DGHO Jahrestagung 2017

Stuttgart 29.09.-03.-10.

Tumorvakzinierung: Aktueller Stand

Cancer vaccination: Present stage

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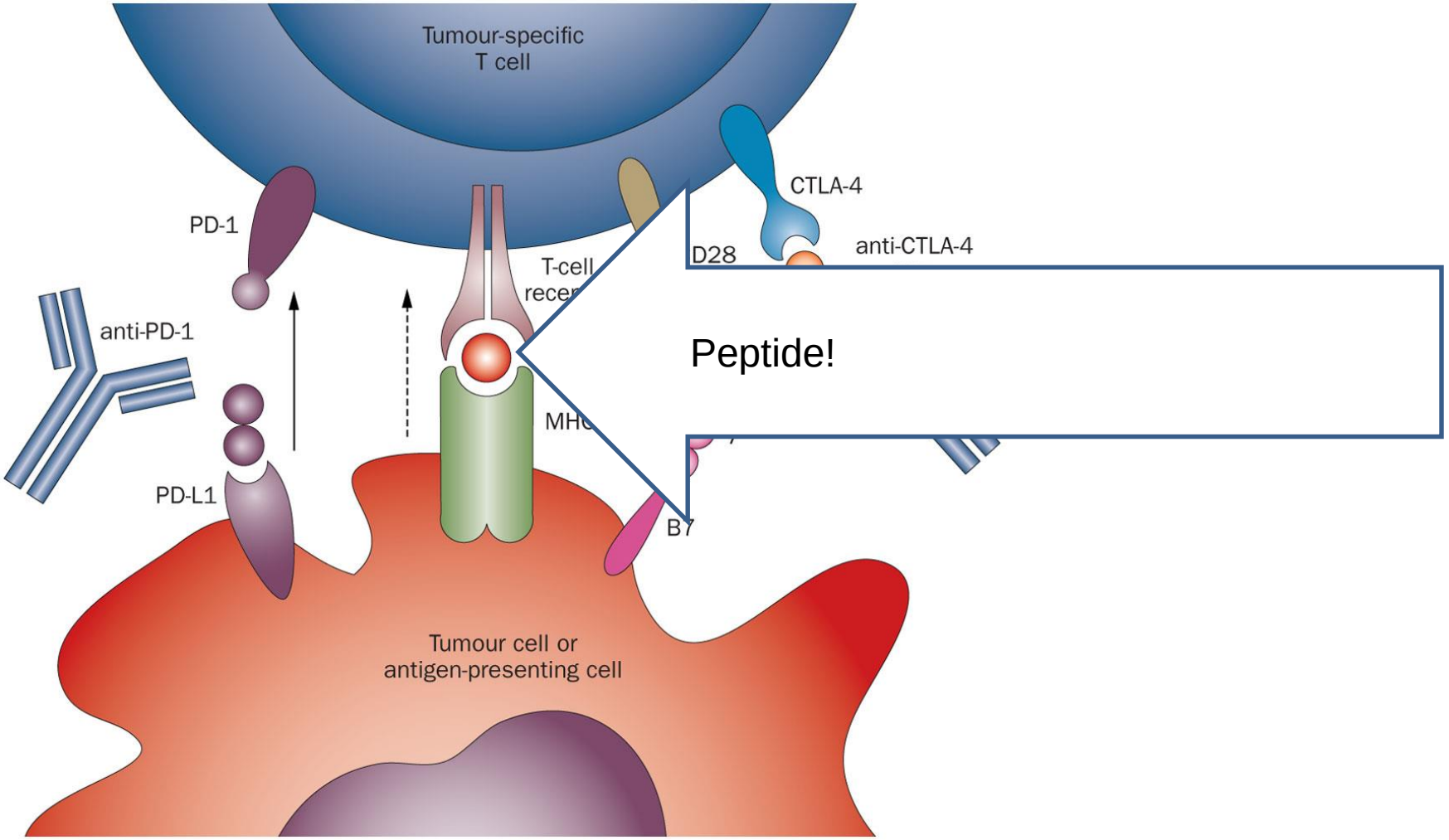
Interfakultäres Institut für Zellbiologie
Abteilung Immunologie
Prof. Dr. Hans-Georg Rammensee

