

**REDACTED VERSION: MAY 14, 2019 FOR NLM**

**A Staged Phase I/II Study, to Assess Safety, Efficacy and Immunogenicity of a New Hepatitis C Prophylactic Vaccine Based on Sequential Use of AdCh3NSmut1 and MVA-NSmut**

**DMID Protocol Number:** 10-0069

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**IND Sponsor:** Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases, National Institutes of Health

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**Version Number:** 9.0

**6 October 2015**

## **STATEMENT OF COMPLIANCE**

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

**SIGNATURE PAGE**

The signatures below constitute the approval of this protocol and appendices, and provide the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

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**LIST OF ABBREVIATIONS**

Ab	Antibody
AdCh	Chimpanzee Derived Adenovirus
AE	Adverse Event/Adverse Experience
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Aminotransferase
ATP	According To Protocol
AUDIT	Alcohol Use Disorders Identification Test
BP	Blood Pressure
BSRI	Blood Systems Research Institute
C	Celsius
CBC	Complete Blood Count
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CDC	Centers for Disease Control and Prevention
CEF	Chicken Embryo Fibroblast
CIN	Cervical Intraepithelial Neoplasia
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
Cl	Chlorine
CLIA	Clinical Laboratory Improvement Amendments
CPM	Clinical Project Manager
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support, DMID
CRO	Contract Research Organization
CTL	Cytotoxic T Lymphocyte
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DMSO	Dimethyl Sulfoxide
DSMB	Data and Safety Monitoring Board
DUIT	Drug Users Intervention Trial
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic Acid
EIA or ELISA	Enzyme Immunoassay or Enzyme-Linked Immunosorbant Assay
ELISpot	Enzyme-Linked Immunospot Assay
F	Fahrenheit
FDA	Food and Drug Administration, DHHS
FDAAA	Food and Drug Administration Amendments Act
FWA	Federalwide Assurance



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GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
GP	General Practitioner
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HEENT	Head, Ears, Eyes, Nose, Throat
HgB	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HIVNET	HIV Network for Prevention Trials
HLA	Human Leukocyte Antigen
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Internet Data Entry System
IDU or IDUs	Injection Drug Use or Injection Drug Users
IEC	Independent or Institutional Ethics Committee
IgG	Immunoglobulin G
IFN	Interferon
IL28B	Interleukin-28B
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IU	International Units
JHU	The Johns Hopkins University
mcg	Microgram
MedDRA®	Medical Dictionary for Regulatory Activities
ME-TRAP	Multiple Epitope - Thrombospondin-Related Adhesion Protein
Mg	Magnesium
mg	Milligram
MHC	Major Histocompatibility Complex
mITT	Modified Intention to Treat
mL	Milliliter
mm	Millimeter
mM	Millimole

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MM	Medical Monitor
mmHg	Millimeter of Mercury
MOP	Manual of Procedures
MVA	Modified Vaccinia Ankara
N	Number (Typically Refers to Subjects)
Na	Sodium
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health, DHHS
NLM	National Library of Medicine
NS	Nonstructural Region
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections, DHHS
OHSR	Office for Human Subjects Research, NIH, DHHS
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD	Protocol Deviation
pfu	Plaque-Forming Units
PI	Principal Investigator
POWs	Peer Outreach Workers/Trained Recruiters
PTT	Partial Thromboplastin Time
PVG	Pharmacovigilance Group
PY	Person-Years
PYO	Person-Years of Observation
QA	Quality Assurance
QC	Quality Control
QM	Quality Management
RBV	Ribavirin
RCA	Replication Competent Adenovirus
RIBA	Recombinant Immunoblot Assay
RNA	Ribonucleic Acid
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SGPT	Serum Glutamic Pyruvic Transaminase
SIV	Simian Immunodeficiency Virus
SFC	Spot-Forming Cell
SMC	Safety Monitoring Committee
SMS	Short Message Service
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

TRAP	Thrombospondin-Related Adhesion Protein
UCSF	University of California San Francisco
UK	United Kingdom
UNM	University of New Mexico
US	United States
vp	Virus Particles
WBC	White Blood Cell
WHO	World Health Organization

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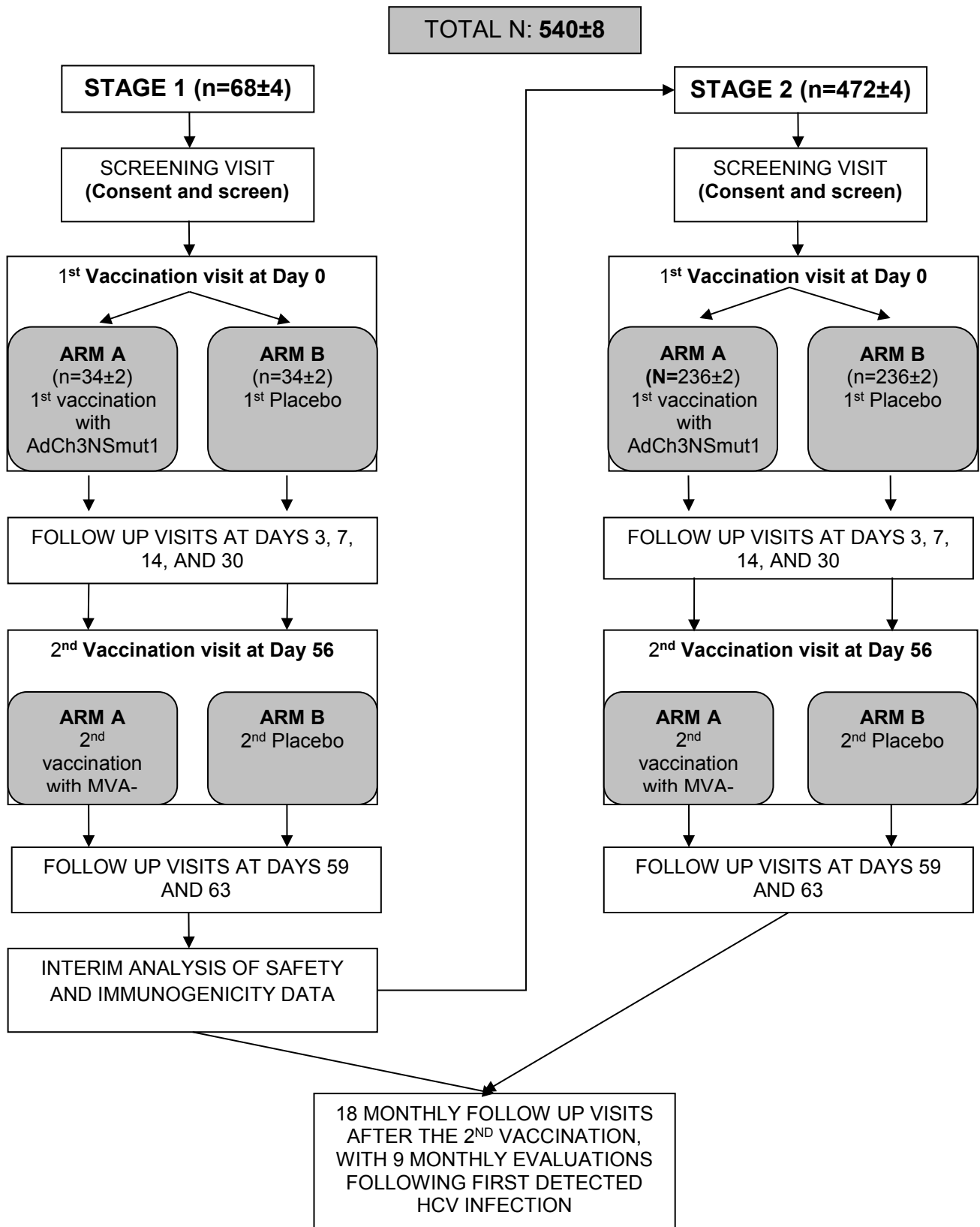
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## PROTOCOL SUMMARY

Title:	A Staged Phase I/II Study, to Assess Safety, Efficacy and Immunogenicity of a New Hepatitis C Prophylactic Vaccine Based on Sequential Use of AdCh3NSmut1 and MVA-NSmut
Phase:	I/II
Design:	<p>Double-blinded, randomized, placebo-controlled two stage study.</p> <p><u>Stage 1: 68±4 evaluable subjects</u></p> <p>Arm A: 34±2 evaluable subjects; 1 dose AdCh3NSmut1 <math>2.5 \times 10^{10}</math> vp at Day 0, and 1 dose MVA-NSmut <math>1.8 \times 10^8</math> pfu at Day 56</p> <p>Arm B: 34±2 evaluable subjects; 1 placebo dose at Day 0, and 1 placebo dose at Day 56</p> <p><u>Stage 2: 472±4 subjects</u></p> <p>Enrollment will be initiated after an interim analysis of the Stage 1 safety and immunogenicity data.</p> <p>Arm A: 236±2 subjects; 1 dose AdCh3NSmut1 <math>2.5 \times 10^{10}</math> vp at Day 0, and 1 dose MVA-NSmut <math>1.8 \times 10^8</math> pfu at Day 56</p> <p>Arm B: 236±2 subjects; 1 placebo dose at Day 0, and 1 placebo dose at Day 56</p> <p>Total number of subjects: 540±8 subjects</p>
Population:	Males and females negative for HCV antibodies and HCV RNA at screening, active injection drug users at high risk for hepatitis C, and 18 to 45 years old.
Number of Sites:	Three Sites (University of California San Francisco; The Johns Hopkins University, Baltimore; University of New Mexico, Albuquerque)
Study Duration:	Approximately 63 months

Subject Participation Duration:	12 months to 29 months. Non-viremic subjects will be followed for 20 months; subjects who are viremic will be followed for 9 months after detection of HCV (up to month 29).
Description of Agent or Intervention:	Two investigational vaccines administered sequentially as a prime and boost:  AdCh3NSmut1: $2.5 \times 10^{10}$ viral particles and MVA-NSmut: $1.8 \times 10^8$ pfu  As intramuscular injections in the deltoid region of the arm.
Objectives:	<u>Primary:</u> <ul style="list-style-type: none"><li>• To assess the safety of the new candidate hepatitis C virus vaccines, AdCh3NSmut1 and MVA-NSmut, compared to placebo when administered to HCV-uninfected injection drug users (IDUs).</li><li>• To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce incidence of chronic HCV infection compared to placebo among HCV-uninfected IDUs.</li></ul> <u>Secondary:</u> <ul style="list-style-type: none"><li>• To evaluate the immunogenicity of the new candidate hepatitis C virus vaccines, AdCh3NSmut1 and MVA-NSmut, compared to placebo when administered to HCV-uninfected IDUs.</li></ul>
Estimated Date of Completion:	2016

### SCHEMATIC OF STUDY DESIGN



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## 2 Background Information and Scientific Rationale

### 2.1 Background Information

#### *Hepatitis C: the need for a vaccine*

HCV is the world's most common blood borne viral infection for which there is no vaccine. The WHO estimates that about 180 million people, some 3% of the world's population, have been infected, 130 million of whom are chronic HCV carriers.<sup>1,2</sup> They estimate that three to four million persons are newly infected each year.<sup>3</sup> HCV is a major etiological agent of liver disease including cirrhosis and hepatocellular carcinoma (HCC) in the U.S. and worldwide and the principal cause of liver transplantation.<sup>4,5</sup> Population estimates of prevalence of HCV infection range from 1-2% in Australia, Canada, US and most of Europe, to between 5% and 10% in many countries of Africa, Latin America, Central and South-Eastern Asia. In the U.S., it is estimated that over four million have been infected, and 3.2 million have chronic infection.<sup>6</sup> In the U.S. in 2008, the CDC estimated that 18,000 new HCV infections occurred,<sup>7</sup> the majority attributed to IDU.<sup>8</sup>

Acute HCV infection is typically asymptomatic and spontaneously resolves in about 20% of cases. Jaundice and fulminant hepatitis occur but are not common. In 70–80% of cases, the immune system is nevertheless unable to eliminate the virus and the disease becomes chronic. Patients chronically infected with HCV typically show some liver function test abnormalities (elevated transaminase levels) and in some cases develop mixed cryoglobulinemia. Chronic infection is associated with progressive liver fibrosis. Although the clinical course of liver disease upon HCV infection is very variable, about 20% of chronically infected individuals will eventually develop cirrhosis, and some of them hepatocellular carcinoma.<sup>4</sup> It has been estimated that only about 50% of HCV-infected persons are diagnosed in most developed countries and that two-thirds of them need to undergo antiviral treatment.<sup>9</sup> However, the proportion of patients who access and complete treatment remains low.<sup>10-13</sup>

The standard of care for patients with chronic HCV infection consists of the combination of pegylated interferon  $\alpha$  (IFN) and ribavirin (RBV).<sup>14,15</sup> Therapy achieves sustained viral clearance in no more than ~40-50% of patients infected with the most common genotype of HCV. More than 20% of the IFN treated patients develop significant adverse effects and RBV frequently causes haemolytic anemia. Current treatment regimens are considered inadequate due to limited efficacy and toxicity resulting in poor tolerability. The various HCV genotypes respond differently to the treatment. Genotype 1 infections are more resistant to IFN therapy than infections with virus of genotypes 2 and 3. Novel therapies based on antiviral drugs that specifically target HCV protease are currently approved for clinical use in combination with IFN and RBV. Recent clinical trials showed an increase in the number of genotype 1 infected individuals responding to the triple antiviral treatment (IFN/RBV and protease inhibitor).<sup>16-18</sup> These new

treatments, however, are associated with the rapid emergence of viral escape variants.<sup>19</sup> Furthermore, many patients are not eligible for therapy because of co-morbid medical or psychiatric conditions or severe liver disease.<sup>10,20</sup> For these reasons, as well as for the cost of the combined standard therapy, it is widely believed that HCV therapy will not have a significant impact on the disease in many parts of the world and will have a minimal impact in blocking the spread of infection within the human population.

A significant epidemiologic impact on the spread of HCV infection is expected to occur only subsequent to the introduction of an effective vaccine. Extensive work in the chimpanzee model suggests that immunity against HCV can be generated by initial infection<sup>21-23</sup> or vaccination, and protects chimpanzees from HCV disease after challenge with homologous or heterologous virus.<sup>22-25</sup> Moreover, prospective studies in acutely infected humans have shown that clearance of the infection is correlated with the type, strength and breadth of the immune T-cell response.<sup>26-28</sup> Recently, Osburn et al. have demonstrated that prior HCV clearance is associated with control of subsequent HCV infections in injection drug users.<sup>29</sup> Reinfection is associated with broadening of the cellular immune response and shorter peak viremia with decreased duration of viremia, suggestive of protective immunity against HCV infection. All these observations provide a rational basis for vaccine design and suggest that an HCV vaccine will be efficacious in prevention of chronic infection if capable of eliciting a T-cell response equivalent or superior to that observed in acute/resolving individuals.

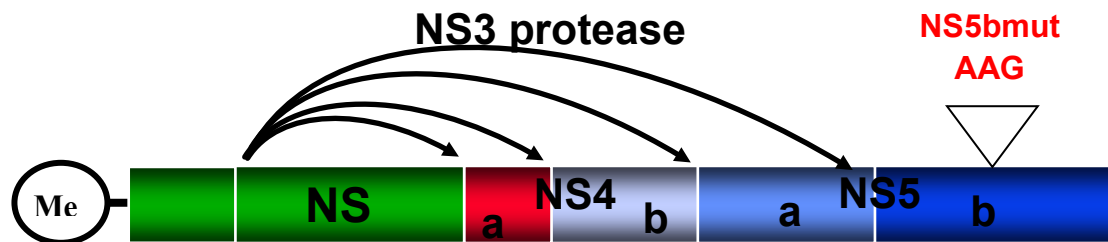
A vaccine is needed to reduce HCV disease especially for groups at high risk of infection. Injection drug users (IDUs) have the highest prevalence of any population, ranging from 25% to 80%, and an incidence rate of new infections ranging from 10% to 30% (annualized rate).<sup>30,31</sup> IDUs are also not considered good candidates for standard therapy due to numerous behavioral and clinical challenges. Few effective prevention modalities are available or implemented in this population.<sup>32</sup> The rates of infection remain highest in this group. Given the asymptomatic nature of infection, it is also likely that the number of infections and the proportion attributed to IDU, are significantly underestimated.<sup>33</sup> Moreover, as many as 30% of the estimated one million HIV-infected people in US (approximately 300,000 subjects in all) are co-infected with HCV; a similar percentage is reported for HIV patients co-infected with HCV in Europe.<sup>9</sup> The course of HCV-related disease is accelerated by HIV co-infection; the US Public Health Service has recognized HCV as an opportunistic infection of HIV. The death rate from HCV is seven times higher among HIV-infected individuals than among patients infected with HCV alone. Despite enormous research efforts, a successful HCV vaccine has not yet been developed. Importantly, a recent study evaluating the potential impact of HCV vaccination predicted that the introduction of a prophylactic HCV vaccine in an IDU population can have substantial impact on the overall incidence of chronic HCV infection.<sup>34</sup> A vaccination program, optimized through targeting strategies and high vaccination rates, is expected to

reduce HCV prevalence of chronic infection to a state where, after several years, other interventions may drive HCV to extinction.<sup>34</sup> The Co-Principal Investigators believe the IDU population represents one group that will benefit greatly if a hepatitis C vaccine is licensed; therefore, we propose to conduct a Phase I/II clinical trial in this population.

***The rationale for using HCV NS polyprotein as antigen for HCV prophylactic vaccine***

HCV belongs to the genus Hepacivirus in the family Flaviviridae. It is an enveloped virus with an icosahedral capsid that contains a 9.6 kb-long, single-stranded, positive sense genomic RNA. Its envelope contains two glycoproteins, E1 and E2, which form heterodimers at the surface of the virion. The genomic RNA is translated into a viral polyprotein which is cleaved by cellular and viral-encoded proteases to generate the capsid protein (C), the two glycoproteins E1 and E2, p7, the viral proteases NS2 and NS3, and nonstructural proteins NS4a, NS4b, NS5a and NS5b, which are required for viral RNA replication. HCV can be classified into a number of distinct genotypes and subtypes, whose distribution varies both geographically and between risk groups.<sup>35,36</sup> Genotype 1 represents the majority of known isolates (88.5% in US, 83.3% in Europe and 62.2% in Asia). In Europe, subtypes 1a and 1b are widely distributed, particularly in older age groups, with those infected through drug use also having significant prevalence of subtype 3a infections. The 1b subtype is also the most frequently found in Japan, while in the United States (US), the closely related 1a subtype is slightly more prevalent. Moreover, HCV displays very high inter- and intra-individual genetic variability, especially in the E1 and E2 envelope glycoproteins where neutralizing determinants have been found. In fact, neutralizing antibody responses have been shown to be effective against only a handful of viral variants. Consistent with this scenario, the role of antibodies in viral clearance is still uncertain. For instance, vaccination protocols in chimpanzees aimed at eliciting neutralizing antibodies have not been able to induce a sterilizing immunity, especially against heterologous viral inocula.<sup>37</sup>

The prophylactic HCV vaccines proposed for this study are based on adenoviral and Modified Vaccinia virus Ankara (MVA) vectors. The vaccines vectors encode the 1985 amino acid-long HCV nonstructural region (NS), with genetically inactivated RNA-dependent RNA polymerase activity (NS5b). The mutated NS region is called NSmut (Figure 1).



**Figure 1. The nonstructural region of the hepatitis C virus encoded by the vaccine.**

The HCV nonstructural region (NS) is commonly conserved between all six HCV genotypes and several of the major subtypes, making this region of HCV a good vaccine candidate. The NS region encompasses about two thirds of the HCV genome and encodes five different proteins. Upon transduction of mammalian cells with the vaccine, the encoded NS fragment is translated into a single polyprotein which is then proteolytically cleaved by the encoded NS3 protease into five mature products (NS3, NS4a, NS4b, NS5a and NS5b). This process recapitulates the same cascade of events occurring *in vivo* upon viral infection and therefore provides for a physiological way of presenting the viral antigens to the host immune system. An effective HCV vaccine will need to induce a broad cellular immune response due to the genetic diversity of human MHC alleles and of the virus. The NS region from a 1b genotype BK strain isolate was chosen as vaccine candidate because its amino acid sequence is well conserved across several genomes covering 14 different subtypes belonging to the 6 major types (between 74 and 79% sequence identity). The HCV database group has mapped a large number of T-cell epitopes in the NS region: more than 90 T helper and more than 70 cytotoxic T lymphocyte (CTL) epitopes.<sup>38</sup> Recent published data in chronically and acutely infected subjects suggest a predominant role for epitopes mapped in the NS region in inducing T-cell immunity.<sup>26,39,40</sup> Poly-specific T-cell response could be detected in human subjects infected by different HCV genotypes by using peptides reproducing the NS 1b/Bk vaccine sequence as antigens in the assay.<sup>26,41</sup> Because of the multiple antigens encoded in this region, it is reasonable to speculate that NS proteins will induce T-cell responses specific for several viral determinants capable of binding to a large panel of different Human Leukocyte Antigen (HLA) molecules.

