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*.

Chronic Hepatitis C: Standard of Care

» **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]

1. Fried M. et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N. Engl. J. Med., Vol. 347, 13, 13 Sep 2002.
2. Manns M.P. et al. PINF alfa-2b plus ribavirin compared with INF alfa-2b plus ribavirin for initial treatment for chronic hepatitis C: a randomized trial. Lancet, Vol. 358 (9286), Sep 2001

HCV: importance of T-cell responses

» **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**

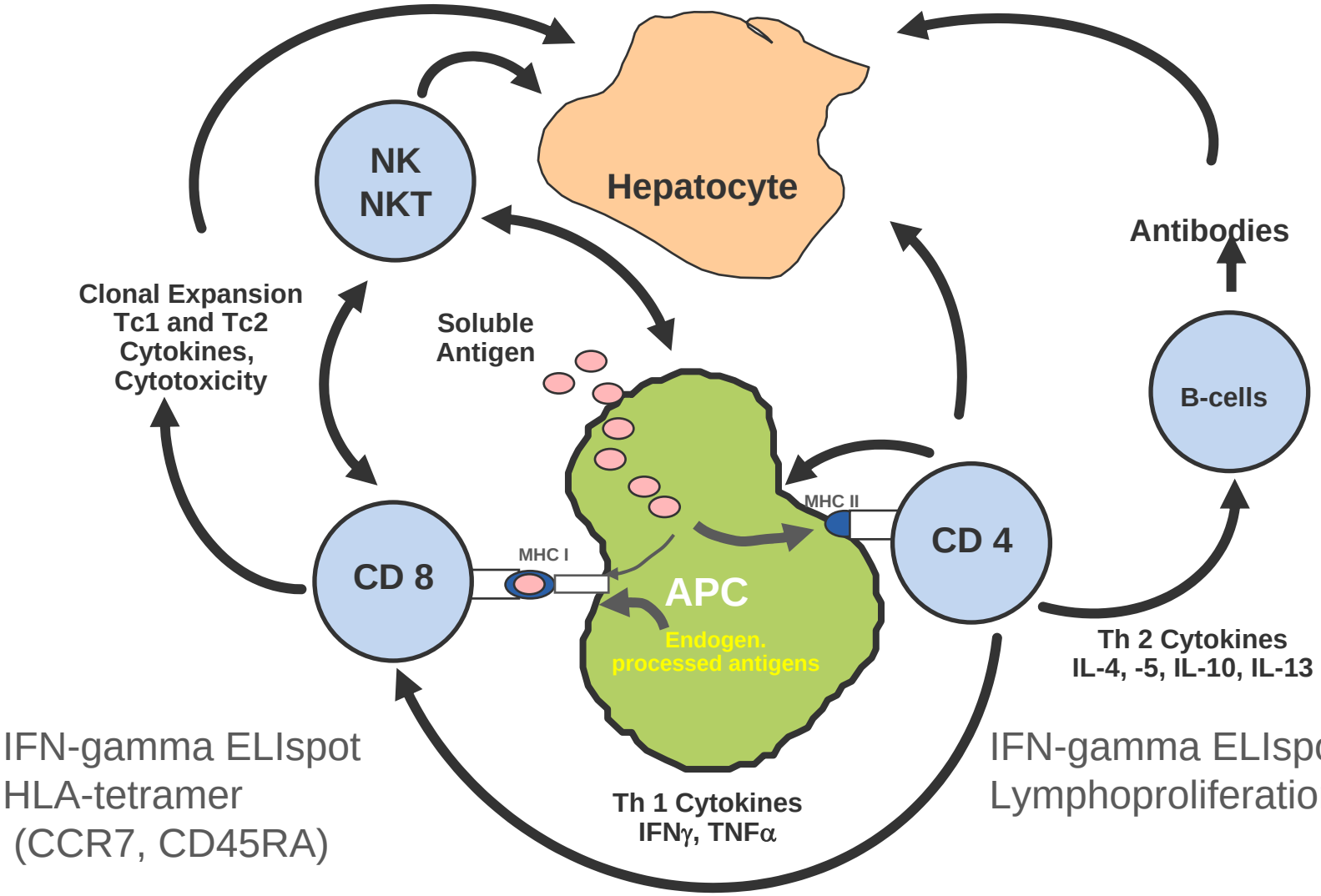
» **Viral persistence in chronic hepatitis C is associated with immune evasion**

- **impaired function of HCV-specific T-cells**
- **mutational T-cell epitope escape**

» **Chimp models**

Diepolder 1995, Missale 1996, Rehermann 1996, Lamonaca 1999, Gruener 2000, Thimme 2001, Wedemeyer 2002, Lauer 2002&2004, Cox 2005, Boettler 2005, Spangenberg 2005,...

The T-cell system and Hepatitis C virus infection



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

Core |--- Envelope ---| |---------- Non-structural proteins ----------|

HCV-Genome

Intercell peptide #	**Core**$_{23\text{-}44}$	**Core**$_{132\text{-}140}$	**NS3**$_{1073\text{-}1081}$	**NS3**$_{1248\text{-}1261}$	**NS4**$_{1764\text{-}1786}$
Class I	**A*0201**	**A*0201**	**A*0201**	**A*0201**	**A*0201**
Class II	**DRB1*1101**			**DRB1*0101** **DRB1*0401** **DRB1*0404** **DRB1*0701** **DRB1*1101** **DRB1*1501**	**DRB1*0101** **DRB1*0401** **DRB1*0404** **DRB1*0701** **DRB1*1101**

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

Sequence variability in the NS3-1073 CTL epitope

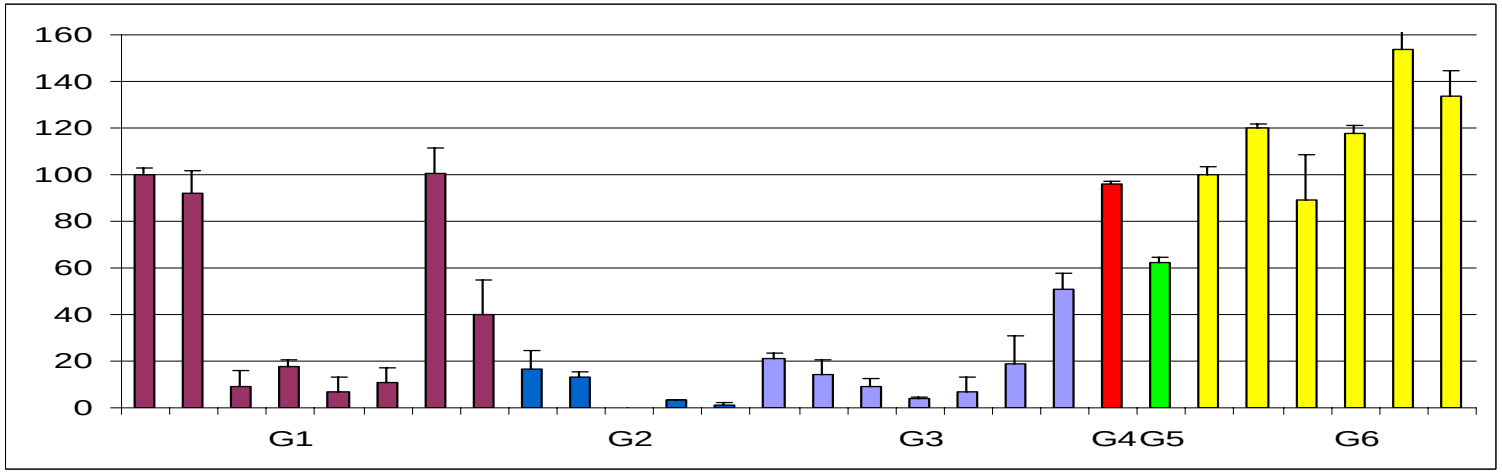
Position	1	2	3	4	5	6	7	8	9
Wild type	C	I	N	G	V	C	W	T	V
HLA binding		*					*		*
TCR receptor			*		*	(*)	*		
Gen. 1		T	S		A		M	S	I
Gen. 2	T,S		S,A		I	L			
Gen. 3	T,S,A		G	D		T,I			
Gen. 4	A					M			
Gen. 5						M			L
Gen. 6	T,S,A					M,L			

