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

VACCINE TECHNOLOGY II
Albufeira, June 1 - 6, 2008

Alexander von Gabain

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

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

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

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

- APC
  - Endogenous processed antigens
  - MHC I
  - MHC II

- CD 4
  - Th 1 Cytokines: IFNγ, TNFα

- CD 8
  - Clonal Expansion
  - Tc1 and Tc2 Cytokines, Cytotoxicity

- NK
  - NKT

- B-cells
  - Antibodies

- Hepatocyte
  - B-cells
  - APC
  - Endogenous processed antigens

- Clonal Expansion
  - Tc1 and Tc2 Cytokines, Cytotoxicity
The IC41 HCV vaccine: 5 synthetic peptides adjuvanted with Poly-L-Arginine

<table>
<thead>
<tr>
<th>HCV-Genome</th>
<th>Intercell peptide #</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>---</td>
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</tr>
<tr>
<td>Envelope</td>
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<tr>
<td>Non-structural proteins</td>
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</table>

<table>
<thead>
<tr>
<th>C</th>
<th>E1</th>
<th>E2</th>
<th>NS2</th>
<th>NS3</th>
<th>NS4</th>
<th>NS5</th>
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Core

<table>
<thead>
<tr>
<th>23-44</th>
<th>132-140</th>
<th>1073-1081</th>
<th>1248-1261</th>
<th>1764-1786</th>
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<tr>
<td>DRB1*1101</td>
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<td>DRB1*0101</td>
<td>DRB1*0101</td>
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<td>DRB1*1501</td>
<td>DRB1*1501</td>
<td>DRB1*1501</td>
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</table>

>80% conserved regions in HCV genotypes 1, 2, 3
**Sequence variability in the NS3-1073 CTL epitope**

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Wild type</td>
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<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>HLA binding</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<tr>
<td>TCR receptor</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>(*)&amp;</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Gen. 1</td>
<td>T</td>
<td>S</td>
<td>A</td>
<td>M</td>
<td>S</td>
<td>I</td>
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<td></td>
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<td>S,A</td>
<td>I</td>
<td>L</td>
<td></td>
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<tr>
<td>Gen. 3</td>
<td>T,S,A</td>
<td>G</td>
<td>D</td>
<td>T,I</td>
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<tr>
<td>Gen. 4</td>
<td>A</td>
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<td>M</td>
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<tr>
<td>Gen. 5</td>
<td>A</td>
<td></td>
<td></td>
<td>M</td>
<td>L</td>
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<tr>
<td>Gen. 6</td>
<td>T,S,A</td>
<td>M,L</td>
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</tbody>
</table>

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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
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>
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<th>8</th>
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</tbody>
</table>

**Study design**

<table>
<thead>
<tr>
<th></th>
<th>5 Hepatitis C Peptides**</th>
<th>Poly-Arginine**</th>
<th>No. of patients</th>
</tr>
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<tbody>
<tr>
<td>Control groups</td>
<td>B</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>C</td>
<td>5.00</td>
<td>0.00</td>
<td>12</td>
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<tr>
<td>Treatment groups</td>
<td>G</td>
<td>2.50</td>
<td>1.25</td>
</tr>
<tr>
<td>H</td>
<td>2.50</td>
<td>2.00</td>
<td>12</td>
</tr>
<tr>
<td>K</td>
<td>5.00</td>
<td>2.00</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
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</table>

* Study period: end 2002 - mid 2004
** Different dose levels

Klade et al. Gastroenterology 2008
Interferon gamma ELISpot using frozen PBMC

ELISpot: > 3x OVER BACKGROUND, AT LEAST 15 PER MIO. PBMC

<table>
<thead>
<tr>
<th>Positive Controls: CMV, EBV, Flu-peptides Con A</th>
</tr>
</thead>
</table>

| Assay standard: control cells HIV vs. CMV peptides |

<table>
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<tr>
<th>IC41102E0096</th>
<th>C.T.L. Cellular Technology Ltd.</th>
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<tbody>
<tr>
<td>1 2 3 4 5 6</td>
<td>7 8 9 10 11 12</td>
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<tr>
<td>A</td>
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<td>CMV</td>
<td>HIV</td>
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<td>Ipep83</td>
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<td>E</td>
<td></td>
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<tr>
<td>F</td>
<td></td>
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<tr>
<td>G</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
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</tbody>
</table>
IC41 induces Th1/Tc1 type immune responses in non-responder patients

Phase II in non-responders

Klade et al. Gastroenterology 2008,
Firbas et al., 2006

* 2.5 mg peptides; 2.0 mg Poly-Arginine

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

<table>
<thead>
<tr>
<th>Dose H*</th>
<th>Peptide only (5 mg)</th>
<th>Poly-Arginine only (2 mg)</th>
<th>IC41/Dose H*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+ and CD4+ T-cells</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.0%</td>
<td>0.0%</td>
<td>25.0%</td>
<td></td>
</tr>
<tr>
<td>CD4+ T-cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0%</td>
<td>0.0%</td>
<td>33.3%</td>
<td></td>
</tr>
</tbody>
</table>
IC41 induces T-cell proliferation in non-responder patients

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

<table>
<thead>
<tr>
<th>Phase II in non-responders</th>
</tr>
</thead>
</table>

Klade et al. Gastroenterology 2008

* 2.5 mg peptides; 2.0 mg Poly-Arginine

---

Dose Group H*
Control Peptide
Control Poly-Arginine

**vaccinations**

**end of vaccination**

**Week of study**

-4 0 4 8 12 16 20 24 32 44

**Median response stimulation index**

4 5 6
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>
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<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*