Okairos researchers have performed a number of immunogenicity studies in non-human primates with adenovirus and DNA-based vectors encoding for HCV NS 1b/Bk sequence, and have shown that a high and broad T-cell response could be induced not only against a set of antigens spanning the 1b sequence,<sup>42,43</sup> but also against antigens derived from the highly divergent genotype 3a which has 23% amino acid diversity.<sup>44</sup> Importantly, genotype 3 is the second most prevalent genotype in IDUs, the specific population we intend to enroll in the Phase I/II trial. Furthermore, Okairos researchers

have demonstrated that genetic vaccination with the 1b/BK NS antigen, inducing broad and long-lasting T-cell responses, protected chimpanzees from HCV disease after challenge with a heterologous virus belonging to the 1a genotype and differing from the vaccine sequence at more than 13% of the amino acid positions.<sup>25</sup>

### ***Chimpanzee Adenovirus as vaccine vectors***

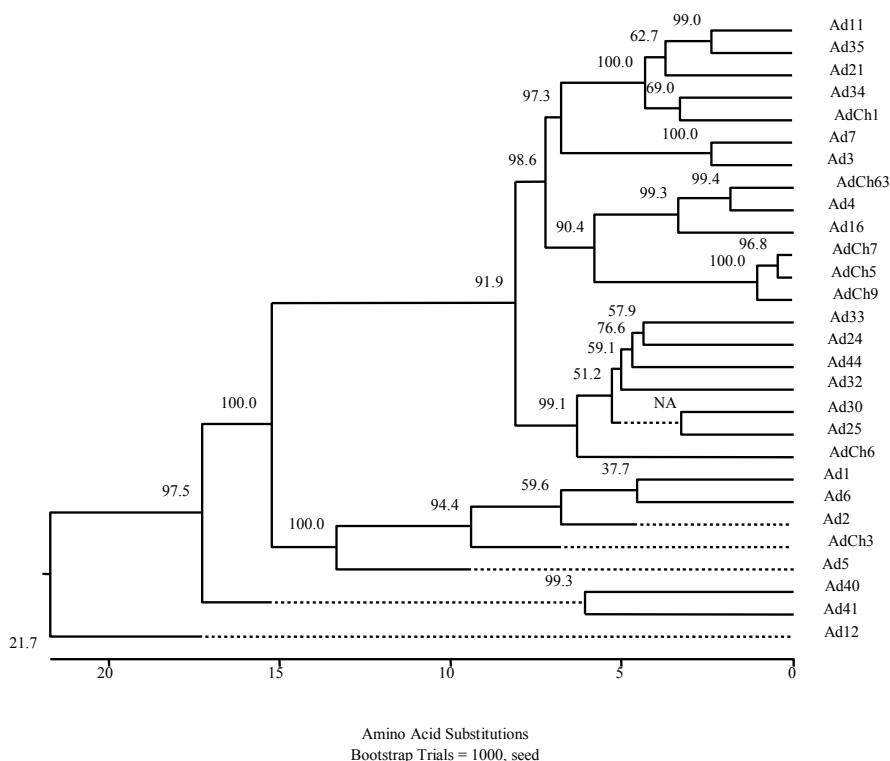
Potent immunogenicity and lack of prolonged transgene expression have made adenovirus attractive viral vectors for vaccine development. Replication defective adenovirus can be engineered by deletion of genes from the E1 locus, which is required for viral replication, and these viruses can be propagated easily with good yields in cell lines such as HEK 293 and PER.C6 (Crucell).<sup>45</sup> They possess a stable virion allowing inserts of foreign genes without deletion. They can infect many different cell types and the transferred information remains epichromosomal thus avoiding the risk of insertional mutagenesis. Preclinical and clinical results conclusively showed superiority of adenovirus-vectored genetic vaccines based on the most common human adenovirus serotype 5 (Ad5) for the induction of T-cell responses in preclinical animal models and in Phase I safety and immunogenicity studies in humans.<sup>46-49</sup> However, recent studies have shown that pre-existing immunity to Ad5 is capable of significantly blunting the immunological response induced by Ad5 vectored vaccines in rodents, in non-human primates and in Phase I clinical trials in humans.<sup>42,46</sup> Various attempts have been made to overcome the problem of pre-existing immunity to Ad5, and thus exploit the full potential of the adenovirus vectors for the development of vaccines. However, alternative adenovirus serotypes to be developed as vaccine vectors have to fulfill a number of requisites including efficient growth in cell lines suitable for GMP production and immunological potency comparable to that of Ad5.

Simian adenoviruses are not known to cause pathological illness in humans and the prevalence of antibodies to chimpanzee derived adenoviruses (AdCh) have low/no seroprevalence (0%-18%) in the human population.<sup>50</sup> In Equatorial Africa, the natural habitat for chimpanzees, seroprevalence is higher, but still significantly below that of Ad5. Okairos performed a large screening of several adenoviruses isolated from chimpanzees and identified several chimpanzee adenoviruses that meet the necessary requirements for vaccine development.<sup>51</sup> In chimpanzee adenoviruses the E1 locus can be deleted to render virus replication deficient and to allow trans-complementation in Ad5 E1 complementing cell line.<sup>51</sup> Moreover, because of lack of sequence homology between the E1 flanking regions of Ad5 and AdCh adenoviruses, no recombination and production of replication competent virus occurs if the AdCh vectors are propagated in HEK 293 cells and PER.C6® (Crucell)<sup>52</sup> eliminating the problem of replication competent adenovirus (RCA) generation during vector manufacturing. Chimpanzee derived adenoviruses exhibit sequence similarity to human adenoviruses. Hexon is the major capsid protein in adenoviruses; adenoviruses can be classified in subgroups based on homology in the hexon amino acid sequence. Phylogenetic analysis of the



hexons of simian and human adenoviruses (Figure 2) shows substantial overlap indicating that there is no clear sequence feature that distinguished a simian from a human adenovirus. In fact, these sequences suggest one large family of higher primate adenoviruses. The potency of chimpanzee derived Ad vectors were assessed in mice, macaques and, recently, in humans. The T-cell immunogenicity of some of these vectors matched or even exceeded the immunogenicity of the standard Ad5 vector used as a comparator. The safety of these vectors has been similar to that of human adenovirus vectors suggesting that these might be suitable for widespread use.

The simian virus AdCh3 has been chosen for the HCV vaccine development. AdCh3 can be classified based on the Hexon protein in subgroup C as human Ad5 and Ad6. The sequences of Ad5 and AdCh3 are clearly divergent in the hypervariable regions of hexons considered the target of neutralizing antibodies.<sup>53</sup>



**Figure 2. The phylogenetic tree of adenovirus' hexons.**

***Modified Vaccinia virus Ankara–based vaccines***

Modified Vaccinia virus Ankara (MVA), an attenuated strain of vaccinia virus that was originally developed as a smallpox vaccine, was obtained following extensive serial passage on primary chicken embryo fibroblasts (CEFs). During this process of attenuation, MVA underwent deletion of 31 kb (~15%) of its genome, as compared to its parental strain, including a number of genes that contribute to viral evasion from host immune responses and that determine virus host range.<sup>54,55</sup> As a result, MVA is unable to replicate productively in most mammalian cell types, including primary human cells. The resultant inability of MVA to undergo more than one infection cycle in a human host has imbued this virus with inherent safety that was demonstrated historically through the immunization of ~120,000 individuals during the smallpox eradication campaign. More recently, the safety of MVA has been demonstrated in pre-clinical studies of immune-deficient mice and immune-suppressed macaques,<sup>56,57</sup> and in Phase I clinical trial evaluations of MVA as a next-generation smallpox vaccine.<sup>58</sup> The desirable safety profile exhibited by MVA, in concert with its ability to express high levels (and large numbers) of foreign genes, has rendered MVA a leading candidate for evaluation as a vaccine vector against an array of infectious diseases and human cancers. MVA-based vaccines against HIV/AIDS,<sup>59</sup> malaria,<sup>60,61</sup> tuberculosis,<sup>62</sup> Human Papilloma Virus-induced cervical intraepithelial neoplasia<sup>63</sup> and melanoma<sup>64</sup> are being evaluated in human clinical trials.

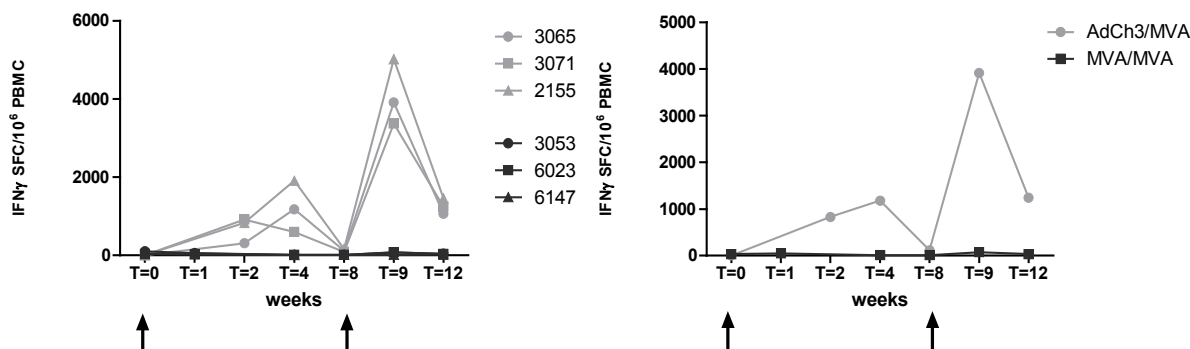
***Heterologous prime-boost vaccination strategy***

The main limitation of vaccination approaches based on viral vectors is linked to the induction of anti-vector immunity after the first immunization. In fact, repeated administration of both recombinant adenoviruses and MVA vaccine vectors typically results in an increasingly diminished efficacy of such booster immunizations due to the elicitation of vector-specific neutralizing antibody responses.<sup>47,65</sup> Several studies have shown that priming/boosting with different viral vector elicits higher immune response than repeated vaccination with an individual viral vector. The combined use of these vectors in a heterologous prime-boost regimen is the best way to overcome the antiviral immunity induced by the first vaccination. The utility of MVA-based vaccines to prime immune responses against foreign antigens appears to be limited due to unfavorable competition for immunodominance between the relatively large number of vector-specific gene products<sup>55</sup> and the much smaller number of intended vaccine antigens.<sup>66</sup> Therefore, an optimal regimen would use adenovirus first to prime and MVA later to boost the previously vaccine induced immune response. An immunization protocol based on adenovirus as prime followed by MVA has demonstrated to be a powerful strategy to induce potent and durable T-cell responses. This strategy enabled induction of protective immune response against mouse malaria<sup>67</sup> and simian immunodeficiency virus (SIV) challenge in rhesus monkeys.<sup>68</sup> Moreover, this approach is now in Phase I and IIa trials in the UK for a malaria vaccine with very promising results.<sup>69</sup>

***Monkeys' pre-clinical data in support of the choice of the vaccination strategy***

Here we summarize the assessments of the immunogenic potency of the heterologous prime-boost regimen based on AdCh3NSmut1 followed by MVA-NSmut conducted in primates. A group of 3 male macaques were primed at Week 0 with AdCh3NSmut1 ( $1 \times 10^{10}$  vp intramuscularly) and boosted at Week 8 with MVA-NSmut ( $2 \times 10^8$  pfu intramuscularly). A second group of 3 male macaques was vaccinated with 2 injections of MVA-NSmut 8 weeks apart. Analysis of HCV specific T-cell responses was performed at various time points following vaccination using an ELISpot assay. Briefly, freshly isolated peripheral blood mononuclear cells (PBMC) were stimulated with 6 pools of peptides encompassing the NS3-NS5B sequence and IFN- $\gamma$  secreting cells were measured.

T-cell responses to the vaccine insert were induced in all 3 animals of the first group after adenovirus vaccination, peaking at Week 4 post prime (Figure 3, below). The sum of responses to the 6 NS pools at peak post-prime, ranged from 598 to 1908 spot forming cells (SFC)/ $10^6$  PBMC. The immune response was contracted at the time of boosting and was readily and efficiently expanded by MVA-NSmut injection, with a fold increase  $>30$  compared to pre-boost residual responses. Moreover, the peak values obtained post-boost were 3-fold higher than those at peak post-prime (sum range: 3372-5021 SFC/ $10^6$  PBMC). Four weeks after MVA-NSmut boost (T=12 weeks), the effector T-cell response is again contracted, as expected. For the MVA-primed/MVA-boosted control group of animals data collected up to four weeks after boost show that no or very weak (58 and 61 SFC/million PBMC against a single peptide pool after boost in two animals) T-cell responses were mounted. We expected, based on previous data performed with MVA vectors carrying other antigens, that the homologous prime-boost strategy would induce a much weaker immune response compared to the heterologous strategy. Results in this study confirm previous findings and highlights the crucial role of AdCh3NSmut1 as priming agent in order to achieve a strong and broad T-cell response to the vaccine.



**Figure 3. Kinetics of IFN- $\gamma$  ELISpot responses over time.**

Data are expressed as IFN- $\gamma$  Spot Forming Cells (SFC)/ $10^6$  PBMC. Individual data points (left panel), represent total NS response in each animal, obtained by summing reactivity to each of the 6 NS pools and subtracting 6 times the (Dimethyl sulfoxide) DMSO background. Right panel shows each group's median. Arrows indicate vaccinations.

## 2.2 Previous Human Experience

### 2.2.1 Chimpanzee Adenovirus Vaccines

The initial clinical experience with Adenoviral vectors as vaccine was based on the use Human Ad5 derived vectors.<sup>50</sup> Ad5 based vectors are safe but unfortunately, pre-existing immunity to Ad5 has been shown to blunt the induction of an immunological response.<sup>42,46</sup> Therefore, novel Adenoviral vectors have been identified and tested for clinical use.

### 2.2.2 MVA Derived Vaccine Vectors

MVA is widely considered as the vaccinia virus strain of choice for clinical investigation because of its high safety profile. The MVA virus can be administered in intracutaneous, subcutaneous, or intramuscular injections. Over 120,000 humans have been inoculated and successfully vaccinated against smallpox with MVA during the campaign for the eradication of smallpox in Germany in the 1970s, including elderly and immunocompromised persons. More recently, the use of MVA in recombinant vaccines is being tested in a number of clinical studies, including those for vaccines against HIV.<sup>59,70-72</sup> An HCV vaccine program is in progress in France (Transgene vaccine TG4040), using MVAHCV vaccine that is similar to the MVA-NSmut vaccine proposed here.<sup>73-75</sup> The safety data obtained from previous trials using other MVA based vaccines have been excellent and no serious adverse events or suspected unexpected serious adverse reactions (SUSARs) have been reported.

### 2.2.3 HCV Vaccine Using NSmut Antigen by Okairos

The HCV NSmut antigen, vectored by either adenoviral vectors or by MVA, is currently being used in three trials (HCV001 HCV002TV and HCV003) (Table 1).

**Table 1. Okairos' on-going clinical trials with NSmut Antigen HCV Vaccine**

Study code(s)	Current status	Phase	Administered products	Subjects Population and Sample Size
HCV001	Completed	I	AdCh3NSmut Ad6NSmut	Healthy subjects (41)
HCV002TV	In progress (Estimated end date: 3rd Qtr, 2012)	I	AdCh3NSmut Ad6NSmut	HCV-infected subjects (36)
HCV003	In progress (Estimated end date: 4th Qtr, 2011)	I	AdCh3NSmut MVA-NSmut	Healthy subjects (9) and HCV subjects (14)

HCV001 is a Phase I study with the candidate HCV vaccines AdCh3NSmut and Ad6NSmut to address safety and immunogenicity of heterologous prime/boost regimen with two different adenovirus vectors in healthy subjects. The same vectors are also under investigation in HCV-infected subjects (HCV002TV). The HCV003 study mimics the immunization schedule proposed in this trial using the AdCh3NSmut to prime, and the MVA-NSmut to boost the immune response.

The AdCh3NSmut vector used in HCV001 is essentially identical to AdCh3NSmut1 proposed here for the Phase I/II clinical trial in IDU. Minimal changes have been introduced in the manufacturing process to improve production. These changes have not been shown to affect the safety profile and immunogenicity as determined by the pre-clinical toxicology assessment as described in more detail in the AdCh3NSmut1 Investigator's Brochure (AdCh3NSmut1 and MVA-NSmut repeated dose toxicity study in rat RTC Study No.: 82930). Immunogenicity data in monkeys for the AdCh3NSmut1 product are shown in Section 2.1 (Monkeys pre-clinical data in support of the choice of the vaccination strategy).

#### AdCh3NSmut safety and immunogenicity data in healthy subjects

Thirty-eight volunteers received AdCh3NSmut at least once during the HCV001 trial. Priming vaccinations of AdCh3NSmut were administered to 22 volunteers at doses of  $5 \times 10^8$  vp (4 volunteers, 8 injections),  $5 \times 10^9$  vp (4 volunteers, 8 injections),  $2.5 \times 10^{10}$  vp (10 volunteers, 15 injections) and  $7.5 \times 10^{10}$  vp (4 volunteers).

A further 16 individuals received a single boosting vaccination of AdCh3NSmut at  $2.5 \times 10^{10}$  vp following priming vaccinations with Ad6NSmut at varying doses. The adverse

events described below have been deemed definitely, probably or possibly related to vaccination. The complete final safety report of the study is shown in the appendix 2 of the Investigator's Brochure (IB). The following safety data pertain to the priming vaccinations with AdCh3NSmut in the HCV001 study.

The observed frequency of local reactions to AdCh3NSmut was 66% overall and appeared to increase with dose; occurring in 25% of volunteers after  $5 \times 10^8$ vp, 75% after  $5 \times 10^9$ vp, 80% after  $2.5 \times 10^{10}$ vp, and 75% after  $7.5 \times 10^{10}$ vp. Local pain (51%), erythema (26%), warmth (14%) and swelling (11%) were the most commonly reported reactions. The frequency of local pain and erythema increased from 25% and 13% in low dose to 75% and 50% in highest dose recipients. Ninety-three percent of the reported local reactions were mild in severity. All were self-limiting, with 86% resolved within 72 hours and 98% within 1 week.

Systemic adverse events were observed in approximately 74% of recipients of AdCh3NSmut, with an observable increase in frequency at highest dose. Fatigue (43%), headache (29%), and malaise (23%) were the most commonly reported systemic adverse events overall; however, in those receiving the highest dose (n=4) the frequencies were myalgia (100%), headache (75%), fever (50%), malaise (50%), and fatigue (50%).

Ninety-six percent of observed systemic adverse events were mild in severity, with the remaining 4% moderate. Eighty-nine percent of the observed systemic adverse events had resolved by 72 hours post-vaccination and 93% by 1 week. No distinct difference in local or systemic reactogenicity following first and second vaccinations with AdCh3NSmut was observed. Immunogenicity was assessed by ex-vivo IFN- $\gamma$  ELISpot using 6 peptide pools spanning the HCV immunogen. Following priming vaccinations with AdCh3NSmut the average peak HCV specific ELISpot responses were 142.5 ( $5 \times 10^8$ vp), 897.8 ( $5 \times 10^9$ vp) and 1863 ( $2.5 \times 10^{10}$ vp) spot forming units/ $10^6$  PBMC. No further increase of induced immune response was observed at the  $7.5 \times 10^{10}$  vp dose. All vaccinees responded to the  $2.5 \times 10^{10}$ vp vaccine dose. HCV specific responses were generally multi-specific; at the highest vaccine doses all individuals responded to at least 2 peptide pools and 7/10 individuals responded to 4 or more peptide pools (data not shown).

Moreover, in the HCV003 study, an additional 5 volunteers received one priming injection with AdCh3NSmut at  $2.5 \times 10^{10}$  doses and showed a safety profile comparable to that observed in the previous HCV001 trial.

