Towards a therapeutic HCV vaccine - a preclinical and clinical learning curve

VACCINE TECHNOLOGY II
Albufeira, June 1 - 6, 2008

Intercell develops vaccines for the prevention and treatment of infectious diseases.

For more information be invited to: www.intercell.com
The treatment of chronic HCV patients is currently based on (pegylated)-Interferon and Ribavirin

- Significant side effects
- Not all infected patients can be treated
- Significant costs of treatment (up to 30,000 USD per year)
- Long duration (up to 48 weeks)

Sustained virus response rates are between 50 and 60%, for genotype 1 only 43-46% \(^1\),\(^2\)

Stronger, broader, quicker and more sustained CD4 and CD8 T-cell responses in self-limited course of acute hepatitis C

Response to antiviral therapy may be associated with increased T-cell responses

Viral persistence in chronic hepatitis C is associated with immune evasion
  - impaired function of HCV-specific T-cells
  - mutational T-cell epitope escape

Chimp models

The T-cell system and Hepatitis C virus infection

Th2 Cytokines
IL-4, -5, IL-10, IL-13

Endogen. processed antigens

Soluble Antigen

Clonal Expansion
Tc1 and Tc2 Cytokines, Cytotoxicity

Hepatocyte

IFN-gamma ELIspot
HLA-tetramer (CCR7, CD45RA)

IFN-gamma ELIspot
Lymphoproliferation

NK
NKT

Antibodies

B-cells

CD 8

CD 4

Th 2 Cytokines
IL-4, -5, IL-10, IL-13

Th 1 Cytokines
IFNγ, TNFα

MHC I

MHC II

Endogen. processed antigens

CD4

MHC II

APC

B-cells

Antibodies

NK
NKT

Clonal Expansion
Tc1 and Tc2 Cytokines, Cytotoxicity

Hepatocyte
The IC41 HCV vaccine: 5 synthetic peptides adjuvanted with Poly-L-Arginine

- **Core**
  - HCV-Genome
  - Intercon peptide #
  - Class I: A*0201, DRB1*1101
  - Class II: A*0201, DRB1*0101, DRB1*0401, DRB1*0404, DRB1*0701, DRB1*1101, DRB1*1501

- **Envelope**
  - >80% conserved regions in HCV genotypes 1, 2, 3

- **Non-structural proteins**
  - NS2
  - NS3
  - NS4
  - NS5
<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<td><strong>Wild type</strong></td>
<td>C</td>
<td>I</td>
<td>N</td>
<td>G</td>
<td>V</td>
<td>C</td>
<td>W</td>
<td>T</td>
<td>V</td>
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<tr>
<td><strong>HLA binding</strong></td>
<td>*</td>
<td>*</td>
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<tr>
<td><strong>TCR receptor</strong></td>
<td>*</td>
<td>*</td>
<td>(※)</td>
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<td>*</td>
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<tr>
<td><strong>Gen. 1</strong></td>
<td>T</td>
<td>S</td>
<td>A</td>
<td>M</td>
<td>S</td>
<td>I</td>
<td></td>
<td></td>
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<tr>
<td><strong>Gen. 2</strong></td>
<td>T, S</td>
<td>S, A</td>
<td>I</td>
<td>L</td>
<td></td>
<td></td>
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<tr>
<td><strong>Gen. 3</strong></td>
<td>T, S, A</td>
<td>G</td>
<td>D</td>
<td>T, I</td>
<td></td>
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<td></td>
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<tr>
<td><strong>Gen. 4</strong></td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
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<tr>
<td><strong>Gen. 5</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>M</td>
<td>L</td>
<td></td>
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<tr>
<td><strong>Gen. 6</strong></td>
<td>T, S, A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>L</td>
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</table>

Conservative (green) and non-conservative (red) amino acid exchanges in each position of the NS2-1073 peptide among the different genotypes of the Hepatitis C Virus.