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

Klade et al. Gastroenterology In revision
Evidence for mutational T-cell epitope escape in a patient responding to IC41-1 vaccination

RESULTS OF PATIENT WITH VIRAL LOAD REDUCTION*

* Published and presented at the EASL Meeting in Vienna, April 2006

**ELISPOT**

Spots per 200,000 PBMC

HCV-RNA

- CINGVCWTV
- CINGVCWSV
- CINGVCWSV
- CINGVCWSV
- Baseline

Week of clinical trial

- 6 vaccinations monthly

Threshold viral load effect

**HCV-RNA (Log IU/ml)**

- 6 vaccinations monthly

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

- IC41 (Ipep89)
- V-variant
- I-variant

**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 \( \gamma \) ELISPOTS IN RELAPSED PATIENTS (N= 8) VS. SVR (N=14)

*Target Population N = 23, for 1 patient missing HCV-RNA data between V8–V10
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 immunicity of IC41 in HLA-transgenic mouse model

**TEST APPLICATION SITES ± IMIQUIMOD**

- **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)

**In vivo CTL assay**

- **CD8⁺ T-cells**
  - HHD.2 mice immunized with
  - 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)

- **CD4⁺ T-cells**
  - HHD.2 mice injected with
  - 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)

**Legend**:
- medium
- Ipep 83
- Ipep 84
- Ipep 87
- Ipep 89
- Ipep 1426
- Ipep 1334
- Ipep 1874
- Ipep 1875
- pR
- Ipep 1274
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

8 vaccinations*

» 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

<table>
<thead>
<tr>
<th>Visits</th>
<th>V0</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
<th>V8</th>
<th>V9</th>
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</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>-2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
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</table>

**Total study group***

<table>
<thead>
<tr>
<th>Change from Baseline***</th>
<th>Log HCV-RNA/copies/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st vaccination</td>
<td></td>
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<tr>
<td>last vaccination</td>
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</table>

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

<table>
<thead>
<tr>
<th>Visits</th>
<th>V0</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
<th>V8</th>
<th>V9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks</td>
<td>-2</td>
<td>0</td>
<td>2</td>
<td>4</td>
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<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
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</table>

* 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

Overlapping set of synthetic peptides derived from viral genome

Defined T-cell epitopes for therapeutic vaccines

Viral genome

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>
</tr>
</thead>
<tbody>
<tr>
<td>lpep 1835</td>
<td>A2, A3, B7</td>
<td>DR11</td>
<td>✓</td>
<td>✓ (B7 / lpep 1506)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>lpep 1829</td>
<td>A2, B7</td>
<td>DR1, 7, 11(?)</td>
<td>✓</td>
<td>✓ B7, (A2)</td>
<td>++(+)</td>
<td>A24</td>
</tr>
<tr>
<td>lpep 1799</td>
<td>B35</td>
<td>DR1, 4</td>
<td>✓</td>
<td>✓ (DR4 / lpep 1563)</td>
<td>++</td>
<td></td>
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<tr>
<td>lpep 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>
</tr>
<tr>
<td>lpep 1827</td>
<td>A24</td>
<td>DR1, 7, 11</td>
<td>✓</td>
<td>Not applicable</td>
<td>+++</td>
<td>B8</td>
</tr>
<tr>
<td>lpep 1846</td>
<td>A2, A11(?) , Cw7</td>
<td>DR1, 4, 7, 11</td>
<td>✓</td>
<td>✓ (DR4 / lpep 1650)</td>
<td>++++</td>
<td>A24</td>
</tr>
<tr>
<td>lpep 1547</td>
<td>A2</td>
<td>DR1, 4, 7, 11</td>
<td>✓ (✓) (from Day et al.)</td>
<td>✓ DR4</td>
<td>++++</td>
<td></td>
</tr>
</tbody>
</table>
| lpep 1624 | B60              | DR7               | ✓                    | (as expected negative for A2, B7, DR4) | + | }

**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

PEPTIDE-SPECIFIC IFN-γ PRODUCTION

- OVA$_{257-264}$
- mTRP-2$_{181-188}$

**CTL - EFFECTOR CELLS**

- naive
- mTRP-2$_{181-188}$
- mTRP-2$_{181-188}$ + IC31®

Specific killing of APC
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®
Naive/Saline

% survival

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

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

Weeks post infection

PAGE 25 VACCINE TECHNOLOGY II JUNE 2, 2008
Protectivity is linked to IFN-γ producing T-cells indicative for Th-1 driven immunity

DEFINITION OF PROTECTION MARKERS (MOUSE MODEL)

**RESIDUAL BACTERIA (lung)**

<table>
<thead>
<tr>
<th></th>
<th>BCG</th>
<th>IC31®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log 10 resistance</td>
<td>1,0</td>
<td>0,5</td>
</tr>
</tbody>
</table>

**IFN-γ production**

<table>
<thead>
<tr>
<th></th>
<th>BCG</th>
<th>IC31®</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>0</td>
<td>6000</td>
</tr>
</tbody>
</table>
Induction of antigen-specific T-cells in humans vaccinated with the novel TB subunit vaccines

DATA FROM TB PHASE I STUDY: STRONG TH-1 INDUCTION

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

Frequency of IFN-\(\gamma\) 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 (inhouse mixture)

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

**IFN-γ production**
- Number of IFN-γ producing cells/million CD4 depleted SPCs

**CD8+ T cell effector function**
- Percentage killing
- **Ipep 89**
Acknowledgments

INTERCELL

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