### MVA-NSmut safety and immunogenicity data in healthy subjects

In the HCV003 study, 9 volunteers in total received MVA-NSmut intramuscularly at the dosage of  $2 \times 10^8$  plaque forming units (pfu). Four subjects received only a single injection of MVA (group A1), while 5 subjects received the injection of MVA 8 weeks after the priming injection with AdCh3NSmut at  $2.5 \times 10^{10}$  (group A2), ipsilateral to the priming injection. No vaccine related serious adverse events occurred. The overall observed percentage of volunteers with one or more local reactions after MVA injection was 100%. Local pain (100%), erythema (67%), warmth (67%) and swelling/induration (56%) were the most commonly reported reactions. 74% of the reported local reactions were mild in severity and 4% were classified as severe. Local reactions persisted for no longer than 7 days. Systemic AEs deemed definitely, probably or possibly related to vaccination were observed in approximately 90% of recipients. Most commonly reported systemic AEs were: malaise (67%), fatigue (56%), myalgia (56%), feverish (56%), nausea (56%) headache (44%), and arthralgia (44%). 66% of observed systemic AEs were classified as mild and 17% as severe. The majority of systemic AEs resolved within 48 hours. Immunogenicity data have been collected up to 22 weeks post MVA vaccination for group A1 and up to 10 weeks post MVA boost for group A2. A single MVA-NSmut administration was unable to prime T-cell responses to NS antigens in any of the 4 volunteers in group A1 during the 22 weeks of follow up. Conversely, and corresponding to previous results, priming with ChAd3NSmut promptly induced T-cell responses 2 weeks after first vaccination in all 5 volunteers. Boosting with MVA-NSmut resulted in great expansion of T-cell responses in all 5 volunteers ranging from 1,503 to 6,147 sum NS SFC/million PBMC.

#### **2.2.4 Prime/boost with Chimpanzee Derived Adenovirus and MVA Vectored Vaccines**

A series of clinical trials have recently been conducted or are in progress at the University of Oxford against malaria using a prime/boost regimen with a chimpanzee adenoviral vector followed by MVA using antigens other than NSmut. For the VAC033 and MAL 034 trials, the candidate vaccines multiple epitope thrombospondin-related adhesion protein, AdCh63 ME-TRAP and MVA ME-TRAP were given to healthy subjects.<sup>76</sup> These vectors encode for ME-TRAP, a surface protein from the sporozoite stage of *P. falciparum* malaria with a selection of T-cell epitopes. 47 subjects have received AdCh63 ME-TRAP at doses from  $1 \times 10^8$  vp to  $2 \times 10^{11}$  vp, followed by MVA ME-TRAP boosting  $2 \times 10^8$  plaque forming units (pfu) administered by intradermal and intramuscular routes. The simian adenovirus/MVA-vectored heterologous prime-boost regimen generates unprecedented cellular and humoral immune responses to TRAP and has recently demonstrated sterile protection against heterologous sporozoite challenge in 2/8 vaccinated subjects.<sup>76</sup> There have been no SAEs following AdCh63 ME-TRAP or MVA ME-TRAP administration to date. These studies also showed that AdCh63 ME-TRAP administered intramuscularly is better tolerated and more

immunogenic than the same dose administered by the intradermal route<sup>76</sup>. Moreover, a significant reduction in the frequency of local injection site reactions and systemic reactions was demonstrated when MVA ME-TRAP is administered intramuscularly compared to intradermally.<sup>76</sup>

### 2.3 Scientific Rationale (including dose selection)

**Route of injection:** The choice of the intramuscular route for AdCh3 is based on the assumption that no co-infection of natural human Adenovirus could occur at this site. Furthermore, there is a large body of data from clinical trials in humans using replication defective Ad5- and Ad6-based HIV vaccines injected intramuscularly showing an excellent safety profile, no viral shedding, and high levels of immunogenicity. MVA-NSmut will also be given by the intramuscular route because it is better tolerated than when administered by subcutaneous route (see Section 2.2.4: Prime/boost with chimpanzee derived Adenovirus and MVA vectored vaccines).

**Vaccine Doses:** In this study AdCh3NSmut1 vaccine will be administered at  $2.5 \times 10^{10}$  vp, and MVA-NSmut at  $1.8 \times 10^8$  pfu.

**AdCh3NSmut1:** Clinical data are not available for the AdCh3NSmut1 vector. AdCh3NSmut1 has minimal changes from AdCh3NSmut vector used in the 3 clinical studies shown in Table 1. Pre-clinical assessment has shown that these changes do not affect the safety or immunogenicity profile. Safety data from the study HCV001 and the ongoing HCV003 have shown that AdCh3NSmut is safe and well tolerated in healthy subjects at the dosage ( $2.5 \times 10^{10}$  vp) selected for this study.

Phase I studies assessing safety and immunogenicity in healthy individuals of Ad5- and Ad6-based HIV vaccine candidates were shown to induce higher rates of responses and more potent responses when they were administered at  $10^{10}$  vp/dose versus  $10^9$  vp/dose.<sup>77</sup> Similarly, there was a greater frequency of detectable cellular responses after 6 months from the first administration of the vector in the higher dose groups (between  $10^{10}$  and  $10^{11}$  vp/dose).<sup>77</sup> These studies have shown that adenoviral vectors are safe and well tolerated up to  $10^{11}$  vp/dose with some more reactogenicity associated with the highest dose.<sup>78-80</sup>

**MVA-NSmut:** In this study, MVA-NSmut will be administered at  $1.8 \times 10^8$  pfu. This dose has been selected based on the use of MVA vectors in primate and human studies. In Oxford, MVA malaria vaccines have been safely administered in multiple Phase I and Phase II studies at  $2 \times 10^8$ . MVA ME-TRAP and MVA-vectored P. falciparum CS protein has been well tolerated with good immunogenicity at  $5 \times 10^8$  pfu and no severe adverse events. In study VAC037, MVA MSP1 was associated with more side effects when administered at  $5 \times 10^8$  pfu compared to studies that have used



MVA vectors at a dose of  $2 \times 10^8$  pfu. In the HCV003 study the MVA-NSmut administered at  $2 \times 10^8$  has shown a good safety profile and the induction of very potent immune responses. A slightly lower dosage ( $1.8 \times 10^8$  pfu) has been chosen for study, aiming to inject a volume of 0.2mL.

**Number of injections and interval between injections:** The vaccine schedule will consist of a single priming injection with AdCh3NSmut1, followed by a single boost with MVA-NSmut 56 days later in a heterologous prime/boost regimen. This is based on promising data from ongoing malaria clinical trials in Oxford, where optimal immunogenicity is achieved with an 8 week interval between vectors and from pre-clinical data shown in the previous section. Subjects randomized to placebo will receive 2 doses of placebo at Day 0 and Day 56 (approximately week 8). As placebo, a volume of the normal saline equal to the volume of the investigational vaccines will be used.

## 2.4 Potential Risks and Benefits

### 2.4.1 Potential Risks

#### *Potential risks*

The general risks to subjects in this study are associated with phlebotomy and with vaccination. The volume of blood drawn over the study period, should not compromise health status. Potential risks include local and systemic reactions as vaccine-related side effects, which are described below. Safety data from the ongoing studies described above predicts for mild to moderate reactogenicity of vaccination.

#### *Local reactions*

Mild tenderness, bruising, light-headedness or, rarely, syncope, may result from venipuncture. Vaccination usually precipitates a local inflammatory reaction. This may include redness, swelling, tenderness, or itching.

#### *Systemic Reactions*

The potential systemic reactions to immunization with vaccines include a flu-like illness with low-grade fever, chills and malaise. As with any vaccine, serious allergic reactions may occur. Monitoring will be ongoing for all of these potential rare events.

#### *Subsequent positivity for hepatitis C antibody (anti-HCV)*

It is expected that after vaccination subjects may become anti-HCV positive. In the previous clinical trial, HCV001, anti-HCV positivity in the absence of documented infection occurred in 10% of vaccinated subjects. Anti-HCV seroconversion may occur as a result of exposure to antigens derived from the hepatitis C virus that are included in the vaccine. In order to reassure subjects and maintain coherent health records, subjects will have blood taken for HCV RNA testing [using polymerase chain reaction

(PCR) methods] prior to the trial to exclude the presence of HCV. No anti-HCV testing will occur systematically during the trial. Only HCV RNA results will be used for determination of HCV infection status during study visits. Anti-HCV testing will be conducted at the end of the trial to examine whether subjects seroconverted (became anti-HCV antibody positive) during the trial. Among subjects who did not have detectable HCV infection, testing will be done to best ascertain whether subjects seroconverted: (1) in association with undetected (HCV RNA) infection, or (2) as a result of vaccination. This testing will be done at the end of the trial, so as not to unblind subjects or investigators during the trial. If the determination is made that subjects became anti-HCV positive as a result of vaccination rather than infection, they will be provided with documentation explaining the reasons for their anti-HCV positivity. These cards will have contact information for study investigators so that providers can communicate as necessary regarding subject's anti-HCV status (with the subject's consent). This will be discussed in detail with subjects at screening and in informed consent forms (ICFs).

### *Confidentiality*

The risk of study participation includes possible loss of confidentiality. Risks to privacy and confidentiality exist with all human research. Subjects will be assigned a unique study identification number which will be used on data collection forms, case report forms (CRFs), and specimens collected for laboratory analyses. No samples or data will be labeled with the subject's name. The key to subjects' names will be accessible only to the study co-principal investigators, and hard-copies and electronic copies of data will be stored in secured locations.

### **2.4.2 Unknown Risks**

The experimental vaccine may have side effects that are not yet known or identified.

For example, a series of experimental HIV vaccine studies (STEP Study - HVTN 502; Phambili Trial - HVTN 503 and HVTN 505) which utilized a human adenovirus serotype 5 (Ad5) vector did not work to prevent HIV infections. The STEP Study data showed an increased risk of HIV infection associated with the active vaccine (adjusted HR, 1.40; 95% CI 1.03-1.92).<sup>81</sup> The HVTN 505 Study was stopped for meeting futility criteria and while not statistically different, there were more infections in the active vaccine arm compared to the placebo arm. The researchers who conducted these HIV vaccine studies have not yet determined the explanation for these findings.

In this prime/boost strategy HCV vaccine study a simian adenovirus serotype 3 (Ad3) vector administered at baseline and a Modified Vaccinia virus Ankara (MVA) vector given at week 8 are utilized. There are significant differences between the HIV studies and this study in the risk of HIV infection that make an increased risk of HIV acquisition

in this trial unlikely. Those differences include exclusion from enrollment in this trial of subjects who are at high risk for HIV acquisition such as men having sex with men and an HIV prevalence of <0.5% among the cohorts from which subjects are drawn. Regarding the potential to increase the risk of HCV infection, for which the subjects in this trial are at risk, there are key differences in HIV and HCV biology that make an increase the risk of HCV infection less likely. Hepatitis C virus infects hepatocytes and not activated T cells. The leading explanation for the increased risk of HIV infection following vaccination is that the vaccine activates T cells, the target of HIV infection. Activation of T cells is not likely to affect HCV transmission given that they are not targets of HCV infection. However, there may be other unknown risks of adenoviral vaccination.

The researchers will inform subjects of any as-yet-unknown risks if they occur. Subjects have the option of not participating in future study activities at any time.

### **2.4.3 Potential Benefits**

There is no previous clinical evidence showing the effectiveness of the AdCh3NSmut1 and MVA-NSmut vaccine for prevention of HCV infection. Subjects in both arms will receive no direct benefit from participating in the trial other than receiving information about their general health status and counseling, education and tools to prevent HCV infection.

Society may benefit from the potential development of a new prophylactic hepatitis C vaccine.

## **3 Study Objectives**

### **3.1 Primary Objectives: Safety and Efficacy**

To assess the safety of new candidate hepatitis C virus vaccines, AdCh3NSmut1 and MVA-NSmut, compared to placebo when administered sequentially to IDUs. To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce incidence of chronic HCV infection compared to placebo among HCV-uninfected IDUs.

#### **3.1.1 Primary Safety Endpoints**

The primary endpoints in the evaluation of safety are as follows: (1) occurrence of vaccine-related serious adverse events (SAEs) from the time of first vaccination through the entire study period; (2) occurrence of severe local and/or systemic solicited reactogenicity signs and symptoms in the 8 days (Day 0-7) after each vaccination; and (3) occurrence of clinical safety laboratory adverse events (AEs) assessed at baseline and 1 month following each vaccination. The occurrence of these safety parameters will be assessed by treatment arm (vaccine and placebo).

Signs and symptoms will be categorized as either local (pain, tenderness, erythema, induration and warmth) or systemic (fever, malaise/fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia and abdominal pain). Clinical laboratory measures will include measures of white blood cell (WBC), hemoglobin (HgB), platelets, ALT, and creatinine.

The protocol includes safety evaluations at multiple time points in order to better characterize the impact of the vaccine on the at-risk population. Although the principal goal is safety signal detection in association with the experimental vaccine, these safety data will be an important resource for future clinical trials in this population.

#### **3.1.2 Primary Efficacy Endpoint**

The primary endpoint for evaluation of efficacy is chronic HCV infection at 6 months defined by persistent viremia over a period of 6 months after initial detection of primary infection. Persistent viremia will be determined by: (1) the presence of the same virus (as confirmed by HCV core-E1 phylogenetic analysis testing) in blood samples collected at the first visit where HCV RNA is detected (initial infection) provided sufficient sample is available, and a subsequent sample collected at month 6 (not less than 159 or more than 201 days following initial infection); and (2) third HCV RNA positive sample taken at a time point in between these two samples (initial and month 6). If there are two or more samples within the 159 to 201 day window for the month 6 collection, the test result from the collection date closest to 180 days after initial infection will be used.

## 3.2 Secondary Objective: Immunogenicity

### 3.2.1 Secondary Immunogenicity Endpoint

The secondary immunogenicity endpoint in the evaluation of immunogenicity is the frequency of positive cell mediated immune response within 14 days after the last vaccination by treatment group (vaccine vs. placebo) measured by interferon gamma (IFN- $\gamma$ ) production by T-cells in response to at least one out of the six HCV genotype 1b (Bk strain) peptide pools representing NS region present in the vaccines, as determined by ELISpot assays.

## 3.3 Exploratory endpoints in the evaluation of efficacy

### *Exploratory objective 1:*

To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce incidence of primary HCV infection compared to placebo among HCV-uninfected IDU.

### *Exploratory endpoint 1:*

An incident HCV infection will be defined as a new confirmed positive HCV RNA test after a previous negative HCV RNA test. The interval during which incident HCV infection occurs will be defined by the date of the last documented negative HCV RNA results, and the date of the first positive HCV RNA result.

In the event that seroconversion is detected in the absence of detectable viremia (no positive HCV RNA results), incident HCV infection date will be defined by results from anti-HCV testing conducted on stored blood samples tested retrospectively.

Seroconversion is defined as a subject developing a positive anti-HCV antibody test after a previous negative anti-HCV antibody test. Detection of infection-induced antibodies to HCV core antigens not present in the vaccine construct will be used to differentiate infection- from vaccine-induced seroconversion. The seroconversion date will be defined by the interval between the date of the last negative anti-HCV and the date of the first positive anti-HCV test result.

### *Exploratory objective 2:*

To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce 9-month incidence of chronic HCV infection compared to placebo among HCV-uninfected IDU.

### *Exploratory endpoint 2:*

The exploratory endpoint for evaluation of efficacy is chronic HCV infection at 9 months defined by persistent viremia over a period of 9 months after initial detection of primary infection. Persistent viremia will be determined by: (1) the presence of the same virus (as confirmed by HCV core-E1 phylogenetic analysis testing) in blood samples collected at the first visit where HCV RNA is detected (initial infection) provided sufficient sample

is available, and a subsequent sample collected at month 9 (not less than 249 or more than 291 days following initial infection); and (2) third HCV RNA positive sample taken at a time point in between these two samples (initial and month 9). If there are two or more samples within the 249 to 291 day window for the month 9 collection, the test result from the collection date closest to 270 days after initial infection will be used. Cases classified as not-chronically infected at 9 months will be considered resolved (cleared of infection).

*Exploratory objective 3:*

To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce peak concentration (magnitude) of HCV RNA compared to placebo, in blood samples of persons with incident HCV infection.

*Exploratory endpoint 3:*

Quantitative viremia measures, including the initial peak and total HCV RNA levels detected, from the date of first HCV RNA detection and at study visits in the first 6 months of viremia will be compared, between vaccinated and placebo arms, using logarithmic IU/mL values.

*Exploratory objective 4:*

To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce duration of HCV viremia (shorter time to spontaneous resolution) during acute infection (6-month period following incident HCV infection) compared to placebo in blood samples of persons with incident HCV infection.

*Exploratory endpoint 4:*

The duration of acute infection will be defined as the interval between incident HCV infection and viral clearance. For subjects who do not clear HCV viremia, the duration will be measured as six months following incident infection. The interval during which incident HCV infection occurs will be defined by the date of the last documented anti-HCV and HCV RNA negative results, and the date of the first positive HCV RNA result. The interval during which viral clearance occurs will be defined by the date of the last test with detectable HCV RNA and the first of two consecutive samples with undetectable HCV RNA.

*Exploratory objective 5:*

To determine if AdCh3NSmut1 and MVA-NSmut HCV vaccines will reduce incidence of chronic HCV infection with genotype 1 compared to non-genotype 1 among HCV-uninfected IDU.

*Exploratory endpoint 5:*

A chronic HCV infection genotype 1 will be defined as a chronic HCV infection at 6 months, as defined in Section 3.1.2, with the additional requirement that the infection is genotype 1 as determined from blood samples obtained at the first HCV viremic visit, provided sufficient sample is available.

## 4 Study Design

### **Overall trial design**

This is a Phase I/II double-blind (subject, investigator, and clinical personnel monitoring safety and laboratory assay results, and laboratory personnel), randomized, and placebo-controlled study in approximately 540 individuals at high risk for HCV infection due to active injection drug use (IDU) between the ages of 18 and 45. HCV-uninfected (negative for both anti-HCV and HCV RNA at screening) male and female adults who are current IDUs will be randomized at a 1:1 ratio, stratified by gender and IL28B status, to receive AdCh3NSmut1 and MVA-NSmut HCV vaccines or placebo at Day 0 and Day 56. The study is designed to be implemented in two stages and enroll a total of 270 subjects/arm.

**Stage 1:** Blinded, randomized, controlled assessment of AdCh3NSmut1 and MVA-NSmut HCV vaccine compared to placebo in  $68 \pm 4$  ( $34 \pm 2$ /arm) evaluable subjects. This study stage will evaluate safety of the combined use of both vectors in a prime/boost regimen in the target population. Each individual will be followed with extensive safety and immunogenicity measures. Interim analysis (safety and immune responses) is planned immediately following the end of stage 1 (at Day 63 follow-up ( $34 \pm 2$ /arm) for all evaluable subjects).

**Stage 2:** After the safety and immunogenicity interim analyses, the study will be extended into a 2<sup>nd</sup> stage, wherein an additional ( $n=472 \pm 4$ ) HCV-uninfected subjects will be enrolled. A 2<sup>nd</sup> interim analysis to evaluate progress (statistical and enrollment) toward the efficacy endpoint will occur when roughly one-third of enrolled and currently per-protocol subjects ( $N = 98$ ) have completed six months of post-vaccination follow-up, but no later than one month prior to the planned completion of enrollment to the trial. Immune responses will be evaluated following each vaccination and HCV viremia status will be monitored monthly for 18 months following the 2<sup>nd</sup> vaccination. This stage is designed as a blinded, randomized placebo-controlled study, with safety and efficacy assessments.

### **Study duration**

The planned duration of the study is approximately 63 months total including accrual time for subjects (assuming 31 months of screening/enrollments, plus 3 months of halted enrollment for the first interim analysis), 2 months vaccination, 18 months follow-up of each enrolled subject, and 9 months extended observation (monthly), from the time of infection, for subjects becoming viremic in the last month of follow-up.

The follow-up period for detection of HCV infection will start after the second injection of vaccine and will last 18 months. Subjects who become viremic will be followed for 9 months after detection of HCV (up to month 29). The trial will be considered closed



when the observation period for the last subject that became viremic during the 18 months follow-up is completed.

## 5 Study Population

All subjects in this study will be adults between the ages of 18 and 45, who are at high risk for HCV infection due to active injection drug use (IDU).