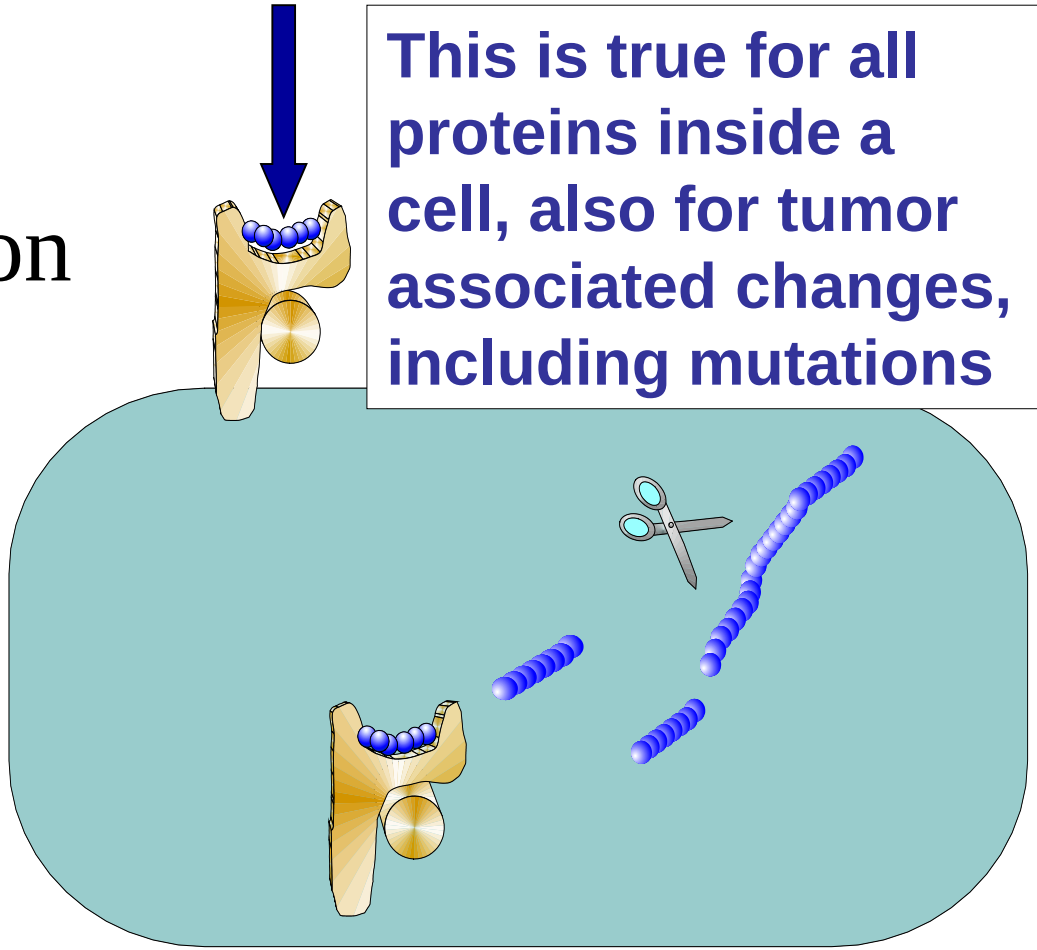
Immunotherapy with checkpoint inhibitors



Drake, C. G. *et al.* (2013) Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.208

