TM7SF3 as a Potential Liver Cancer Stem Cell Marker

DNA vaccine against Chronic hepatitis B: From bench to clinic

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POSTECH
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Origin of Cancer Stem Cells

- mutational transformation of normal stem cells
- mutations that cause differentiated cells to acquire properties of CSCs, such as self-renewal potential

Nat Rev Cancer. 2003 Dec;3(12):895–902
"maturation arrest of tissue-determined stem cells" hypothesis

Defined the hallmark properties of stem cells:
The ability to self-renew & differentiate

The first CSCs in a solid tumor:
- Breast cancer stem cells (CD44^+ CD24^-/low);
- A CSC population in brain tumors (CD133^+)

CSC population identified in the skin (CD20^+)

Milestones in a concept of cancer as a stem cell disorder

1855      1937    Late 1960s–70s  1988  1994  2003 ‘04 ‘05 ‘06 ‘07 ‘08

"embryonal-rest" hypothesis

Leukemia can be transmitted from one mouse to another using a single undifferentiated leukemia cell

Mice as an assay model for human hematopoietic cells

CSC population identified in the prostate (CD44^α2 β1^+ CD133^+)

CSC population identified in the colon (CD133^+ EpCAM^+ CD44^+CD166^+)
Pancreas (CD44^+CD24^-EpCAM^+)
Liver (CD133^+) and head/neck (CD44^+)

The first CSCs:
- Leukemic Stem cells (in patients with AML (CD34^+ CD38^-)
Liver CSCs & Their Cellular Origins

Bone marrow stem cell

Small, proliferating cells w/ oval nuclei

Bile duct cell

Liver CSC

Hepatocyte

Dedifferentiation

Oval/progenitor cell

Hepato-pancreas stem cell

• CSCs are difficult to treat with conventional methods because of their chemo-resistant and radio-resistant properties (high levels of expression of anti-apoptotic genes & ABC transporters).

>>> More definitive CSC-specific markers are required to completely eliminate the self-renewing stem cells.

### Putative Liver CSC Markers – Membrane Proteins

#### Phenotypic Expression of Human LPCs, Cell Lineages, HCC and Putative Liver Stem Cells

<table>
<thead>
<tr>
<th>Protein</th>
<th>Liver Progenitor Cells (LPCs)</th>
<th>Cholangiocyctic Lineage</th>
<th>Hepatocyctic Lineage</th>
<th>HCC</th>
<th>Putative Liver Cancer Stem Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>EpCAM (TACSTD1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Diff.</td>
<td>+/-</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ABCG2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>KIT</td>
<td>+/-</td>
<td>ND</td>
<td>ND</td>
<td>Diff.</td>
<td>+/-</td>
</tr>
<tr>
<td>CD133 (PROM1)</td>
<td>+/-</td>
<td>ND</td>
<td>-</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>CD90 (THY1)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>CD44</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>SMO</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Diff.</td>
<td>+</td>
</tr>
<tr>
<td>GCTM-5</td>
<td>+</td>
<td>low</td>
<td>-</td>
<td>ND</td>
<td>+/-</td>
</tr>
<tr>
<td>MET</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>Diff.</td>
<td>ND</td>
</tr>
<tr>
<td>NCAM1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD34</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>Low</td>
<td>+/-</td>
</tr>
<tr>
<td>CD45</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined/reported; +, positive expression; -, negative; + +, high/over expression; Low expression; diff., positive differential (low or high) expression; +/-, contradictory supporting evidence

**Marked: Reported markers of CSCs**

- **SMO (Smoothened):** No study published on its direct potential as a Liver CSCs; highly expressed in isolated CD133+ CSCs
What is DNA vaccination?