- **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.**

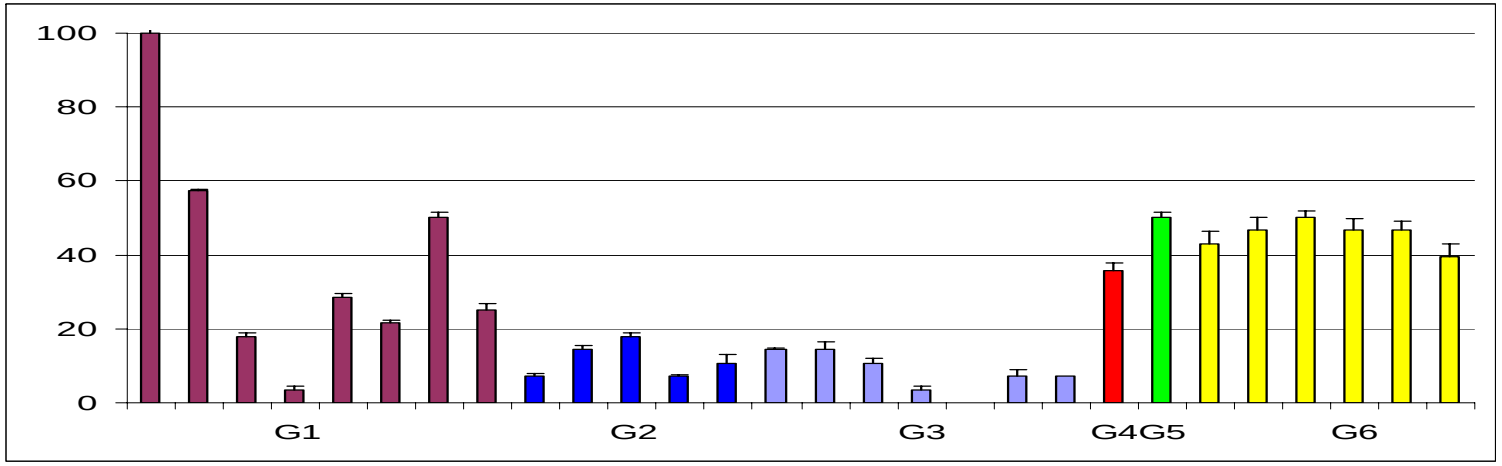
Fytili et al. , Vaccines 2008

Cross-genotype recognition of twenty-eight NS3-1073 peptide variants

IFN-γ ELISPOT USING T-CELLS INDUCED AGAINST WILDTYPE



In vitro T-cell line



Fytili et al. , Vaccines 2008

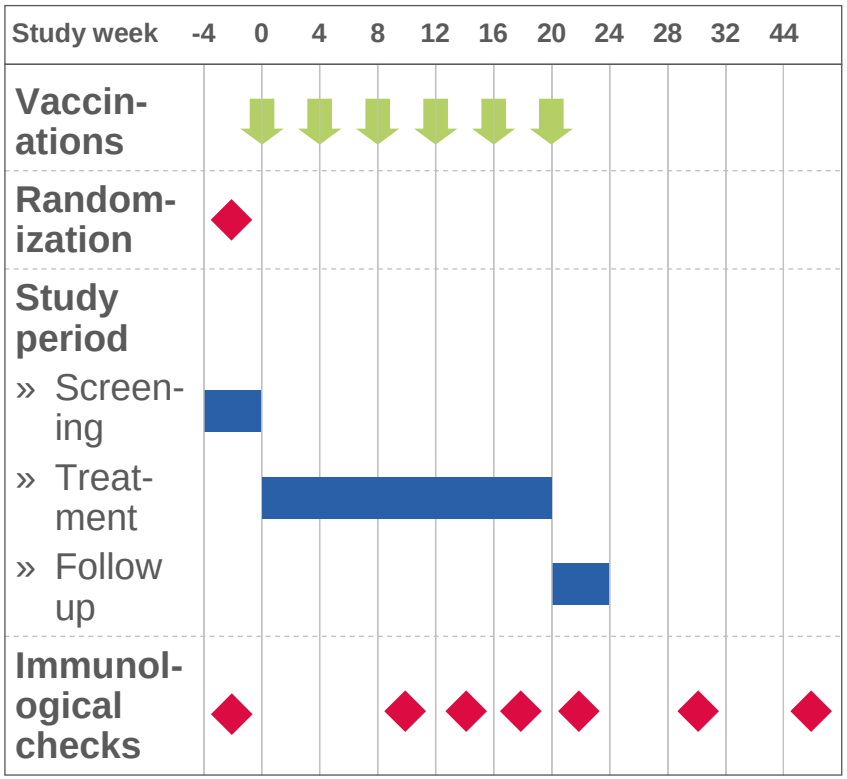
Ex vivo Elispot IC41 vaccinated healthy volunteer

IC41-1: 60 chronic HCV patients, standard IFN/riba therapy non-responders/relapsers

Phase II in non-responders

TREATMENT SCHEDULE AND STUDY DESIGN (IC41-201)

Treatment schedule



Study design

		5 Hepatitis C Peptides**	Poly-Arginine**	No. of patients
Control groups	B	0.00	2.00	12
	C	5.00	0.00	12
Treatment groups	G	2.50	1.25	12
	H	**2.50**	**2.00**	12
	K	5.00	2.00	12
			Total	**60**

