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REVIEW



Aiming for protective T-cell responses: a focus on the first generation conserved-region HIVconsv vaccines in preventive and therapeutic clinical trials

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ABSTRACT

Introduction: Despite life-saving antiretroviral drugs, an effective HIV-1 vaccine is the best solution and likely a necessary component of any strategy for halting the AIDS epidemic. The currently prevailing aim is to pursue antibody-mediated vaccine protection. With ample evidence for the ability of T cells to control HIV-1 replication, their protective potential should be also harnessed by vaccination. The challenge is to elicit not just any, but protective T cells.

Areas covered: This article reviews the clinical experience with the first-generation conserved-region immunogen HIVconsv delivered by combinations of plasmid DNA, simian adenovirus, and poxvirus MVA. The aim of our strategy is to induce strong and broad T cells targeting functionally important parts of HIV-1 proteins common to global variants. These vaccines were tested in eight phase 1/2 preventive and therapeutic clinical trials in Europe and Africa, and induced high frequencies of broadly specific CD8⁺ T cells capable of *in vitro* inhibition of four major HIV-1 clades A, B, C and D, and in combination with latency-reactivating agent provided a signal of drug-free virological control in early treated patients.

Expert opinion: A number of critical T-cell traits have to come together at the same time to achieve control over HIV-1.

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HIV vaccines; T cells; conserved regions; clinical trials; HIVconsv; simian adenovirus; MVA; prime-boost; HIV cure

1. Introduction

1.1. The need for an effective HIV-1 vaccine

In 2017, an estimated 37 million people lived with HIV-1 infection, of whom approximately half received combination antiretroviral treatment (cART) (www.unaids.org). cART effectively suppresses viremia below the clinically detectable level, increases survival, improves quality of life, and decreases onward transmission. Regrettably, cART is not curative and periodically emerging HIV-1 from latent reservoir (LR) has to be drug-controlled for the rest of patients' life. Life-long provision of cART poses not only significant economic and logistical challenges, but heightens concerns for social stigma, unwanted toxicity, and emergence of viral drug-resistance particularly if the use of cART is suboptimal. The use of cART for pre-exposure prophylaxis (PrEP) will further stretch the supply. Thus, even in the broader context of increasing availability of cART for cure and prevention, an effective, affordable, accessible HIV-1 vaccine remains the best means for ending the AIDS epidemic, which would remove continued requirement for access and adherence to prevention tools, although even vaccines will eventually require a degree of adherence.

1.2. CD8⁺ and CD4⁺ T cells shape HIV-1 infection

T cells exert their effector functions by recognizing self HLA molecules presenting a non-self (e.g. viral or bacterial) peptide. Upon sufficiently strong and long stimuli received

through the T-cell receptor supported by co-stimulatory signals, T cells kill infected cells to limit further virus production and produce a number of soluble signaling molecules, which directly or indirectly slow virus reinfection by blocking virus entry and replication by triggering innate antiviral state in the surrounding cells. Despite the currently prevailing focus on antibody-mediated protection, there is an ever growing body of data pointing to the critical role of CD8⁺ and CD4⁺ T cells in the control of HIV-1 replication. This includes genome wide association studies implicating CD8⁺ T cells by linking the control of HIV-1 viremia to the human leukocyte antigen (HLA) class I locus on chromosome 6 [1,2], HLA alleles driving HIV-1 polymorphism on the population level [3,4], identification of protective HLA class I alleles, which differ in different populations and geographical regions [1,5–9], temporal association of CD8⁺ T-cell expansion with control of primary viremia [1,10–14], virus escape by mutations in targeted epitopes, which takes place in every individual during primary viremia [6,7,11,15,16], correlation of the frequency and kinetics of CD8⁺ T-cell activation with viral set point [17], identification of protective CD8⁺ T-cell epitopes in cART-naïve patient cohorts associated with low virus load and high CD4⁺ T-cell counts [18–23], and also association of HIV-1-specific CD4⁺ T cells with a good clinical outcome [24–29]. Model infection of rhesus macaques with simian immunodeficiency virus (SIV) provided a direct demonstration that CD8⁺ T-cell depletion in infected macaques resulted in increased viremia [30,31]. More

Article highlights

- An effective vaccine strategy is the best solution to end the HIV-1 epidemic and holds a lifesaving potential for many people worldwide.
- There is plenty of evidence that T cells limit HIV-1 replication *in vivo* and vaccines should harness T cells for fighting HIV-1.
- HIV-1 is highly variable and easily escapes immune responses.
- T cells, to be protective, need to target multiple HIV-1 sites of vulnerability and do so right from the very first exposure to the incoming or reactivating virus.
- The rationally designed HIVconsv immunogen focuses T cells on the conserved regions of the HIV-1 proteins, the Achilles heel of HIV-1, and uses alternating clade consensus sequences for each segment.
- The HIVconsv immunogen delivered by DNA, simian adenovirus and poxvirus MVA induced in human recipients robust broadly specific T-cell responses inhibiting *in vitro* four major HIV-1 clades and provided a signal of increased viral control in early treated patients.
- Improved second generation of conserved mosaic T-cell vaccines tHIVconsvX is in the pipeline for clinical testing.
- Development of an effective vaccine against HIV-1 may take several iterative improvements to the immunogen design and delivery informed by data generated in human trials.

recently, vaccines vectored by specific molecular clone 68–1 of rhesus cytomegalovirus controlled [32–35] and eventually cleared [36] SIV infection in 50% (now well over 130) of experimentally challenged animals in the absence of Env-specific antibodies. Thus, vaccine induction of highly effective CD8⁺ T cells could significantly contribute to reduction of HIV-1 acquisition by complementing broadly neutralizing antibodies and may be central to an HIV-1 cure by early, rapid elimination of cells with reactivated viruses.