### 5.1 Inclusion Criteria

Inclusion criteria that must met prior to the first vaccination:

1. Comprehension of informed consent.
2. 18-45 year old men or women with acknowledged active IDU in the past 90 days and have no travel plans that would interfere with ability to meet the study visit schedule.
3. In good general health as determined by a participating study physician and results within acceptable ranges for clinical laboratory evaluations as detailed in Appendix A.
4. Negative for antibodies to hepatitis C virus (anti-HCV).
5. Negative for HCV RNA.
6. Negative antibodies to HIV.
7. Negative for HBsAg.
8. Able and willing (in the Investigator's opinion) to comply with all study requirements.
9. Willing to allow the investigators access to their medical records.
10. Willingness to practice continuous effective contraception from the screening visit through 90 days after the last vaccination (males and females).
11. Among females, a negative pregnancy test within 24 hours prior to vaccination.
12. Agreement to refrain from blood donation during the course of the study or after the study.
13. Provide written informed consent prior to initiation of any study procedures.
14. Willing to provide contact information for study follow-up activities, including the address, name and contact information of three people who can be contacted to facilitate follow-up compliance.

## 5.2 Exclusion Criteria

Exclusion criteria that apply prior to the first vaccination:

1. The subject is currently participating in a study that involves an experimental agent (vaccine, drug, biologic device, blood product, or medication) or has received an experimental agent within 30 days prior to enrollment in this study, or expects to receive another experimental agent during participation in this study.
2. Prior receipt of a recombinant simian or human adenoviral vaccine or MVA vaccine.
3. Administration of immunoglobulins and/or any blood products within the 90 days preceding the planned administration of the vaccine candidate.
4. Any confirmed or suspected immunosuppressive or immunodeficient state, including: HIV infection; asplenia; recurrent, severe infections.
5. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine (i.e., known hypersensitivity to aminoglycoside antibiotics or to egg proteins).
6. History of clinically significant contact dermatitis or other significant dermatological conditions such as psoriasis.
7. Any history of anaphylaxis in reaction to vaccination.
8. Pregnancy, lactation or willingness/intention to become pregnant during the study.
9. History of cancer (except for successfully treated basal cell carcinoma of the skin and cervical carcinoma in situ).
10. History of severe psychiatric illness, including severe depression, history of suicidal ideation, suicidal attempts, or psychosis requiring medication. The subject has a diagnosis of schizophrenia, bi-polar disease, or other severe (disabling) chronic psychiatric diagnosis that is uncontrolled and would interfere with the ability to adhere to the protocol.
11. Any other serious chronic illness requiring hospital specialist supervision.
12. Suspected or known current alcohol abuse as defined by a score of 10 or more on the Alcohol Use Disorders Identification Test (AUDIT) C test (a standardized screening tool used to identify hazardous drinkers or those with active alcohol use disorders, including abuse or dependence).<sup>82</sup>
13. At high risk of HIV infection by the following criteria (adapted from HIV Network for Prevention Trials (HIVNET) behavioral criteria for high risk of HIV): (1) sexually active male who has sex with men (MSM), defined as (i) male who has had anal sex with male sexual partner or partners in the past year or (ii) a male

- who exchanged sex with male partner(s) for money or drugs in the past year; and (2) female and in a current relationship with a high risk male (active MSM, HIV positive male).<sup>83</sup>
14. Any other significant disease, disorder or finding, which, in the opinion of the Investigator, may put the subject at risk because of participation in the study, may influence the result of the study, or may influence the subject's ability to participate in the study.
  15. History of or current diagnosis of Diabetes mellitus.
  16. History of or current diagnosis of autoimmune disease.
  17. History of or current cardiac disease including history of myocardial infarction or arrhythmia.
  18. Current diagnosis of active liver disease.
  19. History of seizure disorder or currently taking anti-convulsant therapy that would interfere with safety evaluation.
  20. Uncontrolled hypertension (defined as systolic blood pressure being greater than 140mm Hg or diastolic blood pressure being greater than 90mm Hg).
  21. History of splenectomy.
  22. Long term immunosuppressive use (defined as taken for 14 days or more in total at any time during the past 180 days) of high dose oral or parenteral glucocorticoids (high dose defined as prednisone  $\geq$  20 mg total daily dose, or equivalent dose of other glucocorticoids); or high-dose inhaled steroids (high dose defined as  $>800$  mcg/day of beclomethasone dipropionate or equivalent); or any use of hepatotoxic or non-FDA approved medication.
  23. Have an acute illness, including an oral temperature greater than or equal to 100.4°F, within 7 days prior to the first vaccination.
  24. Immunization against another pathogen within 14 days of planned injection.

***Second vaccination exclusion criteria:***

The following events associated with vaccine immunization constitute absolute contraindications to further administration of vaccine. The subject will not receive additional vaccination, but will continue with scheduled follow-up procedures except vaccination.

1. Anaphylactic reaction following administration of vaccine.
2. Pregnancy. If a woman reports having a positive home urine pregnancy test or a positive in clinic urine pregnancy test prior to a scheduled second vaccination, she is not eligible to receive the study vaccine. If she later returns to clinic and reports

having a negative home urine pregnancy test and this is confirmed by a negative in clinic urine pregnancy test and a negative blood pregnancy test she may be eligible for the second vaccination if she is still within the vaccination window and no other exclusion criteria are met.

The following adverse events constitute contraindications to administration of vaccine at that point in time. If any one of these adverse events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date (within 28 days of scheduled administration) or withdrawn at the discretion of the investigator. The subject will not receive additional vaccination, but will continue with all scheduled follow-up procedures except vaccination.

3. Acute disease at the time of vaccination (acute disease is defined as the presence of a moderate or severe illness with or without fever). The vaccine dose can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection with or without low-grade febrile illness, i.e., temperature of  $<38^{\circ}\text{C}$  ( $100.4^{\circ}\text{F}$ ).
4. Temperature of  $>38^{\circ}\text{C}$  ( $100.4^{\circ}\text{F}$ ) at the time of vaccination.
5. Immunization against another pathogen within 14 days of vaccination.

### **5.3 Randomization and Code Breaking Procedures**

The list of randomized treatment assignments stratified by gender and IL28B status will be prepared by statisticians at The Emmes Corporation and included in the enrollment module of The Emmes Corporation's Internet Data Entry System (IDES). IDES will assign each subject a treatment code from the list after demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

#### ***Unblinding***

Unblinding is unlikely to be necessary for the provision of medical treatment or to otherwise protect the safety of study subjects. In the event that an investigator is concerned that a subject might be put at undue risk by continuing product use, the investigator may discontinue product use by this subject; however, knowledge of the specific product (vaccine or placebo) to which the subject was assigned should not be necessary to guide further follow-up and/or treatment.

The co-principal investigators and/or Independent Safety Monitor (ISM) should be available to review and evaluate any notable reactions. In the case of a medical emergency, the co-principal investigators or ISM may deem it medically necessary to unblind the subject's treatment assignment. If the co-principal investigators or ISM believe that unblinding would benefit the medical care of the subject and time permits, DMID will be consulted prior to unblinding, and concurrence will be obtained.

Otherwise, subjects will be unblinded to their drug only at the close of the trial. Subjects who develop HCV infections or drop out will not be unblinded early. The Data Safety and Monitoring Board (DSMB) may receive data in aggregate and presented by treatment group, but without the treatment group identified. The DSMB may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues.

## **5.4 Withdrawal**

### **5.4.1 Reasons for Withdrawal**

Subjects may withdraw or be withdrawn for any of the reasons given below. The reason for withdrawal will be recorded in the data collection form. The DSMB may recommend withdrawal of subjects.

In accordance with the current revision of the Declaration of Helsinki (amended October 2000, with additional footnotes added 2002 and 2004) and any other applicable regulations, a subject has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the subject at any time in the interests of the subject's health and well-being. In addition the subject may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospective, having been overlooked at screening).
- Significant protocol deviation.
- Subject non-compliance with treatment regime or study requirements.
- An adverse event which requires discontinuation of the vaccination regimen or results in inability to continue to comply with study procedures (see below).

If a subject is withdrawn from the study, all study procedures (except for another vaccination) including safety follow-up will be continued, if possible. If voluntary withdrawal occurs, the subject will be asked to continue scheduled study procedures (except for another vaccination) including safety evaluations, if possible, and be given

appropriate care under medical supervision if symptoms of any AE related to participation in the study are continuing. The subject will be followed until the AE is resolved or until the subject's condition becomes stable. (Note: These plans regarding follow-up only apply if the subject received at least one study dose.)

#### **5.4.2 Handling of Discontinuation of Vaccine Regimen**

Any subject whose vaccinations have been discontinued will be seen for appropriate follow-up visits or medical care will be arranged until the adverse event has resolved or stabilized.

### **5.5 Termination of the Study**

The NIAID/DMID and/or the FDA have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of AEs indicating a potential health hazard.
- Data recording is inaccurate or incomplete.
- The Investigator(s) has not been adhering to the protocol or applicable regulatory guidelines in conducting the study.

## 6 Investigational Products

### 6.1 Study Products Description

Two investigational vaccines will be used in this study, namely AdCh3NSmut1 and MVA-NSmut, along with the 0.9% sodium chloride as placebo.

#### **AdCh3NSmut1 formulation and dose**

The vaccine, AdCh3NSmut1, consists of the attenuated virus, simian adenovirus 3, (AdCh3), encoding the Nonstructural region (NS), from the Hepatitis C virus 1b genotype (BK strain isolate), with a mutation (mut) introduced in the active site of the HCV polymerase.

AdCh3NSmut1 is manufactured under Good Manufacturing Practice (GMP) conditions. The AdCh3NSmut1 vaccine is formulated in A195 buffer (10 mM Tris, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.02% PS80, 5% sucrose, 0.1 mM EDTA, 10 mM Histidine, 0.5% ethanol, pH 7.4). AdCh3NSmut1 has been vialled in 2 mL nominal Type I borosilicate glass vial with a siliconized 13 mm butyl rubber stopper at concentration of  $5 \times 10^{10}$  vp/mL at 0.5 mL extractable volume /vial. **The dose of AdCh3NSmut1 to be used in this study is  $2.5 \times 10^{10}$  vp. This dose is obtained by injecting 0.5 mL of the vaccine without dilution.**

#### **MVA-NSmut formulation and dose**

The vaccine, MVA-NSmut, consists of the attenuated replication-defective orthopoxvirus, modified vaccinia virus Ankara, (MVA), encoding the Nonstructural region (NS) from the Hepatitis C virus 1b genotype (BK strain isolate), with a mutation (mut) introduced in the active site of the HCV polymerase. The investigational medicinal product MVA-NSmut is manufactured under GMP conditions. The investigational medicinal product is supplied as liquid presented in Tris buffer (10 mM Tris, 140 mM NaCl, pH 7.7), at a concentration of  $8.9 \times 10^8$  plaque-forming units (pfu) per mL. The virus suspension is supplied as sterile 0.5mL/vial extractable volume in 2 mL injection vials, made of clear borosilicate glass, FIOLAX® -klar, WBK 1, DIN ISO 8362/1. The vials were sealed with 13 mm bromobutyl rubber stoppers, grey, FM 457/0, V 9128, SAF 1, DIN ISO 8362-2, aluminium injection closures with a 6 mm hole. **The dose of MVA-NSmut to be used in this study will be  $1.8 \times 10^8$  pfu delivered intramuscularly. The volume administered will be 0.2 mL without dilution.**



**0.9% Sodium Chloride for Injection and Dose**

The placebo agent to be utilized will be 0.9% sodium chloride injection, USP which is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. Each mL contains sodium chloride 9 mg. It is supplied as single-dose containers. The volume to be administered will be 0.5 mL and 0.2 mL without dilution delivered intramuscularly.

A pharmacy facility will be located at the study site and staffed by pharmacists who are licensed to practice in the US. The pharmacy will have adequate space to store sufficient quantities of study placebo product to assure continuous access to all study subjects.

Illustrations of the Test Article outer box labels are provided below:

<p><b>Study Drug for DMID Protocol 10-0069</b> <b>Lot # XXXX</b> <b>AdCh3NSmut1 Vaccine</b></p> <p><b>Directions:</b> administer <b>0.5 mL</b> by intramuscular injection</p> <p><b>Store at or below -70°C</b></p> <p><b>Caution: New Drug</b> – limited by United States Law to investigational use.</p>
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<p><b>Study Drug for DMID Protocol 10-0069</b> <b>Lot # XXXX</b> <b>MVA-NSmut Vaccine</b></p> <p><b>Directions:</b> administer <b>0.2 mL</b> by intramuscular injection</p> <p><b>Store at or below -70°C</b></p> <p><b>Caution: New Drug</b> – limited by United States Law to investigational use.</p>
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**Study Drug for DMID Protocol 10-0069**  
**Lot # XXXX**  
**0.9% Sodium Chloride**

**Directions:** administer **0.5 mL** by intramuscular injection

**Store at 20 to 25°C**  
Expires: MM/DD/YYYY

**Caution: New Drug** – limited by United States Law to investigational use.

**Study Drug for DMID Protocol 10-0069**  
**Lot # XXXX**  
**0.9% Sodium Chloride**

**Directions:** administer **0.2 mL** by intramuscular injection

**Store at 20 to 25°C**  
Expires: MM/DD/YYYY

**Caution: New Drug** – limited by United States Law to investigational use.

### 6.1.1 Acquisition

The AdCh3NSmut1 and MVA-NSmut vaccines will be provided by Okairos. The normal saline placebo will be provided by DMID.

Clinical sites will obtain vaccines from the DMID Clinical Agent Repository at Fisher Bioservices.

### 6.1.2 Product Storage, Stability, and Expiration

Vials of both recombinant viruses are stored frozen at -70°C or below in a clearly marked secure, limited-access storage area with temperature-controlled freezers. Access to the area will be controlled and temperature records will be maintained.

A pharmacy facility will be located at the study site and staffed by pharmacists who are licensed to practice in the US. The pharmacy will have adequate space to store sufficient quantities of study product to assure continuous access to all study subjects.

#### *AdCh3NSmut1 Stability*

Research grade purified virus has been stored for 12 months in buffer A195 at -70°C with no significant loss of biological activity. Genetic stability has also been assessed

on a research batch of AdCh3NSmut1, subjected to ten passages in PER.C6 cells and no evidence of genetic instability has been observed.

#### *AdCh3NSmut1 shelf life extension plan*

The AdCh3NSmut1 clinical lot will have an initial expiry date of 12 months from manufacture. A shelf-life extension program is in place to assign new expiry date to the product. Samples of the clinical lot will be tested by a potency assay, based on *in vitro* infectivity in HEK (human embryonic kidney) 293 cells by the AdCh3 hexon immune staining method. Testing will be carried out for a period of 36 months.

#### *MVA-NSmut Stability*

The clinical batch of MVA-NSmut has undergone testing to demonstrate genetic stability of the virus. An initial expiration date of 2 years from date of manufacture was assigned based on the vast stability data for MVA based vectors stored at or below  $-70^{\circ}\text{C}$ . Titrations on chicken embryo fibroblast (CEF) cells will be performed in order to check that the titer does not decrease over time. Long term stability testing for MVA-NSmut stored in a freezer ( $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ ) will be carried out for a period of 36 months to allow the expiration date to be lengthened.

#### *0.9% Sodium chloride for injection*

The 0.9% sodium chloride should be stored at a controlled room temperature of  $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$  ( $68$  to  $77^{\circ}\text{F}$ ). The solution should be inspected visually for particulate matter and discoloration prior to administration.

### **6.1.3 Preparation & Administration**

#### *Vaccine administration*

On vaccination day, both for priming and boosting administration, vaccines will be allowed to thaw to room temperature. The pharmacist or nurse trained to prepare vaccine will wear gloves. Using aseptic technique, the pharmacist or trained nurse will withdraw the volume specified in Section 6.1 into a 1 mL syringe and add a blinding sleeve. The syringe containing the product must be held at room temperature. Once drawn into the syringe, the vaccine should be administered as soon as possible after preparation as intramuscular injection in the deltoid region of the arm. During administration of the vaccines, medicines and resuscitation equipment will be immediately available for the management of anaphylaxis. All subjects will remain on the unit for at least 30 minutes for observation.

#### **6.1.4 Maintaining Blind for Investigational Product**

Vaccine will be prepared by a pharmacist or trained nurse and administered by a blinded vaccine administrator. All study assessments will be performed by blinded study personnel. All other staff, as well as all subjects, will be blinded to treatment assignment.

#### **6.1.5 Accountability/Final Disposition for the Investigational Product(s)**

A pharmacist at the study site will receive the study products and store them in a designated secure area within the pharmacy. Access will be restricted to the pharmacist, nurse trained to prepare vaccine and the site monitor. The pharmacist will be responsible for keeping accurate records of the material received. S/he will keep a daily log of the amount of material received, the amount dispensed, and the amount remaining at the end of the study. At the end of the study, the pharmacist will perform the final drug accounting of unused study material on the proper log documents. At the completion of the study, a clinical monitor will review final accountability records. All unused vials should be returned to the DMID Clinical Repository at Fisher BioServices.

The pharmacist or trained nurse will be responsible for dispensing the drug to the blinded vaccine administrator. The pharmacist will be responsible for storing, managing, and dispensing all drug supplies for the study. The pharmacist will be responsible for tracking the flow of all study product in a log book, including managing and logging all supplies in storage, and logging all study product dispensed to subjects. The on-site investigator will oversee the duties of the pharmacist and will be responsible for ensuring that the pharmacist is accountable for these responsibilities.

## 7 Study Procedures/Evaluations

### 7.1 Clinical Evaluations

*Medical History:* Will be obtained by interview of the subjects. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, and autoimmune disease will be solicited.

*Targeted Physical Examination:* At screening or Day 0 visit, a targeted physical exam will be performed based on subject's clinical history and height and weight will be collected. Vital signs (temperature, heart rate, respiration and blood pressure) will be collected at screening and prior to each vaccination. The exam will be performed by appropriate study personnel to assess general physical condition and will include the following areas/systems: oral cavity, supraclavicular and axillary lymph nodes, cardiovascular, pulmonary, abdomen, skin.

At visits following the first vaccination a targeted physical examination may be conducted based on interim medical history.

*Reactogenicity Assessments:* Will include brief history for assessment of AE/SAEs just prior to and following vaccination, which includes an assessment of pain, tenderness, erythema, induration and warmth at the injection site; fever, chills, arthralgia/joint pain, malaise/fatigue, myalgia/body aches, headache, nausea, vomiting and abdominal pain. The vaccination site will be examined at the end of the 30 minute observation period following each vaccination as well as at the Day 3/7 and Day 59/63 clinic visits following each vaccination.

*Memory Aids:* All subjects will complete a subject memory aid for 8 days following each vaccination (Days 0-7). Subject memory aids will be reviewed with the subject in person or by telephone for AE/SAEs at the Day 1-3 follow-up visit or phone call and at the Day 5-9 clinic visit following each vaccination. Following review, subject memory aids will be discarded.

### 7.2 Concomitant Medications/Treatments

Administration of any concomitant medications, therapies or vaccines will be documented in the subject's data collection form and reported on the subject's data acquisition screen. Concomitant medications will include all current medications and medications taken within the 30 days prior to enrollment (prescription and over-the-

counter drugs, vitamins and supplements, topical products, vaccinations, and allergy shots) through 34 days after the second vaccination (approximately Day 90 for subjects who receive both doses of vaccine or approximately Day 30 for subjects who receive only one dose of vaccine) or early termination (if prior to Day 90), whichever occurs first. Assessment of eligibility also will include a review of permitted and prohibited medications (per the exclusion criteria).

Immunosuppressant medications, as defined by exclusion criterion #22, and medications for the treatment of HCV infection will be collected for the duration of the study.

Medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications in this category include, but are not limited to, glucocorticoids, i.e., oral, parenteral and high-dose inhaled steroids, and immunosuppressive or cytotoxic drugs. Other than from participation in this study, subjects should not receive experimental agents including vaccines during the 20-month study period. The administration of licensed vaccines should be delayed until 14 days after the last study vaccination. Subjects should also not receive licensed products from participation in another clinical trial.

### **7.3 Prevention: Counseling and Risk Reduction**

Prevention counseling for risk avoidance and harm prevention for HCV and other blood borne infections (HIV, HBV) will be provided to all study subjects (in both study arms) at each study visit where HCV testing occurs. Counseling will emphasize the unknown efficacy of the study product in preventing HCV infection (and or other blood borne infections). Subjects will be counseled that stopping injecting will significantly reduce risk of HCV, and that reducing injecting, consistent use of clean needles and drug preparation equipment may also prevent parenteral transmission and acquisition of HCV, if injecting. As well, at each visit study subjects will be provided with free sterile needles and syringes (supplied by local Public Health Departments and/or local syringe exchange programs) and condoms. Members of the Protocol Team will monitor self-reported injection rates, including use of clean and used needles and drug preparation equipment. Risk counseling methods will be updated if needed to address higher than expected rates of reported unsafe behavior and to promote reduction of parenteral risk of HCV by study subjects. In addition to risk reduction information, referral lists of educational resources, community and social service providers, and clinics will be made available. Individuals who are identified as anti-HCV positive at eligibility screening will be referred to established services in each locale. Where possible, medical and social service providers who are experienced in working with the trial population will receive referrals preferentially.