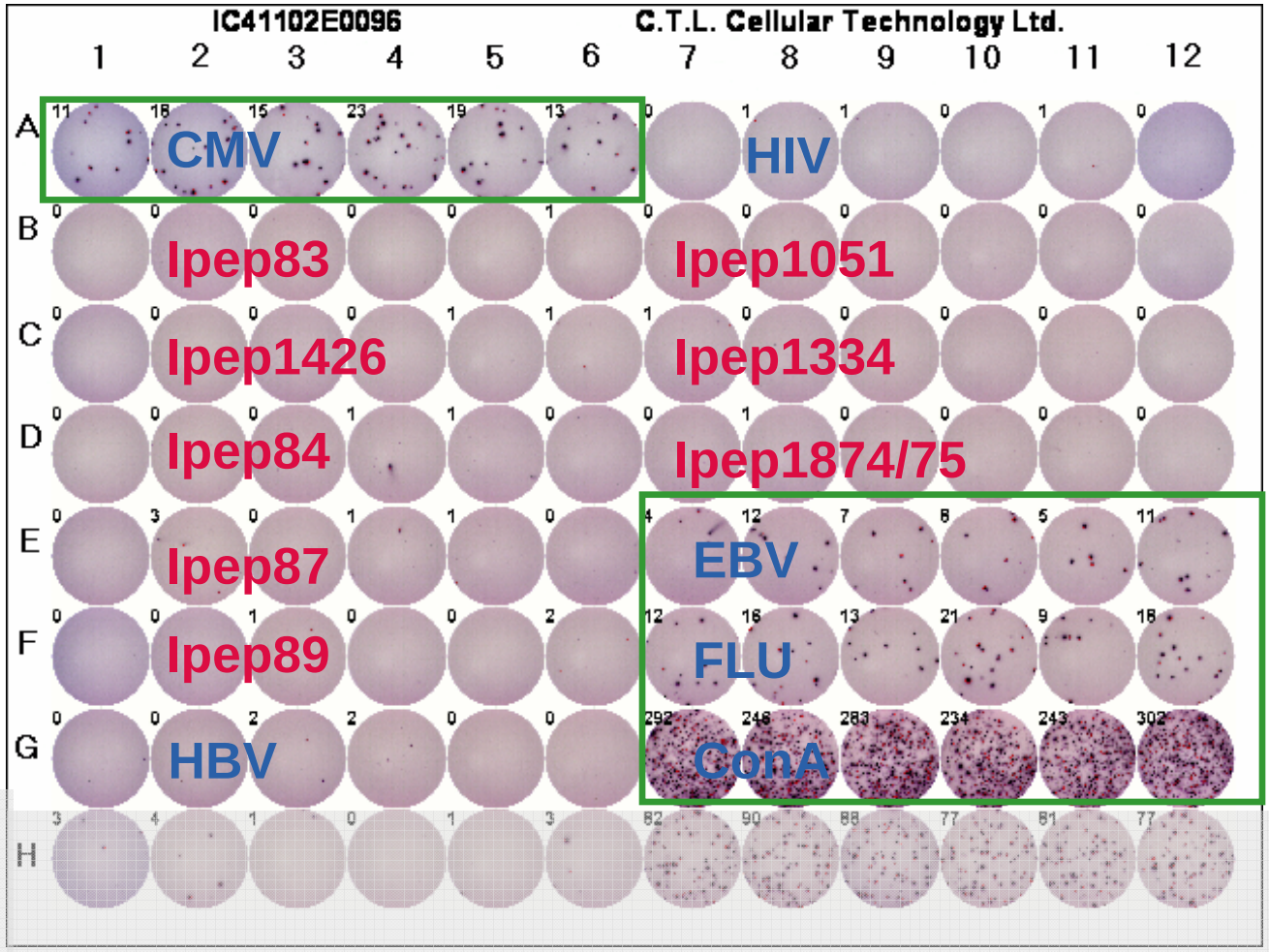
Klade et al. Gastroenterology 2008

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

Interferon gamma ELIspot using frozen PBMC

ELISPOT: ≥ 3x OVER BACKGROUND, AT LEAST 15 PER MIO. PBMC

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

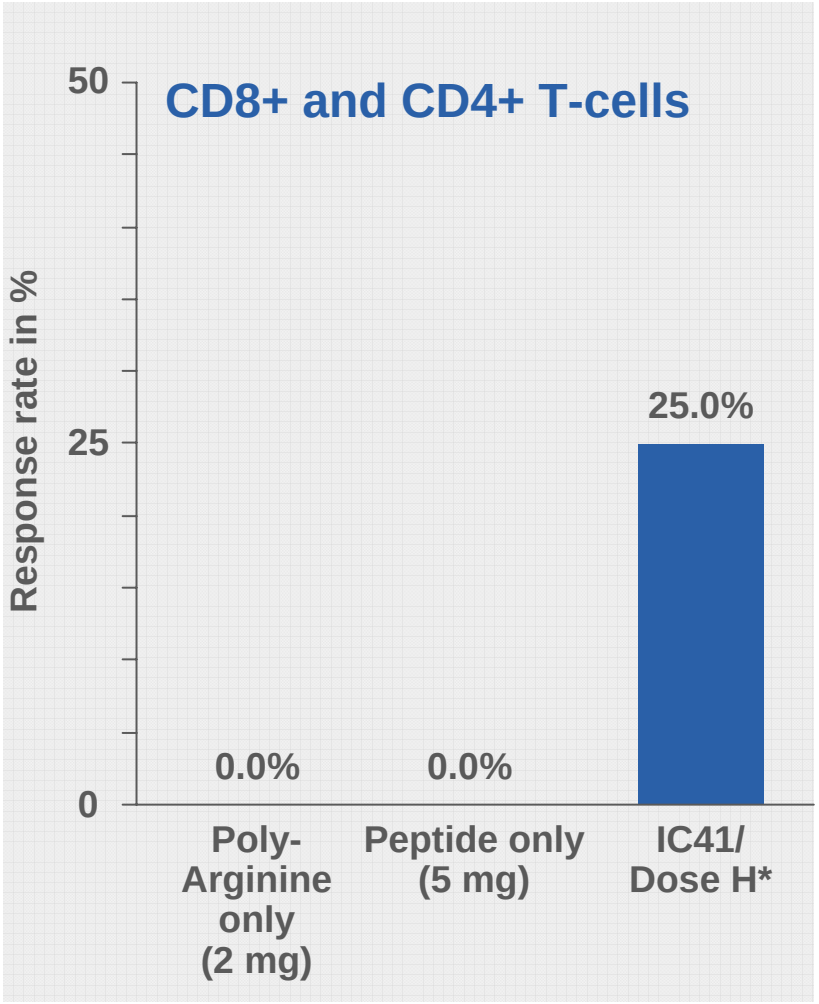


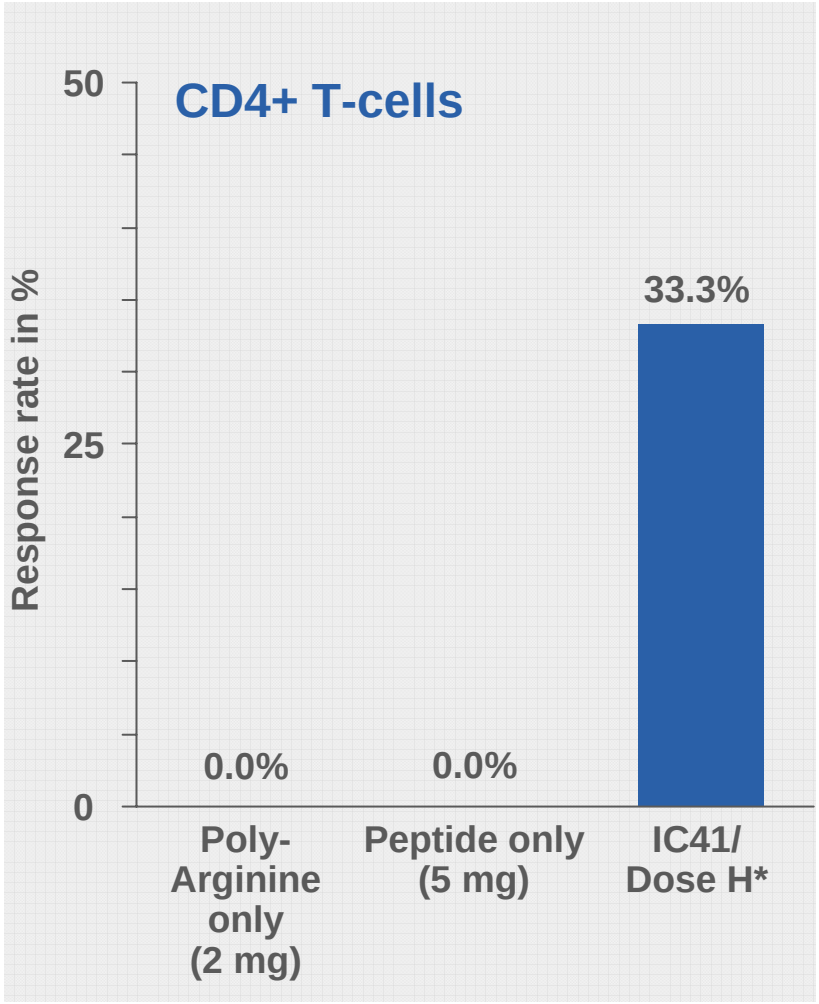
Assay standard:
control cells
HIV vs. CMV peptides

IC41 induces Th1/Tc1 type immune responses in non-responder patients

Phase II in non-responders

CLASS I AND II RESPONSE RATES (ELISPOT)