Antigen presentation by HLA molecule

this can be recognized by T cells

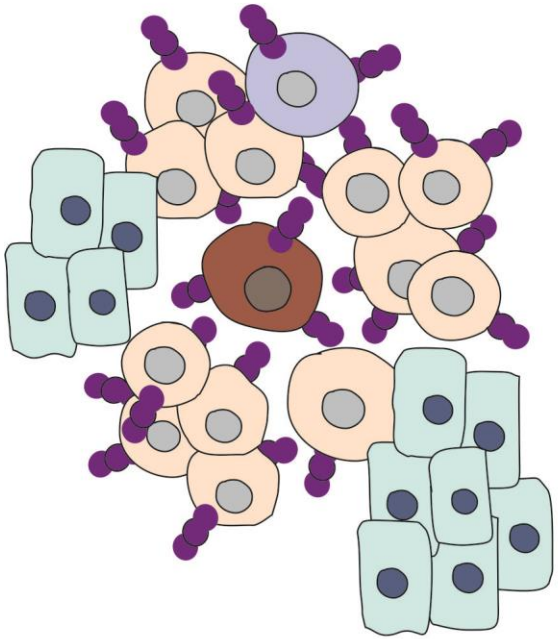


This is true for all proteins inside a cell, also for tumor associated changes, including mutations

Antigen processing

3 categories of tumor antigens:

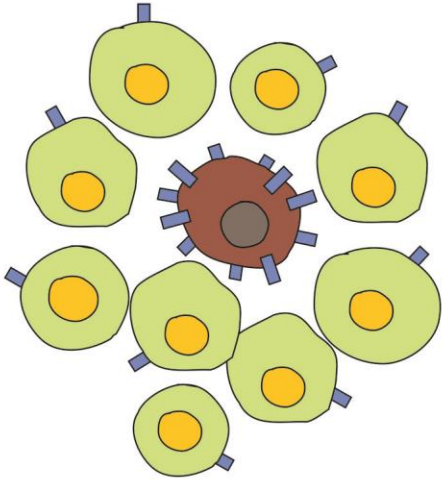
tissue specific
("differentiation antigen")



e.g. Provenge

Limited to few cancer entities
Well established for antibodies (eg., Rituximab)
Popular targets for CARs

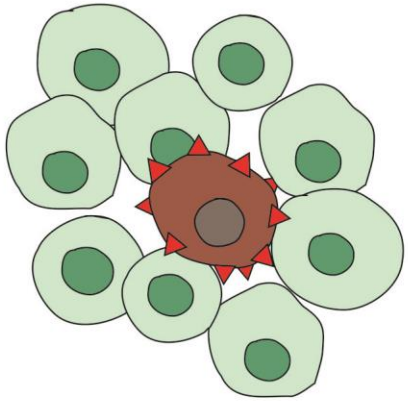
tumor associated
germline antigens



eg., NY-ESO1,
MUC1, IMA 901, ...

We know plenty of such antigens; immune responses tend to be weak with the immunomodulation measures used so far

mutated or viral



for mutated antigens
only possible in
individualized approach

Are mutated peptides presented in all human cancer types?

Thus: the majority of cancer patients will not benefit from checkpoint inhibition (alone)

