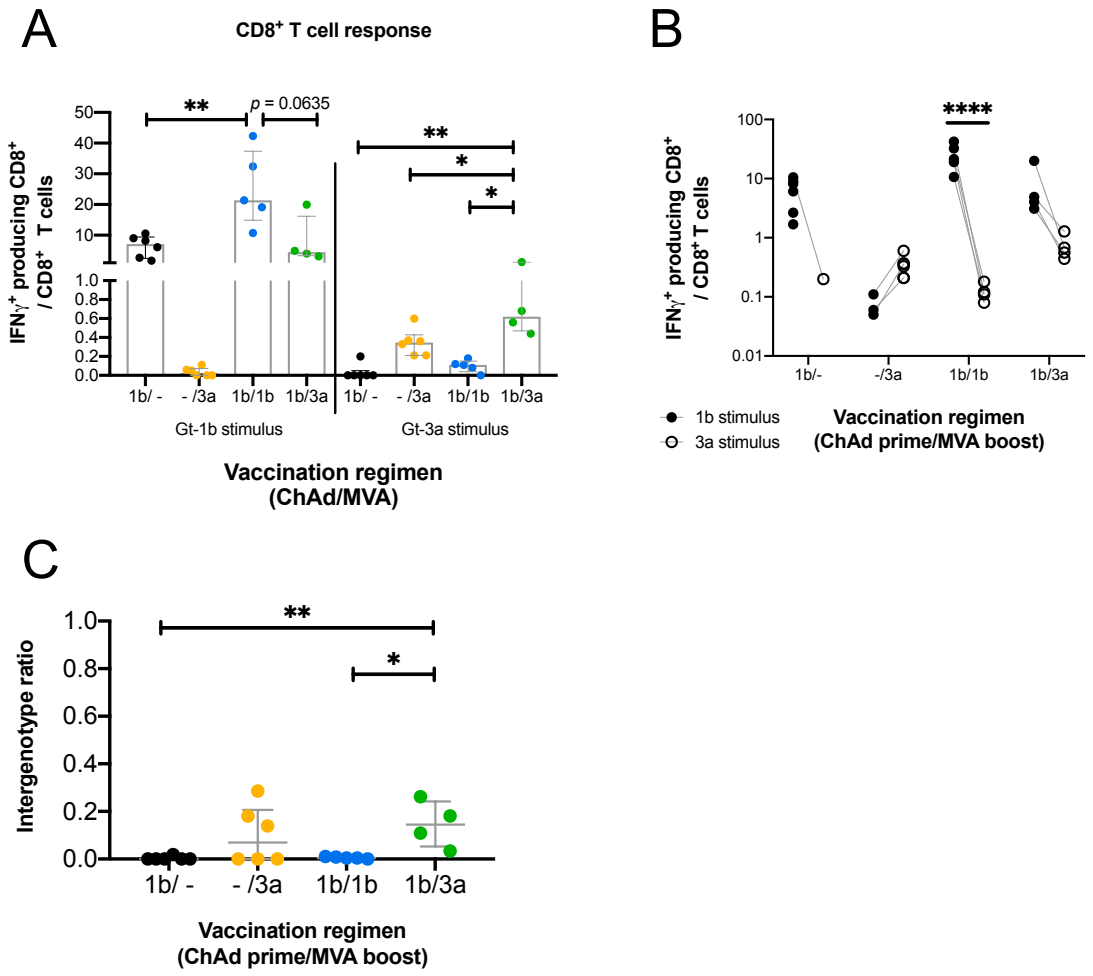


Figure 4-9. The vaccine-induced IFNγ+ CD8+ T cell response: (**A**) The total IFNγ secreting $CD8^+$ T cell response to harvested splenocytes from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-). Splenocytes were stimulated with peptide pools specific to HCV genotype-1b and -3a corresponding to the protein sequence of the HCV non-structural proteins (15-18mers, overlapping by 11aa). (**B**) The total genotype-1b specific and total genotype-3a specific IFNγ secreting $CD8^+$ T cell responses in all vaccination regimens are displayed as linked data points for each individual mouse. (**C**) The intergenotype ratios of the IFNγ secreting $CD8^+$ T cell responses in all vaccination regimens are shown. The total $IFN\gamma^+$ $CD8^+$ T cell responses were assessed using splenocytes stimulated for 6 hours (last 4 hours with BD GolgiPlug™) with HCV genotype-1b and -3a peptide pool (15-18mers that overlap by 11aa) and intracellular cytokine staining (ICS) and

flow cytometry analysis was performed. All T cells are gated on lymphocytes, live, singlets, and $CD3^+$ cells. Data is presented as the sum of the individual responses to peptides minipools. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

4.5.8 MVA vaccines boost a high magnitude polyfunctional $CD8^+$ T cell response that targets genotype-specific epitopes

Spontaneous resolvers of acute HCV infection display a broad polyfunctional HCV-specific $CD8^+$ T cell response (S Cooper et al., 1999; F Lechner et al., 2000). Adenovirus vector vaccines are potent producers of transgene-specific $CD8^+$ T cells (Folgori et al. 2006; Barnes et al. 2012; Ewer et al. 2016)., which are optimally boosted by an MVA vaccine (Alharbi et al., 2017; Swadling et al., 2014).

I assessed the ability of the 1b/3a and 1b/1b prime-boost vaccination regimens to elicit genotype-1b specific $CD8^+$ T cell responses. The 1b/1b vaccination regimen, but not the 1b/3a regimen, generated a significantly higher percentage of total $CD8^+$ T cells that produced IFNγ after a peptide F4-3 and H1-2, compared to the 1b/- prime vaccination regimen (Figure 4-10**A**). The 1b/1b, 1b/3a, and 1b/- vaccination regimens generated a similar cytokine secretion profile which was predominantly mono-functional for IFNγ and dual-functional for IFNγ and TNFα, with some polyfunctionality for IFNγ, TNFα, and IL-2 (Figure 4-10**A**).

A genotype-3a specific $IFN\gamma^+$ $CD8^+$ T cell response was only observed for H1-2 and M2-1 and at a low magnitude of less than 1% of all $CD8^+$ T cells (Figure 4-10**B**). The H1-2 targeted vaccine-induced $IFN\gamma^+$ $CD8^+$ T cell response was <0.2% of all CD8+ T cells and predominantly monofunctional. The -/3a and 1b/3a vaccination regimens generated significantly higher IFNγ producing T cells to M2-1 compared to other vaccination

regimens (Figure 4-10**B**). The 1b/3a regimen induced IFNγ^{+} CD8^{+} T cell response was statistically higher than the -/3a regimen induced IFNγ^{+} CD8^{+} T cell response (Figure 4-10**B**). The -/3a and 1b/3a cytokine secretion profile to M2-1 was similar exhibiting dual-functionality (IFNγ and TNFα) and polyfunctionality (IFNγ, TNFα, IL-2).

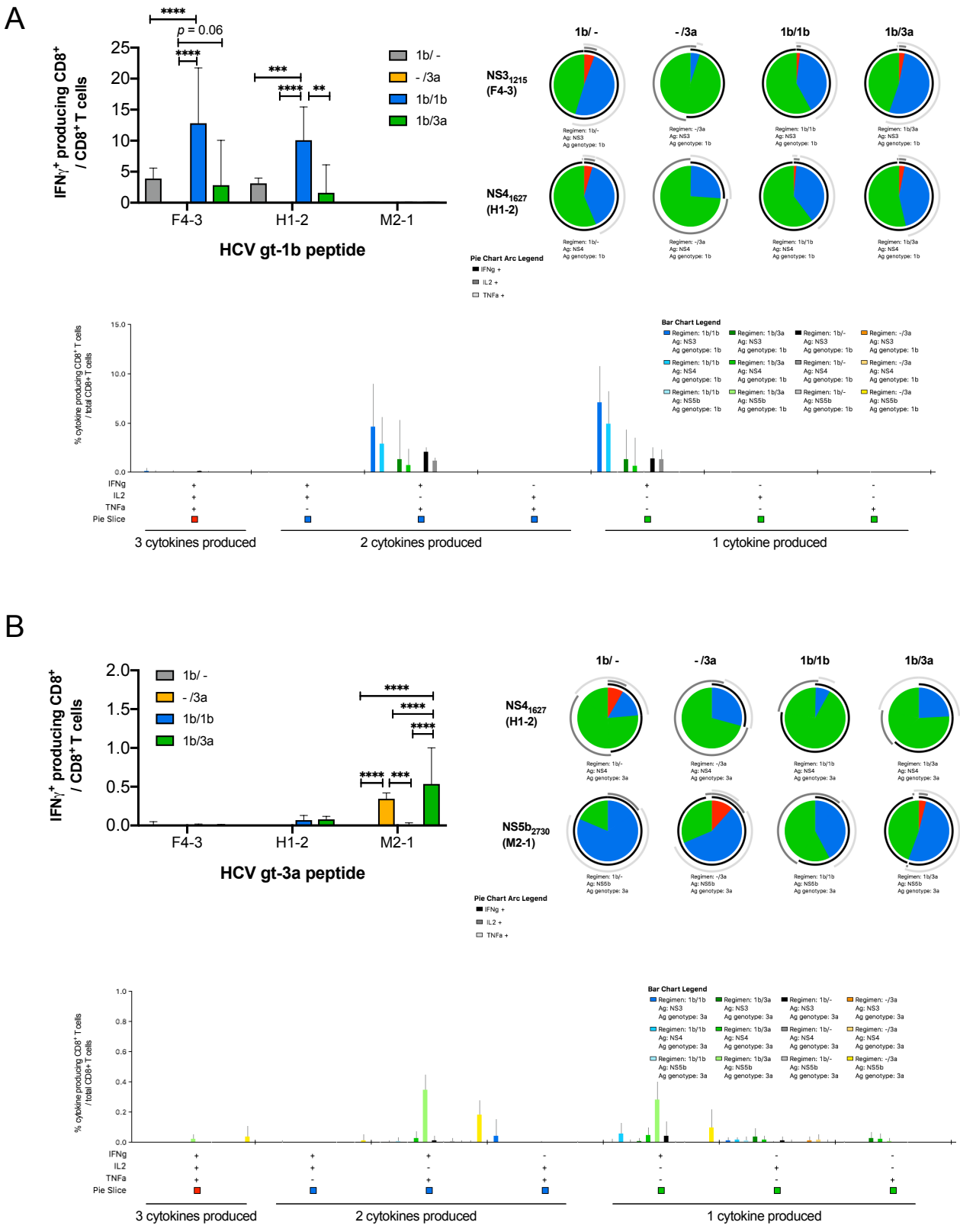


Figure 4-10. The capacity of vaccine-induced genotype-specific CD8+ T cell response to produce cytokines: The percentage of all vaccine-induced $CD8^+$ T cells to secrete IFNγ after stimulation with genotype-1b (**A**) and -3a (**B**) specific peptides: F4-3, H1-2, M2-1. Bars are medians

and interquartile ranges are displayed. The capacity of F4-3, H1-1, and M2-1 specific $CD8^+$ T cell responses to secrete combinations of IFNγ, TNFα, and IL-2. Pie charts represent the proportion of cytokine-secreting $CD8^+$ T cells that produce one, two, or three cytokines. Pie arcs (from dark to light shades) show the proportion of cytokine-producing $CD8^+$ T cells that make a given cytokine. Pie base, mean. The percentage of all $CD8^+$ T cells to secrete combinations of IFNγ, TNFα, and IL-2 for all vaccination regimens is plotted in graph form with bars representing means and standard deviations are displayed. Green indicates mono-functionality, blue indicates dual-functionality, and red indicates triple-functionality and this is reflected in the pie slices and graph. T cells were derived from age-matched female C57BL/6 mice (n=6) vaccinated with a 10^8 IU ChAd prime at day 0 and a $5x10^7$ PFU MVA at day 63 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-1b-NS boost (1b/1b), 2) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), 3) a MVA-gt-3a-NS prime only (-/3a), or 4) a ChAd-gt-1b-NS prime vaccination only (1b/-).

4.5.9 A ChAd-gt-1b-NS, MVA-gt-3a-NS prime-boost vaccination regimen elicits a low magnitude genotype-3a T cell response compared to a conserved sequence HCV T cell vaccine

I have shown that a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost vaccination regimen does not increase the genotype-3a specific T cell response compared to a homologous genotype-1b prime-boost regimen. Yet, it was not assessed if the genotype-3a T cell response was a higher magnitude compared to a genotype-3a T cell response induced by a conserved sequence HCV T cell vaccine prime-boost regimen which was designed to include the most conserved HCV epitopes (ChAd-gt1-6/MVA-gt1-6). In an additional prime-boost experiment, I assessed the prime-boosted genotype-1b and -3a specific T cell response between a 1b/3a and a gt1-6/gt1-6 prime-boost vaccination regimen. Compared to a conserved sequence HCV T cell vaccine prime-boost regimen, the 1b/3a vaccination regimen induced genotype-1b T cell response was a similar magnitude (Figure 4-11**A**), but the genotype-3a T cell response was of a significantly lower magnitude (Figure 4-11**B**). Mapping of the epitope-specific vaccine-induced T cell responses revealed that each prime-boost vaccination regimen induced T cells targeting unique epitopes (Figure 4-11**C**).

The ChAd-gt-1b-NS primed genotype specific T cell epitopes—$NS3_{1215-1230}$, $NS3_{1634-1651}$, $NS3_{1630-1647}$—remained genotype-specific after the MVA-gt-3a-NS boost, as demonstrated in the previous experiment. Conversely, the ChAd-gt1-6 prime, MVA-gt1-6 boost regimen induced T cells targeting epitopes that displayed immunogenicity to both genotype-1b and -3a stimuli: $NS3_{1244-1260}$, $NS4_{1766-1781}$, and the M5 minipool (the epitope was not mapped down in this study yet the minipool was still unique to the gt1-6/gt1-6 prime-boost regimen; data not shown).

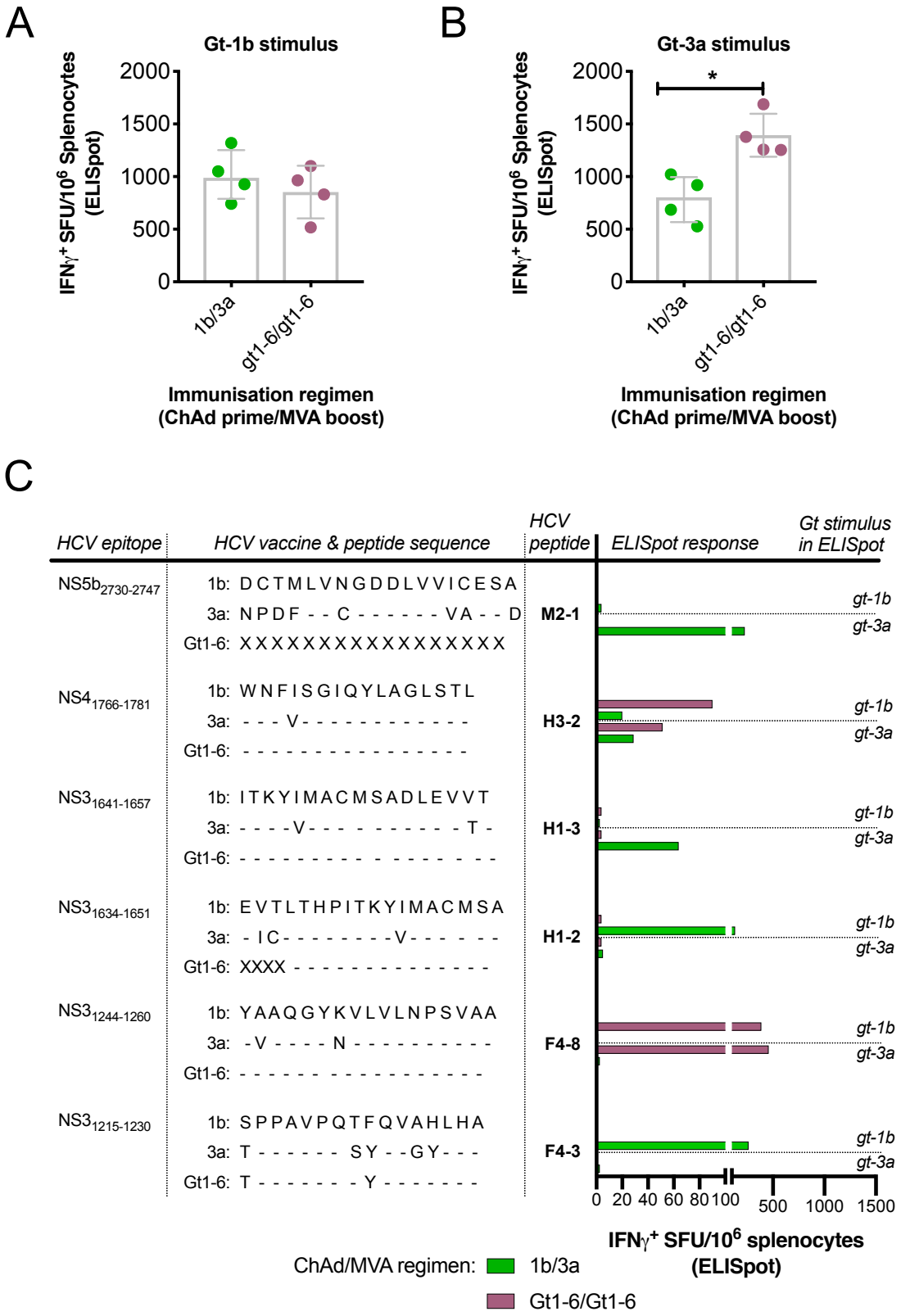


Figure 4-11. The ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost T cell response compared to a conserved sequence T cell prime-boost vaccination: The total magnitude genotype-1b specific (**A**) and total genotype-3a specific (**B**) IFNγ ELISpot response of harvested splenocytes seven days

after boost vaccination from age-matched female C57BL/6 mice (n=4) vaccinated with a 10^8 IU ChAd prime at day 0 and $1x10^6$ PFU MVA at day 14 in the following regimens: 1) a ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a), and 2) a ChAd-gt1-6 prime, MVA-gt1-6 boost (gt1-6/gt1-6), stimulated with HCV genotype specific peptide pool or peptides (15-18mers, overlapping by 11aa) covering the HCV non-structural proteomic sequence. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. (**C**) The epitope specific vaccine-induced T cell response and corresponding HCV amino acid sequence for HCV genotype-1b (J4) and -3a (K3a650) peptide stimuli (the same as the gt-1b and -3a vaccine immunogen sequences). Dashes in the epitope sequence represent sequence homology, crosses indicate no amino acid at that position in the sequence, and one letter amino acids codes represent amino acid substitution mutations in the gt1-6 and -3a sequence compared to the gt-1b sequence. Bars in the graphs represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

4.6 Discussion

The primary hypothesis of the work described in this chapter was that a prime-boost HCV vaccination strategy that employs different HCV genotype-specific immunogens encoded in a ChAd prime, MVA boost viral vector strategy can generate T cells that target conserved epitopes between the two genotypes, therefore eliciting a T cell response against both HCV genotypes.

The experimental data presented in this body of work rejects the primary hypothesis. A ChAd-gt-1b-NS prime, MVA-gt-3a-NS boost (1b/3a) does not boost primed T cells specific for epitopes that display sequence conservation between vaccine genotypes. Epitope mapping revealed that targeted T cell epitopes displayed amino acid sequence

mismatch between prime and boost vaccinal genotypes. The 1b/3a vaccination regimen did not increase the overall genotype-3a specific T cell response but lowered the genotype-1b T cell response compared to a 1b/1b vaccination regimen, inadvertently generating an intergenotypic T cell response. The prime-boost induced $CD8^+$ T cell response was significantly higher than adenovirus primed $CD8^+$ T cell responses, genotype-specific, and predominantly monofunctional with some polyfunctionality. The genotype-3a specific $IFN\gamma^+$ $CD8^+$ T cell response was higher after an MVA-gt-3a-NS boost compared to an MVA-gt-1b-NS boost and this was probably due to the genotype-3a specific T cell response to NS5b not observed in the 1b/1b regimen – possibly a primed T cell response from MVA. The 1b/3a prime boost vaccination elicited a broader T cell response compared to a homologous subtype-1b prime boost (1b/1b) regimen, with genotype-3a specific T cell responses targeting NS5b. Yet, there was no difference in the immunodominant epitope hierarchy between the HCV prime-boost regimens as the three most immunogenic T cell epitopes were the same irrespective of the HCV genotype of the MVA boost vaccine.

4.6.1 MVA vaccines boost the ChAd-gt-1b-NS primed T cell response

Recombinant poxviral vector vaccines such as MVA are potent inducers of the *in vivo* expansion of memory T cells (reviewed by Ewer et al. 2016). Both HCV 1b/1b and 1b/3a ChAd prime, MVA boost vaccination regimens generated genotype-1b and -3a specific T cell responses, including polyfunctional $CD8^+$ T cells, that were significantly higher in magnitude and breadth compared to the ChAd-gt-1b-NS prime vaccine T cell response. The MVA boost mediated increase in the total magnitude T cell response, including the $IFN\gamma^+$ $CD8^+$ T cell subset, is in accordance with data from HCV clinical trials (Swadling et al. 2014) and from other pre-clinical and human trials that demonstrate the immunogenicity and protective efficacy of high magnitude vaccine-induced $CD8^+$ T cell responses using a ChAd prime, MVA boost vaccination regimen (Reyes-Sandoval et al.

2010; Capone et al. 2010; Ewer et al. 2013; Ogwang et al. 2015; Alharbi et al. 2017; Bliss et al. 2018).

4.6.2 The MVA-gt-1b-NS and MVA-gt-3a-NS vaccines boost comparable genotye-3a T cell response

An important requirement of an HCV prophylactic vaccine strategy is the induction of T cells capable of targeting multiple HCV genotypes, in order to control HCV infection regardless of infecting genotype. In this study, a genotype-1b encoding ChAd vaccine and a genotype-3a encoding MVA vaccine were used to generate an increased vaccine-induced prime-boost T cell response against genotypes-3a compared to a homologous genotype-1b prime-boost regimen. The rationale behind this approach was that the MVA-gt-3a-NS would boost T cell response targeting epitopes that are conserved between genotype-1b and -3a and therefore increasing the genotype-3a response. Strikingly, the 1b/3a regimen did not increase the genotype-3a T cell response compared to a homologous genotype-1b prime-boost regimen. Furthermore, the 1b/3a vaccination regimen induced T cells targeting the same immunodominant epitopes—$NS3_{1215-1230}$, $NS3_{1634-1651}$, $NS3_{1641-1657}$—as the 1b/1b regimens' T cell responses, indicating no difference in preferentially recalled memory T cells after an MVA boost, irrespective vaccine genotype. This result is in line with previous reports from in a malaria pre-clinical study that demonstrated that the primed immunodominant T cell epitope remained the main T cell target after an MVA vaccine boost with limited targeting of subdominant epitopes (Rollier et al., 2016).

One exception to the change in vaccine-induced T cell epitope specificity was the increase in the genotype-3a specific $NS5b_{2730-2747}$ $CD8^+$ T cell response by the 1b/3a vaccination regimen which contributed to an overall higher genotye-3a $CD8^+$ T cell response in the 1b/3a vaccination regimen. This result is in line with studies of primary HCV infection which show that genotype-1 and -3 exhibit distinct T cell specificities targeting different

immunodominant epitopes (Humphreys et al., 2012; von Delft et al., 2015). It has been previously shown that subdominant T cell epitopes can be targeted by T cells during adenovirus vaccination if the dominant epitopes are not present or made redundant in the immunogen (Im et al., 2011). Therefore, it's possible that a different genotype in the boosting vaccine (i.e. a heterologous genotype prime-boost) may recall unique primed T cells compared to a homologous genotype prime-boost due to the genotype-specific epitope hierarchies. This is in line with data presented here as the NS5b genotype-3a T cell response was present after an MVA-gt-3a-NS boost but not an MVA-gt-1b-NS boost. While this result suggests that novel T cell epitopes are targeted after a 1b/3a prime-boost, an small yet distinct $NS5b_{2730-2747}$ specific $CD8^+$ T cell response was observed after an MVA prime vaccination making it unclear if the $NS5b_{1730-1747}$ epitope was initially primed by ChAd or MVA.

4.6.3 MVA boosted T cell responses are genotype-specific displaying limited sequence homology between genotype-1b and -3a at targeted epitopes

T cell responses generated by the genotype-1b homologous ChAd, MVA vaccination regimen in human volunteers targeted immunodominant genotype-specific T cell epitopes with a reduced ability to recognise non-subtype-1b epitope variants (Swadling et al. 2014; Kelly et al. 2015). Contrary to this investigation's hypothesis, the 1b/3a prime-boosted T cell response was also genotype-specific displaying no cross-recognition between genotype-specific epitope variants: $NS3_{1215-1230}$ and $NS3_{1634-1651}$ to genotype-1b, and $NS3_{1641-1657}$ and $NS5b_{2730-2747}$ to genotype-3a. Sequence analysis revealed that genotype-1b and -3a epitope variants displayed amino acid sequence mismatch and that recalled memory T cells, regardless of boost vaccine genotype, only targeted the genotype-specific epitope of the prime vaccine: genotype-1b. This result could be explained with a number of theories.

Firstly, the use of full-length HCV non-structural sequence and therefore immunodominant genotype-specific T cell epitopes to prime and boost a T cell response suggests that the dynamics of the T cell epitope hierarchy could be a factor in dictating the specificity of the boosted T cell response. A number of human studies highlight the difference of immunodominant HCV T cell epitopes between genotypes. Patients with multiple HCV infections display limited cross-reactive T cell responses between genotypes at immunodominant epitopes (Harcourt et al., 2003; Sugimoto et al., 2003). A later study revealed that human leukocyte antigens (HLA) exhibit lack of overlap in genotype-specific viral polymorphisms associated with specific HLA types, reflecting the differences in cellular immune pressures on distinct epitopes and subsequent escape profiles between HCV genotypes (Rauch et al., 2009). A large-scale analysis of genotype-1 and genotype-3 HCV-infected patients comprehensively showed limited overlap of genotype-3 specific $CD8^+$ and $CD4^+$ T cell epitopes compared to previously published dominant genotype-1 T cell epitopes (von Delft et al., 2015), a finding recently reported for genotype-1 and -4 epitopes (Lunburger et al. 2018).

The preference for an MVA boosted HCV-specific T cell response to target primed immunodominant genotype-1b epitopes, despite sequence mismatch between MVA-gt-3a-NS vaccine and genotype-1b T cell epitope, could be explained by a phenomenon first described in 1953: ‘original antigen sin’ (Francis, 1953). Upon infection with a novel viral strain, the host immune response targets previous viral strains at the expense of immune responses to the novel, protective antigenic determinants. The term original antigenic sin was first coined to account for the observation in influenza epidemics that antibodies specific for older viral strains were detected after infection of a novel influenza strain. Original antigen sin has since been described in malaria (Good et al., 1993), HIV (Nara et al., 1990), and dengue virus (Sangkawibha et al., 1983; Rothman 2011), and the concept

has been extended to include cytotoxic T lymphocytes (Klenerman & Zinkernagel, 1998). In the context of secondary HCV infection, clearance of subsequent HCV infections is more likely than primary infection presumable due to recognition of viral epitopes by memory T and B cell populations and the corresponding efficacious T and B cell response (Osburn et al., 2010). Yet, persistence of heterologous HCV infection—i.e. a new infecting HCV genotype—is still common despite the presence of T cell responses towards the primary infecting genotype (Prince et al., 2005; Schulze Zur Wiesch et al., 2007). The establishment of a heterologous HCV secondary infection may be, in part, explained by the misdirection of the T cell response to the original HCV epitopes that display mismatched sequence in the novel infecting HCV genotype (Proust et al., 2000). Another study revealed that pre-existing genotype-1a specific $CD4^+$ T cell responses were significantly higher in non-genotype-1a CHC patients compared to genotype-1a CHC patients indicating that pre-existing genotype-1a specific memory $CD4^+$ T cells (from the primary genotype-1a infection) were recalled after a secondary non-genotype-1a infection (Schulze Zur Wiesch et al., 2007). The authors went on to show that the genotype-1a T cell epitopes from the primary infection were located in highly variable HCV regions, lacked cross-recognition to autologous HCV in the secondary non-genotype-1a infection, and were not detected in the T cell epitope repertoire of genotype-1a spontaneous resolvers (Schulze Zur Wiesch et al., 2007). In a therapeutic setting, the HCV subtype-1b adenovirus vector T cell vaccine induced T cell responses that only targeted HCV epitopes that exhibited sequence mismatch between autologous virus and the vaccine immunogen in the epitope, with the T cell displaying a dysfunctional phenotype (Kelly et al., 2015; Swadling et al., 2016). In contrast, there was no vaccine-induced T cell response when the vaccine epitope sequence matched the circulating virus. One explanation is that residual memory T cells specific for the original antigen misdirected the new T cell response—induced by the vaccine—to target the original antigen and therefore did not recognise autologous HCV

epitope variants, increasing the virus' chance of establishing successful infection, as has been described for sequential influenza A virus infections (Kim et al., 2009). As original antigen sin may be at play in the vaccine-boosted T cell response of a prophylactic two-genotype prime-boost vaccination strategy, further investigation of boosted T cell responses and their cognate epitopes in other strains of mice is needed. Furthermore, initially priming the T cell response to conserved HCV epitopes may be preferential as the data shows that a ChAd-gt1-6 prime-boost regimen elicited a higher magnitude genotype-3a T cell response targeting conserved HCV T cell epitopes compared to the 1b/3a prime-boost vaccination regimen.

4.6.4 Limited number of immunogenic T cell epitopes in inbred mice

Inbred mouse strains are important pre-clinical models to map vaccine-targeted T cell epitopes, yet they may not represent immunological trends in an outbred population, such as humans. Inbred strains display a small number of vaccine transgene epitopes in the context of their H antigen peptide-presenting surface proteins due to the low diversity of the mouse H-antigen in inbred strains (for example, H-2K^{b} and H-2D^{b} and no H-2L in C57BL/6 mice). This is useful to facilitate manageable epitope mapping studies in pre-clinical experiments that have large numbers of mice. Overall changes in immunological trends, such as total T cell magnitude and breadth, which may be reflective of the number of immunogenic epitopes, may not be detected in an inbred population. Instead, an outbred population of mice, such as the CD-1 strain, may prove to better detect the effect of a two-genotype heterologous prime-boost vaccination regimen.

4.6.5 MVA primes a robust T cell response in mice but not in humans

MVA vaccine vectors prime very low T cell responses in humans compared to recombinant adenovirus vectors for reasons that are not fully understood, yet MVA vaccines induce

high magnitude T cell responses when used as a boost vaccine (Ewer et al., 2016). However, the data presented here shows that in mice the MVA-gt-3a-NS vaccine can prime a genotype-3a T cell response of a low yet robust magnitude, comparable to the magnitude of a genotype-3a specific response induced by the ChAd-gt-1b-NS vaccine. In addition, no significant difference in the magnitude of the MVA-gt-3a-NS primed and MVA-gt-3a-NS boosted genotype-3a T cell response to $NS5b_{1730-1747}$ was observed. Furthermore, $NS5b_{1730-1747}$ was the only prime-boosted T cell epitope that was not positive after the ChAd-gt-1b-NS prime. These observations confound the question of whether this epitope was boosted or primed by the MVA vaccine. Due to the capacity of MVA to prime T cell responses in mice, testing hypotheses that address changes in the T cell epitope hierarchy using an MVA vaccine may be challenging.

4.7 Conclusion

Whilst HCV viral vector vaccination strategies generate highly immunogenic T cell responses against genotype-1b, the generated T cell response against non-genotype-1b HCV variants is sub-optimal (Swadling et al. 2014). Vaccine-induced T cells target immunodominant epitopes that exhibit genotype-specific variants with limited cross-reactivity and are prone to viral escape (Kelly et al. 2015), supported by sequence analysis of HCV clinical cohorts (Giugliano et al., 2009; Harcourt et al., 2003; Humphreys et al., 2012; Sugimoto et al., 2003; von Delft et al., 2015). To overcome the obstacle of genotype-specific immune responses in a prophylactic HCV vaccine strategy, the vaccine-induced T cell response should target sequences that are conserved across multiple genotypes.

The ChAd prime, MVA boost viral vector vaccine strategy employed in this investigation indicates that a vaccination strategy that utilises the full non-structural sequence of two HCV subtypes does not generate an intergenotypic T cell response that targets conserved T cell epitopes between these genotypes. Instead, the genotype-1b prime vaccination

generates T cell responses to immunodominant epitopes present in full length HCV non-structural sequence—genotype-1b specific or conserved. A subsequent heterologous genotype-3a MVA boost vaccination may target the same immunodominant epitopes, irrespective of sequence conservation or mismatch between epitope genotype variants. This may be explained by the phenomenon of original antigenic sin, previously described for T cell epitopes (Klenerman & Zinkernagel, 1998), and is consistent with vaccine-induced T cell specificity observed in a therapeutic HCV vaccine phase I clinical trial (Swadling et al., 2016). To this end, a prime-boost vaccination strategy that primes naïve T cells to target conserved HCV epitopes across genotypes in the initial prime vaccination may be a preferable approach to generate T cell responses capable of recognising multiple HCV genotypes.

4.8 Summary of results

- Modified vaccinia Ankara (MVA) vector vaccines were generated encoding either the subtype-1b or subtype-3a HCV non-structural protein sequence.
- The MVA-gt-3-NS vaccines primes low magnitude genotype-3a specific T cell responses.
- The MVA vaccines boost high magnitude HCV-specific T cell responses, including $CD8^+$ T cells, that are polyfunctional.
- The MVA-gt-3a-NS boost vaccine does not increase the total magnitude genotype-3a specific T cell response compared to a homologous subtype-1b prime boost regimen.
- Due to a lower genotype-1b T cell response and a high magnitude genotype-3a T cell response, the intergenotype T cell response is significantly higher in the 1b/3a vaccination regimen compared to the 1b/1b regimen.

- The MVA vaccines—irrespective of HCV genotype—boost memory T cells that target the same genotype-specific immunodominant epitopes that were primed by the ChAd-gt-1b-NS vaccine, regardless of any sequence mismatch between epitope (gt-1b) and boost vaccine sequence (gt-3a). An observation akin to the phenomenon of original antigen sin.
- The MVA-gt-3a-NS boost vaccine elicits a T cell response that is genotype-specific targeting T cell epitopes that display mismatched sequence between genotype-1b and 3a.

5 AN HCV VACCINATION STRATEGY TO GENERATE ANTIBODY AND T CELL IMMUNE RESPONSES

5.1 Primary hypothesis

A conserved sequence HCV T cell adenoviral vectored vaccine and the recombinant E2Δ123 protein vaccine can be combined in a mixed modality prime-boost vaccination strategy to generate HCV-specific intergenotypic T cell responses and broadly neutralising antibody responses.

5.2 Introduction

The immune correlates of protection in spontaneously resolved HCV infection are not fully understood. Yet, a number of reports indicated that the innate and adaptive immune responses are important for controlling HCV infection, including T cells and antibodies (reveiewed by Abdel-Hakeem & Shoukry, 2014). Unlike other hepatotropic viruses—namely HBV—an exclusive role of antibodies in HCV resolution has not been shown. However, the early appearance of broadly neutralising antibodies (bNAbs) targeting the viral envelope glycoproteins, E1 and E2, is associated with spontaneous clearance in human infection (Osburn et al., 2014; Pestka et al., 2007). The recent discovery of the two

crystal structures of E2 core fragments in complex with two different antibodies (non-nAb 2A12 and bnAb ARC3) has revealed that E2 contains a central immunoglobulin (Ig)-like β-sandwich with two adjacent layers in both the front and back (Khan et al., 2014; Kong et al., 2013). This discovery of the E2 core crystal structure led to the mapping of contact residues in the conformational epitope of the bnAb, AR3C (Kong et al., 2013). Despite crystallised structural analysis of the immune complex as the gold standard to identify neutralisation epitopes to inform vaccine design, HCV glycoproteins remain difficult to crystallise, have complex conformational epitopes, structural flexibility, and there are functional differences between antibodies that bind closely related binding epitopes (Kinchen & Bailey, 2018; Ströh et al., 2018).

For the prospects of an HCV envelope protein vaccine, studies in chimpanzees and humans demonstrated that a recombinant gpE1E2 protein elicits high-titre antibody responses (Choo et al., 1994; Frey et al., 2010). Vaccine-induced antibodies predominantly targeted variable epitopes linked to immune evasion (Choo et al., 1994; Meunier et al., 2011; Stamataki et al., 2011), and elicited neutralisation of diverse HCVpp in only a minority of healthy volunteers (Law et al., 2013; Ray et al., 2010). In guinea pigs, a modified HCV E2 protein vaccine with deletion of the three hypervariable domains increased the neutralisation of heterologous strains through the antibody-mediated preferential targeting of relatively conserved epitopes found in the neutralising face of the E2 ectodomain (McCaffrey et al., 2016). The neutralising face of E2 is exposed when the protein aggregates into higher molecular weight structures – named by the authors as ‘E2Δ123$_{HMW}$’ (high molecular weight; Vietheer et al. 2016), and this form of E2 is being assessed in for pre-clinical vaccine studies. In contrast to the HCV T cell vaccine, viral vectored gt-1b non-structural sequence (ChAd- and MVA-gt-1b-NS), no HCV protein vaccine candidates have progressed beyond phase I human clinical trials. Nevertheless, there is no licensed HCV vaccine anywhere in the world. The licensure of a prophylactic

HCV vaccine that includes an HCV antibody inducing component remains an important clinical asset to achieve viral hepatitis elimination by 2030.

To date, the genetic diversity exhibited by HCVs genotypes, subtypes, and quasispecies has stalled vaccine development with vaccines eliciting responses to antigens that display high sequence diversity and are prone to mutational escape. In order to overcome the challenge of HCV diversity, vaccines that generate immune responses that target conserved B cell and T cell epitopes between viral genotypes would be optimal in order to generate pan-genotypic immunity. This approach also abrogates viral evasion mechanisms in response to vaccination that may come at the cost of detrimental virus fitness. Considering the evidence that T cell and antibody responses are causally linked with spontaneous clearance of acute HCV infection, an ideal HCV vaccine should generate both HCV-specific intergenotypic T cell and antibody responses targeting multiple HCV genotypes. A 'two-pronged' vaccination strategy that utilises both branches of the immune system— the cellular response and humoral response—is a viable strategy considering the primary aim of vaccination is to prevent the initial HCV infection of the liver and its subsequent persistence that leads to hepatitis C associated disease. Upon intrahepatic infection, HCV persistence is, in part, facilitated through cell-to-cell transmission in addition to secretion of extracellular virus particles indicating that vaccine-induced antibody responses alone would not be sufficient to elicit protection, unless these provide complete sterilising immunity upon HCV exposure.

Adding a T cell component to an HCV antibody vaccine would increase the chances of inducing protective immunity. I have previously demonstrated that a simian adenovirus viral vector vaccine encoding the conserved viral sequence across HCV genotypes 1 to 6 (ChAd-gt1-6L) generates high magnitude, broad, intergenotypic T cell responses that target highly conserved epitopes (see **chapter 0**). In this body of work, I develop a vaccination strategy that combines the conserved sequence HCV T cell vaccine, ChAd-

gt1-6L, with the recombinant E2Δ123 protein vaccine in a heterologous prime-boost regimen to elicit intergenotypic T cell and antibody immune responses. I propose that a single viral vector vaccine candidate encoding both the T cell component, gt1-6L, and the antibody component, E2Δ123, could generate intergenotypic T cell responses and broadly neutralising antibody responses to multiple HCV genotypes. To date, no HCV vaccine strategy has been attempted to combine two HCV vaccine candidates with the primary aim of generating both pan-genotypic T cell responses and neutralising antibody responses.

5.3 Key questions

1. Can an E2Δ123$_{HMW}$ protein vaccine be co-administered in a prime vaccination with a ChAd-gt1-6L T cell vaccine without compromising the ChAd-induced T cell response?
2. Does an E2Δ123$_{HMW}$ protein vaccine that is co-administered with a ChAd-gt1-6L T cell vaccine elicit an E2-specific antibody response that inhibits E2 binding to CD81, and targets neutralisation epitopes and E2-CD81 binding determinants of HCV, comparable to protein vaccine alone?
3. What antibody isotypes are generated from a co-administered protein and T cell vaccine immunisation regimen?
4. Does an E2Δ123 sequence encoded in a ChAd viral vector (ChAd-E2Δ123) vaccine prime an E2-specific antibody response comparable to a E2Δ123$_{HMW}$ protein prime vaccination?
5. Does a heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein boost vaccination regimen generate an E2-specific antibody response that inhibits E2 binding to CD81, and targets neutralisation epitopes and E2-CD81 binding determinants of HCV, comparable to a homologous E2Δ123HMW protein prime boost regimen?

6. Does a ChAd-E2Δ123 prime vaccination in a heterologous E2Δ123 protein boost regimen induce a broadly neutralising antibody response (bNAbs)?
7. Does a ChAd-E2Δ123 prime, $E2\Delta123_{HMW}$ protein heterologous vaccination regimen change the vaccine-induced antibody isotype profile compared to a homologous $E2\Delta123_{HMW}$ prime-boost regimen?

5.4 Chapter specific materials and methods

5.4.1 Vaccine nomenclature

The following vaccines were used in this chapter.

5.4.1.1 ChAd-gt1-6L

The conserved sequence T cell immunogen, gt1-6L, with a 5' genetic adjuvant, truncated shark invariant chain, aa47-72 (sIi), was encoded in the simian adenovirus vector, ChAdOx1.

5.4.1.2 $E2\Delta123_{HMW}$

The recombinant protein, E2Δ123, encodes $E2_{410\text{-}661}$ with deleted sequences of hypervariable region 1 (HVR1; $E2_{384\text{-}411}$), HVR2 ($E2_{460\text{-}480}$), and intergenotypic variable region (igVR; $E2_{570\text{-}580}$) that lie outside and within a core E2 domain that contains the CD81 binding site. The high molecular weight species of E2Δ123 is referred to as $E2\Delta123_{HMW}$ and was used in these vaccine studies. The E2 protein has a C-terminal polyhistine tag for application in assays.

5.4.1.3 $E2_{RBD}$

The recombinant protein, $E2_{RBD}$, encodes $E2_{384\text{-}661}$ and includes hypervariable region 1 (HVR1; $E2_{384\text{-}408}$), HVR2 ($E2_{460\text{-}485}$), and intergenotypic variable region (igVR; $E2_{570\text{-}580}$) sequences, encoding the full length of the E2 receptor binding domain. The low molecular

weight, native species of $E2_{RBD}$ was used. The E2 protein has a C-terminal polyhistine tag for application in assays.

5.4.1.4 ChAd-eGFP

The sequence the green fluorescent protein, eGFP, was encoded in the simian adenovirus vector, ChAdOx1, instead of the HCV immunogen and used as a control T cell vaccine to account for HCV-specific immunogenicity to the ChAdOx1 vector.

5.4.1.5 ChAd-E2Δ123

The E2Δ123 nucleic sequence with a 5' tissue plasminogen activation leader sequence (TPA-LS) was encoded in the simian adenovirus vector, ChAdOx1.

5.4.2 E2 protein vaccine production

E2 vaccine production was performed by Joey McGregor. Transient transfections of FS293FS cells with HCV H77c E2RBD and Δ123 expression plasmids were performed using 293fectin (Invitrogen) according to manufacturer's recommendations. Cultured supernatants contained secreted E2 glycoproteins were filtered, affinity purified, and underwent size exclusion chromatography (SEC) on Superdex 200 16/600 columns (GE Healthcare) using the ÄKTA pure fast protein liquid chromatography (FPLC) system (GE Healthcare).

5.4.3 Animals, immunisations & ethics

All mouse studies were performed at the Biomedical Services Building, Oxford, according to U.K. Home Office Regulations (project license numbers 30/2744 and 30/3293) and approved by the local ethical review board at the University of Oxford. Groups of age-matched female C57BL/6 mice were vaccinated at 8-10 weeks of age.

5.4.3.1 Co-administration of ChAd and protein vaccines

Groups of C57BL/6 mice were vaccinated with either 1) 10^8 infectious units (IU in 40µL intramuscularly) of ChAd-gt1-6L at day 0 or, 2) 20µg of E2Δ123$_{HMW}$ protein in 50µL mixed with 50µL oil-in-water emulsion adjuvant, AddaVax^{TM}, subcutaneously in the scruff of the neck at day 2, or 3) both ChAd-gt1-6L and E2Δ123$_{HMW}$ (and AddaVax^{TM}) at day 0 and day 2, respectively.

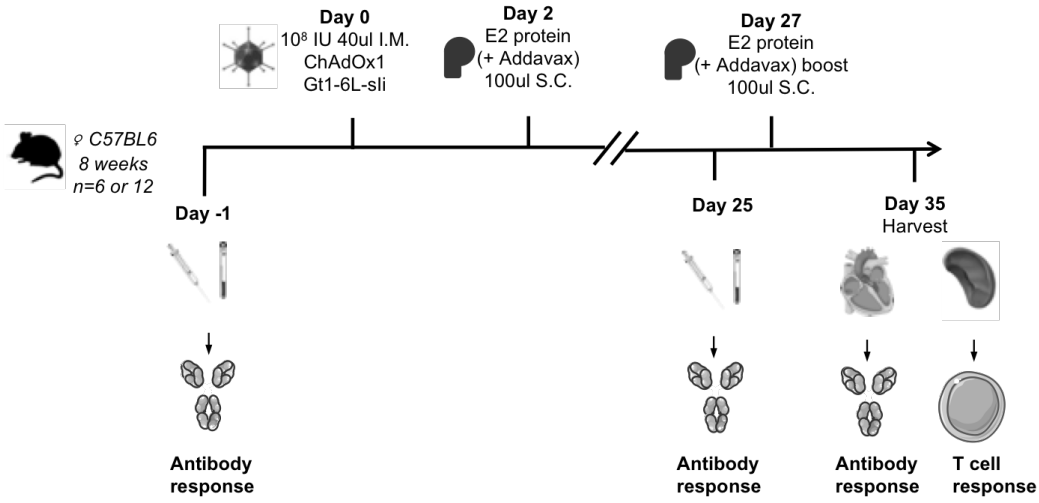
Due to restrictions in the project license issued by the U.K. Home Office, injections must be at least 48 hours apart, therefore the E2Δ123$_{HMW}$ protein prime subcutaneous injection was done 48-hours after the intramuscular ChAd prime vaccination.

For boosting immunisations, mice were vaccinated subcutaneously at three or four-week intervals with 20µg of E2Δ123$_{HMW}$ protein in 50µL mixed with 50µL oil-in-water emulsion adjuvant, AddaVax^{TM}. No mouse received more than three immunisations in accordance with the project license. Mice were culled one week after the last immunisation for collection of blood (via a cardiac bleed) and splenocytes (Figure 5-1**A-B**).

5.4.3.1.1 Power analysis

Two experiments were performed in the investigation of co-administration of ChAd and protein vaccines. The first experiment analysed T cell responses and antibody responses (experiment 1, n=4/vaccination group). The antibody immune response data (not shown) was used to calculate a power analysis to determine the number of animals required to sufficiently assess significant difference (CI=95%) in the mean antibody responses between vaccination groups at 80% power (experiment 2, n=12/vaccination group).

A



B

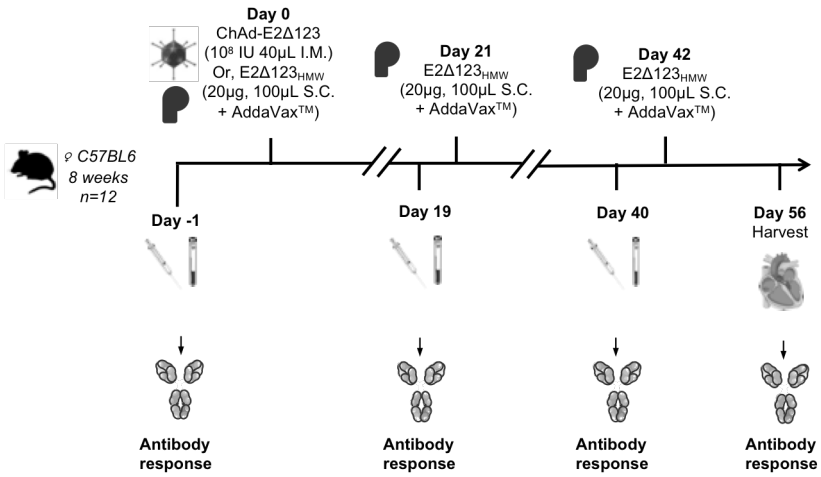
Vaccination schedule	Group 1	Group 2	Group 3	Group 4	Group 5
Day 0	ChAd-gt1-6L	ChAd-gt1-6L	-	ChAd-gt1-6L	ChAd-eGFP
Day 2	$E2\Delta123_{HMW}$	$E2_{RBD}$	$E2\Delta123_{HMW}$	-	$E2\Delta123_{HMW}$
Day 27	$E2\Delta123_{HMW}$	$E2_{RBD}$	$E2\Delta123_{HMW}$	-	$E2\Delta123_{HMW}$

Figure 5-1. Prime-boost vaccination schedule to assess a co-priming vaccination regimen using a ChAd-gt1-6L vaccine and an E2Δ123HMW protein vaccine: A schematic (**A**) and table (**B**) detailing a prime-boost vaccination schedule in 8-week old age-matched female C57BL/6 mice that incorporates a day 0 adenovirus T cell vaccine prime (10^8 IU in 40μL intramuscularly in the left quadricep), and a day 2 protein vaccine prime and 27 protein vaccine boost (20μg of E2 protein in 50μL and mixed with 50μL of the oil-in-water emulsion adjuvant, AddaVax™) administered subcutaneously in the scruff of the neck. Two forms of E2 were administered as the protein vaccine: either 1) the E2Δ123 ($E2_{410-611}$) that has the three hypervariable regions deleted, or 2) the E2RBD which does not have hypervariable region deletions and spans the receptor binding domain of E2 ($E2_{384-661}$). Tail bleeds were performed at day -1 and day 25 prior to the prime and boost vaccinations, respectively. At day 35 after prime vaccination, mice were culled one at a time (schedule 1) and a cardiac bleed was immediately performed followed by extraction of the spleen for assessment of antibodies and T cells, respectively.

5.4.3.2 Administration of ChAd-E2Δ123 prime and an E2Δ123$_{HMW}$ protein boost

A third experiment was undertaken to assess the administration of a ChAd-E2Δ123 vaccination strategy, compared to an E2Δ123$_{HMW}$ protein vaccine. Groups of twelve age-matched female C57BL/6 mice were vaccinated with either 1) 10^8 infectious units (IU in 40µL intramuscularly) of ChAd-gt1-6L or, 2) 20µg of E2Δ123$_{HMW}$ protein in 50µL mixed with 50µL oil-in-water emulsion adjuvant, AddaVax^{TM}, subcutaneously in the scruff of the neck at week 0. For boosting immunisations, mice were vaccinated at three-week intervals (week 3, 6). Tail bleeding was performed prior to vaccinations and a terminal cardiac bleed at end of study at week 8 (Figure 5-2**A-B**). No mouse received more than three immunisations in accordance with the project license.

A



B

Vaccination schedule	Group 1	Group 2	Group 3
Week 0	ChAd- E2Δ123	E2Δ123$_{HMW}$	ChAd-E2Δ123
Week 3	ChAd- E2Δ123	E2Δ123$_{HMW}$	E2Δ123$_{HMW}$
Week 6	ChAd- E2Δ123	E2Δ123$_{HMW}$	E2Δ123$_{HMW}$

Figure 5-2. Vaccination schedule to assess the immunogenicity of a ChAd-E2Δ123 and an E2Δ123$_{HMW}$ protein vaccine homologous and heterologous prime-boost regimen: A

schematic (**A**) and table (**B**) detailing a prime-boost vaccination schedule in 8-week old age-matched female C57BL/6 mice (n=12/group) for a homologous ChAd-E2Δ123 viral vector vaccine and E2Δ123$_{HMW}$ protein vaccine regimen, and a heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein vaccine boost regimen. ChAd-E2Δ123 was administered at 10^8 IU in 40μL intramuscularly in the left quadricep, and E2Δ123$_{HMW}$ protein vaccine with 20μg in 50μL mixed with 50μL of the oil-in-water emulsion adjuvant, AddaVax™, administered subcutaneously in the neck. Tail bleeds were performed at day -1, week 3, and week 6 prior to the prime and the two boost vaccinations, respectively. At week 8 after prime vaccination, mice were culled one at a time (schedule 1) and a cardiac bleed was immediately performed.

5.5 Results

5.5.1 Vaccination with soluble E2Δ123$_{HMW}$ protein and ChAd-gt1-6L does not decrease the T cell response compared to a ChAd vaccination prime vaccine alone

To date, HCV vaccine candidates have primarily focused on inducing either a T cell response or an antibody response (recently reviewed by Shoukry 2018). In this study, I investigated whether two HCV candidates—a conserved sequence T cell vaccine encoded in a simian adenovirus vector, and an E2Δ123$_{HMW}$ protein vaccine—can be combined in a heterologous prime-boost vaccination strategy to elicit T cell and antibody responses comparable to immune responses induced by either vaccine administered alone.

Co-administration of a ChAd-gt1-6L prime vaccine at day 0 with a E2Δ123$_{HMW}$ prime vaccine at day 2 and an E2Δ123$_{HMW}$ boost vaccine at day 27 did not significantly reduce the vaccine-induced HCV-specific T cell response at day 35 post-prime vaccination compared to mice that received ChAd-gt-1-6L alone (medians of 184 [IQR: 185.9] and 115 [IQR:191.22] IFNγ$^+$ SFU/10^6 splenocytes for genotype-1a peptide stimulation, respectfully, Figure 5-3**A**). No difference in the T cell magnitude was observed for genotype-1b and -3a specific T cell responses between groups 1 and 4 (Figure 5-3**B-D**). Vaccination groups that received an HCV T cell ChAd prime vaccine displayed similar T

cell magnitudes, all of which were significantly higher than group 3 (protein only) and group 5 (ChAd-eGFP). Vaccination group 1—that received a combined ChAd-gt1-6L and E2Δ123$_{HMW}$ prime vaccination—generated T cell responses that reached as high as 675 SFU/10^6 splenocytes 5-weeks after prime vaccination, albeit that the T cell magnitude median was not significantly higher than the group 4 T cell magnitude median (Figure 5-3**B**). ChAd-primed mice that received E2$_{RBD}$ prime and boost vaccines induced a lower total T cell magnitude median compared to mice that had received both ChAd-gt1-6L and E2Δ123$_{HMW}$ protein vaccines, an observation only statistically significant with a HCV genotype-3a peptide stimulus (Figure 5-3**C**, p = 0.0485*).

