DNA Vaccine for Chronic Hepatitis C

: Preclinical Evaluation of Immunogenicity and Safety of VGX-6150

Moonsup JEONG, Ph.D.
Director of Pharma R&D Division
GeneOne Life Science
Chronic Hepatitis C

- 130~170 million people are chronically infected with HCV (WHO statistics)
  - 2.2~3.0% of the world’s population

- An estimated 3~4 million people are infected with HCV yearly
  - 75~85% will develop chronic HCV infection
  - 60~70% will develop chronic liver disease
  - 5~20% will develop cirrhosis
  - 1~5% will die from cirrhosis or hepatocellular carcinoma

  *In total, 350,000 individuals die annually from HCV-related disease

- HCV infection is the leading cause for liver transplantation (CDC)
Medical Unmet needs

- Recent treatment (Direct-Acting Antiviral Drugs)
  - Improved SVR rates of 90% in treatment naïve patients

- Medical unmet needs
  - Unaffordable
    : High price ($1,000/dose for Sovaldi (Gilead), total $84,000) per patient
  - Unavailable
    : up to 75% of patients chronically infected with HCV are unaware of infection, precluding from treatment until diagnosed
  - Least effective in patients with advanced liver disease
  - Associated with the development of viral resistance
  - Do not provide protection from re-infection

» Vaccine approach would be considered the most efficacious and cost-effective means
Ideal Vaccine for chronic hepatitis C

- Rationale for T cell vaccine to prevent and treat chronic hepatitis C
  - T cells play a critical role in viral control during primary infection
  - Keeping CD8+ T cells and CD4+ T cells continuously to remove persistent viremia
    → viral eradication and effectively representing long-term clinical cure

- Effective vaccine against HCV
  - not need to provide sterilizing immunity
  - recapitulate and accelerate the immune pathway followed in natural infection
  - “HCV T cell vaccine”
DNA vaccine

Definition

Vaccines for protecting an organism against disease or curing disease by injecting it with genetically engineered naked DNA, plasmid, to produce an immunological response.
DNA vaccine delivery by Electroporation

How Electroporation Delivers DNA Vaccines

1. Syringe and needle electrodes are inserted into selected muscle tissue, and the DNA vaccine is injected.

2. Controlled, milli-second electrical pulses are applied to the needle electrodes, which then form an electric field.

3. Temporary openings in the cell membrane are created by the electrical field, allowing significantly greater amounts of the DNA vaccine to enter cells.

4. The trapped DNA enables cells to produce antigen designed to control cancer & chronic infectious diseases such as HIV. The antigen can also trigger antibody production to prevent diseases.

Characteristics of DNA vaccine delivered by electroporation

- Vaccine toxicity is virtually eliminated
- Early and strong vaccine-specific immune response as evidenced by multiple non-human primate and clinical trials
  : Inducing robust, broad and poly-functional T cell immune responses
- Stable at ambient temperature
- Easy to manipulate
- Quick and inexpensive production
- Multiple immunizations to boost immune response without concerns of pre-existing immunity

DNA based immunization is an attractive vaccine modality for HCV
Antigen expressing plasmids

- **Target antigens**
  
  **HCV Genome**
  
  - **Genotype**: 1a/1b, 70% of all chronic infection
  - **Type of antigens**: 90% of non-structure proteins, 62% of HCV genome
  - **Improving immune response for antigens**
    - Consensus sequence of antigen
    - Engineering antigen gene
    - Codon/RNA optimization, IgE Leader sequence
    - Electroporation delivery

* Backbone: Modified pVAX1 (Invitrogen)
Non-human primate Feasibility Study

- Immunization induces strong and broad HCV specific IFNγ response

<table>
<thead>
<tr>
<th>IMMUNIZATION: I.M. + E.P.</th>
<th>ANTIgen</th>
<th>DOSE (MG)</th>
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<td>pcONNS3/4A</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>pcONNS4B</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>pcONNS5A</td>
<td>1.0</td>
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<tr>
<td>pcONNS5B</td>
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<td></td>
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<tr>
<td>TOTAL</td>
<td>4.0</td>
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- Antigen specific IFNγ ELIspot Analysis

- Strong and broad immunogenicity

- Difference in immuno-dominant domains among outbred monkeys → importance of broad immunity in individuals having genetic diversity

Rhesus macaques (Macaca mulatta)
Non-human primate Feasibility Study

- Intercellular cytokine staining

- Th1 or CTL cells were counted if produced one or more IFNγ, TNFα, or IL-2

- Effective CD4+ and CD8+ T cells activation

- Multi-antigenic vaccination is needed

HCV specific effective CD4+ and CD8+ T cells are elicited by immunization
Non-human primate Feasibility Study

- **Vaccine-induced HCV specific cytolytic CTL remain detectable 6 weeks beyond immunization**

  - Putative cytolytic CTL: positive staining for both surface CD107a and intercellular granzyme B (GrzB)
  - CD8+ T cell activation associated with acute resolution of HCV infection
  - GrzB production in response to antigen stimulation
  - Strong circulatory HCV-specific CTL
Non-human primate Feasibility Study

- Immunization gives rise to circulatory effector memory like CTL

  - Memory-like CD8+ T cells were characterized:
    - CD54RA negative staining,
    - CD27 staining

  - Effector Memory (EM: CD54RA-/CD27-),
    - Central Memory (CM; CD45RA-/CD27+)

  - Phenotype similar to Memory T cells remained detectable in circulation of all monkeys
Conclusions of monkey study