Klade et al. Gastroenterology 2008,

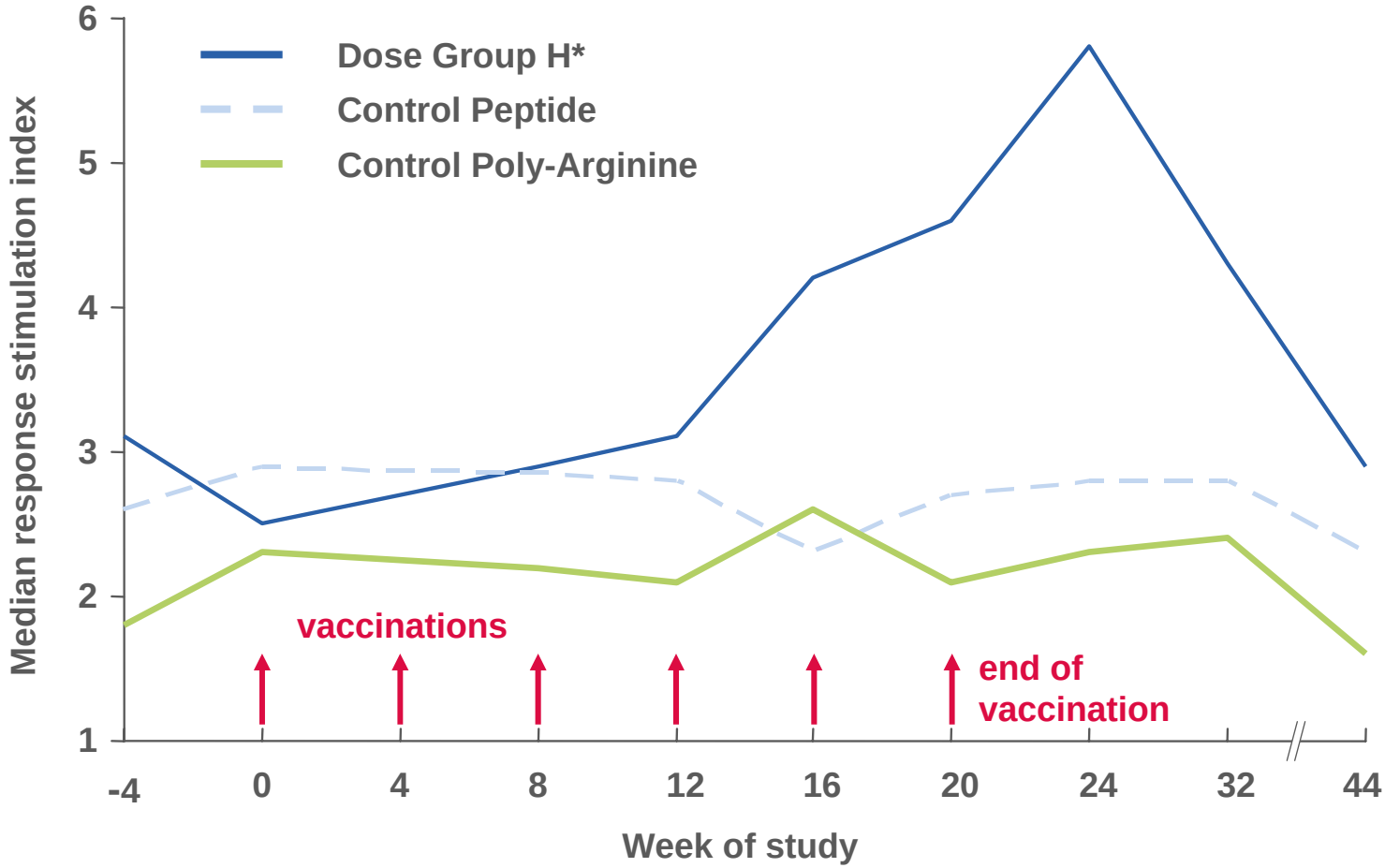
Firbas et al., 2006

* 2.5 mg peptides; 2.0 mg Poly-Arginine

IC41 induces T-cell proliferation in non-responder patients

Phase II in non-responders

MEDIAN CLASS II T-CELL PROLIFERATION: DOSE GROUP H



Klade et al. Gastroenterology 2008

* 2.5 mg peptides; 2.0 mg Poly-Arginine

Results of concluded Phase II study – IC41 already showed trend in efficacy

Phase II in non-responders

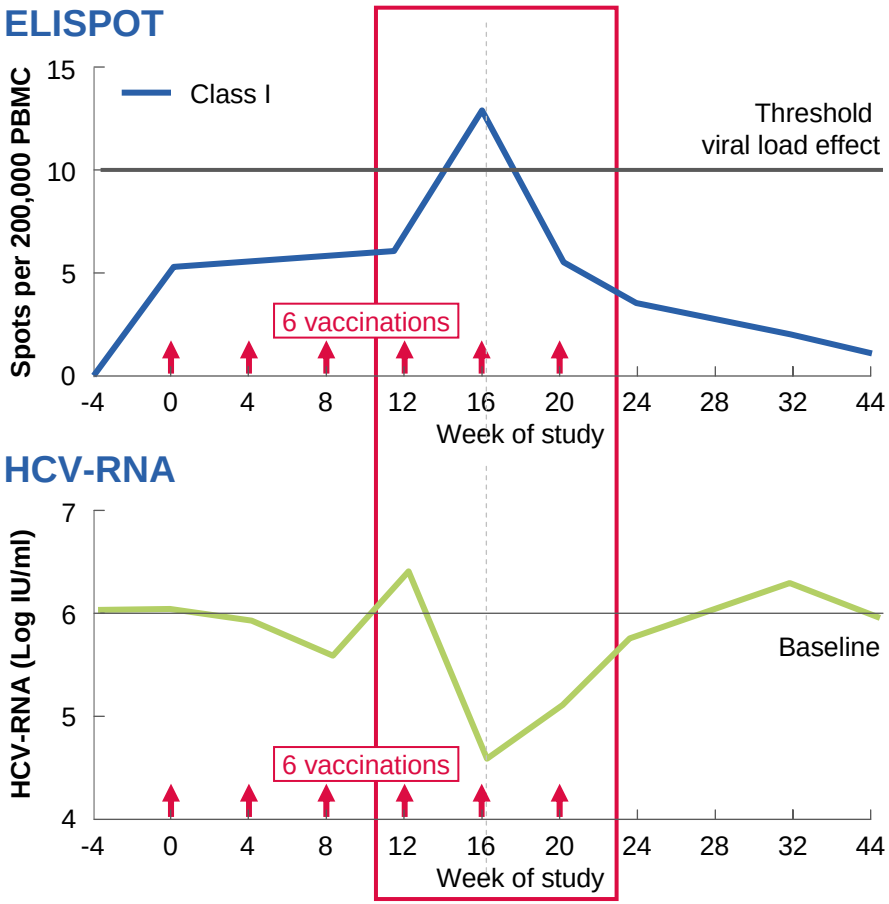
PHASE II NON RESPONDERS (IC41-1)

Group results of 1 Log responders in Phase II trial*

Group	Dosage	N	Resp.
K	5.00/2.00	2	17%
H	2.50/2.00	1	8%
G	2.50/1.25	0	0%
B	0.00/2.00	0	0%
C	5.00/0.00	0	0%

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

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

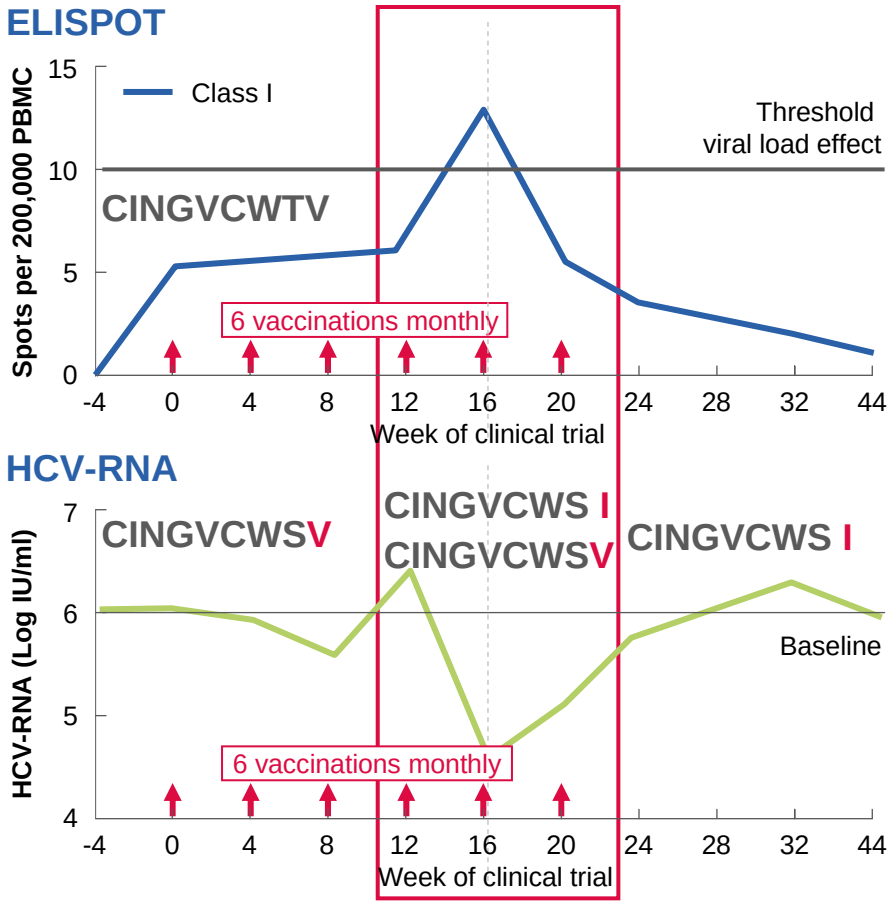


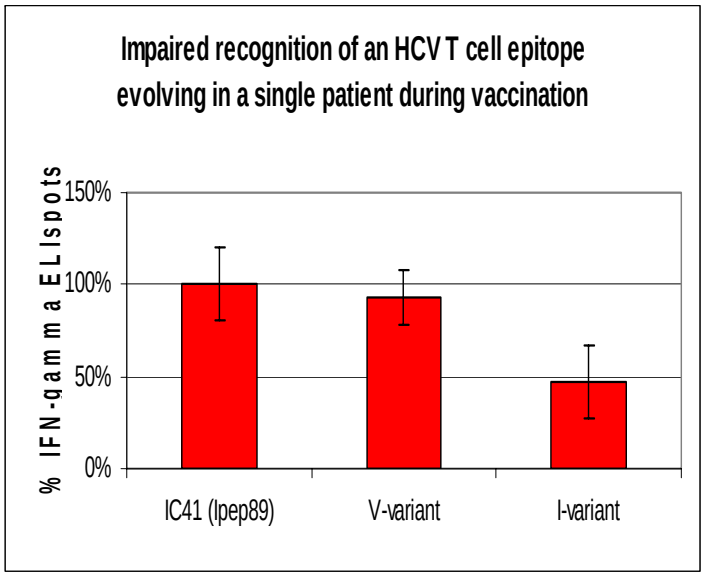
Klade et al. Gastroenterology In revision