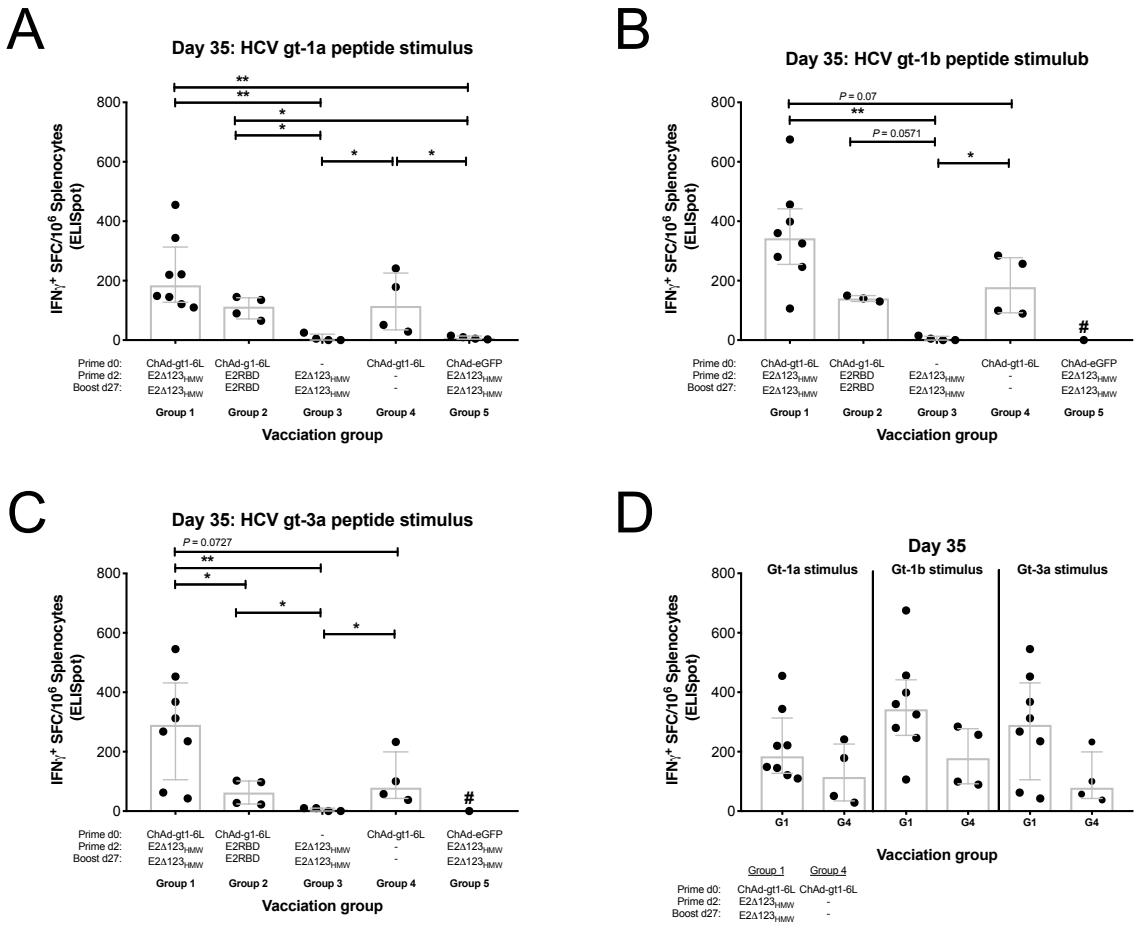


Figure 5-3. T cell responses of mice vaccinated with a ChAd-gt1-6L and E2Δ123$_{HMW}$ co-prime vaccination: The total IFNγ ELISpot response of harvested splenocytes from all vaccination groups stimulated with HCV genotype-1a, (**A**), -1b (**B**), and -3 (**C**) peptide pools that cover the full

length of the HCV proteome (15-18mers overlapping by 11aa). (**D**) The total ELISpot response between groups 1 (T cell vaccine and protein vaccine prime) and 4 (T cell vaccine only) for direct comparison of vaccine-induced genotype-1a, -1b, and -3a T cell responses. A hash indicates no detectable response. Groups of four C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of $E2\Delta123_{HMW}$ or $E2_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and harvested at day 35. The data presented is a combination of two experiments: the first experiment using groups 1-3, and the second experiment using groups 1, 4, and 5, therefore *n*=8 for group 1. Both experiments were performed once. The total IFNγ ELISpot response is defined as the total of positive peptide pools where a positive peptide pool is defined as greater than the DMSO negative control plus three standard deviations, and greater than the median HCV-specific T cell response from a naïve unvaccinated control group. Bars represent the median SFU/10^6 splenocytes, with interquartile ranges displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

Next, I assessed the $CD4^+$ and $CD8^+$ T cell response to HCV proteins E2 and NS4 using intracellular cytokine staining (ICS) and flow cytometry analysis (Figure 5-4**A**). After stimulation with a genotype-1a E2 peptide pool, vaccination group 4 (T cell vaccine alone) generated a significantly greater $IFN\gamma^+$ $CD8^+$ T cell response compared to group 3 (protein vaccine alone, Figure 5-4**B**, $p = 0.0476$*). No significant difference was seen in the E2-specific $IFN\gamma^+$ $CD4^+$ or $IFN\gamma^+$ $CD8^+$ T cell response between any group that had received a ChAd-gt1-6L prime vaccine, albeit all were low magnitude responses. Group 2 (protein vaccine only) displayed a higher $IFN\gamma^+$ $CD4^+$ median but this was not significantly different compared to other vaccination groups (group 2 median = 0.199% of all $CD4^+$ T cells (Figure 5-4**B**).

The $CD4^+$ and $CD8^+$ T cell response against HCV NS4 protein sequence was assessed to investigate if the addition of an E2 protein prime vaccination indirectly effected the T cell

response targeting sequence not included in the protein vaccine. No significant difference in the percentage of NS4-specific IFNγ$^+$ CD8$^+$ T cell responses was detected between vaccination groups that received a ChAd-gt1-6L prime vaccination (Figure 5-4**C**). All groups that received a T cell prime vaccine displayed a significantly higher NS4-specific IFNγ$^+$ CD8$^+$ T cell response compared to group 3 that did not receive a ChAd-gt1-6L T cell prime vaccine (group 3 median of 0.01%, IQR:0). There was a trend for group 4 (T cell vaccine alone) to generate a higher % IFNγ$^+$ CD8$^+$ T cell response compared to group 1 which received a T cell prime vaccine and a protein prime and boost vaccine, but this difference was not statistically significant (group 4 median, 1.94%, IQR: 3.77% v. group 1 median, 0.575%, IQR: 0.1075%). There was no significant difference in the NS4-specific IFNγ$^+$ CD4$^+$ T cell response between vaccination groups 1 and 4 (Figure 5-4**C)**.

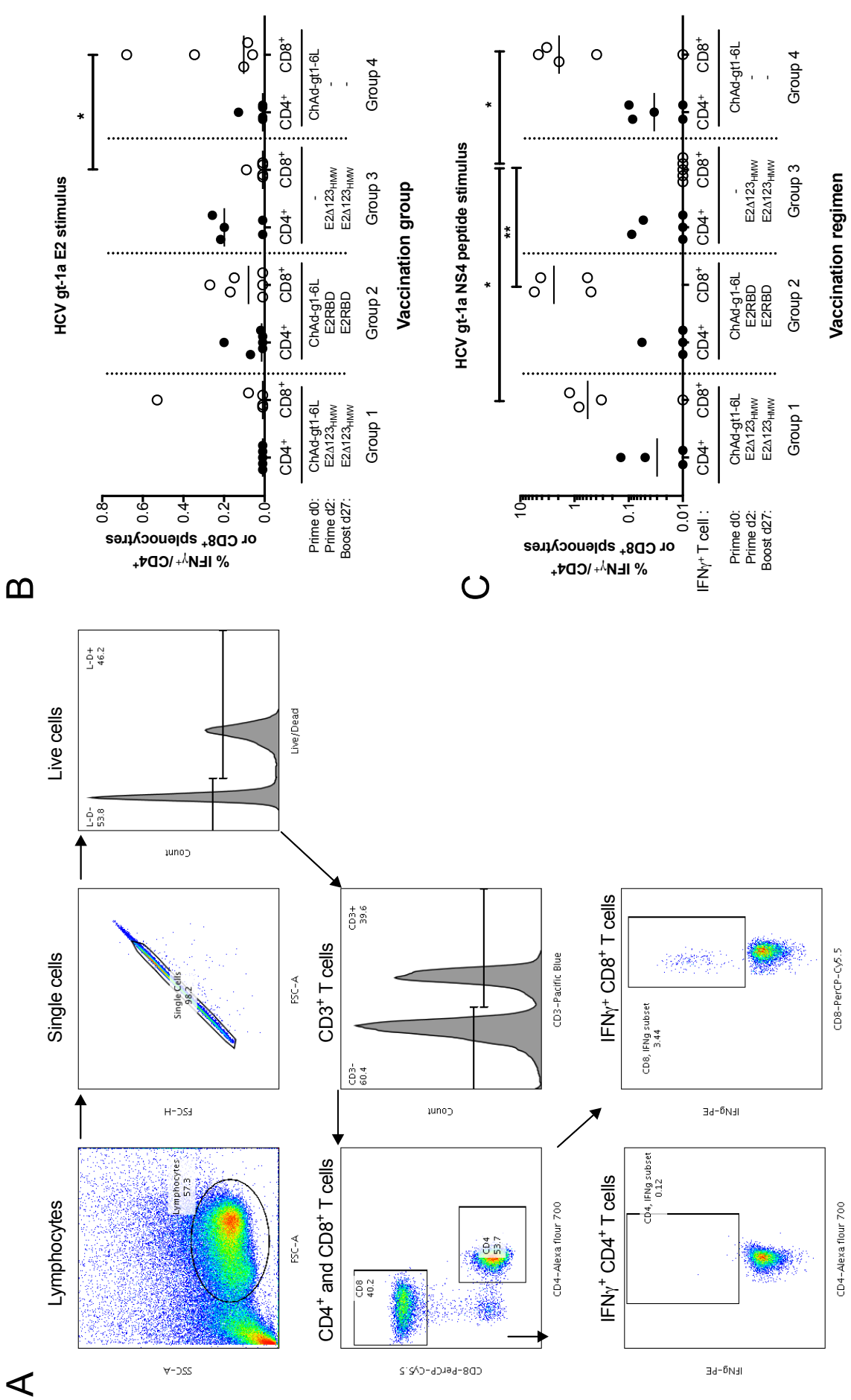

A
Lymphocytes
Single cells
Live cells
CD3+ T cells
CD4+ and CD8+ T cells
IFNγ+ CD8+ T cells
IFNγ+ CD4+ T cells
B
HCV gt-1a E2 stimulus
% IFNγ+ /CD4+ or CD8+ splenocytres
Vaccination group
Group 1
Group 2
Group 3
Group 4
C
HCV gt-1a NS4 peptide stimulus
IFNγ+ T cell :
Vaccination regimen

Figure 5-4. The CD4$^+$ and CD8$^+$ T cell response of mice vaccinated with a ChAd-gt1-6L prime vaccine and an E2123$_{HMW}$ protein prime and boost vaccine: Groups of four 8-week old age-matched female C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of E2Δ123$_{HMW}$ or E2$_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax^{TM}), and harvested at day 35. The total IFNγ$^+$ CD4$^+$ and IFNγ$^+$ CD8$^+$ T cell responses was assessed using splenocytes stimulated for 6 hours (last 4 hours with BD GolgiPlug^{TM}) with HCV genotype-1a peptide pool (E2 or NS4b, 15-18mers that overlap by 11aa) and intracellular cytokine staining (ICS) and flow cytometry analysis was performed. (**A**) The gating strategy is shown, and the arrows indicate the direction of gating. The E2-specific (**B**) and NS4-specific (**C**) CD4$^+$ and CD8$^+$ T cell responses' capacity to secrete IFNγ. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

5.5.2 Co-vaccination with soluble E2Δ123$_{HMW}$ protein and ChAd-gt1-6L elicits a high titre antibody response comparable to a homologous E2Δ123$_{HMW}$ protein prime-boost regimen

Having demonstrated that the magnitude of the ChAd-induced HCV-specific T cell response did not decrease with the addition of an E2Δ123$_{HMW}$ day 2 prime and day 27 E2Δ123$_{HMW}$ boost vaccination, I investigated the vaccine-induced E2-specific antibody response after a heterologous ChAd-gt1-6L, E2Δ123$_{HMW}$ prime-boost regimen compared to a homologous E2Δ123$_{HMW}$ protein prime-boost vaccination. Dilution curves for all vaccine-induced antibody responses were plotted and the antibody titre measured as ten times the background (BSA) and displayed as log reciprocal titres.

All vaccination groups that received an E2 protein—E2Δ123$_{HMW}$ or E2$_{RBD}$—displayed greater than 10^5 antibody titres that were significantly higher than the ChAd-gt1-6L induced E2-specific antibody response (Figure 5-5**A-B**; $p < 0.0005$***). Pre-boost day 25 E2Δ123-specific antibody responses were higher for vaccination groups that received

E2Δ123$_{HMW}$ compared to E2$_{RBD}$ (Figure 5-5**C**; $p < 0.005$**). Day 35 prime-boost E2Δ123-specific antibody responses were significantly higher for all groups when compared to their respective pre-boost day 25 E2Δ123-specific antibody responses, (Figure 5-5**D**; $p < 0.0001$***).

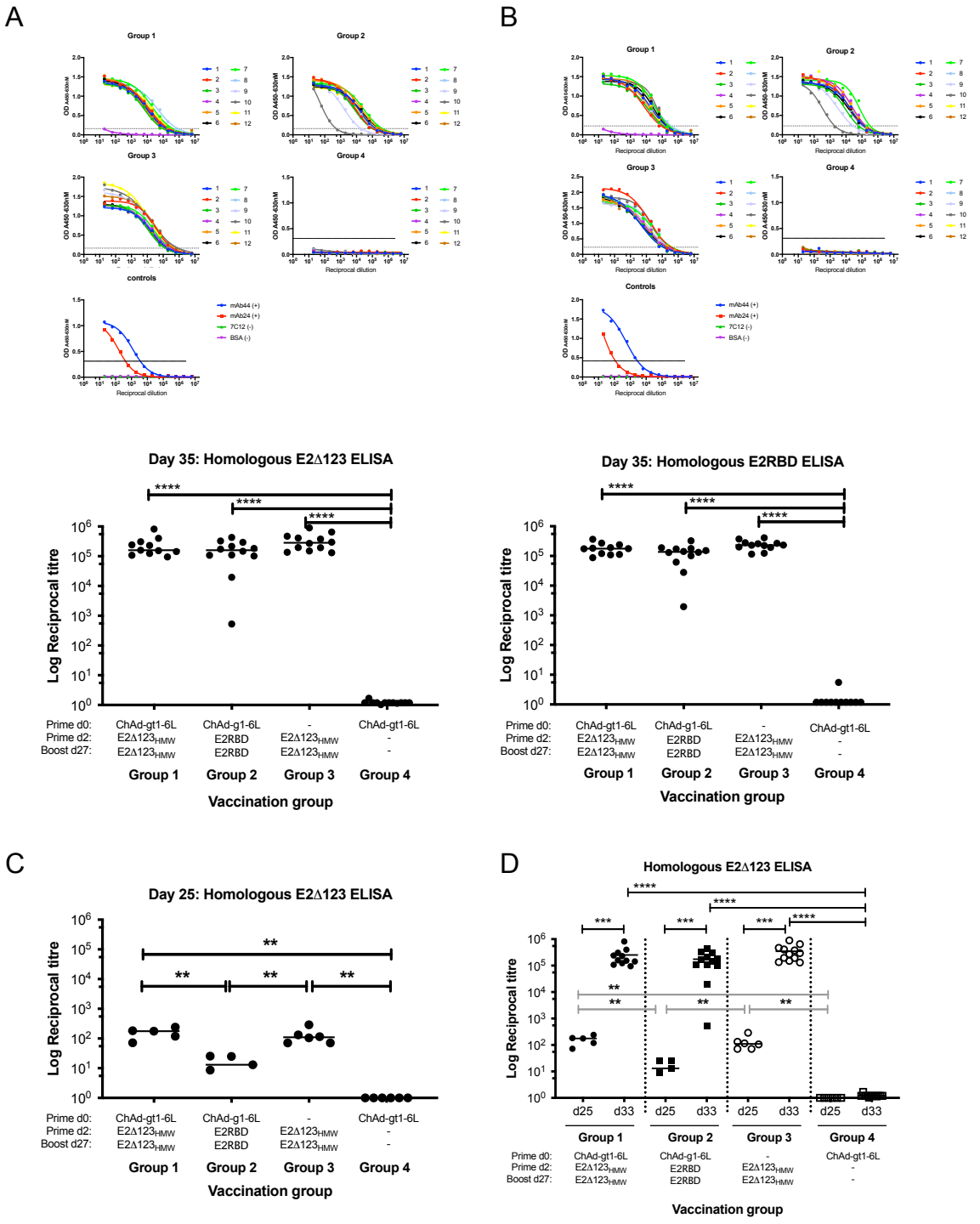


Figure 5-5. The E2Δ123-specific and E2RBD-specific antibody response of mice vaccinated with a ChAd-gt1-6L prime vaccine and an E2123$_{HMW}$ protein prime and boost vaccine: Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of E2Δ123$_{HMW}$ or E2$_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and harvested at day 35. (**A**) Total E2Δ123-specific (monomer) antibody dilution curves and titres. (**B**) E2$_{RBD}$ ELISA dilution curves and total E2$_{RBD}$-specific antibody titres. The E2Δ123-specific antibody titres at day 25, prior to protein vaccine boost (**C**), and in comparison, to end of study, day 35 E2Δ123-specific antibody titres (**D**). Antibody responses are measured at ten times background (BSA) and are plotted as log reciprocal titres. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine significant differences between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

5.5.3 Co-vaccination with soluble E2Δ123$_{HMW}$ protein vaccine and ChAd-gt1-6L vaccine elicits antibodies specific for epitopes in the CD81 binding site of E2

HCV entry into the liver is governed by the interaction of the viral glycoprotein heterodimer E1E2 with a number of host surface receptors including scavenger receptor B1 (SRB1) and CD81 (Bartosch et al., 2003). Therefore, I assessed the ability of vaccine-induced E2Δ123-specific antibodies to bind epitopes found in the CD81 binding site of E2: 'epitope I' (E2$_{408-428}$), 'epitope II' (E2$_{430-451}$), and 'epitope III' (E2$_{523-549}$) which are known CD81-binding determinants.

Vaccination groups that received an E2Δ123$_{HMW}$ protein vaccine generated high antibody titres targeting all three CD81 binding epitopes, with no significant difference between group 1 (T cell and protein vaccine) and group 3 (protein only) antibody titres (Figure 5-6**A-C**). Group 1 and 3 antibody titres were higher than group 2 titres which received a

$E2_{RBD}$ protein vaccine instead of the $E2\Delta123_{HMW}$. The T cell vaccine only group (group 4) displayed no antibody response.

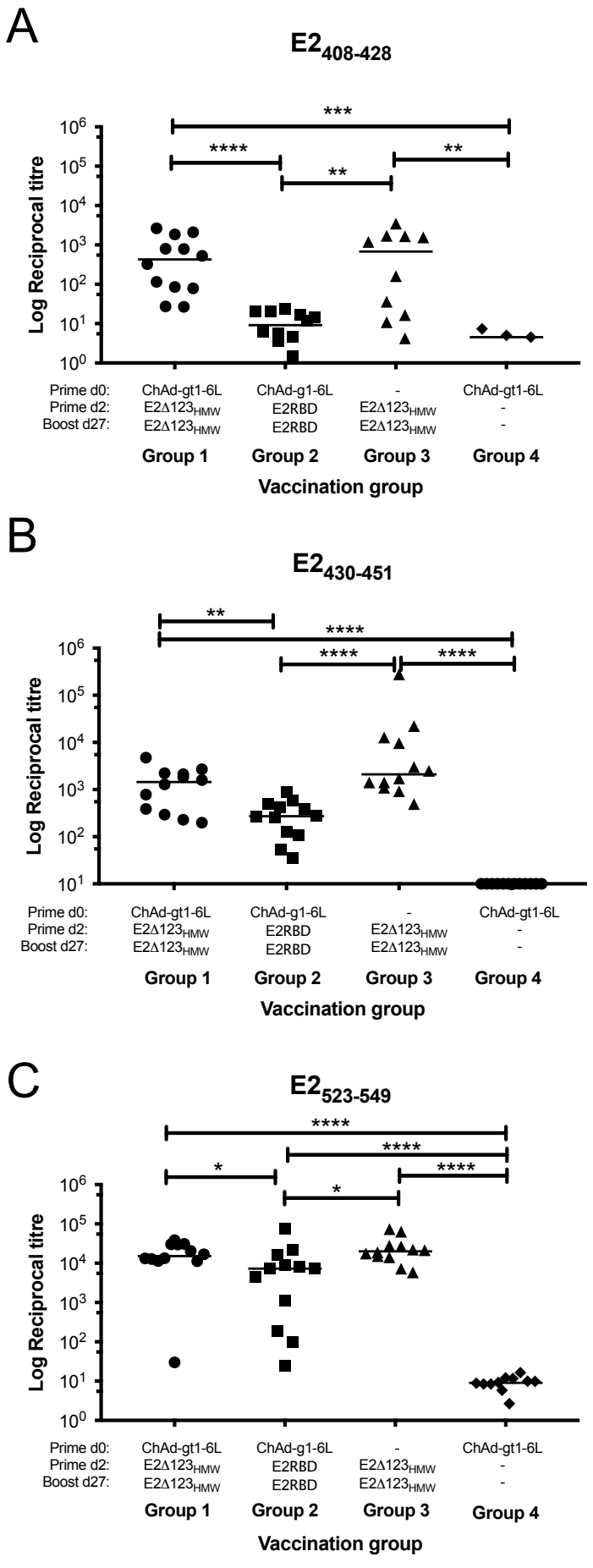

A
E2408-428
***
****
**
**
Log Reciprocal titre
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Prime d0: ChAd-gt1-6L ChAd-g1-6L - ChAd-gt1-6L
Prime d2: E2Δ123HMW E2RBD E2Δ123HMW -
Boost d27: E2Δ123HMW E2RBD E2Δ123HMW -
Group 1 Group 2 Group 3 Group 4
Vaccination group
B
E2430-451
**
****
****
****
Log Reciprocal titre
10^6
10^5
10^4
10^3
10^2
10^1
Prime d0: ChAd-gt1-6L ChAd-g1-6L - ChAd-gt1-6L
Prime d2: E2Δ123HMW E2RBD E2Δ123HMW -
Boost d27: E2Δ123HMW E2RBD E2Δ123HMW -
Group 1 Group 2 Group 3 Group 4
Vaccination group
C
E2523-549
****
****
*
****
*
Log Reciprocal titre
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Prime d0: ChAd-gt1-6L ChAd-g1-6L - ChAd-gt1-6L
Prime d2: E2Δ123HMW E2RBD E2Δ123HMW -
Boost d27: E2Δ123HMW E2RBD E2Δ123HMW -
Group 1 Group 2 Group 3 Group 4
Vaccination group

Figure 5-6. ELISA antibody titres targeting HCV epitopes I, II, and III found in the CD81 binding site of E2: Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of $E2\Delta123_{HMW}$ or $E2_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and harvested at day 35. The vaccine-induced antibody response targeting E2 epitopes I ($E2_{408-428}$, **A**), II ($E2_{430-451}$, **B**), and III ($E2_{523-549}$, **C**) found in the CD81 receptor binding domain of E2 and measured by peptide ELISA. Antibody responses are measured at ten times background (BSA) and are plotted as log reciprocal titres. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

5.5.4 Co-vaccination with soluble $E2\Delta123_{HMW}$ protein and ChAd-gt1-6L elicits antibodies that inhibit E2 binding to CD81

HCV cellular entry is mediated through E2 interaction with CD81. To evaluate the efficacy of the vaccine-induced antibody response specific for the CD81 binding site, I assessed the capacity of the vaccine-induced antibody response to inhibit E2 binding to the long-elongated loop of CD81 (CD81-LEL).

After background subtraction (BSA only), the vaccine-induced ELISA antibody responses were standardised to the negative control (E2; 100% E2 to CD81 binding), and plotted in ELISA dilution curves for IC50 and IC80 as primary readouts (Figure 5-7**A**). Experimental groups that received the $E2\Delta123_{HMW}$ vaccine displayed CD81 inhibiting antibody titres of up to 10^2 for IC50 and IC80, comparable to the HC84.27 positive control and significantly higher compared to vaccination groups that did not receive the $E2\Delta123_{HMW}$ protein vaccine (groups 2 and 4; Figure 5-7**B-C**; $p < 0.0005$***).

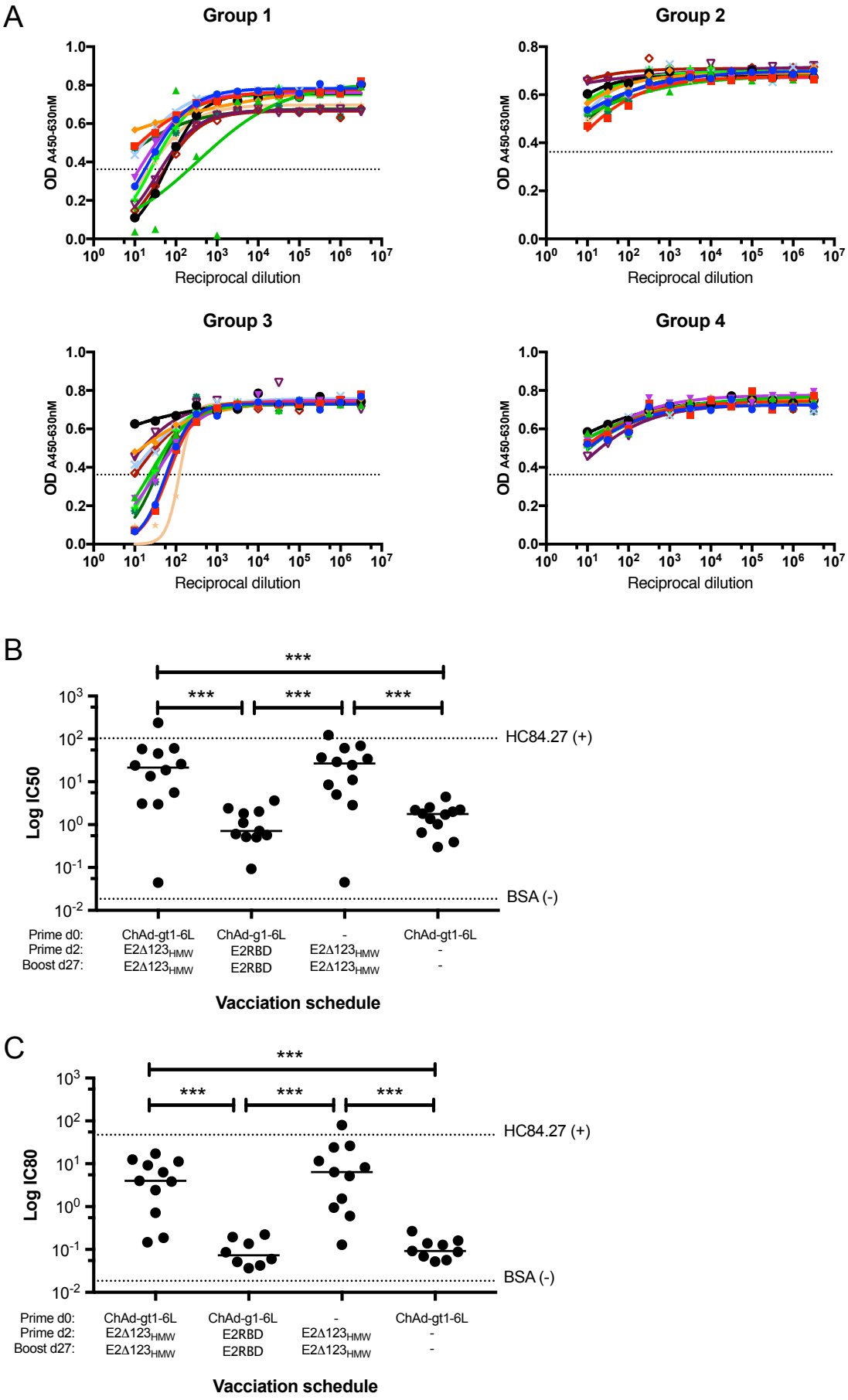

A
Group 1
Group 2
Group 3
Group 4
OD A450-630nM
Reciprocal dilution
B
Log IC50
HC84.27 (+)
BSA (-)
***
Prime d0:
Prime d2:
Boost d27:
ChAd-gt1-6L
E2Δ123HMW
ChAd-g1-6L
E2RBD
-
ChAd-gt1-6L
Vacciation schedule
C
Log IC80
HC84.27 (+)
BSA (-)
Vacciation schedule

Figure 5-7. The capacity of vaccine-induced antibody responses to inhibit E2 binding to CD81-LEL: Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of $E2\Delta123_{HMW}$ or $E2_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and harvested at day 35. (**A**) The vaccine-induced ELISA antibody responses were background subtracted and standardised to the negative control (BSA, which displayed 100% E2 to CD81 binding) and plotted in ELISA dilution curves. (**B-C**) The vaccine-induced antibody titres that inhibit 50% (IC50) and 80% (IC80) of E2 binding to CD81 are plotted as log IC50 and IC80 titres. The log IC50 and IC80 antibody titres of the positive control, the monoclonal antibody HC84.27, are shown (targeting $E2_{610-619}$ residues). Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

5.5.5 The co-vaccination of soluble $E2\Delta123_{HMW}$ protein and ChAd-gt1-6L does not alter the vaccine-induced antibody isotype profile compared to a homologous protein prime-boost regimen

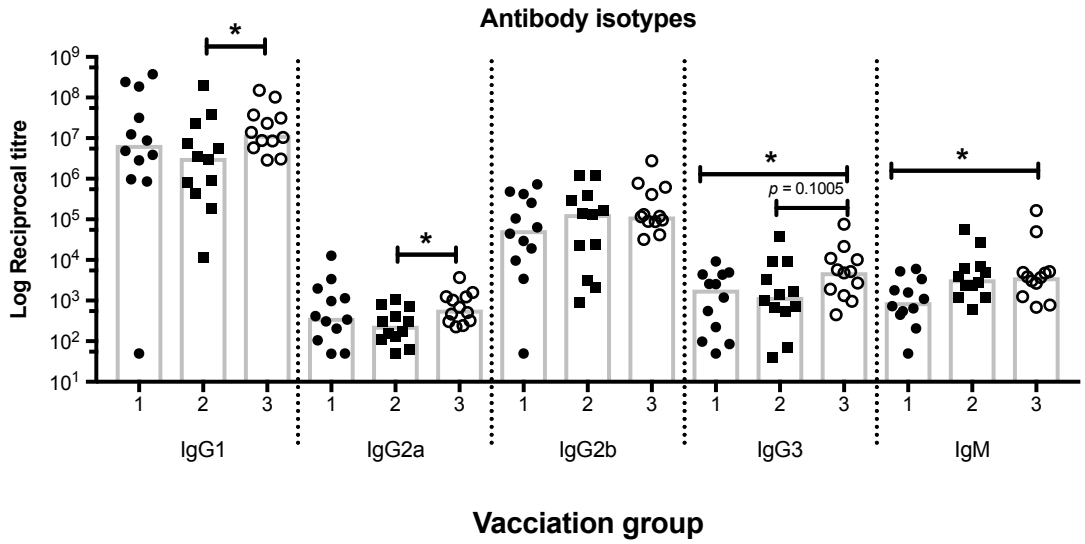
The inclusion of the $E2\Delta123_{HMW}$ protein vaccine is necessary to efficiently bind to epitopes within the CD81 binding site and to generate high titre antibodies that inhibit E2-CD81 binding. To further characterise the vaccine-induce antibody response, antibody isotyping was performed for groups 1, 2, and 3 mouse serum.

All vaccination groups displayed high IgG1 antibody titres, and to a lesser extent IgG2b antibody titres, while IgG2a, IgG3, and IgM were consistently low across all vaccination groups (Figure 5-8**A**). Group 3 had a significantly higher IgG1 and IgG2a antibody titre compared to group 2 and a significantly higher IgG3 and IgM antibody titre compared to group 1 (Figure 5-8**A**). The IgG1 antibody isotype was the most represented isotype as a proportion of the total antibody response in all vaccination regimens, followed by the IgG2b isotype (Figure 5-8**B**). While group 1 produced mostly an IgG1 antibody response,

it did show a greater variety of antibody isotypes induced compared to groups 2 and 3 (Figure 5-8**B**). The ratio of the IgG2a and IgG1 antibody response reflects the skew of the vaccine-induced T cell response towards a Th1 or Th2 phenotype (a high ratio indicates a skewed Th1 response and a low ratio indicates a Th2 response). All vaccination groups displayed low IgG2a: IgG1 ratios that were not significantly different to each other showing that a Th2 T cell phenotype is induced by vaccination (Figure 5-8**C**).

A

Vaccination schedule	Group 1	Group 2	Group 3
Day 0	ChAd-gt1-6L	ChAd-gt1-6L	-
Day 2	E2Δ123$_{HMW}$	E2$_{RBD}$	E2Δ123$_{HMW}$
Day 21	E2Δ123$_{HMW}$	E2$_{RBD}$	E2Δ123$_{HMW}$



B



C

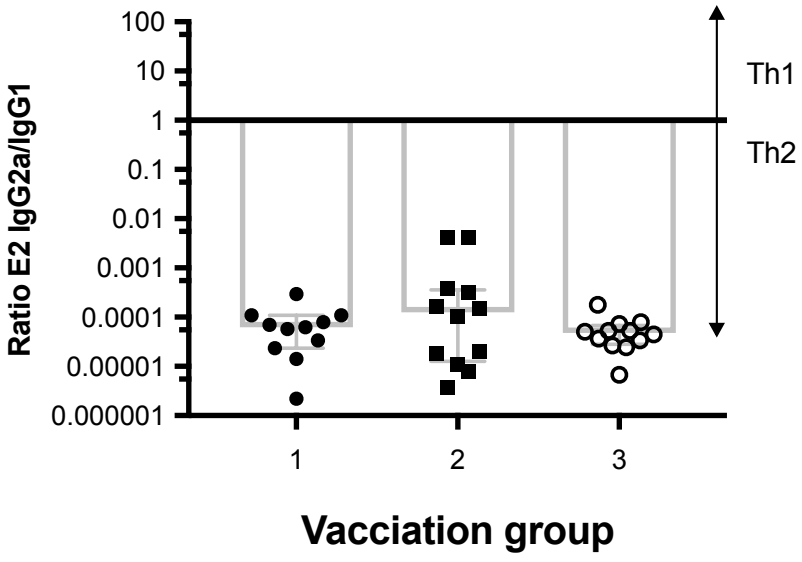


Figure 5-8. The isotype profile of vaccine-induced E2-specific antibodies: Assay performed by Joey McGregor and analysis undertaken by McGregor and Tim Donnison. Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated with a combination of a day 0 ChAd-gt1-6L prime at 10^8 IU in 40uL intramuscularly, a day 2 and day 27 E2 protein vaccine (20μg of $E2\Delta123_{HMW}$ or $E2_{RBD}$ in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and harvested at day 35. (**A**) The vaccine-induced E2-specific antibody isotype titres: IgG1, IgG2a, IgG2b, IgG3, IgM. (**B**) The proportion of each antibody as a fraction of the total antibody response. Pie bases are medians and asterisks, if any, indicate significant difference between groups. (**C**) The ratio of E2-specific IgG2a and IgG1 isotypes to determine preference for Th1 and Th2 T cell subtypes. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when < 0.05*, <0.01**, <0.001***, <0.0001****.

5.5.6 The E2Δ123 sequence encoded in a simian adenovirus vector (ChAd-E2Δ123) primes high titre antibody responses compared to $E2\Delta123_{HMW}$ protein prime vaccine

An ideal prophylactic HCV vaccine strategy should elicit both a T cell and an antibody response, as both are associated with HCV clearance in spontaneous resolvers. I have previously demonstrated that a prime-boost HCV vaccination regimen that incorporates a simian adenovirus T cell vaccine and a $E2\Delta123_{HMW}$ protein co-prime vaccination can generate T cell and antibody responses comparable to the administration of either prime vaccine alone. While mixed modalities have been explored for induction of HCV-specific T cells and antibodies in this study and in other studies for other diseases (de Cassan & Draper, 2013b), immunisation using a single vaccine that can elicit both an antibody and T cell response may prove to be optimal to reduce vaccine manufacturing costs and simplifying immunisations schedules to increase patient adherence to a vaccination programme. In chapter 3, I showed that an adenoviral vectored vaccine encoding a single T cell immunogen (a monovalent viral vector vaccine) induced a high magnitude, broad,

intergenotypic T cell response to multiple HCV antigens. A viral vector that encodes two HCV antigens (a bivalent viral vector vaccine) may be desirable to elicit both HCV-specific T cell and antibody responses using a single vaccine. Therefore, next, I addressed the first step in the development of a bivalent HCV vaccine strategy: to assess the immunogenicity of E2Δ123 sequence when encoded in the ChAdOx1 viral vector compared to the E2Δ123$_{HMW}$ protein-in-adjuvant formulation. Determining the immunogenicity of a monovalent ChAdOx1 vector vaccine that encodes the antibody immunogen—E2Δ123—is a critical prerequisite step before the assessment of the antibody and T cell response from a bivalent HCV vaccine.

ChAd-E2Δ123 vaccine-induced antibody responses were compared to E2Δ123$_{HMW}$ protein vaccine in a single prime vaccination regimen. At 3-weeks post-prime vaccination, ChAd-E2Δ123 induced an antibody titre significantly higher than the antibody titre elicited by an E2Δ123$_{HMW}$ prime vaccine (Figure 5-9; p = 0.0007***).

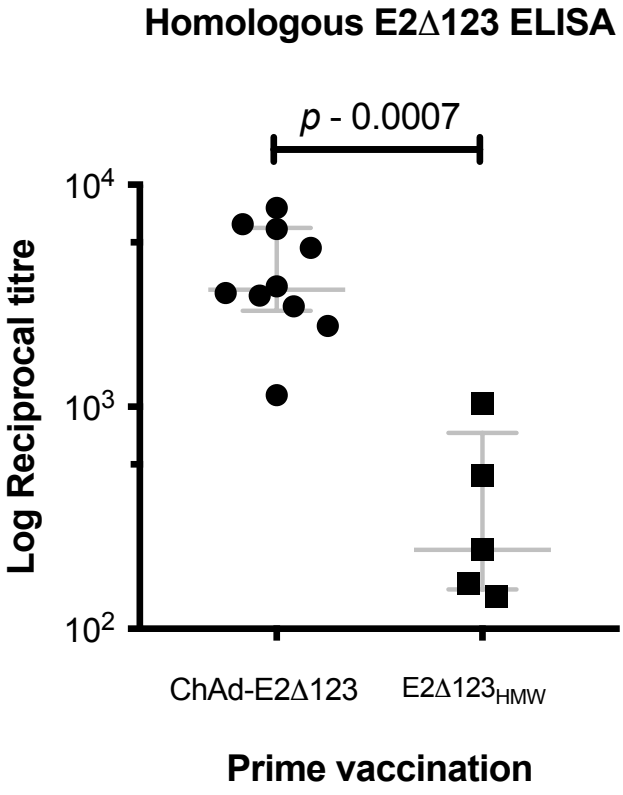


Figure 5-9. Prime vaccine-induced E2Δ123-specific antibody titres: Groups of 8-week old age-matched female C57BL/6 mice were vaccinated with a prime of either ChAd-E2Δ123 at 10^8 IU dose in 40uL intramuscularly, or E2Δ123$_{HMW}$ protein vaccine (20μg in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax™), and blood was taken at week 3. The experiment was performed once. Total vaccine-induced E2Δ123-specific antibody titres after a single prime vaccination is shown. Antibody titres are measured at ten times background (BSA) and are plotted as log reciprocal titres. Bars are medians and interquartile ranges are displayed. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval.

5.5.7 A heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein boost vaccination regimen generates high titre antibody responses comparable to a homologous protein prime-boost regimen

A number of studies support the use of mixed modality vaccination strategies to elicit high vaccine-induced antibody titres (de Cassan and Draper 2013). I assessed the capacity of a ChAd-E2Δ123 prime E2Δ123$_{HMW}$ protein boost heterologous vaccination regimen to generate antibody responses compared to a homologous prime-boost regimen using either vaccine alone (see methods, Figure 5-2**A-B**).

All three vaccination groups demonstrated significantly higher anti-E2Δ123 antibody titres after the second boost vaccination (prime, boost, boost; detonated '*PBB'*; week 8) compared to the prime (*P*; week 3) and prime-boost (*PB*; week 6) vaccination. In group 1, the antibody titre increase between *P* and *PBB* was two-fold, and the group 2 and 3 antibody titre increase was 1000 and 100-fold, respectively (Figure 5-10**A**). A significant increase in the vaccine-induced E2Δ123 antibody titre between *P* and *PB* was only observed for groups 2 and 3 which both received a protein boost (group 2: $p = 0.0079$**; and group 3: $p = 0.0095$** ;Figure 5-10**A**). No increase between *P* and *PB* was observed for group 1. Both the homologous protein prime-boost regimen (group 2) and the heterologous prime-boost (group 3) generated significantly higher antibody responses than

the ChAd prime-boost regimen (group 1) in the *PB* and *PBB* vaccination schedules (Figure 5-10**B-C**). There was no significant difference between the *PB* and *PBB* vaccine-induced anti-E2Δ123 antibody titres between groups 2 and 3 (Figure 5-10**B-C**).

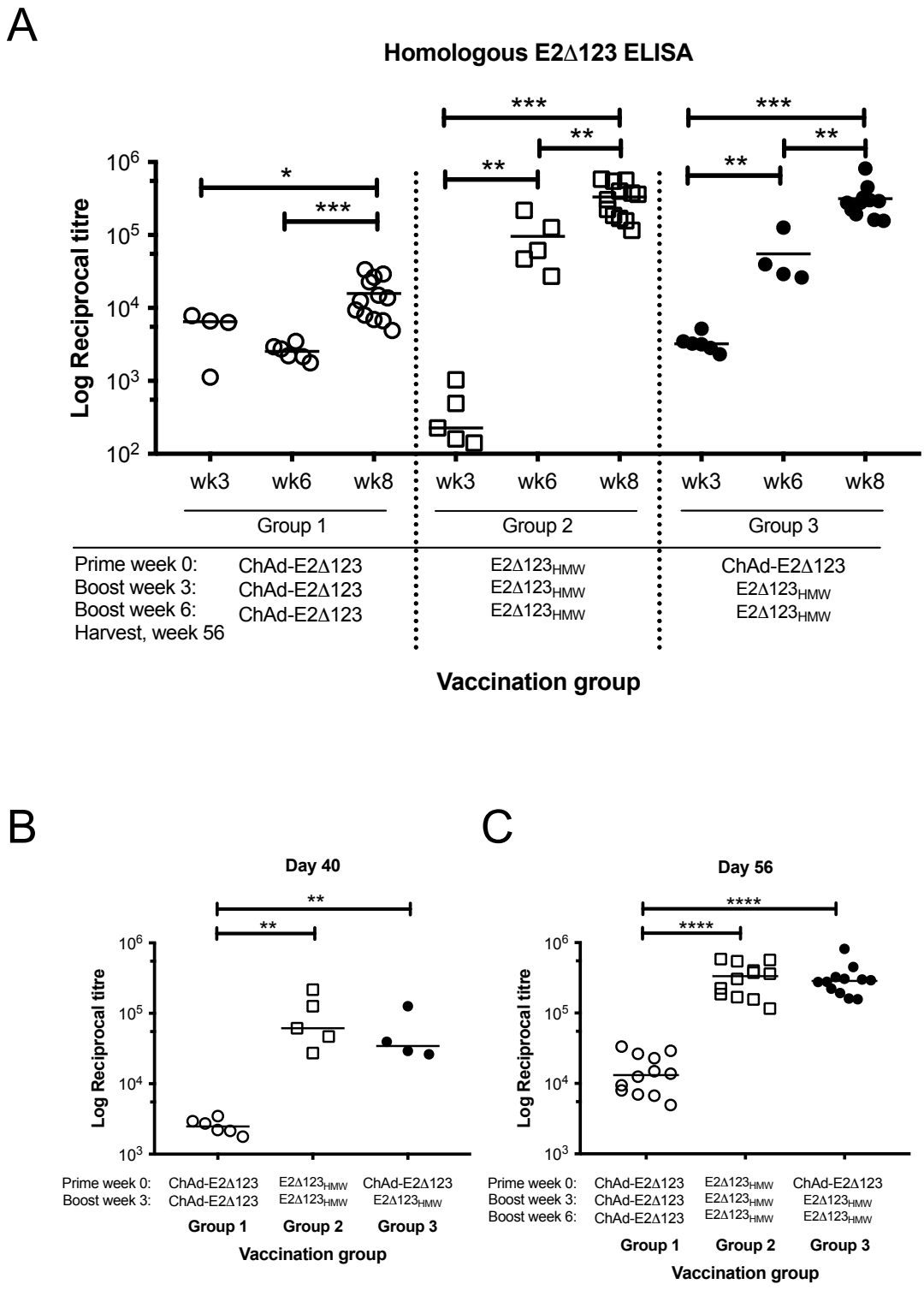


Figure 5-10. Prime-boost vaccine regimen induced E2Δ123-specific antibody titres: Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated in a prime-boost-boost (weeks 0, 3, 6) schedule with 1) ChAd-E2Δ123 vaccine homologous regimen (10^8 IU dose in 40uL intramuscularly), or 2) E2Δ123$_{HMW}$ protein vaccine homologous regimen (20μg in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax^{TM}), or 3) a ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein boost heterologous regimen. Blood was collected prior to every vaccination and at the end of study at week 8 (2-weeks after the last immunisation). The experiment was performed once. (**A**) Comparison of E2Δ123-specific antibody responses between time points within vaccination groups. Comparison of week 6 (**B**) and week 8 (**C**) E2Δ123-specific antibody responses between vaccination groups. Antibody titres are measured at ten times background (BSA) and are plotted as log reciprocal titres. Bars are medians. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when ≤ 0.05*, ≤ 0.001**, ≤ 0.0001***, ≤ 0.0001****.

5.5.8 A ChAd-E2Δ123 prime and soluble E2Δ123$_{HMW}$ protein boost increases the inhibition of CD81-E2 binding compared to a homologous protein vaccination

The E2-CD81 interaction is a key step in HCV cellular entry into the liver. A number of conserved CD81 binding determinants are exposed in the E2Δ123 oligomeric high molecular form and are the targets of the E2Δ123$_{HMW}$ vaccine-induced antibody response (Alhammad et al., 2015; McCaffrey et al., 2016; Vietheer et al., 2016). Therefore, I investigated whether a ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein boost vaccination regimen could elicit antibodies with the capacity to inhibit E2 binding to CD81-LEL (long elongated loop), and if so, which CD81 determinants were targeted.

After background subtraction (BSA only), the vaccine-induced antibody responses were standardised to the negative control (E2; 100% E2 to CD81 binding) and plotted in ELISA dilution curves. The homologous ChAd-E2Δ123 and E2Δ123$_{HMW}$ protein prime-boost regimens generated E2-CD81 inhibiting antibody IC50 and IC80 log reciprocal titres that

were not significantly different between each regimen (Figure 5-11**A-B**). The heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ boost regimen (group 3) generated significantly higher IC50 E2-CD81 inhibiting antibody titres than the group 1 homologous ChAd-E2Δ123 prime-boost regimen (Figure 5-11**A**; $p < 0.0001$****).

Group 3 vaccine-induced antibody responses targeting the CD81 determinant, E2$_{408-428}$, were higher compared to groups 2 and significantly higher compared to group 1 (Figure 5-11**C**; $p = 0.0009$***). Group 2 and 3 vaccine-induced antibody responses to E2$_{430-451}$ and E2$_{523-549}$ epitopes were significantly higher compared to group 1 (Figure 5-11**D-E**; $p < 0.0001$****).

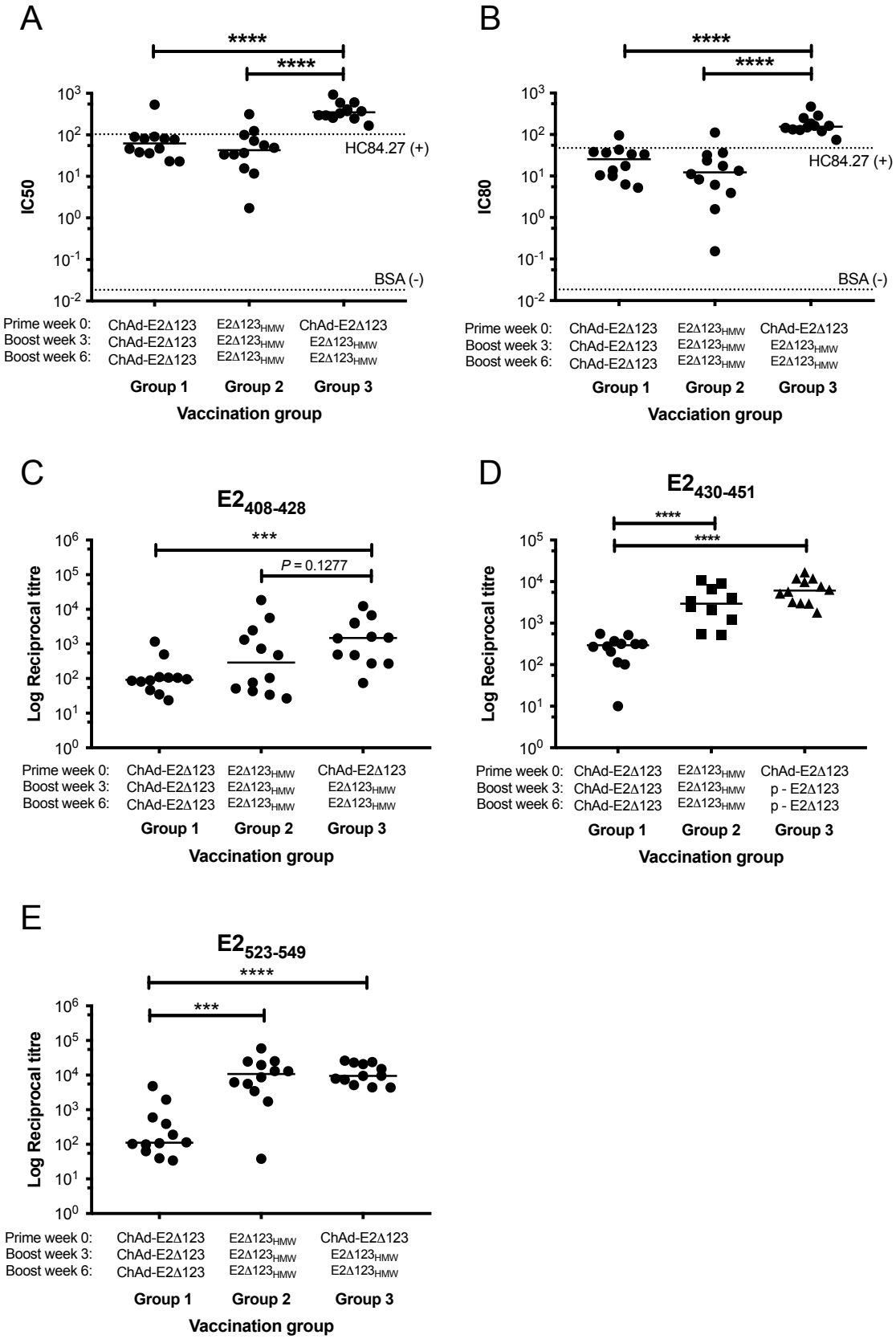


Figure 5-11. Vaccine-induced E2Δ123-specific antibody inhibition of E2 binding to CD81 and specificity to CD81 neutralisation epitopes: Groups of twelve 8-week old age-matched female C57BL/6 mice were vaccinated in a prime-boost-boost (week 0, 3, 6) schedule with 1) ChAd-

E2Δ123 vaccine homologous regimen (10^8 IU dose in 40μL intramuscularly), or 2) E2Δ123$_{HMW}$ protein vaccine homologous regimen (20μg in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax^{TM}), or 3) a ChAd-E2Δ123 prime, E2Δ123HMW protein boost heterologous regimen. Blood was collected prior to every vaccination and at the end of study at week 8 (2-weeks after the last immunisation). The experiment was performed once. The vaccine-induced antibody titre to inhibit 50% (IC50, **A**) and 80% (IC80, **B**) binding of E2 to CD81 is shown. Vaccine-induced antibody titres specific for E2 epitopes I (E2$_{408-428}$, **C**), II (E2$_{430-451}$, **D**), and III (E2$_{523-549}$, **E**) which overlap with the CD81 receptor binding domain of E2. Bars are medians. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when ≤ 0.05*, ≤ 0.001**, ≤ 0.0001***, ≤ 0.0001****.

5.5.9 A heterologous ChAd-E2Δ123 prime and E2Δ123$_{HMW}$ protein boost vaccination regimen increases the broadly neutralising antibody titre compared to a homologous E2Δ123$_{HMW}$ or ChAd-E2Δ123 prime-boost regimen

Hepatitis C virus is difficult to grow in cell culture and this has, until recently, hindered the study of HCV including vaccine development. Retrovirus nucleocapsids pseudotyped with genotype-1a (H77) E1/E2 envelope glycoproteins (HCVpp) and cell culture derived HCV (HCVcc, made by transfecting full-length HCV RNA into Huh 7 cells) have emerged as useful *in vitro* tools to study E1/E2 functionality and vaccine-induced antibody responses (Bartosch et al. 2003; Drummer et al., 2003). HCVpp and HCVcc have emerged as the gold standard for assessing the HCV-specific neutralising antibodies.

Using the HCVpp genotype-1a system, the heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ boost vaccination regimen (group 3) generated IC50 neutralising antibody titres (nAb) 9-fold higher than groups 1 and 2 (Figure 5-12**A**; p = 0.0003*** and p = 0.0105*, respectively). Only four (of twelve) mice in group 3 generated IC80 nAbs compared to two mice in group 2 and none in group 1 (Figure 5-12**B**).

The capacity of the vaccine-induced antibody response to cross-neutralise HCV subtypes (bNAbs) was assessed using the genotype-3a and -5a HCVcc system. Group 3 genotype-3a HCVcc neutralising IC50 log reciprocal antibody titres were 1.5-fold higher than groups 1 and 2, albeit not statistically significant (Figure 5-12**C**). All group genotype-5a HCVcc IC50 nAb titres were high with no statistical difference between group medians (Figure 5-12**D**). No correlation between all HCVpp (1a) and HCVcc genotype-3a and -5a nAbs was observed. However, there was a positive correlation between HCVpp (1a) and HCVcc gt-3a when negative HCVpp nAb responses were removed from the correlation analysis (Figure 5-12**E**; $R = 0.6946$ $p = 0.0442$*)

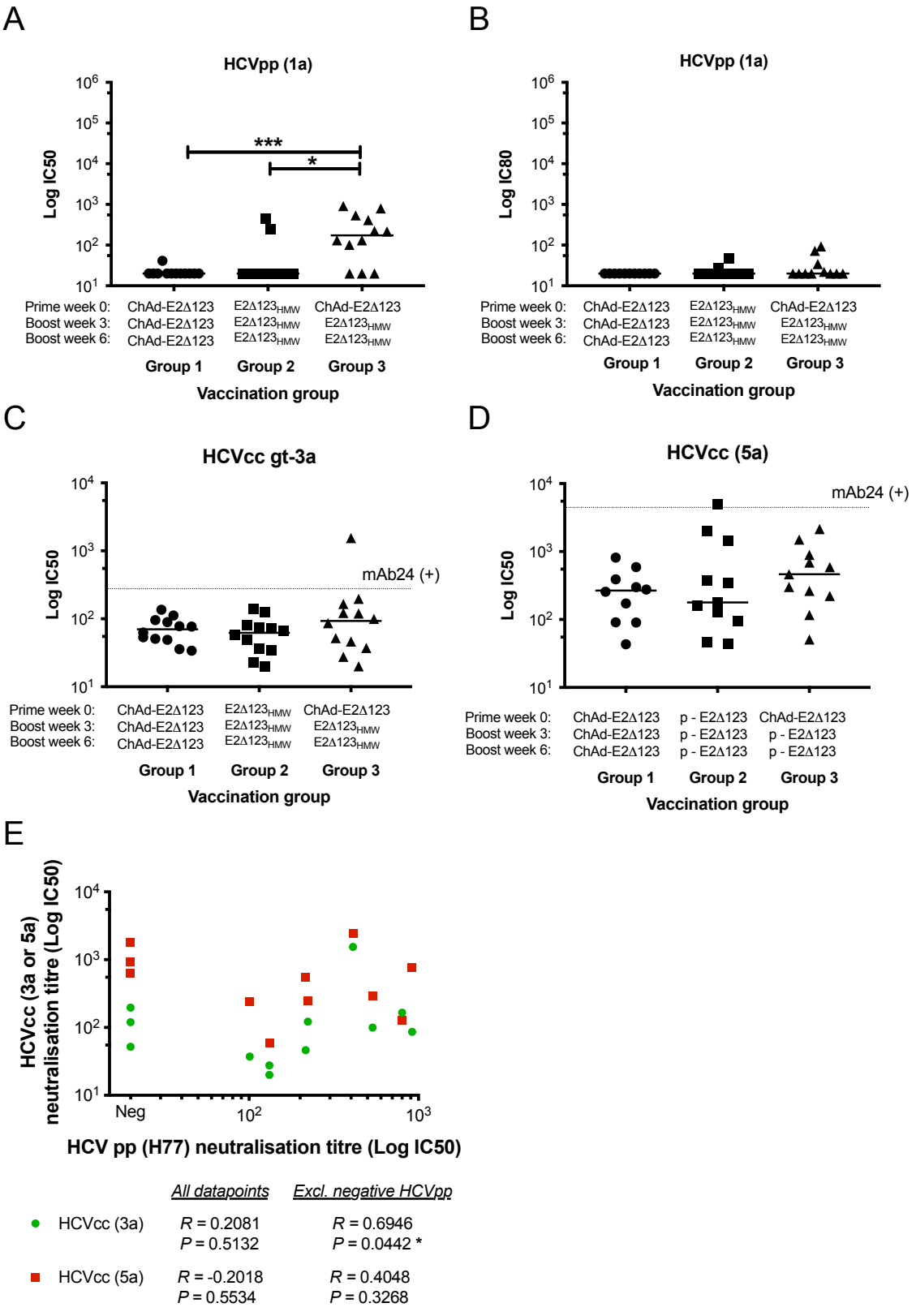


Figure 5-12. The capacity of vaccine-induced E2Δ123-specific antibodies to neutralise HCVpp and HCVcc: HCVcc assay performed by Joey McGregor and analysis performed by McGregor and Tim Donnison. Groups of twelve 8-week old age-matched female C57BL/6 mice

were vaccinated in a prime-boost-boost (week 0, 3, 6) schedule with 1) ChAd-E2Δ123 vaccine homologous regimen (10^8 IU dose in 40μL intramuscularly), or 2) E2Δ123$_{HMW}$ protein vaccine homologous regimen (20μg in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax^{TM}), or 3) a ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ protein boost heterologous regimen. Blood was collected prior to every vaccination and at the end of study at week 8. The experiment was performed once. The vaccine-induced ELISA antibody responses were background subtracted and standardised to the negative control (100% HVCpp/cc entry and luciferase readout) and plotted in ELISA dilution curves (not shown). The vaccine-induced antibody titre to inhibit 50% (IC50, **A**) and 80% (IC80, **B**) HCVpp viral entry into Huh7.5 cells indicated by luciferase readout after Huh7.5 cell lysis, is shown. The capacity of vaccine-induced antibodies to inhibit 50% (IC50) and 80% (IC80) of HCVcc genotype-3a (**C**) and genotype-5a (**D**) virion entry into Huh7.5 cells indicated by luciferase readout after Huh7.5 cell lysis. (**E**) Correlation of the HCVpp (1a) IC50 neutralising antibody titre and the HCVcc genotype-3a and -5 IC50 neutralising antibody titres. Bars are medians. The D'Agostino and Pearson test was used to determine normality of data distribution and Mann Whitney tests were performed to determine a significant difference between two group medians at a 95% confidence interval. *P* values indicate significant difference between groups when ≤ 0.05*, ≤ 0.001**, ≤ 0.0001***, ≤ 0.0001****.