1.3. HIV-1 uses immunodominant, highly variable 'decoy' epitopes

Natural HIV-1 infection induces vigorous T-cell responses, which ultimately do not protect against disease progression. In the vast majority of patients, the earliest T-cell responses typically recognize highly variable and, therefore, non-protective regions of HIV-1 and rapidly select HIV-1 escape variants, which get through the sieve of immune defenses and continue to replicate [6,7,11,16]. These hypervariable epitopes evolved to be immunodominant over the protective, less variable and subdominant ones [37–41], which are consequently underutilized by the immune system. One way to explain the failure of the initially healthy T-cell responses to control a new incoming HIV-1 is that from the infection onset, cytotoxic T-lymphocyte (CTL) responses chase escaping viruses displaying decoy epitopes and by the time CTL redirect to target the subdominant protective determinants, the LR is well established, damage to the CD4⁺ T cells is profound, and the individual is irreversibly on a slippery slope toward AIDS. In chronic infection, protective, neutral, and even 'harmful' responses correlated to the total responses to the least, medium, and the most variable HIV-1 proteins of Gag, Pol, and Env, respectively [42,43]. In individuals durably suppressed with cART, deep sequencing of recovered replication-competent HIV-1 from resting CD4⁺ T cells revealed that most patients already harbored escape variants unrecognized by

their CTL [44,45]. The implications for HIV-1 cure strategies is that just expanding pre-ART HIV-1-specific CD8⁺ T cells will be ineffective due to already present escaped variants [37]. In other words, inducing 'more-of-the-same' CTL responses as the natural infection is not likely to work. Instead, we need to refine the selection of targets on HIV-1 toward small regions of virus vulnerability, which are much shorter than the full-length proteins. HIV-1 control is most likely established from the onset of infection rather than during its chronic phase, because progressors do not spontaneously change into controllers during the chronic disease. Therefore, our working hypothesis is that targeting protective epitopes of HIV-1 from the very first HIV-1 exposure for prevention and/or reactivation from LR for treatment is critical for successful virus control.

2. The first generation of conserved region T-cell vaccines uses alternating HIV-1 clade consensus sequences

The aim of the conserved-region vaccine strategy is to induce not just any, but *effective* cytotoxic T cells. There are two fundamental aspects of every vaccine: the pathogen-derived immunogen, which determines the immune response specificity, and the means of its presentation to the immune system [46]. To halt incoming HIV-1 and achieve cART-free control of the rebound virus, at the very least these two components will need to be optimal.

2.1. Rationale for the HIVconsv immunogen design (the vaccine insert)

HIV-1 genetic plasticity constitutes the biggest roadblock for vaccine development, whereby vaccine-induced responses have to be capable of stopping multiple HIV-1 variants, which may differ in up to 40% of aligned amino acids [47,48]. Our strategy is to focus HIV-1-specific T cells on the functionally conserved regions of HIV-1 proteins essential for replicative fitness [49–53] and, therefore, not easily changed, which are common to most global HIV-1 variants. If successful, the shared targeted regions would make the vaccine universal for major HIV-1 clades and for most global regions.

The HIVconsv immunogen was assembled from 14 (between 27 and 128-amino acid-long) regions totaling 778 amino acid residues, which were selected solely for their sequence conservation [54]. For each region, clade consensus was used and the four major HIV-1 clades A, B, C, and D were alternated to make the clade representation equal (Figure 1). HIVconsv contained 270 (24%) of the 1112 defined CD8⁺ T-cell epitopes present in the Los Alamos National Laboratory HIV Sequence Database (LANL-HSD) as of September 2007. Thus, the fact that the HIVconsv regions represent about one quarter of the HIV-1 proteome and contain about one quarter of known epitopes suggests that the sequence conservation is not caused by a lack of immunological pressure, i.e. absence of epitopes, but by functional constraints.

It is an important feature of the HIVconsv immunogen that it does not employ epitopes as a string of beads [55], and therefore does not rely on immunologically defined epitopes [56], because

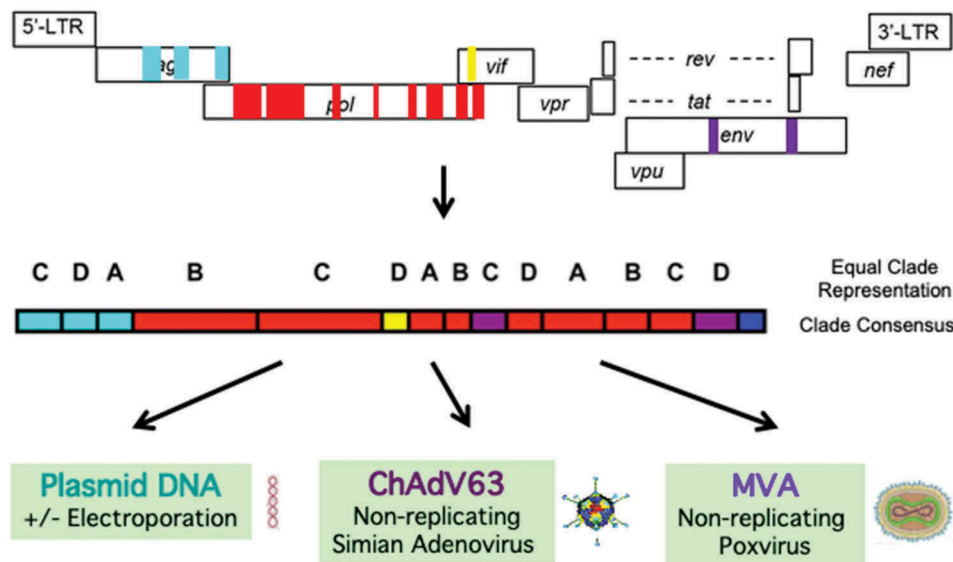


Figure 1. Design of the first generation of the conserved-region HIVconsv vaccines. Fourteen highly conserved regions of the HIV-1 proteome used a clade consensus amino acid sequences, which alternated among the four major global HIV-1 clades and were assembled into one chimeric protein designated HIVconsv. Synthetic gene employing the most frequent codons used in human proteins was synthesized and inserted into three non-replicating vaccine vectors: plasmid pSG2 DNA, simian (chimpanzee) adenovirus, and modified vaccinia virus Ankara.

our knowledge of these is incomplete and skewed toward frequent Caucasian HLA alleles. Furthermore, epitopes as a string of beads inevitably form a large number of junctions, which may inadvertently generate irrelevant epitopes not present on HIV-1-infected cells and thus waste some of the vaccine-induced responses. Having said that, viral vaccine vectors (see below) also induce strong non-HIV-1-specific CD8⁺ T-cell responses to the vector proteins [57,58], which do not necessarily represent a major impediment to efficacy as long as the vaccine induces robust and broad responses to the HIV-1 transgene product. Thus, induction of junctional responses is more annoying than detrimental and is ultimately avoidable by a number of design strategies [22,59,60]. Finally, regions but not CD8⁺ T-cell polyepitopes include conserved epitopes recognized by CD4⁺ T cells, too. This may be important both for the CD4⁺ T-cell cytotoxic activity and their direct effect on CD8⁺ T-cell functions [61].