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

Phase II in non-responders

RESULTS OF PATIENT WITH VIRAL LOAD REDUCTION*

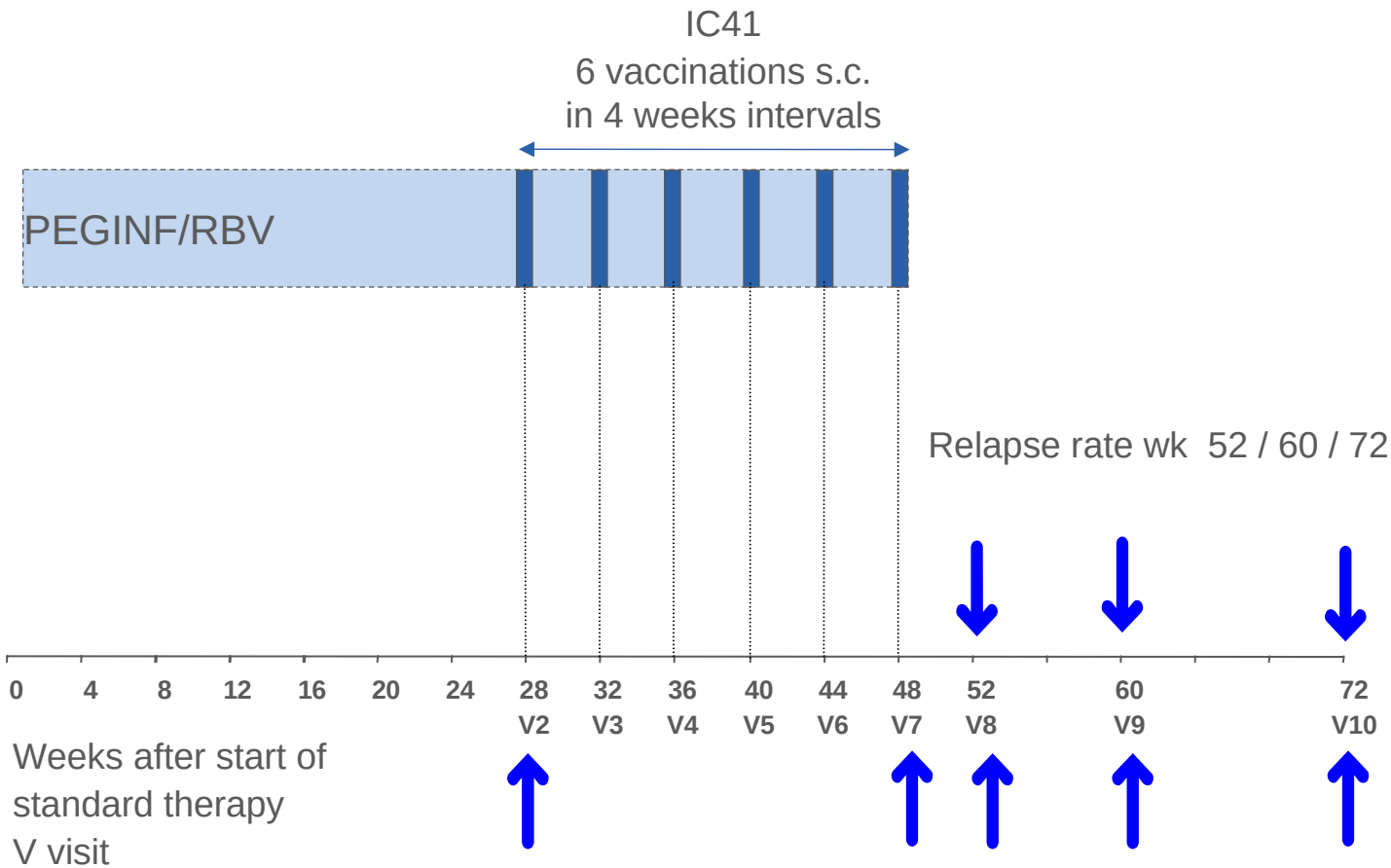




* Published and presented at the EASL Meeting in Vienna, April 2006

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

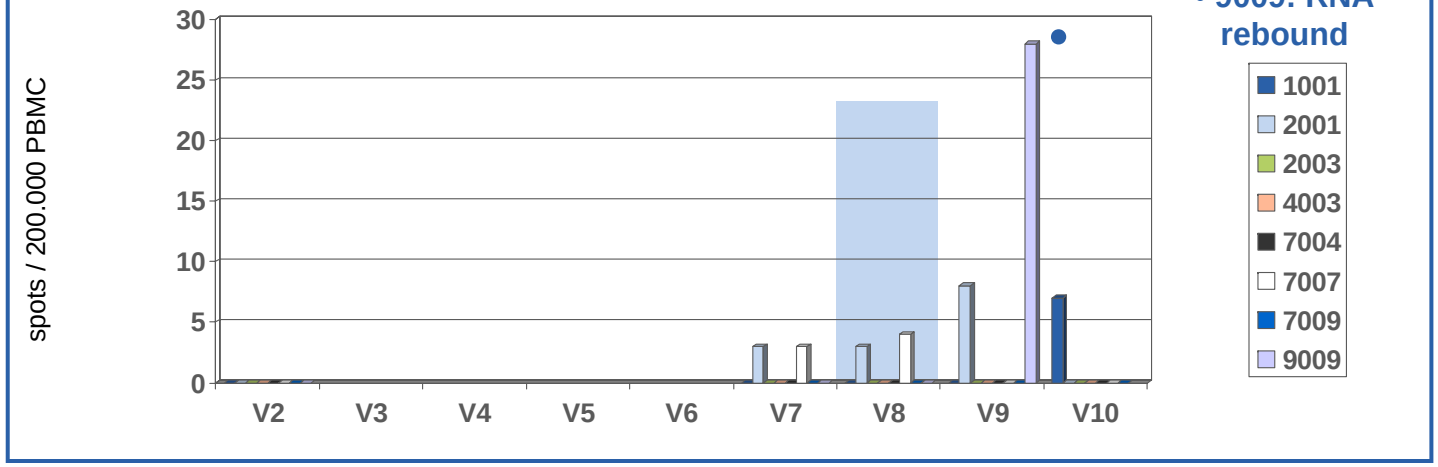


Heiner Wedemeyer
Christoph Klade
et al.
AASLD 2007

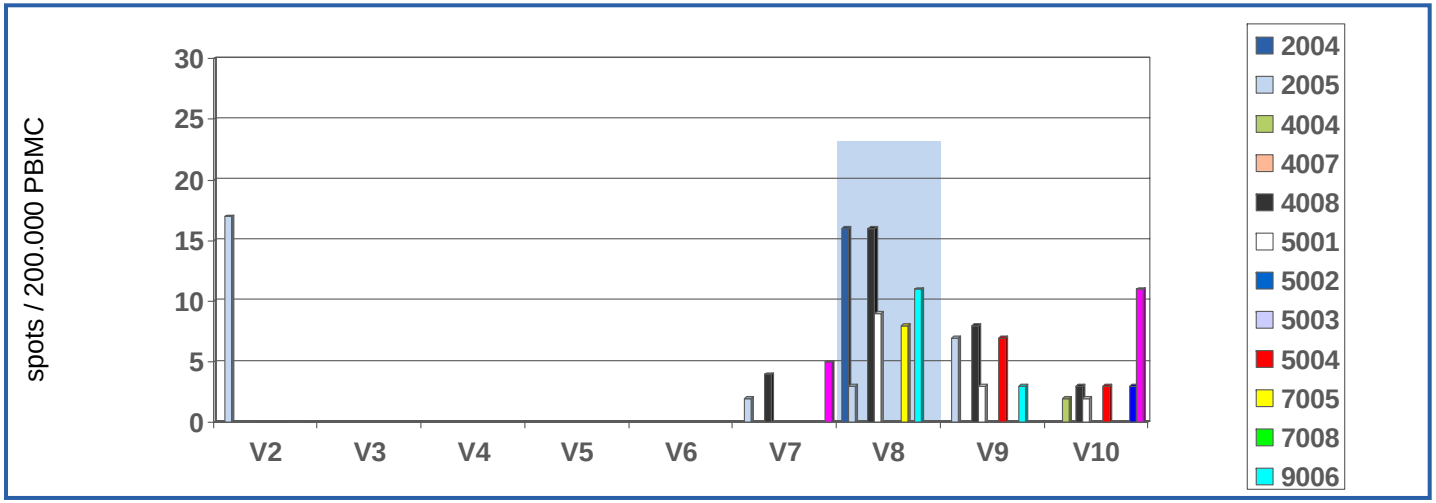
Sustained responders show a stronger and more frequent T-cell response – Target Population*

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 immunicity of IC41 in HLA-transgenic mouse model