※ indicates the positions important for HLA binding or for the TCR receptor recognition.
Cross-genotype recognition of twenty-eight NS3-1073 peptide variants

IFN-γ ELISPOT USING T-CELLS INDUCED AGAINST WILDTYPE

In vitro T-cell line

Ex vivo Elispot IC41 vaccinated healthy volunteer

Fytli et al., Vaccines 2008
IC41-1: 60 chronic HCV patients, standard IFN/riba therapy non-responders/relapsers

TREATMENT SCHEDULE AND STUDY DESIGN (IC41-201)

**Treatment schedule**

<table>
<thead>
<tr>
<th>Study week</th>
<th>-4</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>44</th>
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<tr>
<td>Vaccinations</td>
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<td>Randomization</td>
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<td>Study period</td>
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<tr>
<td>» Screening</td>
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<td>» Treatment</td>
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<tr>
<td>» Follow up</td>
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<tr>
<td>Immunological checks</td>
<td>▲</td>
<td>▲</td>
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</tbody>
</table>

**Study design**

<table>
<thead>
<tr>
<th>5 Hepatitis C Peptides**</th>
<th>Poly-Arginine**</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>C</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Treatment groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>2.50</td>
<td>1.25</td>
</tr>
<tr>
<td>H</td>
<td>2.50</td>
<td>2.00</td>
</tr>
<tr>
<td>K</td>
<td>5.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Study period: end 2002 - mid 2004
** Different dose levels
Interferon gamma ELISPOT using frozen PBMC

Assay standard: control cells
HIV vs. CMV peptides

Positive Controls:
CMV, EBV, Flu-peptides
Con A

ELISPOT: > 3x OVER BACKGROUND, AT LEAST 15 PER MIO. PBMC
IC41 induces Th1/Tc1 type immune responses in non-responder patients

**CLASS I AND II RESPONSE RATES (ELISPOT)**

**CD8+ and CD4+ T-cells**
- Poly-Arginine only (2 mg): 0.0%
- Peptide only (5 mg): 0.0%
- IC41/Dose H*: 25.0%

**CD4+ T-cells**
- Poly-Arginine only (2 mg): 0.0%
- Peptide only (5 mg): 0.0%
- IC41/Dose H*: 33.3%

*2.5 mg peptides; 2.0 mg Poly-Arginine

Klade et al. Gastroenterology 2008,
Firbas et al., 2006
IC41 induces T-cell proliferation in non-responder patients

**MEDIAN CLASS II T-CELL PROLIFERATION: DOSE GROUP H**

<table>
<thead>
<tr>
<th>Dose Group H*</th>
<th>Control Peptide</th>
<th>Control Poly-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mg peptides; 2.0 mg Poly-Arginine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Klade et al. Gastroenterology 2008*
Results of concluded Phase II study – IC41 already showed trend in efficacy

PHASE II NON RESPONDERS (IC41-1)

Group results of 1 Log responders in Phase II trial*

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
<th>N</th>
<th>Resp.</th>
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<tbody>
<tr>
<td>K</td>
<td>5.00/2.00</td>
<td>2</td>
<td>17%</td>
</tr>
<tr>
<td>H</td>
<td>2.50/2.00</td>
<td>1</td>
<td>8%</td>
</tr>
<tr>
<td>G</td>
<td>2.50/1.25</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>B</td>
<td>0.00/2.00</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>C</td>
<td>5.00/0.00</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Results of patient with viral load reduction in high dose group*

ELISPO

Spots per 200,000 PBMC

Week of study

Class I responses of >10 spots/200,000 are associated with transient viral load reductions

Klade et al. Gastroenterology In revision

Threshold viral load effect
Evidence for mutational T-cell epitope escape in a patient responding to IC41-1 vaccination

RESULTS OF PATIENT WITH VIRAL LOAD REDUCTION*

ELISPOT

Spots per 200,000 PBMC

HCV-RNA

Week of clinical trial

Threshold viral load effect

Baseline

Impaired recognition of an HCV T cell epitope evolving in a single patient during vaccination