2.2. The HIVconsv delivery (presentation to the immune system)

The frequency and quality of memory T cells are critically influenced by the employed vaccine modalities [62–65]. A number of vaccine platforms, safe and acceptable for use in humans, were tested for the delivery of the HIVconsv immunogen and all induced robust, multispecific, and plurifunctional cellular responses targeting conserved HIV-1 regions in preclinical models. These modalities include plasmid DNA, engineered strains of BCG, Semliki Forest virus replicons launched as DNA and virus particles, human and simian adenoviruses, and adjuvanted long synthetic peptides [41,54,60,66–73]. Vaccines vectored by three non-replicating platforms plasmid DNA, engineered simian (chimpanzee) adenovirus ChAdV-63, and poxvirus modified vaccinia virus Ankara designated pSG2.HIVconsv (D), ChAdV63.HIVconsv (C), and MVA.HIVconsv (M), respectively, reached clinical

evaluation in humans. ‘Naked’ DNA is an attractive vaccine vector for its simplicity, stability, and well-established manufacturing process. It showed good immunogenicity in some animal species, but it is not sufficiently immunogenic as a stand-alone vaccine in humans and is often used as a prime for other heterologous boosting modalities. Adenoviruses are safe and highly immunogenic vaccine vectors, of which the immunogenicity of human adenovirus serotype 5 (HAdV-5) used to be the gold standard for recombinant genetic subunit vaccines. However, preexisting anti-HAdV-5 antibodies seriously hamper induction of immune responses to the transgene products [74] and prompted by prudence following the Step study safety concerns, the field has moved to rarer HAdV serotypes and simian adenoviruses [75,76]. MVA is a well-tested, safe, and highly immunogenic vector in humans [77]. Because of its over 200 poxvirus proteins, MVA is predominantly used for expansion of already primed immune responses [78]. Single-dose immunogenicity of non-replicating viral vectors cannot be easily enhanced by repeated dosing of the same vaccine; instead, heterologous prime-boost combination regimens are used [46,79,80]. Clearly, complex vaccination regimens are not practical; however, at this stage of HIV-1 vaccine development, any demonstration of a solid vaccine protection by no-matter-how impractical regimen would be hugely significant and encouraging, and would form a platform for further design improvements.

3. HIVconsv vaccines are safe and well tolerated

Safety is the prime concern for every investigational medicinal product. Two separate formal preclinical toxicity studies in the BALB/c mice were conducted using the MMM and DDDC regimens and raised no safety concerns [72]. Approval for the first HIVconsv vaccine trial HIV-CORE (HIV Conserved REgions) 001

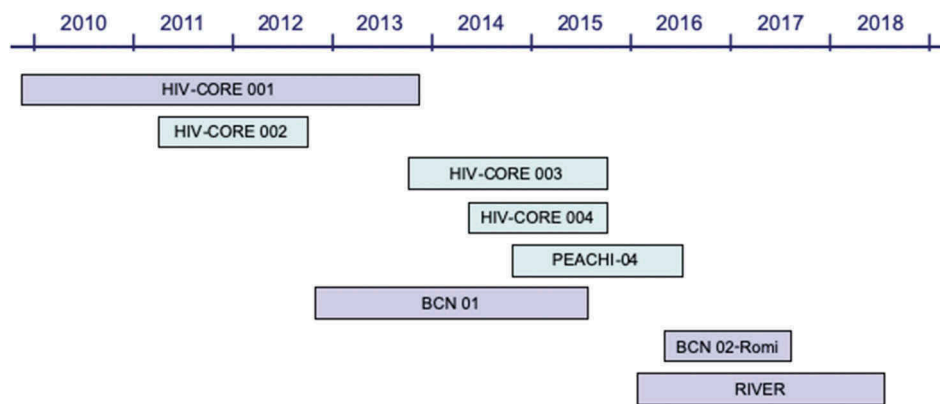


Figure 2. Time lines of clinical trials of the HIVconsv vaccines. Trials recruiting HIV-1-positive and negative individual are color-coded as purple and green, respectively.

by the Medicines and Healthcare products Regulatory Agency of the United Kingdom was granted on 26 October 2009. Since then, the HIVconsv vaccines were tested in eight clinical studies (Figure 2, Table 1). All products were administered to human volunteers using a needle injection into the triceps muscle on both arms. Clinical trials undertook surveillance for

adverse events with medical history, examination and blood tests. Overall, the vaccine safety and tolerability profiles were excellent [81–85] with no suspected unexpected serious adverse reactions (SUSARs) and no serious adverse events (SAEs) judged possibly, probably or definitely related to the vaccine. The majority of local and systemic vaccination

Table 1. Summary of clinical trials with HIVconsv vaccines.