- Direct introduction (i.m.; i.d.; g.g.) of a plasmid DNA into host
  (Host functions as a vaccine factory)
Action mechanism of DNA vaccine

Direct transfection of myocytes

Direct transfection of APC

Afferent lymphatic vessel

Draining lymph node
Direct and cross presentation of antigens in the draining lymph node

Efferent lymphatic vessel

Activated lymphocytes

MHC I

Myocyte

Shed exogenous antigens

Apoptotic or Necrotic bodies

Exogenous antigens

MHC I

MHC II

DNA inoculation induced reporter protein expression in muscle cells

Mice injected with plasmid DNA encoding hGH elicited Ag-specific antibody responses


Early history of DNA vaccine

![Graph showing hGH precipitation per ul serum over days 0 to 131 for different groups A, B, and C.]
DNA vaccine (H1N1) protected mice against H3N2 Influenza virus infection

Early history of DNA vaccine

- V1-NP DNA
- Blank vector
- Uninjected control

Ulmer et al, Science, 1993, 259:1745
Vaccine strategies

- Recombinant subunit
- Synthetic peptide
- Naked DNA
- New & improved technologies
  - Live recombinant (viral, bacterial)
  - Whole inactivated virus
  - Live attenuated virus
<table>
<thead>
<tr>
<th>Characteristics of DNA vaccine</th>
<th>Subunit &amp; Killed Vaccine</th>
<th>Live attenuated vaccine</th>
<th>DNA vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td>Safe*</td>
<td>Potential to revert to pathogenicity; immuno-compromised person?</td>
<td>Safe (No risk of infection &amp; high purity of DNA vaccine)</td>
</tr>
<tr>
<td>CTL response</td>
<td>None</td>
<td>Strong CTL responses</td>
<td>Progressing</td>
</tr>
<tr>
<td>Antibody response</td>
<td>Strong Ab response</td>
<td>Strong Ab response</td>
<td>Needs improvement</td>
</tr>
<tr>
<td>Storage</td>
<td>Refrigeration</td>
<td>Refrigeration</td>
<td>Room temperature; no cold chain required</td>
</tr>
<tr>
<td>Cost of vaccine</td>
<td>Expensive</td>
<td>Moderate to expensive</td>
<td>Relatively inexpensive (easier to manipulate, manufacture, &amp; formulate)</td>
</tr>
</tbody>
</table>
## Potential concerns regarding DNA vaccination

<table>
<thead>
<tr>
<th>Theoretical issues</th>
<th>Concern</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration</td>
<td>Insertional mutagenesis: chromosomal instability, development of autoimmune disorders</td>
<td>US FDA requires integration studies for new DNA products</td>
</tr>
<tr>
<td>Auto-immunity</td>
<td>Development of autoimmune disorders</td>
<td>No anti-nuclear or anti-DNA antibodies have been detected yet</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td>Antibiotic resistance gene is transferred to patients?</td>
<td>Antibiotics are not commonly used to treat human infections</td>
</tr>
<tr>
<td>Low efficacy</td>
<td>Eliciting low levels of T cell and B cell memory</td>
<td>Use novel formulations, adjuvant,s and delivery systems</td>
</tr>
</tbody>
</table>

Michele A. K et al, Nat Rev Genet. 2008, 10: 776-88
Growing interest in the DNA platform

Plasmid DNA vectors make up approximately 27% (354 out of 1,311 trials) of all gene-therapy vector platforms studied in Phase I to Phase III trials in 2007 (4% before 1998).

DNA vaccine-derived mAb Production Scheme

Ag gene → plasmid → CHO cells ( > 6 months ) → Protein purification
Bacteria ( > 1 month )

CHO cell → Fusion with myeloma → spleen → Polyclonal Ab

Strong vector & adjuvant
Hydrodynamic injection

Evaluation of mouse mAb → Sequence analysis & CDR Grafting for humanization

Therapeutic or agonistic mAb

Biomarkers for diagnosis

Strong vector & adjuvant
Hydrodynamic injection

Cell-based screening of hybridoma by FACS
### Comparison of Vaccination Technologies for mAb Production