5.5.10 A ChAd-E2Δ123 prime and soluble E2Δ123$_{HMW}$ protein boost elicits antibodies with a distinct isotype profile

Having demonstrated that a ChAd-E2Δ123 prime vaccination in combination with two E2Δ123$_{HMW}$ protein boost vaccinations elicits a higher neutralising nAb titre compared to both homologous vaccination regimens, I determined the antibody isotype profiles in all vaccination groups.

Vaccination groups 2 and 3 displayed significantly higher antibody titres compared to group 1 for all antibody isotypes (Figure 5-13**A**; $p < 0.0001$****). Groups 2 and 3 had similar antibody titres for all isotypes except for IgG1 which was significantly higher in group 2 ($p = 0.0083$**), and for IgG2a which was significantly higher in group 3 (Figure 5-13**A**; $p < 0.0001$****).

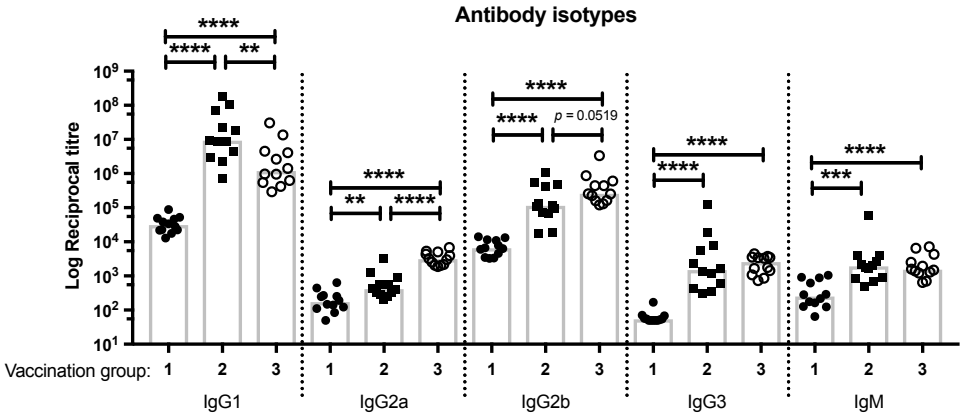
Having demonstrated that group 3 produced higher titres of IgG2a antibodies and a lower titre of IgG1 compared to group 1, I investigated what proportion of the total antibody response each isotype-specific antibody response represented. Vaccination groups that received an adenovirus prime vaccine (groups 1 and 3), generated IgG2a, IgG2b, and IgM antibody responses that represented a significantly higher proportion of the total vaccine-induced antibody response compared to group 2 (Figure 5-13**B**; group 1 v. group 2: p <0.0005***; group 2 v. group 3: $p < 0.0219$*). Conversely, the IgG1 antibody response represented a significantly higher proportion of the group 2 ($E2\Delta123_{HMW}$ only regimen) antibody response compared to groups 1 and 2 (Figure 5-13**B**; $p < 0.0005$***; $p = 0.02$*). The proportion of IgG1 and IgG2b antibody responses was not significantly different between groups 1 and 3, while IgG2a and IgM represented a greater proportion of the total antibody response in group 1 compared to group 3 (Figure 5-13**C**; $p < 0.03$*).

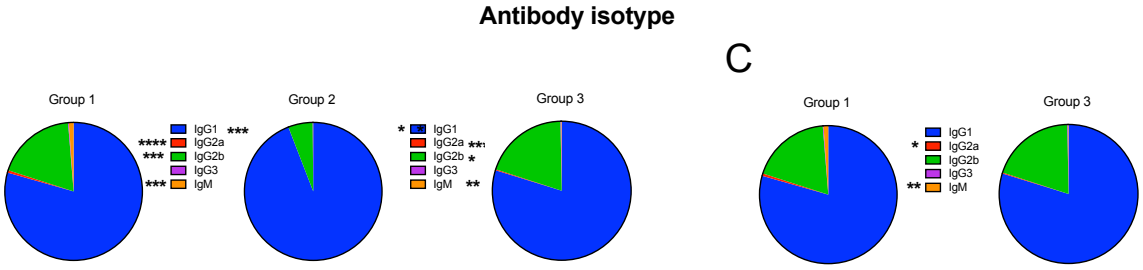
Total isotype antibody titres were compared against respective HCVpp (1a) IC50 nAb titres. Only IgG3 antibody titres displayed a significant positive correlation with HCVpp nAb titres (Figure 5-13**D**; $R = 0.8201$, $p = 0.0096$**).

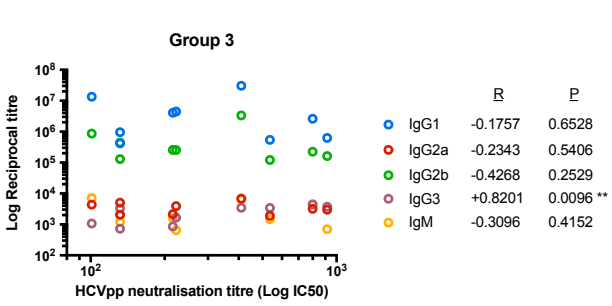
The ratio of the IgG2a to IgG1 has previously been used to indicate the phenotype of the T helper response that is initiated during vaccination (Chmielewska et al., 2014; Coffman et al., 1988; Hutchings, Gilbert, Hill, & Moore, 2005). Group 2 displayed a low IgG2a:IgG1 ratio which was significantly lower than the group 1 and 3 ratios, respectively, skewed towards the Th2 subset (Figure 5-13**E**; $p < 0.0001$****). Conversely, group 1 and 3 IgG2a:IgG1 ratios were closer to 1 and a Th1 skewed response and group 1 displayed a significantly higher IgG2a:IgG1 ratio compared to group 3 (Figure 5-13**E**; $p < 0.0332$*) . A positive correlation between IgG2a:IgG1 ratios and HCVpp IC50 nAb titres was observed (Figure 5-13**F**; $R = 0.4072$, $p = 0.0483$*).

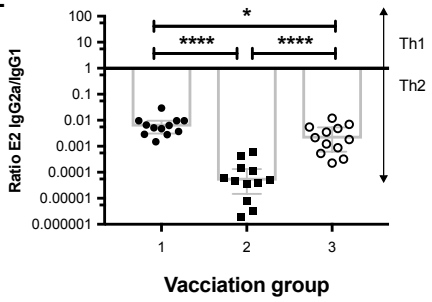
A

Vaccination schedule	Group 1	Group 2	Group 3
Week 0	ChAd- E2Δ123	E2Δ123$_{HMW}$	ChAd-E2Δ123
Week 3	ChAd- E2Δ123	E2Δ123$_{HMW}$	E2Δ123$_{HMW}$
Week 6	ChAd- E2Δ123	E2Δ123$_{HMW}$	E2Δ123$_{HMW}$









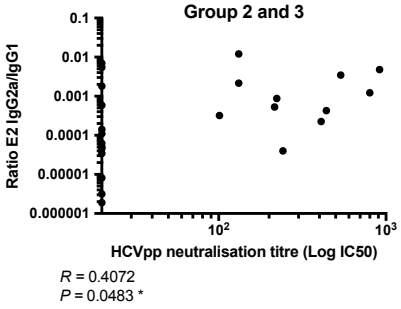


Figure 5-13. The isotype profile of vaccine-induced E2-specific antibodies: Assay performed by Joey McGregor, analysis by both McGregor and Tim Donnison. Groups of twelve 8-week old

age-matched female C57BL/6 mice were vaccinated in a prime-boost-boost (week 0, 3, 6) schedule with 1) ChAd-E2Δ123 vaccine homologous regimen (10^8 IU dose in 40μL intramuscularly), or 2) E2Δ123$_{HMW}$ protein vaccine homologous regimen (20μg in 50μL mixed with 50μL of oil-in-water emulsion, AddaVax^{TM}), or 3) a ChAd-E2Δ123 prime, E2Δ123HMW protein boost heterologous regimen. Blood was collected prior to every vaccination and at the end of study at week 8. The experiment was performed once. (**A**) The vaccine-induced E2-specific antibody isotypes: IgG1, IgG2a, IgG2b, IgG3, IgM. (**B-C**) The proportion of each antibody as a fraction of the total antibody response, comparing vaccination groups 1 and 3 with 2 (**B**), and groups 1 with 3 (**C**). Pie bases are medians and asterisks, if any, indicate significant difference between groups medians. Asterisks to the left of the pie legend indicate a greater proportion of antibody represented in the total antibody response in the group represented by the pie chart to the left (and vice versa for the asterisks and pie chart to the right). (**D**) The correlation of isotype-specific antibody titres against HCVpp IC50 nAb titres. (**E**) The ratio of E2-specific IgG2a and IgG1 isotypes to determine preference for Th1 and Th2 T cell subtypes. (**F**) The correlation of IgG2a/IgG1 ratios against HCVpp IC50 nAb titres when combining groups 2 and 3.

5.5.11 The production of a single bivalent ChAd vector vaccine that encodes the conserved segment T cell immunogen, gt1-6L, and the E2Δ123 sequence

I have demonstrated that a heterologous prime-boost vaccination comprising a monovalent ChAd-E2Δ123 prime and subsequent E2Δ123$_{HMW}$ protein boost induces bNAb titres comparable to homologous prime-boost vaccination regimens.

I next endeavoured to design a simian adenovirus vector vaccine that encodes two HCV antigens in a single immunogen—a 'bivalent' vaccine—to induce a broadly neutralising antibody response and a robust, broad, cross-reactive intergenotypic T cell response targeting multiple HCV genotypes. The immunogen sequences of the conserved sequence T cell immunogen, gt1-6L, and the E2Δ123 sequence were encoded in a transgene immunogen downstream of an ATG start sequence, a Kozak sequence, and a CMV promoter (Figure 5-14**A**). The two HCV antigens were separated by a furin 2 A (F2A) cleavage sequence which causes the ribosome to skip over the F2A sequence to generate

two polypeptides from a single mRNA during translation (Figure 5-14**A**). The HCV bivalent immunogen was inserted into the donor vector via In-Fusion™ cloning to generate the shuttle vector encoding the HCV bivalent immunogen (Figure 5-14**B**). The bivalent immunogen-encoding shuttle vector was cloned into the ChAdOx1 destination vector using ThermoFisher's LR gateway cloning method and linearised using *PmeI* restriction and transfected into T-REx™-293 cells (Thermo Fisher Scientific) for generation of viral vector vaccines. HCV viral vector vaccines were manufactured by the Viral Vector Core Facility (Jenner Institute, University of Oxford, Oxford, U.K.; Figure 5-14**C**).

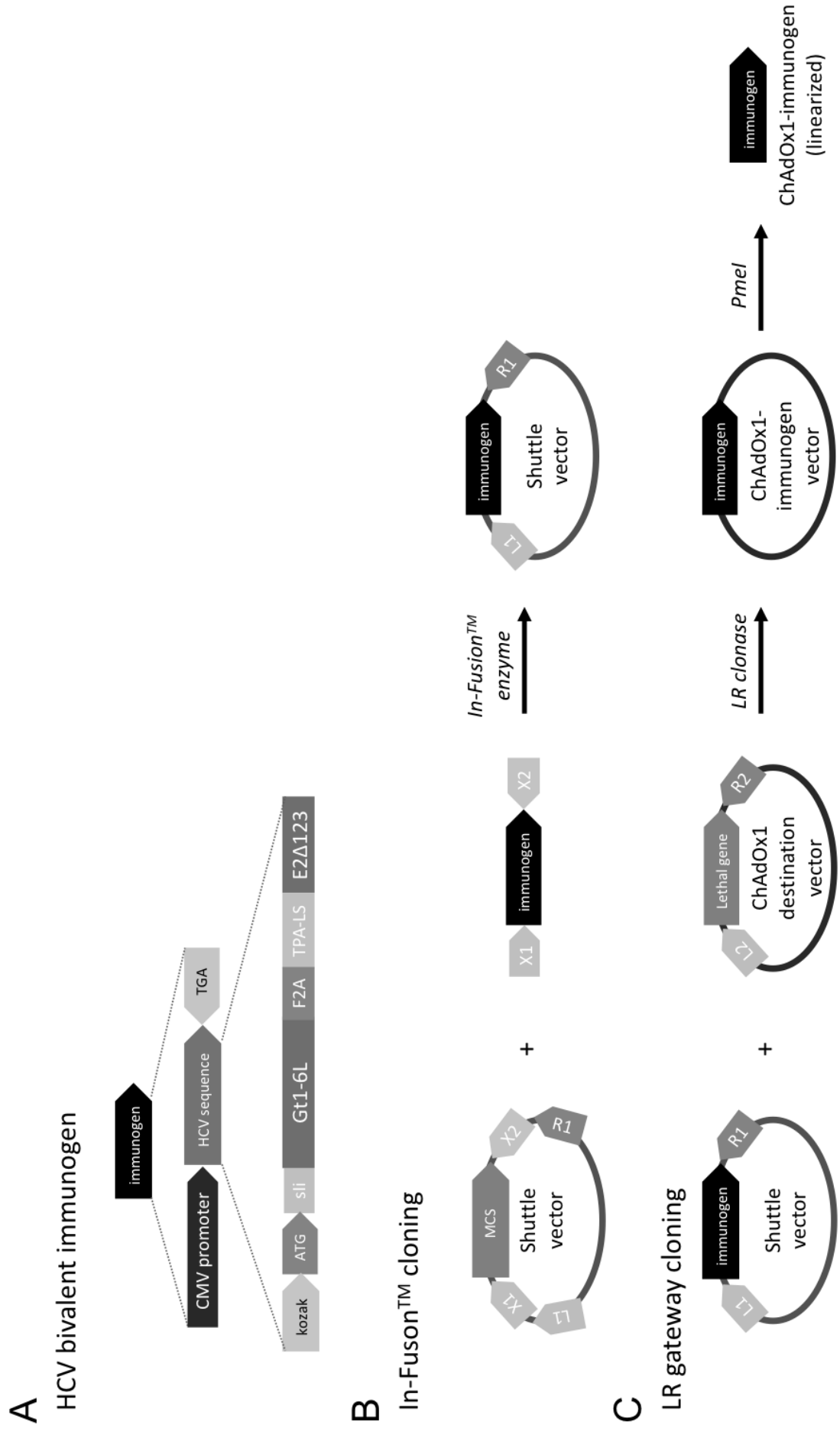

A
HCV bivalent immunogen
immunogen
CMV promoter
HCV sequence
TGA
kozak
ATG
sIi
Gt1-6L
F2A
TPA-LS
E2Δ123
B
In-Fuson™ cloning
MCS
X2
R1
Shuttle vector
X1
L1
+
immunogen
X2
X1
In-Fusion™ enzyme
immunogen
R1
Shuttle vector
L1
C
LR gateway cloning
immunogen
R1
Shuttle vector
L1
+
Lethal gene
R2
ChAdOx1 destination vector
L1
LR clonase
immunogen
ChAdOx1-immunogen vector
PmeI
immunogen
ChAdOx1-immunogen (linearized)

Figure 5-14. Design of a 'bivalent' HCV containing a T cell immunogen and antibody immunogen: (**A**) The HCV bivalent immunogen design contains a CMV promoter, HCV sequence and BGA sequence. The HCV sequence encodes a Kozak sequence, ATG start codon, the truncated shark invariant chain ($sIi_{47\text{-}72}$), the conserved sequence T cell immunogen (gt1-6L), a Furin 2 A cleavage sequence (F2A), and the E2Δ123 protein sequence with a 5' tissue plasminogen activator leader sequence (TPA-LS) and 3' polyhistidine tag. (**B**) Human codon optimised HCV immunogens were inserted into the donor vector via Clontech's In-Fusion™ cloning method to generate the immunogen-contained shuttle vector. (**C**) The immunogen was transferred from the shuttle vector into the ChAdOx1 destination vector via ThermoFisher's LR gateway cloning method. The ChAdOx1-immunogen destination vector product was subsequently linearised (via *PmeI* restriction enzyme) to remove the bacterial backbone of the plasmid for subsequent transfection into a T-REx™293 cell line. HCV viral vector vaccines were submitted to the Viral Vector Core Facility at the University of Oxford for manufacture.

5.6 Discussion

This body of work investigates the integration of two HCV vaccine strategies that activate two different arms of the immune system—the T cell response and the antibody response—into a single vaccination regimen. The conserved sequence HCV T cell vaccine, ChAd-gt1-6L, generates a robust intergenotypic $CD4^+$ and $CD8^+$ T cell response targeting conserved HCV epitopes across multiple HCV genotypes from a single prime vaccination (described in **chapter 0**, von Delft & Donnison et al. 2018). To generate neutralising anti-HCV antibody responses, pre-clinical studies support the use of $E2\Delta123_{HMW}$ protein as an HCV protein vaccine (Alhammad et al. 2015; Vietheer et al. 2016; McCaffrey et al. 2016). The data presented here demonstrated that a conserved sequence HCV T cell vaccine and the recombinant E2Δ123 protein vaccine can be combined in a mixed modality prime-boost vaccination strategy to generate intergenotypic T cell responses and high titre antibody responses comparable to either vaccine alone. Furthermore, a ChAd-E2Δ123 prime and $E2\Delta123_{HMW}$ protein boost increased the neutralising antibody response, eliciting

higher antibody titres targeting CD81 neutralisation epitopes and inhibiting CD81-LEL, compared to a homologous E2Δ123$_{HMW}$ protein prime-boost vaccination regimen. These data provide the rationale for the design and production of a single 'bivalent' HCV viral vector prime vaccine that incorporates the conserved sequence T cell immunogen, gt1-6L, and the antibody component, the E2Δ123 sequence, in a single HCV viral vectored vaccine.

5.6.1 A mixed modality HCV vaccination regimen to generate HCV-specific antibody and T cell immune responses

The co-administration of vaccines is an approved practice in routine immunisation programmes (e.g. measles, mumps, rubella) to reduce number of hospital visits, injections, minimise patient discomfort, and to increase patient compliance and optimise disease prevention. A meta-analysis of combining several routine vaccines revealed that individual assessment of co-administration regimens is critical to compare vaccine efficacy (Bar-On et al., 2009). Thorough evaluation of vaccinations regimens that include distinct immune components from two different vaccine modalities is critical when considering the relative contribution of each vaccine to the overall vaccine-induced immune response, particularly prudent in infections where the correlates of protection are not completely understood, such as HCV.

5.6.1.1 The vaccine-induced T cell response

A combined ChAd-gt1-6L and E2Δ123$_{HMW}$ prime and subsequent E2Δ123$_{HMW}$ boost vaccination regimen generated a high-magnitude T cell response targeting HCV genotype-1a, -1b, and -3a, comparable to those induced by ChAd-gt1-6L alone. Specifically, the IFNγ$^+$ CD8$^+$ T cell response against NS4 and the E2-specific IFNγ$^+$ CD4$^+$ T cell response were not significantly different between groups that received a single ChAd-gt1-6L prime vaccine or a combination of the ChAd-gt1-6L and E2Δ123$_{HMW}$ prime vaccines. The co-

priming of a recombinant protein, E2Δ123$_{HMW}$, with an adenovirus T cell vaccine and subsequent protein boost generated high E2-specific antibody titres, specific towards neutralisation epitopes, and inhibited E2-CD81 binding that was comparable to a homologous protein prime-boost regimen. These data are in line with vaccine studies that have investigated a mixed modality prime vaccination using viral vector vaccines and recombinant proteins (Antrobus et al., 2014; Douglas et al., 2010; Hutchings et al., 2007; Hutchings et al., 2005; Moore & Hutchings, 2007). Strikingly, in this study there was a trend for the HCV ChAd-gt1-6L T cell vaccine and E2Δ123$_{HMW}$ mixed vaccination group to generate a higher magnitude T cell response compared to the protein only vaccination group, albeit not statistically significant. This was also observed in pre-clinical mouse studies previously reported in the influenza field, which demonstrated an increased T cell magnitude after ChAd63 and protein (PfM128) mixed-modality prime vaccination compared to either vaccine administered alone (Douglas et al., 2010).

5.6.1.2 The vaccine-induced antibody response

The HCV envelope heterodimer protein, E1E2, is responsible for mediating HCV entry into the hepatocyte primarily through interactions of E2 with a number of host receptors including the CD81, scavenger receptor B1, claudin-1, and occludin (Evans et al., 2007; Pileri et al., 1998; Ploss et al., 2009; Scarselli et al., 2002). While HCV HCV E2 genetic diversity is up to 30% between genotypes (Smith et al., 2014), X-ray crystallography revealed that E2 contains a core ectodomain (eE2; Kong et al. 2013; Khan et al. 2014) that contains conserved antigenic residues in the CD81 binding site across HCV subtypes that facilitate CD81-mediated HCV cellular entry (Roccasecca et al. 2003; Drummer et al. 2006; Owsianka et al. 2006). A modified E2 protein vaccine: E2Δ123$_{HMW}$—eE2 with hypervariable sequence regions deleted and the protein in a native high molecular weight form—was used in this study to assess vaccine-induced anti-E2 antibody responses. The

administration of E2Δ123$_{HMW}$, compared to E2$_{RBD}$, with the ChAd-gt1-6L vaccine generated higher titre antibodies that inhibited E2 interactions with the CD81-LEL and targeted CD81-E2 binding determinants, epitopes E2$_{408-428}$, E2$_{430-451}$, and E2$_{523-549}$ (Drummer, 2014), demonstrating the preferable induction of antibodies that target the core ectodomain of E2. This is in accordance with previous studies that demonstrate that E2Δ123 exposes the CD81 binding site (Alhammad et al., 2015), particularly in its high molecular weight oligomeric form (Vietheer et al. 2016), and accelerates CD81-E2 binding interactions (McCaffrey et al., 2016). Furthermore, alanine scanning studies mapped importance E2-specific nAb contact residues to eE2 (Pierce et al., 2016), in line with published HCV structures (Khan et al., 2014; Kong et al., 2013). While CD81 binding domain is considered a desirable target of antibodies, conformational flexibility of eE2 and subsequent exposure of neutralising antibody epitopes may govern the success of an HCV vaccine that aims to target CD81-E2 binding determinants (Ströh et al., 2018; Vasiliauskaite et al., 2017). E2 structural plasticity also promotes generation of non-neutralising antibodies towards CD81 binding determinants despite shared contact residues (Deng et al., 2014; Duan et al., 2012; Vasiliauskaite et al., 2017), in addition to allosteric inhibition and beneficial cooperation between antibodies targeting E2 epitopes (Keck et al., 2013; Zhang et al., 2009). These observations convey the complexity of E2 antigenicity in vaccine design.

5.6.1.3 Limitation of this study: prime vaccination time interval

One limitation of this investigation was that the two prime vaccinations were administered 48-hours apart due to the project license which states that an animal can only receive one injection within a 48-hour window. The two prime vaccinations were given using proven injection routes as these were known to elicit vaccine-induced immune responses in previous pre-clinical studies (ChAd-gt1-6L given intramuscularly and E2Δ123$_{HMW}$ given

subcutaneously). It may be that a reduced time interval between prime vaccinations would result in higher magnitude cellular and humoral immune responses compared to either vaccine alone. The cellular and humoral responses are interdependent, and their combined effect dictates the cytokine milieu that skews the immune response towards a pro- or anti-inflammatory state (reviewed by Crotty 2015), an important factor in HCV infection considering the association of both antibodies and T cells with virus resolution (Osburn et al., 2010, 2014). The induction of a pro- or anti-inflammatory cytokine environment is reflective in the Th1 and Th2 T cell subsets, respectively, and the subsequent antibody isotype profile. The latter of which did not differ between mixed modality and single modality vaccinations in this study, unlike other studies (Hutchings et al., 2005), indicating that a 48-hour interval between vaccinations may initiate independent cellular and humoral vaccine-induced responses. Investigation of the immunogenicity of a mixed modality vaccination regimen consisting of both ChAd-gt1-6L and E2Δ123$_{HMW}$ vaccines, either intramuscularly or subcutaneously, is warranted to address the effect on the T cell and antibody response of combining the two prime vaccinations in the same injection route and administered at the same time.

5.6.2 A ChAd-E2Δ123 prime and E2Δ123$_{HMW}$ protein boost regimen to generate anti-HCV antibody responses

Considering both HCV-specific T cell responses and antibody responses are correlates of protection in spontaneous resolvers, an ideal HCV vaccine would generate both antibody and T cell immunity. Mixed modality vaccination regimens are a proven way to elicit both cellular and humoral responses yet overloaded immunisation schedules and high production costs may limit the impact of mixed modality vaccination regimens. These obstacles are particularly relevant for recombinant protein vaccines as multiple doses are required to achieve consistently high efficacious antibody titres. Conversely, viral vectors are potent inducers of high titre antibody responses against many pathogens from fewer

immunisations (de Cassan & Draper, 2013). Viral vectors have the genetic capacity to encode multiple antigens in a single vector suggesting that bivalent and polyvalent vaccines (a combination of two or more antigens in a single vector, respectively) may offer an alternative option to mixed modality vaccination regimens, as recently reported for Influenza A virus (Tully et al., 2017). Reducing vaccinations by administering bivalent vaccines may overcome vaccine production costs and overloaded immunisation schedules that accompany mixed modality vaccinations. Yet, there has been limited investigation of viral vector mediated induction of antibodies in HCV vaccine studies and no bivalent HCV vaccine to induce T cell and antibody immunity has been reported in the field.

In this study, I took the first step in the development of a bivalent HCV vaccine that encodes a T cell immunogen and the E2Δ123 protein sequence of HCV: a monovalent HCV vaccine encoding the E2Δ123 protein sequence encoded in ChAdOx1 viral vector. The data demonstrated that HCV E2-specific antibody responses can be elicited from a single ChAd-E2Δ123 prime vaccination, producing a greater antibody titre compared to a single recombinant E2Δ123$_{HMW}$ protein vaccination. This is in line with previous HCV Ad-E2 and E2 protein vaccine pre-clinical studies (Chmielewska et al., 2014) and human clinical trials for other pathogens (Ewer et al., 2016; Ledgerwood et al., 2017; Rampling et al., 2015; Sheehy et al., 2011). Homologous prime-boost recombinant protein vaccinations regimens are required to generate high titres of HCV-specific antibody responses (Houghton, 2011). The heterologous ChAd-E2Δ123 prime, E2Δ123$_{HMW}$ boost vaccination regimen generated a higher titre E2-specific antibody response compared to the ChAd-E2Δ123 homologous prime-boost regimen. A report by Chmielewska *et al*., found there was no difference in total vaccine-induced antibody titres between adenovirus and protein prime vaccinations in both mice and guinea pigs, if both prime vaccines were boosted with a protein boost vaccine (Chmielewska et al., 2014), in line with data presented

here and with previous HCV vaccine studies using vector vaccines including DNA and measles virus vectors (Li et al., 2006; Reyes-del Valle et al., 2012; Song et al., 2000). Evidence originally from the HIV field supports the use of an adenovirus prime vaccination with subsequent protein boosts to generate high Ab titres (Gómez-Román et al., 2006; Patterson et al., 2003; Shu et al., 2007; Vinner et al., 2006; Zolla-Pazner et al., 1998). The viral vector prime, protein boost regimen has been developed extensively in pre-clinical studies in other fields including liver and blood-stage malaria where mixed vaccine modalities showed comparable antibody and T cell responses to single modality regimens (Wang et al. 2004; Miao et al. 2006), and in some cases increased immune responses when combining modalities compared to administration alone (Douglas et al., 2010; Draper et al., 2010; Hutchings et al., 2007). Mixed modality regimens that include adenovirus prime, and modified vaccinia Ankara (MVA) viral vector and protein boost vaccinations have revealed optimal strategies to induce high titre neutralising antibody responses and high magnitude polyfunctional $CD4^+$ and $CD8^+$ T cell responses in humans (Bliss et al., 2018; Hodgson et al., 2014).

5.6.2.1 Adenovirus vector vaccine generates antibodies that target CD81-E2 binding determinants

E2 contains conserved antibody neutralisation determinants that are located in binding sites for HCV viral entry factors: CD81 and scavenger receptor binding 1 (SRB1; Drummer et al. 2006; Grove et al. 2008), indicating that a successful vaccines' ability to induce antibodies to target these epitopes may be critical to block glycoprotein mediated membrane fusion (Haberstroh et al., 2008). The *APP* regimen generated higher antibody titres that inhibited E2 binding to CD81-LEL compared to homologous *AAA* and *PPP* vaccination regimens. In contrast to the high HCVpp neutralisation antibody titre, only a marginally insignificant increase in antibody responses specific for epitopes $E2_{408-428}$ and $E2_{430-451}$ was observed between *APP* and *PPP* regimens. One explanation is that the

relative contribution of epitope-specific nAb responses to neutralise HCVpp is undetectable when assessed in a peptide ELISA yet is detectable as an accumulative effect in the inhibition of E2 to CD81-LEL, and neutralisation of virus particles pseudotyped with E1E2 (HCVpp).

5.6.3 Adenovirus vector prime vaccine increases anti-E2 nAb titre compared to protein vaccine

In HCV infection, antibodies that appear in early acute resolving infection are characterised by their ability to neutralise HCV (Osburn et al., 2014). To determine the neutralising antibody titre (nAb) of the vaccine-induced antibody response, I determined the ability of immune sera to neutralise the infectivity of HCVpp expressing genotype-1a (H77) glycoprotein and genotype-3a and -5a cell culture-derived HCV (HCVcc-3a, -5a) to Huh7.5 hepatoma cells. The adenovirus prime, protein boost (*APP*) vaccination regimen generated higher nAb titre to HCVpp compared to the homologous prime-boost regimens (*AAA* and *PPP*). Vaccination regimens that included an adenovirus vaccination demonstrated a positive correlation between HCVpp gt-1a nAb and HCVcc gt-3a nAb titres indicating production of broadly neutralising antibodies (bNAbs). In contrast to our results, an adenovirus prime, protein boost heterologous regimen using the antigens $E2_{661}$ and E1E2p7 (denoted '*AAPP*') induced broadly neutralising antibodies targeting HCVpp (H77) of a similar titre to a homologous *PPP* regimen, indicating no benefit of an adenovirus prime vaccination (Chmielewska et al. 2014). One reason for this difference in bNAb titres between regimens in the Chmielewska study may be the use of an additional protein boost vaccination in the *PPP* regimen compared to the *AAPP* regimen or the use of heterologous antigens between the prime and boost vaccines. In line with our observations, a study using measles virus vector encoding HCV core, E1 and E2, as a prime vaccine followed by recombinant protein boost, generated high neutralisation titres targeting homologous (HCVpp) and heterologous (HCVcc) E2 antigens (Reyes-del Valle

et al., 2012). The benefit of enhancing the broadly neutralising antibody response using an *APP* regimen is further supported by studies in the HIV and malaria fields using human and chimpanzee adenovirus vector vaccines. An *APP* regimen generated significantly higher IgG responses and an increased nAb titre compared to a homologous *PPP* regimen in guinea pigs (Shu et al., 2007), improved control of viraemia in rhesus macaques (Patterson et al., 2003), and increased antibody-mediated antibody dependent cellular cytotoxicity (ADCC) in chimpanzees (Gómez-Román et al., 2006). Similarly, a ChAd63 encoding AMA1 malaria blood-stage antigen used as in an *APP* regimen induced high titre functional IgG that inhibited the growth of two divergent strains of *P. falciparum* (Draper et al., 2010).

5.6.4 The ChAd-E2Δ123 vaccine primes Th1 response whereas E2Δ123$_{HMW}$ protein vaccine primes a Th2 response

The antibody isotype profile was evaluated to gain insight into the type of T helper cell response induced with a diverse antibody isotype profile reflecting levels of class-switching in the GC, an indication of the vaccine-induced Tfh response. The *PPP* regimen generated a dominant IgG1 response whilst the *APP* and *AAA* regimens demonstrated greater variety of isotypes as a fraction of the total antibody response indicative of antibody class switching in GC. The IgG2a:IgG1 ratio was used as an indirect marker of a Th1 or Th2-biased vaccine-induced T cell response. Adenovirus vaccinations (both *APP* and *AAA* regimens) displayed a higher IgG2a:IgG1 ratio than the *PPP* regimen, suggesting a biased Th1 response from adenovirus vector vaccination, in line with previous viral vector vaccine studies (Chmielewska et al., 2014; de Cassan et al., 2011; Vinner et al., 2006). Interestingly, the *APP* and *AAA* IgG2a:IgG1 ratios correlated with HCVpp nAb titres suggesting a role for the adenovirus-induced T helper cell response in enhancing the nAb titre of the antibody response, warranting further investigation. While indirect detection of a T cell response towards a Th1-biased phenotype was inferred, the data does not reveal if

increased nAb titres and isotype switching was directly caused by a Tfh or Th1 response, or if this was antigen-independent. A recent report using the OVA antigen system, demonstrated that no effect of innate signal activation by an adenovirus vector lacking the OVA transgene in a mixed empty-adenovirus/protein vaccination was observed on the corresponding OVA-specific B cell and Ab response compared to a protein only regimen, suggesting a transgene-dependent effect on humoral immunity. In the same study, the adenovirus-induced higher magnitude OVA-specific Tfh and B cell responses at seven days after vaccination and exhibited a greater duration lasting 5-weeks after vaccination compared to a protein-in-adjuvant (AddaVax^{TM}) formulation (Wang et al. 2016). While no difference was observed in the overall Ab titre between vaccine modalities by Wang *et al*., the 'quality' of the OVA-specific Ab response was not assessed and no correlation with the GC Tfh and B cell responses was investigated. In two subsequent studies, the *APP* vaccination regimen induced HIV-specific Tfh responses correlated with high B cell responses (Vargas-Inchaustegui et al., 2016) and the secretion of IL-9 by Tfh cells was revealed to be critical in GC memory B cell formation (Wang et al. 2017), both studies highlighting the importance of the Tfh response in developing the humoral immune response against viral infection. In a recent report from the annual international HCV conference, *HCV2018*, the Shoukry group reported that spontaneous HCV resolvers had a greater expansion of circulating Tfh (cTfh) expressing ICOS and IL-21 in early acute infection compared to individuals who went on to develop chronic HCV. In our study, one explanation for increased nAb titres and Ab isotype diversity in the *APP* vaccination group compared to the *PPP* vaccination group, while not investigated, may be the greater induction of Tfh responses and subsequent memory B cell formation by adenovirus vector vaccines compared to protein in adjuvant vaccines. This hypothesis requires assessment in follow-on studies.

The data presented here suggests that the antibody response, if initially primed by an adenovirus vaccine and boosted with a protein vaccine, can generate higher titre neutralising antibodies that target multiple neutralisation epitopes in the CD81 receptor binding domain, than if primed and boosted by a recombinant protein vaccine alone. Nevertheless, the total vaccine-induced antibody titre is unaffected by the different vaccine prime modalities if a protein vaccine is used to boost the immune response. The 'quality' of the vaccine-induced antibody response—in part defined by its ability to cross-neutralise HCV subtypes—may be due to distinct features of adenoviruses to activate the innate and adaptive immune system, to an extent to which recombinant protein vaccines cannot (Tatsis & Ertl, 2004). For example, adenovirus gene expression is prolonged, lasting up to a year (Tatsis et al., 2007) providing extensive opportunity for activation of immune cells (Geiben-Lynn et al. 2008; Wang et al. 2016), including germinal centre B cell, Tfh, and antibody responses via increased antigen availability on APCs in draining lymph nodes (Cubillos-Zapata et al. 2011; Wang et al. 2016). While the important function of the HCV-specific vaccine-induced antibody response is often considered to be the ability of antibodies to neutralise HCV, a recent study in the HIV field indicates that the extent of glycosylation of the antibody's Fc receptor may determine protective efficacy via recruitment of other immune mechanisms highlighting the complexity of immune correlates of protection to chronically-infecting viruses (Lofano et al., 2018).

5.7 Conclusion

Mixed modality prime-boost vaccination regimens have been extensively studied as a means to generate T cell and antibody immunity (reviewed by de Cassan and Draper 2013). The induction of both T cell and antibodies in a successful HCV vaccine is considered optimal, yet contrasting opinions exist in the field on how to achieve this. T cell induction is most effectively induced using viral vector vaccine candidates while, to date, the most

successful antibody vaccine is based on a recombinant gpE1E2 protein (Houghton, 2011; Swadling et al., 2014). We have previous shown that adenovirus prime, MVA boost regimen generated the highest recorded vaccine-induced T cell response to date in a human clinical trial (Swadling et al., 2014), and adenoviruses can elicit robust, broad $CD4^+$ and $CD8^+$ T cells targeting conserved HCV viral segments in pre-clinical studies (**chapter 0**). Using the novel recombinant E2 protein with deletion of the three hypervariable sequence regions (E2Δ123), a mixed adenovirus and protein co-prime vaccination followed by a protein boost does not compromise the T cell or the antibody response compared to either vaccine alone. For the prospects of a single viral vector HCV vaccine that encodes both a T cell immunogen and the E2Δ123 protein sequence (a 'bivalent' vaccine), a monovalent ChAd vector encoding the E2Δ123 sequence was first assessed for its immunogenicity in mice. The ChAd-E2Δ123 prime and $E2\Delta123_{HMW}$ protein boost generated higher titre antibody responses to E2 and CD81 binding determinants, increased inhibition of CD81 binding to E2, and improved neutralising titres to HCVpp and HCVcc, compared to a homologous $E2\Delta123_{HMW}$ prime-boost regimen. The next step is the *in vivo* immunogenic assessment of bivalent viral vector (ChAd and MVA) vaccines that incorporate the conserved T cell immunogen, gt1-6L, and the E2Δ123 protein sequence, and subsequent mixed modality strategies to optimise vaccine-induced immune responses.

Determining the exact vaccination regimen that utilises a bivalent viral vectored HCV vaccine is an important goal. To date, the adenovirus prime MVA boost (*AM*) regimen has consistently produced high magnitude T cell responses and antibody titres against a range of pathogens (Draper et al., 2010; Ewer et al., 2016; Swadling et al., 2014), and now bivalent adenovirus prime, MVA boost regimen are being utilised (Tully et al., 2017).Therefore, the development of a bivalent viral vector HCV vaccination strategy that

includes a ChAd prime and MVA boost may be an optimal strategy and warrants investigation.

5.8 Summary of results

- A ChAd-gt1-6L T cell vaccine prime co-administered with a E2Δ123$_{HMW}$ and subsequent E2Δ123$_{HMW}$ boost did not compromise the magnitude or quality of the ChAd-induced HCV-specific T cell response or E2-specific antibody response to E2 and CD81-E2 binding determinants.
- The ability of E2Δ123$_{HMW}$ prime-boost induced antibodies to inhibit E2 binding to CD81-LEL was not compromised by a ChAd-gt1-6L co-prime vaccination.
- No difference in the vaccine-induced antibody isotype profile was observed between a ChAd-gt1-6L T cell vaccine prime co-administered with a E2Δ123$_{HMW}$ and subsequent E2Δ123$_{HMW}$ boost regimen and a homologous E2Δ123$_{HMW}$ protein prime-boost regimen.
- The E2Δ123 protein sequence encoded in a ChAd vector vaccine (ChAd-E2Δ123) primed higher E2-specific antibody titres compared to an E2Δ123$_{HMW}$ protein prime.
- A heterologous ChAd-E2Δ123 prime E2Δ123$_{HMW}$ boost generated higher titre E2-specific antibody responses compared to a protein or adenovirus prime vaccination and comparable titres compared to a protein homologous prime-boost regimen.
- A heterologous ChAd-E2Δ123 prime E2Δ123$_{HMW}$ boost induced antibodies targeting neutralisation epitopes within the CD81 binding domain of E2 and displayed an increased inhibition of E2 binding to CD81-LEL compared to a homologous protein prime-boost regimen.
- A heterologous ChAd-E2Δ123 prime E2Δ123$_{HMW}$ boost generated a higher titre HCVpp (gt-1a) neutralising antibody titre against and comparable HCVcc (gt-3a, -

5a) neutralising antibody titre compared to a homologous protein prime-boost regimen.

- Vaccination regimens that included an adenovirus vector vaccine displayed a higher IgG2a: IgG1 ratio (indicative of a Th1 response), compared to regimens without an adenovirus vector. The IgG2a: IgG1 ratio positively correlated with HCVpp gt-1a neutralisation titres.
- Novel 'bivalent' HCV viral vector vaccines that contain the conserved sequence gt1-6L T cell sequence and the E2Δ123 sequence, in the same open reading frame and separated by a furin 2 A cleavage sequence in a single immunogen, were designed and produced

6 DISCUSSION

6.1 Vaccine-induced T cells target conserved subdominant HCV epitopes

While HCV infection is self-limiting in approximately 20% of acute infections, the exact immune correlates that facilitate viral clearance are not fully understood. Nevertheless, a substantial body of literature strongly indicates HCV clearance can be achieved if the HCV-specific T cell response exhibits high magnitude, broad, prolonged, and functional $CD4^+$ and $CD8^+$ T cells that act in concert (reviewed by Abdel-Hakeem and Shoukry 2014). Yet, vaccine-induced T cell responses observed in human clinical trials (subtype-1b viral vector vaccines; ChAd- and MVA-gt-1b-NS) are predominantly genotype-specific, targeting immunodominant epitopes with limited cross-reactivity to other genotypes and epitope variants (Swadling et al. 2014; Kelly et al. 2015). By encoding the most conserved HCV sequences across HCV subtypes in simian adenovirus vector vaccine (ChAdOx1; von Delft & Donnison et al. 2018), I have shown that vaccine-induced T cell responses are highly immunogenic to common circulating HCV genotypes: -1a, -1b, and -3a. Importantly, the vaccine-induced T cell response constituted both $CD4^+$ and $CD8^+$ T cells from a single prime vaccination that targeted highly conserved T cell epitopes of up 100% homology across all HCV genotypes. Strikingly, conserved sequence HCV T cell vaccines and the ChAd-gt-1b-NS vaccine displayed a differential epitope hierarchy

suggesting that conserved T cell epitopes are subdominant to genotype-1b epitopes that are targeted by ChAd-gt-1b-NS. Distinct HCV epitope hierarchies have been demonstrated in acute and chronic HCV genotype-1b and -3a infection which exhibit T cell responses targeting different immunodominant epitopes with distinct T cell escape mutations (Rauch et al. 2009; Humphreys et al. 2012; von Delft et al. 2015). Furthermore, patients who had previously resolved HCV have been reported to harbour a new HCV genotype infection suggesting that protective T cell responses in natural infection may also be genotype-specific due to the genotype-specific nature of immunodominant epitopes, distinct between genotypes (Harcourt et al., 2003; Schulze Zur Wiesch et al., 2007). The data presented in this thesis suggest that conserved HCV epitopes are subdominant and cross-reactive and can be targeted via vaccination.

An HCV vaccination approach that utilises conserved, subdominant T cell epitopes is supported by studies from another chronic virus: HIV. In HIV-1 positive individuals, a T cell response that targeted subdominant epitopes correlated with a low viral load and high $CD4^+$ T cell count (Hancock et al., 2015; Masemola et al., 2004; Ondondo et al., 2016), and had the capacity to suppress *in vitro* HIV-1 replication from HIV-1 patient isolates (Murakoshi et al., 2018). A conserved sequence HIV vaccine induced a $CD8^+$ T cell response that targeted novel subdominant epitopes (Ahmed et al., 2016; Borthwick et al., 2014, 2017), suppressed *in vitro* HIV replication, and generated a memory T cell response that lasted up to two-years after vaccination (Moyo et al., 2017). Furthermore, inactivation of immunodominant T cell epitopes enabled subdominant epitopes to dominate naïve T cell induction (Im et al., 2011).

Future work warrants investigation of prime-boost strategies to maximise T cell induction and assessment of distinct T cell subsets in the liver. Recent reports in a range of diseases have identified a memory T cell population with residency to immunological barriers (called resident memory T cells; T_{RM}), such as skin (Cheuk et al., 2017), lung (Van

Braeckel-Budimir, Varga et al., 2018), reproductive organs (Beura et al., 2018), secondary lymphoid organs (Schenkel et al. 2014; Buggert et al. 2018), and liver (Fernandez-Ruiz et al., 2016; Holz et al., 2018; Pallett et al., 2017; Stelma et al., 2017). The role T_{RMs} play in the protective T cell response to chronic viruses and hepatotropic pathogens is an important question. In HIV, T_{RMs} are abundant in the lymph nodes of those who spontaneously clear HIV infection without antiretroviral therapy (elite controllers), and encompass distinct effector T cell genetic signatures compared to HIV-specific non-Trms and chronic progressors (Buggert et al., 2018). In malaria challenge studies in mice, liver T_{RMs} are associated with intrahepatic production of IFNγ, TNFα, granzyme B, and cytotoxicity marker CD107a (Fernandez-Ruiz et al., 2016), suggesting that T_{RMs} are poised at immunological barrier to eliminate infection. The protective capacity of T_{RMs} in HCV infection is not known, although T_{RMs} have been identified in both chronic HBV and HCV infection (Stelma et al., 2017), and shown to confer partial protection in HBV through $IFN\gamma^{+}$ and $IL\text{-}2^{hi}$ production whilst expressing survival markers PD-1 and CD39 (Pallett et al., 2017). Given their presence at the site of infection, T_{RMs} may play an important role in protective immunity to hepatotropic viruses and therefore an HCV vaccination strategy that generates liver resident T cell immunity may be optimal.

6.2 The truncated shark invariant chain as a genetic adjuvant

The magnitude of the T cell response is an important attribute of the immune response in HCV clearance (Lauer & Walker, 2001; Lechner et al., 2000; Missale et al., 1996; Thimme et al., 2001). To this end, vaccine design can utilise genetic adjuvants—sequences encoding adjacent to the transgene in DNA or viral vectors—to induce higher levels of transgene-specific T cell responses. The use of genetic adjuvants is important in disease such as HCV as the exact correlates of protection immunity are not fully understood. Furthermore, a vaccine strategy that utilises subdominant T cell epitopes may have a

reduced propensity for naïve T cell induction due to a lower TCR frequency in the naïve T cell population. For these reasons, technologies, such as genetic adjuvants, that maximise the vaccine-induced T cell response remain an important area of vaccine research. In the data presented here, I show that tethering the truncated shark Ii (sIi_{47-72}) to the N-terminal end of the transgene within the ChAd viral vector can increase the vaccine-induced T cell response. These data add to a growing body of literature that supports the use of Ii as an important component of a viral vector immunogen.

Despite initial hypotheses of using Ii to increase the $CD4^+$ T cell response, the Ii mediated increase in the T cell response was predominantly within the $CD8^+$ T cell subset (Rowe et al. 2006; Holst et al. 2008; Grujic et al. 2009). Subsequent studies using an Ad encoding the HCV protein NS3, revealed an accelerated and prolonged $CD8^+$ T cell response which exhibited increased polyfunctionality and cytolytic capacity compared to non-Ii vaccines (Mikkelsen et al., 2011). Capone and colleagues expanded Ii adjuvant knowledge by demonstrating that ChAd-Ii-NS (HCV) mediated increase of the $CD8^+$ T cell response was not species specific; with a single prime vaccination inducing increased $CD8^+$ T cell responses in outbred mice and non-human primates (Capone et al., 2014). A parallel observation was shown using the malaria antigen, ME-TRAP, with T cell responses significantly higher in mice and NHPs using Ii adjuvanted ME-TRAP in a ChAd prime, MVA boost regimen (Spencer et al., 2014). Follow-up assessment of conserved sequence HCV T cell vaccines that encode sIi should define and characterise the $CD4^+$ and $CD8^+$ T cell response.

The exact mechanism of Ii enhancement of T cell induction is not known and this remains elusive considering Ii has roles in multiple facets of cellular biology including assembly and trafficking MHC class II complexes, endosomal trafficking, cell migration, and signal transduction (reveiewed by Schröder 2016). No difference in the surface expression of MHC class I or co-stimulatory molecules was observed by Holst and colleagues after

transduction of bone marrow-derived dendric cells (DC) with Ii adjuvanted viral vectors compared to non-Ii vaccines despite an increase in Ii-mediated $CD8^+$ T cell proliferation (Holst et al. 2008). Furthermore, the Ii-mediated enhancement of the transgene-specific $CD8^+$ T cell response was shown to be independent of $CD4^+$ T cells in $CD4^{-/-}$ knockout mice (Sorensen et al., 2009). The required sequence for Ii mediated increase of T cell responses was reduced down to amino acids, 47-72, the transmembrane domain (TMD) of the Ii (Ii_{tr}). As Ii is a self-antigen, xenogenised versions of Ii_{tr} and the TMD sequence of other proteins of non-eukaryotic origin retained Ii mediated adjuvanticity of the $CD8^+$ T cell response whilst, in theory, limiting the risk of autoimmunity to Ii (Halbroth et al., 2018). Ii trimerisation was suggested to be important in T cell adjuvantation by Ii as mutations (lll59aaa) in the TMD sequence decreased detection of the trimeric form of Ii_{tr} *in vitro* and reduced $CD8^+$ T cell responses *in vivo* (Halbroth et al., 2018). However, the lll59aaa mutation also decreased expression of the singular form of Ii_{tr} suggesting that the overall decrease in cytosolic expression of Ii_{tr}, not just trimerization, could be responsible for the decreased T cell response. The observation of decreased Ii_{tr} expression could be explained by the reduction in non-AUG initiation of translation as previous studies show that non-AUG codons such as CUG codons (anti-codon Leu-tRNA) initiate translation of antigenic peptides (Norbury et al., 1995). CUG-mediated translation initiation is independent of the canonical AUG (anti-codon Met-tRNA) translation initiation (Malarkannan et al., 1999), eIF2 capping (Schwab et al. 2004; Starck et al. 2012), and is activated by pro-inflammatory stimuli such as a viral or viral vector vaccine infection (Prasad et al. 2016). Therefore, CUG initiation translation may explain, in part, why adenovirus vector vaccines are potent inducer of T cell responses, more so than non-viral vectors. Furthermore, Swadling et al. demonstrated that the Ii mediated enhancement of T cell induction was proteasome dependent with enhanced kinetics of eGFP accumulation in the cell upon proteasome sequestration compared to non-Ii adjuvanted eGFP (personal

communication with Leo Swadling), conducive with the hypothesis of the CUG translation and the defective ribosomal product (DRiP) pathway (Yewdell, 2011). As Halbroth et al. removed three of five leucines in Ii_{tr} via the lll59aa mutation this, in theory, could reduce CUG initiated translation and transgene expression, therefore accounting for a reduced Ad-Ii-ME-TRAP induced $CD8^+$ T cell response. Further investigation of Ii mechanism of increasing transgene-specific T cell responses warrants further research efforts to inform rational vaccine design that aims to induce potent T cell responses.

6.3 The importance of targeting conserved T cell epitopes in the prime vaccination

The most developed prophylactic HCV vaccination strategy along the clinical pathway employs a viral vector encoded immunogen of HCVs genotype-1b non-structural sequence (Folgori et al. 2006; Barnes et al. 2012). This T cell immunogen is in a ChAd prime, MVA boost regimen currently in phase II human clinical trials (Swadling et al. 2014). Despite vaccine-induced high magnitude and broad genotype-1b specific T cell responses in volunteers, the vaccine displayed reduced immunogenicity against non-genotype-1b HCV subtypes. Further studies revealed that vaccine-induced T cells targeted genotype-specific epitopes with lower immunogenicity to genotype-specific epitope variants (Kelly et al. 2015), and this is supported by the observation that T cells in natural infection target distinct epitope repertoires between genotypes (von Delft et al. 2015; Lunburger et al. 2018; Humphreys et al. 2012). These data indicate that a T cell prime-boost vaccine strategy that utilises a single genotype immunogen may only confer T cell responses against a single genotype.

In this study, I have used a genotype-1b ChAd prime vaccination in combination with a genotype-3a MVA boost vaccination to generate a T cell response that targets both genotype-1b and -3a. The rationale behind this approach was that genotype-1b primed T

cells would be boosted if the genotype-1b T cell epitope was conserved in the genotype-3a immunogen sequence, therefore increasing the vaccine-induced genotype-3a specific T cell response. Surprisingly, the prime-boosted T cell response did not display an increased genotype-3a T cell response compared to the homologous genotype-1b prime-boost regimen, and the boosted T cell epitopes were genotype-specific; predominantly immunogenic to the prime HCV genotype. This result is reminiscent of the immunological phenomenon of 'original antigen sin', first described in influenza epidemics (Francis, 1953). Upon infection with a novel viral strain, the host immune response preferentially activates a memory T cell that targets a similar but mismatched antigen sequence at the expense of generating a new response to the new virus. This observation has since been described in malaria (Good et al., 1993), dengue virus (Sangkawibha et al., 1983; Rothman 2011), and chronic viruses that display variant epitopes (Klenerman & Zinkernagel, 1998; Nara et al., 1990). In the context of recurring HCV infections, high magnitude T cell responses that target an original gt-1a antigen after a secondary non-gt-1a HCV infection have been described which displayed no cross-recognition with the non-gt-1a viral sequence, resulting in chronicity of the secondary infection (Harcourt et al., 2003; Schulze Zur Wiesch et al., 2007). This is a possible immune evasion strategy employed by HCV at immunodominant variant T cell epitopes that may account for some secondary heterologous HCV chronic infections (Osburn et al., 2010; Prince et al., 2005; Proust et al., 2000; Schulze Zur Wiesch et al., 2007), and sub-optimal vaccine-induced T cell responses to primary infections in a therapeutic vaccination setting (Kelly et al. 2015; Swadling et al. 2016). In the context of a prime-boost vaccination, the MVA boost HCV gt-3a immunogen recalls the memory T cells specific for the original immunodominant genotype-1b epitopes that were generated after a ChAd prime vaccination.

The study presented here raises important questions around the use of different genotypes in prime and boost viral vector vaccinations. Further assessment of a heterologous

genotype-1b, -3a prime-boost vaccination is required in other strains of mice such as CD-1 outbred mice that may better demonstrate the diversity of the T cell response, and represent the heterogenicity of the vaccine-induced T cell response in humans. Outbred mice exhibit greater diversity of antigen presenting molecules than inbred strains and subsequently have a greater propensity to present a diverse range of HCV epitopes to T cells, therefore potentially capturing immunological trends that may not be observed in an inbred population. Furthermore, comprehensive epitope mapping of vaccine-induced T cell response is warranted to fully assess this vaccination approach. One limitation of using inbred or outbred mice to assess this hypothesis was the observation that in this study, MVA primed a robust T cell response that may potentially confound which T cell epitopes are boosted and which are primed in this investigation.

6.4 A vaccination strategy to generate intergenotypic T cells and broadly neutralising antibodies

HCV vaccines to date primarily focus on generation of the T cell response or the antibody response. The two prophylactic HCV vaccines that have progressed to human clinical trials use a recombinant envelope protein, the glycoprotein heterodimer gpE1E2 (Frey et al., 2010; Law et al., 2013), or the subtype-1b non-structural specific protein sequence encoded in viral vector vaccines (Barnes et al. 2012; Swadling et al. 2014). Data from human clinical trials revealed that the full-length wildtype sequences used in both gpE1E2 recombinant protein vaccines and viral vectored subtype-1b non-structural HCV T cell vaccines have reduced capacity to generate pan-genotype immunity, often producing T cell or antibody responses that are predominantly genotype-specific and target epitopes that lie in variable regions, reflected in high variability at the population level. Exclusion of variable HCV sequences in E2 protein vaccine (E2Δ123) and simian adenovirus vector T cell vaccine design (ChAd-gt1-6L) has been pursued with the aim to direct the vaccine-

induced immune response towards conserved antigens across HCV genotypes that, when mutated, come at a detrimental fitness cost to the virus. The E2Δ123 and ChAd-gt1-6L vaccines have been assessed independently of each other in pre-clinical trials (Vietheer et al. 2016; McCaffrey et al. 2016; von Delft et al. 2018, **chapter 3)**. As protective immunity to HCV is associated with the appearance of a broadly neutralising antibody response and robust $CD4^+$ and $CD8^+$ T cells targeting multiple HCV antigens (Cooper et al., 1999; Lechner et al., 2000; Osburn et al., 2010, 2014; Pestka et al., 2007), a vaccine strategy that utilises both the HCV-specific T cell response and antibody response is desirable.

