GP mAbs that target the GP glycan cap, fusion loop, chalice base, and HR2 region. BALB/c mice were administered individual DMAbs alone or in combination. EVD DMAbs were detected in mouse serum for >100 days, with several candidates reaching 20-80μg/mL C_{max} serum IgG levels. Two EVD DMAbs prevented mortality and morbidity in 100% of animals following a stringent 1000LD50 dose of mouse-adapted Zaire Ebola virus on day 28 post-DMAb administration. Furthermore, 40% of animals survived lethal challenge administered 82 days following DMAb administration, demonstrating long-term systemic circulation of muscle-produced DMAb-IgG. Ongoing studies are evaluating EVD DMAb expression in guinea pigs and non-human primates. DNA-monoclonal antibody delivery is a transformative technology that enables long-term transient IgG expression, with the possibility for repeat DMAb administration. The DMAb platform expands the use of DNA vector technology for gene delivery and other transient gene based therapy applications such as replacement protein or enzyme delivery.

393. Development of Recombinant Adeno-Associated Viral Vector (rAAV) for Passive Immunisation Against Ebola

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The 2014-2016 Ebola outbreak in West Africa was the largest in history leading to 28,639 cases, of which 40% were fatal. Better treatments and effective vaccines against Ebola virus are needed to prevent future recurrence. Multiple, novel, treatment options were assessed during the outbreak, including ZMapp™ monoclonal antibody therapy, which showed promising results in some patients. However, widespread adoption of this approach is hindered by high costs of monoclonal antibody manufacturing and the relatively short half-life of antibody in the blood circulation. To circumvent such limitations, we propose to utilise recombinant adeno-associated virus (rAAV) delivered to the muscle to express the therapeutic monoclonal antibodies. A single intramuscular dose of rAAV encoding lipoprotein lipase has shown clinical efficacy in patients for at least 6 years demonstrating persistent transgene expression, together with a well-established safety profile. During an initial proof-of-principle study, we showed that a single dose of rAAV2/8 carrying secretory reporter gene (*Gaussia* luciferase (GLux)) leads to abundant expression of GLux at day 7 post administration, which increases and persisted in the blood circulation for at least 28 days (20,865 RLU/μl, 43,420 RLU/μl and 6220,000 RLU/μl; $p<0.01$, respectively for 1E9, 1E10 or 1E11 Genome Copies). We then produced rAAV2/8 encoding KZ52 antibody, the first human anti-Ebola (*Zaire* strain) neutralising antibody, isolated from a recovered patient during the 1995 Kikwit Ebola outbreak. A single intramuscular dose of rAAV2/8 encoding KZ52 leads to expression of high human IgG (256.36 μg/ml) in the mouse serum at day 28 post administration, which is ~100-fold higher than the EC_{90} required for *in vitro* neutralisation of the wild-type Ebola virus (J Virol 73:6024-6030,1999). In terms of functioning titre in the serum, we detected high serum binding and neutralising antibody titre against an Ebola (*Zaire*) glycoprotein pseudotyped influenza virus (EC_{50}: 1:7000 & 1:4356, respectively) in BALB/c mice following a single dose of 1E10 or 1E11 Genome Copies of rAAV2/8 expressing KZ52. To test its efficacy in preventing Ebola infections, we are currently undertaking a protection study against Ebola in the guinea pig challenge model. Together, these preliminary data support the generation of rAAV vectors carrying a cocktail of novel antibodies isolated from vaccinated donors for maximum clinical efficacy. In the event of future outbreaks, matched rAAV cocktails which were produced in advance, lyophilised and stockpiled can be used to provide timely prophylaxis among healthcare workers.

394. Influence of Pre-Existing Anti-Capsid Neutralizing and Binding Antibodies on AAV-Mediated Liver Transduction

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The prevalence of pre-existing immunity to adeno-associated virus (AAV) in the human population greatly hinders the global reach of emerging gene-based therapies exploiting this vector platform, particularly when systemic administration is warranted. Beyond the notion of neutralization, the dynamic consequences of such immunity in the form of anti-capsid immunoglobulins on transduction efficiency, vector biodistribution and vector immunogenicity remain largely elusive. Upon screening human serum samples for the presence of anti-AAV8 antibodies, we identified adult donors who harbor circulating anti-capsid binding antibodies devoid of neutralizing activity (BAbs), which were assessed for the ability to influence AAV vector-mediated liver transduction, in comparison to neutralizing antibodies (NAbs). An in vitro virus uptake assay employing imaging flow cytometry revealed that internalization of AAV8 capsid in a murine hepatocyte cell line was detectable both in the presence of anti-AAV8 BAbs and NAbs. However, transgene expression was detectable only when transduction was performed in the presence of BAbs. Animal studies demonstrated that anti-capsid NAbs mediate rapid clearance of systemically-delivered vector from the bloodstream and sequester it to both the parenchymal and non-parenchymal compartment of the liver, leading to lack of transgene expression. Conversely, in mice passively immunized with BAbs prior to intravenous delivery of AAV8 vector, we observed enhanced vector persistence in the circulation and transduction capacity in the liver and skeletal muscle, compared to PBS-injected animals and mice immunized with serum derived from individuals naïve to AAV. The humoral immune response mounted against AAV8 capsid, in the form of vector-specific immunoglobulins, following gene transfer was dampened in mice with pre-existing circulating BAbs and NAbs, compared to that observed in PBS control mice. These results establish the clinical significance of non-neutralizing antibodies directed against the AAV capsid in influencing measures of systemic gene transfer and vector immunogenicity.