<table>
<thead>
<tr>
<th></th>
<th>Peptide Vaccination</th>
<th>Protein Vaccination (E.coli)</th>
<th>Protein Vaccination (CHO)</th>
<th>Gene-based Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein purification</td>
<td>Yes</td>
<td>Yes (&gt; 6 months)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antibodies to impurities</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antibody titer</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Recognition of native form of protein</td>
<td>+/-</td>
<td>+/-</td>
<td>+++ (Diagnosis &amp; Therapy)</td>
<td>+++ (Diagnosis &amp; Therapy)</td>
</tr>
</tbody>
</table>

Gene-based vaccine is efficient for generating mAb to multi-pass membrane Ag which is not easy to purify.
<table>
<thead>
<tr>
<th>#</th>
<th>Antigen</th>
<th>Full Name</th>
<th>Potential Function</th>
<th>Localization</th>
<th>Ab Availability</th>
<th>Current Status</th>
<th>Patent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TM7SF3</td>
<td>TM 7 superfamily 1</td>
<td>N/D</td>
<td>M</td>
<td>N/A</td>
<td>Protein (3 mg)</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>TM6SF1</td>
<td>TM 6 superfamily 1</td>
<td>N/D</td>
<td>M</td>
<td>P</td>
<td>Protein (5 mg)</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>TRO</td>
<td>Trophinin</td>
<td>CAM/signaling</td>
<td>M</td>
<td>M</td>
<td>Protein (14 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>CYYR1</td>
<td>Cys and tyr-rich 1</td>
<td>N/D</td>
<td>M</td>
<td>P</td>
<td>Protein (1 mg)</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>DSG2</td>
<td>Desmoglein 2</td>
<td>CAM/apoptosis regulation</td>
<td>M</td>
<td>M</td>
<td>Protein (1 mg)</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>c14orf1</td>
<td>Chromosome 14 orf 1</td>
<td>Sterol biosynthesis</td>
<td>M</td>
<td>N/A</td>
<td>Ascite</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>MLEC</td>
<td>Malectin precursor (KIAA0152)</td>
<td>Carbohydrate-BP/N-glycosylation</td>
<td>M (ER)</td>
<td>P</td>
<td>Hybridoma</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>CRIM1</td>
<td>Cys rich TM BMP regulator 1</td>
<td>CNS development &amp; organogenesis</td>
<td>M</td>
<td>M</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>ALCAM</td>
<td>Activated leukocyte CAM</td>
<td>T cell development &amp; activation</td>
<td>M</td>
<td>M</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>IGFBP7</td>
<td>Insulin-like growth factor BP 7</td>
<td>CAM; cell proliferation inhibition</td>
<td>M, S</td>
<td>M</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>11</td>
<td>AREG</td>
<td>Amphiregulin</td>
<td>Binds and activates EGFR</td>
<td>M</td>
<td>M</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>12</td>
<td>EMP1</td>
<td>Epithelial membrane protein 1</td>
<td>Cell signaling, communication, CAM</td>
<td>M</td>
<td>P</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>13</td>
<td>PON2</td>
<td>Paraoxonase 2</td>
<td>Cellular antioxidant (cell protection)</td>
<td>M</td>
<td>P</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
<tr>
<td>14</td>
<td>PILRB</td>
<td>Paired Ig-like type 2 receptor beta</td>
<td>Cell signaling activating receptor</td>
<td>M</td>
<td>P</td>
<td>Hybridoma</td>
<td>Y</td>
</tr>
</tbody>
</table>

Antigen (TM: Transmembrane; orf: open reading frame; BMP: Bone Morphogenic Protein; CAM: Cell Adhesion Molecule; BP: Binding Protein)
Localization (M: Membrane; S: Secreted Protein); Ab availability (P: Polyclonal antibody; M: Monoclonal antibody)
Transmembrane 7 superfamily 3 (TM7SF3)