In this body of work, I demonstrate that the ChAd-gt1-6 and E2Δ123 protein vaccines can be combined to elicit T cell and antibody immunity comparable to either vaccine alone. Specifically, a dual ChAd-gt1-6L and $E2\Delta123_{HMW}$ (HMW: high molecular weight form) prime vaccination elicits high magnitude $CD4^+$ and $CD8^+$ T cell responses and E2-specific antibody responses that target CD81-E2 binding determinants and inhibit E2 binding to CD81-LEL, at comparable magnitudes to administration of either vaccine alone.

A number of other fields have demonstrated that viral vector and protein vaccine can be implemented into a mixed modality vaccination strategy, combined with subsequent boost vaccinations to generate T cell and antibody responses, such as HBV (Hutchings et al., 2005), influenza (Antrobus, Berthoud, et al., 2014; Douglas et al., 2010; Lopez et al., 2011), and malaria (Hutchings et al., 2007; Moore & Hutchings, 2007). Interestingly, the data presented in this HCV pre-clinical vaccine study suggests that co-administering a viral vector prime vaccine with a protein prime vaccine may generate high magnitude T cell responses compared to the viral vector vaccination alone, in line with previous studies (Douglas et al., 2010).

Higher neutralising antibody titres and antibodies that targeted the CD81-LEL and CD81-E2 binding determinants were observed in experimental groups that received E2Δ123

(HMW protein vaccine or adenovirus vector vaccine modality) compared to vaccine groups that received $E2_{RBD}$. This result makes sense as the CD81 binding site is exposed after the deletion of HVR1, HVR2, and igVR from $E2_{RBD}$, especially in E2Δ123s high molecular weight form (Alhammad et al., 2015). The $E2\Delta123_{HMW}$ forms a functional homodimer that increases the CD81-E2 binding kinetics and displays increased binding of mAb (Alhammad et al., 2015; McCaffrey et al., 2017). The benefit of using an E2 protein with exposed CD81 binding site was demonstrated in guinea pigs whose sera effectively neutralised diverse HCV genotypes compared to sera from $E2_{RBD}$-immunised animals (Vietheer et al. 2016). Conversely, a recent study demonstrated that the deletion of HVR1 may compromise the homologous neutralisation activity of ΔHVR1 gpE1/E2-immunised animal sera yet displaying comparable heterologous neutralisation titres to sera from wildtype gpE1E2 immunised animals (Law et al., 2018). This observation may be explained by the observation that the deletion of all three: HVR1, HVR2, and igVR in E2Δ123 facilitates the formation of a high molecular weight structure that does not expose the non-neutralising face of E2 (McCaffrey et al. 2016; Vietheer et al. 2016). Furthermore, the deletion of a single variable E2 sequence region significantly decreases mAb binding to CD81 epitopes compared to deletion of multiple E2 sequence regions (Alhammad et al., 2015). Despite preferential targeting of CD81 binding determinants in the core ectodomain of E2 (eE2) by neutralising mAbs and vaccine-induced nAbs, the E2 ectodomain has remarkable conformational flexibility within the core fold of E2 (Kong et al. 2013; Khan et al. 2014; Vasiliauskaite et al. 2017; Ströh et al., 2018). Therefore, the generation of heterologous antibody responses to different conformational epitopes within eE2 (irrespective of shared binding residues; Duan et al. 2012; Deng et al. 2014; Vasiliauskaite et al. 2017) may lead to competitive Ab interference and generation of non-neutralising antibody responses that can abrogate the neutralising antibody response, as previously reported (Keck et al., 2013; Zhang et al., 2009). This may explain, in part, the mixed

homologous and heterologous neutralisation activity of vaccine-induced E2-specific antibodies reported in chimpanzee and human clinical trials (Choo et al., 1994b; Frey et al., 2010; Law et al., 2013; Law et al., 2018). These observations highlight the complexity of variable sequence domains in regulatory exposure of E2 binding determinants and the intrinsic nature of E2s core ectodomain structural plasticity which maintains functional integrity, with consequences for E2 antigenicity.

In summary, a broadly neutralising antibody response that targets the relatively conserved epitopes in the E2 ectodomain core may efficiently neutralise some HCV particles. Yet, the intrinsic conformational flexibility of the receptor binding sites in E2 coupled with the propensity for HCV to form lipoparticles and N-terminal glycan shielding of neutralisation epitopes may render the antibody response only partially effective, leading to intrahepatic infection (Wrensch et al., 2018). In this scenario, a T cell response targeting the infected hepatocytes would be critical to prevent onset of chronic HCV infection.

6.5 Adenovirus vector vaccine to prime an antibody response

While adenoviruses are potent inductors of the HCV specific T cell response, they have been used to induce antibody responses targeting many pathogens (de Cassan & Draper, 2013; Draper et al., 2008; Ewer et al., 2017).

In this study, I assessed the induction of antibody responses from a ChAd-E2Δ123 prime and E2Δ123$_{HMW}$ protein boost and showed that the ChAd-E2Δ123 prime did not reduce the anti-HCV antibody response. Strikingly, the quality of the antibody response—namely the neutralisation titre and antibody titres targeting E2 epitopes that correspond to the binding site of the host receptor CD81 and its long-elongated loop (LEL)—improved when an adenovirus rather than a protein prime was used as part of a prime-boost strategy. The skewed adenovirus-induced Th1 T cell response correlated with nAb titres suggesting a role for adenovirus-induced T cell responses in generations of nAbs. The enhanced

'quality' of the antibody response after an adenovirus prime vaccination may be attributed to a number of features unique to adenovirus infection, not demonstrated in recombinant protein vaccination, such as potent induction of T cell responses.

Viral vector antigen delivery during immunisation is durable and provides prolonged availability of antigen to activate immune cells, in contrast to recombinant protein vaccines (Cubillos-Zapata et al., 2011; Geiben-Lynn et al., 2008). Antigen expression in an adenovirus infected cell is prolonged and persistent, and can last up to a year compared to other viral vectors such as poxviruses (Tatsis et al., 2007). The generation of an adenovirus-mediated antigen 'dose effect' could potentially prime a quantitatively greater B cell response and subsequent increased antigen availability on APCs in draining lymph nodes. A recent report showed that adenovirus and protein-in-adjuvant vaccines generated germinal centre (GC) B cell and antibody responses of a comparable high magnitude between days 7 and 11 post-vaccination, yet an adenovirus-induced antigen-specific GC B cell response was detectable at day 37 after immunisation (Wang et al. 2016). Sustained adenovirus infection, and consequently transgene expression, may improve antigen availability in the draining lymph node and propagate germinal centre (GC) reactions, promoting B cell somatic hypermutation and affinity maturation, and consequently changing the immunogenic profile of the antibody response. For example, in HIV, the extensive rounds of somatic hypermutation is a predominant feature of broadly neutralising antibodies (Stamatatos, 2012).

T follicular helper cells (Tfh) are considered instrumental players in orchestrating GC T cell interactions with B cells to produce high-affinity, class-switched antibodies (Crotty, 2014). Using the OVA antigen model system, the adenovirus-induced GC Tfh response was significantly higher than protein-in-adjuvant induced Tfh responses (Wang et al. 2016). The ability of adenoviruses to effectively activate innate immune signals and the

subsequent T cell response may drive Tfh subset formation and lead to maturation the adenovirus-induced B cell response, a plausible explanation for our data.

For the prospects of eliciting humoral immunity via vaccination, the importance of Tfh responses in formation of GC memory B cells was recently highlighted by a study that identified IL-9 secretion from GC Tfh to recipient GC memory pre-cursor cells (GC-MP cells) as critical for memory B cell maturation (Wang et al. 2017). Furthermore, observations from HCV infection in patients showed that IL-21 secreting $CD4^+$ T cells are apparent in acute resolving infection while loss of IL-21 secretion is linked to viral persistence and associated with induction of Tregs and T cells with an exhaustive phenotype (Kared et al., 2013; Raziorrouh et al., 2016). A recent report at the international HCV2018 conference reported higher induction of circulating Tfh (cTfh) in the circulation of spontaneous resolvers in early acute infection, compared to the same time point in those who developed chronic HCV, indicating a potential role for Tfh responses in HCV clearance. In vaccine trials, viral vector induction of Tfh responses were associated with SIV-specific B cell responses and control of viraemia in pre-clinical HIV vaccine studies (Helmold Hait et al., 2018; Vargas-Inchaustegui et al., 2016). These data indicate that the induction of Tfh responses to promote antibody responses and B cell memory is plausible and may be a vital component of an efficacious HCV antibody vaccination strategy.

Overall, the data indicates that the inclusion of an adenovirus vector prime vaccination is desirable in a prophylactic HCV vaccine regimen that aims to generate HCV-specific antibody responses.

6.6 The next generation of prophylactic HCV vaccine design

The licensure of an HCV vaccine is an important aim for the research field and funding bodies as global prevalence targets are unlikely to be reached by 2030, particularly in high-risk populations (WHO, 2017). In the minority of patients who spontaneously clear HCV

(20% of infections), the immune response elicits both a broad, high-magnitude anti-viral T cell response and broadly neutralising antibodies targeting HCVs glycoprotein, E2. Therefore, an ideal HCV vaccine candidate should elicit both T cell and antibody responses to overcome genotype-specific immunity by targeting epitopes that have immunogenicity across multiple HCV genotypes and limited capacity for immune escape. Utilising the antibody response in the host periphery as the first line of defence of the adaptive immune response whilst harnessing the cellular immune response to target infected hepatocytes provides a two-tiered adaptive immune response. The induction of two arms of the adaptive immune response, instead of either alone, is the optimal vaccination approach to prevent onset of persistent HCV infection.

Worldwide vaccine implementation must consider a number of obstacles which may limit the employability and success of a prophylactic HCV vaccine programme: the cost of GMP manufacturing, scalability, and intensity of the vaccination regimen which affects patient coherence. Therefore, the expression of two HCV antigens in a single modality (a 'bivalent' vaccine)—rather than multiple vaccines—may be the optimal approach. A bivalent vaccine approach is given merit by a recent report from the influenza field (Tully et al., 2017). To this end, I have designed and developed a bivalent HCV viral vector vaccine that encodes both a conserved sequence T cell immunogen and an E2Δ123 protein sequence.

6.7 Future work

HCV vaccine development has been hindered by lack of a small animal challenge model and the recently discovered 'HCV-like' rat hepacivirus (RHV) as a small animal model of viral hepatitis provides an novel method to assess vaccine candidates (Billerbeck et al., 2017). We aim to assess the bivalent HCV vaccine strategy using RHV vaccine analogues in the rat model of viral hepatitis. These proof-of-principle challenge studies will

investigate vaccine-induced T cell and antibody mediated protective efficacy, a study which will be the first of its kind in the HCV field. The proposed research will de-risk the bivalent HCV vaccine for progression to human trials.

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APPENDIX 1: GENETIC ADJUVANT AND HCV VACCINE SEQUENCES

Here, the vaccine sequences used through this thesis are shown. For conserved sequence HCV vaccines, the first eight amino acids of a gene segment are in green and the last eight are in red. Where linkers are present between gene segments, they are highlighted in blue.

>Tissue plasminogen activation leader signal (TPA-LS)

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR

>shark Invariant chain (sIi) and Linker

SLLWGGVTVLAAMLIAGQVASVVFLV

>GT1&3S_TPA-LS

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR

kgggpgggkSTNPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRA

TRKTSERSQPRGRRQPIPKARRPEGRSWAQPGYPWPLYGNEGCGWAGWLLSPR

GSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARALAHGV

RVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASgkggTTELAILPCSFTPLPALST

GLIHLHQNIVDVQYLYGVGSGMMGWRLLAPITAYAQQTRGLLGTIVTSLTGRDK

NVVTGEVQVLSTAgsgPCTCGSADLYLVTRDADVIPARRRGDSTASLLSPRPLACL

KGSSGGPVMCPSGHVAGIFRAAVCTRGVAKALQFIPVETLRSPVFSDNSSPPAVP

QSYQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAT

GNRTITTGAKLTYSTYGKFLADGGCSGGAYDVIICDECHAQDATSILGIGTVLDQ

AETAGVRLTVLATATPPGSITVPHSNIEEVALSVIPTAGDVVVCATDALMTGFTG

DFDSVIDCNVAVEQYVDFSLDPTFSIETRTAPQDAVSRSQRRGRTGRGRLGTYRY

VGPGERPSGMFDSVVLCECYDAGCAWYELQPAETTVRLRAYLSTPGLPVCQDH

LDFWESVFTGLTHIDAHFLSQTKQQGLNFPYLTAYQATVCARAQAPPPSWDEM

WKCLVRLKPTLHGPTPLLYRLGPVQNggsgkggIGSVGLGKVLVDILAGYGAGVA

GALVAFKIMSGEIPSTEDLVNLLPAILSPGALVVGVVCAAILRRHVGPGEGAVQW

MNRLIAFASRGNHVSPTHYVPESDAAARVTAILSSLTsgQSVVCCSMSYSWTGAL

ITPCSAEEEKLPINPLSNSLLRHHNLVYSTSSRSASQRQKKVTFDRLQVLDDHYK

KGKRYYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMYAPTIWVRMVMgkgpg

sYGATYSVTPLDLPAIIERLHGLSAFTLHSYSPVELNRVAGTLRKLGCPPLRAWRH

R

>GT1&3L_TPA-LS

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VIDTLTCGFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFS

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YPYRLWHYPCTAACNWTRGERCDIEDRDRSELggsgTTELAILPCSFTPLPALSTG

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GSSGGPVMCPSGHVAGIFRAAVCTRGVAKALQFIPVETLRSPVFSDNSSPPAVPQ

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FDSVIDCNVAVEQYVDFSLDPTFSIETRTAPQDAVSRSQRRGRTGRGRLGTYRYV

GPGERPSGMFDSVVLCECYDAGCAWYELQPAETTVRLRAYLSTPGLPVCQDHL

DFWESVFTGLTHIDAHFLSQTKQQGLNFPYLTAYQATVCARAQAPPPSWDEMW

KCLVRLKPTLHGPTPLLYRLGPVQNgppMACMSADLEVTTSTWVLLGGVLAALA

AYCLSVGCVVIVGHFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAgpIGS
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VCAAILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTAILSS
LTCPCQVPAPEFFTEVDGVRLHRgggppgggIGSQLPCEPEPDVSVLTSMLpTAARRL
ARGSPPSEASSSASQLSAPSLKATCQTHRESDSESCSSMPPLEGEPGDPDLSCDSW
STVSDQSVVCCSMSYSWTGALITPCSAEEEKLPINPLSNSLLRHHNLVYSTSSRSA
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>GT1-6S_TPA-LS

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AVDFIPVESLEMRSPVFTDNSTPPAVPQTYQVAHLHAPTGSGKSTKVPAAYAAQ
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MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRkgggpgggTKRNTNRRPMDVK
FPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIPKARRPEGRSWAQ
PGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPNDPRRRSRNLGKVIDTLTCGFA
DLMGYIPLVGAPVGGVARALAHGVRALEDGINYATGNLPGCSFSIFLLALLSCLT
VPASCNCSIYPGHITGHRMAWDMMMNWSPTTNGSWHINRTALNCNDSLNTGFI
SVCGPVYCFTPSPVVVGTTDRCPTDCFRKHPEATYTKCGSGPWLTPRCLVDYPY
RLWHYPCTVNFLLLSTTEWQILPCSFTTLPALSTGLIHLHQNIVDVQYLYGVGSM
GWRLLAPITAYAQQTRGLLGTIVTSLTGRDKNPCTCGSSDLYLVTRHADVIPVRR
RGDSRGSLLSPRPISYLKGSSGGPVLCPSGHAVGIFRAAVCTRGVAKAVDFIPVES
LEMRSPVFTDNSTPPAVPQTYQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLN
PSVAATLGFGAYMSKAYGIRSGVRTITTGAPITYSTYGKFLADGGCSGGAYDIIIC
DECHSTDSTTILGIGTVLDQAETAGVRLVVLATATPPGIKGGRHLIFCHSKKKCD
ELANAVAYYRGLDVSVIPTSGDVVVVATDALMTGFTGDFDSVIDCNTCVTQTV
DFSLDPTFTIETTTVPQDAVSRSQRRGRTGRGRRGIYRFVTPGERPSGMFDSSVLC
ECYDAGCAWYELTPAETSVRLRAYLNTPGLPVCQDHLEFWEGVFTGLTHIDAHF

LSQTKQAGDNFPYLVAYQATVCARAQAPPPSWDQMWTHPITKYIMACMSADLE

VVTSTWVLVGGVLAALAAYCLSVGSVVIVGFWAKHMWNFISGIQYLAGLSTLP

GNPAIASLMAFTAAVTSPLGAAVGSVGLGKVLVDILAGYGAGVAGALVAFKIM

SGEVPSTEDLVNLLPAILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRLIAFA

SRGNHVSPTHYVPESDAAARVTAETAARRLARGSPPSLASSSASQLSAPSLKATC

TVCCSMSYSWTGALITPCAAEEEKLPINPLSNSLIRHHNMVYSTTSRSASLRQKK

VTFDRPSKGGRKPARLIVYPDLGVRVCEKRALYDVKKTPMGFSYDTRCFDSTVT

ERDIRTECGYRRCRASGVLTTSMGNTITCYIKALAAEAMTRYSAPPGDPPQPEYD

LELITSCSSNVSVAHDTPLARAAWETARHTPVNSWLGNIIMYAPTIWVRMVLMT

HFFSILQYGATYSVTPLDLPAIIERLHGLSAFTLHSYSELNRVGACLRKLGVPPLR

AWRHRARAVRAKLIAQGGKAAICGKYLFNWAV

>GT1b-NS (with sIi)

M**SLLWGGVTVLAAMLIAGQVASVVFLV**kggggpggg APITAYSQQTRGLLGCIITS

LTGRDKNQVEGEVQVVSTATQSFLATCVNGVCWTVYHGAGSKTLAGPKGPITQ

MYTNVDQDLVGWQAPPGARSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSL

LSPRPVSYLKGSSGGPLLCPSGHAVGIFRAAVCTRGVAKAVDFVPVESMETTMR

SPVFTDNSSPPAVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAA

TLGFGAYMSKAHGIDPNIRTGVRTITTGAPVTYSTYGKFLADGGCSGGAYDIIICD

ECHSTDSTTILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSNTGE

IPFYGKAIPIEAIRGGRHLIFCHSKKKCDELAAKLSGLGINAVAYYRGLDVSVIPTI

GDVVVVATDALMTGYTGDFDSVIDCNTCVTQTVDFSLDPTFTIETTTVPQDAVS

RSQRRGRTGRGRRGIYRFVTPGERPSGMFDSSVLCECYDAGCAWYELTPAETSV

RLRAYLNTPGLPVCQDHLEFWESVFTGLTHIDAHFLSQTKQAGDNFPYLVAYQA

TVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEVTLTHPITKYI

MACMSADLEVVTSTWVLVGGVLAALAAYCLTTGSVVIVGRIILSGRPAIVPDRE

FLYQEFDEMEECASHLPYIEQGMQLAEQFKQKALGLLQTATKQAEAAAPVVES

KWRALETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTASITSPLTTQSTL
LFNILGGWVAAQLAPPSAASAFVGAGIAGAAVGSIGLGKVLVDILAGYGAGVAG
ALVAFKVMSGEMPSTEDLVNLLPAILSPGALVVGVVCAAILRRHVGPGEGAVQ
WMNRLIAFASRGNHVSPTHYVPESDAAARVTQILSSLTITQLLKRLHQWINEDCS
TPCSGSWLRDVWDWICTVLTDFKTWLQSKLLPQLPGVPFFSCQRGYKGVWRGD
GIMQTTCPCGAQITGHVKNGSMRIVGPKTCSNTWHGTFPINAYTTGPCTPSPAPN
YSRALWRVAAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRL
HRYAPACRPLLREEVTFQVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAET
AKRRLARGSPPSLASSSASQLSAPSLKATCTTHHVSPDADLIEANLLWRQEMGG
NITRVESENKVVVLDSFDPLRAEEDEREVSVPAEILRKSKKFPAAMPIWARPDYN
PPLLESWKDPDYVPPVVHGCPLPPIKAPPIPPPRRKRTVVLTESSVSSALAELATK
TFGSSESSAVDSGTATALPDQASDDGDKGSDVESYSSMPPLEGEPGDPDLSDGS
WSTVSEEASEDVVCCSMSYTWTGALITPCAAEESKLPINALSNSLLRHHNMVYA
TTSRSAGLRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKAKLLSVEEACKLT
PPHSAKSKFGYGAKDVRNLSSKAVNHIHSVWKDLLEDTVTPIDTTIMAKNEVFC
VQPEKGGRKPARLIVFPDLGVRVCEKMALYDVVSTLPQVVMGSSYGFQYSPGQ
RVEFLVNTWKSKKNPMGFSYDTRCFDSTVTENDIRVEESIYQCCDLAPEARQAIK
SLTERLYIGGPLTNSKGQNCGYRRCRASGVLTTSCGNTLTCYLKASAACRAAKL
QDCTMLVNGDDLVVICESAGTQEDAASLRVFTEAMTRYSAPPGDPPQPEYDLEL
ITSCSSNVSVAHDASGKRVYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMYA
PTLWARMILMTHFFSILLAQEQLEKALDCQIYGACYSIEPLDLPQIIERLHGLSAFS
LHSYSPGEINRVASCLRKLGVPPLRVWRHRARSVRARLLSQGGRAATCGKYLFN
WAVKTKLKLTPIPAASQLDLSGWFVAGYSGGDIYHSLSRARPRWFMLCLLLLSV
GVGIYLLPNR

>GT3a-NS (with sIi)

MSLLWGGVTVLAAMLIAGQVASVVFLVSKAPITAYAQQTRGLLGTIVTSLTGR

DKNVVAGEVQVLSTATQTFLGTTVGGVMWTVYHGAGSRTLAGVKHPALQMY

TNVDQDLVGWPAPPGAKSLEPCTCGSADLYLVTRDADVIPARRRGDSTASLLSP

RPLARLKGSSGGPVMCPSGHVAGIFRAAVCTRGVAKALQFIPVETLSTQARSPSF

SDNSTPPAVPQSYQVGYLHAPTGSGKSTKVPAAYVAQGYNVLVLNPSVAATLG

FGSFMSRAYGIDPNIRTGNRTVTTGAKLTYSTYGKFLAGGGCSGGAYDVIICDDC

HAQDATSILGIGTVLDQAETAGVRLTVLATATPPGSITVPHSNIEEVALGSEGEIPF

YGKAIPIACIKGGRHLIFCHSKKKCDKMASKLRGMGLNAVAYYRGLDVSVIPTT

GDVVVCATDALMTGFTGDFDSVIDCNVAVEQYVDFSLDPTFSIETCTAPQDAVS

RSQRRGRTGRGRLGTYRYVTPGERPSGMFDSVVLCECYDAGCSWYDLQPAETT

VRLRAYLSTPGLPVCQDHLDLWESVFTGLTHIDAHFLSQTKQAGLNFSYLTAYQ

ATVCARAQAPPPSWDETWKCLVRLKPTLHGPTPLLYRLGPVQNEICLTHPITKYV

MACMSADLEVTTSTWVLLGGVLAAVAAYCLSVGCVVIVGHIELGGKPALVPDK

EVLYQQYDEMEECSQARPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVVS

NWQKLEVLWHKHMWNFVSGIQYLAGLSTLPGNPAVASLMAFTASVTSPLTTNQ

TMFFNILGGWVATHLAGPQASSAFVVSGLAGAAIGGIGLGRVLLDILAGYGAGV

SGALVAFKIMGGEPPTTEDMVNLLPAILSPGALVVGVICAAILRRHVGPGEGPVQ

WMNRLIAFASRGNHVSPAHYVPESDAAARVTALLSSLTVTSLLRRLHQWINEDY

PSPCSGDWLRIIWDWVCSVVSDFKTWLSAKIMPALPGLPFISCQKGYKGVWRGD

GVMSTRCPCGASIAGHVKNGSMRLAGPRTCANMCHGTFPINEYTTGPSTPCPPP

NYTRALWRVAANSYVEVRRVGDFHYITGATEDGLKCPCQVPATEFFTEVDGVRI

HRYAPPCRPLLRDEITFMVGLNSYAIGSQLPCEPEPDVSVLTSMLRDPSHITAETA

ARRLARGSPPSEASSSASQLSAPSLKATCQTHRPHPDAELVDANLLWRQEMGSNI

TRVESETKVVILDSFEPLRAETDDAELSAAAECFKKPPKYPPALPIWARPDYNPPL

LDRWKSPDYVPPTVHGCALPPKGAPPVPPPRRKRTIQLDGSNVSAALAALAEKSF

PSSKPQEENSSSSGVDTQSSTASKVLPSPGEESDSESCSSMPPLEGEPGDPDLSCDS

WSTVSDSEEQSVVCCSMSYSWTGALITPCSAEEEKLPISPLSNSLLRHHNLVYSTS
SRSASQRQKKVTFDRLQVLDDHYKTALQEVKERASRVKARMLSIEEACALVPPH
SARSKFGYSAKDVRSLSSKAINQIRSVWEDLLEDTTTPIPTTIMAKNEVFCVDPAK
GGRKAARLIVYPDLGVRVCEKRALYDVIQRLSIETMGSAYGFQYSPRQRVERLL
KMWTSKKTPLGFSYDTRCFDSTVTGQDIRVEEAVYQCCNLEPEPGQAISSLTERL
YCGGPMNNSKGAQCGYLRCRASGVLPTSFGNTITCYIKATAAARAAGLRNPDFL
VCGDDLVVVAESDGVDEDRATLRAFTEAMTRYSAPPGDAPQPTYDLELITSCSS
NVSVARDDKGKRYYYLTRDATTPLARAAWETARHTPVNSWLGSIIMYAPTIWV
RMVMMTHFFSILQSQEILDRPLDFEMYGATYSVTPLDLPAIIERLHGLSAFSVHSY
SPVELNRVAGTLRKLGCPPLRAWRHRARAVRAKLIAQGGRAKICGLYLFNWAV
RTKTKLTPLPAAGQLDLSSWFTVGVGGNDIYHSVSRARTRYLLLCLLLLTVGVGI
FLLPAR

>GT1-6L-sIi [Revised segment order for MVA]

1,14,3,16,5,19,22,21,7,23,11,25,13,2,15,4,18,6,20,8,9,24,10,12

M**SLLWGGVTVLAAMLIA**<u>**GQVASVVFLV**</u>TKRNTNRRPMDVKFPGGGQIVGGVYLLPR
RGPRLGVRATRKTSERSQPRGRRQPIPKARRPEGRSWAQPGYPWPLYGNEGCGWAGWL
LSPRGSRPSWGPNDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPVGGVARALAHGVR
ALEDGINYATGNLPGCSFSIFLLALLSCLTVPASFWAKHMWNFISGIQYLAGLSTLPGNPA
IASLMAFTAAVTSPLNGSWHINRTALNCNDSLNTGFITAETAARRLARGSPPSLASSSASQ
LSAPSLKATCTkkCPTDCFRKHPEATYTKCGSGPWLTPRCLVDYPYRLWHYPCTVNFPS
KGGRKPARLIVYPDLGVRVCEKRALYDVgpdEAMTRYSAPPGDPPQPEYDLELITSCSSN
VSVAHDggpCGYRRCRASGVLTTSMGNTITCYIKALAAkgggkMGWRLLAPITAYAQQTR
GLLGTIVTSLTGRDKNTPLARAAWETARHTPVNSWLGNIIMYAPTIWVRMVLMTHFFSI
LQIKGGRHLIFCHSKKKCDELAELNRVGACLRKLGVPPLRAWRHRARAVRAKLIAQGG
KAAICGKYLFNWAVTHPITKYIMACMSADLEVVTSTWVLVGGVLAALAAYCLSVGSVV
IVGCNCSIYPGHITGHRMAWDMMMNWSPTTgskGAAVGSVGLGKVLVDILAGYGAGVA
GALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRL

IAFASRGNHVSPTHYVPESDAAARVSVCGPVYCFTPSPVVVGTTDRVCCSMSYSWTGAL

ITPCAAEEEKLPINPLSNSLIRHHNMVYSTTSRSASLRQKKVTFDRLLLSTTEWQILPCSFT

TLPALSTGLIHLHQNIVDVQYLYGVGSKKTPMGFSYDTRCFDSTVTERDIRTEggpggPCT

CGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPVLCPSGHAVGIFRAAVC

TRGVAKAVDFIPVESLEMRSPVFTDNSTPPAVPQTYQVAHLHAPTGSGKSTKVPAAYAA

QGYKVLVLNPSVAATLGFGAYMSKAYGIYGATYSVTPLDLPAIIERLHGLSAFTLHSYSk

pgRSGVRTITTGAPITYSTYGKFLADGGCSGGAYDIIICDECHSTDSTTILGIGTVLDQAET

AGVRLVVLATATPPGNAVAYYRGLDVSVIPTSGDVVVVATDALMTGFTGDFDSVIDCN

TCVTQTVDFSLDPTFTIETTTVPQDAVSRSQRRGRTGRGRRGIYRFVTPGERPSGMFDSSV

LCECYDAGCAWYELTPAETSVRLRAYLNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQ

TKQAGDNFPYLVAYQATVCARAQAPPPSWDQM

APPENDIX 2: HCV VACCINES AND PEPTIDE SEQUENCE COMPARISON

1a (H77) HCV Peptide pools and minipools

Red and underlined indicates an amino acid in vaccine sequence not present in peptide set

Blue indicates amino acids absent in the vaccine sequence

BEI Peptides				Gt1-6				Gt1/3			
Peptide Length Sequence	**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Seg pool***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***
Pool A										
Core										
1 of 28 18 1 MSTNPKPQRKTKRNTNRR 18	40mg/ml	25ul	A1	MSTLPKPQRKTKRNTIRR	Seg 1	4Q	A1	MSTNPKPQRKTKRNTNRR	Seg 1	1a
2 of 28 18 7 PQRKTKRNTNRRPQDVKF 24	40mg/ml	25ul	A1	PQRKTKRNTNRRPMDVKF	Seg 1	4Q	A1	PQRKTKRNTNRRPQDVKF	Seg 1	1a
3 of 28 18 14 NTNRRPQDVKFPGGGQIV 31	40mg/ml	25ul	A1	NTNRRPMDVKFPGGGQIV	Seg 1	4Q	A1	NTNRRPQDVKFPGGGQIV	Seg 1	1a
4 of 28 17 21 DVKFPGGGQIVGGVYLL 37	40mg/ml	25ul	A1	DVKFPGGGQIVGGVYLL	Seg 1	4Q	A1	DVKFPGGGQIVGGVYLL	Seg 1	1a
5 of 28 18 27 GGQIVGGVYLLPRRGPRL 44	40mg/ml	25ul	A1	GGQIVGGVYLLPRRGPRL	Seg 1	4Q	A1	GGQIVGGVYLLPRRGPRL	Seg 1	1a
6 of 28 18 34 VYLLPRRGPRLGVRATRK 51	40mg/ml	25ul	A2	VYLLPRRGPRLGVRATRK	Seg 1	4Q	A2	VYLLPRRGPRLGVRATRK	Seg 1	1a
7 of 28 15 41 GPRLGVRATRKTSER 55	40mg/ml	25ul	A2	PRLGVRATRKTSER	Seg 1	4Q	A2	PRLGVRATRKTSER	Seg 1	1a
8 of 28 18 45 GVRATRKTSERSQPRGRR 62	40mg/ml	25ul	A2	GVRATRKTSERSQPRGRR	Seg 1	4Q	A2	GVRATRKTSERSQPRGRR	Seg 1	1a
9 of 28 18 52 TSERSQPRGRRQPIPKAR 69	40mg/ml	25ul	A2	TSERSQPRGRRQPIPKAR	Seg 1	4Q	A2	TSERSQPRGRRQPIPKAR	Seg 1	1a
10 of 28 18 59 RGRRQPIPKARRPEGRTW 76	40mg/ml	25ul	A2	RGRRQPIPKARRPEGRSW	Seg 1	4Q	A2	RGRRQPIPKARRPEGRSW	Seg 1	1a
11 of 28 18 66 PKARRPEGRTWAQPGYPW 83	40mg/ml	25ul	A3	PKARRPEGRSWAQPGYPW	Seg 1	4Q	A3	PKARRPEGRSWAQPGYPW	Seg 1	1a
12 of 28 18 73 GRTWAQPGYPWPLYGNEG 90	40mg/ml	25ul	A3	GRSWAQPGYPWPLYGNEG	Seg 1	4Q	A3	GRSWAQPGYPWPLYGNEG	Seg 1	1a
13 of 28 18 80 GYPWPLYGNEGCGWAGWL 97	40mg/ml	25ul	A3	GYPWPLYGNEGCGWAGWL	Seg 1	4Q	A3	GYPWPLYGNEGCGWAGWL	Seg 1	1a
14 of 28 18 87 GNEGCGWAGWLLSPRGSR 104	40mg/ml	25ul	A3	GNEGCGWAGWLLSPRGSR	Seg 1	4Q	A3	GNEGCGWAGWLLSPRGSR	Seg 1	1a
15 of 28 18 94 AGWLLSPRGSRPSWGPTD 111	40mg/ml	25ul	A3	AGWLLSPRGSRPSWGPND	Seg 1	4Q	A3	AGWLLSPRGSRPSWGPTD	Seg 1	1a
16 of 28 17 101 RGSRPSWGPTDPRRRSR 117	40mg/ml	25ul	A4	RGSRPSWGPNDPRRRSR	Seg 1	4Q	A4	RGSRPSWGPTDPRRRSR	Seg 1	1a
17 of 28 17 107 WGPTDPRRRSRNLGKVI 123	20mg/ml	50ul	A4	WGPNDPRRRSRNLGKVI	Seg 1	4Q	A4	WGPTDPRRRSRNLGKVI	Seg 1	1a
18 of 28 18 113 RRRSRNLGKVIDTLTCGF 130	40mg/ml	25ul	A4	RRRSRNLGKVIDTLTCGF	Seg 1	4Q	A4	RRRSRNLGKVIDTLTCGF	Seg 1	1a
19 of 28 18 120 GKVIDTLTCGFADLMGYI 137	40mg/ml	25ul	A4	GKVIDTLTCGFADLMGYI	Seg 1	4Q	A4	GKVIDTLTCGFADLMGYI	Seg 1	1a
20 of 28 18 127 TCGFADLMGYIPLVGAPL 144	40mg/ml	25ul	A4	TCGFADLMGYIPLVGAPV	Seg 1	4Q	A4	TCGFADLMGYIPLVGAPL	Seg 1	1a
21 of 28 18 134 MGYIPLVGAPLGGAARAL 151	40mg/ml	25ul	A5	MGYIPLVGAPVGGVARAL	Seg 1	4Q	A5	MGYIPLVGAPLGGAARAL	Seg 1	1a
22 of 28 18 141 GAPLGGAARALAHGVRVL 158	40mg/ml	25ul	A5	GAPVGGVARALAHGVRAL	Seg 1	4Q	A5	GAPLGGAARALAHGVRVL	Seg 1	1a
23 of 28 18 148 ARALAHGVRVLEDGVNYA 165	40mg/ml	25ul	A5	ARALAHGVRALEDGINYA	Seg 1	4Q	A5	ARALAHGVRVLEDGVNYA	Seg 1	1a
24 of 28 15 155 VRVLEDGVNYATGNL 169	20mg/ml	50ul	A5	VRALEDGINYATGNL	Seg 1	4Q	A5	VRVLEDGVNYATGNL	Seg 1	1a
25 of 28 18 159 EDGVNYATGNLPGCSFSI 176	13.3mg/ml	75ul	A6	EDGINYATGNLPGCSFSI	Seg 1	4Q	A6	EDGVNYATGNLPGCSFSI	Seg 1	1a
26 of 28 17 166 TGNLPGCSFSIFLLALL 182	20mg/ml	50ul	A6	TGNLPGCSFSIFLLALL	Seg 1	4Q	A6	TGNLPGCSFSIFLLALL	Seg 1	1a
27 of 28 18 172 CSFSIFLLALLSCLTVPA 189	13.3mg/ml	75ul	A6	CSFSIFLLALLSCLTVPA	Seg 1	4Q	A6	CSFSIFLLALLSCLTVPA	Seg 1	1a
28 of 28 13 179 LALLSCLTVPASA 191	13.3mg/ml	75ul	A6	LALLSCLTVPASA	Seg 1	4Q	A6	LALLSCLTVPASA	Seg 1	1a
Pool B										
E1										
Peptide Length Sequence	**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Seg pool***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***
1 of 29 18 1 YQVRNSSGLYHVTNDCPN 18	40mg/ml	25ul								
2 of 29 18 8 GLYHVTNDCPNSSIVYEA 25	40mg/ml	25ul								
3 of 29 17 15 DCPNSSIVYEAADAILH 31	40mg/ml	25ul								

4 of 29	16 21 IVYEAADAILHTPGCV 36	40mg/ml	25ul										
5 of 29	15 26 ADAILHTPGCVPCVR 40	40mg/ml	25ul										
6 of 29	17 30 LHTPGCVPCVREGNASR 46	40mg/ml	25ul										
7 of 29	16 36 VPCVREGNASRCWVAV 51	40mg/ml	25ul										
8 of 29	18 41 EGNASRCWVAVTPTVATR 58	40mg/ml	25ul										
9 of 29	15 48 WVAVTPTVATRDGKL 62	40mg/ml	25ul										
10 of 29	18 52 TPTVATRDGKLPTTQLRR 69	40mg/ml	25ul										
11 of 29	17 59 DGKLPTTQLRRHIDLLV 75	40mg/ml	25ul										
12 of 29	16 65 TQLRRHIDLLVGSATL 80	40mg/ml	25ul							TQLRRHVDLLVGAATL	Seg 2	3a	
13 of 29	17 70 HIDLLVGSATLCSALYV 86	20mg/ml	50ul							HVDLLVGAATLCSALYV	Seg 2	3a	
14 of 29	18 76 GSATLCSALYVGDLCGSV 93	40mg/ml	25ul							GAATLCSALYVGDMCGSV	Seg 2	3a	
15 of 29	18 83 ALYVGDLCGSVFLVGQLF 100	40mg/ml	25ul							ALYVGDMCGSVFLVGQLF	Seg 2	3a	
16 of 29	18 90 CGSVFLVGQLFTFSPRRH 107	40mg/ml	25ul										
17 of 29	18 97 GQLFTFSPRRHWTTQDCN 114	40mg/ml	25ul										
18 of 29	18 104 PRRHWTTQDCNCSIYPGH 121	40mg/ml	25ul										
19 of 29	18 110 TQDCNCSIYPGHITGHRM 127	40mg/ml	25ul		TQDCNCSIYPGHITGHRM	Seg 2	1a						
20 of 29	17 117 IYPGHITGHRMAWDMMM 133	40mg/ml	25ul		IYPGHITGHRMAWDMMM	Seg 2	1a						
21 of 29	18 123 TGHRMAWDMMMNWSPTAA 140	40mg/ml	25ul		TGHRMAWDMMMNWSPTAA	Seg 2	1a						
22 of 29	18 130 DMMMNWSPTAALVVAQLL 147	20mg/ml	50ul										
23 of 29	18 137 PTAALVVAQLLRIPQAIM 154	40mg/ml	25ul										
24 of 29	18 144 AQLLRIPQAIMDMIAGAH 161	40mg/ml	25ul										
25 of 29	17 150 PQAIMDMIAGAHWGVLA 166	20mg/ml	50ul										
26 of 29	18 156 MIAGAHWGVLAGIAYFSM 173	40mg/ml	25ul							MIAGAHWGVLAGLAYYSM	Seg 3	3a	
27 of 29	18 163 GVLAGIAYFSMVGNWAKV 180	20mg/ml	50ul							GVLAGLAYYSMQGNWAKV	Seg 3	3a	
28 of 29	18 170 YFSMVGNWAKVLVVLLLF 187	20mg/ml	50ul							YYSMQGNWAKVLVVLLLF	Seg 3	3a	
29 of 29	16 177 WAKVLVVLLLFAGVDA 192	20mg/ml	50ul										

Pool C

E2

Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 56 17 1 ETHVTGGNAGRTTAGLV 17	40mg/ml	25ul								
2 of 56 18 7 GNAGRTTAGLVGLLTPGA 24	40mg/ml	25ul								
3 of 56 18 14 AGLVGLLTPGAKQNIQLI 31	40mg/ml	25ul								
4 of 56 18 21 TPGAKQNIQLINTNGSWH 38	40mg/ml	25ul								
5 of 56 17 28 IQLINTNGSWHINSTAL 44	40mg/ml	25ul	C1	IQLINTNGSWHINRTAL	Seg 3	2b				
6 of 56 17 34 NGSWHINSTALNCNESL 50	40mg/ml	25ul	C1	NGSWHINRTALNCNDSL	Seg 3	2b				
7 of 56 17 40 NSTALNCNESLNTGWLA 56	40mg/ml	25ul	C1	NRTALNCNDSLNTGFIA	Seg 3	2b				
8 of 56 18 46 CNESLNTGWLAGLFYQHK 63	40mg/ml	25ul	C1	CNDSLNTGFIAGLFYQHK	Seg 3	2b				
9 of 56 18 53 GWLAGLFYQHKFNSSGCP 70	40mg/ml	25ul								
10 of 56 18 60 YQHKFNSSGCPERLASCR 77	40mg/ml	25ul								
11 of 56 17 67 SGCPERLASCRRLTDFA 83	40mg/ml	25ul								
12 of 56 17 73 LASCRRLTDFAQGWGPI 89	40mg/ml	25ul								
13 of 56 19 79 LTDFAQGWGPISYANGSGL 97	40mg/ml	25ul								
14 of 56 18 87 GPISYANGSGLDERPYCW 104	40mg/ml	25ul								
15 of 56 16 94 GSGLDERPYCWHYPPR 109	40mg/ml	25ul								
16 of 56 18 99 ERPYCWHYPPRPCGIVPA 116	40mg/ml	25ul								

17 of 56	18	106	YPPRPCGIVPAKSVCGPV	123	40mg/ml	25ul								
18 of 56	19	113	IVPAKSVCGPVYCFTPSPV	131	40mg/ml	25ul	C2	IVPAKSVCGPVYCFTPSPV	Seg 4	4a	C2	IVPAKSVCGPVYCFTPSPV	Seg 4	1a
19 of 56	18	121	GPVYCFTPSPVVVGTTDR	138	40mg/ml	25ul	C2	GPVYCFTPSPVVVGTTDR	Seg 4	4a	C2	GPVYCFTPSPVVVGTTDR	Seg 4	1a
20 of 56	17	128	PSPVVVGTTDRSGAPTY	144	40mg/ml	25ul	C2	PSPVVVGTTDRSGAPTY	Seg 4	4a	C2	PSPVVVGTTDRSGAPTY	Seg 4	1a
21 of 56	15	134	GTTDRSGAPTYSWGA	148	40mg/ml	25ul								
22 of 56	18	138	RSGAPTYSWGANDTDVFV	155	40mg/ml	25ul								
23 of 56	16	145	SWGANDTDVFVLNNTR	160	20mg/ml	50ul								
24 of 56	18	150	DTDVFVLNNTRPPLGNWF	167	40mg/ml	25ul								
25 of 56	16	157	NNTRPPLGNWFGCTWM	172	40mg/ml	25ul								
26 of 56	18	162	PLGNWFGCTWMNSTGFTK	179	40mg/ml	25ul								
27 of 56	15	169	CTWMNSTGFTKVCGA	183	40mg/ml	25ul								
28 of 56	16	173	NSTGFTKVCGAPPCVI	188	40mg/ml	25ul								
29 of 56	19	178	TKVCGAPPCVIGGVGNNTL	196	40mg/ml	25ul								
30 of 56	18	186	CVIGGVGNNTLLCPTDCF	203	40mg/ml	25ul								
31 of 56	17	193	NNTLLCPTDCFRKHPEA	209	40mg/ml	25ul	C3	NNTLLCPTDCFRKHPEA	Seg 5	1b				
32 of 56	15	199	PTDCFRKHPEATYSR	213	40mg/ml	25ul	C3	PTDCFRKHPEATYTK	Seg 5	1b				
33 of 56	18	203	FRKHPEATYSRCGSGPWI	220	40mg/ml	25ul	C3	FRKHPEATYTKCGSGPWL	Seg 5	1b	C3	FRKHPEATYSRCGSGPWI	Seg 5	1a
34 of 56	17	210	TYSRCGSGPWITPRCMV	226	40mg/ml	25ul	C3	TYTKCGSGPWLTPRCLV	Seg 5	1b	C3	TYSRCGSGPWITPRCMV	Seg 5	1a
35 of 56	18	216	SGPWITPRCMVDYPYRLW	233	40mg/ml	25ul	C3	SGPWLTPRCLVDYPYRLW	Seg 5	1b	C3	SGPWITPRCMVDYPYRLW	Seg 5	1a
36 of 56	17	223	RCMVDYPYRLWHYPCTI	239	40mg/ml	25ul	C3	RCLVDYPYRLWHYPCTV	Seg 5	1b	C3	RCMVDYPYRLWHYPCTI	Seg 5	1a
37 of 56	18	229	PYRLWHYPCTINYTIFKV	246	40mg/ml	25ul		PYRLWHYPCTVNFTIFKV	Seg 5	1b		PYRLWHYPCTINYTIFKV	Seg 5	1a
38 of 56	18	236	PCTINYTIFKVRMYVGGV	253	40mg/ml	25ul								
39 of 56	18	243	IFKVRMYVGGVEHRLEAA	260	40mg/ml	25ul								
40 of 56	16	250	VGGVEHRLEAACNWTR	265	40mg/ml	25ul								
41 of 56	17	255	HRLEAACNWTRGERCDL	271	40mg/ml	25ul						HRLEAACNWTRGERCDI	Seg 6	3a
42 of 56	16	261	CNWTRGERCDLEDRDR	276	40mg/ml	25ul						CNWTRGERCDIEDRDR	Seg 6	3a
43 of 56	18	266	GERCDLEDRDRSELSPLL	283	40mg/ml	25ul						GERCDIEDRDRSELSPLL	Seg 6	3a
44 of 56	17	273	DRDRSELSPLLLSTTQW	289	40mg/ml	25ul	C4	DRDRSELSPLLLSTTEW	Seg 6	1b				
45 of 56	18	279	LSPLLLSTTQWQVLPCSF	296	40mg/ml	25ul	C4	LSPLLLSTTEWQILPCSF	Seg 6	1b		LSPLLLSTTELAILPCSF	Seg 7	3a
46 of 56	17	286	TTQWQVLPCSFTTLPAL	302	40mg/ml	25ul	C4	TTEWQILPCSFTTLPAL	Seg 6	1b		TTELAILPCSFTPLPAL	Seg 7	3a
47 of 56	18	292	LPCSFTTLPALSTGLIHL	309	40mg/ml	25ul	C4	LPCSFTTLPALSTGLIHL	Seg 6	1b		LPCSFTPLPALSTGLIHL	Seg 7	3a
48 of 56	18	299	LPALSTGLIHLHQNIVDV	316	40mg/ml	25ul	C4	LPALSTGLIHLHQNIVDV	Seg 6	1b		LPALSTGLIHLHQNIVDV	Seg 7	3a
49 of 56	17	306	LIHLHQNIVDVQYLYGV	322	40mg/ml	25ul	C4	LIHLHQNIVDVQYLYGV	Seg 6	1b		LIHLHQNIVDVQYLYGV	Seg 7	3a
50 of 56	18	312	NIVDVQYLYGVGSSIASW	329	40mg/ml	25ul	C4	NIVDVQYLYGVGSSIASW	Seg 6	1b		NIVDVQYLYGVGSGMASW	Seg 7	3a

Pool C continued

E2

Peptide	Length	Sequence		Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
51 of 56	18	319	LYGVGSSIASWAIKWEYV	336	40mg/ml	25ul					LYGVGSGMASWAIKWEYV	Seg 7	3a
52 of 56	18	326	IASWAIKWEYVVLLFLLL	343	20mg/ml	50ul							
53 of 56	16	333	WEYVVLLFLLLADARV	348	20mg/ml	50ul							
54 of 56	18	338	LLFLLLADARVCSCLWMM	355	40mg/ml	25ul							
55 of 56	17	345	DARVCSCLWMMLLISQA	361	40mg/ml	25ul							
56 of 56	13	351	CLWMMLLISQAEA	363	40mg/ml	25ul							

Pool D

p7

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 8	17 1	ALENLVILNAASLAGTH 17	20mg/ml	50ul								
2 of 8	18 7	ILNAASLAGTHGLVSFLV 24	40mg/ml	25ul								
3 of 8	18 14	AGTHGLVSFLVFFCFAWY 31	40mg/ml	25ul								
4 of 8	17 21	SFLVFFCFAWYLKGRWV 37	40mg/ml	25ul								
5 of 8	18 27	CFAWYLKGRWVPGAVYAL 44	40mg/ml	25ul								
6 of 8	18 34	GRWVPGAVYALYGMWPLL 51	40mg/ml	25ul								
7 of 8	17 41	VYALYGMWPLLLLLLAL 57	40mg/ml	25ul								
8 of 8	17 47	MWPLLLLLLALPQRAYA 63	40mg/ml	25ul								
NS2												
Peptide	Length	Sequence	Concentration	Volume								
1 of 32	18 1	LDTEVAASCGGVVLVGLM 18	20mg/ml	50ul								
2 of 32	18 8	SCGGVVLVGLMALTLSPY 25	20mg/ml	50ul								
3 of 32	18 15	VGLMALTLSPYYKRYISW 32	40mg/ml	25ul								
4 of 32	18 22	LSPYYKRYISWCMWWLQY 39	40mg/ml	25ul								
5 of 32	18 29	YISWCMWWLQYFLTRVEA 46	40mg/ml	25ul								
6 of 32	17 36	WLQYFLTRVEAQLHVWV 52	40mg/ml	25ul								
7 of 32	17 42	TRVEAQLHVWVPPLNVR 58	40mg/ml	25ul								
8 of 32	18 48	LHVWVPPLNVRGGRDAVI 65	40mg/ml	25ul								
9 of 32	18 55	LNVRGGRDAVILLMCVVH 72	40mg/ml	25ul								
10 of 32	18 62	DAVILLMCVVHPTLVFDI 79	40mg/ml	25ul								
11 of 32	18 69	CVVHPTLVFDITKLLLAI 86	40mg/ml	25ul								
12 of 32	18 76	VFDITKLLLAIFGPLWIL 93	40mg/ml	25ul								
13 of 32	18 83	LLAIFGPLWILQASLLKV 100	40mg/ml	25ul								
14 of 32	17 90	LWILQASLLKVPYFVRV 106	40mg/ml	25ul								
15 of 32	17 96	SLLKVPYFVRVQGLLRI 112	40mg/ml	25ul								
16 of 32	18 102	YFVRVQGLLRICALARKI 119	40mg/ml	25ul								
17 of 32	17 109	LLRICALARKIAGGHYV 125	40mg/ml	25ul								
18 of 32	18 115	LARKIAGGHYVQMAIIKL 132	40mg/ml	25ul								
19 of 32	18 122	GHYVQMAIIKLGALTGTY 139	40mg/ml	25ul								
20 of 32	16 129	IIKLGALTGTYVYNHL 144	40mg/ml	25ul								
21 of 32	18 134	ALTGTYVYNHLTPLRDWA 151	40mg/ml	25ul								
22 of 32	18 141	YNHLTPLRDWAHNGLRDL 158	40mg/ml	25ul								
23 of 32	18 148	RDWAHNGLRDLAVAVEPV 165	40mg/ml	25ul								
24 of 32	16 155	LRDLAVAVEPVVFSRM 170	40mg/ml	25ul								
25 of 32	18 160	VAVEPVVFSRMETKLITW 177	40mg/ml	25ul								
26 of 32	17 167	FSRMETKLITWGADTAA 183	40mg/ml	25ul								
27 of 32	16 173	KLITWGADTAACGDII 188	40mg/ml	25ul								
28 of 32	18 178	GADTAACGDIINGLPVSA 195	40mg/ml	25ul								
29 of 32	18 185	GDIINGLPVSARRGQEIL 202	40mg/ml	25ul								
30 of 32	18 192	PVSARRGQEILLGPADGM 209	40mg/ml	25ul								
31 of 32	18 198	GQEILLGPADGMVSKGWR 215	40mg/ml	25ul								
32 of 32	13 205	PADGMVSKGWRLL 217	40mg/ml	25ul		PADGMVSMGWRLL	Seg 7	3a		PADGMVSMGWRLL	Seg 8	3a

Pool F

NS3

Peptide	Length	Sequence	Conc.	Vol.	Minipool	Vacc Seq.	Seg pool	Vacc GT	Minipool	Vacc Seq.	Segment	Vacc GT
1 of 98	18	1 APITAYAQQTRGLLGCII 18	40mg/ml	25ul	F1	APITAYAQQTRGLLGTIV	Seg 7	3a	F1	APITAYAQQTRGLLGTIV	Seg 8	3a
2 of 98	18	7 AQQTRGLLGCIITSLTGR 24	40mg/ml	25ul	F1	AQQTRGLLGTIVTSLTGR	Seg 7	3a	F1	AQQTRGLLGTIVTSLTGR	Seg 8	3a
3 of 98	16	14 LGCIITSLTGRDKNQV 29	20mg/ml	50ul	F1	LGTIVTSLTGRDKNQV	Seg 7	3a	F1	LGTIVTSLTGRDKNVV	Seg 8	3a
4 of 98	18	19 TSLTGRDKNQVEGEVQIV 36	40mg/ml	25ul					F1	SLTGRDKNVVTGEVQVL	Seg 8	3a
5 of 98	18	26 KNQVEGEVQIVSTATQTF 43	40mg/ml	25ul	F2				F1	KNVVTGEVQVLSTATQTF	Seg 8	3a
6 of 98	16	33 VQIVSTATQTFLATCI 48	20mg/ml	50ul	F2							
7 of 98	18	38 TATQTFLATCINGVCWTV 55	40mg/ml	25ul	F2				F2			
8 of 98	18	45 ATCINGVCWTVYHGAGTR 62	40mg/ml	25ul	F2				F2			
9 of 98	17	52 CWTVYHGAGTRTIASPK 68	40mg/ml	25ul	F2				F2			
10 of 98	18	58 GAGTRTIASPKGPVIQMY 75	40mg/ml	25ul	F2				F2			
11 of 98	18	65 ASPKGPVIQMYTNVDQDL 82	40mg/ml	25ul	F2				F2			
12 of 98	16	72 IQMYTNVDQDLVGWPA 87	40mg/ml	25ul	F2				F2			
13 of 98	18	77 NVDQDLVGWPAPQGSRSL 94	40mg/ml	25ul	F2				F2			
14 of 98	18	84 GWPAPQGSRSLTPCTCGS 101	20mg/ml	50ul	F2				F2			
15 of 98	17	91 SRSLTPCTCGSSDLYLV 107	40mg/ml	25ul	F3	SRSLTPCTCGSSDLYLV	Seg 8	1a	F3	SRSLTPCTCGSADLYLV	Seg 9	3a
16 of 98	18	97 CTCGSSDLYLVTRHADVI 114	40mg/ml	25ul	F3	CTCGSSDLYLVTRHADVI	Seg 8	1a	F3	CTCGSSDLYLVTRDADVI	Seg 9	3a
17 of 98	16	104 LYLVTRHADVIPVRRR 119	40mg/ml	25ul	F3	LYLVTRHADVIPVRRR	Seg 8	1a	F3	LYLVTRDADVIPARRR	Seg 9	3a
18 of 98	18	109 RHADVIPVRRRGDSRGSL 126	40mg/ml	25ul	F3	RHADVIPVRRRGDSRGSL	Seg 8	1a	F3	RDADVIPARRRGDSTASL	Seg 9	3a
19 of 98	17	116 VRRRGDSRGSLLSPRPI 132	40mg/ml	25ul	F3	VRRRGDSRGSLLSPRPI	Seg 8	1a	F3	ARRRGDSTASLLSPRPI	Seg 9	3a
20 of 98	15	122 SRGSLLSPRPISYLK 136	40mg/ml	25ul	F3	SRGSLLSPRPISYLK	Seg 8	1a	F3	STASLLSPRPLACLK	Seg 9	3a
21 of 98	18	126 LLSPRPISYLKGSSGGPL 143	40mg/ml	25ul	F3	LLSPRPISYLKGSSGGPV	Seg 8	1a	F3	LLSPRPLACLKGSSGGPV	Seg 9	3a
22 of 98	18	133 SYLKGSSGGPLLCPAGHA 150	40mg/ml	25ul	F3	SYLKGSSGGPVLCPSGHA	Seg 8	1a	F3	ACLKGSSGGPVMCPSGHV	Seg 9	3a
23 of 98	18	140 GGPLLCPAGHAVGLFRAA 157	40mg/ml	25ul	F3	GGPVLCPSGHAVGIFRAA	Seg 8	1a	F3	GGPVMCPSGHVAGIFRAA	Seg 9	3a
24 of 98	18	147 AGHAVGLFRAAVCTRGVA 164	40mg/ml	25ul	F3	SGHAVGIFRAAVCTRGVA	Seg 8	1a	F3	SGHVAGIFRAAVCTRGVA	Seg 9	3a
25 of 98	17	154 FRAAVCTRGVAKAVDFI 170	40mg/ml	25ul	F3	FRAAVCTRGVAKAVDFI	Seg 8	1a	F3	FRAAVCTRGVAKALQFI	Seg 9	3a
26 of 98	16	160 TRGVAKAVDFIPVENL 175	40mg/ml	25ul	F3	TRGVAKAVDFIPVESL	Seg 8	1a	F3	TRGVAKALQFIPVETL	Seg 9	3a
27 of 98	16	165 KAVDFIPVENLGTTMR 180	40mg/ml	25ul		KAVDFIPVESLGTEMR	Seg 8	1a	F3	KALQFIPVETLGTTMR	Seg 9	3a
28 of 98	15	170 IPVENLGTTMRSPVF 184	40mg/ml	25ul		IPVESLGTEMRSPVF						
29 of 98	19	174 NLGTTMRSPVFTDNSSPPA 192	40mg/ml	25ul	F4	SLGTEMRSPVFTDNSTPPA	Seg 9	4a	F4	NLGTTMRSPVFSDNSSPPA	Seg 10	1a
30 of 98	18	182 PVFTDNSSPPAVPQSFQV 199	40mg/ml	25ul	F4	PVFTDNSTPPAVPQTYQV	Seg 9	4a	F4	PVFSDNSSPPAVPQSYQV	Seg 10	1a
31 of 98	16	189 SPPAVPQSFQVAHLHA 204	40mg/ml	25ul	F4	TPPAVPQTYQVAHLHA	Seg 9	4a	F4	SPPAVPQSYQVAHLHA	Seg 10	1a
32 of 98	17	194 PQSFQVAHLHAPTGSGK 210	40mg/ml	25ul	F4	PQTYQVAHLHAPTGSGK	Seg 9	4a	F4	PQSYQVAHLHAPTGSGK	Seg 10	1a
33 of 98	18	200 AHLHAPTGSGKSTKVPAA 217	40mg/ml	25ul	F4	AHLHAPTGSGKSTKVPAA	Seg 9	4a	F4	AHLHAPTGSGKSTKVPAA	Seg 10	1a
34 of 98	18	207 GSGKSTKVPAAYAAQGYK 224	40mg/ml	25ul	F4	GSGKSTKVPAAYAAQGYK	Seg 9	4a	F4	GSGKSTKVPAAYAAQGYK	Seg 10	1a
35 of 98	15	214 VPAAYAAQGYKVLVL 228	40mg/ml	25ul	F4	VPAAYAAQGYKVLVL	Seg 9	4a	F4	VPAAYAAQGYKVLVL	Seg 10	1a
36 of 98	17	218 YAAQGYKVLVLNPSVAA 234	40mg/ml	25ul	F4	YAAQGYKVLVLNPSVAA	Seg 9	4a	F4	YAAQGYKVLVLNPSVAA	Seg 10	1a
37 of 98	18	224 KVLVLNPSVAATLGFGAY 241	40mg/ml	25ul	F4	KVLVLNPSVAATLGFGAY	Seg 9	4a	F4	KVLVLNPSVAATLGFGAY	Seg 10	1a
38 of 98	18	231 SVAATLGFGAYMSKAHGV 248	40mg/ml	25ul	F4	SVAATLGFGAYMSKAYGI	Seg 9	4a	F4	SVAATLGFGAYMSKAHGV	Seg 10	1a
39 of 98	16	238 FGAYMSKAHGVDPNIR 253	40mg/ml	25ul	F4	FGAYMSKAYGIDPNIR	Seg 9	4a				
40 of 98	17	243 SKAHGVDPNIRTGVRTI 259	40mg/ml	25ul		SKAYGIDPNIRSGVRTI						
41 of 98	17	249 DPNIRTGVRTITTGSPI 265	40mg/ml	25ul	F5	DPNIRSGVRTITTGAPI	Seg 10	4a	F5	DPNIRTGNRTITTGAKL	Seg 11	3a
42 of 98	18	255 GVRTITTGSPITYSTYGK 272	40mg/ml	25ul	F5	GVRTITTGAPITYSTYGK	Seg 10	4a	F5	GVRTITTGAKLTYSTYGK	Seg 11	3a
43 of 98	18	262 GSPITYSTYGKFLADGGC 279	40mg/ml	25ul	F5	GAPITYSTYGKFLADGGC	Seg 10	4a	F5	GAKLTYSTYGKFLADGGC	Seg 11	3a
44 of 98	18	269 TYGKFLADGGCSGGAYDI 286	40mg/ml	25ul	F5	YGKFLADGGCSGGAYDI	Seg 10	4a	F5	TYGKFLADGGCSGGAYDV	Seg 11	3a