Table 1: Summary of clinical trials with HIV-1 test-negative vaccinees							
Arm	Regimen ^a	Subjects (V + P) ^b	pSG2.HIVconsv Subjects/Doses	ChAdV63.HIVconsv Subjects/Doses	MVA.HIVconsv Subjects/Doses	Funder Sponsor	Ref.
HIV-CORE 002 (HIV-1-negative adults, UK)							
1	c	2 (2 + 0)	-	2/2	-	MRC-DFID UK/ UOXF	[83,87,88,90]
2	CM	8 (7 + 1)	-	8/8	7/7		
3	DDDCM	10 (8 + 2)	8/24	8/8	8/8		
4	DDDMC	10 (8 + 2)	8/24	8/8	8/8		
HIV-CORE 003 (HIV-1-negative adults, UK)							
1a	cDcDcDC	4	4/12	4/4	-	MRC-DFID UK/ UCL	[81]
1b	cDcDcDCM	16	16/48	16/16	16/16		
2a	DDDC	3	3/9	3/3	-		
2b	DDDCM	17	17/51	17/17	17/17		
HIV-CORE 004 (HIV-1-negative adults, Kenya)							
1	AM	20	-	-	19/19	EDCTP-IAVI/ UOXF	[85]
2	DDAM	20	20/60	-	19/19		
3	DeDeDeAM	20	20/60	-	19/19		
4	PP/PPPPP	12	-	-	-		
PEACHI 04 (HIV-1-negative adults, UK)							
1	HCV CM	8	-	-	-	European Commission/ UOXF	[101]
2	HIV CM	8	-	8/8	8/8		
3	HCV+HIV CM	16	-	16/16	16/16		
HIV-CORE 001 (HIV-1-positive adults, UK)							
1	mmm	10 (8 + 2)	-	-	8/24	MRC-DFID UK/ UOXF	[82]
2	MMM	9 (7 + 2)	-	-	7/21		
BCN 01 (HIV-1-positive adults, Spain)							
1	C24M ^c	12	-	12/12	12/12	IrsiCaixa/ IrsiCaixa	[84]
2	C8M ^c	12	-	12/12	12/12		
3	-	24	-	-	-		
BCN 02 (HIV-1-positive adults, Spain)							
1	(CM)-MM +Romidepsin	15 (BCN 01)	-	-	15/30	IrsiCaixa/ IrsiCaixa	[86]
RIVER (HIV-1-positive adults, UK)							
1	CM+Vorinostat	30	-	30/30	30/30	MRC-DFID UK/ ICL	[104, 105]
2	-	30	-	-	-		
CUMULATIVE TOTAL		219 HIVconsv	96/288	144/144	206/266		

a – Regimens, whereby D – 4 mg of pSG2.HIVconsv plasmid DNA; cD – D with pre-injection CPHPC treatment; De – D with electroporation using the TriGrid Delivery System device of ICOR Medical Systems; c – 5×10^3 vp of ChAdV63.HIVconsv; C – 5×10^{10} vp of ChAdV63.HIVconsv; m – 5×10^7 PFU of MVA.HIVconsv; M – 2×10^8 PFU of MVA.HIVconsv; A – 5×10^{10} vp of Ad35-GRIN, an HAdV-35 expressing a fusion protein of Gag-reverse transcriptase-integrase-Nef of HIV-1 clade A [100], which contains 604 amino acids (78%) of HIVconsv with 97.6% homology. Ad35-GRIN replaced ChAdV63.HIVconsv after we lost access to the ChAdV-63 vector. b – V for vaccine and P for placebo recipients; c – Numbers between the C and M indicate the interval in weeks between vaccine administrations. MRC-DFID UK – The work was jointly funded by the UK Medical Research Council and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreements; UOXF – University of Oxford; EDCTP – European and Developing Countries Clinical Trials Partnership; IAVI – The International AIDS Vaccine Initiative; IrsiCaixa – Institut de Recerca de la Sida; UCL – University College London; ICL – Imperial College of Science, Technology and Medicine.

reactions were grade 1–2 (erythema/pain at injection site, headache, malaise myalgia, and ‘flu’-like symptoms). It was expected that recipients of MVA.HIVcons_v may occasionally develop symptoms such as pain and swelling and transient limitation of limb movement related to inflammation at the site of vaccination. All symptoms resolved spontaneously within days.

4. HIVcons_v prophylactic vaccine studies

Trials with the aim to develop an effective prevention of HIV-1 infection tested the HIVcons_v vaccines in healthy, low-risk, HIV-1-negative adults in UK and Kenya.

4.1. Optimizing HIVcons_v delivery (HIV-CORE 002)

Trial HIV-CORE 002 (NCT01151319) was a single-blind, placebo-controlled trial in healthy volunteers ($n = 32$) in Oxford, UK, whereby the immunological laboratory remained blinded throughout the analyses. The first two volunteers received one vaccination with a low dose of ChAdV63.HIVcons_v for initial safety evaluation. Thirty volunteers were then recruited and assigned sequentially into groups of 10 to one of three vaccination regimens: CM (weeks 0 and 8), DDDCM (weeks 0, 4, 8, 12, and 20) or DDDMC (weeks 0, 4, 8, 12, and 16) and were randomized to receive a vaccine or placebo in a 4:1 ratio (Table 1).

All vaccine recipients (100%) developed HIV-1-specific T-cell responses [87]. The vaccine regimens induced high frequencies of effector T-cells in the hierarchy of DDDCM>CM>DDDMC reaching for the two most immunogenic arms median 5,790 and 5,150 IFN- γ SFU/10⁶ PBMC, respectively. These responses were plurifunctional and each vaccine recipient responded to an estimated 10 different target specificities. On average out of 10 recognized 15-mer peptides, 6 contained a known CD8⁺ T-cell epitope, and 2 were junctional epitopes spanning two adjacent conserved regions (14 conserved regions created 13 junctions). Vaccine-elicited responses persisted for a prolonged period of time, whereby 12/12 returning subjects after 1 year and 8/8 after 2 years had detectable responses with median frequencies of 990 and 763 IFN- γ SFU/10⁶ PBMC, respectively, and half of the HIV-1-specific T-cell population displayed at least 3 functions and were capable of proliferation to HIVcons_v-derived peptides [88].