- Membrane protein
- Function & antigen-expressing cell type unknown
- Chromosomal location 12q11.2 → q12
  (amplification of 12q11.2 → q12 band reported in HCC)
- Polyclonal & monoclonal antibody commercially are not available
### TM7SF3 & CD133 Expression in various cell types including HCC & CCC Cell Lines

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Cell line</th>
<th>CD133 (%)</th>
<th>TM7SF3 (%)</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>JCK</td>
<td>34.0</td>
<td>5.9</td>
<td>Yes</td>
</tr>
<tr>
<td>CCC</td>
<td>Choi-CK</td>
<td>0.48</td>
<td>2.5</td>
<td>Yes</td>
</tr>
<tr>
<td>CCC</td>
<td>Cho-CK</td>
<td>2.0</td>
<td>8.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Sarcomatoid CC</td>
<td>SCK</td>
<td>0</td>
<td>1.57</td>
<td>Yes</td>
</tr>
<tr>
<td>HCC</td>
<td>SNU475</td>
<td>8.0</td>
<td>25</td>
<td>No</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>HepG2</td>
<td>30.0</td>
<td>6.5</td>
<td>No</td>
</tr>
<tr>
<td>HCC</td>
<td>Hep3B</td>
<td>98.0</td>
<td>40.0</td>
<td>Yes</td>
</tr>
<tr>
<td>HCC</td>
<td>Huh7</td>
<td>83.0</td>
<td>40.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>U87</td>
<td>0.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Lovo</td>
<td>58.7</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

#### Characteristics of HCC & CCC Cell Lines

- Differentiation status among 3 CCC cell lines: JCK < Cho-CK < Choi-CK
- Tumorigenicity: HepG2 < Hep3B < Huh7
Potential Liver CSC Marker Expression in HCC Cell Lines

- SNU475
- HepG2
- HuH7
- Hep3B

Potential Liver CSC Marker Expression in HCC Cell Lines
Growth Inhibition Effect of Anti-TM7SF3 Ab

In *vitro* Growth Inhibition by MTS Assay

Inhibition (%) = \( \frac{\text{absorbance of mAb-untreated cells} - \text{absorbance of mAb-treated cells}}{\text{absorbance of mAb-untreated cells} - \text{absorbance of culture medium}} \times 100 \)

- **Effector cell** = mouse splenocytes
- **E:T** = 10:1

- **TM7SF3 High**
- **TM7SF3 Low**
DNA vaccine against Chronic hepatitis B
: From bench to clinic

Young chul Sung, Ph.D
Dept. of Life Science
POSTECH
Genome of HBV

- Relaxed circular partial ds 3.2 kb DNA
- Four different ORFs
  - ORF S / PreS: HBsAg
  - ORF P: Polymerase
  - ORF C: HBeAg (soluble protein)
    Core (27nm particle)
  - ORF X: X protein
Replication cycle of HBV

Non-cytolytic hepatotropic virus

Nucleus

Cytoplasm

Endoplasmic Reticulum

Plasma membrane

Partially double-strand DNA

(-)-DNA

cccDNA

Pg RNA

Encapsulated pregenomic mRNA

PreS1/S2/S

PreS2/S

Subgenomic mRNA

HBsAg particle

HBeAg

HBsAg

(hepatotropic virus)
Six multiple genotypes geographically predominant genotypes

Miyakawa Y, Mizokami M. *Intervirology* 2003
Cumulative Infections of HBV & Mortality

- 350 million chronic infections, worldwide
- 50 million new HBV cases annually
- Nearly 75% of HBV chronic carriers are Asian.
- 3 million people lived with HBV in South Korea.