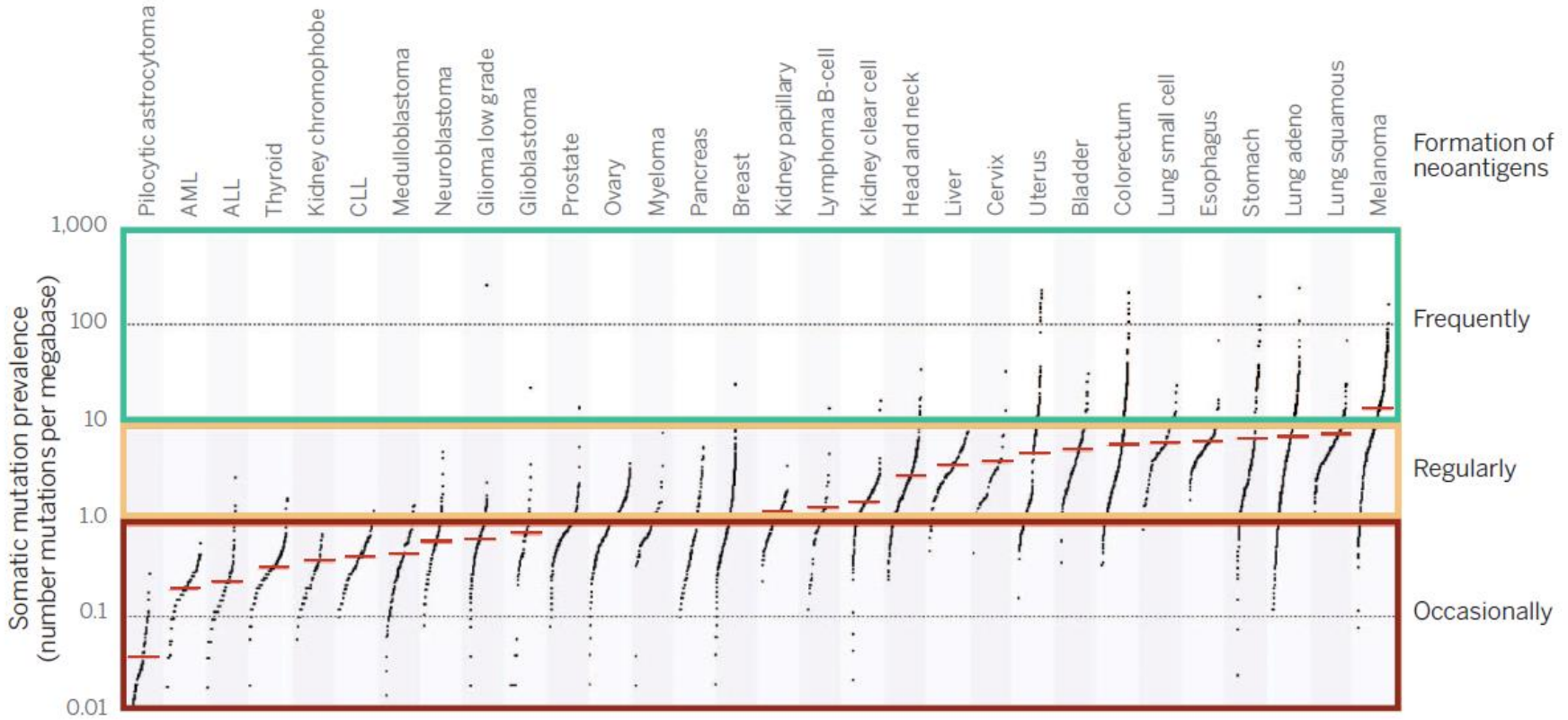


Fig. 2. Estimate of the neoantigen repertoire in human cancer. Data depict the number of somatic mutations in individual tumors. Categories on the right indicate current estimates of the likelihood of neoantigen formation in different tumor types. Adapted from (*50*). It is possible that the immune system in melanoma patients picks up on only a fraction of the available neoantigen repertoire, in which case the current analysis will be an underestimate. A value of 10 somatic mutations per Mb of coding DNA corresponds to ~150 nonsynonymous mutations within expressed genes.

T. Schumacher & R. Schreiber

Thus: the majority of cancer patients will not benefit from checkpoint inhibition (alone)

Is therapeutic vaccination an option for this majority?

So far no real successful therapeutic vaccination in phase III trials.

Neither with peptides nor with anything else.