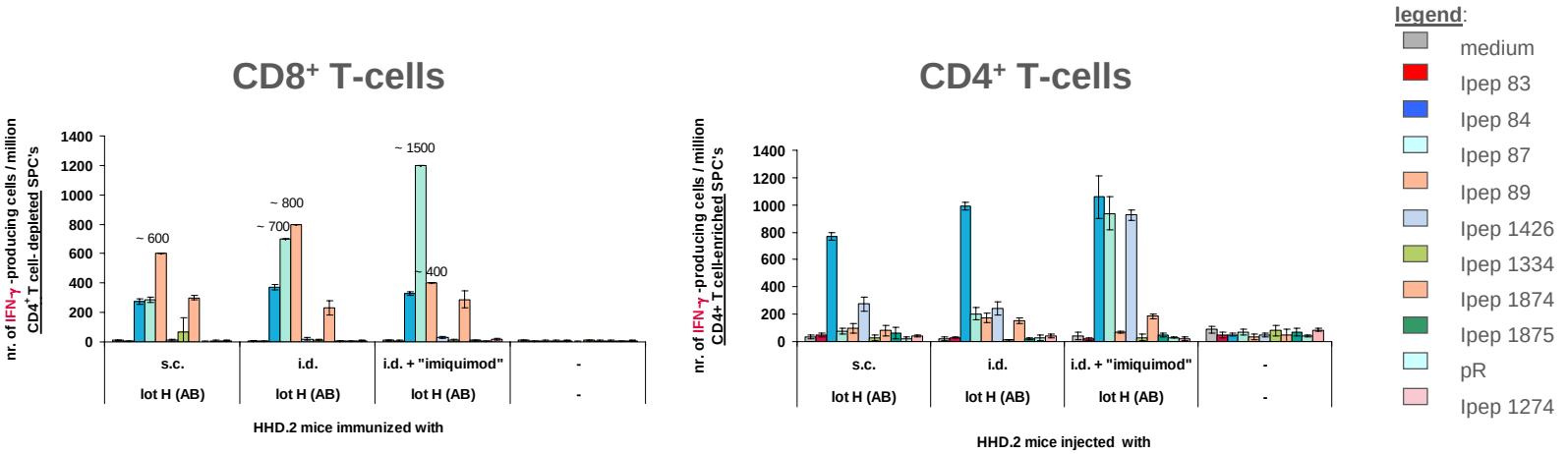
TEST APPLICATION SITES ± IMIQUIMOD

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

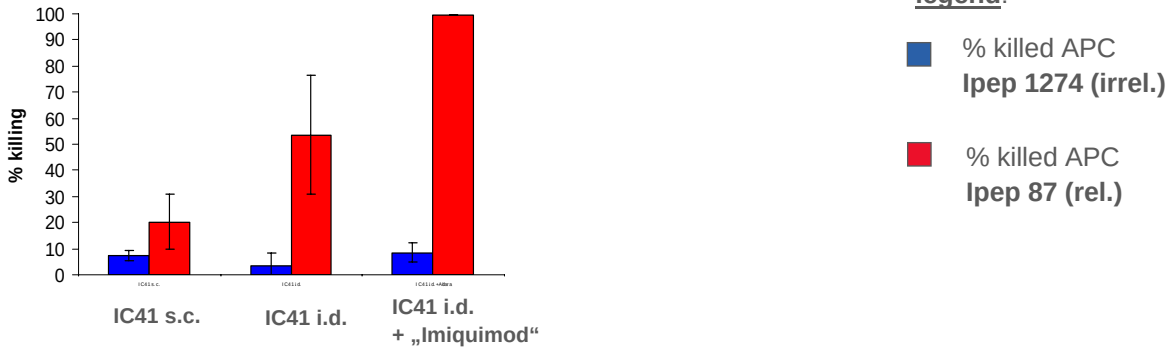
exp. scheme:
day 0, 14, 28,
42, 56, 70

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

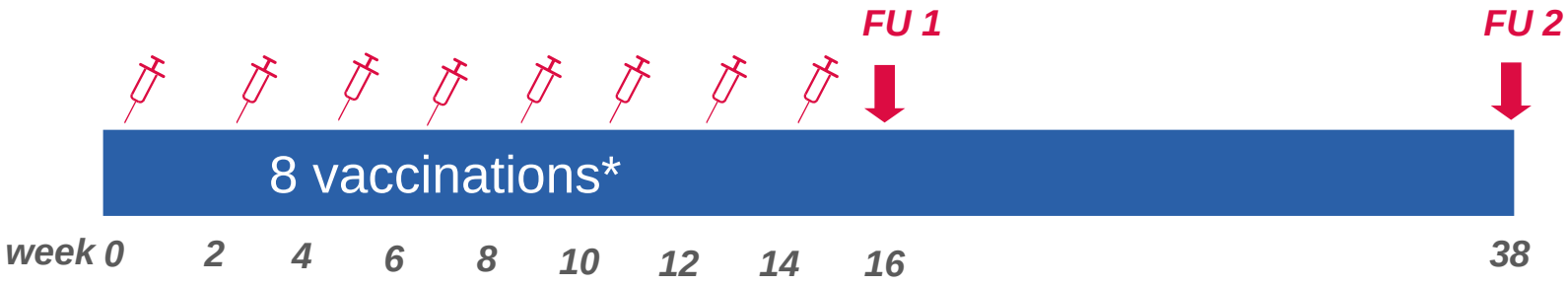


IC41-3 Study concluded January 2008

Phase II in naïve patients

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

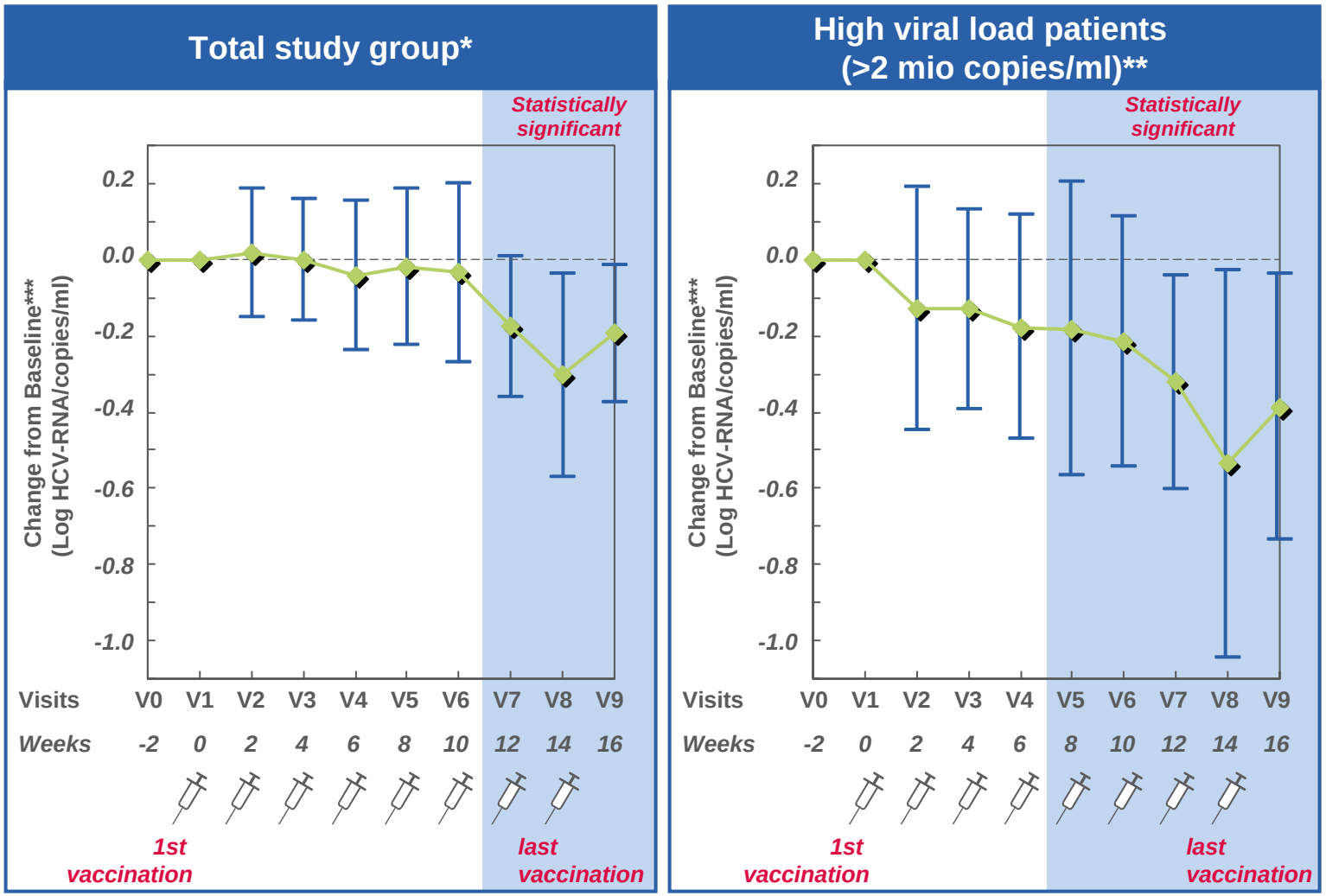
» **Status**
- Participating countries: Romania, Poland, Germany
- End of recruitment on track for February 2007

* Bi-weekly; intradermal; topical Aldara® (3M)

Primary endpoint met – a weak, but statistically significant HCV-RNA reduction

Point Estimate

OVERVIEW IC41-3 PHASE II DATA



* 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

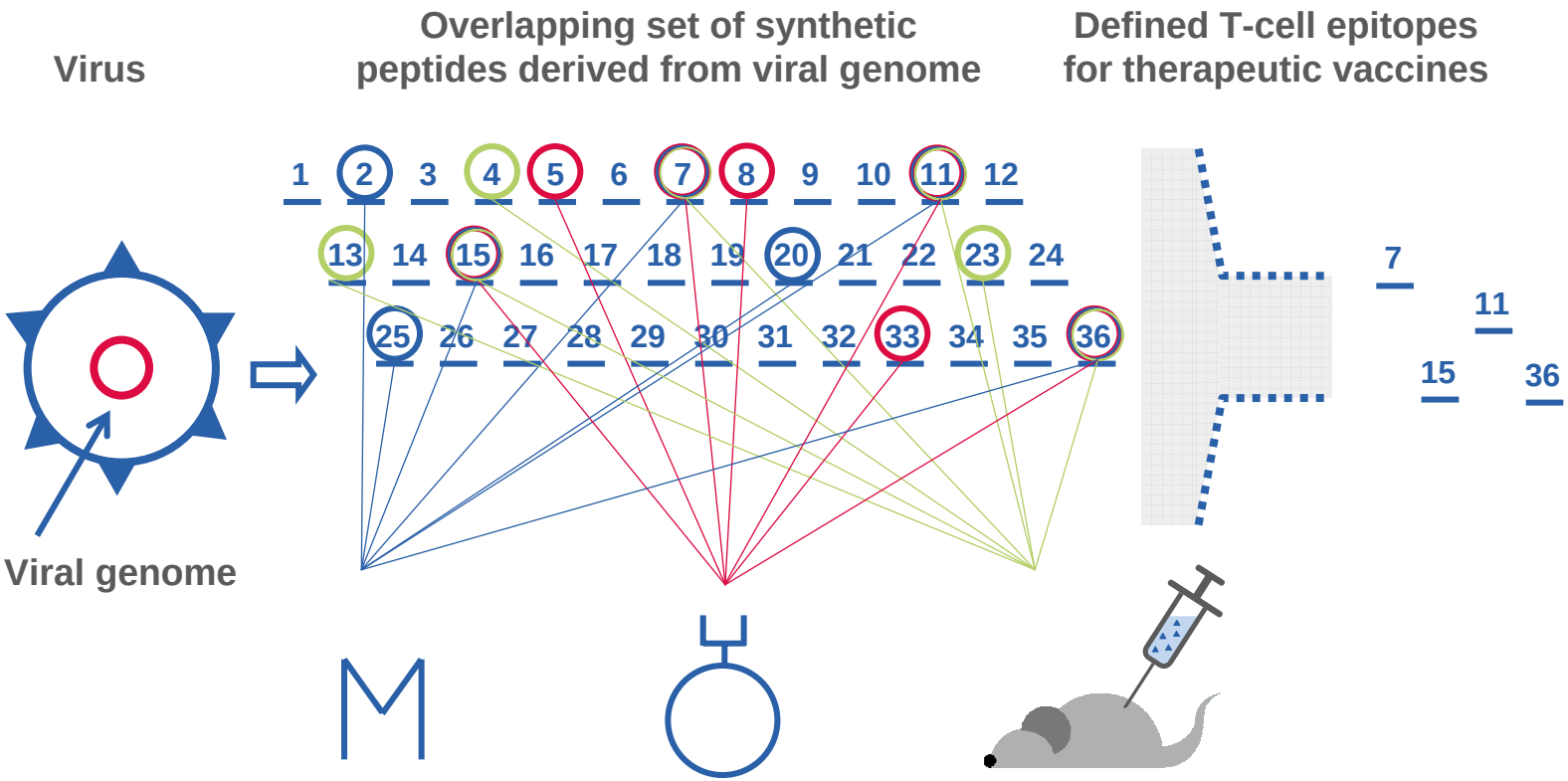
- » More & better peptides (HLA-restriction, efficacy)
- » Improved T-cell adjuvant (IC31®)

Future plans: combination therapy

- » plus PEG-IFN/RBV
- » plus novel small molecule inhibitor

Identification of further T-cell peptides

T-CELL EPITOPE IDENTIFICATION PROGRAM



Reviewed in Scharnagl & Klade 2007, Exp Rev Vaccines 6:605-15.