- 15 - 40% of HBV carriers will develop serious hepatic complications

: HBV infection remains a serious health problem

*~7 million susceptible to liver cancer

*~70 million Susceptible to LC

Source: WHO
Consequence of HBV infection

HBV infection

Acute hepatitis

Resolution

Persistent infection

HBV protein vaccine

Baby: 90%
Early childhood: 30%
Adult: 5~10%

Chronic hepatitis

20-25 years (20~40%)
5-10 years (upto 30%)

Healthy carriers

Cirrhosis & HCC
Resolution after acute hepatitis

- Elevated ALT & HBV DNA are not overlapped
- >>> Viremia control without Liver injury
Two immunological mechanisms for HBV clearance

Cytopathic antiviral mechanism

( Destructive effect ; during chronic infection)

: CTLs & byproducts of the secondary inflammatory responses

( TNF-a, free radicals, & proteases ) can eliminate HBV by killing

HBV-infected hepatocytes >>> elevated ALT

Noncytolytic antiviral mechanism

Curative effect; during natural resolution )

: Cytokines ( IFN-a/-beta, & IFN-r ) can exert antiviral functions w/out

destruction of virus-infected hepatocytes via the degradation of viral

RNA.................................................................

“Developing therapeutic vaccine ?”
Course of chronic HBV infection after vertical transmission

Outcome:
- Chronic Hepatitis
- Serum HBV DNA
- Serum HBeAg
- Serum HBsAg

Increase (% of maximum)

Time after infection (years)
- Immuno-tolerance
- Immuno-active phase
- Low replicative phase
- Reactivation

HBcAg specific antibodies
HBeAg specific antibodies
Serum HBeAg
Serum HBsAg
Serum ALR activity
Currently approved drugs

1. Cytokines (Protein drug; IFN-a)
   - inhibits protein synthesis in uninfected hepatocytes
   - stimulates immune system nonspecifically in an antigen-independent manner

2. Nucleoside Analogues (Chemical drug)
   - a potent & selective inhibitor targeting HBV DNA polymerase
     (ex) - Lamivudine (deoxycytosine analogue)
     - Adefovir (adenosine monophosphate analogue)
     - Entecavir
     - Tenofovir & L-deoxythymididine?

>>> beneficial impact on HBV-infected individuals & global epidemic
Obstacles of current anti-HBV drugs

(1) Side effects of therapy with IFN-a
   (Flulike symptoms, fever, myalgias, thrombocytopenia, depression, headache, ALT flare, ... etc)

(2) Development of drug-resistant variants during long-term treatment
   (LAM > Adefovir....)

(3) Costs

(4) Limited efficacy in sustained response.
   (viral relapse rate: 80 - 90% after LAM treatment for a year & 70% after IFN-a therapy)

>>> another novel approach urgently needed to fight against CHB more effectively!
Therapeutic vaccine as a future novel drug?

- Vaccination has been proved to be a cost-effective medical strategy.
- Action mechanism is different from current therapy,
- Induction of Ag-specific immune responses, resulting in increased safety
- Vaccine-induced memory cells, long-lived cells for several decades, leading to long-lasting efficacy & eventually *making the patients cured*
Which type of HBV-specific immunity in viral clearance?

Lessons from “Nature”

1. Strong HBV Ag-specific CTL may contribute to virus elimination in chronic inflammation. (JID. 168:1133 ’93; JCI. 97:1655, ’96).

2. The polyclonal, multi-specific CTL and Th1 responses to HBV Ag were weak or absent in patients with CHB. (Vaccine. 19: 2395, ’01; J. Exp. Med. 181:1047, ’95).

3. Clinically recovered individuals who are positive for anti-Hbe & anti-HBs might experience reactivation of HBV, when treated with immuno-suppressive drugs. (Eur J. Haematol. 67,45,’01)

4. HBV-specific T cell responses have been detected in the blood just before seroconversion. (JCI. 102, 968, ’92)
- Multigene vaccine (most HBV genes)* to induce broad immunity
- IL-12N222L to induce stronger type 1 T cell responses*
IL-12 as a Th1-producing cytokine is a jump-starter of cell-mediated immunity. It plays important roles on antiviral, antibacterial, & anti-tumor activity.
IL-12p40 is also produced with IL-12* : major obstacles of genetic vaccine adjuvants

Viruses, Bacteria, Fungi, Protozoa

APC

Inhibition

Stimulation

IL-12

Th1 immunity

CTL Response

NK

CD4+ (Th0)

CD8+ (Tc)

IFN-g

(p35)

(p40)

IL-12p40

IL-12
IL12N222L*, as a genetic adjuvant, induced Th1 & CTL responses > IL-12

Stronger Th1 immunity?