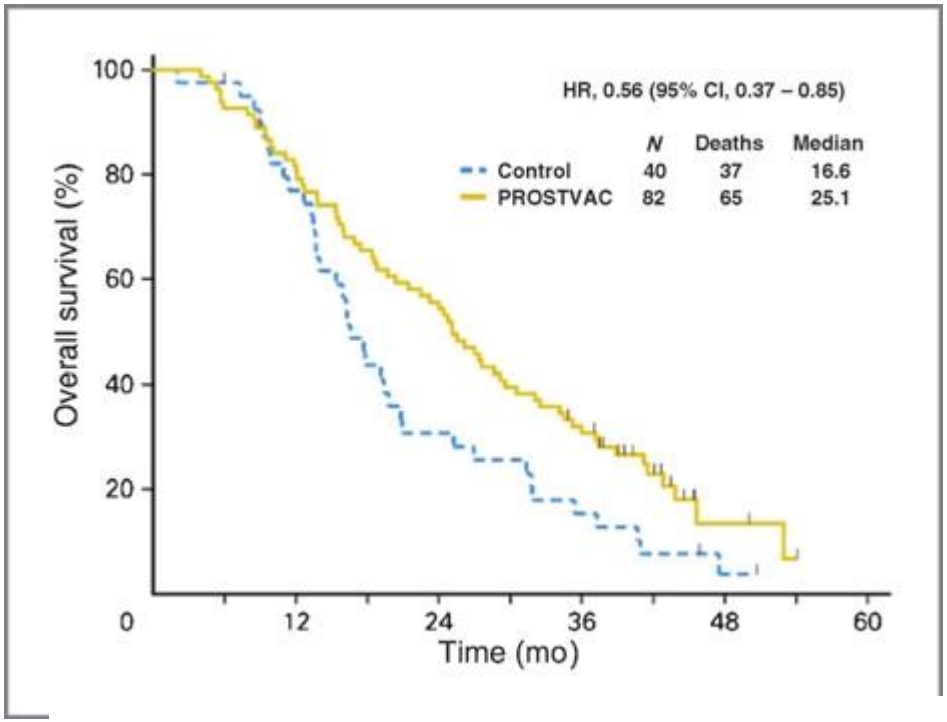
In contrast, there is a large number of phase II or earlier clinical trials, and case reports, reporting therapeutic vaccination with tumor antigens

- viral, mutated, tumor-associated or tissue specific –

leading to antigen specific T cell responses associated with clinical benefit.

Some selected examples for therapeutic vaccination approaches

cf. Provenge



Immune Impact Induced by PROSTVAC (PSA-TRICOM), a Therapeutic Vaccine for Prostate Cancer

James L. Gulley[1], Ravi A. Madan[1], Kwong Y. Tsang[1], Caroline Jochems[1], Jennifer L. Marté[1], Benedetto Farsaci[1], Jo A. Tucker[1], James W. Hodge[1], David J. Liewehr[2], Seth M. Steinberg[2], Christopher R. Heery[1], and Jeffrey Schlom[1]

Cancer Immunol Res; 2(2) February 2014

mRNA-Vaccination in prostate carcinoma patients / CureVac

Kübler *et al. Journal for ImmunoTherapy of Cancer* (2015) 3:26
DOI 10.1186/s40425-015-0068-y

Journal for ImmunoTherapy of Cancer

RESEARCH ARTICLE Open Access

Self-adjuvanted mRNA vaccination in advanced prostate cancer patients: a first-in-man phase I/IIa study

Hubert Kübler[1†], Birgit Scheel[2*†], Ulrike Gnad-Vogt[2], Kurt Miller[3], Wolfgang Schultze-Seemann[4], Frank vom Dorp[5], Giorgio Parmiani[6], Christian Hampel[7], Steffen Wedel[8], Lutz Trojan[9], Dieter Jocham[10], Tobias Maurer[1], Gerd Rippin[11], Mariola Fotin-Mleczek[2], Florian von der Mülbe[2], Jochen Probst[2], Ingmar Hoerr[2], Karl-Josef Kallen[2], Thomas Lander[2] and Arnulf Stenzl[12]

uicc
global cancer control

2012

IJC
International Journal of Cancer

Vaccination-induced functional competence of circulating human tumor-specific CD8 T-cells

Petra Baumgaertner[1], Camilla Jandus[1], Jean-Paul Rivals[2], Laurent Derré[1], Tanja Lövgren[1], Lukas Baitsch[1], Philippe Guillaume[1], Immanuel F. Luescher[1], Gregoire Berthod[2], Maurice Matter[2], Nathalie Rufer[1], Olivier Michielin[1,2] and Daniel E. Speiser[1]