## 7.4 Contraception Status and Pregnancy

Women of childbearing potential (not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy or who have not been postmenopausal for  $\geq 1$  year) must agree to practice adequate contraception from the day of the screening visit through 90 days after the second vaccination. Acceptable birth control methods for the purposes of this study may include, but are not limited to: abstinence; monogamous relationship with vasectomized partner; barrier methods such as condoms, diaphragms, spermicides, and intrauterine devices; and licensed hormonal methods.

Contraceptive status is assessed and documented at every scheduled clinic visit for subjects who were born female and who are sexually active in a way that could lead them to becoming pregnant. Prior to enrollment and throughout the study, staff will ask subjects to verbally confirm their use of adequate contraceptive methods. A subject who was born female and is sexually active in a way that could cause that subject to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. This reminder should be documented in the subject's study record. Self-reported infertility—including having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation—must be documented in the subject's study record.

Vaccinations will be discontinued for subjects who become pregnant during the course of the study. Pregnant subjects will attend the remaining visits and study procedures should be completed unless medically contraindicated. If the subject terminates from the study prior to the pregnancy outcome, the site must keep in touch with the subject in order to ascertain the pregnancy outcome.

## 7.5 Behavioral Risk Assessment Questionnaire

A clinic interviewer/clinician will administer a Behavioral Risk Assessment Questionnaire as specified in the study schedule (Section 8). The Behavioral Risk Assessment will query subjects' self-reported HCV risk including injection frequency, use of clean and used needles and drug preparation equipment, drugs used, and behaviors injecting partners. All subjects will receive risk reduction counseling as detailed in Section 7.3.

## 7.6 Laboratory Evaluations

All laboratory testing will be conducted at CLIA certified laboratories at each site. Blood specimens and volumes will be obtained in accordance with the schedule (Appendix C). Blood specimens that will be collected as part of the standard specimen collection protocol will include serum and anticoagulated plasma. These specimens may be used for (1) repeat analysis of safety and efficacy parameters; (2) analyses of anti-HCV; (3) HCV viral nucleic acids; and (4) antibodies to adenovirus (anti-ChAd3). Blood samples

for isolation and cryopreservation of peripheral blood mononuclear cells (PBMCs) are collected for use in immunology studies. Blood samples collected for immunology analyses at the San Francisco site will be processed for PBMC at Blood Systems Research Institute (BSRI) using a standardized research operating protocol, and shipped to the HCV Immunology Laboratory of Dr. Andrea Cox at Johns Hopkins University in Baltimore for laboratory analyses of host antiviral immune responses. PBMC for the UNM site will be processed at the UNM Division of Epi/Biostat Molecular Epidemiology Lab and shipped to JHU.

### **7.6.1 Clinical Laboratory Evaluations**

Urine or serum pregnancy tests will be performed within 24 hours prior to each vaccination on all female subjects of childbearing potential. Results must be negative and known prior to vaccination.

Subjects will be tested for HIV antibodies and Hepatitis B surface antigen at screening and the last visit.

Clinical laboratory evaluations for safety will be performed by local (clinical) laboratories. Venous blood samples (approximately 23 mL) will be collected from each subject prior to each vaccination and according to the Study Schedule in Appendix C. Clinical safety lab parameters to be followed include WBC, HgB, platelets, ALT, and creatinine. Urine will be collected to evaluate glucose and protein, which will be performed at screening only.

Human Leukocyte Antigen (HLA) and IL28B typing will be performed for all subjects. IL28B testing and typing will be done at screening/enrollment to inform the randomization process. HLA typing will be done on collected blood samples from vaccinated subjects to inform immunogenicity assessments.

### **7.6.2 Endpoint Assays: HCV serology and virology**

#### HCV RNA

HCV RNA will be monitored using both qualitative and quantitative assays.

#### *Qualitative HCV RNA testing:*

A standardized HCV RNA qualitative assay (Procleix<sup>®</sup> HIV-1/HCV assay, by Gen-Probe Inc., San Diego, CA) with 50% limit of detection of 12.1 copies/mL of HCV RNA when using the recommended 0.5 mL specimen input (95% CI, 11.1 to 13.2) will be used to screen for HCV RNA at monitoring visits. HCV reactive samples will be confirmed using a quantitative HCV RNA assay based on real-time PCR (Procleix HCV assay; Gene-Probe). (This assay will not be used for HIV screening).



### *Quantitative HCV RNA testing:*

Quantitative HCV RNA testing (based on real-time PCR (Procleix HCV assay; Gene-Probe) will be used to confirm HCV infection. Monitoring of HCV infection relies on sensitive and accurate HCV RNA detection by quantitative assays. Recently developed tests based on real time PCR have a high sensitivity (based on the lower limit for detecting HCV RNA) and a great dynamic range. The principle of real-time PCR techniques is to detect amplicon synthesis and to deduce the number of viral genomes in the start of the clinical sample during the PCR rather than at the end. The main advantage of tests based on real time PCR is that each has been standardized, which makes results by different laboratories comparable through reporting in international units (IU).

### *HCV subtypes*

HCV genotype will be determined by reverse transcription/nested PCR (nt 85--1326 core-E1 region amplicon) following nucleic acid extraction on automated instrumentation. Genotype is determined by sequence comparison with GenBank using BLAST. Viral sequencing of HCV Core-E1 sequences of initial infections will be conducted to determine HCV subtype on the first quantitative measurement of HCV RNA, dependent on specimen availability and RNA level. In patients who demonstrate alternating HCV RNA positive and negative results or who have missed more than two follow up study visits, viral sequencing will be used to confirm reinfection with a different virus or intercalating viremia with the same virus.<sup>29,84</sup>

### *HCV antibodies*

Anti-HCV testing (counseling will be given prior to testing blood for these blood-borne viruses), will be performed at screening and at indicated time points during the study. Anti-HCV antibody testing will be done using a commercial enzyme immunoassay (EIA). Following a positive EIA test in individuals who are not documented to have evidence of an HCV infection (positive HCV RNA), additional testing for HCV antibodies will be done at the end of the study to differentiate infection-induced antibodies from vaccine-induced HCV antibodies (anti-HCV). Differentiation will be based on detection or not of antibodies to antigens not present in the vaccine.

### *Anti ChAd3 antibodies*

Antibodies specific for the ChAd3 virus will be monitored at indicated time points (Appendix C) at Okairos laboratories by using an experimental assay.

### 7.6.3 Immunogenicity Evaluations

Interferon-gamma (IFN- $\gamma$ ) ELISpot will be used to examine HCV specific T-cell responses following vaccination.

#### ELISpot Assay

Samples for assessment of vaccine immunogenicity will be collected at the indicated time points (Appendix C). The primary immunogenicity assay is the *ex vivo* ELISpot assay for IFN- $\gamma$  with peptide pools derived from the HCV genotype 1b BK virus used in the vaccine as antigen. IFN- $\gamma$  is a cytokine critical in mediating the adaptive T-cell response to viral infections. It is secreted by activated antigen-specific CD8 T-cells and by the CD4 helper T-cells required to sustain effective CD8 T-cell responses. The ELISpot assay is an efficient, simple, and sensitive method of assessing the total T-cell (sum of CD8+ and CD4+) response to viral antigens. Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood and frozen according to validated SOPs. PBMC will be thawed according to Okairos SOP IU008 (Human PBMCs triage) and assessed for HCV specific immune responses via a human IFN- $\gamma$  ELISpot assay based on Okairos' standard operating procedure. This Okairos SOP has been already used to measure anti-HCV immune responses in vaccinated volunteers in previous trials: HCV001 (EudraCT number 2007-004259-12), HCV002TV (EudraCT number 2008-006127-32), HCV003 (EudraCT number 2009-018260-10) and HCV004 (EudraCT number 2010-022700-49). Thawed PBMCs will be incubated for 16 hours with HCV antigens in 15-mer peptide pools (overlapping by 11 amino acids) covering the entire NS3-NS5b sequence contained in the vaccine. Peptides 15 amino acid-long have been shown to optimally allow stimulation, and consequently detection, of both CD8+ and CD4+ T-cell responses.<sup>85</sup> To facilitate the analysis the 494 nonstructural HCV peptides, the peptides are dissolved in DMSO, and divided into 6 mixtures, or pools for screening: pool NS3p (NS3 protease, aa 1027-1349), pool NS3h (NS3 helicase, aa 1339-1661), pool NS4 (NS4, aa 1655-1977), pool NS5a (NS5a, aa 1971-2425), pool NS5bl (NS5b aa 2415-2725) and pool NS5bII (NS5b aa 2715-3011)

Depending on availability of PBMCs, additional assays will be performed on selected samples to further characterize cross-reactivity, function, and phenotype of the induced response as detailed in Sections 7.6.4-5.

### 7.6.4 Exploratory Assays

Exploratory studies may include measurements of serum or plasma cytokine and chemokine profiles, intracellular cytokine staining to determine if HCV specific T-cells can secrete multiple cytokines in addition to IFN- $\gamma$  and if they degranulate, or additional T-cell assays for function based on specimen availability.

### **7.6.5 Ancillary Assays**

Ancillary studies may include but are not limited to epitope mapping, tetramer analysis by flow cytometry, and vector-specific immunogenicity. Vector-specific studies may be performed to better understand the immune responses to the vector and vaccine insert. This information may help further product development. Cryopreserved samples may also be used to perform assays to support standardization and validation. Additional cell surface markers, cytokines or functional markers may also be analyzed.

### **7.6.6 Future use of stored specimens**

HCV vaccine researchers aim not only to test vaccine candidates but also to continue to explore the correlates of immunity to HCV. In order to do so, the research team intends to store plasma, serum, and PBMC samples from subjects at UCSF and JHU. These stored samples will be used for future testing and research related to furthering the understanding of HCV or vaccines, such as immunologic analyses in addition to those outlined in sections 7.6.3-7.6.5 and future potential bridging studies.

The protocol ICF is written so that the subject is informed that by agreeing to participate in the study they are also agreeing to have samples stored for future additional research studies. Some of these samples will be sent to a DMID central storage facility. Subjects will not be contacted with the results of these future research studies. Future testing on specimens will only occur to the extent authorized in each study site's ICF or as otherwise authorized under applicable law and after review and approval by the DMID and the IRB of the researcher requesting the specimens.

## **7.7 Specimen Preparation, Handling, and Shipping**

Biological specimens are to be collected in accordance with protocol specifications, as outlined in the Protocol Schedule of Activities. Study staff will follow all safety guidelines of local safety committee and/or institutional policies for handling biological specimens. Blood samples will be labeled and processed according to the protocol-specific MOP. Samples will be barcoded and entered into the GlobalTrace Inventory system before shipping, as indicated in the protocol-specific MOP.

The blood test for detection of anti-HCV, urine test for glucose and protein, and urine pregnancy test will be performed on site. All other specimens will be collected and shipped as indicated in the protocol-specific MOP.

### **7.7.1 Instructions for Specimen Preparation, Handling, and Storage**

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP.

### **7.7.2 Specimen Shipment**

Instructions for specimen shipment are included in the protocol-specific MOP.

## 8 Study Schedule

### 8.1 Screening and Enrollment

#### 8.1.1 Anti-HCV Pre-Screening Day -30 to -1

- Potential subjects will be provided with a description of the anti-HCV test and will provide consent for anti-HCV testing. Anti-HCV screening will be conducted for detection of anti-HCV obtained using finger stick or venipuncture whole blood sample.
- Potential subjects who test positive for anti-HCV at this test will not proceed to the next screening step. Those who test negative for anti-HCV will be asked if they wish to continue to the Screening Visit Procedures.

#### 8.1.2 Day -30 to -1, Visit 00, Study Screening

- Potential subjects with previous negative anti-HCV test may be screened for eligibility. All pre-screening and screening activities outlined in Sections 8.1.1 and 8.1.2 must be completed within 30 days prior to enrollment. The following activities will be performed at the screening visit:
  - Potential subjects will be provided with a description of the study (purpose and study procedures). Subjects will be asked to read and sign the informed consent form (ICF). The ICF will be signed prior to performing any other screening procedures.
  - Eligibility criteria for study entry and receipt of Dose 1 will be reviewed with the subject – see Section 5.1 and 5.2.
  - Vital signs will be obtained, including oral temperature, pulse, and blood pressure.
  - Height and weight will be obtained.
  - Medical history will be reviewed.
  - All concomitant medications will be recorded.
  - A targeted physical examination will be performed by appropriate study personnel to assess general physical condition. Additional physical assessments may be performed as indicated.
  - Urine sample will be obtained to evaluate glucose and protein.
  - Review of acceptable contraceptive methods (male and female subjects) and recent menstrual history (female subjects only).

- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- A urine or serum pregnancy test must be performed within 24 hours prior to vaccination for all female subjects of childbearing potential. Results must be negative and known prior to vaccination. If the screening visit is Day -1, a negative pregnancy test obtained at Day -1 may be used for eligibility at Day 0. If the pregnancy test is > 24 hours prior to vaccination, it must be repeated to determine eligibility.
- Approximately 5 mL of venous blood will be collected for IL28B, as described in Section 7.6.1.
- Approximately 23 mL of venous blood will be collected for serological studies and chemistry (see hematology and biochemistry parameters, as described in Section 7.6.1).
- Approximately 20 mL of venous blood will be collected for anti-HCV, HbsAg, anti-HIV, and HCV viremia.

### **8.1.3 Day 0, Visit 1, Dose 1**

- Eligibility criteria for study entry and receipt of Dose 1 will be reviewed with the subject – see Section 5.1 and 5.2.
- Vital signs will be obtained, including oral temperature, pulse, and blood pressure.
- Medical history will be reviewed to assure continued eligibility.
- All concomitant medications will be reviewed for accuracy and completeness. Any new medications will be recorded and assessed for continuing eligibility.
- A targeted physical examination may be performed, as indicated, based on subject's recent clinical history since the screening visit.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- A urine or serum pregnancy test must be performed within 24 hours prior to vaccination for all female subjects of childbearing potential. Results must be negative and known prior to vaccination. If the screening visit is Day -1, a negative pregnancy test obtained at Day -1 may be used for eligibility at Day 0. If the pregnancy test is > 24 hours prior to vaccination, it must be repeated to determine eligibility.
- Approximately 8 mL of venous blood will be collected for HLA typing/genetic studies, as described in Section 7.6.1.

- Approximately 23 mL of venous blood will be collected prior to vaccination for hematology, coagulation and chemistry/metabolic biochemistry parameters, as described in Section 7.6.1. Clinical safety laboratory assessments will be performed by the local laboratory. The results from this blood draw will not be available or reviewed prior to vaccination, and will serve as a baseline safety assessment only.
- Approximately 160 mL of venous blood will be collected prior to vaccination for baseline immunology assessments.
- Plasma saved from the blood collected for immunology assessments at this visit will be used for anti-adenovirus Ab testing.
- Subjects will be enrolled in AdvantageEDC (IDES) and randomly assigned to a treatment arm.
- Subjects will receive a single dose of AdCh3NSmut1 vaccine or placebo per randomized assignment via intramuscular injection in the deltoid muscle of the preferred arm. Following vaccination the injection site will be covered with a sterile dressing and subjects will be observed in the clinic for at least 30 minutes. The vaccination site will be examined, and any AE/SAEs will be assessed prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study related materials to record daily oral temperature and systemic and local AE/SAEs. Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record AE/SAEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions following vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

## 8.2 Subject Follow-Up

Follow-up visits are scheduled in reference to dosing dates as indicated for each visit window.

### **8.2.1 Day 3, Visit 2**

#### **(Window: Day 1 to 5)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review the memory aid information with the subject and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

### **8.2.2 Day 7, Visit 3**

#### **(Window: Day 5 to 9)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review the memory aid information with the subject and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

### **8.2.3 Day 14, Visit 4**

#### **(Window: Day 7 to 21)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined if ongoing Memory Aid symptoms are reported after Visit 3.



- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review and record all AE/SAEs and concomitant medications on the appropriate data collection form
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

#### **8.2.4 Day 30, Visit 5**

##### **(Window: Day 23 to 37)**

- Current health status will be reviewed and any changes since the last visit will be recorded.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Approximately 160 mL of venous blood will be collected for immunologic assays.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

#### **8.2.5 Day 56, Visit 6, Dose 2**

##### **(Window: Day 49 to 63)**

- Eligibility criteria for receipt of Dose 2 will be reviewed with the subject – see Section 5.1 and 5.2.
- Current health status will be reviewed and any changes since the last visit will be recorded.
- The vaccination site will be examined.

- Vital signs will be obtained, including oral temperature, pulse, and blood pressure.
- All concomitant medications will be reviewed for accuracy and completeness. Any new medications will be recorded and assessed for continuing eligibility.
- A targeted physical examination may be performed, as indicated, based on subject's recent clinical history since the screening visit.
- A urine or serum pregnancy test must be performed within 24 hours prior to vaccination for all female subjects of childbearing potential. Results must be negative and known prior to vaccination. If the screening visit is Day -1, a negative pregnancy test obtained at Day -1 may be used for eligibility at Day 0. If the pregnancy test is > 24 hours prior to vaccination, it must be repeated to determine eligibility.
- Approximately 23 mL of venous blood will be collected prior to vaccination for hematology, coagulation and chemistry/metabolic biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Approximately 10 mL of venous blood will be collected prior to vaccination for HCV viremia.
- Approximately 100 mL of venous blood will be collected prior to vaccination for baseline immunology assessments.
- Subjects will receive a single dose of MVA-NSmut vaccine or placebo per randomized assignment via intramuscular injection in the deltoid muscle of the preferred arm. Following vaccination the injection site will be covered with a sterile dressing and subjects will be observed in the clinic for at least 30 minutes. The vaccination site will be examined, and any AE/SAEs will be assessed prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study related materials to record daily oral temperature and systemic and local AE/SAEs. Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the memory aid and how to measure and record AE/SAEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions following vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

- Subjects who become HCV PCR+ after the 1st vaccine may receive a 2nd vaccine dose. These subjects will return for their Visit 7 and 8 assessments and follow the Confirmed HCV Infection visit schedule outlined in Appendix D.

### **8.2.6 Day 59 [Target 3 Days Post Second Vaccination], Visit 7**

#### **(Window: 1-5 days after receipt of second dose of vaccine)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review the memory aid information with the subject and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

### **8.2.7 Day 63 [Target 7 Days Post Second Vaccination], Visit 8**

#### **(Window: 7-21 days after receipt of second dose of vaccine)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Study personnel will review the memory aid information with the subject and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Approximately 100 mL of venous blood will be collected for immunologic assays.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

### **8.2.8 Day 90, Visit 9**

#### **(Window: Day 83 to 97)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- Study personnel will review and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Approximately 160 mL of venous blood will be collected for immunologic assays.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

### **8.2.9 Day 120, Visit 10**

#### **(Window: Day 113 to 127)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

**8.2.10 Days 150–270, Visits 11–15**

Visit	Target (Day)	Window (Days)
11	150	$\pm 7$
12	180	$\pm 7$
13	210	$\pm 7$
14	240	$\pm 7$
15	270	$\pm 7$

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire at visits 12 and 15.
- Approximately 100 mL of venous blood will be collected for immunologic assays at Visit 14.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

**8.2.11 Day 300, Visit 16****(Window: Day 293 to 307)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the local laboratory.

- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

#### **8.2.12 Day 330, Visit 17**

##### **(Window: Day 323 to 337)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

#### **8.2.13 Day 360, Visit 18**

##### **(Window: Day 353 to 367)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAES that have occurred since the last contact will be collected.
- Approximately 20 mL of venous blood will be collected for anti-adenovirus Ab and HCV viremia testing.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

**8.2.14 Days 390–570, Visits 19–25**

<b>Visit</b>	<b>Target (Day)</b>	<b>Window (Days)</b>
19	390	$\pm 7$
20	420	$\pm 7$
21	450	$\pm 7$
22	480	$\pm 7$
23	510	$\pm 7$
24	540	$\pm 7$
25	570	$\pm 7$

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire at visits 21 and 24.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 10 mL of venous blood will be collected for HCV viremia.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.