Careful definition of optimum peptides can critically inform future vaccine designs and increase the predictive power of a vaccine success or failure. Thus, PBMCs from 12 vaccine recipients were utilized for fine definition of 14 previously unreported optimal CD8⁺ T-cell epitopes and their four-digit HLA allele restriction, which were candidates for the ‘A’ list of well-defined epitopes [89], and another 13 novel epitopes with incomplete definition, which were added to the ‘B’ list [90]. The ability to ‘mine’ novel T-cell epitopes in recipients of the conserved-region vaccines supports the subdominance of conserved epitopes and their underutilization by the immune system in natural HIV-1 infection.

A valuable collective assessment of anti-HIV-1 T-cell functions prior to efficacy in humans provides the virus inhibition

assay (VIA), which quantifies *in vitro* reduction of HIV-1 replication in autologous CD4⁺ cells and does so in the context of evasive HIV-1 mechanisms [91–94]. Antigen-nonspecifically expanded CD8⁺ T-cell effectors [92] inhibited replication of up to 8 HIV-1 isolates covering clades A, B and C in autologous CD4⁺ cells *in vitro* [87]. Specific peptide-expanded effectors demonstrated that the virus inhibition was mediated by both Gag- and Pol-specific effector CD8⁺ T cells with equal efficiency [95].

4.2. Improving the plasmid DNA vaccine take (HIV-CORE 003)

There are licensed veterinary DNA vaccines for horses, dogs and salmon, but for reasons not well understood, DNA vaccines are only very weakly immunogenic in humans. Indeed in our hands, previous intramuscular injection of DNA to humans primed immune responses, but this was only demonstrated by increased magnitude of a subsequent heterologous boost [87,96–98]; DNA vaccines alone induced barely detectable frequencies of HIVcons_v-specific T cells. Serum amyloid P component (SAP) is the single normal DNA-binding protein in human plasma and drug called CPHPC or miridesap, developed for treatment of systemic amyloidosis and Alzheimer’s disease, potently and safely depletes circulating SAP for as long as the drug is administered [99]. Transgenic mouse model demonstrated that DNA immunogenicity was attenuated by DNA binding to human SAP. The proof-of-concept trial HIV-CORE 003 (NCT02425241) was a randomized, double-blind, placebo-controlled clinical study ($n = 40$) in London, UK, which tested whether or not a 24-hour SAP depletion by CPHPC prior to intramuscular DNA administration enhances the immune induction in humans. Note that oral delivery of CPHPC is now possible. Volunteers in each arm received DDDC(M) regimens with CPHPC or placebo pre-DNA treatment (Table 1) and, while the HIVcons_v vaccine immunogenicity was confirmed, no benefit to DNA priming or subsequent boosts was detected [81].

4.3. Translating from north to south and DNA electroporation (HIV-CORE 004)

HIV-CORE 004 (NCT02099994) was a randomized, double-blind, placebo-controlled trial ($n = 74$) evaluating the two most immunogenic regimens of HIV-CORE 002 in Nairobi, Kenya. The regimens tested were AM, DDDAM and DeDeDeAM administered to healthy, low-risk, HIV-1-uninfected adults in Nairobi, whereby in the regimen description, the A stands for the Ad35-GRIN vaccine and De indicates intramuscular DNA delivery enhanced by electroporation using the TriGrid Delivery System of Ichor Medical Systems (Table 1). Ad35-GRIN replaced ChAdV63.HIVcons_v after we lost the freedom to operate for the ChAdV63 vector. HAdV-35 expressed the HIV-1 clade A Gag, reverse transcriptase, integrase, and Nef fusion protein GRIN [100], which contained 604 amino acids (78%) of HIVcons_v with 97.6% homology and thus worked very well with the pSG2.HIVcons_v DNA and MVA.HIVcons_v vaccines.

All (100%) vaccine recipients developed HIVcons_v-specific T-cell responses. Although not statistically separable, the

highest peak ELISPOT frequencies over median 3,500 SFU/10⁶ PBMC were induced by DDDAM, followed by DeDeDeAM and AM with median of 6/6 recognized peptide pools used for the primary read out in all three trial arms. Electroporation of DNA significantly improved peak DNA responses from median 81 to 393 SFU/10⁶ PBMC, however, T-cell frequencies did not differ 24 weeks later. Vaccine-induced T cells were plurifunctional, capable of proliferation and inhibited significantly *in vitro* replication of 6/8 tested viruses from 4 major HIV-1 clades A, B, C, and D [85].

4.4. Combining HIV-1 and HCV vaccines into one regimen (PEACHI-04)

An estimated 71 million people are currently living with hepatitis C virus (HCV) infection, and both HCV and HIV-1 are responsible for a significant global burden of disease and premature death. PEACHI-04 (NCT02362217) was an open-label study ($n = 32$) directly addressing the need for innovative combined vaccine strategies to prevent HCV and HIV-1 infections. It used the CM vaccination regimen using serologically distinct simian adenoviruses ChAdV-3 and -63 in parallel for priming to deliver HCV non-structural (NSmut) and HIVconsv immunogens, respectively, to healthy HCV- and HIV-1-negative adults allocated into 3 trial arms: NSmut alone, HIVconsv alone, and NSmut+HIVconsv together. Both vaccines were co-administered without compromising immunogenicity of the other vaccine [101].

5. HIVconsv studies toward HIV cure

Curative strategies for HIV-1 infection have experienced increased interest and investments. As for sterile cure, it will never be possible to routinely purge every single integrated functional provirus from the body and no assay will ever be sensitive enough to definitely rule out the provirus presence. In contrast, a functional cure (immune control without LR eradication) or a hybrid cure (LR reduction with improved immune control), which would prevent both AIDS and onward HIV-1 transmission, might be more a realistic goal [102]. An HIV cure explores many approaches and the currently most pursued is the 'kick-&-kill' strategy [103]. It aims to expose the latent HIV-1 through latency reverting agents (LRA) and kill the cells with reactivated virus by augmented immune effector functions. Induction of effective killer T cells is, therefore, high on the priority list.