( Ha SJ et al., Nature Biotechnology, 2002 )
The first objective:
- evaluate a safety & tolerability of HBV DNA vaccine in combination with LAM treatment in HBV chronic carriers

The second objective:
- Generation of HBV DNA vaccine-induced immunity & its possible relationship with efficacy such as
  - HBe & HBs seroconversion,
  - Decrease in viral load,
  - ALT normalization
Criteria to enroll volunteers in Lithuania and Ukraine

1) Presence of serum HBsAg for at least 6 months
2) Absence of IgM anti-HBc & presence of IgG anti-HBc
3) Baseline serum ALT level > 1.5X the upper limit of normal
4) Positive serum HBV DNA ( >10,000 copies / ml )
5) Negative anti-HIV & anti-HCV status
6) Absence of treatment with anti-viral drugs, interferon, corticosteroid or other immune-based therapy
9) Absence of decompensated cirrhosis or HCC
Study protocol of GHB-02

- injected with 8 mg of GX-100 i. m. 12 times at 4-week interval
- *treated with LAM orally (100mg once daily) for 52 weeks
- followed up for another 52 weeks

Serum & PBMCs prepared
- ALT levels
- Serum HBV DNA levels by sensitive PCR method
- Serological responses
- serum cytokines
- IFN-r ELISPOT activity
**DNA vaccination itself did not cause adverse events in patients under LAM**

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>On-treatment</th>
<th>Off-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>2 (17%)*</td>
<td>1 (8%)*</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (17%)*</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>2 (17%)*</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>1 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Sleep disorder</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALT flare</td>
<td>2 (17%)</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Rash in injection sites</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pain in injection sites</td>
<td>1 (8%)**</td>
<td>0</td>
</tr>
<tr>
<td>Reaction in injection sites</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
**Autoantibody induction during and after combined treatment**

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA Ab</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-nuclear Ab</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-IL-12N222L Ab</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
HBV DNA vaccination itself does not cause liver injury
## Virological & serological characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Sex</th>
<th>Pretreatment (T0)</th>
<th>T12</th>
<th>F13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VL (X1000)</td>
<td>ALT (U/L)</td>
<td>HBe/ antiHBe</td>
</tr>
<tr>
<td>#103</td>
<td>44</td>
<td>M</td>
<td>108</td>
<td>154+/-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#106</td>
<td>20</td>
<td>F</td>
<td>601</td>
<td>372+/-</td>
<td>3</td>
</tr>
<tr>
<td>#113</td>
<td>20</td>
<td>M</td>
<td>12</td>
<td>84-/+</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#116</td>
<td>40</td>
<td>F</td>
<td>24,333</td>
<td>80+/-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#118</td>
<td>29</td>
<td>M</td>
<td>13</td>
<td>94-/+</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#121</td>
<td>49</td>
<td>M</td>
<td>3,966</td>
<td>165-/+</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td></td>
<td></td>
<td>33.7 ± 12.5</td>
<td>4.500</td>
<td>158 ± 111</td>
</tr>
<tr>
<td>#105</td>
<td>46</td>
<td>M</td>
<td>9,466</td>
<td>180+/-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#107</td>
<td>20</td>
<td>M</td>
<td>986</td>
<td>100+/-</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#109</td>
<td>48</td>
<td>M</td>
<td>2,200</td>
<td>126-/+</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#110</td>
<td>50</td>
<td>M</td>
<td>14,013</td>
<td>390-/+</td>
<td>&lt;1</td>
</tr>
<tr>
<td>#112</td>
<td>47</td>
<td>M</td>
<td>103</td>
<td>466-/+</td>
<td>720</td>
</tr>
<tr>
<td>#114</td>
<td>30</td>
<td>M</td>
<td>161</td>
<td>63+/-</td>
<td>40</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td></td>
<td></td>
<td>40.1 ± 12.2</td>
<td>4.400</td>
<td>221 ± 167</td>
</tr>
</tbody>
</table>
### Comparison of combined therapy* with LAM std treatment previously reported