**8.2.15 Day 600, Visit 26****(Window: Day 593 to 607)**

- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- Immunosuppressant concomitant medications and medications for the treatment of HCV infection will be recorded.
- SAEs that have occurred since the last contact will be collected.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1, and performed by the

local laboratory.

- Approximately 20 mL of venous blood will be collected for anti-HCV, HbsAg, anti-HIV and HCV viremia.
- Approximately 100 mL of venous blood will be collected for immunologic assays.
- Counseling on avoidance of HCV and HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- Subjects will be thanked for participating in the study and contact information for end of study results will be confirmed.

### **8.3 Follow-up for Subjects with HCV Infection**

Subjects who are confirmed HCV RNA positive will be followed for approximately 9 months after their first HCV-positive quantitative RNA result. The schedule of assessments for HCV RNA positive subjects is outlined in Appendix D: Study Visit Chart – Confirmed HCV Infection. Subjects will exit the study after visit H09, approximately Day 270 after initial HCV detection.

Subjects who are confirmed HCV RNA positive prior to the 2nd dose of vaccine will be followed for safety as scheduled from the date of their first HCV-positive quantitative RNA result. Subjects will exit the study after visit H09, approximately Day 270 after initial HCV detection.

Subjects who are confirmed HCV RNA positive prior to the 2nd dose of vaccine and choose to terminate early will do the full schedule of assessments for HCV RNA positive subjects for visit H09 (exit visit).

Trial subjects who test positive for HCV RNA will be provided with a referral to providers external to the study for evaluation for appropriate care and treatment of HCV infection. Treatment may include interferon alpha with or without ribavirin. Referrals will be provided at every visit at which a participant tests positive for HCV RNA. The providers' lists will include primary care and specialist resources with experience working with and providing care to those at high risk for HCV infection, including injecting populations. This approach is based upon the NIH Consensus Statement on Management of Hepatitis C: 2002 regarding acute hepatitis C treatment which states, "it is recommended that treatment of active injection drug use be considered on a case-by-case basis, and that active injection drug use in and of itself not be used to exclude such patients from antiviral therapy."<sup>86</sup>



Follow-up visits are scheduled in reference to the first positive HCV RNA quantitative result.

### 8.3.1 HCV Day 30, Visit H01

#### (Window: HCV Day 23 to 37)

- Study personnel will inform subjects that they have tested positive for the hepatitis C virus.
- Counseling and written information on acute hepatitis C treatment options will be provided along with a list of healthcare providers who are separate from this study; a referral to a community-based healthcare provider will be offered.
- Counseling on avoidance of HCV transmission, HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- Current health status will be reviewed and any changes since the last visit will be noted.
- The vaccination site will be examined.
- A targeted physical examination may be performed, if indicated, based on review of the current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire, if applicable.
- SAEs that have occurred since the last contact will be collected
- Approximately 138 mL of venous blood for hematology, biochemistry, HCV genotyping, HBsAg, anti-HIV, HCV quantitative viremia, and immunology will be collected.

### 8.3.2 HCV Days 60–210, Visits H02–H07

Visit	Target (Day)	Window (Days)
H02	60	± 7
H03	90	± 7
H04	120	± 7
H05	150	± 7
H06	180	± 21
H07	210	± 7

- Counseling and written information on acute hepatitis C treatment options will be provided along with a list of healthcare providers who are separate from this study; a referral to a community-based healthcare provider will be offered.

- Counseling on avoidance of HCV transmission, HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of the current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire, if applicable.
- SAEs that have occurred since the last contact will be collected.
- Approximately 133 mL of venous blood for hematology, biochemistry, HCV genotyping, HCV quantitative viremia, and immunology will be collected.

### **8.3.3 HCV Day 240, Visit H08**

#### **(Window: HCV Day 233 to 247)**

- Counseling and written information on acute hepatitis C treatment options will be provided along with a list of healthcare providers who are separate from this study; a referral to a community-based healthcare provider will be offered.
- Counseling on avoidance of HCV transmission, HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of the current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire, if applicable.
- SAEs that have occurred since the last contact will be collected.
- Approximately 33 mL of venous blood for hematology, biochemistry, HCV genotyping, and HCV quantitative viremia will be collected.

### 8.3.4 HCV Day 270, Visit H09

#### (Window: HCV Day 263 to 277)

- Counseling and written information on acute hepatitis C treatment options will be provided along with a list of healthcare providers who are separate from this study; a referral to a community-based healthcare provider will be offered.
- Counseling on avoidance of HCV transmission, HIV infection, and pregnancy will be conducted.
- Referral to drug treatment and needle exchange programs will be provided.
- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of the current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- SAEs that have occurred since the last contact will be collected.
- Approximately 143 mL of venous blood for hematology, biochemistry, HCV genotyping, anti-HCV, HbsAg, anti-HIV, HCV quantitative viremia, and immunology will be collected.
- Subjects will be thanked for participating in the study and contact information for end of study results will be confirmed.

### 8.4 Unscheduled Visit(s)

Unscheduled visits may occur at any time during the study. They may occur for the following reasons:

- (1) For operational reasons, e.g., a subject may request to reschedule, or to ask questions;
- (2) For AE related reasons. When interim contacts or visits are completed in response to subject reports of AEs, study staff will assess the reported event clinically and provide or refer the subject to appropriate medical care. All AEs will be evaluated and reported as required (Section 9.3);
- (3) If a subject presents to the study site after having missed a scheduled visit (e.g., in response to locator/tracing efforts) on a day that does not fall within a scheduled visit window;
- (4) For other reasons at subject request. All interim contacts and visits will be documented in subjects' study records and on applicable data collection forms.

## 8.5 Early Termination

Subjects may voluntarily withdraw their consent for participation in the study at any time and for any reason, without penalty. Subjects may also withdraw voluntarily from receiving the study intervention for any reason. A site principal investigator may also withdraw a subject from receiving further study intervention. Follow-up safety assessments and immunogenicity evaluations will be completed, if possible, whether the subject withdraws from the study or is withdrawn from receiving further study product. Subjects will be encouraged to permit continued follow-up of AE/SAEs and to donate scheduled venous blood samples for clinical safety laboratory and immunogenicity evaluations, if possible – see the protocol-specific MOP for alternate follow-up requirements. For subjects who withdraw within 180 days of their last dose of vaccine, a 6-month follow-up phone call should be conducted to collect any new SAEs.

### 8.5.1 For HCV-uninfected subjects

- Current health status will be reviewed and any changes since the last visit will be noted.
- All concomitant medications will be recorded (if prior to Day 90 visit). Immunosuppressant medications and medications for the treatment of HCV infection will be collected throughout the study as applicable.
- Approximately 23 mL of venous blood will be collected for hematology and biochemistry parameters, as described in Section 7.6.1.
- Approximately 100 mL of venous blood will be collected for immunologic assays.
- Approximately 20 mL of venous blood will be collected for anti-HCV, HbsAg, anti-HIV, and HCV viremia.
- A targeted physical examination may be performed, if indicated based on review of current health status.
- Information regarding adverse events will be collected (if before Day 90). All SAEs that have occurred since the last contact will be collected.
- Subjects will be thanked for participating in the study and contact information for end of study results will be confirmed.

### 8.5.2 For HCV-infected subjects

- Counseling and written information on acute hepatitis C treatment options will be provided along with a list of health care providers who are separate from this study; a referral to a community-based healthcare provider will be offered.
- Counseling on avoidance of HCV transmission, HIV infection, and pregnancy will be conducted.

- Referral to drug treatment and needle exchange programs will be provided.
- Current health status will be reviewed and any changes since the last visit will be noted.
- A targeted physical examination may be performed, if indicated, based on review of the current health status.
- Behavioral risk will be assessed using an interviewer administered questionnaire.
- SAEs that have occurred since the last contact will be collected.
- Approximately 138 mL of venous blood for hematology, biochemistry, HCV genotyping, anti-HCV, HCV quantitative viremia, and immunology will be collected.
- Approximately 5 mL of venous blood will be collected for HbsAg and anti-HIV for subjects who become HCV-infected prior to the 2nd dose of vaccine and choose to terminate early.
- Subjects will be thanked for participating in the study and contact information for end of study results will be confirmed.

## **8.6 Referral for Acute HCV Infection**

All trial subjects with positive HCV RNA test results will be provided a referral to providers external to the study for evaluation for appropriate care and treatment of HCV infection. Treatment may include interferon alpha with or without ribavirin. Referrals will be provided at every visit at which a participant tests HCV RNA positive.

## **8.7 Subsequent Positivity for Anti-HCV: Distinguishing Intercurrent HCV Infection from Vaccine Induced Positive HCV Serology**

It is expected that after vaccination some subjects may become positive for anti-HCV in the absence of infection as a result of exposure to antigens derived from HCV that are included in the vaccine. In the previous clinical trial, HCV001, seroconversion to HCV in absence of documented infection occurred only in 10% of vaccinated subjects. Anti-HCV testing will be done using a commercial enzyme immunoassay (EIA) on subjects who do not develop HCV infection during the trial. Following a positive EIA test, additional testing will be done to differentiate infection- from vaccine-induced antibodies (See Section 7.6). Testing will be conducted on stored blood samples obtained from monthly visits. Differentiation will be based on detection or not of antibodies to antigens not present in the vaccine. Subjects who develop vaccine induced anti-HCV will be informed at the end of the study and be provided with documentation explaining the reasons for their anti-HCV positivity for communication with their medical providers (with the subjects' consents). This will be discussed in detail at screening and in ICFs.

## 9 DMID Safety Reporting and Safety Assessment Monitoring

Regulatory requirements, including the FDA regulations and the ICH Guidelines for Good Clinical Practice, define safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

### 9.1 Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of adverse events (AEs) for seriousness, severity, and causality
- Notify the sponsor (DMID) through their Pharmacovigilance contractor of SAEs immediately
- Provide detailed written reports promptly following immediate initial reports, including necessary documentation requested by the sponsor or IRB
- Inform the IRB of AEs as required by applicable regulatory requirements

### 9.2 Definitions

#### Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical investigation subject who has received a study intervention and that does not necessarily have to have a causal relationship with the study product. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of the study product, whether or not considered related to the study product.

#### *Unexpected*

An unexpected AE is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure [IB] for an unapproved investigational medicinal product). Unexpected refers to an experience that has not been previously observed. This includes events that are more serious than expected or occur more frequently than expected.

#### *Expected*

Any adverse experience, the nature, severity or frequency of which is consistent with the current IB; or with the risk information described in the investigational plan or protocol or consent form.

### Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening adverse event\*,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

*\* Life-threatening adverse event. An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the subject or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.*

## **9.3 Safety Reporting Requirements**

### **9.3.1 Reporting Interval**

All SAEs and new onset chronic illnesses will be documented from randomization through subject’s last visit (Visit 26 or H09)/subject completion of all study assessments.

- New-onset chronic illnesses will be collected from randomization through a subject’s last visit (Visit 26 or Visit H09). New-onset chronic illnesses are defined as any new ICD-10 diagnosis that is applied to the subject during the duration of the study, after receipt of the study agent, that is expected to continue for at least 6 months and requires continued health care intervention. To reference the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10): <http://www.cdc.gov/nchs/about/otheract/icd9/abtcd10.htm>
- Non-serious Adverse Events will be collected from randomization through 34 days post-vaccination 2 (approximately Day 90). All SAEs and AEs will be followed until they are resolved or determined by the PI to be medically stable,

even if this extends beyond the study-reporting period. Resolution of an adverse event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

At any time after completion of the study, if the investigator becomes aware of a serious adverse event that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance contractor, DMID CROMS Pharmacovigilance Group.

### 9.3.2 Notification of the Sponsor of Serious Adverse Events

Any AE that meets a protocol-defined serious criterion must be submitted on an SAE form to the DMID CROMS Pharmacovigilance Group (PVG), the DMID Pharmacovigilance contractor **immediately** (within 24 hours of site awareness), at the following address:

DMID CROMS Pharmacovigilance Group (PVG)  
Technical Resources International, Inc  
6500 Rock Spring Dr., Suite 650  
Bethesda, MD 20817 USA  
Email: XXXXXXXXXXXX  
Fax: XXXXXXXXXXXX  
SAE line: XXXXXXXXXXXX

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance contractor and should be provided as soon as possible.

The DMID Medical Monitor (MM) and Clinical Project Manager (CPM) will be notified of the SAE. The DMID MM will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

Questions about SAE reporting can be referred to the SAE line  
(available 24 hours a day/7 days a week).

### 9.3.3 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies



for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

### **9.3.4 Reporting of Pregnancy**

Although not AEs, pregnancies are reportable events. Any pregnancies that occur during the study period in women who have received any study product will be reported via the Emmes Internet Data Entry System (IDES) on the Pregnancy Report form within 5 days of site awareness. The report will include pregnancy outcome (e.g., any premature terminations, elective or therapeutic, and any spontaneous abortions or stillbirths), as well as the health status of the mother and child, including date of delivery and infant's gender and weight. Pregnancies will be followed until birth. All pregnancies will be reported to the clinical data management group. If the database is locked at time of pregnancy, a supplemental report will be generated and completed after birth and 3 month follow up, which will be appended to the database.

## **9.4 Investigator's Assessment of Adverse Events**

The determination of seriousness, severity, and causality will be made by an onsite investigator who is qualified (licensed in the state) to diagnose adverse event information, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners. Laboratory abnormalities will be reported as AEs based on the normal range of values for the laboratory. The grading of the laboratory AEs will be based on the DMID toxicity table (Appendix B).

### **9.4.1 Assessment of Seriousness**

Event seriousness will be determined according to the protocol definition of an SAE (Section 9.2).

### **9.4.2 Assessment of Severity**

Event severity will be graded according to parameters shown in Appendix B or if not, noted in the tables using the definitions below:

- 1 = Mild (awareness of a symptom but the symptom is easily tolerated; the symptom requires minimal or no treatment)
- 2 = Moderate (discomfort enough to cause interference with usual activity)
- 3 = Severe (incapacitating; unable to perform usual activities; requires absenteeism/bed rest)
- 4 = Life-threatening
- 5 = Death

### 9.4.3 Assessment of Association

**Relationship to Study Products:** The clinician's assessment of an AE's relationship to test article (vaccine or study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

Not Related – There is **not** a reasonable possibility that the administration of the study product caused the event.

### 9.4.4 Assessment of Reactogenicity

Subjects will be asked to record events daily on an 8-day subject memory aid. The subject will record temperature and the presence and intensity of post-vaccination reactogenicity events, including pain, tenderness, erythema, induration and warmth at the at injection site; fever, chills, arthralgia/joint pain, malaise/fatigue, myalgia/body aches, headache, nausea, vomiting, abdominal pain and any other symptoms they might be experiencing after vaccination. Erythema and induration at the vaccination site will be measured in millimeters. Any symptoms still present at Day 8 will continue to be followed until symptom resolution. Subjects will also be asked to record any medications taken and any emergency room or physician visits (other than routine check-ups). The subject memory aid will be reviewed with the subject at subsequent clinic visits and phone calls as needed.

Symptoms will be graded using the following scale:

- Grade 0: Not present
- Grade 1: Mild (present but easily tolerated)

- Grade 2: Moderate (able to tolerate routine activity with effort)
- Grade 3: Severe (unable to continue routine activity)

This information will be reviewed by study staff during follow-up subject contacts.

In the event a subject experiences an adverse event or injection site reaction that is still present at the end of the subject's participation in the study, the subject must be followed until resolution or until the event or abnormality resolves to the investigator's and/or sponsor's satisfaction. Follow-up procedures, evaluations, and outcomes will be recorded on the subject's data collection forms.

## **9.5 Safety Monitoring by the DMID Safety Oversight Mechanism**

### **9.5.1 Data Safety and Monitoring Board (DSMB)**

Safety oversight will be under the direction of a DSMB. The DSMB will meet at least annually (to be determined by the DSMB) to assess safety and enrollment data in addition to the planned interim analysis. If halting rules are initiated or the DSMB requests additional meetings, more frequent meetings may be held. The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. The DSMB will advise DMID of its findings. The DSMB will review blinded aggregate safety data for increased rate of occurrence of serious suspected adverse reactions

### **9.5.2 Independent Safety Monitor (ISM)**

Safety oversight for this study will include an ISM in close proximity to each of the clinical research sites. The ISM will receive and evaluate SAEs blinded and provide an independent written summary to the DMID Clinical Project Manager and DMID Medical Monitor. The ISM may be asked by the DMID Medical Monitor to review additional safety events (i.e. significantly increased frequency of non-serious adverse events).

### **9.5.3 Interruption or Discontinuation of Study Enrollment and Study Product Administration for all Subjects in the Study**

DMID, as the study sponsor, may interrupt study dosing and/or study entry at any time if medically indicated. To minimize risk, cumulative safety data will be reviewed by the Data and Safety Monitoring Board (DSMB), as expeditiously as is reasonable. The DSMB may recommend terminating the study if deemed necessary following a vaccine-related SAE.

## 9.6 Halting Criteria/Rules

If the trial is halted, study drug will not be administered to any additional subjects, and enrollment will stop until the DSMB has reviewed the event.

After their review, the DSMB will make a recommendation to DMID whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group or for the entire study) is another potential outcome of a safety review.

Subsequent review of serious, unexpected, and related AEs by the medical monitor, DSMB, IRB, the Sponsor, the FDA, or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site. The FDA and study Sponsor retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable. The following events will trigger a safety review:

- If two subjects experience a systemic adverse event that is judged to be severe (Grade 3) and related to vaccination. Number of events contributing to halting rule is the sum of a and b:
  - a. Solicited severe (Grade 3) systemic reaction that is judged to be related to the vaccination. Solicited reactions are assumed to be associated with vaccination.
  - b. Severe (Grade 3) adverse event that is judged to be related to the vaccination.
- If 1 subject experiences an SAE judged by an investigator to be related to vaccination.
- If 1 subject develops a laboratory value that is considered Severe (Grade 3) and that is judged to be related to vaccination. Includes all lab values that are considered severe. Relatedness must be determined by the medical monitor.

## **10 Clinical Monitoring**

### **10.1 Site Monitoring Plan**

Clinical monitoring at the site will be conducted to verify that the study is conducted according to the approved protocol, human subjects are protected, and data collection processes are of high quality and meet sponsor SOPs, GCP/ICH guidelines, and federal regulations. DMID, the sponsoring agency, or its designee will conduct site monitoring visits as detailed in the monitoring plan or in the Manual of Procedures (MOP).

Site visits will be made at standard intervals or more frequently as defined by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, study product accountability records, data collection forms, case report forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions. Clinical monitoring reports will be submitted to DMID.

## **11 Statistical Considerations**

### **11.1 Study Hypotheses**

The primary objective of the trial is to assess the safety and evaluate the efficacy of new candidate hepatitis C virus vaccines, AdCh3NSmut1 and MVA-NSmut, compared to placebo when administered sequentially to IDU. This Phase I/II study is intended primarily to uncover any safety issues that occur at a sufficiently high rate that they might be observed in a study of this size, and to obtain meaningful estimates of the efficacy of the vaccine against chronic HCV infection. While the limited sample precludes reaching definitive conclusions, descriptive analyses will be supplemented by hypothesis testing and estimation of vaccination effects when feasible. For the primary safety and efficacy endpoints, the null hypothesis of no difference in vaccine versus placebo arms will be tested against a two-sided alternative at the  $p = 0.05$  level.

### **11.2 Sample Size Considerations**

Sample size calculations are based on the assumption of detecting a 60% reduction in incidence of chronic infection over 18 months of post-vaccination follow-up among vaccinated subjects compared to unvaccinated controls in an according to protocol (ATP) analysis. A total of 43 observed events of chronic infection in the ATP cohort would provide power of 85% for a two-sided log rank test conducted at the significance level (alpha) of 0.05 to detect a 60% reduction in hazard rate. The incidence of chronic infection among controls is assumed to be 14% annually.<sup>30</sup> Assuming 14 cases/100 person-years (PY) in the control arm, and 5.6 cases/100 PY in the vaccine arm, a total of 292.5 subjects in the ATP cohort followed for 1.5 years would provide on average 43 events. We further assume that 65% of enrolled subjects will be retained in the ATP cohort. Therefore, we inflate the required number of subjects by a factor of  $1/0.65$  and obtain a target enrollment of 540 subjects, or 270 subjects per vaccination arm.

As described in Section 11.3, the DSMB will conduct a blinded examination of progress towards the goal of 43 observed events of chronic infection in the ATP cohort, and decide whether to recommend an extension of the enrollment period. At the September 2, 2015 DSMB meeting, the DSMB reviewed the progress towards this goal and recommended increasing the target enrollment from 450 to 540 subjects, or 270 subjects per vaccination arm.