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

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

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

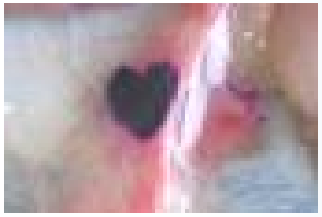
PCT/EP2003/009482

Otava & Klade AASLD 2004

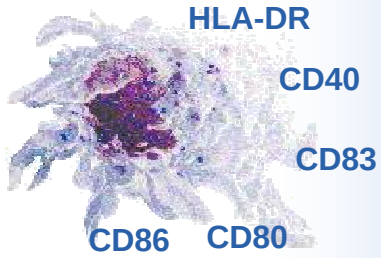
Kubitschke & Klade in preparation

IC31®: a TLR agonist comprising two chemically defined biodegradable components

» **KLK:**
antimicrobial peptide H-KLKL$_5$KLK-OH
- Type 2 immune responses (+ proteins)
- Depot formation at injection site

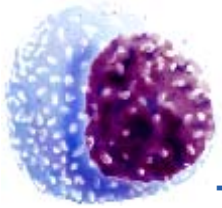


- Enhancement of antigen and ODN1a uptake by APC

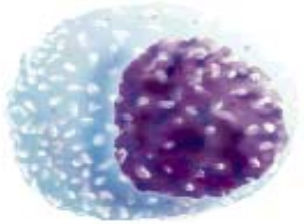


» **ODN1a:**
oligodeoxynucleotide oligo-(dIdC)$_{13}$ phosphodiester, ssDNA
- Type 1 induction
- Activation of APC (Dendritic Cells)
- TLR-9 / MyD88-dependent signaling

Potent and sustained Th-1 / type 2 responses



T cell



B cell

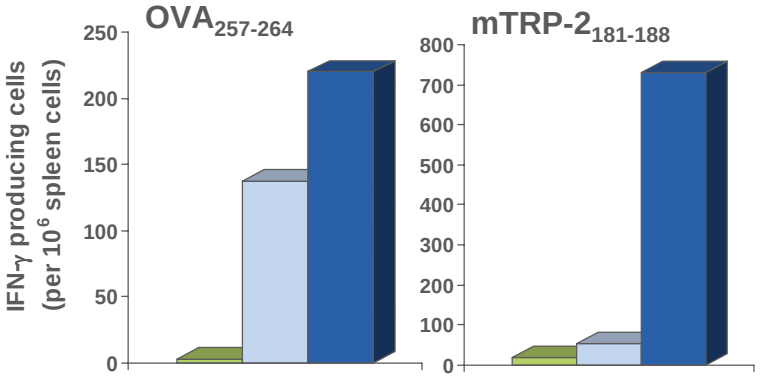
IC31®: Induction of potent type 1 cellular immune responses

EXAMPLE: IMMUNIZATION WITH MODEL PEPTIDES

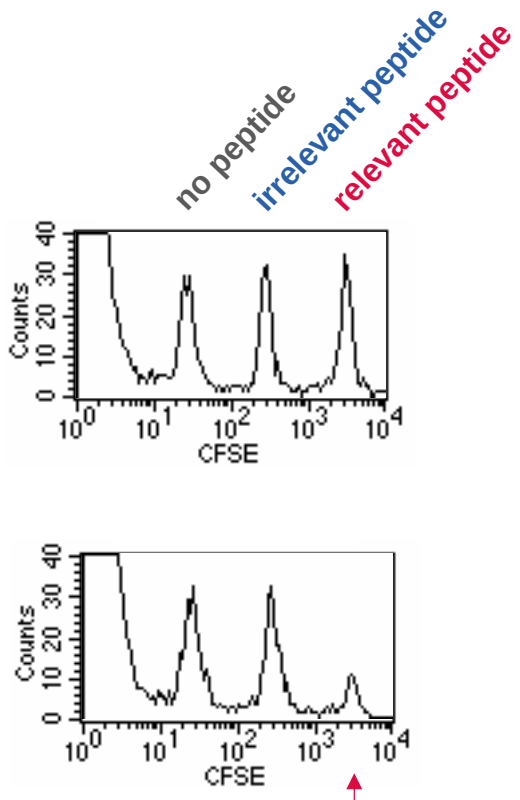
day 7 after single injection
IFN-γ ELISpot