Phase II in non-responders

* Published and presented at the EASL Meeting in Vienna, April 2006
IC41-2: Combination with standard therapy

Patients with chronic hepatitis C of genotype I scheduled for standard treatment for 48 weeks already treated for 28 weeks and responded at week 12

IC41
6 vaccinations s.c. in 4 weeks intervals

PEGINF/RBV

Weeks after start of standard therapy V visit

Relapse rate wk 52 / 60 / 72

Immunological Assays wk 28 / 48 / 52 / 60 / 72

Heiner Wedemeyer Christoph Klade et al. AASLD 2007
Sustained responders show a stronger and more frequent T-cell response – Target Population*

INTERFERON γ ELISPOTS IN RELapsed PATients (N= 8) VS. SVR (N=14)

- Relapse patients
- Sustained responders

*Target Population N = 23, for 1 patient missing HCV-RNA data between V8–V10

Heiner Wedemeyer, Christoph Klade et al. AASLD 2007
Conclusions from non-responder patients (IC41-1) and late add-on to PEG-IFN/RBV (IC41-2)

- Favorable safety profile in chronic HCV patients with or without PEG-IFN/RBV standard therapy

- Optimal vaccine dose (2.5 mg peptides / 2.0 mg poly-L-Arg)

- Th1/Tc1 type responses in chronic HCV patients, no apparent negative influence through PEG-IFN/RBV

- Several transient 1 log Hepatitis C - RNA responders at optimal dose

- RNA responses associated with strongest CD8+ responses achieved

- **T-cell immunogenicity requires optimization** (rate, strength, breadth, sustainability)
Improving immunogenicity of IC41 in HLA-transgenic mouse model

**TEST APPLICATION SITES ± IMIQUIMOD**

**CD8⁺ T-cells**

<table>
<thead>
<tr>
<th></th>
<th>s.c.</th>
<th>i.d.</th>
<th>i.d. + “Imiquimod”</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>lot H (AB)</td>
<td>600</td>
<td>700</td>
<td>1500</td>
<td>-</td>
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</table>

**CD4⁺ T-cells**

<table>
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<tr>
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<th>s.c.</th>
<th>i.d.</th>
<th>i.d. + “Imiquimod”</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>lot H (AB)</td>
<td></td>
<td></td>
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</tbody>
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**In vivo CTL assay**

<table>
<thead>
<tr>
<th></th>
<th>% killing</th>
</tr>
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<tbody>
<tr>
<td>IC41 s.c.</td>
<td>10%</td>
</tr>
<tr>
<td>IC41 i.d.</td>
<td>30%</td>
</tr>
<tr>
<td>IC41 i.d. + “Imiquimod”</td>
<td>70%</td>
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**Legend:**
- medium
- Ipep 83
- Ipep 84
- Ipep 87
- Ipep 89
- Ipep 1426
- Ipep 1334
- Ipep 1874
- Ipep 1875
- pR
- Ipep 1274

HHD.2 mice
Dose/100µl/mouse: 100µg/peptide + 400µg pR (lot H in-house mixture AB)

exp. scheme:
day 0, 14, 28, 42, 56, 70
s.c. or i.d. injection
day 7 after 6th inj.
IFN-g ELIspot (spleen cells)
day 29 after 6th inj.
APC transfer
day 30 after 6th inj.
FACS analysis spleen cells
IC41-3 Study concluded January 2008

OPTIMAL VACCINATION SCHEDULE IN TREATMENT NAIVE PATIENTS

» 50 Chronic HCV patients, treatment naive, HCV Genotype 1. Desired subset with low viral load at baseline

- First vaccination on September 26 2006, first data Q2/2007
- Endpoints: Decline in HCV-RNA, T-cell response
- Status: Participating countries: Romania, Poland, Germany, End of recruitment on track for February 2007