### **11.3 Planned Interim Analyses**

The DSMB will meet and review safety data and enrollment data at least annually as outlined in the DSMB charter. The study will be monitored to determine if any of the

safety halting rules described above in Section 9.6 are met. Additionally, there will be two planned interim analyses.

The first interim analysis is planned when study Day 63 (approximately week 9) follow-up data is available on the last of approximately 68 subjects enrolled in Stage 1 of the trial. The second interim analysis is planned to occur when roughly one-third of enrolled and currently per-protocol subjects (N = 98) have completed six months of post-vaccination follow-up, but no later than one month prior to the planned completion of enrollment to the trial. Both the first and second interim analysis will be fully blinded and will report on trial data aggregated across both treatment arms.

The first stage of the study is designed to establish the safety and immunogenicity of the sequential administration of the AdCh3NSmut1 and MVA-NSmut vaccines in a prime/boost regimen in the target population. The first interim analysis will focus on presenting descriptive tabulations of the safety parameters to the DSMB along with the ELISpot assay immunological data. The immunological data analysis will not require study un-blinding. A positive immune response is defined by two criteria: i) more than 48 spot forming cells per million PBMC; ii) at least three times the mean background spots per million PBMC found in ELISpot wells containing cells and peptide diluent (DMSO).<sup>39,87,88</sup> A subject will be considered to be a responder if a positive immune response is detected in at least one of six different pools of peptides utilized from at least one of the blood samples drawn after the first or second vaccination. Please note that results obtained from blood samples drawn after the onset of HCV infection will not be used in this first interim analysis. A subject will be considered evaluable for the first interim immunological analysis if ELISpot assay data is available from a sample drawn 7-21 days after receipt of the second vaccination, the anticipated timeframe in which a positive response to vaccine will be detected if present. Note that this requirement does not alter the definition of a responder provided above, and in particular, a positive ELISpot result after the first vaccination still suffices to define a responder. The study will proceed to Stage 2 in the absence of any safety concerns and if at least 30% of the evaluable subjects in Stage 1 of the study ( $n = 68 \pm 4$ ) have a positive immune response to HCV. If this threshold is not met, then a more complete unblinded analysis of immunogenicity responses will be undertaken by an independent assessor appointed by DMID.

As noted in the section 11.2 (Sample Size Considerations), the statistical information goal for the efficacy endpoint of prevention of chronic HCV is 43 cases of chronic HCV in the per protocol analysis cohort. At the second interim analysis, the DSMB will do a blinded examination of progress towards this goal, and decide whether to recommend an extension of the enrollment period, while it is still possible to do so without halting enrollment and losing momentum in accrual.

## **11.4 Final Analysis Plan**

### **11.4.1 Definition of Analysis Cohorts**

The safety analysis cohort will consist of all randomized subjects who receive at least one vaccination.

The mITT efficacy analysis cohort will consist of all randomized subjects who receive at least one vaccination, are not HCV infected at the first vaccination, and have sufficient follow-up to be evaluable for efficacy. For the primary efficacy endpoint of chronic HCV infection, sufficient follow-up will consist of at least three clinic visits following the second vaccination. If the second dose of vaccine was not administered, the target date for the second dose will be used in this calculation. Subjects who receive treatment for acute HCV infection will be censored from time to event analyses of chronic infection at the date of first treatment.

The according to protocol (ATP) efficacy analysis cohort is a subset of the mITT analysis cohort, excluding subjects with major protocol deviations that would compromise the assessment of vaccine efficacy. Subjects who meet any of the criteria listed below will be censored from time to event analyses according to the date of first occurrence of the criteria, and will be excluded from all other types of analyses. The ATP cohort will exclude subjects who: a) receive treatment for acute HCV infection; b) were enrolled but subsequently found to have been ineligible at enrollment based on a criterion that could reasonably be expected to affect vaccine efficacy; c) did not receive both doses of vaccine or control; d) acquired HCV infection prior to receipt of the second vaccination; e) received the wrong (non-randomized) product at either dose; f) received the second dose fewer than 42 days or more than 70 days after the first dose; g) received an immunosuppressant other than inhaled or topical steroids; h) were immunized against another pathogen or received immunoglobulins or other blood products within 14 days of either dose of study vaccine; i) have autoimmune disease; and j) have a confirmed or suspected immunosuppressive or immunodeficient state. Membership in the ATP efficacy cohort will be assessed by a centralized clinical review prior to unblinding of the study data.

### **11.4.2 Safety Analyses**

In safety analyses, if a subject receives the wrong (non-randomized) study product at either dose, he/she will be included in the vaccine group if they received one or more doses of vaccine and in the placebo group otherwise.

Solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none or mild versus moderate or severe) and using standard techniques, such as exact confidence intervals, to summarize the



reactogenicity rates. Analyses will be conducted separately for each dose, and both doses will be compared to see if a reaction to the first dose is predictive of a reaction to the second dose.

Results of safety laboratories assessed prior to and following each vaccination will be summarized by treatment group and grade. Changes in laboratory parameters pre- and post-vaccination will also be examined. Standard summary statistics, including 95% confidence intervals will be computed. Additionally, graded results will either be analyzed as a binary variable (normal, abnormal) or multinomial variable (Grade 0, Grade 1, Grade 2, Grade 3 and above) using either logistic regression or ordinal logistic regression respectively to make treatment group comparisons.

Unsolicited AEs will be coded by MedDRA® for preferred term and system organ class. The rate and exact 95% confidence intervals of AEs in aggregate, as well as by MedDRA® categories, will be computed. The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (vaccinations and AEs), severity, relatedness, and outcome for each event.

#### *Local Laboratory Values*

Analyses of local laboratory values may include boxplots or scatterplots. These analyses will be conducted for baseline values and for values measured during the course of the study.

### **11.4.3 Evaluation of the Primary Efficacy Endpoint**

*Reduction in the incidence of chronic HCV infection associated with AdCh3NSmut1 and MVA-NSmut HCV vaccines.*

HCV RNA assays will be used to classify chronic vs. cleared infection, using the definition described in Section 3.1.2 (page 19). Based on previous analysis performed in IDU cohorts in Baltimore and San Francisco, we expect very few cases of re-infection to occur during the trial time.<sup>29,30</sup> Blood samples from individuals who demonstrate alternating positive and negative HCV RNA results, suggestive of reinfection will be analyzed to determine infection status using genotyping tests and viral sequencing where necessary to confirm infection outcome. A small number of cases may occur in which individuals will clear HCV viremia after 6 months of infection. All possible cases, including cases with uncertain classification for chronicity, will be reviewed by a blinded independent case review committee to make a determination of infection outcome.

The primary efficacy analysis will be performed in the ATP cohort, and repeated in the mITT cohort, as defined in Section 11.4.1 above. A time to event analysis will be performed as follows. For subjects in the mITT (ATP) cohort without acute HCV

infection, the 6-month chronic infection endpoint will be coded as censored, with time to event defined as the elapsed time from the first (second) vaccination, to six months post completion of follow-up for acute HCV infection (26 months), or to the first occurrence of loss to follow-up or of a criterion listed under the censoring criteria for the mITT (ATP) cohort. For subjects in the mITT (ATP) cohort with acute HCV infection, the 6-month chronic infection endpoint will be coded as an observed event if the 6-month chronic infection endpoint definition is met, and as censored otherwise, with time to event defined as the elapsed time from the first (second) vaccination to the date of the sample collection with available test result nearest to the target assessment date of six months after primary acute infection. Time to event and censoring status for 9-month chronic infection are defined analogously. The reduction in chronic HCV incidence will be calculated from the hazard ratio of a Cox model stratified by gender and IL28B status fit to the time to event data which includes a binary indicator for membership in the vaccine as compared to the placebo arm. A sensitivity analysis will be performed, in which subjects with acute infection who do not go on to develop chronic infection will be treated as experiencing a competing risk in the Fine-Gray proportional hazards regression model.

#### **11.4.4 Immunogenicity Analyses**

The primary assay to monitor vaccine immunogenicity is the IFN- $\gamma$  ELISpot, which will be performed at time points indicated in the study visit flow chart in Appendix C. A positive immune response is defined by two criteria: i) more than 48 spot forming cells per million PBMC; ii) at least three times the mean background spots per million PBMC found in ELISpot wells containing cells and peptide diluent (DMSO).<sup>39,87,88</sup> An individual will be considered a responder if a positive response to at least one in 6 mixtures (pools) of peptides is detected. Additional immunological parameters will be evaluated. These parameters include: (1) the strength of the total anti-NS immune response defined as the sum of positive responses (total SFC/million PBMC) against pools; and (2) the breadth of the immune response defined as the number of individual pools recognized by each positive subjects. The peptides recognized in the matrix will be then identified and tested individually in duplicate to confirm recognition and to measure the number of SFC produced. If additional cells remain, T-cells specific for antigen will be assessed for the ability to secrete other cytokines via intracellular cytokine staining and for the ability to respond to peptides spanning the NS region from other HCV genotypes by IFN- $\gamma$  ELISpot and potentially other tests if cells permit. All immunological parameters listed will be correlated with the presence of HCV infection and HCV persistent infection. Both cross-sectional and longitudinal analyses of this data will be performed. Analyses will consider the geometric mean level of response, and the proportion of responders. The analysis will be primarily descriptive, with tables and graphs displaying the results by treatment arm. Additionally, the null hypotheses of no difference in peak immune response, and of no difference in proportion responding, in

the vaccine versus placebo arms will be tested against two-sided alternatives at the  $p = 0.05$  level. Analysis of immunogenicity data collected will be performed at the first interim analysis and the end of the study.

#### **11.4.5 Evaluation of Exploratory Efficacy Endpoints**

1. Reduction in incidence of primary HCV infection associated with AdCh3NSmut1 and MVA-NSmut HCV vaccines

HCV RNA and and/or anti-HCV will be used to classify incident infection, using the definition described in Section 3.3 (page 20). The observed cumulative incidence of primary HCV infection in the IDU populations participating to the study has been observed at 27%/100 PYO<sup>30</sup>. Analyses will be performed in both ATP and mITT populations. The analyses will be based on comparisons of incidence of HCV infection between study groups using a log-rank test for time to midpoint in both ATP and mITT populations. A sensitivity analysis will also be performed using interval censoring methods.

2. Reduction of HCV chronic infection with a 9 month chronic cut-off associated with AdCh3NSmut1 and MVA-NSmut HCV vaccines

Previous data have shown that acute HCV infection in individuals followed for two years is cleared in 86% of cases by 6 months, but in a small number, clearance occurs after 6 months (95% at 12 months<sup>30</sup>). We will assess the reduction in incidence of chronic infection based on 9 months follow up after incident infection. HCV RNA assays will be used to classify chronic infections at 9 months using the definition described in Section 3.3 (page 20). Analysis will be performed in both ATP and mITT populations.

3. Reduction of peak concentration (magnitude) of HCV RNA associated with AdCh3NSmut1 and MVA-NSmut HCV vaccines

Quantitative measures of HCV RNA concentration will be compared between vaccinated and controls. We expect to observe a reduction of 3 log values of the maximum concentration of RNA detected in the blood in vaccinated subjects who develop HCV viremia (similar to that observed in subjects with HCV reinfection<sup>29</sup>), compared to the placebo group. Quantitative viremia measures, including the initial peak and total HCV RNA levels (described in Section 3.3 page 20) be compared, between vaccinated and placebo arms, using logarithmic IU/mL values, by Kruskal-Wallis test, and a non-parametric equality of median tests. Analysis will performed in both on ATP, and mITT populations.

4. Reduction in the duration of HCV viremia following incidence HCV infection associated with AdCh3NSmut and MVA-NSmut HCV vaccines

The duration of acute infection is expected to decrease 4-fold in vaccinated subjects who develop viremia (similar to that observed in subjects with HCV reinfection<sup>29</sup>), compared to the placebo group, after 6 months of observation following initial infection. The midpoint

between sample collection dates will be used to determine duration as previously described (Section 3.3 page 21). Time to event analyses using interval based censoring will be performed in both ATP and mITT populations.

5. Reduction in the incidence of chronic HCV infection with genotype 1, compared to other HCV genotypes associated with AdCh3NSmut and MVA-NSmut HCV vaccines

Because the vaccine is developed from HCV genotype 1b sequences, we hypothesize that we may see greater reduction in chronic infection with HCV genotype 1, compared to other genotypes as a result of stronger genotype specific immune responses: 75% reduction in chronic infection with genotype 1 compared to 45% reduction in chronic infection with non-1 genotypes. These exploratory analyses will be based on comparisons of incidence of HCV genotype 1 infection and non-1 genotypes between study groups using methods for censored survival regression (Cox proportional hazards).

## **12 Data Handling/Record Keeping/Source Documents**

The principal investigator at each site is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Forms for use as source documents will be derived from the electronic case report forms (eCRFs) and provided by the SDCC to the sites to record and maintain data for each subject enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the source documents or the discrepancies should be documented.

The sponsor will provide guidance to investigators on making corrections to the source documents and eCRFs.

### **12.1 Source Documents and Access to Source Documents**

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Forms for use as source documents will be derived from the eCRFs and be provided by the Statistical and Data Coordinating Center (SDCC).

### **12.2 Data Management Responsibilities**

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must

maintain complete and accurate documentation for the study. All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee.

The Emmes Corporation will serve as the Statistical and Data Coordinating Center for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

### **12.3 Data Capture Methods**

Clinical data (including AEs, concomitant medications, and reactogenicity data) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

### **12.4 Types of Data**

Data for this study will include safety, laboratory, and outcome measures (e.g., reactogenicity, immunogenicity, infection status).

### **12.5 Timing/Reports**

Safety data will be provided to the DSMB and investigators as per the DSMB charter. Immunogenicity reports will be provided to the investigators following preliminary analysis by the SDCC.

A final clinical study report will be prepared following the last subject visit and upon completion of assays related to the immunogenicity endpoints.

### **12.6 Study Records Retention**

Records and documents pertaining to the conduct of this study, including CRFs, source documents, ICFs, laboratory test results, and medication inventory records, must be retained in a secure storage location by the investigator for at least 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. No study records will be destroyed without prior authorization from NIAID.

## **13 Quality Control and Quality Assurance**

Following a written DMID-accepted site quality management plan, the investigational site is responsible for conducting routine, quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator(s) will provide direct access to all trial-related sites, source data/documents and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The Statistical and Data Coordinating Center (SDCC) will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

## **14 Ethics/Protection of Human Subjects**

### **14.1 Ethical Standard/Declaration of Helsinki**

The investigator(s) will ensure that this study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The investigator's Institution will hold a current Federalwide Assurance (FWA) issued by OHRP for federally funded research.

### **14.2 Institutional Review Board**

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents, by an appropriate ethics review committee or Institutional Review Board (IRB) listed on their Federal Wide Assurance. Any amendments to the protocol or informed consent materials must also be approved before they are placed into use unless change is for the safety of the subject. Only those IRB members who are independent of the investigators and the sponsor should provide an opinion on study related matters. Verification of IRB approval of the protocol and the written informed consent will be transmitted by the investigator or designee prior to the shipment of vaccine. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment unless change is for the safety of the subject.

### **14.3 Informed Consent Process**

The written informed consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice. Informed consent should be implemented before any protocol-specified procedures or interventions are carried out. Informed consent will be obtained in accordance with 21 CFR 50.25 and 45 CFR 46. Information should be presented both orally and in written form.

All subjects will sign and date the informed consent form before any study specific procedures are performed. An information sheet will also be made available to all potential subjects prior to screening. At the screening visit, the subject will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasized:

- Participation in the study is entirely voluntary



- Refusal to participate involves no penalty or loss of medical benefits
- The subject may withdraw from the study at any time
- The subject is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- The study involves research of an investigational vaccine
- There is no direct benefit for participating
- The subject's general practitioner (GP) will be contacted to corroborate their medical history

The aims of the study and all tests to be carried out will be explained. The subject will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date two copies of the ICF, one for them to take away and keep, and one to be kept by the investigator. These forms will also be signed and dated by the study staff person obtaining the informed consent.

An investigator or designee will describe the protocol to potential subjects face-to-face. The ICF may be read to the subjects, but, in any event, the investigator shall give the subjects ample opportunity to inquire about details of the study and ask any questions before the signing and dating the ICF.

Study staff must inform subjects that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for random assignment to treatment groups. Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They must also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects must be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They must be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Information will also include the foreseeable

circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects must be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or continue to participate in the trial. The extent of the confidentiality of the subjects' records must be defined, and subjects must be informed that applicable data protection legislation will be followed. Subjects must be informed that the monitor(s), auditor(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access. Subjects must be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential.

Informed consent forms must be in a language fully comprehensible to the prospective subjects. Comprehension of informed consent will be verified using a brief comprehension tool. Informed consent shall be documented by the use of a written ICF approved by the IRB and signed and dated by the subject and the person who conducted the informed consent discussion. The signature confirms that the consent is based on information that has been provided and all questions have been answered to the prospective subject's satisfaction. Each subject's signed ICF must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or the sponsor and Regulatory Compliance persons. The subject should receive a copy of the signed and dated written ICF and any other written information provided to the subjects, and should receive copies of any signed and dated ICF updates and any amendments to the written information provided to subjects.

#### **14.4 Subject Confidentiality**

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the sponsor and the subject.

The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

#### **14.5 PI Responsibility When Subject Withdraws or Is Discontinued**

See Section 5.4

#### **14.6 Referrals for HCV Treatment and Care**

See Section 8.6.

## 15 Protocol Conduct

The protocol will be conducted in compliance with federal regulations, the principles of GCP and DMID standards, policies and procedures, including the following processes:

- Protocol registration, activation, and implementation;
- Informed consent, screening, and enrollment;
- Clinical and safety assessments;
- Safety monitoring and reporting;
- Data collection and documentation;
- Study follow-up and close-out;
- Unblinding of staff and subjects;
- Quality management;
- Protocol monitoring and compliance;
- Risk reduction counseling; and
- Specimen collection, processing, and analysis.

Any policies or procedures that vary from DMID standards or require additional instructions will be described in the study Manual of Procedures.

### 15.1 Subject Confidentiality

The investigators will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor. The principal investigators will obtain a Certificate of Confidentiality from NIH to further protect subjects' privacy.

### 15.2 Data Collection, Handling, Source Documents

Clinical research data will be collected as described in detail in Section 12.

Standard GCP will be followed to ensure accurate, reliable, and consistent data collection. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH, GCP, regulatory, and institutional requirements for the protection of confidentiality of subjects.

## 15.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with Good Clinical Practice:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1 and 5.20.2d

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The Emmes Corporation's IDES.

All deviations from the protocol must be addressed in study source documents. A completed copy of the DMID Protocol Deviation (PD) Form must be maintained in the Regulatory File. Protocol deviations must be submitted to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

## 15.4 Recruitment and Retention of Study Subjects

All subjects in this study will be adult subjects who are part of the cohort maintained by the study sites.

Recruitment and retention of young adult IDUs is challenging and requires an experienced culturally sensitive operational structure. The two original sites, Baltimore (MD) and San Francisco (CA) have been studying HCV in IDUs for over 10 years each and have developed successful accrual and follow up methods. Both established study sites have experience recruiting and retaining IDUs in prospective studies. An urban IDU cohort has been established in Baltimore under the supervision of Dr. Cox to create and maintain a cohort of persons at risk for acute HCV infection. This cohort will be continued and expanded. The infrastructure was originally established during parallel research on injection drug use that was funded by CDC and NIDA under the leadership of several principal investigators in the Bloomberg School of Public Health.<sup>89-93</sup> At the San Francisco study site, prospective studies (under the collective name of The UFO Study) of young IDUs at risk of HCV and with acute HCV have been ongoing for 10 years.<sup>30,94</sup> The study is directed and supervised by Dr. Page; and currently has over

100 high risk HCV negative subjects in active follow up. The infrastructure is in existence, including trained personnel and a community-clinic based research site. Dr. Page has recently initiated studies of HCV in young adult users in New Mexico where there is a significant HCV epidemic. The clinical study site and supporting laboratory infrastructure for conducting the study is located in Albuquerque, NM, proximal to the University of New Mexico Health Sciences Center.