45 of 98	18	276	DGGCSGGAYDIIICDECH 293	40mg/ml	25ul	F5	DGGCSGGAYDIIICDECH	Seg 10	4a	F5	DGGCSGGAYDVIICDECH	Seg 11	3a
46 of 98	18	283	AYDIIICDECHSTDATSI 300	40mg/ml	25ul	F5	AYDIIICDECHSTDSTTI	Seg 10	4a	F5	AYDVIICDECHAQDATSI	Seg 11	3a
47 of 98	18	290	DECHSTDATSILGIGTVL 307	40mg/ml	25ul	F5	DECHSTDSTTILGIGTVL	Seg 10	4a	F5	DECHAQDATSILGIGTVL	Seg 11	3a
48 of 98	17	297	ATSILGIGTVLDQAETA 313	40mg/ml	25ul	F5	STTILGIGTVLDQAETA	Seg 10	4a	F5	ATSILGIGTVLDQAETA	Seg 11	3a
49 of 98	18	303	IGTVLDQAETAGARLVVL 320	20mg/ml	50ul	F5	IGTVLDQAETAGVRLVVL	Seg 10	4a	F5	IGTVLDQAETAGVRLTVL	Seg 11	3a
50 of 98	18	310	AETAGARLVVLATATPPG 327	13.3mg/ml	75ul	F5	AETAGVRLVVLATATPPG	Seg 10	4a	F5	AETAGVRLTVLATATPPG	Seg 11	3a

Pool G

NS3

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
51 of 98	17	317 LVVLATATPPGSVTVSH 333	40mg/ml	25ul						TVLATATPPGSITVPH	Seg 11	3a
52 of 98	18	323 ATPPGSVTVSHPNIEEVA 340	40mg/ml	25ul						ATPPGSITVPHSNIEEVA	Seg 11	3a
53 of 98	18	330 TVSHPNIEEVALSTTGEI 347	40mg/ml	25ul						TVPHSNIEEVALSTTGEI	Seg 11	3a
54 of 98	18	337 EEVALSTTGEIPFYGKAI 354	40mg/ml	25ul								
55 of 98	17	344 TGEIPFYGKAIPLEVIK 360	40mg/ml	25ul								
56 of 98	18	350 YGKAIPLEVIKGGRHLIF 367	40mg/ml	25ul		YGKAIPLEVIKGGRHLIF	Seg 11	1a				
57 of 98	17	357 EVIKGGRHLIFCHSKKK 373	40mg/ml	25ul		EVIKGGRHLIFCHSKKK	Seg 11	1a				
58 of 98	18	363 RHLIFCHSKKKCDELAAK 380	40mg/ml	25ul		RHLIFCHSKKKCDELAAK	Seg 11	1a				
59 of 98	17	370 SKKKCDELAAKLVALGI 386	40mg/ml	25ul		SKKKCDELAAKLVALGI	Seg 11	1a				
60 of 98	18	376 ELAAKLVALGINAVAYYR 393	20mg/ml	50ul								
61 of 98	18	383 ALGINAVAYYRGLDVSVI 400	20mg/ml	50ul		ALGINAVAYYRGLDVSVI	Seg 12	1b				
62 of 98	18	390 AYYRGLDVSVIPTSGDVV 407	20mg/ml	50ul		AYYRGLDVSVIPTSGDVV	Seg 12	1b		AYYRGLDVSVIPTAGDVV	Seg 12	3a
63 of 98	18	397 VSVIPTSGDVVVVSTDAL 414	40mg/ml	25ul		VSVIPTSGDVVVVATDAL	Seg 12	1b		VSVIPTAGDVVVCATDAL	Seg 12	3a
64 of 98	15	404 GDVVVVSTDALMTGF 418	40mg/ml	25ul		GDVVVVATDALMTGF	Seg 12	1b		GDVVVCATDALMTGF	Seg 12	3a
65 of 98	18	408 VVSTDALMTGFTGDFDSV 425	40mg/ml	25ul		VVATDALMTGFTGDFDSV	Seg 12	1b		VCATDALMTGFTGDFDSV	Seg 12	3a
66 of 98	18	415 MTGFTGDFDSVIDCNTCV 432	40mg/ml	25ul		MTGFTGDFDSVIDCNTCV	Seg 12	1b		MTGFTGDFDSVIDCNVAV	Seg 12	3a
67 of 98	17	422 FDSVIDCNTCVTQTVDF 438	20mg/ml	50ul		FDSVIDCNTCVTQTVDF	Seg 12	1b		FDSVIDCNVAVEQYVDF	Seg 12	3a
68 of 98	17	428 CNTCVTQTVDFSLDPTF 444	20mg/ml	50ul		CNTCVTQTVDFSLDPTF	Seg 12	1b		CNVAVEQYVDFSLDPTF	Seg 12	3a
69 of 98	19	433 TQTVDFSLDPTFTIETTTL 451	20mg/ml	50ul		TQTVDFSLDPTFTIETTTV	Seg 12	1b		EQYVDFSLDPTFSIETRTA	Seg 12	3a
70 of 98	18	441 DPTFTIETTTLPQDAVSR 458	40mg/ml	25ul		DPTFTIETTTVPQDAVSR	Seg 12	1b		DPTFSIETRTAPQDAVSR	Seg 12	3a
71 of 98	17	448 TTTLPQDAVSRTQRRGR 464	40mg/ml	25ul		TTTVPQDAVSRSQRRGR	Seg 12	1b		TRTAPQDAVSRSQRRGR	Seg 12	3a
72 of 98	16	454 DAVSRTQRRGRTGRGK 469	40mg/ml	25ul		DAVSRSQRRGRTGRGR	Seg 12	1b		DAVSRSQRRGRTGRGR	Seg 12	3a
73 of 98	18	459 TQRRGRTGRGKPGIYRFV 476	40mg/ml	25ul		SQRRGRTGRGRRGIYRFV	Seg 12	1b		SQRRGRTGRGRLGTYRYV	Seg 12	3a
74 of 98	16	466 GRGKPGIYRFVAPGER 481	40mg/ml	25ul		GRGRRGIYRFVTPGER	Seg 12	1b		GRGRLGTYRYVGPGER	Seg 12	3a
75 of 98	16	471 GIYRFVAPGERPSGMF 486	40mg/ml	25ul		GIYRFVTPGERPSGMF	Seg 12	1b		GTYRYVGPGERPSGMF	Seg 12	3a
76 of 98	16	476 VAPGERPSGMFDSSVL 491	40mg/ml	25ul		VTPGERPSGMFDSSVL	Seg 12	1b		VAPGERPSGMFDSVVL	Seg 12	3a
77 of 98	17	481 RPSGMFDSSVLCECYDA 497	40mg/ml	25ul		RPSGMFDSSVLCECYDA	Seg 12	1b		RPSGMFDSVVLCECYDA	Seg 12	3a
78 of 98	18	487 DSSVLCECYDAGCAWYEL 504	40mg/ml	25ul		DSSVLCECYDAGCAWYEL	Seg 12	1b		DSVVLCECYDAGCAWYEL	Seg 12	3a
79 of 98	18	494 CYDAGCAWYELTPAETTV 511	40mg/ml	25ul		CYDAGCAWYELTPAETSV	Seg 12	1b		CYDAGCAWYELQPAETTV	Seg 12	3a
80 of 98	17	501 WYELTPAETTVRLRAYM 517	40mg/ml	25ul		WYELTPAETSVRLRAYL	Seg 12	1b		WYELQPAETTVRLRAYL	Seg 12	3a
81 of 98	18	507 AETTVRLRAYMNTPGLPV 524	40mg/ml	25ul		AETSVRLRAYLNTPGLPV	Seg 12	1b		AETTVRLRAYLSTPGLPV	Seg 12	3a
82 of 98	18	514 RAYMNTPGLPVCQDHLEF 531	40mg/ml	25ul		RAYLNTPGLPVCQDHLEF	Seg 12	1b		RAYLSTPGLPVCQDHLDF	Seg 12	3a
83 of 98	16	521 GLPVCQDHLEFWEGVF 536	40mg/ml	25ul		GLPVCQDHLEFWEGVF	Seg 12	1b		GLPVCQDHLDFWESVF	Seg 12	3a
84 of 98	18	525 CQDHLEFWEGVFTGLTHI 542	40mg/ml	25ul		CQDHLEFWEGVFTGLTHI	Seg 12	1b		CQDHLDFWESVFTGLTHI	Seg 12	3a
85 of 98	16	532 WEGVFTGLTHIDAHFL 547	40mg/ml	25ul		WEGVFTGLTHIDAHFL	Seg 12	1b		WESVFTGLTHIDAHFL	Seg 12	3a
86 of 98	15	537 TGLTHIDAHFLSQTK 551	40mg/ml	25ul		TGLTHIDAHFLSQTK	Seg 12	1b		TGLTHIDAHFLSQTK	Seg 12	3a

87 of 98	17 541 HIDAHFLSQTKQSGENF 557	40mg/ml	25ul		HIDAHFLSQTKQAGDNF	Seg 12	1b		HIDAHFLSQTKQQGLNF	Seg 12	3a
88 of 98	17 547 LSQTKQSGENFPYLVAY 563	40mg/ml	25ul		LSQTKQAGDNFPYLVAY	Seg 12	1b		SQTKQQGLNFPYLTAY	Seg 12	3a
89 of 98	18 553 SGENFPYLVAYQATVCAR 570	40mg/ml	25ul		AGDNFPYLVAYQATVCAR	Seg 12	1b		QGLNFPYLTAYQATVCAR	Seg 12	3a
90 of 98	19 560 LVAYQATVCARAQAPPPSW 578	40mg/ml	25ul		LVAYQATVCARAQAPPPSW	Seg 12	1b		LTAYQATVCARAQAPPPSW	Seg 12	3a
91 of 98	18 568 CARAQAPPPSWDQMWKCL 585	40mg/ml	25ul		CARAQAPPPSWDQMWKCL	Seg 12	1b		CARAQAPPPSWDEMWKCL	Seg 12	3a
92 of 98	18 575 PPSWDQMWKCLIRLKPTL 592	40mg/ml	25ul		PPSWDQMWKCLIRLKPTL	Seg 12	1b		PPSWDEMWKCLVRLKPTL	Seg 12	3a
93 of 98	18 582 WKCLIRLKPTLHGPTPLL 599	40mg/ml	25ul						WKCLVRLKPTLHGPTPLL	Seg 12	3a
94 of 98	17 589 KPTLHGPTPLLYRLGAV 605	40mg/ml	25ul						KPTLHGPTPLLYRLGPV	Seg 12	3a
95 of 98	17 595 PTPLLYRLGAVQNEVTL 611	20mg/ml	50ul						PTPLLYRLGPVQNEVTL	Seg 12	3a
96 of 98	18 601 RLGAVQNEVTLTHPITKY 618	40mg/ml	25ul	H1-01	RLGAVQNEVTLTHPITKY	Seg 13	4a				
97 of 98	18 608 EVTLTHPITKYIMTCMSA 625	40mg/ml	25ul	H1-02	EVTLTHPITKYIMACMSA	Seg 13	4a				
98 of 98	17 615 ITKYIMTCMSADLEVVT 631	40mg/ml	25ul	H1-03	ITKYIMACMSADLEVVT	Seg 13	4a		ITKYIMACMSADLEVTT	Seg 13	3a

Pool H

NS4A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 7	18 1 STWVLVGGVLAALAAYCL 18	20mg/ml	50ul	H1-04	STWVLVGGVLAALAAYCL	Seg 13	4a	H1	STWVLLGGVLAALAAYCL	Seg 13	3a
2 of 7	18 8 GVLAALAAYCLSTGCVVI 25	20mg/ml	50ul	H1-05	GVLAALAAYCLSVGSVVI	Seg 13	4a	H1	GVLAALAAYCLSVGCVVI	Seg 13	3a
3 of 7	17 15 AYCLSTGCVVIVGRIVL 31	40mg/ml	25ul	H1-06	AYCLSVGSVVIVGRIVL	Seg 13	4a	H1	AYCLSVGCVVIVGHIVL	Seg 13	3a
4 of 7	18 21 GCVVIVGRIVLSGKPAII 38	20mg/ml	50ul								
5 of 7	18 28 RIVLSGKPAIIPDREVLY 45	40mg/ml	25ul	H2				H2			
6 of 7	17 35 PAIIPDREVLYQEFDEM 51	40mg/ml	25ul	H2				H2			
7 of 7	14 41 REVLYQEFDEMEEC 54	40mg/ml	25ul	H2				H2			

NS4B

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 40	18 1 SQHLPYIEQGMMLAEQFK 18	40mg/ml	25ul	H2				H2			
2 of 40	18 8 EQGMMLAEQFKQKALGLL 25	40mg/ml	25ul	H2				H2			
3 of 40	18 15 EQFKQKALGLLQTASRHA 32	40mg/ml	25ul	H2				H2			
4 of 40	18 22 LGLLQTASRHAEVITPAV 39	40mg/ml	25ul	H2				H2			
5 of 40	18 29 SRHAEVITPAVQTNWQKL 46	40mg/ml	25ul	H2				H2			
6 of 40	18 36 TPAVQTNWQKLEVFWAKH 53	40mg/ml	25ul								
7 of 40	16 43 WQKLEVFWAKHMWNFI 58	40mg/ml	25ul		WQKLEVFWAKHMWNFI	Seg 14	1a	H3	WQKLEVFWAKHMWNFI	Seg 14	1a
8 of 40	18 48 VFWAKHMWNFISGIQYLA 65	20mg/ml	50ul	H3	VFWAKHMWNFISGIQYLA	Seg 14	1a	H3	VFWAKHMWNFISGIQYLA	Seg 14	1a
9 of 40	16 55 WNFISGIQYLAGLSTL 70	40mg/ml	25ul	H3	WNFISGIQYLAGLSTL	Seg 14	1a	H3	WNFISGIQYLAGLSTL	Seg 14	1a
10 of 40	18 60 GIQYLAGLSTLPGNPAIA 77	40mg/ml	25ul	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a
11 of 40	18 67 LSTLPGNPAIASLMAFTA 84	40mg/ml	25ul	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a
12 of 40	17 74 PAIASLMAFTAAVTSPL 90	20mg/ml	50ul	H3	PAIASLMAFTAAVTSPL	Seg 14	1a	H3	PAIASLMAFTAAVTSPL	Seg 14	1a
13 of 40	18 80 MAFTAAVTSPLTTGQTLL 97	40mg/ml	25ul	H3	MAFTAAVTSPLTTGQTLL	Seg 14	1a	H3			
14 of 40	18 87 TSPLTTGQTLLFNILGGW 104	40mg/ml	25ul								
15 of 40	18 93 GQTLLFNILGGWVAAQLA 110	40mg/ml	25ul	H4				H4			
16 of 40	18 100 ILGGWVAAQLAAPGAATA 117	40mg/ml	25ul	H4				H4			
17 of 40	18 107 AQLAAPGAATAFVGAGLA 124	40mg/ml	25ul	H4				H4			
18 of 40	18 114 AATAFVGAGLAGAAIGSV 131	20mg/ml	50ul								
19 of 40	18 121 AGLAGAAIGSVGLGKVLV 138	40mg/ml	25ul	H5	AGLAGAAVGSVGLGKVLV	Seg 15	1a	H5	AGLAGAAIGSVGLGKVLV	Seg 15	1a
20 of 40	17 128 IGSVGLGKVLVDILAGY 144	40mg/ml	25ul	H5	IGSVGLGKVLVDILAGY	Seg 15	1a	H5	IGSVGLGKVLVDILAGY	Seg 15	1a
21 of 40	18 134 GKVLVDILAGYGAGVAGA 151	13.3mg/ml	75ul	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a

22 of 40	18	141	LAGYGAGVAGALVAFKIM 158	13.3mg/ml	75ul	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a
23 of 40	15	148	VAGALVAFKIMSGEV 162	13.3mg/ml	75ul	H5	VAGALVAFKIMSGEV	Seg 15	1a	H5	VAGALVAFKIMSGEI	Seg 15	1a
24 of 40	18	152	LVAFKIMSGEVPSTEDLV 169	40mg/ml	25ul	H5	LVAFKIMSGEVPSTEDLV	Seg 15	1a	H5	LVAFKIMSGEIPSTEDLV	Seg 15	1a
25 of 40	18	159	SGEVPSTEDLVNLLPAIL 176	40mg/ml	25ul	H5	SGEVPSTEDLVNLLPAIL	Seg 15	1a	H5	SGEIPSTEDLVNLLPAIL	Seg 15	1a
26 of 40	18	166	EDLVNLLPAILSPGALVV 183	40mg/ml	25ul	H5	EDLVNLLPAILSPGALVV	Seg 15	1a	H5	EDLVNLLPAILSPGALVV	Seg 15	1a
27 of 40	18	173	PAILSPGALVVGVVCAAI 190	40mg/ml	25ul	H5	AILSPGALVVGVVCAAI	Seg 15	1a	H5	PAILSPGALVVGVVCAAI	Seg 15	1a
28 of 40	16	180	ALVVGVVCAAILRRHV 195	40mg/ml	25ul	H5	ALVVGVVCAAILRRHV	Seg 15	1a	H5	ALVVGVVCAAILRRHV	Seg 15	1a
29 of 40	18	185	VVCAAILRRHVGPGEGAV 202	40mg/ml	25ul	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a
30 of 40	18	192	RRHVGPGEGAVQWMNRLI 209	40mg/ml	25ul	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a
31 of 40	16	199	EGAVQWMNRLIAFASR 214	40mg/ml	25ul	H5	EGAVQWMNRLIAFASR	Seg 15	1a	H5	EGAVQWMNRLIAFASR	Seg 15	1a
32 of 40	15	204	WMNRLIAFASRGNHV 218	40mg/ml	25ul	H5	WMNRLIAFASRGNHV	Seg 15	1a	H5	WMNRLIAFASRGNHV	Seg 15	1a
33 of 40	17	208	LIAFASRGNHVSPTHYV 224	40mg/ml	25ul	H5	LIAFASRGNHVSPTHYV	Seg 15	1a	H5	LIAFASRGNHVSPTHYV	Seg 15	1a
34 of 40	18	214	RGNHVSPTHYVPESDAAA 231	40mg/ml	25ul	H5	RGNHVSPTHYVPESDAAA	Seg 15	1a	H5	RGNHVSPTHYVPESDAAA	Seg 15	1a
35 of 40	17	221	THYVPESDAAARVTAIL 237	40mg/ml	25ul	H5	THYVPESDAAARVTAIL	Seg 15	1a	H5	THYVPESDAAARVTAIL	Seg 15	1a
36 of 40	16	227	SDAAARVTAILSSLTV 242	40mg/ml	25ul					H5	SDAAARVTAILSSLTV	Seg 15	1a
37 of 40	18	232	RVTAILSSLTVTQLLRRL 249	40mg/ml	25ul	H6				H5	RVTAILSSLTVTQLLRRL	Seg 15	1a
38 of 40	15	239	SLTVTQLLRRLHQWI 253	40mg/ml	25ul	H6							
39 of 40	18	243	TQLLRRLHQWISSECTTP 260	40mg/ml	25ul	H6				H6			
40 of 40	12	250	HQWISSECTTPC 261	40mg/ml	25ul	H6				H6			

Pool I

NS5A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 71	16	1 SGSWLRDIWDWICEVL 16	40mg/ml	25ul								
2 of 71	18	6 RDIWDWICEVLSDFKTWL 23	40mg/ml	25ul								
3 of 71	16	13 CEVLSDFKTWLKAKLM 28	40mg/ml	25ul								
4 of 71	17	18 DFKTWLKAKLMPQLPGI 34	40mg/ml	25ul								
5 of 71	18	24 KAKLMPQLPGIPFVSCQR 41	40mg/ml	25ul								
6 of 71	18	31 LPGIPFVSCQRGYRGVWR 48	40mg/ml	25ul								
7 of 71	17	38 SCQRGYRGVWRGDGIMH 54	40mg/ml	25ul								
8 of 71	18	44 RGVWRGDGIMHTRCHCGA 61	40mg/ml	25ul								
9 of 71	18	51 GIMHTRCHCGAEITGHVK 68	40mg/ml	25ul								
10 of 71	18	58 HCGAEITGHVKNGTMRIV 75	40mg/ml	25ul								
11 of 71	17	65 GHVKNGTMRIVGPRTCR 81	40mg/ml	25ul								
12 of 71	18	71 TMRIVGPRTCRNMWSGTF 88	40mg/ml	25ul								
13 of 71	16	78 RTCRNMWSGTFPINAY 93	40mg/ml	25ul								
14 of 71	19	83 MWSGTFPINAYTTGPCTPL 101	40mg/ml	25ul								
15 of 71	18	91 NAYTTGPCTPLPAPNYKF 108	40mg/ml	25ul								
16 of 71	18	98 CTPLPAPNYKFALWRVSA 115	40mg/ml	25ul								
17 of 71	18	105 NYKFALWRVSAEEYVEIR 122	20mg/ml	50ul								
18 of 71	18	112 RVSAEEYVEIRRVGDFHY 129	40mg/ml	25ul								
19 of 71	15	119 VEIRRVGDFHYVSGM 133	40mg/ml	25ul								
20 of 71	17	123 RVGDFHYVSGMTTDNLK 139	40mg/ml	25ul								
21 of 71	16	129 YVSGMTTDNLKCPCQI 144	40mg/ml	25ul								
22 of 71	17	134 TTDNLKCPCQIPSPEFF 150	40mg/ml	25ul						TTDNLKCPCQVPAPEFF	Seg 16	1b
23 of 71	18	140 CPCQIPSPEFFTELDGVR 157	40mg/ml	25ul						CPCQVPAPEFFTEVDGVR	Seg 16	1b
24 of 71	16	147 PEFFTELDGVRLHRFA 162	40mg/ml	25ul						PEFFTELDGVRLHRFA	Seg 16	1b

25 of 71	18 152 ELDGVRLHRFAPPCKPLL 169	40mg/ml	25ul						ELDGVRLHRFAPPCKPLL	Seg 16		1b	
26 of 71	18 159 HRFAPPCKPLLREEVSFR 176	40mg/ml	25ul										
27 of 71	17 166 KPLLREEVSFRVGLHEY 182	40mg/ml	25ul										
28 of 71	17 172 EVSFRVGLHEYPVGSQL 188	40mg/ml	25ul										
29 of 71	19 178 GLHEYPVGSQLPCEPEPDV 196	40mg/ml	25ul						GLHEYPIGSQLPCEPEPDV	Seg 17		3a	
30 of 71	18 186 SQLPCEPEPDVAVLTSML 203	40mg/ml	25ul						SQLPCEPEPDVSVLTSML	Seg 17		3a	
31 of 71	17 193 EPDVAVLTSMLTDPSHI 209	40mg/ml	25ul						EPDVAVLTSMLTDPSHI	Seg 17		3a	
32 of 71	18 199 LTSMLTDPSHITAEAAGR 216	40mg/ml	25ul										
33 of 71	15 206 PSHITAEAAGRRLAR 220	40mg/ml	25ul		PSHITAETAARRLAR	Seg 16	6d						
34 of 71	18 210 TAEAAGRRLARGSPPSMA 227	40mg/ml	25ul		TAETAARRLARGSPPSLA	Seg 16	6d		TAETAARRLARGSPPSEA	Seg 18		3a	
35 of 71	18 217 RLARGSPPSMASSSASQL 234	40mg/ml	25ul		RLARGSPPSLASSSASQL	Seg 16	6d		RLARGSPPSEASSSASQL	Seg 18		3a	
36 of 71	18 224 PSMASSSASQLSAPSLKA 241	40mg/ml	25ul		PSLASSSASQLSAPSLKA	Seg 16	6d		PSEASSSASQLSAPSLKA	Seg 18		3a	
37 of 71	17 231 ASQLSAPSLKATCTANH 247	40mg/ml	25ul		ASQLSAPSLKATCTANH	Seg 16	6d		ASQLSAPSLKATCQTHR	Seg 18		3a	
38 of 71	18 237 PSLKATCTANHDSPDAEL 254	40mg/ml	25ul		PSLKATCTANHDSPDAEL	Seg 16	6d		PSLKATCQTHRDSPDAEL	Seg 18		3a	
39 of 71	18 244 TANHDSPDAELIEANLLW 261	40mg/ml	25ul										
40 of 71	15 251 DAELIEANLLWRQEM 265	13.3mg/ml	75ul										
41 of 71	18 255 IEANLLWRQEMGGNITRV 272	40mg/ml	25ul										
42 of 71	18 262 RQEMGGNITRVESENKVV 279	40mg/ml	25ul										
43 of 71	16 269 ITRVESENKVVILDSF 284	40mg/ml	25ul										
44 of 71	16 274 SENKVVILDSFDPLVA 289	40mg/ml	25ul										
45 of 71	18 279 VILDSFDPLVAEEDEREV 296	40mg/ml	25ul										
46 of 71	18 286 PLVAEEDEREVSVPAEIL 303	40mg/ml	25ul										

Pool I continued

NS5A

Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Seg pool*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
47 of 71 18 293 EREVSVPAEILRKSRRFA 310	40mg/ml	25ul								
48 of 71 18 300 AEILRKSRRFARALPVWA 317	40mg/ml	25ul								
49 of 71 15 307 RRFARALPVWARPDY 321	40mg/ml	25ul								
50 of 71 16 311 RALPVWARPDYNPPLV 326	40mg/ml	25ul								
51 of 71 16 316 WARPDYNPPLVETWKK 331	40mg/ml	25ul								
52 of 71 18 321 YNPPLVETWKKPDYEPPV 338	40mg/ml	25ul								
53 of 71 17 328 TWKKPDYEPPVVHGCPL 344	40mg/ml	25ul								
54 of 71 15 334 YEPPVVHGCPLPPPR 348	40mg/ml	25ul								
55 of 71 15 338 VVHGCPLPPPRSPPV 352	40mg/ml	25ul								
56 of 71 18 342 CPLPPPRSPPVPPPRKKR 359	40mg/ml	25ul								
57 of 71 15 349 SPPVPPPRKKRTVVL 363	40mg/ml	25ul								
58 of 71 16 353 PPPRKKRTVVLTESTL 368	40mg/ml	25ul								
59 of 71 18 358 KRTVVLTESTLSTALAEL 375	20mg/ml	50ul								
60 of 71 16 365 ESTLSTALAELATKSF 380	20mg/ml	50ul								
61 of 71 19 370 TALAELATKSFGSSSTSGI 388	20mg/ml	50ul								
62 of 71 18 378 KSFGSSSTSGITGDNTTT 395	40mg/ml	25ul								
63 of 71 16 385 TSGITGDNTTTSSEPA 400	40mg/ml	25ul								
64 of 71 18 390 GDNTTTSSEPAPSGCPPD 407	40mg/ml	25ul								
65 of 71 17 397 SEPAPSGCPPDSDVESY 413	40mg/ml	25ul								
66 of 71 17 403 GCPPDSDVESYSSMPPL 419	40mg/ml	25ul						GCPPESDSESCSSMPPL	Seg 19	3a
67 of 71 18 409 DVESYSSMPPLEGEPGDP 426	40mg/ml	25ul						SESCSSMPPLEGEPGDP	Seg 19	3a

68 of 71	18 416 MPPLEGEPGDPDLSDGSW 433	40mg/ml	25ul						MPPLEGEPGDPDLSCDSW	Seg 19	3a
69 of 71	18 423 PGDPDLSDGSWSTVSSGA 440	40mg/ml	25ul						PGDPDLSCDSWSTVSDGA	Seg 19	3a
70 of 71	17 430 DGSWSTVSSGADTEDVV 446	40mg/ml	25ul						CDSWSTVSDGADTQSVV	Seg 19	3a
71 of 71	13 436 VSSGADTEDVVCC 448	40mg/ml	25ul		VSSGADTEDVVCC				VSDGADTQSVVCC	Seg 20	3a

Pool L

NS5B

Peptide	Length	Sequence	Conc.	Vol.	Minipool	Vacc Seq.	Seg pool	Vacc GT	Minipool	Vacc Seq.	Segment	Vacc GT
1 of 91	16	1 SMSYSWTGALVTPCAA 16	40mg/ml	25ul		SMSYSWTGALITPCAA	Seg 18	6a		SMSYSWTGALITPCSA	Seg 20	3a
2 of 91	18	6 WTGALVTPCAAEEQKLPI 23	40mg/ml	25ul		WTGALITPCAAEEEKLPI	Seg 18	6a		WTGALITPCSAEEEKLPI	Seg 20	3a
3 of 91	18	13 PCAAEEQKLPINALSNSL 30	40mg/ml	25ul		PCAAEEEKLPINPLSNSL	Seg 18	6a		PCSAEEEKLPINPLSNSL	Seg 20	3a
4 of 91	18	20 KLPINALSNSLLRHHNLV 37	40mg/ml	25ul		KLPINPLSNSLIRHHNMV	Seg 18	6a		KLPINPLSNSLLRHHNLV	Seg 20	3a
5 of 91	17	27 SNSLLRHHNLVYSTTSR 43	40mg/ml	25ul		SNSLIRHHNMVYSTTSR	Seg 18	6a		SNSLLRHHNLVYSTSSR	Seg 20	3a
6 of 91	18	33 HHNLVYSTTSRSACQRQK 50	40mg/ml	25ul		HHNMVYSTTSRSASLRQK	Seg 18	6a		HHNLVYSTSSRSASQRQK	Seg 20	3a
7 of 91	18	40 TTSRSACQRQKKVTFDRL 57	40mg/ml	25ul		TTSRSASLRQKKVTFDRL	Seg 18	6a		TSSRSASQRQKKVTFDRL	Seg 20	3a
8 of 91	18	46 CQRQKKVTFDRLQVLDSH 63	40mg/ml	25ul		CQRQKKVTFDRLQVLDSH	Seg 18	6a		SQRQKKVTFDRLQVLDDH	Seg 20	3a
9 of 91	17	53 TFDRLQVLDSHYQDVLK 69	40mg/ml	25ul						TFDRLQVLDDHYKDVLK	Seg 20	3a
10 of 91	17	59 VLDSHYQDVLKEVKAAA 75	20mg/ml	50ul								
11 of 91	17	64 YQDVLKEVKAAASKVKA 80	13.3mg/ml	75ul								
12 of 91	16	70 EVKAAASKVKANLLSV 85	13.3mg/ml	75ul								
13 of 91	17	75 ASKVKANLLSVEEACSL 91	20mg/ml	50ul								
14 of 91	18	81 NLLSVEEACSLTPPHSAK 98	40mg/ml	25ul								
15 of 91	18	88 ACSLTPPHSAKSKFGYGA 105	40mg/ml	25ul								
16 of 91	18	95 HSAKSKFGYGAKDVRCHA 112	40mg/ml	25ul								
17 of 91	18	102 GYGAKDVRCHARKAVAHI 119	40mg/ml	25ul								
18 of 91	18	109 RCHARKAVAHINSVWKDL 126	40mg/ml	25ul								
19 of 91	16	116 VAHINSVWKDLLEDSV 131	40mg/ml	25ul								
20 of 91	18	121 SVWKDLLEDSVTPIDTTI 138	40mg/ml	25ul								
21 of 91	18	128 EDSVTPIDTTIMAKNEVF 145	40mg/ml	25ul								
22 of 91	17	135 DTTIMAKNEVFCVQPEK 151	40mg/ml	25ul								
23 of 91	18	141 KNEVFCVQPEKGGRKPAR 158	40mg/ml	25ul		KNEVFCVQPAKGGRKPAR	Seg 19	3a		KNEVFCVQPEKGGRKPAR	Seg 21	1a
24 of 91	16	147 VQPEKGGRKPARLIVF 162	40mg/ml	25ul		VQPAKGGRKPARLIVY	Seg 19	3a		VQPEKGGRKPARLIVY	Seg 21	1a
25 of 91	18	152 GGRKPARLIVFPDLGVRV 169	40mg/ml	25ul		GGRKPARLIVYPDLGVRV	Seg 19	3a		GGRKPARLIVYPDLGVRV	Seg 21	1a
26 of 91	18	159 LIVFPDLGVRVCEKMALY 176	20mg/ml	50ul		LIVYPDLGVRVCEKRALY	Seg 19	3a		LIVYPDLGVRVCEKMALY	Seg 21	1a
27 of 91	17	166 GVRVCEKMALYDVVSKL 182	40mg/ml	25ul		GVRVCEKRALYDVVSKL	Seg 19	3a		GVRVCEKMALYDVVSKL	Seg 21	1a
28 of 91	16	172 KMALYDVVSKLPLAVM 187	40mg/ml	25ul								
29 of 91	17	177 DVVSKLPLAVMGSSYGF 193	40mg/ml	25ul								
30 of 91	18	183 PLAVMGSSYGFQYSPGQR 200	40mg/ml	25ul								
31 of 91	18	190 SYGFQYSPGQRVEFLVQA 207	40mg/ml	25ul								
32 of 91	16	197 PGQRVEFLVQAWKSKK 212	40mg/ml	25ul								
33 of 91	18	202 EFLVQAWKSKKTPMGFSY 219	40mg/ml	25ul		EFLVQAWKSKKTPMGFSY	Seg 20	6h		EFLVQAWTSKKTPMGFSY	Seg 22	3a
34 of 91	16	209 KSKKTPMGFSYDTRCF 224	40mg/ml	25ul		KSKKTPMGFSYDTRCF	Seg 20	6h		KSKKTPMGFSYDTRCF	Seg 22	3a
35 of 91	15	214 PMGFSYDTRCFDSTV 228	40mg/ml	25ul		PMGFSYDTRCFDSTV	Seg 20	6h		PMGFSYDTRCFDSTV	Seg 22	3a
36 of 91	17	218 SYDTRCFDSTVTESDIR 234	40mg/ml	25ul		SYDTRCFDSTVTERDIR	Seg 20	6h		SYDTRCFDSTVTEQDIR	Seg 22	3a
37 of 91	17	224 FDSTVTESDIRTEEAIY 240	20mg/ml	50ul		DSTVTERDIRTEEAIY	Seg 20	6h		DSTVTEQDIRVEEEIY	Seg 22	3a
38 of 91	16	230 ESDIRTEEAIYQCCDL 245	40mg/ml	25ul						EQDIRVEEEIYQCCDL	Seg 22	3a
39 of 91	18	235 TEEAIYQCCDLDPQARVA 252	40mg/ml	25ul								

40 of 91	18 242	CCDLDPQARVAIKSLTER 259	20mg/ml	50ul										
41 of 91	18 249	ARVAIKSLTERLYVGGPL 266	13.3mg/ml	75ul										
42 of 91	15 256	LTERLYVGGPLTNSR 270	40mg/ml	25ul										
43 of 91	18 260	LYVGGPLTNSRGENCGYR 277	40mg/ml	25ul										
44 of 91	18 267	TNSRGENCGYRRCRASGV 284	40mg/ml	25ul		TNSRGENCGYRRCRASGV	Seg 21	2a	M1	TNSRGENCGYRRCRASGV	Seg 23	1a		
45 of 91	18 274	CGYRRCRASGVLTTSCGN 291	40mg/ml	25ul	M1	CGYRRCRASGVLTTSMGN	Seg 21	2a	M1	CGYRRCRASGVLTTSCGN	Seg 23	1a		
46 of 91	18 281	ASGVLTTSCGNTLTCYIK 298	40mg/ml	25ul	M1	ASGVLTTSMGNTITCYIK	Seg 21	2a		ASGVLTTSCGNTLTCYIK	Seg 23	1a		

Pool M

NS5B

Peptide Length Sequence			Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
47 of 91	18 288	SCGNTLTCYIKARAACRA 305	40mg/ml	25ul	M1	SMGNTITCYIKALAACRA	Seg 21	2a	M1	SCGNTLTCYIKARAACRA	Seg 23	1a
48 of 91	19 295	CYIKARAACRAAGLQDCTM 313	40mg/ml	25ul	M1	CYIKALAACRAAGLQDCTM	Seg 21	2a	M1	CYIKARAACRAAGLQDCTM	Seg 23	1a
49 of 91	18 303	CRAAGLQDCTMLVCGDDL 320	20mg/ml	50ul								
50 of 91	18 310	DCTMLVCGDDLVVICESA 327	20mg/ml	50ul	M2				M2			
51 of 91	18 317	GDDLVVICESAGVQEDAA 334	20mg/ml	50ul	M2				M2			
52 of 91	16 324	CESAGVQEDAASLRAF 339	20mg/ml	50ul	M2				M2			
53 of 91	18 329	VQEDAASLRAFTEAMTRY 346	20mg/ml	50ul	M2				M3	VQEDAAALRAFTEAMTRY	Seg 24	3a
54 of 91	18 336	LRAFTEAMTRYSAPPGDP 353	20mg/ml	50ul	M3	LRAFTEAMTRYSAPPGDP	Seg 22	1a	M3	LRAFTEAMTRYSAPPGDA	Seg 24	3a
55 of 91	18 343	MTRYSAPPGDPPQPEYDL 360	20mg/ml	50ul	M3	MTRYSAPPGDPPQPEYDL	Seg 22	1a	M3	MTRYSAPPGDAPQPEYDL	Seg 24	3a
56 of 91	18 350	PGDPPQPEYDLELITSCS 367	20mg/ml	50ul	M3	PGDPPQPEYDLELITSCS	Seg 22	1a				
57 of 91	18 357	EYDLELITSCSSNVSVAH 374	40mg/ml	25ul	M3	EYDLELITSCSSNVSVAH	Seg 22	1a	M4			
58 of 91	18 364	TSCSSNVSVAHDGAGKRV 381	40mg/ml	25ul	M3	TSCSSNVSVAHDGAGKRV	Seg 22	1a	M4			
59 of 91	16 371	SVAHDGAGKRVYYLTR 386	40mg/ml	25ul						SVAHDGKGKRYYYLTR	Seg 25	3a
60 of 91	18 376	GAGKRVYYLTRDPTTPLA 393	40mg/ml	25ul	M4				M5	AGKRYYYLTRDPTTPLA	Seg 25	3a
61 of 91	18 383	YLTRDPTTPLARAAWETA 400	40mg/ml	25ul		YLTRDPTTPLARAAWETA	Seg 23	6a	M5	YLTRDPTTPLARAAWETA	Seg 25	3a
62 of 91	16 390	TPLARAAWETARHTPV 405	40mg/ml	25ul	M5	TPLARAAWETARHTPV	Seg 23	6a	M5	TPLARAAWETARHTPV	Seg 25	3a
63 of 91	18 395	AAWETARHTPVNSWLGNI 412	40mg/ml	25ul	M5	AAWETARHTPVNSWLGNI	Seg 23	6a	M5	AAWETARHTPVNSWLGNI	Seg 25	3a
64 of 91	18 402	HTPVNSWLGNIIMFAPTL 419	40mg/ml	25ul	M5	HTPVNSWLGNIIMYAPTI	Seg 23	6a	M5	HTPVNSWLGNIIMYAPTI	Seg 25	3a
65 of 91	18 409	LGNIIMFAPTLWARMILM 426	40mg/ml	25ul	M5	LGNIIMYAPTIWVRMVLM	Seg 23	6a	M5	LGNIIMYAPTIWVRMVMM	Seg 25	3a
66 of 91	18 416	APTLWARMILMTHFFSVL 433	20mg/ml	50ul	M5	APTIWVRMVLMTHFFSIL	Seg 23	6a		APTIWVRMVMMTHFFSVL	Seg 25	3a
67 of 91	17 423	MILMTHFFSVLIARDQL 439	20mg/ml	50ul	M5	MVLMTHFFSILQARDQL	Seg 23	6a	M6			
68 of 91	15 429	FFSVLIARDQLEQAL 443	20mg/ml	50ul					M6			
69 of 91	18 433	LIARDQLEQALNCEIYGA 450	20mg/ml	50ul	M6				M6			
70 of 91	18 440	EQALNCEIYGACYSIEPL 457	40mg/ml	25ul		EQALNCEIYGATYSVTPL	Seg 24	3a		EQALNCEIYGATYSVTPL	Seg 26	3a
71 of 91	17 447	IYGACYSIEPLDLPPII 463	40mg/ml	25ul	M7	YGATYSVTPLDLPAII	Seg 24	3a	M7	YGATYSVTPLDLPAII	Seg 26	3a
72 of 91	17 453	SIEPLDLPPIIQRLHGL 469	40mg/ml	25ul	M7	SVTPLDLPAIIQRLHGL	Seg 24	3a	M7	SVTPLDLPAIIERLHGL	Seg 26	3a
73 of 91	17 459	LPPIIQRLHGLSAFSLH 475	40mg/ml	25ul	M7	LPAIIQRLHGLSAFSLH	Seg 24	3a	M7	LPAIIERLHGLSAFTLH	Seg 26	3a
74 of 91	18 465	RLHGLSAFSLHSYSPGEI 482	40mg/ml	25ul	M7	RLHGLSAFSLHSYSPGEI	Seg 24	3a	M7	RLHGLSAFTLHSYSPVEL	Seg 26	3a
75 of 91	18 472	FSLHSYSPGEINRVAACL 489	40mg/ml	25ul		FSLHSYSPGEINRVAACL			M7	FTLHSYSPVELNRVAGTL	Seg 26	3a
76 of 91	16 479	PGEINRVAACLRKLGV 494	40mg/ml	25ul	M8	PGEINRVAACLRKLGV	Seg 25	6a	M7	PVELNRVAGTLRKLGC	Seg 26	3a
77 of 91	18 484	RVAACLRKLGVPPLRAWR 501	40mg/ml	25ul	M8	RVAACLRKLGVPPLRAWR	Seg 25	6a	M7	RVAGTLRKLGCPPLRAWR	Seg 26	3a
78 of 91	18 491	KLGVPPLRAWRHRARSVR 508	20mg/ml	50ul	M8	KLGVPPLRAWRHRARSVR	Seg 25	6a	M7	KLGCPPLRAWRHRARSVR	Seg 26	3a
79 of 91	17 498	RAWRHRARSVRARLLSR 514	20mg/ml	50ul	M8	RAWRHRARSVRARLIAR	Seg 25	6a				
80 of 91	17 504	ARSVRARLLSRGGRAAI 520	40mg/ml	25ul	M8	ARSVRARLIARGGKAAI	Seg 25	6a	M8	ARSVRAKLLSQGGRAAI	Seg 27	1b
81 of 91	17 510	RLLSRGGRAAICGKYLF 526	40mg/ml	25ul	M8	RLIARGGKAAICGKYLF	Seg 25	6a	M8	KLLSQGGRAAICGKYLF	Seg 27	1b

82 of 91	18	516	GRAAICGKYLFNWAVRTK	533	20mg/ml	50ul	M8	GKAAICGKYLFNWAVRTK	Seg 25	6a	M8	GRAAICGKYLFNWAVRTK	Seg 27	1b
83 of 91	18	523	KYLFNWAVRTKLKLTPIA	540	40mg/ml	25ul					M8	KYLFNWAVRTKLKLTPIA	Seg 27	1b
84 of 91	18	530	VRTKLKLTPIAAAGRLDL	547	40mg/ml	25ul	M9							
85 of 91	17	537	TPIAAAGRLDLSGWFTA	553	40mg/ml	25ul	M9				M9			
86 of 91	18	543	GRLDLSGWFTAGYSGGDI	560	40mg/ml	25ul	M9				M9			
87 of 91	18	550	WFTAGYSGGDIYHSVSHA	567	40mg/ml	25ul	M9				M9			
88 of 91	18	557	GGDIYHSVSHARPRWFWF	574	40mg/ml	25ul	M9				M9			
89 of 91	18	564	VSHARPRWFWFCLLLLAA	581	40mg/ml	25ul	M9				M9			
90 of 91	18	571	WFWFCLLLLAAGVGIYLL	588	20mg/ml	50ul	M9				M9			
91 of 91	14	578	LLAAGVGIYLLPNR	591	40mg/ml	25ul	M9				M9			

1b (J4) HCV Peptide pools and minipools

BEI Peptides				Gt1-6				Gt1/3			
Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
Pool A										
Core										
1 of 28 18 1 MSTNPKPQRKTKRNTNRR 18	40mg/ml	25ul	A1	MSTNPKPQRKTKRNTIRR	Seg 1	4Q	A1	MSTNPKPQRKTKRNTNRR	Seg 1	1a
2 of 28 18 7 PQRKTKRNTNRRPQDVKF 24	40mg/ml	25ul	A1	PQRKTKRNTNRRPMDVKF	Seg 1	4Q	A1	PQRKTKRNTNRRPQDVKF	Seg 1	1a
3 of 28 18 14 NTNRRPQDVKFPGGGQIV 31	40mg/ml	25ul	A1	NTNRRPMDVKFPGGGQIV	Seg 1	4Q	A1	NTNRRPQDVKFPGGGQIV	Seg 1	1a
4 of 28 17 21 DVKFPGGGQIVGGVYLL 37	40mg/ml	25ul	A1	DVKFPGGGQIVGGVYLL	Seg 1	4Q	A1	DVKFPGGGQIVGGVYLL	Seg 1	1a
5 of 28 18 27 GGQIVGGVYLLPRRGPRL 44	40mg/ml	25ul	A1	GGQIVGGVYLLPRRGPRL	Seg 1	4Q	A1	GGQIVGGVYLLPRRGPRL	Seg 1	1a
6 of 28 18 34 VYLLPRRGPRLGVRATRK 51	40mg/ml	25ul	A2	VYLLPRRGPRLGVRATRK	Seg 1	4Q	A2	VYLLPRRGPRLGVRATRK	Seg 1	1a
7 of 28 15 41 GPRLGVRATRKASER 55	40mg/ml	25ul	A2	GPRLGVRATRKTSER	Seg 1	4Q	A2	GPRLGVRATRKTSER	Seg 1	1a
8 of 28 18 45 GVRATRKASERSQPRGRR 62	40mg/ml	25ul	A2	GVRATRKTSERSQPRGRR	Seg 1	4Q	A2	GVRATRKTSERSQPRGRR	Seg 1	1a
9 of 28 18 52 ASERSQPRGRRQPIPKAR 69	40mg/ml	25ul	A2	TSERSQPRGRRQPIPKAR	Seg 1	4Q	A2	TSERSQPRGRRQPIPKAR	Seg 1	1a
10 of 28 18 59 RGRRQPIPKARRPEGRAW 76	40mg/ml	25ul	A2	RGRRQPIPKARRPEGRSW	Seg 1	4Q	A2	RGRRQPIPKARRPEGRSW	Seg 1	1a
11 of 28 18 66 PKARRPEGRAWAQPGYPW 83	40mg/ml	25ul	A3	PKARRPEGRSWAQPGYPW	Seg 1	4Q	A3	PKARRPEGRSWAQPGYPW	Seg 1	1a
12 of 28 19 73 GRAWAQPGYPWPLYGNEGL 91	40mg/ml	25ul	A3	GRSWAQPGYPWPLYGNEGC	Seg 1	4Q	A3	GRSWAQPGYPWPLYGNEGC	Seg 1	1a
13 of 28 18 81 YPWPLYGNEGLGWAGWLL 98	10mg/ml	100ul	A3	YPWPLYGNEGCGWAGWLL	Seg 1	4Q	A3	YPWPLYGNEGCGWAGWLL	Seg 1	1a
14 of 28 17 88 NEGLGWAGWLLSPRGSR 104	40mg/ml	25ul	A3	NEGCGWAGWLLSPRGSR	Seg 1	4Q	A3	NEGCGWAGWLLSPRGSR	Seg 1	1a
15 of 28 18 94 AGWLLSPRGSRPSWGPTD 111	40mg/ml	25ul	A3	AGWLLSPRGSRPSWGPND	Seg 1	4Q	A3	AGWLLSPRGSRPSWGPTD	Seg 1	1a
16 of 28 17 101 RGSRPSWGPTDPRRRSR 117	40mg/ml	25ul	A4	RGSRPSWGPNDPRRRSR	Seg 1	4Q	A4	RGSRPSWGPTDPRRRSR	Seg 1	1a
17 of 28 17 107 WGPTDPRRRSRNLGKVI 123	40mg/ml	25ul	A4	WGPNDPRRRSRNLGKVI	Seg 1	4Q	A4	WGPTDPRRRSRNLGKVI	Seg 1	1a
18 of 28 18 113 RRRSRNLGKVIDTLTCGF 130	40mg/ml	25ul	A4	RRRSRNLGKVIDTLTCGF	Seg 1	4Q	A4	RRRSRNLGKVIDTLTCGF	Seg 1	1a
19 of 28 18 120 GKVIDTLTCGFADLMGYI 137	40mg/ml	25ul	A4	GKVIDTLTCGFADLMGYI	Seg 1	4Q	A4	GKVIDTLTCGFADLMGYI	Seg 1	1a
20 of 28 18 127 TCGFADLMGYIPLVGAPL 144	40mg/ml	25ul	A4	TCGFADLMGYIPLVGAPV	Seg 1	4Q	A4	TCGFADLMGYIPLVGAPL	Seg 1	1a
21 of 28 18 134 MGYIPLVGAPLGGAARAL 151	40mg/ml	25ul	A5	MGYIPLVGAPVGGVARAL	Seg 1	4Q	A5	MGYIPLVGAPLGGAARAL	Seg 1	1a
22 of 28 18 141 GAPLGGAARALAHGVRVL 158	40mg/ml	25ul	A5	GAPVGGVARALAHGVRAL	Seg 1	4Q	A5	GAPLGGAARALAHGVRVL	Seg 1	1a
23 of 28 18 148 ARALAHGVRVLEDGVNYA 165	40mg/ml	25ul	A5	ARALAHGVRALEDGINYA	Seg 1	4Q	A5	ARALAHGVRVLEDGVNYA	Seg 1	1a
24 of 28 15 155 VRVLEDGVNYATGNL 169	13.3mg/ml	75ul	A5	VRALEDGINYATGNL	Seg 1	4Q	A5	VRVLEDGVNYATGNL	Seg 1	1a
25 of 28 18 159 EDGVNYATGNLPGCSFSI 176	40mg/ml	25ul	A6	EDGINYATGNLPGCSFSI	Seg 1	4Q	A6	EDGVNYATGNLPGCSFSI	Seg 1	1a
26 of 28 17 166 TGNLPGCSFSIFLLALL 182	40mg/ml	25ul	A6	TGNLPGCSFSIFLLALL	Seg 1	4Q	A6	TGNLPGCSFSIFLLALL	Seg 1	1a
27 of 28 18 172 CSFSIFLLALLSCLTIPA 189	40mg/ml	25ul	A6	CSFSIFLLALLSCLTVPA	Seg 1	4Q	A6	CSFSIFLLALLSCLTVPA	Seg 1	1a
28 of 28 13 179 LALLSCLTIPASA 191	10mg/ml	100ul	A6	LALLSCLTVPASA	Seg 1	4Q	A6	LALLSCLTVPASA	Seg 1	1a
Pool B										
E1										
Peptide Length Sequence	**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***
1 of 28 18 1 YEVRNVSGIYHVTNDCSN 18	40mg/ml	25ul								
2 of 28 18 8 GIYHVTNDCSNSSIVYEA 25	40mg/ml	25ul								
3 of 28 17 15 DCSNSSIVYEAADVIMH 31	40mg/ml	25ul								
4 of 28 16 21 IVYEAADVIMHTPGCV 36	40mg/ml	25ul								
5 of 28 18 26 ADVIMHTPGCVPCVQEGN 43	40mg/ml	25ul								
6 of 28 18 33 PGCVPCVQEGNSSRCWVA 50	40mg/ml	25ul								
7 of 28 18 39 VQEGNSSRCWVALTPTLA 56	40mg/ml	25ul								
8 of 28 17 46 RCWVALTPTLAARNASV 62	40mg/ml	25ul								