* Bi-weekly; intradermal; topical Aldara® (3M)
Primary endpoint met – a weak, but statistically significant HCV-RNA reduction

OVERVIEW IC41-3 PHASE II DATA

Total study group*

High viral load patients (>2 mio copies/ml)**

* 46 patients  
** 25 patients  
*** 95% confidence intervals  

1st vaccination  
last vaccination

Statistically significant

Change from Baseline***  
(Log HCV-RNA/copies/ml)

Visits  
V0  V1  V2  V3  V4  V5  V6  V7  V8  V9

Weeks  
-2  0  2  4  6  8  10  12  14  16

Change from Baseline***  
(Log HCV-RNA/copies/ml)

Visits  
V0  V1  V2  V3  V4  V5  V6  V7  V8  V9

Weeks  
-2  0  2  4  6  8  10  12  14  16

1st vaccination  
last vaccination

Statistically significant

* 46 patients  
** 25 patients  
*** 95% confidence intervals
Conclusions from IC41 trials

- Favorable safety profile in chronic HCV patients with or without PEG-IFN/RBV standard therapy
- Optimal vaccine dose / schedule identified
- Th1/Tc1 type responses in chronic HCV patients, no apparent negative influence through PEG-IFN/RBV
- Antiviral activity demonstrated in patients with strongest CD8+ responses, and treatment group with optimal vaccination
HCV therapeutic vaccination: Forward Strategy

**Development of second generation vaccine**

- More & better peptides (HLA-restriction, efficacy)
- Improved T-cell adjuvant (IC31®)

**Future plans: combination therapy**

- plus PEG-IFN/RBV
- plus novel small molecule inhibitor
Identification of further T-cell peptides

T-CELL EPITOPE IDENTIFICATION PROGRAM

Virus

Overlapping set of synthetic peptides derived from viral genome

Defined T-cell epitopes for therapeutic vaccines

Epitope Capture: Binding to human HLA receptors

Reacting with T-cell derived from humans with positive disease outcome

Induced in HLA-transgenic mice


Schalich & Klade 2008, Biol Chem

Kubitschke & Klade 2008, in preparation
Identification of HCV vaccine candidate peptides beyond IC41

HLA-COVERAGE: 80-90% IN EUROPE, USA AND JAPAN

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Class I epitopes</th>
<th>Class II epitopes</th>
<th>Human PBMC screening</th>
<th>tg mice screening</th>
<th>Epitope Capture</th>
<th>Additional predicted epitopes</th>
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<tbody>
<tr>
<td>Ipep 1835</td>
<td>A2, A3, B7</td>
<td>DR11</td>
<td>✓</td>
<td>✓ (B7 / Ipep 1506)</td>
<td>+</td>
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<tr>
<td>Ipep 1829</td>
<td>A2, B7</td>
<td>DR1, 7, 11(?)</td>
<td>✓</td>
<td>✓ (Ipep1605, IVS)</td>
<td>++(+)</td>
<td>A24</td>
</tr>
<tr>
<td>Ipep 1799</td>
<td>B35</td>
<td>DR1, 4</td>
<td>✓</td>
<td>✓ (DR4 / Ipep 1563)</td>
<td>++</td>
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<tr>
<td>Ipep 1798</td>
<td>A2, A3, A11</td>
<td>DR1, 4, 7</td>
<td>✓</td>
<td>✓ (✓) (A2 no final data)</td>
<td>+++</td>
<td>A24</td>
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<tr>
<td>Ipep 1827</td>
<td>A24</td>
<td>DR1, 7, 11</td>
<td>✓ (Ipep1801)</td>
<td>Not applicable</td>
<td>+++</td>
<td>B8</td>
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<tr>
<td>Ipep 1846</td>
<td>A2, A11(?) , Cw7</td>
<td>DR1, 4, 7, 11</td>
<td>✓ (Ipep1800, IVS)</td>
<td>✓ (DR4 / Ipep 1650)</td>
<td>++++</td>
<td>A24</td>
</tr>
<tr>
<td>Ipep 1547</td>
<td>A2</td>
<td>DR1, 4, 7, 11</td>
<td>✓ (from Day et al.)</td>
<td>✓ DR4</td>
<td>++++</td>
<td></td>
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<tr>
<td>Ipep 1624</td>
<td>B60</td>
<td>DR7</td>
<td>✓</td>
<td>(as expected negative for A2, B7, DR4)</td>
<td>+</td>
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</table>