5.1. Modest, but significant increase in HIV-1 inhibition by administration of MVA.HIVconsv alone to late-treated patients (HIV-CORE 001)

The very first clinical study of the first-generation HIVconsv vaccines was phase 1 trial HIV-CORE 001 (NCT01024842) in Oxford. It was a randomized, placebo-controlled study ($n = 20$) administering three low or standard doses of MVA.HVconsv (weeks 0, 4, and 12) to HIV-1-positive subjects (Table 1), who received cART late during chronic infection, were on cART between 24 and 58 months and had ≥ 350 CD4⁺ cells/ μ l.

Overall, three doses of MVA.HIVconsv alone administered to HIV-1-positive cART-treated subjects induced a modest increase in CD8⁺ T-cell antiviral activity, which might be an indication of irreversibly compromised CD4⁺ T-cell function due to the late cART, with no impact on the size of the viral LR. Although the trial was small in size and used only one assay to quantify the LR, to our knowledge, this was the first study to investigate the relationship between the HIV-1 reservoir size and CD8⁺ T-cell viral-inhibitory activity in chronic cART-treated patients [82].

5.2. Refocusing T cells to conserved regions by heterologous CM regimen (BCN 01)

BCN 01 (NCT01712425) was a phase I, open-label, multicenter trial ($n = 48$) in Barcelona and Badalona, Spain. In BCN 01, HIV-1-positive subjects diagnosed and treated during early acute HIV-1 infection and stable on cART including Raltegravir for 6 months were administered the CM regimen using either an 8- or 24-week interval or received no vaccine (Table 1).

At baseline prior to the cART initiation, half of the subjects displayed weak, but detectable responses recognizing conserved regions of the HIVconsv immunogen, which represented median 4% of the subjects' total anti-HIV-1 IFN- γ ELISPOT assay responses [84]. After vaccination, all (100%) vaccine recipients developed broadly specific T cells responding to HIVconsv peptides, which peaked after MVA.HIVconsv and were comparable to those in HIV-negative volunteers in trial HIV-CORE 002. After the ChAdV63.HIVconsv-MVA. HIVconsv vaccination, the HIVconsv responses represented median 58% of the total volunteers' anti-HIV-1 T cells. No T-cell parameter was statistically separable between the two regimens using different prime-boost intervals. Notably, in contrast to HIV-1-negative participants of HIV-CORE 002, vaccines in BCN 01 did not develop detectable junctional responses. Thus, either priming in HIV-1-positive subjects is less efficient compared to healthy uninfected volunteers, or vaccination in HIV-1-positive subjects mainly expands pre-existing responses even if they were miniscule due to immunodominance. The HIVconsv-specific CD8⁺ T cells displayed high levels of HIV-1-inhibitory capacity *in vitro*, nevertheless, the decay in the size of the HIV-1 LR was consistent with the first year of early cART initiation without any evidence for a further vaccine-induced decrease [84].

5.3. Signal of ART-free control of rebound HIV-1 (BCN 02)

The BCN 02 study (NCT02616874) was a pilot, open-label study ($n = 15$) assessing the effect of combined early cART treatment, vaccination and Romidepsin, an LRA in the histone deacetylase inhibitor (HDACi) family, on the size of LR and viremic control during antiretroviral treatment interruption (ATI). Two to three years after BCN 01, 15 participants were rolled over to BCN 02 and received 2 additional doses of MVA.HIVconsv (CM-MM in total) before and after 3 doses of Romidepsin. Both MVA. HIVconsv boosts increased the response magnitude so that the volunteers' HIVconsv-specific T cells represented median 85% of

their total anti-HIV-1 responses. Upon pausing cART 8 weeks after the second MVA.HIVconsv, 4/13 (31%) individuals controlled HIV-1 beyond the typical 2–3 weeks of stopping cART and 3/13 (23%) showed a durable viremic control over the scheduled 32 weeks of ATI. Changes in both acetylation and cell-associated HIV-1 RNA were detected. pVL before ART initiation was the only factor significantly associated with controlled rebound during ATI. The only other feature common to the small group of controllers pointed to, but did not reach significance, a high percentage (>75%) of refocusing on the HIVconsv regions relative to the overall anti-HIV-1 T-cell responses. There was a non-significant decrease in LR beyond what was expected from the time being on ART and no evidence for reservoir re-seeding during ATI (manuscript submitted). While we fully acknowledge the open-label design, small sample size and absence of a control arm interrupting cART, but not receiving any vaccine/LRA to indicate spontaneous control of viremia in this cohort of HIV-1-positive individuals, the BCN 02 study provided a signal of durable post-treatment control, which warrants further investigations.

5.4. The first randomized controlled ‘kick-&-kill’ study in early treated patients (RIVER)

RIVER (Research in Viral Eradication of HIV Reservoirs) (NCT02336074) was a randomized proof-of-concept study ($n = 60$) in six UK centers, whereby recruited patients with confirmed recent HIV-1 infection (within a maximum 12 weeks of estimated date of HIV-1 infection) commenced early 4-drug cART including Raltegravir and were randomized on a 1:1 basis to either receive a combination of the HIVconsv vaccine regimen of ChAdV63.HIVconsv-MVA.HIVconsv plus LRA Vorinostat, the same class of LRA as Romidepsin working through the histone deacetylase inhibition, or cART alone. The primary study endpoint was quantification of total HIV-1 DNA in peripheral blood CD4⁺ T cells at weeks 16 and 18 post vaccination. Thus, the ChAdV63.HIVconsv-MVA.HIVconsv administration increased the frequencies of HIVconsv-specific CD8⁺ and CD4⁺ T cells, which were capable of viral inhibition *in vitro*, and Vorinostat treatment increased over 3-fold the histone acetylation, but not HIV-1 RNA in PBMC. Neither the total CD4-associated HIV-1 DNA nor the virus outgrowth assay suggested a significant decrease in latent virus reservoir in the cART+Vaccine+Vorinostat arm relative to the cART-alone treated controls [104,105].