<table>
<thead>
<tr>
<th>End-of-treatment</th>
<th>LAM</th>
<th>LAM + GX-100</th>
<th>Fisher’s Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>eAg seroconversion</td>
<td>4/12(33%)$^a$</td>
<td>4/6(67%)</td>
<td>$P = 0.2$</td>
</tr>
<tr>
<td>Virological responses</td>
<td>15/27(56%)$^{a+b}$</td>
<td>10/12(83%)</td>
<td>$P = 0.09$</td>
</tr>
</tbody>
</table>

#### Follow-up

<table>
<thead>
<tr>
<th></th>
<th>LAM</th>
<th>LAM + GX-100</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>eAg seroconversion</td>
<td>2/12(17%)$^a$</td>
<td>3/6(50%)</td>
<td>$0.17$</td>
</tr>
<tr>
<td>sAg seroconversion</td>
<td>0/27</td>
<td>1/12(8%)</td>
<td></td>
</tr>
<tr>
<td>Long-term antiviral effect</td>
<td>3/27(11%)$^{a+b}$</td>
<td>6/12(50%)</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

---

*a* Boni et al. J Hepatol, 12 HBeAg+ patients, follow-up 24 weeks after LAM treatment for 1 year

*b* Santantonio et al. J Hepatol, 15 HBeAg- patients, follow-up 12 months after standard LAM treatment for 1 year
DNA vaccine-induced IFN-\(r\) Elispot activity is higher in VRs than in NVRs

HBV-specific cultured Elispot activity is mainly CD4+ T cell-dependent

- ISC5/10^6 PBMCs
- V#116
- Env, core, Pol
- V#118
- total
- CD4 depleted
- CD8 depleted
VRs do not have higher ALT levels than NVRs.
DNA vaccination* eliminate HBV mainly via noncytolytic clearance mechanism (hypothesis)

HBV-specific T cells?

Type 1 cytokines

CD8 T

Perforin & Granzyme

Fas

Fas L

TcR

HBV

Intracellular inactivation

MACRO-PHAGE

NO

TNF alpha

NK

>>> ALT

†:

: Chronic liver disease
Summary

(1) HBV DNA vaccine is safe even in patients with CHB being treated with LAM clinically, pathologically, and immunologically.

(2) DNA vaccination has a beneficial effect on enhancing long-term suppression of HBV replication up to 50% of vaccinee.

(3) DNA vaccination can restore HBV-specific type 1 T cell responses (memory CD4+ T cells) in combination with chemotherapy.

(4) Long-term viremia control (VRs) correlates with HBV-specific IFN-γ ELISPOT activity, which is not associated with ALT elevation, supporting noncytolytic mechanism.
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- N. Opanasyuk, Ph.D.
LAM alone

LAM + HB-100

P < 0.01

ISCs/10^6 PBMCs

- Env-specific
- core-specific
- Pol-specific

P < 0.01
Liver Development

- Oval cell (mouse)
- Hepatic progenitor cell (human)
1st Evidence of CSCs – Acute Myeloid Leukemia

- Evidence of CSCs
  - CD34
    - sialomucin (glycoprotein antigen)
    - invariably expressed by human HSCs
  - CD38
    - lymphocyte differentiation antigen
    - starts in committed precursor

- Leukemia Initiating Cells
  - “uncommitted precursor”
  - 0.01~1% of total population