9 of 28	18 52 TPTLAARNASVPTTTIRR 69	40mg/ml	25ul								
10 of 28	17 59 NASVPTTTIRRHVDLLV 75	40mg/ml	25ul								
11 of 28	16 65 TTIRRHVDLLVGTAAF 80	40mg/ml	25ul						TTIRRHVDLLVGAATM	Seg 2	3a
12 of 28	17 70 HVDLLVGTAAFCSAMYV 86	40mg/ml	25ul						HVDLLVGAATMCSALYV	Seg 2	3a
13 of 28	18 76 GTAAFCSAMYVGDLCGSI 93	40mg/ml	25ul						GAATLCSALYVGDMCGSV	Seg 2	3a
14 of 28	18 83 AMYVGDLCGSIFLVSQLF 100	40mg/ml	25ul						ALYVGDMCGSIFLVSQLF	Seg 2	3a
15 of 28	18 90 CGSIFLVSQLFTFSPRRH 107	40mg/ml	25ul								
16 of 28	18 97 SQLFTFSPRRHETVQDCN 114	40mg/ml	25ul								
17 of 28	18 104 PRRHETVQDCNCSIYPGH 121	40mg/ml	25ul								
18 of 28	18 110 VQDCNCSIYPGHVSGHRM 127	40mg/ml	25ul		VQDCNCSIYPGHITGHRM	Seg 2	1a				
19 of 28	17 117 IYPGHVSGHRMAWDMMM 133	40mg/ml	25ul		IYPGHITGHRMAWDMMM	Seg 2	1a				
20 of 28	18 123 SGHRMAWDMMMNWSPTTA 140	40mg/ml	25ul		TGHRMAWDMMMNWSPTTA	Seg 2	1a				
21 of 28	18 130 DMMMNWSPTTALVVSQLL 147	40mg/ml	25ul								
22 of 28	18 137 PTTALVVSQLLRIPQAVV 154	40mg/ml	25ul								
23 of 28	18 144 SQLLRIPQAVVDMVAGAH 161	40mg/ml	25ul								
24 of 28	17 150 PQAVVDMVAGAHWGVLA 166	40mg/ml	25ul								
25 of 28	18 156 MVAGAHWGVLAGLAYYSM 173	40mg/ml	25ul						MVAGAHWGVLAGLAYYSM	Seg 3	3a
26 of 28	18 163 GVLAGLAYYSMVGNWAKV 180	40mg/ml	25ul						GVLAGLAYYSMQGNWAKV	Seg 3	3a
27 of 28	18 170 YYSMVGNWAKVLIVALLF 187	40mg/ml	25ul						YYSMQGNWAKVLIVALLF	Seg 3	3a
28 of 28	16 177 WAKVLIVALLFAGVDG 192	10mg/ml	100ul								

Pool C

E2

Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 55 16 1 ETHTTGRVAGHTTSGF 16	40mg/ml	25ul								
2 of 55 15 6 GRVAGHTTSGFTSLF 20	40mg/ml	25ul								
3 of 55 18 10 GHTTSGFTSLFSSGASQK 27	40mg/ml	25ul								
4 of 55 15 17 TSLFSSGASQKIQLV 31	40mg/ml	25ul								
5 of 55 18 21 SSGASQKIQLVNTNGSWH 38	40mg/ml	25ul								
6 of 55 17 28 IQLVNTNGSWHINRTAL 44	40mg/ml	25ul	C1	IQLVNTNGSWHINRTAL	Seg 3	2b				
7 of 55 17 34 NGSWHINRTALNCNDSL 50	40mg/ml	25ul	C1	NGSWHINRTALNCNDSL	Seg 3	2b				
8 of 55 18 40 NRTALNCNDSLQTGFFAA 57	40mg/ml	25ul	C1	NRTALNCNDSLNTGFIAA	Seg 3	2b				
9 of 55 18 47 NDSLQTGFFAALFYAHKF 64	40mg/ml	25ul	C1	NDSLNTGFIAALFYAHKF	Seg 3	2b				
10 of 55 19 54 FFAALFYAHKFNSSGCPER 72	40mg/ml	25ul								
11 of 55 18 62 HKFNSSGCPERMASCRPI 79	40mg/ml	25ul								
12 of 55 18 69 CPERMASCRPIDWFAQGW 86	40mg/ml	25ul								
13 of 55 18 76 CRPIDWFAQGWGPITYTK 93	40mg/ml	25ul								
14 of 55 18 83 AQGWGPITYTKPNSSDQR 100	40mg/ml	25ul								
15 of 55 18 90 TYTKPNSSDQRPYCWHYA 107	40mg/ml	25ul								
16 of 55 18 97 SDQRPYCWHYAPRPCGVV 114	40mg/ml	25ul								
17 of 55 16 104 WHYAPRPCGVVPASQV 119	40mg/ml	25ul								
18 of 55 18 109 RPCGVVPASQVCGPVYCF 126	40mg/ml	25ul								
19 of 55 18 116 ASQVCGPVYCFTPSPVVV 133	40mg/ml	25ul	C2	ASSVCGPVYCFTPSPVVV	Seg 4	4a	C2	ASSVCGPVYCFTPSPVVV	Seg 4	1a
20 of 55 16 123 VYCFTPSPVVVGTTDR 138	40mg/ml	25ul	C2	VYCFTPSPVVVGTTDR	Seg 4	4a	C2	VYCFTPSPVVVGTTDR	Seg 4	1a
21 of 55 17 128 PSPVVVGTTDRSGVPTY 144	40mg/ml	25ul	C2	PSPVVVGTTDRSGVPTY	Seg 4	4a	C2	PSPVVVGTTDRSGVPTY	Seg 4	1a
22 of 55 18 134 GTTDRSGVPTYSWGENET 151	40mg/ml	25ul								

23 of 55	16	141	VPTYSWGENETDVMLL 156	40mg/ml	25ul								
24 of 55	15	146	WGENETDVMLLNNTR 160	40mg/ml	25ul								
25 of 55	18	150	ETDVMLLNNTRPPQGNWF 167	40mg/ml	25ul								
26 of 55	16	157	NNTRPPQGNWFGCTWM 172	40mg/ml	25ul								
27 of 55	18	162	PQGNWFGCTWMNSTGFTK 179	40mg/ml	25ul								
28 of 55	18	169	CTWMNSTGFTKTCGGPPC 186	40mg/ml	25ul								
29 of 55	16	176	GFTKTCGGPPCNIGGV 191	40mg/ml	25ul								
30 of 55	17	181	CGGPPCNIGGVGNRTLI 197	40mg/ml	25ul								
31 of 55	18	187	NIGGVGNRTLICPTDCFR 204	40mg/ml	25ul								
32 of 55	18	194	RTLICPTDCFRKHPEATY 211	40mg/ml	25ul	C3	RTLICPTDCFRKHPEATY	Seg 5	1b				
33 of 55	19	201	DCFRKHPEATYTKCGSGPW 219	40mg/ml	25ul	C3	DCFRKHPEATYTKCGSGPW	Seg 5	1b				
34 of 55	18	209	ATYTKCGSGPWLTPRCLV 226	40mg/ml	25ul	C3	ATYTKCGSGPWLTPRCLV	Seg 5	1b	C3	ATYTRCGSGPWLTPRCLV	Seg 5	1a
35 of 55	18	216	SGPWLTPRCLVDYPYRLW 233	40mg/ml	25ul	C3	SGPWLTPRCLVDYPYRLW	Seg 5	1b	C3	SGPWLTPRCLVDYPYRLW	Seg 5	1a
36 of 55	17	223	RCLVDYPYRLWHYPCTL 239	40mg/ml	25ul	C3	CLVDYPYRLWHYPCTV	Seg 5	1b	C3	CLVDYPYRLWHYPCTL	Seg 5	1a
37 of 55	18	229	PYRLWHYPCTLNFSIFKV 246	40mg/ml	25ul	C3	PYRLWHYPCTVNFSIFKV	Seg 5	1b	C3	PYRLWHYPCTLNFSIFKV	Seg 5	1a
38 of 55	18	236	PCTLNFSIFKVRMYVGGV 253	40mg/ml	25ul								
39 of 55	18	243	IFKVRMYVGGVEHRLNAA 260	40mg/ml	25ul								
40 of 55	16	250	VGGVEHRLNAACNWTR 265	40mg/ml	25ul								
41 of 55	17	255	HRLNAACNWTRGERCNL 271	40mg/ml	25ul						HRLNAACNWTRGERCDI	Seg 6	3a
42 of 55	16	261	CNWTRGERCNLEDRDR 276	40mg/ml	25ul						CNWTRGERCDIEDRDR	Seg 6	3a
43 of 55	18	266	GERCNLEDRDRSELSPLL 283	40mg/ml	25ul						GERCDIEDRDRSELSPLL	Seg 6	3a
44 of 55	17	273	DRDRSELSPLLLSTTEW 289	40mg/ml	25ul	C4	DRDRSELSPLLLSTTEW	Seg 6	1b				
45 of 55	18	279	LSPLLLSTTEWQILPCAF 296	40mg/ml	25ul	C4	LSPLLLSTTEWQILPCSF	Seg 6	1b		LSPLLLSTTELAILPCSF	Seg 7	3a
46 of 55	17	286	TTEWQILPCAFTTLPAL 302	40mg/ml	25ul	C4	TTEWQILPCSFTTLPAL	Seg 6	1b		TTELAILPCSFTPLPAL	Seg 7	3a
47 of 55	18	292	LPCAFTTLPALSTGLIHL 309	40mg/ml	25ul	C4	LPCSFTTLPALSTGLIHL	Seg 6	1b		LPCSFTPLPALSTGLIHL	Seg 7	3a
48 of 55	18	299	LPALSTGLIHLHQNIVDV 316	40mg/ml	25ul	C4	LPALSTGLIHLHQNIVDV	Seg 6	1b		LPALSTGLIHLHQNIVDV	Seg 7	3a

Pool C continued

E2

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
49 of 55	17	306 LIHLHQNIVDVQYLYGV 322	40mg/ml	25ul	C4	LIHLHQNIVDVQYLYGV	Seg 6	1b		LIHLHQNIVDVQYLYGV	Seg 7	3a
50 of 55	18	312 NIVDVQYLYGVGSAFVSF 329	40mg/ml	25ul	C4	NIVDVQYLYGVGSAFVSF	Seg 6	1b		NIVDVQYLYGVGSGMVSF	Seg 7	3a
51 of 55	18	319 LYGVGSAFVSFAIKWEYI 336	40mg/ml	25ul						LYGVGSGMVSFAIKWEYV	Seg 7	3a
52 of 55	18	326 FVSFAIKWEYILLLFLLL 343	40mg/ml	25ul								
53 of 55	18	333 WEYILLLFLLLADARVCA 350	40mg/ml	25ul								
54 of 55	18	340 FLLLADARVCACLWMMLL 357	40mg/ml	25ul								
55 of 55	17	347 RVCACLWMMLLIAQAEA 363	20mg/ml	50ul								

Pool D

p7

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 8	17	1 ALENLVVLNAASVAGAH 17	40mg/ml	25ul								
2 of 8	18	7 VLNAASVAGAHGILSFLV 24	40mg/ml	25ul								
3 of 8	18	14 AGAHGILSFLVFFCAAWY 31	40mg/ml	25ul								
4 of 8	17	21 SFLVFFCAAWYIKGRLA 37	40mg/ml	25ul								
5 of 8	18	27 CAAWYIKGRLAPGAAYAF 44	40mg/ml	25ul								

6 of 8	18 34 GRLAPGAAYAFYGVWPLL 51	40mg/ml	25ul									
7 of 8	17 41 AYAFYGVWPLLLLLLAL 57	40mg/ml	25ul									
8 of 8	17 47 VWPLLLLLLALPPRAYA 63	40mg/ml	25ul									
NS2												
Peptide Length Sequence		Concentration										
1 of 32	18 1 LDREMAASCGGAVLVGLV 18	40mg/ml	25ul									
2 of 32	18 8 SCGGAVLVGLVFLTLSPY 25	40mg/ml	25ul									
3 of 32	18 15 VGLVFLTLSPYYKVFLTR 32	40mg/ml	25ul									
4 of 32	18 22 LSPYYKVFLTRLIWWLQY 39	40mg/ml	25ul									
5 of 32	18 29 FLTRLIWWLQYFITRAEA 46	40mg/ml	25ul									
6 of 32	17 36 WLQYFITRAEAHMQVWV 52	40mg/ml	25ul									
7 of 32	17 42 TRAEAHMQVWVPPLNVR 58	40mg/ml	25ul									
8 of 32	18 48 MQVWVPPLNVRGGRDAII 65	40mg/ml	25ul									
9 of 32	18 55 LNVRGGRDAIILLTCAVH 72	40mg/ml	25ul									
10 of 32	18 62 DAIILLTCAVHPELIFDI 79	40mg/ml	25ul									
11 of 32	18 69 CAVHPELIFDITKLLLAI 86	40mg/ml	25ul									
12 of 32	18 76 IFDITKLLLAILGPLMVL 93	20mg/ml	50ul									
13 of 32	18 83 LLAILGPLMVLQAGITRV 100	40mg/ml	25ul									
14 of 32	17 90 LMVLQAGITRVPYFVRA 106	40mg/ml	25ul									
15 of 32	17 96 GITRVPYFVRAQGLIRA 112	40mg/ml	25ul									
16 of 32	18 102 YFVRAQGLIRACMLVRKV 119	40mg/ml	25ul									
17 of 32	17 109 LIRACMLVRKVAGGHYV 125	40mg/ml	25ul									
18 of 32	18 115 LVRKVAGGHYVQMVFMKL 132	40mg/ml	25ul									
19 of 32	18 122 GHYVQMVFMKLGALTGTY 139	40mg/ml	25ul									
20 of 32	16 129 FMKLGALTGTYVYNHL 144	40mg/ml	25ul									
21 of 32	18 134 ALTGTYVYNHLTPLRDWA 151	40mg/ml	25ul									
22 of 32	18 141 YNHLTPLRDWAHAGLRDL 158	40mg/ml	25ul									
23 of 32	18 148 RDWAHAGLRDLAVAVEPV 165	40mg/ml	25ul									
24 of 32	16 155 LRDLAVAVEPVVFSAM 170	13.3mg/ml	75ul									
25 of 32	18 160 VAVEPVVFSAMETKVITW 177	40mg/ml	25ul									
26 of 32	17 167 FSAMETKVITWGADTAA 183	40mg/ml	25ul									
27 of 32	17 173 KVITWGADTAACGDIIL 189	40mg/ml	25ul									
28 of 32	18 179 ADTAACGDIILGLPVSAR 196	40mg/ml	25ul									
29 of 32	18 186 DIILGLPVSARRGKEIFL 203	40mg/ml	25ul									
30 of 32	17 193 VSARRGKEIFLGPADSL 209	40mg/ml	25ul									
31 of 32	18 199 KEIFLGPADSLEGQGWRL 216	40mg/ml	25ul									
32 of 32	12 206 ADSLEGQGWRLL 217	40mg/ml	25ul		PADSLEGMGWRLL	Seg 7	3a		PADSLEGMGWRLL	Seg 8	3a	
	Pool F											
NS3												
Peptide Length Sequence		**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***	
1 of 98	18 1 APITAYSQQTRGVLGCII 18	40mg/ml	25ul	F1	APITAYAQQTRGLLGTIV	Seg 7	3a	F1	APITAYAQQTRGLLGTIV	Seg 8	3a	
2 of 98	18 7 SQQTRGVLGCIITSLTGR 24	40mg/ml	25ul	F1	AQQTRGLLGTIVTSLTGR	Seg 7	3a	F1	AQQTRGLLGTIVTSLTGR	Seg 8	3a	
3 of 98	16 14 LGCIITSLTGRDKNQV 29	20mg/ml	25ul	F1	LGTIVTSLTGRDKNQV	Seg 7	3a	F1	LGTIVTSLTGRDKNVV	Seg 8	3a	
4 of 98	18 19 TSLTGRDKNQVEGEVQVV 36	40mg/ml	25ul					F1	SLTGRDKNVVTGEVQVL	Seg 8	3a	
5 of 98	18 26 KNQVEGEVQVVSTATQSF 43	40mg/ml	25ul	F2				F1	KNVVTGEVQVLSTATQSF	Seg 8	3a	
6 of 98	16 33 VQVVSTATQSFLATCI 48	40mg/ml	25ul	F2								

7 of 98	18 38	TATQSFLATCINGVCWTV 55	40mg/ml	25ul		F2					F2			
8 of 98	18 45	ATCINGVCWTVYHGAGSK 62	40mg/ml	25ul		F2					F2			
9 of 98	17 52	CWTVYHGAGSKTLAGPK 68	40mg/ml	25ul		F2					F2			
10 of 98	18 58	GAGSKTLAGPKGPITQMY 75	40mg/ml	25ul		F2					F2			
11 of 98	18 65	AGPKGPITQMYTNVDLDL 82	40mg/ml	25ul		F2					F2			
12 of 98	16 72	TQMYTNVDLDLVGWQA 87	40mg/ml	25ul		F2					F2			
13 of 98	18 77	NVDLDLVGWQAPPGARSM 94	40mg/ml	25ul		F2					F2			
14 of 98	18 84	GWQAPPGARSMTPCSCGS 101	40mg/ml	25ul		F2					F2			
15 of 98	17 91	ARSMTPCSCGSSDLYLV 107	40mg/ml	25ul	F3		ARSMTPCTCGSSDLYLV	Seg 8	1a	F3		ARSMTPCTCGSADLYLV	Seg 9	3a
16 of 98	18 97	CSCGSSDLYLVTRHADVI 114	40mg/ml	25ul	F3		CTCGSSDLYLVTRHADVI	Seg 8	1a	F3		CTCGSSDLYLVTRDADVI	Seg 9	3a
17 of 98	16 104	LYLVTRHADVIPVRRR 119	40mg/ml	25ul	F3		LYLVTRHADVIPVRRR	Seg 8	1a	F3		LYLVTRDADVIPARRR	Seg 9	3a
18 of 98	18 109	RHADVIPVRRRGDSRGSL 126	20mg/ml	25ul	F3		RHADVIPVRRRGDSRGSL	Seg 8	1a	F3		RDADVIPARRRGDSTASL	Seg 9	3a
19 of 98	17 116	VRRRGDSRGSLLSPRPV 132	40mg/ml	25ul	F3		VRRRGDSRGSLLSPRPI	Seg 8	1a	F3		ARRRGDSTASLLSPRPI	Seg 9	3a
20 of 98	15 122	SRGSLLSPRPVSYLK 136	40mg/ml	25ul	F3		SRGSLLSPRPISYLK	Seg 8	1a	F3		STASLLSPRPLACLK	Seg 9	3a
21 of 98	18 126	LLSPRPVSYLKGSSGGPL 143	40mg/ml	25ul	F3		LLSPRPISYLKGSSGGPV	Seg 8	1a	F3		LLSPRPLACLKGSSGGPV	Seg 9	3a
22 of 98	18 133	SYLKGSSGGPLLCPSGHV 150	40mg/ml	25ul	F3		SYLKGSSGGPVLCPSGHA	Seg 8	1a	F3		ACLKGSSGGPVMCPSGHV	Seg 9	3a
23 of 98	18 140	GGPLLCPSGHVVGVFRAA 157	40mg/ml	25ul	F3		GGPVLCPSGHAVGIFRAA	Seg 8	1a	F3		GGPVMCPSGHVAGIFRAA	Seg 9	3a
24 of 98	18 147	SGHVVGVFRAAVCTRGVA 164	40mg/ml	25ul	F3		SGHAVGIFRAAVCTRGVA	Seg 8	1a	F3		SGHVAGIFRAAVCTRGVA	Seg 9	3a
25 of 98	17 154	FRAAVCTRGVAKAVDFI 170	40mg/ml	25ul	F3		FRAAVCTRGVAKAVDFI	Seg 8	1a	F3		FRAAVCTRGVAKALQFI	Seg 9	3a
					F3		TRGVAKAVDFIPVESL	Seg 8	1a	F3		TRGVAKALQFIPVETL	Seg 9	3a
27 of 98	16 165	KAVDFIPVESMETTMR 180	40mg/ml	25ul			KAVDFIPVESLETEMR	Seg 8	1a	F3		KALQFIPVETLETTMR	Seg 9	3a
28 of 98	15 170	IPVESMETTMRSPVF 184	40mg/ml	25ul										
29 of 98	19 174	SMETTMRSPVFTDNSTPPA 192	40mg/ml	25ul	F4		SLETEMRSPVFTDNSTPPA	Seg 9	4a	F4		TLETTMRSPVFTDNSSPPA	Seg 10	1a
30 of 98	18 182	PVFTDNSSPPAVPQTFQV 199	40mg/ml	25ul	F4		PVFTDNSTPPAVPQTYQV	Seg 9	4a	F4		PVFTDNSSPPAVPQSYQV	Seg 10	1a
31 of 98	16 189	SPPAVPQTFQVAHLHA 204	40mg/ml	25ul	F4		TPPAVPQTYQVAHLHA	Seg 9	4a	F4		SPPAVPQSYQVAHLHA	Seg 10	1a
32 of 98	17 194	PQTFQVAHLHAPTGSGK 210	40mg/ml	25ul	F4		PQTYQVAHLHAPTGSGK	Seg 9	4a	F4		PQSYQVAHLHAPTGSGK	Seg 10	1a
33 of 98	18 200	AHLHAPTGSGKSTKVPAA 217	40mg/ml	25ul	F4		AHLHAPTGSGKSTKVPAA	Seg 9	4a	F4		AHLHAPTGSGKSTKVPAA	Seg 10	1a
34 of 98	18 207	GSGKSTKVPAAYAAQGYK 224	40mg/ml	25ul	F4		GSGKSTKVPAAYAAQGYK	Seg 9	4a	F4		GSGKSTKVPAAYAAQGYK	Seg 10	1a
35 of 98	15 214	VPAAYAAQGYKVLVL 228	20mg/ml	50ul	F4		VPAAYAAQGYKVLVL	Seg 9	4a	F4		VPAAYAAQGYKVLVL	Seg 10	1a
36 of 98	17 218	YAAQGYKVLVLNPSVAA 234	40mg/ml	25ul	F4		YAAQGYKVLVLNPSVAA	Seg 9	4a	F4		YAAQGYKVLVLNPSVAA	Seg 10	1a
37 of 98	18 224	KVLVLNPSVAATLGFGAY 241	40mg/ml	25ul	F4		KVLVLNPSVAATLGFGAY	Seg 9	4a	F4		KVLVLNPSVAATLGFGAY	Seg 10	1a
38 of 98	18 231	SVAATLGFGAYMSKAHGI 248	40mg/ml	25ul	F4		SVAATLGFGAYMSKAYGI	Seg 9	4a	F4		SVAATLGFGAYMSKAHGI	Seg 10	1a
39 of 98	16 238	FGAYMSKAHGIDPNIR 253	40mg/ml	25ul			FGAYMSKAYGIDPNIR	Seg 9	4a					
40 of 98	17 243	SKAHGIDPNIRTGVRTI 259	40mg/ml	25ul										
41 of 98	17 249	DPNIRTGVRTITTGGSI 265	40mg/ml	25ul	F5		DPNIRSGVRTITTGAPI	Seg 10	4a	F5		DPNIRTGNRTITTGAKL	Seg 11	3a
42 of 98	18 255	GVRTITTGGSITYSTYGK 272	40mg/ml	25ul	F5		GVRTITTGAPITYSTYGK	Seg 10	4a	F5		GVRTITTGAKLTYSTYGK	Seg 11	3a
43 of 98	18 262	GGSITYSTYGKFLADGGC 279	40mg/ml	25ul	F5		GAPITYSTYGKFLADGGC	Seg 10	4a	F5		GAKLTYSTYGKFLADGGC	Seg 11	3a
44 of 98	18 269	TYGKFLADGGCSGGAYDI 286	40mg/ml	25ul	F5		YGKFLADGGCSGGAYDI	Seg 10	4a	F5		TYGKFLADGGCSGGAYDV	Seg 11	3a
45 of 98	18 276	DGGCSGGAYDIIICDECH 293	40mg/ml	25ul	F5		DGGCSGGAYDIIICDECH	Seg 10	4a	F5		DGGCSGGAYDVIICDECH	Seg 11	3a
46 of 98	18 283	AYDIIICDECHSTDSTTI 300	40mg/ml	25ul	F5		AYDIIICDECHSTDSTTI	Seg 10	4a	F5		AYDVIICDECHAQDATSI	Seg 11	3a
47 of 98	18 290	DECHSTDSTTILGIGTVL 307	20mg/ml	50ul	F5		DECHSTDSTTILGIGTVL	Seg 10	4a	F5		DECHAQDATSILGIGTVL	Seg 11	3a
48 of 98	17 297	STTILGIGTVLDQAETA 313	40mg/ml	25ul	F5		STTILGIGTVLDQAETA	Seg 10	4a	F5		ATSILGIGTVLDQAETA	Seg 11	3a
49 of 98	18 303	IGTVLDQAETAGARLVVL 320	40mg/ml	25ul	F5		IGTVLDQAETAGVRLVVL	Seg 10	4a	F5		IGTVLDQAETAGVRLTVL	Seg 11	3a
50 of 98	18 310	AETAGARLVVLATATPPG 327	40mg/ml	25ul	F5		AETAGVRLVVLATATPPG	Seg 10	4a	F5		AETAGVRLTVLATATPPG	Seg 11	3a

Pool G

NS3											
Peptide Length Sequence			Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
51 of 98	17 317	LVVLATATPPGSVTVPH 333	40mg/ml	25ul	G1					TVLATATPPGSITVPH	Seg 11	3a
52 of 98	17 323	ATPPGSVTVPHPNIEEI 339	40mg/ml	25ul	G1					ATPPGSITVPHSNIEEI	Seg 11	3a
53 of 98	19 329	VTVPHPNIEEIGLSNNGEI 347	40mg/ml	25ul	G1					ITVPHSNIEEIGLSNNGEI	Seg 11	3a
54 of 98	18 337	EEIGLSNNGEIPFYGKAI 354	40mg/ml	25ul	G1							
55 of 98	17 344	NGEIPFYGKAIPIEAIK 360	40mg/ml	25ul	G1							
56 of 98	18 350	YGKAIPIEAIKGGRHLIF 367	40mg/ml	25ul		YGKAIPIEAIKGGRHLIF	Seg 11	1a				
57 of 98	17 357	EAIKGGRHLIFCHSKKK 373	40mg/ml	25ul	G2	EVIKGGRHLIFCHSKKK	Seg 11	1a				
58 of 98	18 363	RHLIFCHSKKKCDELAAK 380	40mg/ml	25ul	G2	RHLIFCHSKKKCDELAAK	Seg 11	1a				
59 of 98	17 370	SKKKCDELAAKLTGLGL 386	40mg/ml	25ul	G2	SKKKCDELAAKLTGLGL	Seg 11	1a				
60 of 98	18 376	ELAAKLTGLGLNAVAYYR 393	40mg/ml	25ul								
61 of 98	18 383	GLGLNAVAYYRGLDVSVI 400	40mg/ml	25ul	G3	GLGLNAVAYYRGLDVSVI	Seg 12	1b				
62 of 98	18 390	AYYRGLDVSVIPPIGDVV 407	40mg/ml	25ul	G3	AYYRGLDVSVIPTSGDVV	Seg 12	1b		AYYRGLDVSVIPTAGDVV	Seg 12	3a
63 of 98	18 397	VSVIPPIGDVVVVATDAL 414	40mg/ml	25ul	G3	VSVIPTSGDVVVVATDAL	Seg 12	1b		VSVIPTAGDVVVCATDAL	Seg 12	3a
64 of 98	15 404	GDVVVVATDALMTGF 418	40mg/ml	25ul	G3	GDVVVVATDALMTGF	Seg 12	1b		GDVVVCATDALMTGF	Seg 12	3a
65 of 98	18 408	VVATDALMTGFTGDFDSV 425	40mg/ml	25ul	G3	VVATDALMTGFTGDFDSV	Seg 12	1b		VCATDALMTGFTGDFDSV	Seg 12	3a
66 of 98	18 415	MTGFTGDFDSVIDCNTCV 432	40mg/ml	25ul	G3	MTGFTGDFDSVIDCNTCV	Seg 12	1b		MTGFTGDFDSVIDCNVAV	Seg 12	3a
67 of 98	17 422	FDSVIDCNTCVTQTVDF 438	40mg/ml	25ul	G3	FDSVIDCNTCVTQTVDF	Seg 12	1b		FDSVIDCNVAVEQYVDF	Seg 12	3a
68 of 98	17 428	CNTCVTQTVDFSLDPTF 444	40mg/ml	25ul	G3	CNTCVTQTVDFSLDPTF	Seg 12	1b		CNVAVEQYVDFSLDPTF	Seg 12	3a
69 of 98	19 433	TQTVDFSLDPTFTIETTTV 451	40mg/ml	25ul	G3	TQTVDFSLDPTFTIETTTV	Seg 12	1b		EQYVDFSLDPTFSIETRTA	Seg 12	3a
70 of 98	18 441	DPTFTIETTTVPQDAVSR 458	40mg/ml	25ul	G3	DPTFTIETTTVPQDAVSR	Seg 12	1b		DPTFSIETRTAPQDAVSR	Seg 12	3a
71 of 98	17 448	TTTVPQDAVSRSQRRGR 464	40mg/ml	25ul	G3	TTTVPQDAVSRSQRRGR	Seg 12	1b		TRTAPQDAVSRSQRRGR	Seg 12	3a
72 of 98	16 454	DAVSRSQRRGRTGRGR 469	40mg/ml	25ul	G3	DAVSRSQRRGRTGRGR	Seg 12	1b		DAVSRSQRRGRTGRGR	Seg 12	3a
73 of 98	18 459	SQRRGRTGRGRSGIYRFV 476	40mg/ml	25ul	G3	SQRRGRTGRGRRGIYRFV	Seg 12	1b		SQRRGRTGRGRLGTYRYV	Seg 12	3a
74 of 98	16 466	GRGRSGIYRFVTPGER 481	40mg/ml	25ul	G3	GRGRRGIYRFVTPGER	Seg 12	1b		GRGRLGTYRYVGPGER	Seg 12	3a
75 of 98	16 471	GIYRFVTPGERPSGMF 486	40mg/ml	25ul	G3	GIYRFVTPGERPSGMF	Seg 12	1b		GTYRYVGPGERPSGMF	Seg 12	3a
76 of 98	16 476	VTPGERPSGMFDSSVL 491	40mg/ml	25ul	G3	VTPGERPSGMFDSSVL	Seg 12	1b		VAPGERPSGMFDSVVL	Seg 12	3a
77 of 98	17 481	RPSGMFDSSVLCECYDA 497	40mg/ml	25ul	G4	RPSGMFDSSVLCECYDA	Seg 12	1b		RPSGMFDSVVLCECYDA	Seg 12	3a
78 of 98	18 487	DSSVLCECYDAGCAWYEL 504	40mg/ml	25ul	G4	DSSVLCECYDAGCAWYEL	Seg 12	1b		DSVVLCECYDAGCAWYEL	Seg 12	3a
79 of 98	18 494	CYDAGCAWYELTPAETSV 511	40mg/ml	25ul	G4	CYDAGCAWYELTPAETSV	Seg 12	1b		CYDAGCAWYELQPAETTV	Seg 12	3a
80 of 98	17 501	WYELTPAETSVRLRAYL 517	40mg/ml	25ul	G4	WYELTPAETSVRLRAYL	Seg 12	1b		WYELQPAETTVRLRAYL	Seg 12	3a
81 of 98	18 507	AETSVRLRAYLNTPGLPV 524	40mg/ml	25ul	G4	AETSVRLRAYLNTPGLPV	Seg 12	1b		AETTVRLRAYLSTPGLPV	Seg 12	3a
82 of 98	18 514	RAYLNTPGLPVCQDHLEF 531	40mg/ml	25ul	G4	RAYLNTPGLPVCQDHLEF	Seg 12	1b		RAYLSTPGLPVCQDHLDF	Seg 12	3a
83 of 98	16 521	GLPVCQDHLEFWESVF 536	40mg/ml	25ul	G4	GLPVCQDHLEFWEGVF	Seg 12	1b		GLPVCQDHLDFWESVF	Seg 12	3a
84 of 98	18 525	CQDHLEFWESVFTGLTHI 542	40mg/ml	25ul	G4	CQDHLEFWEGVFTGLTHI	Seg 12	1b		CQDHLDFWESVFTGLTHI	Seg 12	3a
85 of 98	16 532	WESVFTGLTHIDAHFL 547	40mg/ml	25ul	G4	WEGVFTGLTHIDAHFL	Seg 12	1b		WESVFTGLTHIDAHFL	Seg 12	3a
86 of 98	17 537	TGLTHIDAHFLSQTKQA 553	40mg/ml	25ul	G4	TGLTHIDAHFLSQTK	Seg 12	1b		TGLTHIDAHFLSQTK	Seg 12	3a
87 of 98	18 543	DAHFLSQTKQAGDNFPYL 560	40mg/ml	25ul	G4	DAHFLSQTKQAGDNFPY	Seg 12	1b		DAHFLSQTKQQGLNFPY	Seg 12	3a
88 of 98	18 550	TKQAGDNFPYLVAYQATV 567	40mg/ml	25ul	G4	TKQAGDNFPYLVAYQATV	Seg 12	1b		TKQQGLNFPYLTAYQATV	Seg 12	3a
89 of 98	17 557	FPYLVAYQATVCARAQA 573	40mg/ml	25ul	G4	FPYLVAYQATVCARAQA	Seg 12	1b		FPYLTAYQATVCARAQA	Seg 12	3a
90 of 98	16 563	YQATVCARAQAPPPSW 578	40mg/ml	25ul	G4	YQATVCARAQAPPPSW	Seg 12	1b		QATVCARAQAPPPSW	Seg 12	3a
91 of 98	18 568	CARAQAPPPSWDQMWKCL 585	40mg/ml	25ul	G4	CARAQAPPPSWDQMWKCL	Seg 12	1b		CARAQAPPPSWDEMWKCL	Seg 12	3a
92 of 98	18 575	PPSWDQMWKCLIRLKPTL 592	40mg/ml	25ul	G4	PPSWDQMWKCLIRLKPTL	Seg 12	1b		PPSWDEMWKCLVRLKPTL	Seg 12	3a
93 of 98	18 582	WKCLIRLKPTLHGPTPLL 599	40mg/ml	25ul	G5					WKCLVRLKPTLHGPTPLL	Seg 12	3a
94 of 98	17 589	KPTLHGPTPLLYRLGAV 605	40mg/ml	25ul	G5 (Gh1)					KPTLHGPTPLLYRLGPV	Seg 12	3a

95 of 98	17 595 PTPLLYRLGAVQNEVIL 611	40mg/ml	25ul	(Gh2)					PTPLLYRLGPVQNEVIL	Seg 12	3a
96 of 98	18 601 RLGAVQNEVILTHPITKY 618	40mg/ml	25ul	H1 (Gh3)	RLGAVQNEVILTHPITKY	Seg 13	4a				
97 of 98	18 608 EVILTHPITKYIMACMSA 625	40mg/ml	25ul	H1 (Gh4)	EVTLTHPITKYIMACMSA	Seg 13	4a				
98 of 98	17 615 ITKYIMACMSADLEVVT 631	40mg/ml	25ul	H1 (Gh5)	ITKYIMACMSADLEVVT	Seg 13	4a	H1	ITKYIMACMSADLEVTT	Seg 13	3a

Pool H

NS4A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 7	18 1	STWVLVGGVLAALAAYCL 18	13.3mg/ml	75ul	H1	STWVLVGGVLAALAAYCL	Seg 13	4a	H1	STWVLLGGVLAALAAYCL	Seg 13	3a
2 of 7	18 8	GVLAALAAYCLTTGSVVI 25	20mg/ml	50ul	H1	GVLAALAAYCLSVGSVVI	Seg 13	4a	H1	GVLAALAAYCLSVGCVVI	Seg 13	3a
3 of 7	17 15	AYCLTTGSVVIVGRIIL 31	40mg/ml	25ul	H1	AYCLSVGSVVIVGRIIL	Seg 13	4a	H1	AYCLSVGCVVIVGHIIL	Seg 13	3a
4 of 7	18 21	GSVVIVGRIILSGKPAVV 38	40mg/ml	25ul								
5 of 7	18 28	RIILSGKPAVVPDREVLY 45	40mg/ml	25ul	H2							
6 of 7	17 35	PAVVPDREVLYQEFDEM 51	40mg/ml	25ul	H2							
7 of 7	14 41	REVLYQEFDEMEEC 54	20mg/ml	50ul	H2							

NS4B

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 40	18 1	ASQLPYIEQGMQLAEQFK 18	40mg/ml	25ul	H2				H2			
2 of 40	18 8	EQGMQLAEQFKQKALGLL 25	40mg/ml	25ul	H2				H2			
3 of 40	18 15	EQFKQKALGLLQTATKQA 32	40mg/ml	25ul	H2				H2			
4 of 40	18 22	LGLLQTATKQAEAAAPVV 39	40mg/ml	25ul	H2				H2			
5 of 40	18 29	TKQAEAAAPVVESKWRAL 46	40mg/ml	25ul	H2				H2			
6 of 40	18 36	APVVESKWRALETFWAKH 53	40mg/ml	25ul								
7 of 40	16 43	WRALETFWAKHMWNFI 58	40mg/ml	25ul		WRALETFWAKHMWNFI	Seg 14	1a		WRALETFWAKHMWNFI	Seg 14	1a
8 of 40	18 48	TFWAKHMWNFISGIQYLA 65	40mg/ml	25ul	H3	TFWAKHMWNFISGIQYLA	Seg 14	1a	H3	TFWAKHMWNFISGIQYLA	Seg 14	1a
9 of 40	16 55	WNFISGIQYLAGLSTL 70	40mg/ml	25ul	H3	WNFISGIQYLAGLSTL	Seg 14	1a	H3	WNFISGIQYLAGLSTL	Seg 14	1a
10 of 40	18 60	GIQYLAGLSTLPGNPAIA 77	40mg/ml	25ul	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a
11 of 40	18 67	LSTLPGNPAIASLMAFTA 84	40mg/ml	25ul	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a
12 of 40	17 74	PAIASLMAFTASITSPL 90	20mg/ml	25ul	H3	PAIASLMAFTAAVTSPL	Seg 14	1a	H3	PAIASLMAFTASITSPL	Seg 14	1a
13 of 40	18 80	MAFTASITSPLTTQNTLL 97	40mg/ml	25ul		MAFTAAVTSPLTTQNTLL	Seg 14	1a	H3			
14 of 40	18 87	TSPLTTQNTLLFNILGGW 104	40mg/ml	25ul								
15 of 40	17 94	NTLLFNILGGWVAAQLA 110	40mg/ml	25ul	H4				H4			
16 of 40	18 100	ILGGWVAAQLAPPSAASA 117	40mg/ml	25ul	H4				H4			
17 of 40	18 107	AQLAPPSAASAFVGAGIA 124	40mg/ml	25ul	H4				H4			
18 of 40	18 114	AASAFVGAGIAGAAVGSI 131	10mg/ml	25ul								
19 of 40	18 121	AGIAGAAVGSIGLGKVLV 138	40mg/ml	25ul	H5	AGIAGAAVGSVGLGKVLV	Seg 15	1a		AGIAGAAIGSVGLGKVLV	Seg 15	1a
20 of 40	17 128	VGSIGLGKVLVDILAGY 144	40mg/ml	25ul	H5	IGSVGLGKVLVDILAGY	Seg 15	1a	H5	IGSVGLGKVLVDILAGY	Seg 15	1a
21 of 40	18 134	GKVLVDILAGYGAGVAGA 151	40mg/ml	25ul	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a
22 of 40	18 141	LAGYGAGVAGALVAFKVM 158	20mg/ml	25ul	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a
23 of 40	15 148	VAGALVAFKVMSGEV 162	20mg/ml	25ul	H5	VAGALVAFKIMSGEV	Seg 15	1a	H5	VAGALVAFKIMSGEI	Seg 15	1a
24 of 40	18 152	LVAFKVMSGEVPSTEDLV 169	40mg/ml	25ul	H5	LVAFKIMSGEVPSTEDLV	Seg 15	1a	H5	LVAFKIMSGEIPSTEDLV	Seg 15	1a
25 of 40	18 159	SGEVPSTEDLVNLLPAIL 176	40mg/ml	25ul	H5	SGEVPSTEDLVNLLPAIL	Seg 15	1a	H5	SGEIPSTEDLVNLLPAIL	Seg 15	1a
26 of 40	18 166	EDLVNLLPAILSPGALVV 183	40mg/ml	25ul	H5	EDLVNLLPAILSPGALVV	Seg 15	1a	H5	EDLVNLLPAILSPGALVV	Seg 15	1a
27 of 40	18 173	PAILSPGALVVGVVCAAI 190	40mg/ml	25ul	H5	AILSPGALVVGVVCAAI	Seg 15	1a	H5	PAILSPGALVVGVVCAAI	Seg 15	1a
28 of 40	16 180	ALVVGVVCAAILRRHV 195	40mg/ml	25ul	H5	ALVVGVVCAAILRRHV	Seg 15	1a	H5	ALVVGVVCAAILRRHV	Seg 15	1a
29 of 40	18 185	VVCAAILRRHVGPGEGAV 202	40mg/ml	25ul	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a
30 of 40	18 192	RRHVGPGEGAVQWMNRLI 209	40mg/ml	25ul	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a

31 of 40	16	199	EGAVQWMNRLIAFASR 214	40mg/ml	25ul	H5	EGAVQWMNRLIAFASR	Seg 15	1a	H5	EGAVQWMNRLIAFASR	Seg 15	1a
32 of 40	15	204	WMNRLIAFASRGNHV 218	40mg/ml	25ul	H5	WMNRLIAFASRGNHV	Seg 15	1a	H5	WMNRLIAFASRGNHV	Seg 15	1a
33 of 40	17	208	LIAFASRGNHVSPTHYV 224	40mg/ml	25ul	H5	LIAFASRGNHVSPTHYV	Seg 15	1a	H5	LIAFASRGNHVSPTHYV	Seg 15	1a
34 of 40	18	214	RGNHVSPTHYVPESDAAA 231	40mg/ml	25ul	H5	RGNHVSPTHYVPESDAAA	Seg 15	1a	H5	RGNHVSPTHYVPESDAAA	Seg 15	1a
35 of 40	17	221	THYVPESDAAARVTQIL 237	40mg/ml	25ul	H5	THYVPESDAAARVTQIL	Seg 15	1a	H5	THYVPESDAAARVTAIL	Seg 15	1a
36 of 40	16	227	SDAAARVTQILSSLTI 242	20mg/ml	50ul					H5	SDAAARVTAILSSLTV	Seg 15	1a
37 of 40	18	232	RVTQILSSLTITQLLKRL 249	40mg/ml	25ul	H6				H5	RVTAILSSLTITQLLKRL	Seg 15	1a
38 of 40	15	239	SLTITQLLKRLHQWI 253	40mg/ml	25ul	H6							
39 of 40	18	243	TQLLKRLHQWINEDCSTP 260	40mg/ml	25ul	H6				H6			
40 of 40	12	250	HQWINEDCSTPC 261	40mg/ml	25ul	H6				H6			

Pool I

NS5A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 71	16	1 SGSWLRDVWDWICTVL 16	40mg/ml	25ul	I1							
2 of 71	18	6 RDVWDWICTVLTDFKTWL 23	40mg/ml	25ul	I1							
3 of 71	18	13 CTVLTDFKTWLQSKLLPR 30	40mg/ml	25ul	I1							
4 of 71	18	20 KTWLQSKLLPRLPGVPFL 37	40mg/ml	25ul	I1							
5 of 71	18	27 LLPRLPGVPFLSCQRGYK 44	40mg/ml	25ul	I1							
6 of 71	15	34 VPFLSCQRGYKGVWR 48	40mg/ml	25ul	I1							
7 of 71	16	38 SCQRGYKGVWRGDGIM 53	40mg/ml	25ul	I1							
8 of 71	19	43 YKGVWRGDGIMQTTCPCGA 61	40mg/ml	25ul	I1							
9 of 71	18	51 GIMQTTCPCGAQIAGHVK 68	40mg/ml	25ul	I1							
10 of 71	18	58 PCGAQIAGHVKNGSMRIV 75	40mg/ml	25ul	I1							
11 of 71	18	65 GHVKNGSMRIVGPRTCSN 82	40mg/ml	25ul	I1							
12 of 71	17	72 MRIVGPRTCSNTWHGTF 88	40mg/ml	25ul	I1							
13 of 71	16	78 RTCSNTWHGTFPINAY 93	40mg/ml	25ul	I1							
14 of 71	18	83 TWHGTFPINAYTTGPCTP 100	40mg/ml	25ul	I1							
15 of 71	17	90 INAYTTGPCTPSPAPNY 106	40mg/ml	25ul	I1							
16 of 71	18	96 GPCTPSPAPNYSRALWRV 113	40mg/ml	25ul	I2							
17 of 71	17	103 APNYSRALWRVAAEEYV 119	40mg/ml	25ul	I2							
18 of 71	16	109 ALWRVAAEEYVEVTRV 124	20mg/ml	50ul	I2							
19 of 71	17	114 AAEEYVEVTRVGDFHYV 130	40mg/ml	25ul	I2							
20 of 71	19	120 EVTRVGDFHYVTGMTTDNV 138	40mg/ml	25ul	I2							
21 of 71	17	128 HYVTGMTTDNVKCPCQV 144	40mg/ml	25ul	I2							
22 of 71	17	134 TTDNVKCPCQVPAPEFF 150	40mg/ml	25ul	I2					TTDNVKCPCQVPAPEFF	Seg 16	1b
23 of 71	18	140 CPCQVPAPEFFTEVDGVR 157	40mg/ml	25ul	I2					CPCQVPAPEFFTEVDGVR	Seg 16	1b
24 of 71	18	147 PEFFTEVDGVRLHRYAPA 164	40mg/ml	25ul	I2					PEFFTELDGVRLHRYAPA	Seg 16	1b
25 of 71	17	154 DGVRLHRYAPACKPLLR 170	40mg/ml	25ul	I2					DGVRLHRYAPACKPLLR	Seg 16	1b
26 of 71	18	160 RYAPACKPLLREDVTFQV 177	40mg/ml	25ul	I2							
27 of 71	18	167 PLLREDVTFQVGLNQYLV 184	40mg/ml	25ul	I2							
28 of 71	15	174 TFQVGLNQYLVGSQL 188	40mg/ml	25ul	I2							
29 of 71	19	178 GLNQYLVGSQLPCEPEPDV 196	40mg/ml	25ul	I2					GLNQYLIGSQLPCEPEPDV	Seg 17	3a
30 of 71	18	186 SQLPCEPEPDVTVLTSML 203	40mg/ml	25ul	I2					SQLPCEPEPDVSVLTSML	Seg 17	3a
31 of 71	17	193 EPDVTVLTSMLTDPSHI 209	40mg/ml	25ul	I2					EPDVAVLTSMLTDPSHI	Seg 17	3a
32 of 71	18	199 LTSMLTDPSHITAETAKR 216	40mg/ml	25ul								
33 of 71	15	206 PSHITAETAKRRLAR 220	40mg/ml	25ul		PSHITAETAARRLAR	Seg 16	6d				

34 of 71	18 210	TAETAKRRLARGSPPSLA 227	40mg/ml	25ul	I3		TAETAARRLARGSPPSLA	Seg 16	6d		TAETAARRLARGSPPSEA	Seg 18	3a
35 of 71	18 217	RLARGSPPSLASSSASQL 234	40mg/ml	25ul	I3		RLARGSPPSLASSSASQL	Seg 16	6d		RLARGSPPSEASSSASQL	Seg 18	3a
36 of 71	18 224	PSLASSSASQLSAPSLKA 241	40mg/ml	25ul	I3		PSLASSSASQLSAPSLKA	Seg 16	6d		PSEASSSASQLSAPSLKA	Seg 18	3a
37 of 71	17 231	ASQLSAPSLKATCTTHH 247	40mg/ml	25ul	I3		ASQLSAPSLKATCTANH	Seg 16	6d		ASQLSAPSLKATCQTHR	Seg 18	3a
38 of 71	18 237	PSLKATCTTHHDSPDADL 254	40mg/ml	25ul			PSLKATCTTHHDSPDAEL	Seg 16	6d		PSLKATCQTHRDSPDAEL	Seg 18	3a
39 of 71	18 244	TTHHDSPDADLIEANLLW 261	40mg/ml	25ul		I4							
40 of 71	15 251	DADLIEANLLWRQEM 265	40mg/ml	25ul		I4							
41 of 71	18 255	IEANLLWRQEMGGNITRV 272	40mg/ml	25ul		I4							
42 of 71	18 262	RQEMGGNITRVESENKVV 279	40mg/ml	25ul		I4							
43 of 71	16 269	ITRVESENKVVILDSF 284	20mg/ml	50ul		I4							
44 of 71	16 274	SENKVVILDSFEPLHA 289	40mg/ml	25ul	I4	(Ih)							
45 of 71	18 279	VILDSFEPLHAEGDEREI 296	40mg/ml	25ul	I4	(Ih)							
46 of 71	18 286	PLHAEGDEREISVAAEIL 303	40mg/ml	25ul	I4	(Ih)							

Pool I continued

NS5A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
47 of 71	17 293	EREISVAAEILRKSRKF 309	40mg/ml	25ul	I5	(Ih)						
48 of 71	18 299	AAEILRKSRKFPSALPIW 316	40mg/ml	25ul	I5	(Ih)						
49 of 71	16 306	SRKFPSALPIWARPDY 321	40mg/ml	25ul	I5	(Ih)						
50 of 71	16 311	SALPIWARPDYNPPLL 326	40mg/ml	25ul	I5	(Ih)						
51 of 71	15 316	WARPDYNPPLLESWK 330	40mg/ml	25ul		I5						
52 of 71	16 320	DYNPPLLESWKDPDYV 335	40mg/ml	25ul		I5						
53 of 71	16 325	LLESWKDPDYVPPVVH 340	40mg/ml	25ul		I5						
54 of 71	15 330	KDPDYVPPVVHGCPL 344	40mg/ml	25ul		I5						
55 of 71	16 334	YVPPVVHGCPLPPTKA 349	40mg/ml	25ul	I6							
56 of 71	18 339	VHGCPLPPTKAPPIPPPR 356	40mg/ml	25ul	I6							
57 of 71	18 346	PTKAPPIPPPRRKRTVVL 363	40mg/ml	25ul	I6							
58 of 71	16 353	PPPRRKRTVVLTESNV 368	20mg/ml	50ul	I6							
59 of 71	18 358	KRTVVLTESNVSSALAEL 375	40mg/ml	25ul	I6							
60 of 71	16 365	ESNVSSALAELATKTF 380	20mg/ml	50ul	I6							
61 of 71	18 370	SALAELATKTFGSSGSSA 387	40mg/ml	25ul	I6							
62 of 71	17 377	TKTFGSSGSSAVDSGTA 393	40mg/ml	25ul	I6							
63 of 71	18 383	SGSSAVDSGTATALPDLA 400	40mg/ml	25ul	I6							
64 of 71	17 390	SGTATALPDLASDDGDK 406	40mg/ml	25ul	I6							
65 of 71	18 396	LPDLASDDGDKGSDVESY 413	40mg/ml	25ul	I6							
66 of 71	17 403	DGDKGSDVESYSSMPPL 419	40mg/ml	25ul	I6					DGDKESDSESCSSMPPL	Seg 19	3a
67 of 71	18 409	DVESYSSMPPLEGEPGDP 426	40mg/ml	25ul	I6					DSESCSSMPPLEGEPGDP	Seg 19	3a
68 of 71	18 416	MPPLEGEPGDPDLSDGSW 433	40mg/ml	25ul	I6					MPPLEGEPGDPDLSCDSW	Seg 19	3a
69 of 71	18 423	PGDPDLSDGSWSTVSEEA 440	40mg/ml	25ul	I6					PGDPDLSCDSWSTVSDGA	Seg 19	3a
70 of 71	16 430	DGSWSTVSEEASEDVV 445	40mg/ml	25ul	I6					DSWSTVSDEASQSVV	Seg 19	3a
71 of 71	13 435	TVSEEASEDVVCC 447	10mg/ml	100ul	I6		TVSSGADTEDVVCC			TVSDEASQSVVCC	Seg 20	3a

Pool L

NS5B

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 90	16 1	SMSYTWTGALITPCAA 16	20mg/ml	50ul	L1	SMSYSWTGALITPCAA	Seg 18	6a		SMSYSWTGALITPCSA	Seg 20	3a
2 of 90	18 6	WTGALITPCAAEESKLPI 23	40mg/ml	25ul	L1	WTGALITPCAAEEEKLPI	Seg 18	6a		WTGALITPCSAEEEKLPI	Seg 20	3a

3 of 90	18 13 PCAAEESKLPINPLSNSL 30	40mg/ml	25ul	L1		PCAAEEEKLPINPLSNSL	Seg 18	6a		PCSAEEEKLPINPLSNSL	Seg 20	3a
4 of 90	18 20 KLPINPLSNSLLRHHNMV 37	40mg/ml	25ul	L1		KLPINPLSNSLIRHHNMV	Seg 18	6a		KLPINPLSNSLLRHHNLV	Seg 20	3a
5 of 90	17 27 SNSLLRHHNMVYATTSR 43	40mg/ml	25ul	L1		SNSLIRHHNMVYATTSR	Seg 18	6a		SNSLLRHHNLVYSTSSR	Seg 20	3a
6 of 90	18 33 HHNMVYATTSRSASLRQK 50	40mg/ml	25ul	L1		HHNMVYATTSRSASLRQK	Seg 18	6a		HHNLVYSTSSRSASQRQK	Seg 20	3a
7 of 90	18 40 TTSRSASLRQKKVTFDRL 57	40mg/ml	25ul	L1		TTSRSASLRQKKVTFDRL	Seg 18	6a		TSSRSASQRQKKVTFDRL	Seg 20	3a
8 of 90	18 47 LRQKKVTFDRLQVLDDHY 64	40mg/ml	25ul	L1		QRQKKVTFDRLQVLDDHY	Seg 18	6a		QRQKKVTFDRLQVLDDHY	Seg 20	3a
9 of 90	18 54 FDRLQVLDDHYRDVLKEM 71	40mg/ml	25ul		L2					FDRLQVLDDHYKDVLKEM	Seg 20	3a
10 of 90	18 61 DDHYRDVLKEMKAKASTV 78	40mg/ml	25ul		L2							
11 of 90	18 68 LKEMKAKASTVKAKLLSI 85	10mg/ml	100ul		L2							
12 of 90	17 75 ASTVKAKLLSIEEACKL 91	10mg/ml	100ul		L2							
13 of 90	18 81 KLLSIEEACKLTPPHSAK 98	40mg/ml	25ul		L2							
14 of 90	18 88 ACKLTPPHSAKSKFGYGA 105	40mg/ml	25ul		L2							
15 of 90	17 95 HSAKSKFGYGAKDVRNL 111	40mg/ml	25ul		L2							
16 of 90	18 101 FGYGAKDVRNLSSRAVNH 118	40mg/ml	25ul		L2							
17 of 90	16 108 VRNLSSRAVNHIRSVW 123	40mg/ml	25ul		L2							
18 of 90	15 113 SRAVNHIRSVWEDLL 127	40mg/ml	25ul		L2							
19 of 90	18 117 NHIRSVWEDLLEDTETPI 134	13.3mg/ml	75ul		L2							
20 of 90	18 124 EDLLEDTETPIDTTIMAK 141	10mg/ml	100ul		L2							
21 of 90	17 131 ETPIDTTIMAKSEVFCV 147	40mg/ml	25ul		L2							
22 of 90	18 137 TIMAKSEVFCVQPEKGGR 154	40mg/ml	25ul		L2							
23 of 90	18 144 VFCVQPEKGGRKPARLIV 161	40mg/ml	25ul	L3		VFCVQPAKGGRKPARLIV	Seg 19	3a		VFCVQPEKGGRKPARLIV	Seg 21	1a
24 of 90	18 151 KGGRKPARLIVFPDLGVR 168	40mg/ml	25ul	L3		KGGRKPARLIVYPDLGVR	Seg 19	3a		KGGRKPARLIVYPDLGVR	Seg 21	1a
25 of 90	18 158 RLIVFPDLGVRVCEKMAL 175	40mg/ml	25ul	L3		RLIVYPDLGVRVCEKRAL	Seg 19	3a		RLIVYPDLGVRVCEKMAL	Seg 21	1a
26 of 90	18 165 LGVRVCEKMALYDVVSTL 182	40mg/ml	25ul	L3		LGVRVCEKRALYDVVSTL	Seg 19	3a		LGVRVCEKMALYDVVSTL	Seg 21	1a
27 of 90	16 172 KMALYDVVSTLPQAVM 187	40mg/ml	25ul									
28 of 90	17 177 DVVSTLPQAVMGSSYGF 193	40mg/ml	25ul		L4							
29 of 90	18 183 PQAVMGSSYGFQYSPKQR 200	40mg/ml	25ul		L4							
30 of 90	16 190 SYGFQYSPKQRVEFLV 205	40mg/ml	25ul		L4							
31 of 90	18 195 YSPKQRVEFLVNTWKSKK 212	40mg/ml	25ul		L4							
32 of 90	18 202 EFLVNTWKSKKCPMGFSY 219	40mg/ml	25ul			EFLVQAWKSKKTPMGFSY	Seg 20	6h		EFLVQAWTSKKTPMGFSY	Seg 22	3a
33 of 90	16 209 KSKKCPMGFSYDTRCF 224	40mg/ml	25ul	L5		KSKKTPMGFSYDTRCF	Seg 20	6h		TSKKTPMGFSYDTRCF	Seg 22	3a
34 of 90	15 214 PMGFSYDTRCFDSTV 228	40mg/ml	25ul	L5		PMGFSYDTRCFDSTV	Seg 20	6h		PMGFSYDTRCFDSTV	Seg 22	3a
35 of 90	18 218 SYDTRCFDSTVTESDIRV 235	40mg/ml	25ul	L5		SYDTRCFDSTVTERDIR	Seg 20	6h		SYDTRCFDSTVTEQDIR	Seg 22	3a
36 of 90	16 225 DSTVTESDIRVEESIY 240	13.3mg/ml	75ul	L5		DSTVTERDIRTEEAIY	Seg 20	6h		DSTVTEQDIRVEEEIY	Seg 22	3a
37 of 90	17 230 ESDIRVEESIYQCCDLA 246	40mg/ml	25ul							EQDIRVEEEIYQCCDL	Seg 22	3a
38 of 90	18 236 EESIYQCCDLAPEARQAI 253	40mg/ml	25ul		L6							
39 of 90	18 243 CDLAPEARQAIRSLTERL 260	40mg/ml	25ul		L6							
40 of 90	17 250 RQAIRSLTERLYIGGPL 266	40mg/ml	25ul		L6							
41 of 90	15 256 LTERLYIGGPLTNSK 270	40mg/ml	25ul		L6							
42 of 90	18 260 LYIGGPLTNSKGQNCGYR 277	40mg/ml	25ul		L6							
43 of 90	18 267 TNSKGQNCGYRRCRASGV 284	40mg/ml	25ul			TNSRGEMCGYRRCRASGV	Seg 21	2a		TNSRGENCGYRRCRASGV	Seg 23	1a
44 of 90	18 274 CGYRRCRASGVLTTSCGN 291	40mg/ml	25ul	M1		CGYRRCRASGVLTTSMGN	Seg 21	2a	M1	CGYRRCRASGVLTTSCGN	Seg 23	1a
45 of 90	18 281 ASGVLTTSCGNTLTCYLK 298	40mg/ml	25ul	M1		ASGVLTTSMGNTITCYIK	Seg 21	2a	M1	ASGVLTTSCGNTLTCYIK	Seg 23	1a