6. Conclusions and future plans for the conserved region strategy

6.1. HIVconsv delivered by ChAdV63 and MVA induced robust responses against naturally subdominant epitopes

The first generation of vaccines against HIV-1 utilizing functionally conserved protein regions as targets of the T-cell immune attack were tested in 8 clinical trials recruiting both healthy and HIV-1-infected subjects. These trials demonstrated that taking naturally subdominant CD8⁺ T-cell epitopes out of the context of the full-length proteins and indeed the whole-virus infection allowed induction of robust CD8⁺ T-cell

responses of high frequencies, which were broadly specific for conserved epitopes, plurifunctional, rapidly proliferating to a specific antigenic stimulus and were capable of slowing HIV-1 replication *in vitro* and possibly *in vivo*. These results support further development of this T-cell vaccine strategy toward efficacy evaluations.

6.2. The second generation conserved mosaic tHIVconsvX vaccines

In May 2013, we lost the freedom to operate for the ChAdV63 vector and had to switch to University of Oxford fully owned vaccine vector ChAdOx1, which is derived from simian adenovirus Y25 [106]. This provided an opportunity to redesign and upgrade the HIV-1 conserved immunogens based on the ever advancing understanding of T-cell protection [107]. The main competitive advantage of the second-generation tHIVconsvX vaccines is in combining the four currently leading T-cell strategies into one vaccine design: the focus on functionally conserved regions, which may offer a cross-protection of many strains of globally circulating viruses [46], rational bioinformatics-assisted computer-optimized bivalent mosaic immunogen design (see below) with perfect match of 9-amino acid-long T-cell epitopes to 80% of the group M variants [108], inclusion of epitopes protective in treatment-naïve HIV-1-positive individuals on four continents [19,20,22] and in-human proven highly immunogenic delivery of the immunogens by the ChAdV-MVA regimen [46,109]. The tHIVconsvX concept is now in the pipeline for a number of clinical evaluations with two US trials already recruiting. While responses to epitopes in tHIVconsvX regions correlated with good virus control and preservation of the CD4⁺ T-cell counts and thus the vaccine-elicited T cells have the right specificity, it remains to be seen whether the vaccine modalities presenting the tHIVconsvX immunogens to the immune system elicit T-cell responses, which are robust enough not to be redirected to the non-protective decoy epitopes by an incoming or reactivating virus before the virus becomes controlled.

7. The T-cell vaccine perspective

7.1. Historical efficacy studies of T-cell vaccines

For prevention, the first T-cell vaccine tested in two phase 2b efficacy trials was designed around the beginning of this millennium before the HIV-1 diversity was fully appreciated. Natural sequences of HIV-1 clade B Gag, Pol and, Nef full-length proteins delivered by three repeated administrations of the same vector HAdV-5 (a vaccine called MRKAd5) were tested in HVTN 502 (Step) [110,111] and prematurely terminated HVTN 503 (Phambili) [112] trials in populations in high risk of clade B and C (mismatched) infections, respectively. In the Step study, 77% of vaccine recipients developed detectable HIV-1-specific T-cell responses with peak frequencies between 163 and 686 IFN- γ -producing SFU/10⁶ PBMC [111]. According to the pre-specified data analysis plan, there was no statistically significant effect of the vaccine on HIV-1 acquisition or virus load upon infection, although in the Step study mainly, post-hoc subgroup analyses associated increased

acquisition with the MRKAd5 vaccine administration among uncircumcised and/or HAdV-5-seropositive men [113–115]. HVTN 505 improved both the delivery and Env clade match by priming 3x with plasmid DNA delivering Gag, Pol, and Nef of clade B before boosting 1x with HAdV-5 expressing Gag/Pol of clade B and Env of clades A, B, and C, but, similarly to the previous studies, showed no overall protection [79]. Follow-up investigations pointed to the limited capacity of these early vaccines to induce broad and circulating virus-matching CD8⁺ T-cell responses [107,116,117], which supported the more likely and generally accepted interpretation that the lack of efficacy was due to the tested products rather than an intrinsic inability of robust, broad and well matched CD8⁺ T-cell responses to stop and control HIV-1. In contrast to the overall lack of protection, detailed analyses of CD8⁺ T-cell responses and breakthrough viruses in infected individuals revealed association of reduced risk of infection with Env-specific CD8⁺ T-cell frequencies [118,119], increased genetic distances from the vaccines of breakthrough viruses in vaccine compared to placebo recipients suggesting a selective pressure imposed by the vaccine-elicited T cells [120,121] and decreased virus load associated with 3 or more CD8⁺ T-cell responses to Gag [117]. However, there was only a small number of individuals and their protective T-cell responses were too rare to impact the trials' endpoints. Collectively, these efficacy trials were highly informative and encouraged further vaccine refinements toward increased breadth and potency of induced CD8⁺ T-cell responses.

For immunotherapy, a variety of vaccine modalities including the use of mRNA for the immunogen gene delivery were tested and achieved variable immunogenicity and no or very limited viremic control [103,122]. At least 13 studies in HIV-1-positive subjects employing various types of dendritic cells (DC), several DC maturation strategies and autologous virus or whole viral proteins as immunogens [122] were conducted, of which many induced HIV-1-specific T cells and some a virologic control of 0.5–1 log₁₀ of plasma virus load [123].

7.2. T-cell vaccine concepts in translation

As of July 2019, a search on clinicaltrials.gov for HIV-1 vaccines found 756 studies. Currently, the main focus of the HIV-1-vaccine field is on active induction or passive introduction of broadly neutralizing Abs, whereby many of these vaccines, maybe for historical reasons, also deliver Gag and Pol and therefore aim to elicit T cells. Thus, Pox-Protein Public-Private Partnership (5Ps) builds on the marginal, but significant 31% protection achieved in Thai trial RV144 with the goal of licensure, should efficacy be repeated, and testing vaccine variations to improve protection. Studies assessing full-length mosaic proteins of Gag-Pol and Env gp140 delivered by HAdV-26, MVA plus gp140 clade C protein reported good T-cell and B-cell immunogenicities [124], vaccines designated Ad26.Mos4.HIV plus clade C and bivalent mosaic gp140 progressed to phase 2b prevention study HVTN 705 (Imbokodo) in sub-Saharan Africa and phase 3 trial HVTN 706 (Mosaico) is scheduled for September 2019.