- Immunization gave rise to IFNγ-secreting CD8+ T cells
  → immunological determinant highly associated with acute HCV clearance
- Upon recruitment of HCV-specific T cells to the liver late in acute infection, HCV replication is strongly inhibited due to IFNγ secretion
  → effective means of controlling HCV replication w/o tissue damage
- Significant frequencies of circulating cytolytic CTL, and significant increase of GrzB production within CD8+ population
- HCV specific memory T cells have been shown to expand and could control secondary infection in human acute resolvers
  → crucial for protection against reinfection

⇒ Supporting clinical investigation of our HCV DNA vaccine
VGX-6150 Drug Product

- **Composition**
  Mixture of pGX8005, pGX8006, pGX8007 and pGX6005 with concentration of 10 mg/mL in 1 mL

- **One vial contains**

<table>
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<tr>
<th>Components</th>
<th>Description</th>
<th>Quantity</th>
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<tr>
<td>pGX8005:</td>
<td>plasmid DNA expressing HCV NS3/4A</td>
<td>3mg</td>
</tr>
<tr>
<td>pGX8006:</td>
<td>plasmid DNA expressing HCV NS4B</td>
<td>3mg</td>
</tr>
<tr>
<td>pGX8007:</td>
<td>plasmid DNA expressing HCV NS5A</td>
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<tr>
<td>pGX6005:</td>
<td>plasmid DNA expressing human IL-28B</td>
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<tr>
<td>Formulation</td>
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Immune-Adjuvant

■ Target cytokine

- IL-28B
  - Type III Interferon (recently discovered, 2003)
    : IL-29 (IFN λ1), IL-28A (IFN λ2), IL-28B (IFN λ3)
  - Produced by DCs in response to viral proteins or Toll-like receptor agonist
  - Same signaling pathways as type I IFNs to induce expression of IFN-responsive genes
    → inhibiting replication of various viruses (HBV, HCV)

- Immuno-Adjuvant function
  - Typical T cell-stimulating better than cytokine IL-12
  - Good T cell benefit in antigen-specific IFN γ production
  - Significant impact on long-lived antigen specific immune responses in CD8+ T cells (monkey study)
VGX-6150-01 clinical study synopsis

Summary (ClinicalTrials.gov Identifier: NCT02027116)

<table>
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<tr>
<th>Title of study</th>
<th>Multi-center, open-label, dose-escalation, phase I trial to evaluate the safety, tolerability and immunogenicity of VGX-6150 as second-line therapy in chronic hepatitis C patients</th>
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</table>
| Principal Investigators               | Sang-Hoon Ahn, MD, PhD, Severance Hospital  
                                 Jeong Heo, MD, PhD, Pusan National University Hospital |
| Sponsor                               | GeneOne Life Science, Inc. |
| Endpoints                             | 1. Primary endpoints  
    - Adverse Events (NCI-CTCAE 4.0, Lab test, Vital signs and ECG)  
    - Pain during vaccination (VAS)  
  2. Secondary endpoints  
    - Immunological response  
      • Antigen specific IFN-γ secreting T-cell response  
      • CD4+, CD8+ T-cell response,  • Antigen-specific IgG titer  
    - Virologic response  
      • Serum HCV-RNA level |
VGX-6150-01 clinical study synopsis

- **Study flow per subject**
  - Viral load
  - Humoral/Cellular immune response
  - Week: 0, 4, 8, 12, 16, 28, 36
  - Vaccinations with DNA and *in vivo* EP
  - F/U 24 weeks
  - Termination of clinical study

- **Dose escalation**
  - **Cohort 3** (6mg/dose)
    - N=6
    - S - V1 - V2 - V3 - V4 - VF1 - VF2 - VF3(T)
  - **Cohort 2** (3mg/dose)
    - N=6
    - 4wks
    - S - V1 - V2 - V3 - V4 - VF1 - VF2 - VF3(T) 24 weeks
  - **Cohort 1** (1mg/dose)
    - N=6
    - 4wks
    - S - V1 - V2 - V3 - V4 - VF1 - VF2 - VF3(T) 24 weeks
**VGX-6150-01 Follow-on clinical study synopsis**

### Study flow per subject

- **Viral load**
- **Humoral/Cellular immune response**

**Screening**

Week 0, 4, 8, 12, 16, 28, 36, +4, +12, +24

F/U 24 weeks (Main study)

F/U 24 weeks (Follow-on study)

### Overall schedule

- **Cohort 3**
  - (6mg/dose)
  - N=6

- **Cohort 2**
  - (3mg/dose)
  - N=6

- **Cohort 1**
  - (1mg/dose)
  - N=6

Follow on study (6mg/dose)

V1 - V2 - V3 - V4 - VF1 - VF2 - VF3(T)

V1 - VF1 - VF2 - VF3(T)

same day
Conclusions

- Our HCV DNA vaccine, VGX-6150, in non-primate study demonstrated strong immunogenicity, broad T cell reactivity, and T cell phenotypes closely associated with protection against chronic HCV infection.

- VGX-6150 showed a favorable safety profile and was judged as well-tolerated.

- VGX-6150 induced robust and strong T cell immune response dose-dependently.

- IL-28B expressed from plasmid enhanced antigen specific T cell immune response more strongly as immune adjuvant.

- We are now conducting phase I clinical study against chronic hepatitis C patients who failed SOC and DAA in Korea.

- We are also preparing phase I clinical study in health adults as preventive vaccine against chronic hepatitis C in Korea.
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