- Alum
- CpG 1668
- IC31®

PEPTIDE-SPECIFIC IFN-γ PRODUCTION

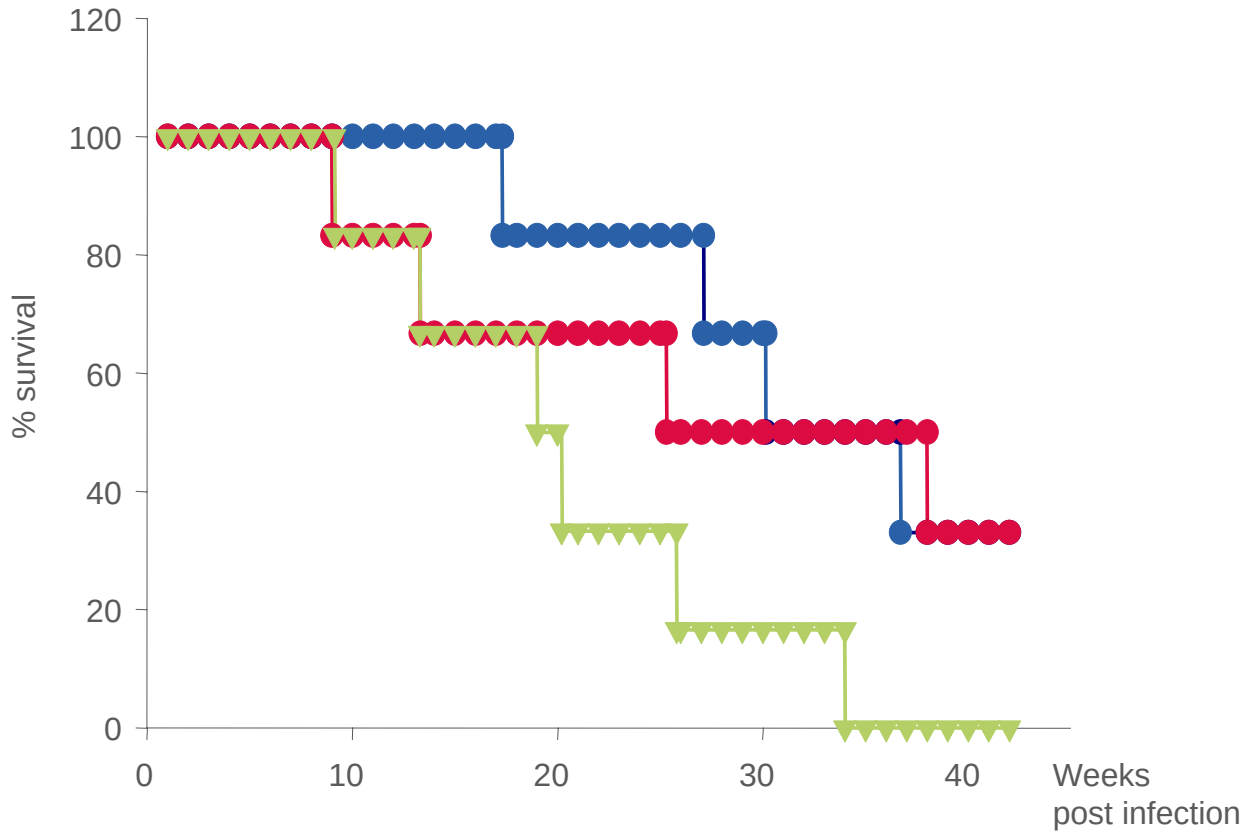


CTL - EFFECTOR CELLS



Protective immunity of a novel TB subunit vaccine adjuvanted with IC31®

PRECLINICAL EVALUATION – SURVIVAL (GUINEA PIG)*



- BCG
- Ag85B/ ESAT-6 + IC31®
- Naive/ Saline

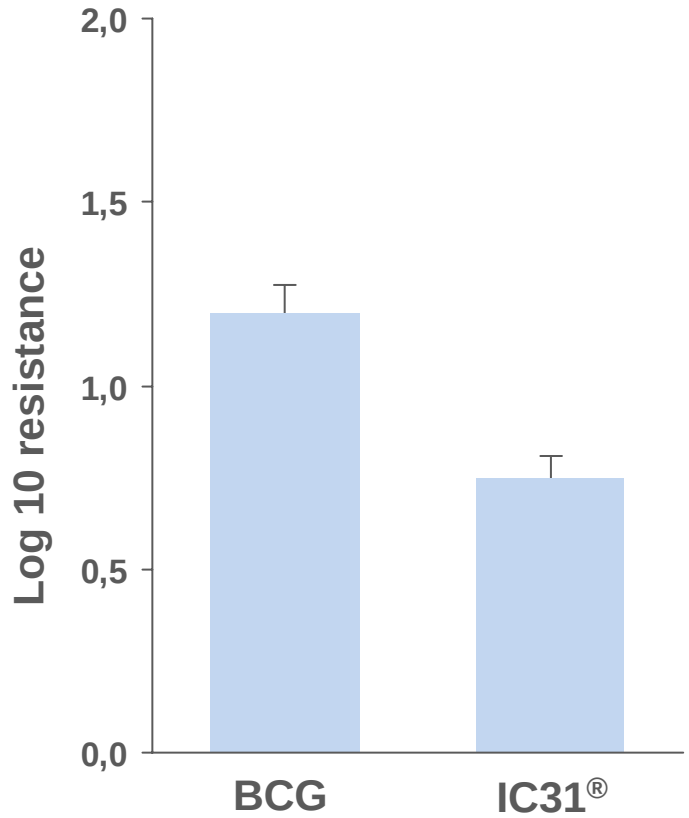
* 3x i.m. injection, 4-week interval

Aerosol infection; 16 weeks after first injection

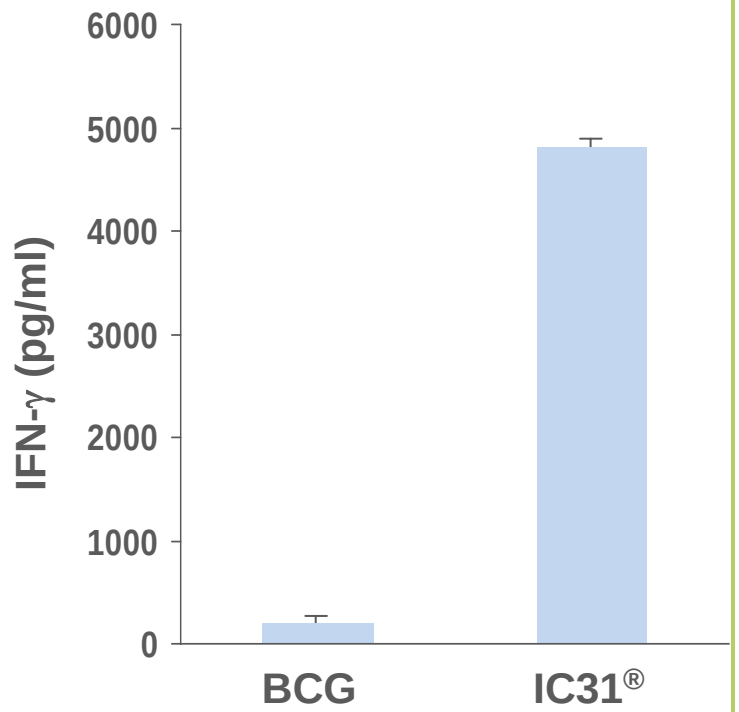
Protectivity is linked to IFN- γ producing T-cells indicative for Th-1 driven immunity

DEFINITION OF PROTECTION MARKERS (MOUSE MODEL)

RESIDUAL BACTERIA (lung)



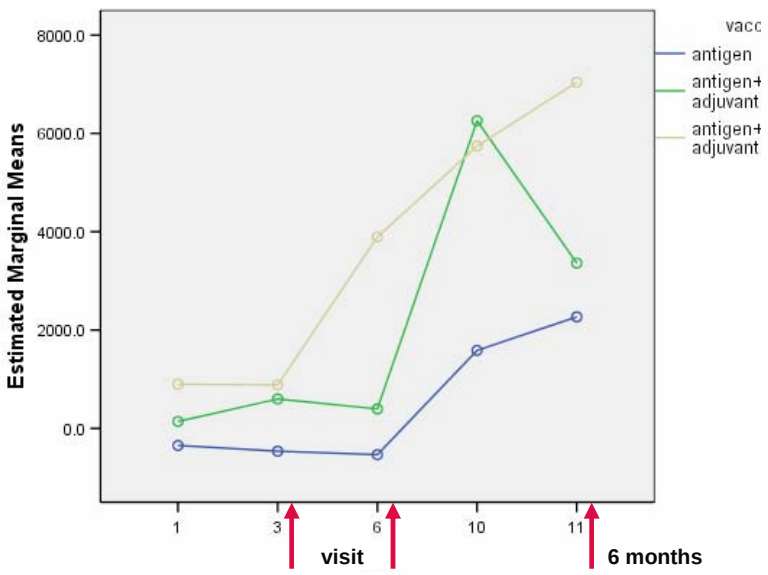
IFN-γ production



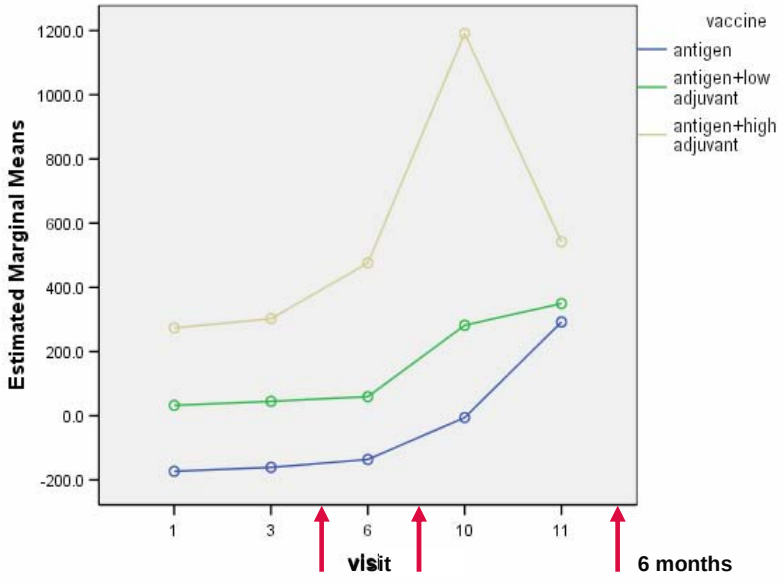
Induction of antigen-specific T-cells in humans vaccinated with the novel TB subunit vaccines

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)

Dose/100µl/mouse:

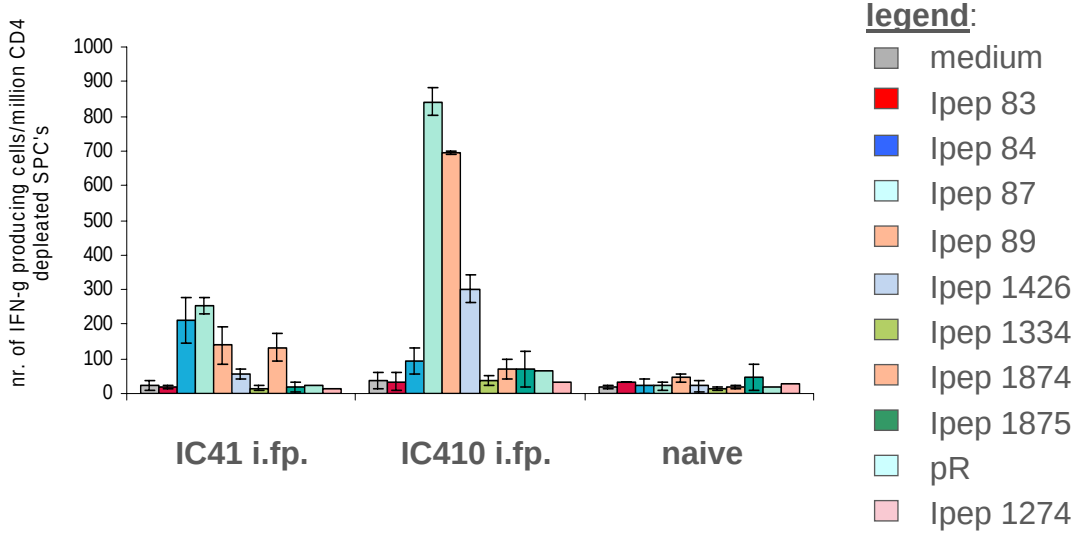
IC41:

200µg/peptide +
400µg pR43
(lot K, batch PD03126)

IC410:

50µg/peptide +
35nmol KLK+
1.4nmol ODN1a
(inhouse mixture)

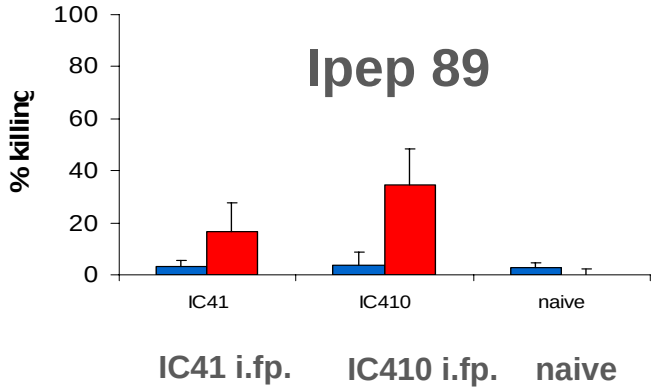
IFN-γ PRODUCTION



CD8+ T CELL EFFECTOR FUNCTION

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

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



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INTERCELL

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