IVS: *in vitro* stimulation of PBMC from HLA-matched healthy donors

PCT/EP2003/009482
Otava & Klade
AASLD 2004
Kubitschke & Klade: in preparation
IC31®: a TLR agonist comprising two chemically defined biodegradable components

**KLK:**
- Antimicrobial peptide H-KLKL₅KLK-OH
  - Type 2 immune responses (+ proteins)
  - Depot formation at injection site
  - Enhancement of antigen and ODN1a uptake by APC

**ODN1a:**
- Oligodeoxynucleotide oligo-(dIdC)₁₃ phosphodiester, ssDNA
  - Type 1 induction
  - Activation of APC (Dendritic Cells)
  - TLR-9 / MyD88-dependent signaling

Potent and sustained Th-1 / type 2 responses

T cell
B cell
IC31®: Induction of potent type 1 cellular immune responses

EXAMPLE: IMMUNIZATION WITH MODEL PEPTIDES

CTL - EFFECTOR CELLS

PEPTIDE-SPECIFIC IFN-γ PRODUCTION

- OVA<sub>257-264</sub>
- mTRP-2<sub>181-188</sub>

naive
or
mTRP-2<sub>181-188</sub>

mTRP-2<sub>181-188</sub>
+ IC31®

Specific killing of APC

day 7 after single injection
IFN-γ ELISPOT
- Alum
- Cpg 1668
- IC31®
Protective immunity of a novel TB subunit vaccine adjuvanted with IC31®

PRECLINICAL EVALUATION – SURVIVAL (GUINEA PIG)*

* 3x i.m. injection, 4-week interval
Aerosol infection; 16 weeks after first injection

BCG
Ag85B/ESAT-6 + IC31®
Naïve/Saline

% survival

0 10 20 30 40 50 60 70 80 90 100 110 120

0 10 20 30 40

Weeks post infection
Protectivity is linked to IFN-γ producing T-cells indicative for Th-1 driven immunity

DEFINITION OF PROTECTION MARKERS (MOUSE MODEL)

RESIDUAL BACTERIA (lung)

IFN-γ production

- Log 10 resistance
- IFN-γ (pg/ml)

BCG | IC31® | BCG | IC31®
Induction of antigen-specific T-cells in humans vaccinated with the novel TB subunit vaccines

DATA FROM TB PHASE I STUDY: STRONG $T_H$-1 INDUCTION

**IFN-γ in T-cell supernatants**  
(Ag85B/ESAT-6-specific ELISA; Estimated Marginal Means)

**Frequency of IFN-γ prod. T-cells**  
(Ag85B/ESAT-6-specific ELISpot; Estimated Marginal Means)
Dramatic improvement of IC41 by replacing poly(Arg) with IC31® (IC410)

**exp. scheme:**
- day 0, 14, 28 i. fp. injection
- day 34 APC transfer
- day 35 FACS analysis (LNC) ELIspot (spleen cells)

**Dose/100µl/mouse:**
- **IC41:**
  - 200µg/peptide + 400µg pR43 (lot K, batch PD03126)
- **IC410:**
  - 50µg/peptide + 35nmol KLK+ 1.4nmol ODN1a (inhhouse mixture)

**legend:**
- % killed APC
- Ipep 1274 (irrel.)
- Ipep 87, 89 (rel.)
Acknowledgments

INTERCELL

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HCV study group

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Hubert Blum
Ulrich Spengler
Rudolf Stauber
Bernd Jilma