Pool M

NS5B

Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
46 of 90 18 288 SCGNTLTCYLKATAACRA 305	40mg/ml	25ul	M1	SMGNTITCYIKALAACRA	Seg 21	2a	M1	SCGNTLTCYIKARAACRA	Seg 23	1a
47 of 90 19 295 CYLKATAACRAAKLQDCTM 313	40mg/ml	25ul	M1	CYIKALAACRAAGLQDCTM	Seg 21	2a	M1	CYIKARAACRAAGLQDCTM	Seg 23	1a
48 of 90 18 303 CRAAKLQDCTMLVNGDDL 320	40mg/ml	25ul								
49 of 90 18 310 DCTMLVNGDDLVVICESA 327	40mg/ml	25ul	M2				M2			
50 of 90 18 317 GDDLVVICESAGTQEDAA 334	40mg/ml	25ul	M2				M2			
51 of 90 16 324 CESAGTQEDAAALRAF 339	40mg/ml	25ul	M2				M2			
52 of 90 18 329 TQEDAAALRAFTEAMTRY 346	40mg/ml	25ul	M2				M3	VQEDAAALRAFTEAMTRY	Seg 24	3a
53 of 90 18 336 LRAFTEAMTRYSAPPGDP 353	40mg/ml	25ul	M3	LRAFTEAMTRYSAPPGDP	Seg 22	1a	M3	LRAFTEAMTRYSAPPGDA	Seg 24	3a
54 of 90 18 343 MTRYSAPPGDPPQPEYDL 360	40mg/ml	25ul	M3	MTRYSAPPGDPPQPEYDL	Seg 22	1a	M3	MTRYSAPPGDAPQPEYDL	Seg 24	3a
55 of 90 18 350 PGDPPQPEYDLELITSCS 367	40mg/ml	25ul	M3	PGDPPQPEYDLELITSCS	Seg 22	1a				
56 of 90 18 357 EYDLELITSCSSNVSVAH 374	40mg/ml	25ul	M3	EYDLELITSCSSNVSVAH	Seg 22	1a	M4			
57 of 90 18 364 TSCSSNVSVAHDASGKRV 381	40mg/ml	25ul	M3	TSCSSNVSVAHDGAGKRV	Seg 22	1a	M4			
58 of 90 16 371 SVAHDASGKRVYYLTR 386	40mg/ml	25ul						SVAHDAKGKRYYYLTR	Seg 25	3a
59 of 90 18 376 ASGKRVYYLTRDPTTPLA 393	40mg/ml	25ul	M4				M5	KGKRYYYLTRDPTTPLA	Seg 25	3a
60 of 90 18 383 YLTRDPTTPLARAAWETA 400	40mg/ml	25ul		YLTRDPTTPLARAAWETA	Seg 23	6a	M5	YLTRDPTTPLARAAWETA	Seg 25	3a
61 of 90 16 390 TPLARAAWETARHTPI 405	40mg/ml	25ul	M5	TPLARAAWETARHTPV	Seg 23	6a	M5	TPLARAAWETARHTPV	Seg 25	3a
62 of 90 18 395 AAWETARHTPINSWLGNI 412	40mg/ml	25ul	M5	AAWETARHTPVNSWLGNI	Seg 23	6a	M5	AAWETARHTPVNSWLGNI	Seg 25	3a
63 of 90 18 402 HTPINSWLGNIIMYAPTL 419	40mg/ml	25ul	M5	HTPVNSWLGNIIMYAPTI	Seg 23	6a	M5	HTPVNSWLGNIIMYAPTI	Seg 25	3a
64 of 90 18 409 LGNIIMYAPTLWARMILM 426	40mg/ml	25ul	M5	LGNIIMYAPTIWVRMVLM	Seg 23	6a	M5	LGNIIMYAPTIWVRMVMM	Seg 25	3a
65 of 90 18 416 APTLWARMILMTHFFSIL 433	40mg/ml	25ul	M5	APTIWVRMVLMTHFFSIL	Seg 23	6a		APTIWVRMVMMTHFFSVL	Seg 25	3a
66 of 90 17 423 MILMTHFFSILLAQEQL 439	40mg/ml	25ul	M5	MVLMTHFFSILQAQEQL	Seg 23	6a	M6			
67 of 90 15 429 FFSILLAQEQLEKAL 443	40mg/ml	25ul					M6			
68 of 90 18 433 LLAQEQLEKALDCQIYGA 450	40mg/ml	25ul	M6				M6			
69 of 90 18 440 EKALDCQIYGACYSIEPL 457	40mg/ml	25ul		EKALDCQIYGATYSVTPL	Seg 24	3a		EKALDCQIYGATYSVTPL	Seg 26	3a
70 of 90 17 447 IYGACYSIEPLDLPQII 463	40mg/ml	25ul	M7	YGATYSVTPLDLPAII	Seg 24	3a	M7	YGATYSVTPLDLPAII	Seg 26	3a
71 of 90 17 453 SIEPLDLPQIIERLHGL 469	40mg/ml	25ul	M7	SVTPLDLPAIIQRLHGL	Seg 24	3a	M7	SVTPLDLPAIIERLHGL	Seg 26	3a
72 of 90 17 459 LPQIIERLHGLSAFTLH 475	40mg/ml	25ul	M7	LPAIIQRLHGLSAFSLH	Seg 24	3a	M7	LPAIIERLHGLSAFTLH	Seg 26	3a
73 of 90 18 465 RLHGLSAFTLHSYSPGEI 482	40mg/ml	25ul	M7	RLHGLSAFSLHSYSPGEI	Seg 24	3a	M7	RLHGLSAFTLHSYSPVEL	Seg 26	3a
74 of 90 18 472 FTLHSYSPGEINRVASCL 489	40mg/ml	25ul		FSLHSYSPGELNRVGACL			M7	FTLHSYSPVELNRVAGTL	Seg 26	3a
75 of 90 16 479 PGEINRVASCLRKLGV 494	40mg/ml	25ul	M8	PGELNRVGACLRKLGV	Seg 25	6a	M7	PVELNRVAGTLRKLGC	Seg 26	3a
76 of 90 18 484 RVASCLRKLGVPPLRTWR 501	40mg/ml	25ul	M8	RVGACLRKLGVPPLRAWR	Seg 25	6a	M7	RVAGTLRKLGCPPLRAWR	Seg 26	3a
77 of 90 18 491 KLGVPPLRTWRHRARSVR 508	40mg/ml	25ul	M8	KLGVPPLRAWRHRARAVR	Seg 25	6a	M7	KLGCPPLRAWRHRARSVR	Seg 26	3a
78 of 90 15 498 RTWRHRARSVRAKLL 512	40mg/ml	25ul	M8	RAWRHRARAVRARLLSR	Seg 25	6a				
79 of 90 18 502 HRARSVRAKLLSQGGRAA 519	40mg/ml	25ul	M8	ARAVRAKLIAQGGKAAI	Seg 25	6a	M8	ARSVRAKLLSQGGRAAI	Seg 27	1b
80 of 90 18 509 AKLLSQGGRAATCGRYLF 526	40mg/ml	25ul	M8	RLIAQGGRAAICGKYLF	Seg 25	6a	M8	KLLSQGGRAAICGKYLF	Seg 27	1b
81 of 90 18 516 GRAATCGRYLFNWAVRTK 533	40mg/ml	25ul	M8	GRAAICGKYLFNWAVRTK	Seg 25	6a	M8	GRAAICGKYLFNWAVRTK	Seg 27	1b
82 of 90 17 523 RYLFNWAVRTKLKLTPI 539	40mg/ml	25ul					M8	KYLFNWAVRTKLKLTPIA	Seg 27	1b
83 of 90 17 529 AVRTKLKLTPIPAASQL 545	40mg/ml	25ul	M9							
84 of 90 18 535 KLTPIPAASQLDLSGWFV 552	40mg/ml	25ul	M9				M9			
85 of 90 19 542 ASQLDLSGWFVAGYSGGDI 560	40mg/ml	25ul	M9				M9			
86 of 90 18 550 WFVAGYSGGDIYHSLSRA 567	40mg/ml	25ul	M9				M9			
87 of 90 18 557 GGDIYHSLSRARPRWFPL 574	40mg/ml	25ul	M9				M9			
88 of 90 18 564 LSRARPRWFPLCLLLLSV 581	40mg/ml	25ul	M9				M9			
89 of 90 18 571 WFPLCLLLLSVGVGIYLL 588	40mg/ml	25ul	M9				M9			
90 of 90 14 578 LLSVGVGIYLLPNR 591	40mg/ml	25ul	M9				M9			

3a (K3a650) HCV Peptide pools and minipools

BEI Peptides				Gt1-6				Gt1/3			
Peptide Length Sequence	**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***
Pool A										
Core										
1 of 29 18 1 MSTLPKPQRKTKRNTIRR 18	40mg/ml	25ul	A1	MSTLPKPQRKTKRNTNRR	Seg 1	4Q	A1	MSTNPKPQRKTKRNTNRR	Seg 1	1a
2 of 29 18 7 PQRKTKRNTIRRPQDVKF 24	40mg/ml	25ul	A1	PQRKTKRNTNRRPMDVKF	Seg 1	4Q	A1	PQRKTKRNTNRRPQDVKF	Seg 1	1a
3 of 29 18 14 NTIRRPQDVKFPGGGVIY 31	40mg/ml	25ul	A1	NTNRRPMDVKFPGGGQIV	Seg 1	4Q	A1	NTNRRPQDVKFPGGGQIV	Seg 1	1a
4 of 29 17 21 DVKFPGGGVIYVGVYVL 37	40mg/ml	25ul	A1	DVKFPGGGQIVGGVYLL	Seg 1	4Q	A1	DVKFPGGGQIVGGVYLL	Seg 1	1a
5 of 29 18 27 GGVIYVGVYVLPRRGPRL 44	40mg/ml	25ul	A1	GGQIVGGVYLLPRRGPRL	Seg 1	4Q	A1	GGQIVGGVYLLPRRGPRL	Seg 1	1a
6 of 29 18 34 VYVLPRRGPRLGVRATRK 51	40mg/ml	25ul	A2	VYLLPRRGPRLGVRATRK	Seg 1	4Q	A2	VYLLPRRGPRLGVRATRK	Seg 1	1a
7 of 29 15 41 GPRLGVRATRKTSER 55	40mg/ml	25ul	A2	PRLGVRATRKASER	Seg 1	4Q	A2	PRLGVRATRKASER	Seg 1	1a
8 of 29 18 45 GVRATRKTSERSQPRGRR 62	40mg/ml	25ul	A2	GVRATRKASERSQPRGRR	Seg 1	4Q	A2	GVRATRKASERSQPRGRR	Seg 1	1a
9 of 29 18 52 TSERSQPRGRRKPIPKAR 69	40mg/ml	25ul	A2	TSERSQPRGRRQPIPKAR	Seg 1	4Q	A2	TSERSQPRGRRQPIPKAR	Seg 1	1a
10 of 29 18 59 RGRRKPIPKARRSEGRSW 76	40mg/ml	25ul	A2	RGRRQPIPKARRPEGRSW	Seg 1	4Q	A2	RGRRQPIPKARRPEGRSW	Seg 1	1a
11 of 29 18 66 PKARRSEGRSWAQPGYPW 83	40mg/ml	25ul	A3	PKARRPEGRSWAQPGYPW	Seg 1	4Q	A3	PKARRPEGRSWAQPGYPW	Seg 1	1a
12 of 29 18 73 GRSWAQPGYPWPLYGNEG 90	40mg/ml	25ul	A3	GRSWAQPGYPWPLYGNEG	Seg 1	4Q	A3	GRSWAQPGYPWPLYGNEG	Seg 1	1a
13 of 29 18 80 GYPWPLYGNEGCGWAGWL 97	40mg/ml	25ul	A3	GYPWPLYGNEGCGWAGWL	Seg 1	4Q	A3	GYPWPLYGNEGCGWAGWL	Seg 1	1a
14 of 29 18 87 GNEGCGWAGWLLSPRGSR 104	40mg/ml	25ul	A3	GNEGCGWAGWLLSPRGSR	Seg 1	4Q	A3	GNEGCGWAGWLLSPRGSR	Seg 1	1a
15 of 29 15 94 AGWLLSPRGSRPNWA 108	40mg/ml	25ul	A3	AGWLLSPRGSRPSWGPND	Seg 1	4Q	A3	AGWLLSPRGSRPSWGPTD	Seg 1	1a
16 of 29 18 98 LSPRGSRPNWAPNDPRRR 115	40mg/ml	25ul	A4	LSPRGSRPSWGPNDPRRR	Seg 1	4Q	A4	LSPRGSRPSWGPTDPRRR	Seg 1	1a
17 of 29 18 105 PNWAPNDPRRRSRNLGKV 122	40mg/ml	25ul	A4	PNWGPNDPRRRSRNLGKV	Seg 1	4Q	A4	PNWGPTDPRRRSRNLGKV	Seg 1	1a
18 of 29 15 112 PRRRSRNLGKVIDTL 126	40mg/ml	25ul	A4	PRRRSRNLGKVIDTL	Seg 1	4Q	A4	PRRRSRNLGKVIDTL	Seg 1	1a
19 of 29 18 116 SRNLGKVIDTLTCGFADL 133	40mg/ml	25ul	A4	SRNLGKVIDTLTCGFADL	Seg 1	4Q	A4	SRNLGKVIDTLTCGFADL	Seg 1	1a
20 of 29 18 123 IDTLTCGFADLMGYIPLV 140	40mg/ml	25ul	A4	IDTLTCGFADLMGYIPLV	Seg 1	4Q	A4	IDTLTCGFADLMGYIPLV	Seg 1	1a
21 of 29 18 130 FADLMGYIPLVGAPLGGA 147	40mg/ml	25ul	A5	FADLMGYIPLVGAPVGGV	Seg 1	4Q	A5	FADLMGYIPLVGAPVGGV	Seg 1	1a
22 of 29 17 137 IPLVGAPLGGAARALAH 153	40mg/ml	25ul	A5	IPLVGAPVGGVARALAH	Seg 1	4Q	A5	IPLVGAVGGVARALAH	Seg 1	1a
23 of 29 16 143 PLGGAARALAHGVRAL 158	40mg/ml	25ul	A5	PVGGVARALAHGVRAL	Seg 1	4Q	A5	PVGGVARALAHGVRVL	Seg 1	1a
24 of 29 18 148 ARALAHGVRALEDGINFA 165	40mg/ml	25ul	A5	ARALAHGVRALEDGINYA	Seg 1	4Q	A5	ARALAHGVRVLEDGVNYA	Seg 1	1a
25 of 29 15 155 VRALEDGINFATGNL 169	40mg/ml	25ul	A6	VRALEDGINYATGNL	Seg 1	4Q	A6	VRVLEDGVNYATGNL	Seg 1	1a
26 of 29 18 159 EDGINFATGNLPGCSFSI 176	40mg/ml	25ul	A6	EDGINYATGNLPGCSFSI	Seg 1	4Q	A6	EDGVNYATGNLPGCSFSI	Seg 1	1a
27 of 29 17 166 TGNLPGCSFSIFLLALF 182	40mg/ml	25ul	A6	TGNLPGCSFSIFLLALL	Seg 1	4Q	A6	TGNLPGCSFSIFLLALL	Seg 1	1a
28 of 29 18 172 CSFSIFLLALFSCLIHPA 189	20mg/ml	50ul	A6	CSFSIFLLALLSCLTVPA	Seg 1	4Q	A6	CSFSIFLLALLSCLTVPA	Seg 1	1a
29 of 29 13 179 LALFSCLIHPAAS 191	40mg/ml	25ul		LALLSCLTVPAAS	Seg 1	4Q		LALLSCLTVPAAS	Seg 1	1a
Pool B										
E1										
Peptide Length Sequence	**Conc.**	**Vol.**	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***	***Minipool***	***Vacc Seq.***	***Segment***	***Vacc GT***
1 of 28 18 1 LEWRNTSGLYVLTNDCSN 18	40mg/ml	25ul								
2 of 28 18 8 GLYVLTNDCSNSSIVYEA 25	20mg/ml	50ul								
3 of 28 17 15 DCSNSSIVYEADDVILH 31	40mg/ml	25ul								
4 of 28 16 21 IVYEADDVILHTPGCI 36	40mg/ml	25ul								
5 of 28 18 26 DDVILHTPGCIPCVQDGN 43	40mg/ml	25ul								
6 of 28 16 33 PGCIPCVQDGNTSTCW 48	40mg/ml	25ul								
7 of 28 18 38 CVQDGNTSTCWTPVTPTV 55	40mg/ml	25ul								

8 of 28	18 45 STCWTPVTPTVAVRYVGA 62	40mg/ml	25ul									
9 of 28	17 52 TPTVAVRYVGATTASIR 68	40mg/ml	25ul									
10 of 28	18 58 RYVGATTASIRSHVDLLV 75	40mg/ml	25ul									
11 of 28	16 65 ASIRSHVDLLVGAGTM 80	40mg/ml	25ul						ASIRSHVDLLVGAATM	Seg 2	3a	
12 of 28	17 70 HVDLLVGAGTMCSALYV 86	40mg/ml	25ul						HVDLLVGAATMCSALYV	Seg 2	3a	
13 of 28	18 76 GAGTMCSALYVGDMCGPV 93	40mg/ml	25ul						GAATMCSALYVGDMCGSV	Seg 2	3a	
14 of 28	18 83 ALYVGDMCGPVFLVGQAF 100	40mg/ml	25ul						ALYVGDMCGPVFLVGALF	Seg 2	3a	
15 of 28	18 90 CGPVFLVGQAFTFRPRRH 107	40mg/ml	25ul									
16 of 28	18 97 GQAFTFRPRRHRTVQTCN 114	40mg/ml	25ul									
17 of 28	18 104 PRRHRTVQTCNCSLYPGH 121	40mg/ml	25ul									
18 of 28	18 110 VQTCNCSLYPGHLSGQRM 127	40mg/ml	25ul		TQSCNCSIYPGHITGHRM	Seg 2	1a					
19 of 28	17 117 LYPGHLSGQRMAWDMMM 133	40mg/ml	25ul		IYPGHITGHRMAWDMMM	Seg 2	1a					
20 of 28	17 123 SGQRMAWDMMMNWSPAV 139	40mg/ml	25ul		TGHRMAWDMMMNWSPTAA	Seg 2	1a					
21 of 28	18 129 WDMMMNWSPAVGMVVAHI 146	40mg/ml	25ul									
22 of 28	18 136 SPAVGMVVAHILRLPQTL 153	40mg/ml	25ul									
23 of 28	18 143 VAHILRLPQTLFDVVAGA 160	40mg/ml	25ul									
24 of 28	17 150 PQTLFDVVAGAHWGIIA 166	40mg/ml	25ul									
25 of 28	18 156 VVAGAHWGIIAGLAYYSM 173	40mg/ml	25ul						VAAGAHWGVLAGLAYYSM	Seg 3	3a	
26 of 28	18 163 GIIAGLAYYSMQGNWAKV 180	40mg/ml	25ul						GVLAGLAYYSMQGNWAKV	Seg 3	3a	
27 of 28	18 170 YYSMQGNWAKVAIIMVMF 187	40mg/ml	25ul						YYSMQGNWAKVAIIMVMF	Seg 3	3a	
28 of 28	16 177 WAKVAIIMVMFSGVDA 192	10mg/ml	100ul									

Pool C

E2

Peptide Length Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 57 16 1 STHVTAGQAARNAYGI 16	40mg/ml	25ul								
2 of 57 17 6 AGQAARNAYGITSLFSV 22	40mg/ml	25ul								
3 of 57 17 12 NAYGITSLFSVGAKQNL 28	40mg/ml	25ul								
4 of 57 18 18 SLFSVGAKQNLQLINTNG 35	10mg/ml	100ul								
5 of 57 17 25 KQNLQLINTNGSWHINR 41	40mg/ml	25ul	C1	KWNLQLINTNGSWHINR	Seg 3	2b				
6 of 57 18 31 INTNGSWHINRTALNCNE 48	40mg/ml	25ul	C1	INTNGSWHINRTALNCND	Seg 3	2b				
7 of 57 18 38 HINRTALNCNESINTGFI 55	40mg/ml	25ul	C1	HINRTALNCNDSLNTGFI	Seg 3	2b				
8 of 57 18 45 NCNESINTGFIAGLFYYH 62	40mg/ml	25ul	C1	NCNDSLNTGFIAGLFYYH	Seg 3	2b				
9 of 57 18 52 TGFIAGLFYYHKFNSTGC 69	40mg/ml	25ul								
10 of 57 15 59 FYYHKFNSTGCPQRL 73	40mg/ml	25ul								
11 of 57 17 63 KFNSTGCPQRLSSCKPI 79	40mg/ml	25ul								
12 of 57 18 69 CPQRLSSCKPITFFKQGW 86	40mg/ml	25ul								
13 of 57 17 76 CKPITFFKQGWGPLTDA 92	40mg/ml	25ul								
14 of 57 18 82 FKQGWGPLTDANITGPSD 99	40mg/ml	25ul								
15 of 57 18 89 LTDANITGPSDDKPYCWH 106	40mg/ml	25ul								
16 of 57 15 96 GPSDDKPYCWHYAPR 110	40mg/ml	25ul								
17 of 57 18 100 DKPYCWHYAPRPCGIVPA 117	40mg/ml	25ul								
18 of 57 18 107 YAPRPCGIVPALNVCGPV 124	40mg/ml	25ul								
19 of 57 19 114 IVPALNVCGPVYCFTPSPV 132	40mg/ml	25ul	C2	IVPALSVCGPVYCFTPSPV	Seg 4	4a	C2	IVPAKSVCGPVYCFTPSPV	Seg 4	1a
20 of 57 18 122 GPVYCFTPSPVVVGTTDA 139	40mg/ml	25ul	C2	GPVYCFTPSPVVVGTTDR	Seg 4	4a	C2	GPVYCFTPSPVVVGTTDR	Seg 4	1a
21 of 57 17 129 PSPVVVGTTDAKGAPTY 145	40mg/ml	25ul	C2	PSPVVVGTTDRSGAPTY	Seg 4	4a	C2	PSPVVVGTTDRSGAPTY	Seg 4	1a

22 of 57	17	135	GTTDAKGAPTYTWGANK	151	40mg/ml	25ul								
23 of 57	17	141	GAPTYTWGANKTDVFLL	157	40mg/ml	25ul								
24 of 57	15	147	WGANKTDVFLLESLR	161	40mg/ml	25ul								
25 of 57	18	151	KTDVFLLESLRPPSGRWF	168	40mg/ml	25ul								
26 of 57	16	158	ESLRPPSGRWFGCTWM	173	40mg/ml	25ul								
27 of 57	18	163	PSGRWFGCTWMNSTGFVK	180	40mg/ml	25ul								
28 of 57	15	170	CTWMNSTGFVKTCGA	184	40mg/ml	25ul								
29 of 57	17	174	NSTGFVKTCGAPPCNIY	190	40mg/ml	25ul								
30 of 57	17	180	KTCGAPPCNIYGDGRDA	196	40mg/ml	25ul								
31 of 57	18	186	PCNIYGDGRDAQNESDLF	203	40mg/ml	25ul								
32 of 57	18	193	GRDAQNESDLFCPTDCFR	210	40mg/ml	25ul								
33 of 57	18	200	SDLFCPTDCFRKHPEATY	217	40mg/ml	25ul	C3	SDLFCPTDCFRKHPEATY	Seg 5	1b				
34 of 57	16	207	DCFRKHPEATYSRCGA	222	40mg/ml	25ul	C3	PTDCFRKHPEATYTKCGS	Seg 5	1b				
35 of 57	18	212	HPEATYSRCGAGPWLTPR	229	40mg/ml	25ul	C3	HPEATYTKCGSGPWLTPR	Seg 5	1b	C3	HPEATYSRCGSGPWITPR	Seg 5	1a
36 of 57	18	219	RCGAGPWLTPRCLVDYPY	236	40mg/ml	25ul	C3	KCGSGPWLTPRCLVDYPY	Seg 5	1b	C3	CGSGPWITPRCLVDYPY	Seg 5	1a
37 of 57	16	226	LTPRCLVDYPYRLWHY	241	40mg/ml	25ul	C3	LTPRCLVDYPYRLWHY	Seg 5	1b	C3	ITPRCLVDYPYRLWHY	Seg 5	1a
38 of 57	17	231	LVDYPYRLWHYPCTVNF	247	40mg/ml	25ul	C3	LVDYPYRLWHYPCTVNF	Seg 5	1b	C3	LVDYPYRLWHYPCTIVNY	Seg 5	1a
39 of 57	18	237	RLWHYPCTVNFTLFKVRM	254	40mg/ml	25ul		RLWHYPCTVNFTLFKVRM	Seg 5	1b		PYRLWHYPCTVNYTLFKV	Seg 5	1a
40 of 57	18	244	TVNFTLFKVRMFVGGFEH	2	40mg/ml	25ul								
41 of 57	16	251	KVRMFVGGFEHRFTAA	266	40mg/ml	25ul								
42 of 57	16	256	VGGFEHRFTAACNWTR	271	40mg/ml	25ul								
43 of 57	17	261	HRFTAACNWTRGERCDI	277	40mg/ml	25ul						HRLEAACNWTRGERCDI	Seg 6	3a
44 of 57	16	267	CNWTRGERCDIEDRDR	282	40mg/ml	25ul						CNWTRGERCDIEDRDR	Seg 6	3a
45 of 57	18	272	GERCDIEDRDRSEQHPLL	289	40mg/ml	25ul						GERCDIEDRDRSELHPLL	Seg 6	3a
46 of 57	18	279	DRDRSEQHPLLHSTTELA	296	40mg/ml	25ul	C4	DRDRSEQHPLLLSTTEWQ	Seg 6	1b				
47 of 57	17	286	HPLLHSTTELAILPCSF	302	40mg/ml	25ul	C4	HPLLLSTTEWQILPCSF	Seg 6	1b		HPLLHSTTELAILPCSF	Seg 7	3a

Pool C continued

E2

Peptide	Length	Sequence		Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
48 of 57	17	292	TTELAILPCSFTPMPAL	308	40mg/ml	25ul	C4	TTEWQILPCSFTTLPAL	Seg 6	1b		TTELAILPCSFTPLPAL	Seg 7	3a
49 of 57	18	298	LPCSFTPMPALSTGLIHL	315	40mg/ml	25ul	C4	LPCSFTTLPALSTGLIHL	Seg 6	1b		LPCSFTPLPALSTGLIHL	Seg 7	3a
50 of 57	18	305	MPALSTGLIHLHQNIVDV	322	40mg/ml	25ul	C4	LPALSTGLIHLHQNIVDV	Seg 6	1b		LPALSTGLIHLHQNIVDV	Seg 7	3a
51 of 57	17	312	LIHLHQNIVDVQYLYGI	328	40mg/ml	25ul	C4	LIHLHQNIVDVQYLYGV	Seg 6	1b		LIHLHQNIVDVQYLYGV	Seg 7	3a
52 of 57	18	318	NIVDVQYLYGIGSGMVGW	335	40mg/ml	25ul	C4	NIVDVQYLYGVGSGMVGW	Seg 6	1b		NIVDVQYLYGVGSGMVGW	Seg 7	3a
53 of 57	18	325	LYGIGSGMVGWALKWEFV	342	40mg/ml	25ul						LYGVGSGMVGWALKWEFV	Seg 7	3a
54 of 57	18	332	MVGWALKWEFVILIFLLL	349	40mg/ml	25ul								
55 of 57	18	339	WEFVILIFLLLADARVCV	356	40mg/ml	25ul								
56 of 57	17	346	FLLLADARVCVALWLIL	362	40mg/ml	25ul								
57 of 57	18	352	ARVCVALWLILTISQAEA	369	40mg/ml	25ul								

Pool D

p7

Peptide	Length	Sequence		Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*

1 of 8	17 1 ALENLVTLNAVAAAGTH 17	40mg/ml	25ul
2 of 8	18 7 TLNAVAAAGTHGIGWYLV 24	40mg/ml	25ul
3 of 8	18 14 AGTHGIGWYLVAFCAAWY 31	40mg/ml	25ul
4 of 8	17 21 WYLVAFCAAWYVRGKLV 37	40mg/ml	25ul
5 of 8	18 27 CAAWYVRGKLVPLVTYSL 44	40mg/ml	25ul
6 of 8	18 34 GKLVPLVTYSLTGLWSLA 51	40mg/ml	25ul
7 of 8	17 41 TYSLTGLWSLALLVLLL 57	20mg/ml	50ul
8 of 8	17 47 LWSLALLVLLLPQRAYA 63	40mg/ml	25ul

NS2

Peptide	Length	Sequence	Concentration	Volume
1 of 33	17 1	WSGEDSATLGAGILVLF 17	40mg/ml	25ul
2 of 33	16 7	ATLGAGILVLFGFFTL 22	20mg/ml	50ul
3 of 33	18 12	GILVLFGFFTLSPWYKHW 29	40mg/ml	25ul
4 of 33	18 19	FFTLSPWYKHWIARLIWW 36	40mg/ml	25ul
5 of 33	18 26	YKHWIARLIWWNQYTICR 43	40mg/ml	25ul
6 of 33	18 33	LIWWNQYTICRCESALHV 50	40mg/ml	25ul
7 of 33	18 40	TICRCESALHVWVPPLLA 57	40mg/ml	25ul
8 of 33	18 47	ALHVWVPPLLARGGRDGV 64	40mg/ml	25ul
9 of 33	18 54	PLLARGGRDGVILLTSLL 71	40mg/ml	25ul
10 of 33	17 61	RDGVILLTSLLYPSLIF 77	40mg/ml	25ul
11 of 33	18 67	LTSLLYPSLIFDITKLLI 84	40mg/ml	25ul
12 of 33	18 74	SLIFDITKLLIAALGPLY 91	40mg/ml	25ul
13 of 33	17 81	KLLIAALGPLYLIQATI 97	20mg/ml	50ul
14 of 33	18 87	LGPLYLIQATITATPYFV 104	40mg/ml	25ul
15 of 33	18 93	IQATITATPYFVRAHVLV 110	40mg/ml	25ul
16 of 33	18 100	TPYFVRAHVLVRLCMLVR 117	40mg/ml	25ul
17 of 33	18 107	HVLVRLCMLVRSVMGGKY 124	40mg/ml	25ul
18 of 33	17 114	MLVRSVMGGKYFQMIIL 130	40mg/ml	25ul
19 of 33	18 120	MGGKYFQMIILSLADGSN 137	40mg/ml	25ul
20 of 33	18 127	MIILSLADGSNTYLYDHL 144	40mg/ml	25ul
21 of 33	18 134	DGSNTYLYDHLAPMQHWA 151	40mg/ml	25ul
22 of 33	18 141	YDHLAPMQHWAAAGLKDL 158	40mg/ml	25ul
23 of 33	15 147	MQHWAAAGLKDLAVA 161	40mg/ml	25ul
24 of 33	17 151	AAAGLKDLAVATEPVIF 167	40mg/ml	25ul
25 of 33	18 157	DLAVATEPVIFSPMEIKV 174	20mg/ml	50ul
26 of 33	16 164	PVIFSPMEIKVITWGA 179	40mg/ml	25ul
27 of 33	15 169	PMEIKVITWGADTAA 183	20mg/ml	50ul
28 of 33	16 173	KVITWGADTAACGDIL 188	40mg/ml	25ul
29 of 33	18 178	GADTAACGDILCGLPVSA 195	40mg/ml	25ul
30 of 33	18 185	GDILCGLPVSARLGREVL 202	40mg/ml	25ul
31 of 33	18 192	PVSARLGREVLLGPADDY 209	40mg/ml	25ul
32 of 33	18 199	REVLLGPADDYREMGWRL 216	40mg/ml	25ul
33 of 33	12 206	ADDYREMGWRLL 217	40mg/ml	25ul

Pool F

NS3

F1	PADDYREMGWRLL	Seg 7	3a		PADDYREMGWRLL	Seg 8	3a		

Peptide	Length	Sequence	Conc.	Vol.	Minipool	Vacc Seq.	Segment	Vacc GT	Minipool	Vacc Seq.	Segment	Vacc GT
1 of 97	18 1	APITAYAQQTRGLLGTIV 18	40mg/ml	25ul	F1	APITAYAQQTRGLLGTIV	Seg 7	3a	F1	APITAYAQQTRGLLGTIV	Seg 8	3a
2 of 97	18 7	AQQTRGLLGTIVTSLTGR 24	40mg/ml	25ul	F1	AQQTRGLLGTIVTSLTGR	Seg 7	3a	F1	AQQTRGLLGTIVTSLTGR	Seg 8	3a
3 of 97	17 14	LGTIVTSLTGRDKNVVA 30	40mg/ml	25ul	F1	LGTIVTSLTGRDKNVVA	Seg 7	3a	F1	LGTIVTSLTGRDKNVV	Seg 8	3a
4 of 97	17 20	SLTGRDKNVVAGEVQVL 36	40mg/ml	25ul					F1	SLTGRDKNVVTGEVQVL	Seg 8	3a
5 of 97	18 26	KNVVAGEVQVLSTATQTF 43	20mg/ml	50ul	F2				F1	KNVVTGEVQVLSTATQTF	Seg 8	3a
6 of 97	16 33	VQVLSTATQTFLGTTV 48	40mg/ml	25ul	F2							
7 of 97	18 38	TATQTFLGTTVGGVMWTV 55	40mg/ml	25ul	F2				F2			
8 of 97	18 45	GTTVGGVMWTVYHGAGSR 62	40mg/ml	25ul	F2				F2			
9 of 97	18 52	MWTVYHGAGSRTLAGVKH 69	40mg/ml	25ul	F2				F2			
10 of 97	17 59	AGSRTLAGVKHPALQMY 75	40mg/ml	25ul	F2				F2			
11 of 97	18 65	AGVKHPALQMYTNVDQDL 82	40mg/ml	25ul	F2				F2			
12 of 97	16 72	LQMYTNVDQDLVGWPA 87	40mg/ml	25ul	F2				F2			
13 of 97	18 77	NVDQDLVGWPAPPGAKSL 94	40mg/ml	25ul	F2				F2			
14 of 97	19 84	GWPAPPGAKSLEPCTCGSA 102	40mg/ml	25ul	F2				F3	GMPAPPGSRSLTPCTCGSA	Seg 9	3a
15 of 97	18 92	KSLEPCTCGSADLYLVTR 109	40mg/ml	25ul	F3	KSLEPCTCGSSDLYLVTR	Seg 8	1a	F3	KSLEPCTCGSSDLYLVTR	Seg 9	3a
16 of 97	18 99	CGSADLYLVTRDADVIPA 116	40mg/ml	25ul	F3	CGSSDLYLVTRHADVIPA	Seg 8	1a	F3	CGSADLYLVTRDADVIPA	Seg 9	3a
17 of 97	19 106	LVTRDADVIPARRRGDSTA 124	40mg/ml	25ul	F3	LVTRHADVIPVRRRGDSRG	Seg 8	1a	F3	LVTRDADVIPARRRGDSTA	Seg 9	3a
18 of 97	17 114	IPARRRGDSTASLLSPR 130	40mg/ml	25ul	F3	IPVRRRGDSRGSLLSPR	Seg 8	1a	F3	IPARRRGDSTASLLSPR	Seg 9	3a
19 of 97	17 120	GDSTASLLSPRPLARLK 136	40mg/ml	25ul	F3	GDSRGSLLSPRPISYLK	Seg 8	1a	F3	GDSTASLLSPRPLACLK	Seg 9	3a
20 of 97	18 126	LLSPRPLARLKGSSGGPV 143	40mg/ml	25ul	F3	LLSPRPISYLKGSSGGPV	Seg 8	1a	F3	LLSPRPLACLKGSSGGPV	Seg 9	3a
21 of 97	18 133	ARLKGSSGGPVMCPSGHV 150	40mg/ml	25ul	F3	SYLKGSSGGPVLCPSGHA	Seg 8	1a	F3	ACLKGSSGGPVMCPSGHV	Seg 9	3a
22 of 97	18 140	GGPVMCPSGHVAGIFRAA 157	40mg/ml	25ul	F3	GGPVLCPSGHAVGIFRAA	Seg 8	1a	F3	GGPVMCPSGHVAGIFRAA	Seg 9	3a
23 of 97	18 147	SGHVAGIFRAAVCTRGVA 164	40mg/ml	25ul	F3	SGHAVGIFRAAVCTRGVA	Seg 8	1a	F3	SGHVAGIFRAAVCTRGVA	Seg 9	3a
24 of 97	17 154	FRAAVCTRGVAKALQFI 170	40mg/ml	25ul	F3	FRAAVCTRGVAKAVDFI	Seg 8	1a	F3	FRAAVCTRGVAKALQFI	Seg 9	3a
25 of 97	16 160	TRGVAKALQFIPVETL 175	40mg/ml	25ul	F3	TRGVAKAVDFIPVESL	Seg 8	1a	F3	TRGVAKALQFIPVETL	Seg 9	3a
26 of 97	16 165	KALQFIPVETLSTQAR 180	40mg/ml	25ul	F3	KAVDFIPVESLSTEMR	Seg 8	1a	F3	KALQFIPVETLSTQAR	Seg 9	3a
27 of 97	15 170	IPVETLSTQARSPSF 184	40mg/ml	25ul		IPVESLSTEMRSPVF						
28 of 97	19 174	TLSTQARSPSFSDNSTPPA 192	40mg/ml	25ul	F4	SLSTEMRSPVFTDNSTPPA	Seg 9	4a	F4	TLSTQARSPVFSDNSSPPA	Seg 10	1a
29 of 97	18 182	PSFSDNSTPPAVPQSYQV 199	40mg/ml	25ul	F4	PVFTDNSTPPAVPQTYQV	Seg 9	4a	F4	PVFSDNSSPPAVPQSYQV	Seg 10	1a
30 of 97	16 189	TPPAVPQSYQVGYLHA 204	40mg/ml	25ul	F4	TPPAVPQTYQVAHLHA	Seg 9	4a	F4	SPPAVPQSYQVAHLHA	Seg 10	1a
31 of 97	17 194	PQSYQVGYLHAPTGSGK 210	40mg/ml	25ul	F4	PQTYQVAHLHAPTGSGK	Seg 9	4a	F4	PQSYQVAHLHAPTGSGK	Seg 10	1a
32 of 97	18 200	GYLHAPTGSGKSTKVPAA 217	40mg/ml	25ul	F4	AHLHAPTGSGKSTKVPAA	Seg 9	4a	F4	AHLHAPTGSGKSTKVPAA	Seg 10	1a
33 of 97	17 207	GSGKSTKVPAAYVAQGY 223	40mg/ml	25ul	F4	GSGKSTKVPAAYAAQGYK	Seg 9	4a	F4	GSGKSTKVPAAYAAQGYK	Seg 10	1a
34 of 97	16 213	KVPAAYVAQGYNVLVL 228	40mg/ml	25ul	F4	KVPAAYAAQGYKVLVL	Seg 9	4a	F4	VPAAYAAQGYKVLVL	Seg 10	1a
35 of 97	17 218	YVAQGYNVLVLNPSVAA 234	40mg/ml	25ul	F4	YAAQGYKVLVLNPSVAA	Seg 9	4a	F4	YAAQGYKVLVLNPSVAA	Seg 10	1a
36 of 97	18 224	NVLVLNPSVAATLGFGSF 241	40mg/ml	25ul	F4	KVLVLNPSVAATLGFGAY	Seg 9	4a	F4	KVLVLNPSVAATLGFGAY	Seg 10	1a
37 of 97	18 231	SVAATLGFGSFMSRAYGI 248	40mg/ml	25ul	F4	SVAATLGFGAYMSKAYGI	Seg 9	4a	F4	SVAATLGFGAYMSKAYGI	Seg 10	1a
38 of 97	16 238	FGSFMSRAYGIDPNIR 253	40mg/ml	25ul	F4	FGAYMSKAYGIDPNIR	Seg 9	4a				
39 of 97	17 243	SRAYGIDPNIRTGNRTV 259	40mg/ml	25ul		SKAYGIDPNIRSGVRTI						
40 of 97	17 249	DPNIRTGNRTVTTGAKL 265	40mg/ml	25ul	F5	DPNIRSGVRTITTGAPI	Seg 10	4a	F5	DPNIRTGNRTITTGAKL	Seg 11	3a
41 of 97	18 255	GNRTVTTGAKLTYSTYGK 272	40mg/ml	25ul	F5	GVRTITTGAPITYSTYGK	Seg 10	4a	F5	GVRTITTGAKLTYSTYGK	Seg 11	3a
42 of 97	18 262	GAKLTYSTYGKFLAGGGC 279	40mg/ml	25ul	F5	GAPITYSTYGKFLADGGC	Seg 10	4a	F5	GAKLTYSTYGKFLADGGC	Seg 11	3a
43 of 97	18 269	TYGKFLAGGGCSGGAYDV 286	40mg/ml	25ul	F5	YGKFLADGGCSGGAYDI	Seg 10	4a	F5	TYGKFLADGGCSGGAYDV	Seg 11	3a
44 of 97	18 276	GGGCSGGAYDVIICDDCH 293	40mg/ml	25ul	F5	DGGCSGGAYDIIICDECH	Seg 10	4a	F5	DGGCSGGAYDVIICDECH	Seg 11	3a
45 of 97	18 283	AYDVIICDDCHAQDATSI 300	40mg/ml	25ul	F5	AYDIIICDECHSTDSTTI	Seg 10	4a	F5	AYDVIICDECHAQDATSI	Seg 11	3a

46 of 97	18	290 DDCHAQDATSILGIGTVL 307	40mg/ml	25ul	F5	DECHSTDSTTILGIGTVL	Seg 10	4a	F5	DECHAQDATSILGIGTVL	Seg 11	3a
47 of 97	17	297 ATSILGIGTVLDQAETA 313	40mg/ml	25ul	F5	STTILGIGTVLDQAETA	Seg 10	4a	F5	ATSILGIGTVLDQAETA	Seg 11	3a
48 of 97	18	303 IGTVLDQAETAGVRLTVL 320	40mg/ml	25ul	F5	IGTVLDQAETAGVRLVVL	Seg 10	4a	F5	IGTVLDQAETAGVRLTVL	Seg 11	3a
49 of 97	18	310 AETAGVRLTVLATATPPG 327	20mg/ml	50ul	F5	AETAGVRLVVLATATPPG	Seg 10	4a	F5	AETAGVRLTVLATATPPG	Seg 11	3a

Pool G

NS3

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
50 of 97	17	317 LTVLATATPPGSITVPH 333	40mg/ml	25ul	G1					TVLATATPPGSITVPH	Seg 11	3a
51 of 97	18	323 ATPPGSITVPHSNIEEVA 340	40mg/ml	25ul	G1					ATPPGSITVPHSNIEEVA	Seg 11	3a
52 of 97	18	330 TVPHSNIEEVALGSEGEI 347	40mg/ml	25ul	G1					TVPHSNIEEVALSTTGEI	Seg 11	3a
53 of 97	18	337 EEVALGSEGEIPFYGKAI 354	40mg/ml	25ul	G1							
54 of 97	17	344 EGEIPFYGKAIPIACIK 360	40mg/ml	25ul	G1							
55 of 97	18	350 YGKAIPIACIKGGRHLIF 367	40mg/ml	25ul		YGKAIPIACIKGGRHLIF	Seg 11	1a				
56 of 97	17	357 ACIKGGRHLIFCHSKKK 373	40mg/ml	25ul	G2	EVIKGGRHLIFCHSKKK	Seg 11	1a				
57 of 97	18	363 RHLIFCHSKKKCDKMASK 380	40mg/ml	25ul	G2	RHLIFCHSKKKCDELAAK	Seg 11	1a				
58 of 97	17	370 SKKKCDKMASKLRGMGL 386	40mg/ml	25ul	G2	SKKKCDELASKLRGMGL	Seg 11	1a				
59 of 97	18	376 KMASKLRGMGLNAVAYYR 393	40mg/ml	25ul								
60 of 97	18	383 GMGLNAVAYYRGLDVSVI 400	40mg/ml	25ul	G3-I	GMGLNAVAYYRGLDVSVI	Seg 12	1b				
61 of 97	18	390 AYYRGLDVSVIPTTGDVV 407	40mg/ml	25ul	G3-I	AYYRGLDVSVIPTSGDVV	Seg 12	1b		AYYRGLDVSVIPTAGDVV	Seg 12	3a
62 of 97	18	397 VSVIPTTGDVVVCATDAL 414	40mg/ml	25ul	G3-I	VSVIPTSGDVVVVATDAL	Seg 12	1b		VSVIPTAGDVVVCATDAL	Seg 12	3a
63 of 97	15	404 GDVVVCATDALMTGF 418	40mg/ml	25ul	G3-I	GDVVVVATDALMTGF	Seg 12	1b		GDVVVCATDALMTGF	Seg 12	3a
64 of 97	18	408 VCATDALMTGFTGDFDSV 425	40mg/ml	25ul	G3-I	VVATDALMTGFTGDFDSV	Seg 12	1b		VCATDALMTGFTGDFDSV	Seg 12	3a
65 of 97	18	415 MTGFTGDFDSVIDCNVAV 432	20mg/ml	50ul	G3-I	MTGFTGDFDSVIDCNTCV	Seg 12	1b		MTGFTGDFDSVIDCNVAV	Seg 12	3a
66 of 97	17	422 FDSVIDCNVAVEQYVDF 438	40mg/ml	25ul	G3-I	FDSVIDCNTCVTQTVDF	Seg 12	1b		FDSVIDCNVAVEQYVDF	Seg 12	3a
67 of 97	17	428 CNVAVEQYVDFSLDPTF 444	40mg/ml	25ul	G3-I	CNTCVTQTVDFSLDPTF	Seg 12	1b		CNVAVEQYVDFSLDPTF	Seg 12	3a
68 of 97	19	433 EQYVDFSLDPTFSIETCTA 451	40mg/ml	25ul	G3-I	TQTVDFSLDPTFTIETTTV	Seg 12	1b		EQYVDFSLDPTFSIETRTA	Seg 12	3a
69 of 97	18	441 DPTFSIETCTAPQDAVSR 458	40mg/ml	25ul	G3-I	DPTFTIETTTVPQDAVSR	Seg 12	1b		DPTFSIETRTAPQDAVSR	Seg 12	3a
70 of 97	17	448 TCTAPQDAVSRSQRRGR 464	40mg/ml	25ul	G3-I	TTTVPQDAVSRSQRRGR	Seg 12	1b		TRTAPQDAVSRSQRRGR	Seg 12	3a
71 of 97	17	454 DAVSRSQRRGRTGRGRL 470	40mg/ml	25ul	G3-I	DAVSRSQRRGRTGRGR	Seg 12	1b		DAVSRSQRRGRTGRGR	Seg 12	3a
72 of 97	18	459 SQRRGRTGRGRLGTYRYV 476	40mg/ml	25ul	G3-I	SQRRGRTGRGRRGIYRFV	Seg 12	1b		SQRRGRTGRGRLGTYRYV	Seg 12	3a
73 of 97	16	466 GRGRLGTYRYVTPGER 481	40mg/ml	25ul	G3-I	GRGRRGIYRFVTPGER	Seg 12	1b		GRGRLGTYRYVGPGER	Seg 12	3a
74 of 97	16	471 GTYRYVTPGERPSGMF 486	40mg/ml	25ul	G3-I	GIYRFVTPGERPSGMF	Seg 12	1b		GTYRYVGPGERPSGMF	Seg 12	3a
75 of 97	16	476 VTPGERPSGMFDSVVL 491	40mg/ml	25ul	G3-I	VTPGERPSGMFDSSVL	Seg 12	1b		VAPGERPSGMFDSVVL	Seg 12	3a
76 of 97	17	481 RPSGMFDSVVLCECYDA 497	40mg/ml	25ul	G3-II	RPSGMFDSSVLCECYDA	Seg 12	1b		RPSGMFDSVVLCECYDA	Seg 12	3a
77 of 97	18	487 DSVVLCECYDAGCSWYDL 504	40mg/ml	25ul	G3-II	DSSVLCECYDAGCAWYEL	Seg 12	1b		DSVVLCECYDAGCAWYEL	Seg 12	3a
78 of 97	18	494 CYDAGCSWYDLQPAETTV 511	40mg/ml	25ul	G3-II	CYDAGCAWYELTPAETSV	Seg 12	1b		CYDAGCAWYELQPAETTV	Seg 12	3a
79 of 97	17	501 WYDLQPAETTVRLRAYL 517	40mg/ml	25ul	G3-II	WYELTPAETSVRLRAYL	Seg 12	1b		WYELQPAETTVRLRAYL	Seg 12	3a
80 of 97	18	507 AETTVRLRAYLSTPGLPV 524	40mg/ml	25ul	G3-II	AETSVRLRAYLNTPGLPV	Seg 12	1b		AETTVRLRAYLSTPGLPV	Seg 12	3a
81 of 97	18	514 RAYLSTPGLPVCQDHLDL 531	40mg/ml	25ul	G3-II	RAYLNTPGLPVCQDHLEF	Seg 12	1b		RAYLSTPGLPVCQDHLDF	Seg 12	3a
82 of 97	16	521 GLPVCQDHLDLWESVF 536	40mg/ml	25ul	G3-II	GLPVCQDHLEFWEGVF	Seg 12	1b		GLPVCQDHLDFWESVF	Seg 12	3a
83 of 97	18	525 CQDHLDLWESVFTGLTHI 542	40mg/ml	25ul	G3-II	CQDHLEFWEGVFTGLTHI	Seg 12	1b		CQDHLDFWESVFTGLTHI	Seg 12	3a
84 of 97	16	532 WESVFTGLTHIDAHFL 547	40mg/ml	25ul	G3-II	WEGVFTGLTHIDAHFL	Seg 12	1b		WESVFTGLTHIDAHFL	Seg 12	3a
85 of 97	17	537 TGLTHIDAHFLSQTKQA 553	40mg/ml	25ul	G3-II	TGLTHIDAHFLSQTKQA	Seg 12	1b		TGLTHIDAHFLSQTKQQ	Seg 12	3a
86 of 97	18	543 DAHFLSQTKQAGLNFSYL 560	40mg/ml	25ul	G3-II	DAHFLSQTKQAGDNFPYL	Seg 12	1b		DAHFLSQTKQQGLNFPYL	Seg 12	3a
87 of 97	18	550 TKQAGLNFSYLTAYQATV 567	40mg/ml	25ul	G3-II	TKQAGDNFPYLVAYQATV	Seg 12	1b		TKQQGLNFPYLTAYQATV	Seg 12	3a

88 of 97	17 557 FSYLTAYQATVCARAQA 573	40mg/ml	25ul		G3-II	FPYLVAYQATVCARAQA	Seg 12	1b		FPYLTAYQATVCARAQA	Seg 12	3a
89 of 97	16 563 YQATVCARAQAPPPSW 578	40mg/ml	25ul		G3-II	YQATVCARAQAPPPSW	Seg 12	1b		YQATVCARAQAPPPSW	Seg 12	3a
90 of 97	18 568 CARAQAPPPSWDETWKCL 585	40mg/ml	25ul		G3-II	CARAQAPPPSWDQMWKCL	Seg 12	1b		CARAQAPPPSWDEMWKCL	Seg 12	3a
91 of 97	18 575 PPSWDETWKCLVRLKPTL 592	40mg/ml	25ul		G3-II	PPSWDQMWKCLVRLKPTL	Seg 12	1b		PPSWDEMWKCLVRLKPTL	Seg 12	3a
92 of 97	18 582 WKCLVRLKPTLHGPTPLL 599	40mg/ml	25ul	G4						WKCLVRLKPTLHGPTPLL	Seg 12	3a
93 of 97	17 589 KPTLHGPTPLLYRLGPV 605	40mg/ml	25ul	G4						KPTLHGPTPLLYRLGPV	Seg 12	3a
94 of 97	17 595 PTPLLYRLGPVQNEICL 611	40mg/ml	25ul							PTPLLYRLGPVQNEVTL	Seg 12	3a
95 of 97	18 601 RLGPVQNEICLTHPITKY 618	40mg/ml	25ul	H1		RLGAVQNEVTLTHPITKY	Seg 13	4a				
96 of 97	18 608 EICLTHPITKYVMACMSA 625	40mg/ml	25ul	H1		EVTLTHPITKYIMACMSA	Seg 13	4a				
97 of 97	17 615 ITKYVMACMSA---VTT 631	40mg/ml	25ul	H1		ITKYIMACMSADLEVVT	Seg 13	4a		ITKYVMACMSADLEVTT	Seg 13	3a

Pool H

NS4A

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 7	18 1	STWVLLGGVLAAVAAYCL 18	13.3mg/ml	75ul	H1	STWVLVGGVLAALAAYCL	Seg 13	4a	H1	STWVLLGGVLAALAAYCL	Seg 13	3a
2 of 7	18 8	GVLAAVAAYCLSVGCVVI 25	20mg/ml	50ul	H1	GVLAALAAYCLSVGSVVI	Seg 13	4a	H1	GVLAALAAYCLSVGCVVI	Seg 13	3a
3 of 7	17 15	AYCLSVGCVVIVGHIEL 31	40mg/ml	25ul	H1	AYCLSVGSVVIVGHIEL	Seg 13	4a	H1	AYCLSVGCVVIVGHEVL	Seg 13	3a
4 of 7	18 21	GCVVIVGHIELGGKPALV 38	40mg/ml	25ul								
5 of 7	18 28	HIELGGKPALVPDKEVLY 45	40mg/ml	25ul	H2							
6 of 7	17 35	PALVPDKEVLYQQYDEM 51	40mg/ml	25ul	H2							
7 of 7	14 41	KEVLYQQYDEMEEC 54	40mg/ml	25ul	H2							

NS4B

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 39	18 1	SQARPYIEQAQVIAHQFK 18	40mg/ml	25ul	H2				H2			
2 of 39	18 8	EQAQVIAHQFKEKVLGLL 25	40mg/ml	25ul	H2				H2			
3 of 39	19 15	HQFKEKVLGLLQRATQQQA 33	40mg/ml	25ul	H2				H2			
4 of 39	18 23	GLLQRATQQQAVIEPIVV 40	13.3mg/ml	75ul	H2				H2			
5 of 39	18 29	TQQQAVIEPIVVSNWQKL 46	13.3mg/ml	75ul	H2				H2			
6 of 39	18 36	EPIVVSNWQKLEVLWHKH 53	40mg/ml	25ul								
7 of 39	16 43	WQKLEVLWHKHMWNFV 58	40mg/ml	25ul		WQKLEVFWAKHMWNFI	Seg 14	1a	H3	WQKLEVFWAKHMWNFI	Seg 14	1a
8 of 39	18 48	VLWHKHMWNFVSGIQYLA 65	40mg/ml	25ul	H3	VFWAKHMWNFISGIQYLA	Seg 14	1a	H3	VFWAKHMWNFISGIQYLA	Seg 14	1a
9 of 39	16 55	WNFVSGIQYLAGLSTL 70	40mg/ml	25ul	H3	WNFISGIQYLAGLSTL	Seg 14	1a	H3	WNFISGIQYLAGLSTL	Seg 14	1a
10 of 39	18 60	GIQYLAGLSTLPGNPAVA 77	40mg/ml	25ul	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a	H3	GIQYLAGLSTLPGNPAIA	Seg 14	1a
11 of 39	18 67	LSTLPGNPAVASLMAFTA 84	40mg/ml	25ul	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a	H3	LSTLPGNPAIASLMAFTA	Seg 14	1a
12 of 39	17 74	PAVASLMAFTASVTSPL 90	20mg/ml	50ul	H3	PAIASLMAFTAAVTSPL	Seg 14	1a	H3	PAIASLMAFTASVTSPL	Seg 14	1a
13 of 39	18 80	MAFTASVTSPLTTNQTMF 97	20mg/ml	50ul	H3	MAFTAAVTSPLTTNQTMF	Seg 14	1a	H4			
14 of 39	18 87	TSPLTTNQTMFFNILGGW 104	40mg/ml	25ul					H4			
15 of 39	18 93	NQTMFFNILGGWVATHLA 110	40mg/ml	25ul	H4				H4			
16 of 39	18 100	ILGGWVATHLAGPQASSA 117	40mg/ml	25ul	H4				H4			
17 of 39	18 107	THLAGPQASSAFVVSGLA 124	40mg/ml	25ul	H4				H4			
18 of 39	18 114	ASSAFVVSGLAGAAIGGI 131	13.3mg/ml	75ul					H4			
19 of 39	18 121	SGLAGAAIGGIGLGRVLL 138	40mg/ml	25ul	H5	AGLAGAAVGSVGLGKVLV	Seg 15	1a		AGLAGAAIGSVGLGKVLV	Seg 15	1a
20 of 39	17 128	IGGIGLGRVLLDILAGY 144	40mg/ml	25ul	H5	IGSVGLGKVLVDILAGY	Seg 15	1a	H5	IGSVGLGKVLVDILAGY	Seg 15	1a
21 of 39	18 134	GRVLLDILAGYGAGVSGA 151	40mg/ml	25ul	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a	H5	GKVLVDILAGYGAGVAGA	Seg 15	1a
22 of 39	18 141	LAGYGAGVSGALVAFKIM 158	40mg/ml	25ul	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a	H5	LAGYGAGVAGALVAFKIM	Seg 15	1a