The Co-Principal Investigators are leaders in the field of acute HCV research in the US and both have been studying HCV infection in injection drug users and other at-risk populations for over 10 years. They have extensive professional networks to which they refer research participants for care, including HCV treatment, vaccinations and primary care, drug treatment, mental health, housing, and other health resources. These investigators have extensive experience working with, and in referral and treatment of, HCV in injection drug users. They have working relationships with local providers who can evaluate, assess available resources for care, and treat HCV, including acute HCV, on a case-by-case basis. Both investigators are leaders in advocacy for access to HCV testing and treatment in injection drug users and other at-risk populations. In addition, Dr. Cox is an expert in the treatment of acute HCV infections and clinically manages therapy of HCV in IDUs.

We estimate that 2160 persons will need to be screened to enroll the target study population size of 540 (screening: enrollment ratio, 4:1); corresponding to screening 70 subjects/month and enrolling 17.5/month, corresponding to per site screening of 23 subjects/month and enrollment of 6/month. The San Francisco study had an average screening of 45/month and enrollment of 25/month during peak operations and operating study site three nights per week (screening for and enrolling only anti-HCV negative subjects). Baltimore has had similar accrual numbers. These estimates are based on a previous intervention study conducted with HCV negative IDUs (the DUIT Study<sup>95</sup>). The vaccine trial will include resources for enhanced outreach to achieve the required 90% follow-up rate including: monthly visits, expanded clinic hours, and subject reimbursement, facilitated by a subject tracking database to ensure targeted rates.

All procedures for retention are described during informed consent. Retention is a coordinated activity involving several groups within the study team, including POWs, subject advocates, physicians, pharmacists, and counselors. Once a subject enrolls in this study, the study site will make every effort to retain him/her in follow-up to minimize possible bias associated with loss-to-follow-up. This study aims to achieve a minimum of 90% retention. Retention procedures and methods will include:

- (1) Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit, and at study termination.

- (2) Compilation of detailed contact tracing and locator information at the study screening visits, and active review and updating of this information at each subsequent visit.
- (3) Use of mapping techniques to establish the location of subject residences and other locator venues.
- (4) Use of appropriate and timely visit reminder mechanisms.
- (5) Immediate and multi-targeted follow-up on missed visits.
- (6) Use of trained outreach workers to complete in-person contact with subjects at their homes and/or other community locations.
- (7) The study site will have a subject tracking database to facilitate visit scheduling and timely identification and follow-up on missed visits. The study team will generate weekly reports on the number and percentage of subjects completing the follow-up visits throughout the course of the study. Members of the Protocol Team will track retention rates closely and work with the study site as needed to take any required action to address below-target retention rates.

The key role for POWs in retention: Retention of IDU in prospective studies is extremely challenging and at all three sites, significant effort will be made to maximize retention. Every enrolled subject will be assigned to a POW, in accordance to a POW's geographical coverage during fieldwork. It is estimated that there will be approximately 50 subjects assigned to each POW. During enrollment, the counselor will notify the subject that he will be assigned a POW and the counselor will give the subject the POW's name. POWs will verify all the addresses of the subjects. Throughout the study, the recruitment coordinator or POW or their designee will remind his/her subjects by e-mail, text short message service (SMS) messaging, or by telephone of their upcoming appointments and invite them to any activities hosted at the study sites. Retention and follow-up rate will be assessed and reported periodically. Study coordinating meetings will include review and discussion of recruitment and retention, with case reviews and problem solving aimed at maximizing both.

## **15.5 Study Subject Reimbursement**

Reimbursement of study subjects for attendance at study visits is at the discretion of each study site. Reimbursement should be comparable to the reimbursement offered for similar research in the local community, if possible. The study site is encouraged to confer with its local resources (including Community Advisory Boards, and other researchers) in deciding appropriate reimbursement.

The study informed consent submitted to the site IRB/IEC will state the plan for reimbursement (if any). The DMID relies upon local IRBs to determine whether the proposed plan for reimbursement meets ethical requirements in the local context. The

exact amounts may be modified during the course of the study in consideration of changes in costs such as bus fares, exchange rates, child care, or other factors that affect the ability of a subject to comply with study visit requirements. Reviewing IRBs must be made aware of the changes in reimbursement before they occur. Study subjects will not be charged for study injections, research clinic visits, research-related examinations, or research-related laboratory tests.



## 16 Publication Policy

All manuscripts resulting from this trial will be reviewed by representatives from the site(s), DMID and the product owner(s) (Okairos). Each institution will have thirty days to review the publication prior to submission.

Following completion of the study, the co-principal investigators will publish the results of this research in a scientific journal. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires that all investigators submit or have submitted for them an electronic version of their final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive of the National Library of Medicine's PubMed Central upon acceptance for publication. Further the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after the official date of publication.

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The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication.<sup>96</sup> This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. This trial will be registered in NLM in accordance with the new NLM requirements under the FDAAA.

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## **SUPPLEMENTS/APPENDICES**

## Appendix A: Acceptable Ranges for Clinical Laboratory Evaluations at Screening

**NOTE:** When the site-specific laboratory normal range is available, if there is a discrepancy between the laboratory value in the Appendix and the site specific laboratory normal range, the site normal range will take precedence in eligibility determination.

<b>HEMATOLOGY</b>	
	<i>Acceptable Range</i>
Hemoglobin F (g/dl)	11.5-15.0
Hemoglobin M (g/dl)	12.5-17.0
Platelet (K)	120-499
WBCs (thou/mcl)	3.0-10.8

<b>CHEMISTRIES</b>	
	<i>Acceptable Range</i>
Creatinine <sup>A</sup>	Within or below the normal range

<sup>A</sup> Values lower than the normal range are not exclusionary.

<b>ENZYMES</b>	
	<i>Acceptable Range</i>
ALT (SGPT)	$\leq 1.25 \times \text{ULN}$

<b>URINALYSIS</b>	
	<i>Acceptable Range</i>
Glucose (mg/dl)	$\leq 1+$
Protein (mg/dl)	$\leq 1+$

## Appendix B: Toxicity Table

### ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal
Rx = Therapy	Req = Required
Mod = Moderate	IV = Intravenous
ADL = Activities of Daily Living	Dec = Decreased

**NOTE:** For any of the lab values listed below, if the Grade 1 value conflicts with the LLN value, the LLN value takes precedence and the event will not be considered a Grade 1 Adverse Event.

When the site-specific laboratory normal range is available, if there is a discrepancy between the laboratory value in the Appendix and the site specific laboratory normal range, the site normal range will take precedence in determining Grade 1 Adverse Events.

### ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

- GRADE 1: **Mild** (events require minimal or no treatment and do not interfere with the subject's daily activities.)
- GRADE 2: **Moderate** (events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning)
- GRADE 3: **Severe** (events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.)
- GRADE 4: **Life-threatening** (Any adverse drug experience that places the subject or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).

### SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

<b>HEMATOLOGY</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Hemoglobin (g/dl)				
Male:	11.0-12.4	9.6-10.9	8.3-9.5	<8.3
Female:	9.6-10.8	8.4-9.5	7.2-8.3	<7.2
Platelets (per cumm)	84,500-117,000	65,000-84,499	25,000-64,999	< 25,000
WBCs (thou/mcl)				
Lower Limit	2.5-2.9	1.9-2.4	1.0-1.8	<1.0
Upper Limit	11.9-15.1	15.2-21.6	21.7-25.0	>25

<b>CHEMISTRIES</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	<2.0 mEq/L or abnormal potassium <i>with</i> paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	>7.0 mEq/L or abnormal potassium <i>with</i> life-threatening arrhythmia
Hyperglycemia (nonfasting and no prior diabetes) <b>NOTE:</b> (normal range for Glucose, Random, Serum is 60-199 mg/dL for one site laboratory)	200 - 274 mg/dL	275 - 349 mg/dL	350 - 499 mg/dL	>500 mg/dL or abnormal glucose <i>with</i> ketoacidosis or seizures
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate <i>with</i> life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	>1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	>3.0 x ULN

<b>CHEMISTRIES cont.</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	>10 x ULN
Creatinine*	1.2 - 1.5 x ULN	>1.5- 2 x ULN	>2 x ULN	Dialysis required
CPK	2.0-3.4 x ULN	3-5.9 x ULN	6-9.9 x ULN	≥10 x ULN

\* Use age and gender appropriate values

<b>ENZYMES</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
AST (SGOT)	>1.25 -2.5 x ULN	>2.5 – 4 x ULN	>4 -8 x ULN	>8 x ULN
ALT (SGPT)	>1.25 - 2.5 x ULN	>2.5 – 4 x ULN	>4 -8 x ULN	> 8 xULN
LDH	>1.25 - 2.5 x ULN	>2.5 – 4 x ULN	>4-8xULN	> 8 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	>8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	>8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	>5.0 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	>5.0 x ULN

<b>URINALYSIS</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Proteinuria	1+ or 200 mg – 1 gm loss/day	2-3 + or 1-2 gm loss/day	4+ or 2-3.5 gm loss/day	Nephritic syndrome or >3.5 gm loss/day
Hematuria	Microscopic only <10 rbc/hpf	Gross, no clots >10rbc/hpf	Gross, with or without clots, OR red blood cell casts	Obstructive or required transfusion
Glucose	1+	2+	3+	4+

<b>CARDIOVASCULAR</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Cardiac Rhythm	Not defined	asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrhythmia; hospitalization and treatment required

<b>CARDIOVASCULAR cont.</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Hypertension	Asymptomatic transient (<24 hrs) increase >20 mmHg in diastolic or to >140/90 and ≤150/95 mmHg; no treatment	recurrent or persistent (>24 hrs) or symptomatic increase >20 mmHg in diastolic or to >151/96 and ≤155/100 mmHg; monotherapy may be indicated	requiring more than one drug or more intensive therapy than previously or to >155/100 mmHg	Life-threatening consequences (e.g. hypertensive crisis)
Hypotension	non applicable	Symptomatic corrected with oral fluids	Symptomatic IV fluids indicated	Shock—requiring use of vasopressors or mechanical assistance to maintain blood pressure
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; >3 units transfused

<b>SKIN</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	<25mm	25 - 50 mm	>50mm	
Erythema	<25mm	25 - 50 mm	>50mm	
Edema	<25mm	25 - 50 mm	>50mm	
Rash at Injection Site	<25mm	25 - 50 mm	>50mm	
Regional lymphadenopathy	Soft/spongy	Firm	Hard	

<b>SKIN cont.</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	

<b>SYSTEMIC</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	Anaphylaxis
Fever: oral	38.0 – 39.0 °C 100.4 F - 102.2 °F	>39.0 – 40.0 °C 102.3F - 104.0 °F	>40.0 °C >104.0 °F	

<b>MUSCULOSKELETAL</b>				
	<i>Grade 1</i>	<i>Grade 2</i>	<i>Grade 3</i>	<i>Grade 4</i>
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling – and interfering with activities of daily living	permanent and/or disabling joint destruction



**Appendix C: Study Visit Chart**

<b>Arms A and B</b>	<b>S</b>	<b>V</b>					<b>V</b>							
<b>Timeline from enrollment (months)</b>	<-1	0	0	0	0	1	2	2	2	3	4	5	6	7
<b>Timeline from enrollment (days)</b>	<-30	0	3	7	14	30	56	59	63	90	120	150	180	210
<b>Window (days)</b>	-30	0	±2	±2	±7	±7	±7	±2 <sup>C</sup>	+14 <sup>C</sup>	±7	±7	±7	±7	±7
<b>Visit Number</b>	00	1	2	3	4	5	6	7	8	9	10	11	12	13
Review Inclusion/Exclusion criteria	X	X					X							
Informed consent	X													
Behavioral Risk Assessment Questionnaire		X								X			X	
Medical history	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Examine vaccination site		X	X	X	(X)		X	X	X					
Targeted Physical Examination	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Vital Signs (Temp, Pulse, BP)	X	X					X							
Height/Weight	X													
Pregnancy Test*	X	X					X							
Counseling on avoidance of HIV, HCV, and pregnancy	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>
Vaccination		X					X							
Distribute Memory Aid		X					X							
Review Memory Aid			X	X				X	X					
AE/SAE Assessment		X	X	X	X	X	X	X	X	X	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>
Urinalysis	X													
Hematology (mL)	9	9			9	9	9		9	9	9			
Biochemistry (mL)	14	14			14	14	14		14	14	14			
IL28B (mL)	5													
Anti-HCV (mL)	5													
HBsAg, anti-HIV (mL)	5													
HCV qualitative viremia (mL)	5					5	5			5	5	5	5	5
HCV quantitative viremia (mL)	5					5	5			5	5	5	5	5
Anti-adenovirus Ab (mL)		X <sup>D</sup>												
HLA typing/genetic studies (mL)		8												
Immunology (mL)		160				160	100		100	160				
Blood per visit (mL)	48	191	0	0	23	193	133	0	123	193	33	10	10	10
Cumulative blood volume (mL)	48	239	239	239	262	455	588	588	711	904	937	947	957	967

<b>Arms A and B (continued)</b>														
<b>Timeline from enrollment (months)</b>	8	9	10	11	12	13	14	15	16	17	18	19	20	
<b>Timeline from enrollment (days)</b>	240	270	300	330	360	390	420	450	480	510	540	570	600	
<b>Window (days)</b>	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
<b>Visit Number</b>	14	15	16	17	18	19	20	21	22	23	24	25	26	<b>Early Termination</b>
Review Inclusion/Exclusion criteria														
Medical history	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Examine vaccination site														
Targeted Physical Examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Behavioral Risk Assessment Questionnaire		X			X			X			X		X	X
Counseling on avoidance of HIV, HCV, and pregnancy	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X <sup>B</sup>	X or X <sup>B</sup>
AE/SAE Assessment	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X <sup>A</sup>	X
Hematology (mL)			9										9	9
Biochemistry (mL)			14										14	14
Anti-HCV (mL)													5	5
HBsAg, anti-HIV (mL)													5	5
HCV Qualitative viremia (mL)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
HCV Quantitative viremia (mL)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Anti-adenovirus Ab (mL)					10									
Immunology (mL)	100												100	100
Blood per visit (mL)	110	10	33	10	20	10	10	10	10	10	10	10	143	143
Cumulative blood volume (mL)	1077	1087	1120	1130	1150	1160	1170	1180	1190	1200	1210	1220	1363	

S, screening visit;

V, vaccination visit;

(X), if considered necessary, emphasizing any acute complaint;

A, only SAE's collected;

B, All concomitant medications will be recorded through Day 90 (Day 30 if only receiving 1 vaccination).

At visits after Day 90 (Day 30 if only receiving 1 vaccination), only immunosuppressants and medications for the treatment of HCV infection will be recorded;

C, windows dependent on Visit 6, Dose 2

D, Plasma saved from the blood collected for immunology assessments at this visit will be used for anti-adenovirus Ab testing.

\* Pregnancy test must be performed and documented as negative within 24 hours prior to each vaccination.

**Appendix D: Study Visit Chart – Confirmed HCV Infection**

	HCV + test										
Timeline from HCV infection (months)	0	1	2	3	4	5	6	7	8	9	
Timeline from first positive HCV Quantitative RNA result (days)	0	30	60	90	120	150	180	210	240	270	
Window (days)		±7	±7	±7	±7	±7	±21	±7	±7	±21	
Visit Number		H01	H02	H03	H04	H05	H06	H07	H08	H09	Early Termination <sup>G</sup>
Review confirmed HCV infection		X	X	X	X	X	X	X	X	X	X
Medical history		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Examine vaccination site		(X) <sup>C</sup>									
Targeted Physical Examination		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Behavioral Risk Assessment Questionnaire		X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>	X <sup>D</sup>
Concomitant Medications		X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>	X <sup>E</sup>
AE/SAE Assessment		X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>	X <sup>F</sup>
Counseling on avoidance of HIV, HCV transmission and reinfection		X	X	X	X	X	X	X	X	X	X
Hematology (mL)		9	9	9	9	9	9	9	9	9	9
Biochemistry (mL)		14	14	14	14	14	14	14	14	14	14
HCV genotyping (mL)		5	5	5	5	5	5	5	5	5	5
Anti-HCV (mL)										5	5
HBsAg, anti-HIV (mL)		5								5	
HCV Quantitative viremia (mL)		5	5	5	5	5	5	5	5	5	5
Immunology (mL)		100	100	100	100	100	100	100		100	100
Blood per visit (mL)		138	133	133	133	133	133	133	33	143	138
Cumulative blood volume (mL) post HCV Infection		138	271	404	537	670	803	936	969	1112	

(X), if considered necessary, emphasizing any acute complaint;

C, Vaccination site is only to be examined at Day 59/63;

D, For confirmed HCV infected subjects Behavioral Risk Assessment Questionnaire is to be administered at Day 90, 180, 270, 360, 450, and 540 (and Early Termination Visit, if conducted) if applicable, as the follow-up period allows; if the subject's follow-up period is extended as a result of confirmed HCV infection, the Behavioral Risk Assessment Questionnaire will be administered at Day 630, 720, 810, and the final study visit, as the follow-up period allows.

E, All concomitant medications will be recorded through Day 90 (Day 30 if only receiving 1 vaccination). At visits after Day 90 (Day 30 if only receiving 1 vaccination), only immunosuppressants and medications for the treatment of HCV infection will be recorded;

F, AE's and SAE's recorded through Day 90. At visits following Day 90, only SAE's recorded.

G, Subjects who become HCV-infected prior to the 2nd dose of vaccine and choose to terminate early will follow the full schedule of tests for visit H09.

## Appendix E: Alcohol Use Disorders Identification Test (AUDIT) C test

### AUDIT-C- Overview

The AUDIT-C is a 3-item alcohol screen that can help identify persons who are hazardous drinkers or have active alcohol use disorders (including alcohol abuse or dependence). The AUDIT-C is a modified version of the 10 question AUDIT instrument.

### Clinical Utility

The AUDIT-C is a brief alcohol screen that reliably identifies patients who are hazardous drinkers or have active alcohol use disorders.

### Scoring

The AUDIT-C is scored on a scale of 0-12.

Each AUDIT-C question has 5 answer choices. Points allotted are:

A = 0 points, b = 1 point, c = 2 points, d = 3 points, e = 4 points

- **In men**, a score of 4 or more is considered positive, optimal for identifying hazardous drinking or active alcohol use disorders.
- **In women**, a score of 3 or more is considered positive (same as above).
- However, when the points are all from Question #1 alone (#2 & #3 are zero), it can be assumed that the patient is drinking below recommended limits and it is suggested that the provider review the patient's alcohol intake over the past few months to confirm accuracy.<sup>1</sup>
- Generally, the higher the score, the more likely it is that the patient's drinking is affecting his or her safety.

### Psychometric Properties

For identifying patients with heavy/hazardous drinking and/or Active-DSM alcohol abuse or dependence:

	<b>Men<sup>2</sup></b>	<b>Women<sup>3</sup></b>
$\geq 3$	Sens: 0.95 / Spec. 0.60	Sens: 0.66 / Spec. 0.94
$\geq 4$	Sens: 0.86 / Spec. 0.72	Sens: 0.48 / Spec. 0.99

For identifying patients with active alcohol abuse or dependence:

>3	Sens: 0.90 / Spec. 0.45	Sens: 0.80 / Spec. 0.87
>4	Sens: 0.79 / Spec. 0.56	Sens: 0.67 / Spec. 0.94

<sup>1</sup> Frequently Asked Questions guide to using the AUDIT-C can be found via the website:

<http://www.queri.research.va.gov/tools/alcohol-misuse/alcohol-faqs.cfm>

<sup>2</sup> Bush K, Kivlahan DR, McDonnell MB, et al. The AUDIT Alcohol Consumption Questions (AUDIT-C): An effective brief screening test for problem drinking. *Arch Internal Med.* 1998 (3): 1789-1795.

<sup>3</sup> Bradley KA, Bush KR, Epler AJ, et al. Two brief alcohol-screening tests from the Alcohol Use Disorders Identification Test (AUDIT): Validation in a female veterans affairs patient population. *Arch Internal Med Vol 163, April 2003: 821-829.*

**AUDIT-C Questionnaire**

Patient Name \_\_\_\_\_ Date of Visit \_\_\_\_\_

Now I'm going to ask you about your use of alcohol beverages during this past year.

1. How often did you have a drink containing alcohol?

0	1	2	3	4	-8	-9
never	monthly or less	2 to 4 times/month	2 to 3 times/week	4 or more times/week	Don't Know	Decline

2. How many drinks containing alcohol do you have on a typical day when you are drinking?

0	0	2	3	4	5	-8	-9
none	1 or 2	3 or 4	5 or 6	7 to 9	10 or more	Don't Know	Decline

3. How often do you have six or more drinks on one occasion?

0	1	2	3	4	-8	-9
never	less than monthly	monthly	weekly	daily or almost daily	Don't Know	Decline