HIV-1 variability is astonishing and a number of groups have come to a realization that not all epitopes are equally

protective and that it may be beneficial not to use full-length proteins, but instead to direct the vaccine elicited responses to the vulnerable regions of HIV-1, or to 'hit the virus more precisely where it hurts' [125]. Novel immunogens avoiding decoy epitopes were recently reviewed [107] and include the HTI (HIVACAT T-cell Immunogen) human-data-informed immunogen assembled from 16 regions with emphasis on responses correlated with lower virus load in natural untreated infections rather than sequence conservation delivered by DNA, MVA, and ChAdOx1 [59], Ultra Conserved Gag and Pol peptides of at least 14 amino acids selected by highly stringent, tighter conservation criteria intended for DC vaccination [107], p24gagCE (conserved elements) assembled as a string of 6 very short conserved Gag regions [126] and immunogens built around conserved (networked) amino acids [127]. A match of full proteins and shorter protein regions to the circulating viruses can be enhanced by bioinformatics-assisted algorithms used for the polyvalent mosaic [108] and epigraph [128] designs, because even the shared regions retain a degree of variability. These algorithms maximize vaccine match to HIV-1 variants, and minimize redundancy and use of rare epitopes within the vaccine cocktails. All these vaccine immunogens have a more developed rationale and data from human volunteers will tease them apart keeping in mind that also their delivery is critical (e.g. 'the best immunogen in the world' delivered by DNA alone will not protect).

8. Expert opinion

8.1. What T cells are we aiming for?

Several of the strongest correlates of control of HIV-1 infection are associated with T-cell responses and, at least during the primary infection, some T-cell responses are capable of lowering the level of established infection. To date, no simple functional or phenotypic T-cell marker has been consistently associated with HIV-1 control. This is because an antiviral CD8⁺ T-cell response is mediated by a heterogeneous population of cells with individual antigenic and stimulatory histories, which are capable of performing multiple, mutually overlapping functions. In natural HIV-1 infection, CTL target both protective and non-protective epitopes, and this alone is sufficient to blur any simple attempts to associate a single T-cell parameter with HIV-1 control. To be beneficial, CD8⁺ T cells will have to display individually and as a population multiple attributes, many of which have to come together at the same time and at the right place within the body to achieve control of HIV-1. These T-cell traits include specificity for protective epitopes, parallel recognition of multiple protective epitopes, optimal interaction of TCR with HLA-presented peptides, rapid proliferation upon exposure of cognate peptides to reach protective frequencies, homing profiles allowing the T cells to reach cells with replicating HIV-1 as soon as possible, cytotoxicity and production of soluble antiviral molecules. For immunotherapy, all of these features should be acquired with minimum proinflammatory signals not to 'fuel' the infection [129]. One key protective parameter, if suboptimal, may be sufficient to cause the T-cell response/vaccine to fail. For a pathogen as challenging as HIV-1, achieving protection may require several

rounds of iterative refinement of the vaccine formula and combinations with other immunomodulating or LR-targeting drugs. Iterative improvements will be best based on human data, the only species that matters.

8.2. Issues in HIV-1 vaccine prevention

There are now a number of antibody and T-cell vaccine approaches, which show promise in pre-clinical models and/or early clinical trials that are gradually progressing through human testing with results emerging in not too distant future. The most promising antibodies and T-cell vaccines should be rationally combined into single regimens. For prevention, more efficient adaptive trial protocols with multiple built-in review/decision points will be employed increasingly to accelerate prophylactic vaccine testing. Novel ethical models will be needed for testing of preventive vaccines in the context of improved efficiency and availability of cART as prevention. Basic research, novel technologies, an ever-growing list of protective epitopes in different global populations, and big data/integrative approaches will improve early prediction of a vaccine success/failure.

8.3. Issues in HIV-1 cure

Also for prevention, basic research, novel technologies, and big data/integrative approaches will keep moving the field of HIV-1 cure forward. Investigations into processes such as structure and regulation of chromosomes, mechanisms of establishment and maintenance of LR, biomarkers for the LR size, novel nontoxic and more efficient strategies for purging integrated provirus or 'locking' it, i.e. preventing its reactivation, and better understanding of mechanisms and prediction of post-treatment control will be key. cART will need to be paused safely while leaving sufficient time to establish, assess and detect virological remission [130]. The first vaccine trials should start with 'easy' populations to not give up a potentially useful vaccine strategy too early. Thus, vaccine tests should take advantage of the relatively preserved immune system of early-treated cohorts of HIV-1-positive individuals rather than going straight into chronically infected subjects (this is by no means to say that subjects first treated in chronic infection should be excluded from trials). Another special population is perinatally infected children and young adults, who were put on cART within a few hours to days after delivery. For these subjects, cART within the first three months is life-saving, but it has to be continued for many decades, which magnifies all cART-associated complications and challenges. Similarly to adults treated during acute infection, early treatment limits the reservoir size, decreases HIV-1 diversity, and minimizes preexisting HIV-1-specific responses, which together with the young immune system and functioning thymus form a good basis toward the HIV-1 remission and cure [131]. As such, children, adolescents, and young adults, who acquired HIV-1 perinatally, are unique populations, which may allow to assess an effective immunotherapy inducing broadly specific

protective T cells and nAbs with a better chance of sustained periods of HIV-1 remission compared to adults [132,133].

8.4. Growing optimism for the future

In five years' time, we may see the first more significant signal of prevention from efficacy studies in high risk populations and increasing proportions of patients experiencing sustained periods of post-cART control. The T-cell and B-cell vaccine strategies focusing on vulnerable sites on HIV-1 will be central to these efforts. Patients, the public, scientists, funders, policy makers, and all other stakeholders have to be prepared for stumbles on the way, but progress is inevitable and the future is promising.

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Declaration of interest

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 - **This article summarizes the outcome of a meeting of scientists, clinicians, ethicists, social scientists, regulators, people living with HIV-1 and advocacy groups, which aimed to discuss the main challenges concerning antiretroviral treatment interruption studies and formulate recommendations with an emphasis on strategies for risk mitigation and monitoring, cART resumption criteria, and ethical considerations.**
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