23 of 39	18	148	VSGALVAFKIMGGEPPTT	165	40mg/ml	25ul	H5	VAGALVAFKIMSGEVPST	Seg 15	1a	H5	VAGALVAFKIMSGEIPST	Seg 15	1a
24 of 39	18	155	FKIMGGEPPTTEDMVNLL	172	40mg/ml	25ul	H5	FKIMSGEVPSTEDLVNLL	Seg 15	1a	H5	LVAFKIMSGEIPSTEDLV	Seg 15	1a
25 of 39	15	162	PPTTEDMVNLLPAIL	176	40mg/ml	25ul	H5	PSTEDLVNLLPAIL	Seg 15	1a	H5	PSTEDLVNLLPAIL	Seg 15	1a
26 of 39	18	166	EDMVNLLPAILSPGALVV	183	40mg/ml	25ul	H5	EDLVNLLPAILSPGALVV	Seg 15	1a	H5	EDLVNLLPAILSPGALVV	Seg 15	1a
27 of 39	18	173	PAILSPGALVVGVICAAI	190	40mg/ml	25ul	H5	PAILSPGALVVGVVCAAI	Seg 15	1a	H5	PAILSPGALVVGVVCAAI	Seg 15	1a
28 of 39	16	180	ALVVGVICAAILRRHV	195	40mg/ml	25ul	H5	ALVVGVVCAAILRRHV	Seg 15	1a	H5	ALVVGVVCAAILRRHV	Seg 15	1a
29 of 39	18	185	VICAAILRRHVGPGEGPV	202	40mg/ml	25ul	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a	H5	VVCAAILRRHVGPGEGAV	Seg 15	1a
30 of 39	18	192	RRHVGPGEGPVQWMNRLI	209	40mg/ml	25ul	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a	H5	RRHVGPGEGAVQWMNRLI	Seg 15	1a
31 of 39	16	199	EGPVQWMNRLIAFASR	214	40mg/ml	25ul	H5	EGAVQWMNRLIAFASR	Seg 15	1a	H5	EGAVQWMNRLIAFASR	Seg 15	1a
32 of 39	18	204	WMNRLIAFASRGNHVSPA	221	40mg/ml	25ul	H5	WMNRLIAFASRGNHVSPT	Seg 15	1a	H5	WMNRLIAFASRGNHVSPT	Seg 15	1a
33 of 39	19	211	FASRGNHVSPAHYVPESDA	229	40mg/ml	25ul	H5	FASRGNHVSPTHYVPESDA	Seg 15	1a	H5	FASRGNHVSPTHYVPESDA	Seg 15	1a
34 of 39	18	219	SPAHYVPESDAAARVTAL	236	40mg/ml	25ul	H5	SPTHYVPESDAAARVTAL	Seg 15	1a	H5	SPTHYVPESDAAARVTAI	Seg 15	1a
35 of 39	17	226	ESDAAARVTALLSSLTV	242	40mg/ml	25ul	H5	ESDAAARVTALLSSLTV	Seg 15	1a	H5	ESDAAARVTAILSSLTV	Seg 15	1a
36 of 39	18	232	RVTALLSSLTVTSLLRRL	249	40mg/ml	25ul	H6				H5	RVTALLSSLTVTSLLRRL	Seg 15	1a
37 of 39	15	239	SLTVTSLLRRLHQWI	253	40mg/ml	25ul	H6				H6			
38 of 39	15	243	TSLLRRLHQWINEDY	257	40mg/ml	25ul	H6				H6			
39 of 39	15	247	RRLHQWINEDYPSPC	261	40mg/ml	25ul	H6				H6			

Pool I

NS5A

Peptide	Length	Sequence		Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 72	16	1	SGDWLRIIWDWVCSVV	16	40mg/ml	25ul	I1						
2 of 72	18	6	RIIWDWVCSVVSDFKTWL	23	40mg/ml	25ul	I1						
3 of 72	18	13	CSVVSDFKTWLSAKIMPA	30	40mg/ml	25ul	I1						
4 of 72	18	20	KTWLSAKIMPALPGLPFI	37	40mg/ml	25ul	I1						
5 of 72	18	27	IMPALPGLPFISCQKGYK	44	40mg/ml	25ul	I1						
6 of 72	15	34	LPFISCQKGYKGVWR	48	40mg/ml	25ul	I1						
7 of 72	16	38	SCQKGYKGVWRGDGVM	53	40mg/ml	25ul	I1						
8 of 72	19	43	YKGVWRGDGVMSTRCPCGA	61	40mg/ml	25ul	I1						
9 of 72	18	51	GVMSTRCPCGASIAGHVK	68	40mg/ml	25ul	I1						
10 of 72	18	58	PCGASIAGHVKNGSMRLA	75	40mg/ml	25ul	I1						
11 of 72	17	65	GHVKNGSMRLAGPRTCA	81	40mg/ml	25ul	I1						
12 of 72	18	71	SMRLAGPRTCANMCHGTF	88	40mg/ml	25ul	I1						
13 of 72	16	78	RTCANMCHGTFPINEY	93	40mg/ml	25ul	I1						
14 of 72	18	83	MCHGTFPINEYTTGPSTP	100	40mg/ml	25ul	I1						
15 of 72	17	90	INEYTTGPSTPCPPPNY	106	40mg/ml	25ul	I1						
16 of 72	18	96	GPSTPCPPPNYTRALWRV	113	40mg/ml	25ul	I2						
17 of 72	17	103	PPNYTRALWRVAANSYV	119	40mg/ml	25ul	I2						
18 of 72	16	109	ALWRVAANSYVEVRRV	124	40mg/ml	25ul	I2						
19 of 72	17	114	AANSYVEVRRVGDFHYI	130	40mg/ml	25ul	I2						
20 of 72	19	120	EVRRVGDFHYITGATEDGL	138	40mg/ml	25ul	I2						
21 of 72	17	128	HYITGATEDGLKCPCQV	144	40mg/ml	25ul	I2						
22 of 72	17	134	TEDGLKCPCQVPATEFF	150	40mg/ml	25ul	I2				TTDNLKCPCQVPAPEFF	Seg 16	1b
23 of 72	18	140	CPCQVPATEFFTEVDGVR	157	40mg/ml	25ul	I2				CPCQVPAPEFFTEVDGVR	Seg 16	1b
24 of 72	16	147	TEFFTEVDGVRIHRYA	162	40mg/ml	25ul	I2				PEFFTELDGVRLHRFA	Seg 16	1b
25 of 72	18	152	EVDGVRIHRYAPPCRPLL	169	40mg/ml	25ul	I2				ELDGVRLHRYAPPCRPLL	Seg 16	1b

26 of 72	18	159	HRYAPPCRPLLRDEITFM	176	40mg/ml	25ul		I2						
27 of 72	18	166	RPLLRDEITFMVGLNSYA	183	40mg/ml	25ul		I2						
28 of 72	16	173	ITFMVGLNSYAIGSQL	188	40mg/ml	25ul		I2						
29 of 72	19	178	GLNSYAIGSQLPCEPEPDV	196	40mg/ml	25ul		I2				GLNSYAIGSQLPCEPEPDV	Seg 17	3a
30 of 72	18	186	SQLPCEPEPDVSVLTSML	203	40mg/ml	25ul		I2				SQLPCEPEPDVSVLTSML	Seg 17	3a
31 of 72	17	193	EPDVSVLTSMLRDPSHI	209	40mg/ml	25ul		I2				EPDVAVLTSMLRDPSHI	Seg 17	3a
32 of 72	18	199	LTSMLRDPSHITAETAAR	216	40mg/ml	25ul								
33 of 72	15	206	PSHITAETAARRLAR	220	40mg/ml	25ul			PSHITAETAARRLAR	Seg 16	6d			
34 of 72	18	210	TAETAARRLARGSPPSEA	227	40mg/ml	25ul	I2		TAETAARRLARGSPPSLA	Seg 16	6d	TAETAARRLARGSPPSEA	Seg 18	3a
35 of 72	18	217	RLARGSPPSEASSSASQL	234	40mg/ml	25ul	I2		RLARGSPPSLASSSASQL	Seg 16	6d	RLARGSPPSAESSSASQL	Seg 18	3a
36 of 72	18	224	PSEASSSASQLSAPSLKA	241	40mg/ml	25ul	I2		PSLASSSASQLSAPSLKA	Seg 16	6d	PSEASSSASQLSAPSLKA	Seg 18	3a
37 of 72	17	231	ASQLSAPSLKATCQTHR	247	40mg/ml	25ul	I2		ASQLSAPSLKATCTANH	Seg 16	6d	ASQLSAPSLKATCQTHR	Seg 18	3a
38 of 72	18	237	PSLKATCQTHRPHPDAEL	254	40mg/ml	25ul			PSLKATCTQTHRPHPDAEL	Seg 16	6d	PSLKATCQTHRPHPDAEL	Seg 18	3a
39 of 72	18	243	CQTHRPHPDAELVDANLL	260	40mg/ml	25ul		I3						
40 of 72	16	250	PDAELVDANLLWRQEM	265	40mg/ml	25ul		I3						
41 of 72	18	255	VDANLLWRQEMGSNITRV	272	40mg/ml	25ul		I3						
42 of 72	18	262	RQEMGSNITRVESETKVV	279	40mg/ml	25ul		I3						
43 of 72	16	269	ITRVESETKVVILDSF	284	20mg/ml	50ul		I3						
44 of 72	16	274	SETKVVILDSFEPLRA	289	40mg/ml	25ul		I3						
45 of 72	18	279	VILDSFEPLRAETDDAEL	296	40mg/ml	25ul		I3						
46 of 72	18	286	PLRAETDDAELSAAAECF	303	40mg/ml	25ul		I3						
47 of 72	17	293	DAELSAAAECFKKPPKY	309	40mg/ml	25ul		I3						
48 of 72	18	299	AAECFKKPPKYPPALPIW	316	40mg/ml	25ul		I3						

Pool I continued

Peptide	Length	Sequence		Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
49 of 72	16	306	PPKYPPALPIWARPDY	321	40mg/ml	25ul		I3						
50 of 72	18	311	PALPIWARPDYNPPLLDR	328	40mg/ml	25ul		I3						
51 of 72	18	318	RPDYNPPLLDRWKSPDYV	335	40mg/ml	25ul		I3						
52 of 72	16	325	LLDRWKSPDYVPPTVH	340	40mg/ml	25ul		I3						
53 of 72	18	330	KSPDYVPPTVHGCALPPK	347	40mg/ml	25ul		I3						
54 of 72	16	337	PTVHGCALPPKGAPPV	352	40mg/ml	25ul		I3						
55 of 72	18	342	CALPPKGAPPVPPPRRKR	359	40mg/ml	25ul		I3						
56 of 72	15	349	APPVPPPRRKRTIQL	363	40mg/ml	25ul	I4							
57 of 72	18	353	PPPRRKRTIQLDGSNVSA	370	40mg/ml	25ul	I4							
58 of 72	17	360	TIQLDGSNVSAALAALA	376	40mg/ml	25ul	I4							
59 of 72	15	366	SNVSAALAALAEKSF	380	40mg/ml	25ul	I4							
60 of 72	15	370	AALAALAEKSFPSSK	384	20mg/ml	50ul	I4							
61 of 72	18	374	ALAEKSFPSSKPQEENSS	391	40mg/ml	25ul	I4							
62 of 72	15	381	PSSKPQEENSSSSGV	395	40mg/ml	25ul	I4							
63 of 72	18	385	PQEENSSSSGVDTQSSTA	402	40mg/ml	25ul	I4							
64 of 72	15	392	SSGVDTQSSTASKVL	406	40mg/ml	25ul	I4							
65 of 72	18	396	DTQSSTASKVLPSPGEES	413	40mg/ml	25ul	I4							
66 of 72	19	403	SKVLPSPGEESDSESCSSM	421	40mg/ml	25ul	I4				SKVLPSPGBESDSESCSSM	Seg 19	3a	
67 of 72	18	411	EESDSESCSSMPPLEGEP	428	40mg/ml	25ul	I4				EESDSESCSSMPPLEGEP	Seg 19	3a	
68 of 72	16	418	CSSMPPLEGEPGDPDL	433	40mg/ml	25ul	I4				CSSMPPLEGEPGDPDL	Seg 19	3a	

69 of 72	16	423	PLEGEPGDPDLSCDSW 438	40mg/ml	25ul	I4						PLEGEPGDPDLSCDSW	Seg 19	3a
70 of 72	18	428	PGDPDLSCDSWSTVSDSE 445	40mg/ml	25ul	I4						PGDPDLSCDSWSTVSDSE	Seg 19	3a
71 of 72	16	435	CDSWSTVSDSEEQSVV 450	40mg/ml	25ul							CDSWSTVSDSEEQSVV	Seg 19	3a
72 of 72	13	440	TVSDSEEQSVVCC 452	40mg/ml	25ul			TVSDSEEQSTVCC	Seg 18	6a		TVSDSEEQSVVCC	Seg 20	3a

Pool L

NS5B

Peptide	Length		Sequence	Conc.	Vol.	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*	*Vacc Seq.*	*Segment*	*Vacc GT*
1 of 90	16	1	SMSYSWTGALITPCSA 16	40mg/ml	25ul	L1	SMSYSWTGALITPCAA	Seg 18	6a		SMSYSWTGALITPCSA	Seg 20	3a
2 of 90	18	6	WTGALITPCSAEEEKLPI 23	40mg/ml	25ul	L1	WTGALITPCAAEEEKLPI	Seg 18	6a		WTGALITPCSAEEEKLPI	Seg 20	3a
3 of 90	18	13	PCSAEEEKLPISPLSNSL 30	40mg/ml	25ul	L1	PCAAEEEKLPINPLSNSL	Seg 18	6a		PCSAEEEKLPINPLSNSL	Seg 20	3a
4 of 90	18	20	KLPISPLSNSLLRHHNLV 37	40mg/ml	25ul	L1	KLPINPLSNSLIRHHNMV	Seg 18	6a		KLPINPLSNSLLRHHNLV	Seg 20	3a
5 of 90	17	27	SNSLLRHHNLVYSTSSR 43	40mg/ml	25ul	L1	SNSLIRHHNMVYSTTSR	Seg 18	6a		SNSLLRHHNLVYSTSSR	Seg 20	3a
6 of 90	18	33	HHNLVYSTSSRSASQRQK 50	40mg/ml	25ul	L1	HHNMVYSTTSRSASLRQK	Seg 18	6a		HHNLVYSTSSRSASQRQK	Seg 20	3a
7 of 90	18	40	TSSRSASQRQKKVTFDRL 57	40mg/ml	25ul	L1	TTSRSASLRQKKVTFDRL	Seg 18	6a		TSSRSASQRQKKVTFDRL	Seg 20	3a
8 of 90	18	46	SQRQKKVTFDRLQVLDDH 63	40mg/ml	25ul	L1	CQRQKKVTFDRLQVLDDH	Seg 18	6a		SQRQKKVTFDRLQVLDDH	Seg 20	3a
9 of 90	16	53	TFDRLQVLDDHYKTAL 68	40mg/ml	25ul	L2					TFDRLQVLDDHYKTALQ	Seg 20	3a
10 of 90	18	57	LQVLDDHYKTALQEVKER 74	40mg/ml	25ul	L2							
11 of 90	18	64	YKTALQEVKERASRVKAR 81	40mg/ml	25ul	L2							
12 of 90	18	71	VKERASRVKARMLSIEEA 88	40mg/ml	25ul	L2							
13 of 90	18	78	VKARMLSIEEACALVPPH 95	40mg/ml	25ul	L2							
14 of 90	17	85	IEEACALVPPHSARSKF 101	40mg/ml	25ul	L2							
15 of 90	18	91	LVPPHSARSKFGYSAKDV 108	40mg/ml	25ul	L2							
16 of 90	18	98	RSKFGYSAKDVRSLSSKA 115	40mg/ml	25ul	L2							
17 of 90	18	105	AKDVRSLSSKAINQIRSV 122	40mg/ml	25ul	L2							
18 of 90	16	112	SSKAINQIRSVWEDLL 127	40mg/ml	25ul	L2							
19 of 90	18	117	NQIRSVWEDLLEDTTTPI 134	40mg/ml	25ul	L2							
20 of 90	18	124	EDLLEDTTTPIPTTIMAK 141	40mg/ml	25ul	L2							
21 of 90	17	131	TTPIPTTIMAKNEVFCV 147	40mg/ml	25ul	L2							
22 of 90	18	137	TIMAKNEVFCVDPAKGGR 154	40mg/ml	25ul	L2							
23 of 90	18	144	VFCVDPAKGGRKAARLIV 161	40mg/ml	25ul	L3	VFCVDPAKGGRKPARLIV	Seg 19	3a		VFCVDPEKGGRKPARLIV	Seg 21	1a
24 of 90	18	151	KGGRKAARLIVYPDLGVR 168	40mg/ml	25ul	L3	GGRKPARLIVYPDLGVR	Seg 19	3a		GGRKPARLIVYPDLGVR	Seg 21	1a
25 of 90	18	158	RLIVYPDLGVRVCEKRAL 175	40mg/ml	25ul	L3	RLIVYPDLGVRVCEKRAL	Seg 19	3a		RLIVYPDLGVRVCEKMAL	Seg 21	1a
26 of 90	18	165	LGVRVCEKRALYDVIQRL 182	40mg/ml	25ul	L3	LGVRVCEKRALYDVIQRL	Seg 19	3a		LGVRVCEKMALYDVIQRL	Seg 21	1a
27 of 90	16	172	KRALYDVIQRLSIETM 187	40mg/ml	25ul								
28 of 90	17	177	DVIQRLSIETMGSAYGF 193	40mg/ml	25ul	L4							
29 of 90	18	183	SIETMGSAYGFQYSPRQR 200	40mg/ml	25ul	L4							
30 of 90	18	190	AYGFQYSPRQRVERLLKM 207	40mg/ml	25ul	L4							
31 of 90	16	197	PRQRVERLLKMWTSKK 212	40mg/ml	25ul	L4							
32 of 90	18	202	ERLLKMWTSKKTPLGFSY 219	40mg/ml	25ul		ERLLKMWTSKKCPMGFSY	Seg 20	6h		ERLLKMWTSKKTPMGFSY	Seg 22	3a
33 of 90	16	209	TSKKTPLGFSYDTRCF 224	40mg/ml	25ul	L5	KSKKTPMGFSYDTRCF	Seg 20	6h		KSKKTPMGFSYDTRCF	Seg 22	3a
34 of 90	15	214	PLGFSYDTRCFDSTV 228	40mg/ml	25ul	L5	PMGFSYDTRCFDSTV	Seg 20	6h		PMGFSYDTRCFDSTV	Seg 22	3a
35 of 90	18	218	SYDTRCFDSTVTGQDIRV 235	40mg/ml	25ul	L5	SYDTRCFDSTVTERDIR	Seg 20	6h		SYDTRCFDSTVTEQDIR	Seg 22	3a
36 of 90	16	225	DSTVTGQDIRVEEAVY 240	40mg/ml	25ul	L5	DSTVTERDIRTEEAVY	Seg 20	6h		DSTVTEQDIRVEEEIY	Seg 22	3a
37 of 90	16	230	GQDIRVEEAVYQCCNL 245	40mg/ml	25ul						EDQDIRVEEEVYQCCNL	Seg 22	3a
38 of 90	18	235	VEEAVYQCCNLEPEPGQA 252	40mg/ml	25ul	L6							

39 of 90	18	242	CCNLEPEPGQAISSLTER 259	40mg/ml	25ul		L6							
40 of 90	18	249	PGQAISSLTERLYCGGPM 266	40mg/ml	25ul		L6							
41 of 90	17	256	LTERLYCGGPMNNSKGA 272	40mg/ml	25ul		L6							
42 of 90	17	262	CGGPMNNSKGAQCGYLR 278	40mg/ml	25ul		L6							
43 of 90	18	268	NSKGAQCGYLRCRASGVL 285	40mg/ml	25ul	M1		NSKGAQCGYRRCRASGVL	Seg 21	2a	M1		NSKGAQCGYRRCRASGVL	Seg 23	1a
44 of 90	15	275	GYLRCRASGVLPTSF 289	40mg/ml	25ul	M1		GYRRCRASGVLTTSM	Seg 21	2a	M1		GYRRCRASGVLTTSF	Seg 23	1a
45 of 90	18	279	CRASGVLPTSFGNTITCY 296	40mg/ml	25ul	M1		CRASGVLTTSMGNTITCY	Seg 21	2a	M1		CRASGVLTTSCGNTLTCY	Seg 23	1a

Pool M

NS5B

Peptide	Length	Sequence	Conc.	Vol.	*Minipool*		*Vacc Seq.*	*Segment*	*Vacc GT*	*Minipool*		*Vacc Seq.*	*Segment*	*Vacc GT*
46 of 90	18	286 PTSFGNTITCYIKATAAA 303	20mg/ml	50ul	M1		TTSMGNTITCYIKATAAA	Seg 21	2a	M1		TTSCGNTLTCYIKARAAA	Seg 23	1a
47 of 90	17	293 ITCYIKATAAARAAGLR 309	40mg/ml	25ul	M1		ITCYIKALAAARAAGLR	Seg 21	2a			LTCYIKARAACRAAGLR	Seg 23	1a
48 of 90	17	299 ATAAARAAGLRNPDFLV 315	40mg/ml	25ul							M2			
49 of 90	18	305 AAGLRNPDFLVCGDDLVV 322	40mg/ml	25ul		M2					M2			
50 of 90	18	312 DFLVCGDDLVVVAESDGV 329	40mg/ml	25ul		M2					M2			
51 of 90	18	319 DLVVVAESDGVDEDRATL 336	40mg/ml	25ul		M2					M2			
52 of 90	18	326 SDGVDEDRATLRAFTEAM 343	40mg/ml	25ul		M2								
53 of 90	16	333 RATLRAFTEAMTRYSA 348	40mg/ml	25ul						M3		AALRAFTEAMTRYSA	Seg 24	3a
54 of 90	16	338 AFTEAMTRYSAPPGDA 353	40mg/ml	25ul	M3		AFTEAMTRYSAPPGDP	Seg 22	1a	M3		AFTEAMTRYSAPPGDA	Seg 24	3a
55 of 90	18	343 MTRYSAPPGDAPQPTYDL 360	40mg/ml	25ul	M3		MTRYSAPPGDPPQPEYDL	Seg 22	1a	M3		MTRYSAPPGDAPQPEYDL	Seg 24	3a
56 of 90	18	350 PGDAPQPTYDLELITSCS 367	40mg/ml	25ul	M3		PGDPPQPEYDLELITSCS	Seg 22	1a					
57 of 90	18	357 TYDLELITSCSSNVSVAR 374	40mg/ml	25ul	M3		EYDLELITSCSSNVSVAH	Seg 22	1a		M4			
58 of 90	18	364 TSCSSNVSVARDDKGKRY 381	40mg/ml	25ul	M3		TSCSSNVSVAHDGAGKRV	Seg 22	1a		M4			
59 of 90	18	371 SVARDDKGKRYYYLTRDA 388	40mg/ml	25ul						M5		SVARDGKGKRYYYLTRDP	Seg 25	3a
60 of 90	18	378 GKRYYYLTRDATTPLARA 395	40mg/ml	25ul		M4				M5		GKRYYYLTRDPTTPLARA	Seg 25	3a
61 of 90	18	385 TRDATTPLARAAWETARH 402	40mg/ml	25ul	M5		TRDPTTPLARAAWETARH	Seg 23	6a	M5		TRDPTTPLARAAWETARH	Seg 25	3a
62 of 90	18	392 LARAAWETARHTPVNSWL 409	40mg/ml	25ul	M5		LARAAWETARHTPVNSWL	Seg 23	6a	M5		LARAAWETARHTPVNSWL	Seg 25	3a
63 of 90	18	399 TARHTPVNSWLGSIIMYA 416	40mg/ml	25ul	M5		TARHTPVNSWLGNIIMYA	Seg 23	6a	M5		TARHTPVNSWLGNIIMYA	Seg 25	3a
64 of 90	18	406 NSWLGSIIMYAPTIWVRM 423	40mg/ml	25ul	M5		NSWLGNIIMYAPTIWVLM	Seg 23	6a	M5		NSWLGNIIMYAPTIWVRM	Seg 25	3a
65 of 90	18	413 IMYAPTIWVRMVMMTHFF 430	40mg/ml	25ul	M5		IMYAPTIWVRMVLMTHFF	Seg 23	6a	M5		IMYAPTIWVRMVMMTHFF	Seg 25	3a
66 of 90	19	420 WVRMVMMTHFFSILQSQEI 438	40mg/ml	25ul	M5		WVRMVLMTHFFSILQSQEI	Seg 23	6a		M6			
67 of 90	18	428 HFFSILQSQEILDRPLDF 445	40mg/ml	25ul	M5		HFFSILQSQEILDRPLDF	Seg 23	6a		M6			
68 of 90	18	435 SQEILDRPLDFEMYGATY 452	40mg/ml	25ul		M6					M6			
69 of 90	18	442 PLDFEMYGATYSVTPLDL 459	40mg/ml	25ul			PLDFEMYGATYSVTPLDL	Seg 24	3a	M7		PLDFEMYGATYSVTPLDL	Seg 26	3a
70 of 90	18	449 GATYSVTPLDLPAIIERL 466	40mg/ml	25ul	M7		GATYSVTPLDLPAIIERL	Seg 24	3a	M7		GATYSVTPLDLPAIIERL	Seg 26	3a
71 of 90	17	456 PLDLPAIIERLHGLSAF 472	40mg/ml	25ul	M7		PLDLPAIIERLHGLSAF	Seg 24	3a	M7		PLDLPAIIERLHGLSAF	Seg 26	3a
72 of 90	16	462 IIERLHGLSAFSVHSY 477	40mg/ml	25ul	M7		IERLHGLSAFTLHSY	Seg 24	3a	M7		IERLHGLSAFTLHSY	Seg 26	3a
73 of 90	18	467 HGLSAFSVHSYSPVELNR 484	40mg/ml	25ul	M7		HGLSAFTLHSYSPVELNR	Seg 24	3a	M7		HGLSAFSVHSYSPVELNR	Seg 26	3a
74 of 90	18	474 VHSYSPVELNRVAGTLRK 491	40mg/ml	25ul			VHSYSPVELNRVGACLRK	Seg 25	6a	M7		VHSYSPVELNRVAGTLRK	Seg 26	3a
75 of 90	18	481 ELNRVAGTLRKLGCPPLR 498	40mg/ml	25ul	M8		ELNRVGACLRKLGVPPLR	Seg 25	6a	M7		ELNRVAGTLRKLGCPPLR	Seg 26	3a
76 of 90	18	488 TLRKLGCPPLRAWRHRAR 505	40mg/ml	25ul	M8		CLRKLGVPPLRAWRHRAR	Seg 25	6a	M7		TLRKLGCPPLRAWRHRAR	Seg 26	3a
77 of 90	18	495 PPLRAWRHRARAVRAKLI 512	40mg/ml	25ul	M8		PPLRAWRHRARAVRAKLI	Seg 25	6a			PPLRAWRHRARAVRAKLL	Seg 26	3a
78 of 90	18	502 HRARAVRAKLIAQGGRAK 519	40mg/ml	25ul	M8		HRARAVRAKLIAQGGKAA	Seg 25	6a	M8		HRARAVRAKLLSQGGRAA		
79 of 90	18	509 AKLIAQGGRAKICGLYLF 526	40mg/ml	25ul	M8		AKLIAQGGKAAICGKYLF	Seg 25	6a	M8		AKLLSQGGRAAICGKYLF	Seg 27	1b
80 of 90	18	516 GRAKICGLYLFNWAVRTK 533	40mg/ml	25ul	M8		GKAAICGKYLFNWAVRTK	Seg 25	6a	M8		GRAAICGKYLFNWAVRTK	Seg 27	1b

81 of 90	17	523	LYLFNWAVRTKTKLTPL	539	40mg/ml	25ul		M8	KYLFNWAVRTKTKLTPL	Seg 27	1b
82 of 90	17	529	AVRTKTKLTPLPAAGQL	545	40mg/ml	25ul	M9	M8	AVRTKTKLTPLPAAGQL	Seg 27	1b
83 of 90	17	535	KLTPLPAAGQLDLSSWF	551	40mg/ml	25ul	M9				
84 of 90	15	541	AAGQLDLSSWFTVGV	555	40mg/ml	25ul	M9				
85 of 90	18	545	LDLSSWFTVGVGGNDIYH	562	40mg/ml	25ul	M9	M9			
86 of 90	17	552	TVGVGGNDIYHSVSRAR	568	40mg/ml	25ul	M9	M9			
87 of 90	17	558	NDIYHSVSRARTRYLLL	574	40mg/ml	25ul	M9	M9			
88 of 90	18	564	VSRARTRYLLLCLLLLTV	581	40mg/ml	25ul	M9	M9			
89 of 90	18	571	YLLLCLLLLTVGVGIFLL	588	10mg/ml	100ul	M9	M9			
90 of 90	14	578	LLTVGVGIFLLPAR	591	40mg/ml	25ul	M9	M9			

APPENDIX 3: HLA-A2.1 RESTRICTED TRANSGENIC MOUSE T CELL EPITOPES

A2.1 epitope				Gt1-6L		Gt1/3		Gt1b	
Peptide sequence	Protein	Start	End	Present?	Vaccine sequence	Present?	Vaccine sequence	Present?	Vaccine sequence
YLLPRRGPRL	Core	35	44	yes	YLLPRRGPRL	yes		no	
DLMGYIPLV	Core	132	140	yes	DLMGYIPLV	yes		no	
IMHTPGCV	E1	220	227	no		no		no	
TIRRHVDLLV	E1	257	266	no		no		no	
SMVGNWAKV	E1	363	371	no		no		no	
RLWHYPCTI	E2	614	622	mismatch	RLWHYPCTV	partial	RLWHYPCT x	no	
ALSTGLIHL	E2	684	692	yes	ALSTGLIHL	yes	ALSTGLIHL	no	ALSTGLIHL
FLLLADARV	E2	723	731	no		no		no	FLLLADARV
GLLGCIITSL	NS3	1038	1047	no		no		yes	GLLGCIITSL
CVNGVCWTV	NS3	1073	1081	no		no		yes	CVNGVCWTV
LLCPSGHVV	NS3	1169	1177	no		no		mismatch	LLCPSGHAV

ATLGFGAYM	NS3	1260	1268	yes	ATLGFGAYM	yes	ATLGFGAYM	yes	ATLGFGAYM
KLTGLGLNAV	NS3	1406	1415	no		no		mismatch	KL**S**GLG**I**NAV
YLVAYQATV	NS3	1585	1593	yes	YLVAYQATV	mismatch	YL**T**AYQATV	yes	YLVAYQATV
TLHGPTPLL	NS3	1617	1625	no		yes	TLHGPTPLL	yes	TLHGPTPLL
HMWNFITGI	NS4b	1764	1772	mismatch	HMWNFI**S**GI	mismatch	HMWNFI**S**GI	mismatch	HMWNFI**S**GI
SLMAFTASI	NS4b	1789	1797	mismatch	SLMAFTA**AA**	mismatch	SLMAFTA**A** x	yes	SLMAFTASI
ILAGYGAGV	NS4b	1851	1859	yes	ILAGYGAGV	yes	ILAGYGAGV	yes	ILAGYGAGV
SPDADLIEANL	NS5a	2221	2231	no		no		yes	SPDADLIEANL
ILDSFDPLR	NS5a	2252	2260	no		no		mismatch	**V**LDSFDPLR
RLIVFPDLGV	NS5b	2578	2587	no		no		yes	RLIVFPDLGV
ALYDVVSTL	NS5b	2594	2602	no		partial	ALYDV x x x x	yes	ALYDVVSTL
KLQDCTMLV	NS5b	2727	2735	no		no		yes	KLQDCTMLV

APPENDIX 4: GENOTYPE-1B AND -3A VACCINE SEQUENCES

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              10        20        30        40        50        60
GT-1b  APITAYSQQTRGLLGCIITSLTGRDKNQVDGEVQVLSTATQSFLATCVNGVCWTVYHGAG
       ::::::.:::::::: :.:::::::::: : :::::::::::.::.: :.:: ::::::::
GT-3a  APITAYAQQTRGLLGTIVTSLTGRDKNVVAGEVQVLSTATQTFLGTTVGGVMWTVYHGAG
              10        20        30        40        50        60

              70        80        90       100       110       120
GT-1b  SKTLAGPKGPITQMYTNVDQDLVGWPAPPGARSMTPCTCGSSDLYLVTRHADVVPVRRRG
       :.:::: : :  ::::::::::::::::::::.:. ::::::.::::::: :::.:.::::
GT-3a  SRTLAGVKHPALQMYTNVDQDLVGWPAPPGAKSLEPCTCGSADLYLVTRDADVIPARRRG
              70        80        90       100       110       120

             130       140       150       160       170       180
GT-1b  DSRGSLLSPRPISYLKGSSGGPLLCPSGHVVGIFRAAVCTRGVAKAVDFIPVESMETTMR
       :: .::::::::.. ::::::::..:::::::::::::::::::..::::::.. :  :
GT-3a  DSTASLLSPRPLARLKGSSGGPVMCPSGHVAGIFRAAVCTRGVAKALQFIPVETLSTQAR
             130       140       150       160       170       180

             190       200       210       220       230       240
GT-1b  SPVFTDNSSPPAVPQTFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGA
       :: :.:::.:::::::..::..:::::::::::::::::::.::::.:::::::::::::::.
GT-3a  SPSFSDNSTPPAVPQSYQVGYLHAPTGSGKSTKVPAAYVAQGYNVLVLNPSVAATLGFGS
             190       200       210       220       230       240

             250       260       270       280       290       300
GT-1b  YMSKAHGIEPNIRTGVRTITTGGPITYSTYCKFLADGGCSGGAYDIIICDECHSTDSTTI
       .::.:.::.:::::: ::.:::. .::::: :::: ::::::::::.::::.::. :.:.:
GT-3a  FMSRAYGIDPNIRTGNRTVTTGAKLTYSTYGKFLAGGGCSGGAYDVIICDDCHAQDATSI
             250       260       270       280       290       300

             310       320       330       340       350       360
GT-1b  LGIGTVLDQAETAGARLVVLATATPPGSITVPHPNIEEVALSNTGEIPFYGKAIPIEAIK
       ::::::::::::::.::.::::::::::::::: ::::::::.. :::::::::::::  ::
GT-3a  LGIGTVLDQAETAGVRLTVLATATPPGSITVPHSNIEEVALGSEGEIPFYGKAIPIACIK
             310       320       330       340       350       360

             370       380       390       400       410       420
GT-1b  GGRHLIFCHSKKKCDELAAKLTGLGLNAVAYYRGLDVSVIPTSGDVVVVATDALMTGFTG
       ::::::::::::::..:.:: :.:::::::::::::::::::.::::: ::::::::::::
GT-3a  GGRHLIFCHSKKKCDKMASKLRGMGLNAVAYYRGLDVSVIPTTGDVVVCATDALMTGFTG
             370       380       390       400       410       420

             430       440       450       460       470       480
GT-1b  DFDSVIDCNTCVTQTVDFSLDPTFTIETTTLPQDAVSRAQRRGRTGRGRSGIYRFVTPGE
       ::::::::::. : : ::::::::::.::: : :::::::.:::::::::: : ::.:::::
GT-3a  DFDSVIDCNVAVEQYVDFSLDPTFSIETCTAPQDAVSRSQRRGRTGRGRLGTYRYVTPGE
             430       440       450       460       470       480

             490       500       510       520       530       540
GT-1b  RPSGMFDSSVLCECYDAGCAWYELTPAETSVRLRAYLNTPGLPVCQDHLEFWESVFTGLT
       :::::::: ::::::::::.::.: ::::.::::::::.::::::::::..:::::::::
GT-3a  RPSGMFDSVVLCECYDAGCSWYDLQPAETTVRLRAYLSTPGLPVCQDHLDLWESVFTGLT
             490       500       510       520       530       540

             550       560       570       580       590       600
GT-1b  HIDAHFLSQTKQAGDNLPYLVAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLY
       :::::::::::::: :. ::.:::::::::::::::::. ::::.::::::::::::::
GT-3a  HIDAHFLSQTKQAGLNFSYLTAYQATVCARAQAPPPSWDETWKCLVRLKPTLHGPTPLLY
             550       560       570       580       590       600
```

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             610       620       630       640       650       660
GT-1b  RLGAVQNEVTLTHPITKYIMACMSADLEVVTSTWVLVGGVLAALAAYCLTTGSVVIVGRI
       ::: :::::. ::::::::.:::::::::.:::::.:::::.:::::..: ::::::.:
GT-3a  RLGPVQNEICLTHPITKYVMACMSADLEVTTSTWVLLGGVLAAVAAYCLSVGCVVIVGHI
             610       620       630       640       650       660

             670       680       690       700       710       720
GT-1b  ILSGRPAVIPDREVLYQEFDEMEECASHLPYIEQGMQLAEQFKQKALGLLQTATKQAEAA
        :.:.::..::.::::::.:::::::..  ::::::.. .:.:::.:.::::: ::.:  .
GT-3a  ELGGKPALVPDKEVLYQQYDEMEECSQARPYIEQAQVIAHQFKEKVLGLLQRATQQQAVI
             670       680       690       700       710       720

             730       740       750       760       770       780
GT-1b  APVVESKWRALEVFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTASITSPLTTQNT
        :.: :.:. :::.: ::::::.:::::::::::::::::::.:::::::::.:::::..:
GT-3a  EPIVVSNWQKLEVLWHKHMWNFVSGIQYLAGLSTLPGNPAVASLMAFTASVTSPLTTNQT
             730       740       750       760       770       780

             790       800       810       820       830       840
GT-1b  LLFNILGGWVAAQLAPPSAASAFVGAGIAGAAVGSIGLGKVLVDILAGYGAGVAGALVAF
       ..:::::::::..:: :.:.:::: .:.::::.:.::::.::.::::::::::.::::::
GT-3a  MFFNILGGWVATHLAGPQASSAFVVSGLAGAAIGGIGLGRVLLDILAGYGAGVSGALVAF
             790       800       810       820       830       840

             850       860       870       880       890       900
GT-1b  KVMSGEMPSTEDLVNLLPAILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRLIAFASRG
       :.:.:: :.::::::::::::::::::.:::::::::::: ::::::::::::::
GT-3a  KIMGGEPPTTEDMVNLLPAILSPGALVVGVICAAILRRHVGPGEGPVQWMNRLIAFASRG
             850       860       870       880       890       900

             910       920       930       940       950       960
GT-1b  NHVSPTHYVPESDAAARVTQILSSLTITQLLKRLHQWINEDCSTPCSGSWLKDVWDWICT
       :::::.:::::::::::::: .::::::.:.::.:::::::::  .::::.::. .::::.:.
GT-3a  NHVSPAHYVPESDAAARVTALLSSLTVTSLLRRLHQWINEDYPSPCSGDWLRIIWDWVCS
             910       920       930       940       950       960

             970       980       990      1000      1010      1020
GT-1b  VLSDFKTWLQSKLLPRLPGLPFLSCQRGYKGVWRGDGIMQTTCPCGAQITGHVKNGSMRI
       :.:::::::..:..: :::::::.:::.:::::::::::.:.: ::::::.:.::::::::.
GT-3a  VVSDFKTWLSAKIMPALPGLPFISCQKGYKGVWRGDGVMSTRCPCGASIAGHVKNGSMRL
             970       980       990      1000      1010      1020

            1030      1040      1050      1060      1070      1080
GT-1b  VGPKTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRVAAEEYVEVTRVGDFHYVTGMT
       .::.::.:  ::::::: ::::: :: : :::.:::::::::. :::: ::::::::.:: :
GT-3a  AGPRTCANMCHGTFPINEYTTGPSTPCPPPNYTRALWRVAANSYVEVRRVGDFHYITGAT
            1030      1040      1050      1060      1070      1080

            1090      1100      1110      1120      1130      1140
GT-1b  TDNVKCPCQVPAPEFFTEVDGVRLHRYAPVCKPLLREEVVFQVGLNQYLVGSQLPCEPEP
        :..::::::::: :::::::::::::::: :.:::::.:..:.::::.: .::::::::::
GT-3a  EDGLKCPCQVPATEFFTEVDGVRIHRYAPPCRPLLRDEITFMVGLNSYAIGSQLPCEPEP
            1090      1100      1110      1120      1130      1140

            1150      1160      1170      1180      1190      1200
GT-1b  DVAVLTSMLTDPSHITAETAKRRLARGSPPSLASSSASQLSAPSLKATCTTHHDSPDADL
       ::.::::::: :::::::::: :::::::::: ::::::::::::::::: ::.  :::.:
GT-3a  DVSVLTSMLRDPSHITAETAARRLARGSPPSEASSSASQLSAPSLKATCQTHRPHPDAEL
            1150      1160      1170      1180      1190      1200

            1210      1220      1230      1240      1250      1260
GT-1b  IEANLLWRQEMGGNITRVESENKVVILDSFDPIRAVEDEREISVPAEILRKPRKFPPALP
```

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       ..:::::::::::.:::::::::.:::::::.:.::  :. :.:. :: ..:: :.:::::
GT-3a  VDANLLWRQEMGSNITRVESETKVVILDSFEPLRAETDDAELSAAAECFKKPPKYPPALP
            1210      1220      1230      1240      1250      1260

            1270      1280      1290      1300      1310      1320
GT-1b  IWARPDYNPPLLESWKDPDYVPPVVHGCPLPSTKAPPIPPPRRKRTVVLTESTVSSALAE
       ::::::::::::. ::.:::::::.:::: ::   :::.:::::::::. :  :.::.:::
GT-3a  IWARPDYNPPLLDRWKSPDYVPPTVHGCALPPKGAPPVPPPRRKRTIQLDGSNVSAALAA
            1270      1280      1290      1300      1310      1320

                   1330      1340      1350      1360      1370
GT-1b  LATKTFGSS-------GSSAVDSGTATGPPDQASDDGDKGSDVESYSSMPPLEGEPGDPD
       :: :.: ::       .::.::. ..:.     :  :.. :: :: ::::::::::::::
GT-3a  LAEKSFPSSKPQEENSSSSGVDTQSSTASKVLPSP-GEE-SDSESCSSMPPLEGEPGDPD
            1330      1340      1350        1360      1370

         1380      1390      1400      1410      1420      1430
GT-1b  LSDGSWSTVSGEAGEDVVCCSMSYTWTGALITPCAAEESKLPINPLSNSLLRHHSMVYST
       ::  ::::::    ..:::::::.:::::::::.::: ::::.::::::::::..::::
GT-3a  LSCDSWSTVSDSEEQSVVCCSMSYSWTGALITPCSAEEEKLPISPLSNSLLRHHNLVYST
     1380      1390      1400      1410      1420      1430

         1440      1450      1460      1470      1480      1490
GT-1b  TSRSASLRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKARLLSIEEACKLTPPHSAKSK
       .::::: ::::::::::::::::. .:.:.: .:: ::::.:::::::: :.::::::.::
GT-3a  SSRSASQRQKKVTFDRLQVLDDHYKTALQEVKERASRVKARMLSIEEACALVPPHSARSK
     1440      1450      1460      1470      1480      1490

         1500      1510      1520      1530      1540      1550
GT-1b  FGYGAKDVRSLSSRAVNHIRSVWEDLLEDTETPIDTTIMAKNEVFCVQPEKGGRKPARLI
       :::.:::::::::.:.:.:::::::::::: ::: ::::::::::::.: ::::: ::::
GT-3a  FGYSAKDVRSLSSKAINQIRSVWEDLLEDTTTPIPTTIMAKNEVFCVDPAKGGRKAARLI
     1500      1510      1520      1530      1540      1550

         1560      1570      1580      1590      1600      1610
GT-1b  VFPDLGVRVCEKMALYDVVSTLPQAVMGPSYGFQYSPGQRVEFLVNTWKSKKCPMGFSYD
       :.:::::::::: :::::.. :   .:: .::::::: :::: :.. : ::: :.::::::
GT-3a  VYPDLGVRVCEKRALYDVIQRLSIETMGSAYGFQYSPRQRVERLLKMWTSKKTPLGFSYD
     1560      1570      1580      1590      1600      1610

         1620      1630      1640      1650      1660      1670
GT-1b  TRCFDSTVTENDIRTEESIYQCCDLAPEARQAIRSLTERLYVGGPLTNSKGQNCGYRRCR
       ::::::::: .:::.::..:::::.: ::  ::: ::::::: :::..:::: .::: :::
GT-3a  TRCFDSTVTGQDIRVEEAVYQCCNLEPEPGQAISSLTERLYCGGPMNNSKGAQCGYLRCR
     1620      1630      1640      1650      1660      1670

         1680      1690      1700      1710      1720      1730
GT-1b  ASGVLTTSCGNTLTCYLKATAACRAAKLQDCTMLVNGDDLVVICESAGTQEDAAALRAFT
       ::::: :: :::.:::.:::::: ::: :..  .:: ::::::. :: :..:: :.::::::
GT-3a  ASGVLPTSFGNTITCYIKATAAARAAGLRNPDFLVCGDDLVVVAESDGVDEDRATLRAFT
     1680      1690      1700      1710      1720      1730

         1740      1750      1760      1770      1780      1790
GT-1b  EAMTRYSAPPGDPPQPEYDLELITSCSSNVSVAHDASGKRVYYLTRDPTTPLARAAWETV
       :::::::::::: ::: ::::::::::::::::.: .::: :::::: ::::::::::::.
GT-3a  EAMTRYSAPPGDAPQPTYDLELITSCSSNVSVARDDKGKRYYYLTRDATTPLARAAWETA
     1740      1750      1760      1770      1780      1790

         1800      1810      1820      1830      1840      1850
GT-1b  RHTPVNSWLGNIIMYAPTLWARMILMTHFFSILLAQEQLEKALDCQIYGACYSIEPLDLP
       ::::::::::.:::::::.:.::..:::::::: .:: :.. :: ..::: ::. :::::
GT-3a  RHTPVNSWLGSIIMYAPTIWVRMVMMTHFFSILQSQEILDRPLDFEMYGATYSVTPLDLP
     1800      1810      1820      1830      1840      1850
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          1860      1870      1880      1890      1900      1910
GT-1b  QIIERLHGLSAFSLHSYSPGEINRVASCLRKLGVPPLRVWRHRARSVRAKLLSQGGRAAT
        :::::::::::.::::: :.::::. ::::: ::::.::::::.::::::..:::::
GT-3a  AIIERLHGLSAFSVHSYSPVELNRVAGTLRKLGCPPLRAWRHRARAVRAKLIAQGGRAKI
     1860      1870      1880      1890      1900      1910

          1920      1930      1940      1950      1960      1970
GT-1b  CGKYLFNWAVKTKLKLTPIPAASQLDLSGWFVAGYNGGDIYHSLSRARPRWFMLCLLLLS
       :: ::::::::.:: ::::.:::.::::::.::..: .:.::::::.:::: :...:::::::.
GT-3a  CGLYLFNWAVRTKTKLTPLPAAGQLDLSSWFTVGVGGNDIYHSVSRARTRYLLLCLLLLT
     1920      1930      1940      1950      1960      1970

          1980
GT-1b  VGVGIYLLPNR
       ::::::::: :
GT-3a  VGVGIFLLPAR
     1980
```

APPENDIX 5: HCV GENOTYPE NOMENCLATURE

Genotypes used for sequence analysis in the thesis were obtained from the International Committee on the Taxonomy of Viruses (IVTU, June 2017).

Genotype[1]	Locus/Isolate(s)[2]	Accession number(s)	Reference(s)
Genotype 1			
1a	HPCPLYPRE, HPCCGAA	M62321, M67463	(Choo *et al.*, 1991)(Inchauspe *et al.*, 1991)
1b	HPCJCG, HPCHUMR	D90208, M58335	(Kato *et al.*, 1990)(Takamizawa *et al.*, 1991)
1c	HPCCGS, AY051292	D14853, AY051292	(Okamoto *et al.*, 1994)**(Guntaka et al. unpublished 2001)**
1d	QC103	KJ439768	(Lu *et al.*, 2014)
1e	148636, QC172	KC248194, KJ439769	(Li *et al.*, 2013),(Lu *et al.*, 2014)

1g	1804, QC78	AM910652, KJ439770	(Bracho *et al.*, 2008),(Lu et al., 2014)
1h	EBW443, EBW9	KC248198, KC248199	(Li *et al.*, 2013)
1i	QC181	KJ439772	(Lu *et al.*, 2014)
1j	QC329	KJ439773	(Lu *et al.*, 2014)
1k	QC82	KJ439774	(Lu *et al.*, 2014)
1l	136142, EBW424	KC248193, KC248197	(Li *et al.*, 2013)
1m	QC196, QC87	KJ439778, KJ439782	(Lu *et al.*, 2014)
1n	QC113, QC74	KJ439775, KJ439781	(Lu *et al.*, 2014)

Genotype 2			
2a	HPCPOLP, JFH-1	D00944, AB047639	(Okamoto *et al.*, 1991)(Kato *et al.*, 2001)
2b	HPCJ8G, JPUT971017	D10988, AB030907	(Okamoto *et al.*, 1992)(Murakami *et al.*, 2001)
2c	BEBE1	D50409	(Nakao *et al.*, 1996)
2d	QC259	JF735114	(Li *et al.*, 2012)
2e	QC64	JF735120	(Li *et al.*, 2012)
2f	ZS542, GZ98799	KC844042, KC844050	(Xu *et al.*, 2013)
2i	D54	DQ155561	(Noppornpanth *et al.*, 2006)
2j	C1799, QC232	HM777358 JF735113	(Sulbarán *et al.*, 2010)(Li *et al.*, 2012)

2k	VAT96	AB031663	(Samokhvalov *et al.*, 2000)
2l	MRS89, PTR7904	KC197235, KC197240	(Jordier *et al.*, 2013)
2m	QC178, BID-G1314	JF735111, JX227967	(Li *et al.*, 2012)(Newman *et al.*, 2013)
2q	963, 852	FN666428, FN666429	(Martró *et al.*, 2011)
2r	QC283	JF735115	(Li *et al.*, 2012)
2t	MRS40	KC197238	(Jordier *et al.*, 2013)
2u	QC182	JF735112	(Li *et al.*, 2012)

Genotype 3

3a	HPCEGS, HPCK3A	D17763, D28917	(Sakamoto *et al.*, 1994)(Yamada *et al.*, 1994)
3b	HPCFG	D49374	(Chayama *et al.*, 1994)
3d	NE274	KJ470619	(Li *et al.*, 2014a)
3e	NE145	KJ470618	(Li *et al.*, 2014a)
3g	BID-G1243, QC260	JX227954, JF735123	(Newman *et al.*, 2013)(Lu *et al.*, 2013)
3h	QC29	JF735121	(Lu *et al.*, 2013)
3i	IND-HCV, BID-G1244	FJ407092, JX227955	(Arankalle & Gupte 2008 unpublished)(Newman *et al.*, 2013)
3k	HPCJK049E1, **QC105**	D63821, **JF735122**	(Tokita *et al.*, 1996) (Lu *et al.*, 2013)

Genotype 4			
4a	ED43	Y11604	(Chamberlain *et al.*, 1997a)
4b	QC264	FJ462435	(Li *et al.*, 2009b)
4c	QC381	FJ462436	(Li *et al.*, 2009b)
4d	03-18, QC382	DQ418786, FJ462437	(Timm *et al.*, 2007)(Li *et al.*, 2009b)
4f	IFBT88, PS6	EF589161, EU392175	(Hmaied *et al.*, 2007)(Kuntzen *et al.*, 2008)
4g	QC193	FJ462432	(Li *et al.*, 2009b)
4k	PS3, QC383	EU392173, FJ462438	(Kuntzen *et al.*, 2008) (Li *et al.*, 2009b)
4l	QC274	FJ839870	(Li *et al.*, 2009b)

4m	QC249	FJ462433	(Li *et al.*, 2009b)
4n	QC97	FJ462441	(Li *et al.*, 2009b)
4o	QC93	FJ462440	(Li *et al.*, 2009b)
4p	QC139	FJ462431	(Li *et al.*, 2009b)
4q	QC262	FJ462434	(Li *et al.*, 2009b)
4r	QC384	FJ462439	(Li *et al.*, 2009b)
4s	QC361	JF735136	(Lu *et al.*, 2015)
4t	QC155	FJ839869	(Li *et al.*, 2009b)
4v	CYHCV073, BID-G1248	HQ537009, JX227959	(Demetriou & Kostrikis, 2011) (Newman *et al.*, 2013)

4w3	P212, P245		FJ025855, FJ025856	(Koletzki *et al.*, 2009)	
Genotype 5					
5a	EUH1480, SA134	Y13184	,AF064490	(Chamberlain *et al.*, 1997b) (Bukh *et al.*, 1998)	
Genotype 6					
6a	EUHK2, 6a33	Y12083, **AY859526**		(Adams *et al.*, 1997), (Zhou *et al.*, 2010)	
6b	Th580	D84262		(Tokita *et al.*, 1998)	
6c	Th846	EF424629		(Lu *et al.*, 2007a)	
6d	VN235	D84263		(Tokita *et al.*, 1998)	

6e	GX004	DQ314805	(Li *et al.*, 2006)
6f	C-0044	DQ835760	(Lu *et al.*, 2007b)
6g	HPCJK046E2	D63822	(Tokita *et al.*, 1996)
6h	VN004	D84265	(Tokita *et al.*, 1998)
6i	Th602	DQ835770	(Lu *et al.*, 2007b)
6j	Th553	DQ835769	(Lu *et al.*, 2007b)
6k	VN405	D84264	(Tokita *et al.*, 1998)
6l	537796	EF424628	(Lu *et al.*, 2007a)
6m	B4/92	DQ835767	(Lu *et al.*, 2007b)

6n	KM42, D86/93	DQ278894, DQ835768	(Lu *et al.*, 2006)(Lu *et al.*, 2007b)
6o	QC227	EF424627	(Lu *et al.*, 2007a)
6p	QC216	EF424626	(Lu *et al.*, 2007a)
6q	QC99	EF424625	(Lu *et al.*, 2007a)
6r	QC245	EU408328	(Li *et al.*, 2009a)
6s	QC66	EU408329	(Li *et al.*, 2009a)
6t	VT21, D49	EF632071, EU246939	(Lu *et al.*, 2008) (Noppornpanth *et al.*, 2008)
6u	D83	EU246940	(Noppornpanth *et al.*, 2008)
6v	NK46, KMN-02	EU158186, EU798760	(Lu *et al.*, 2008) (Wang *et al.*, 2009)

6w	GZ52557, D140	DQ278892, EU643834	(Lu *et al.*, 2006)(Lee *et al.*, 2010)
6xa5	DH012, DH028	EU408330, EU408332	(Xia *et al.*, 2008)
6xb	TV476, VN110	JX183552, KJ567645	(Wang *et al.*, 2013)
6xc	TV520	KJ567651	(Wang *et al.*, 2013)
6xd	L23, L347	KM252789, KM252790	(Li *et al.*, 2015)
6	DH027**, KM98**	JX183557, **KM252792**	(Wang *et al.*, 2013)(Li *et al.*, 2015)
6xf	VN214, TV469	KJ567647, KJ567646	(Li *et al.*, 2014b)

Genotype 7

7a	QC69	EF108306	(Murphy *et al.*, 2015)
7b	BAK1	KX092342	(Salmona *et al.*, 2016)

[1] Consensus proposed genotype/subtype names. Where multiple sequences of an HCV genotype are available, two sequences have been listed, prioritised by (a) publication date or (b) submission date when unpublished.

[2] Locus (or isolate name if locus is the same as the accession number).

[3] Previously described as 4b, (Koletzki *et al.*, 2009; Nakano *et al.*, 2012).

[4] Sequence obtained from acute phase plasma of a chimpanzee experimentally infected with (human-derived) isolate SA13.

[5] Previously described as 6u (Xia *et al.*, 2008).

APPENDIX 6: HCV NOMENCLATURE REFERENCES

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