Nature. 1994 Feb 17;367(6464):645-8
<table>
<thead>
<tr>
<th>Grade</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Safety</td>
</tr>
<tr>
<td>B</td>
<td>Delivery efficiency</td>
</tr>
<tr>
<td>C+</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; T cell response</td>
</tr>
<tr>
<td>D</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; T cell response</td>
</tr>
<tr>
<td>F</td>
<td>Ab response</td>
</tr>
</tbody>
</table>
Strategies for improving immunogenicity of DNA vaccines

- Vector & Gene optimization
- Antigen Engineering
- Vaccine Candidates
- Adjuvants
- Delivery system
- Evaluation in mouse model
- Evaluation in nonhuman primates
- Clinical study in humans
- Novel Vaccines
- Mechanisms of Immunopathology & protective immunity

DNA vaccine commercially available?
DNA vaccine-derived mAb - Assay System

In Vitro Cell-based Assay System

Transfection → Cos7 Cell

Cell-based Ab detection plasmid

Serum from the immunized mice

Murine Ab specific Ab-dye for FACS analysis

EGFP

Target protein (DNA vaccine-induced antigen)

FACS Analysis

(EGFP expression → Confirm Ab binding to the antigen)
<table>
<thead>
<tr>
<th>#</th>
<th>Antigen</th>
<th>Full Name</th>
<th>Potential Function</th>
<th>Localization</th>
<th>Ab Availability</th>
<th>Current Status</th>
<th>Patent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TERT</td>
<td>Telomerase RT</td>
<td>Telomere elongation / antiapoptotic</td>
<td>N</td>
<td>M</td>
<td>Protein (4 clones, 3mg each)</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>Gankyrin</td>
<td>Gankyrin</td>
<td>Cell cycle regulation / antiapoptotic</td>
<td>C, N</td>
<td>M</td>
<td>Protein (2 clones, 3mg each)</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>RPS14</td>
<td>Ribosomal protein S14 (SKY17)</td>
<td>Protein synthesis</td>
<td>C</td>
<td>M</td>
<td>Protein</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>RPL36</td>
<td>Ribosomal protein L36 (SKY19)</td>
<td>Protein synthesis</td>
<td>C</td>
<td>P</td>
<td>Protein (4mg)</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>c19orf56</td>
<td>Chromosome 19 orf 56</td>
<td>N/D</td>
<td>M</td>
<td>P</td>
<td>Protein (2 clones, 8 &amp; 15mg)</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>IL-17R</td>
<td>Interleukin 17 Receptor</td>
<td>Regulates Inflammatory response</td>
<td>M</td>
<td>M</td>
<td>Ascite</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>IL-23R</td>
<td>Interleukin 23 Receptor</td>
<td>Regulates Inflammatory response</td>
<td>M</td>
<td>M</td>
<td>Ascite</td>
<td>Y</td>
</tr>
</tbody>
</table>

Antigen (RT: Reverse Transcriptase; orf: open reading frame); Localization (C: Cytoplasm; N: Nucleus; M: Membrane); Ab availability (P: Polyclonal antibody; M: Monoclonal antibody)
Three different forms of HBV particle

- 40 nm Dane particles
- Filament particles
- 20 nm HBsAg particles.

(Phosphotungstic Acid–Negative Stain)

( 1 million/ml )

( 10,000 million/ml )
Transmission of HBV

- Transfusion: transplant recipients
- Needle sharing: i.v. drug users, healthcare workers
- Sexually: individuals with multiple partners
- Vertically: newborns of carriers
Cross-presentation of exogenous antigens

Plasma membrane

MHC I - peptide

Endocytosed antigens

MHC II - peptide

Endoplasmic reticulum

Nature Reviews/Immunology
Today’s menu:

1. HBV as a causative agent of Chronic hepatitis B

2. Therapeutic approach for chronic hepatitis B.

3. Pilot clinical trial of HBV DNA vaccine under LAM treatment