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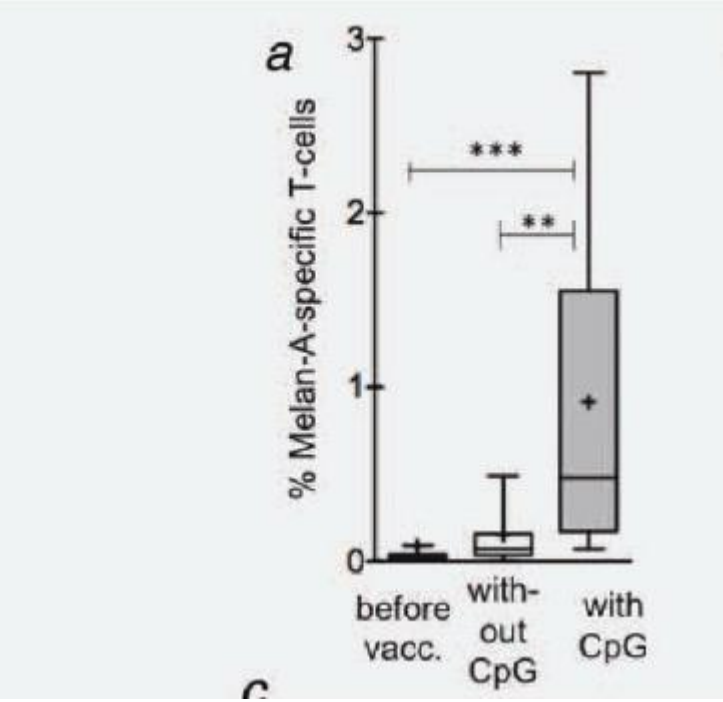
[2] University Hospital Center and University of Lausanne (CHUV), Lausanne, Switzerland



vaccination of peptide + CpG
in IFA (Montanide ISA51)

uicc
global cancer control

IJC
International Journal of Cancer

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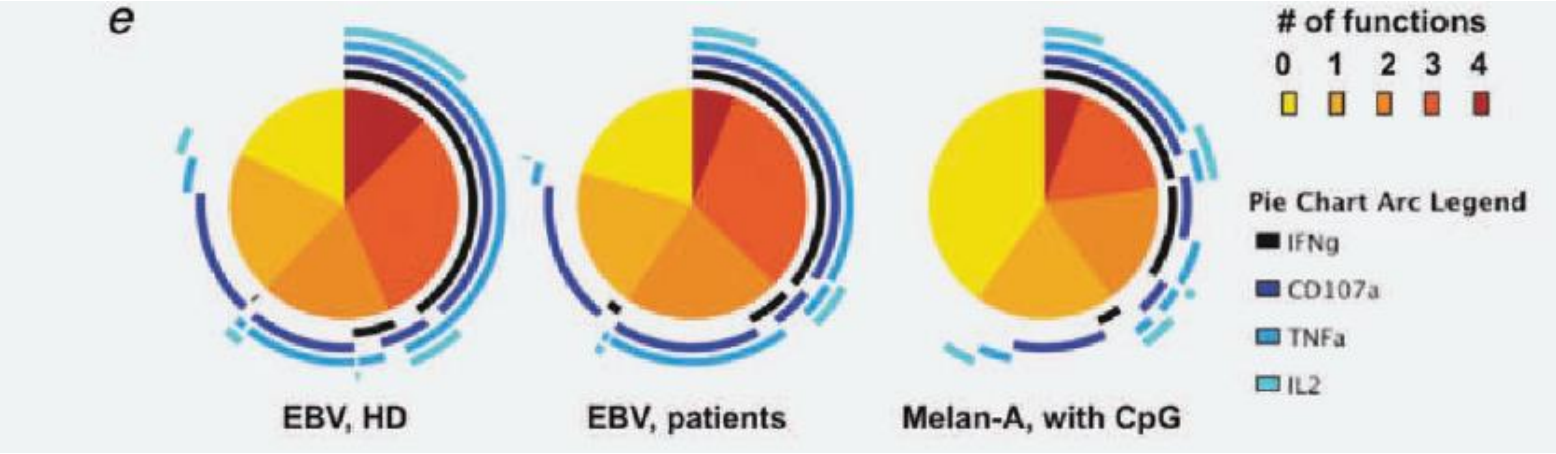
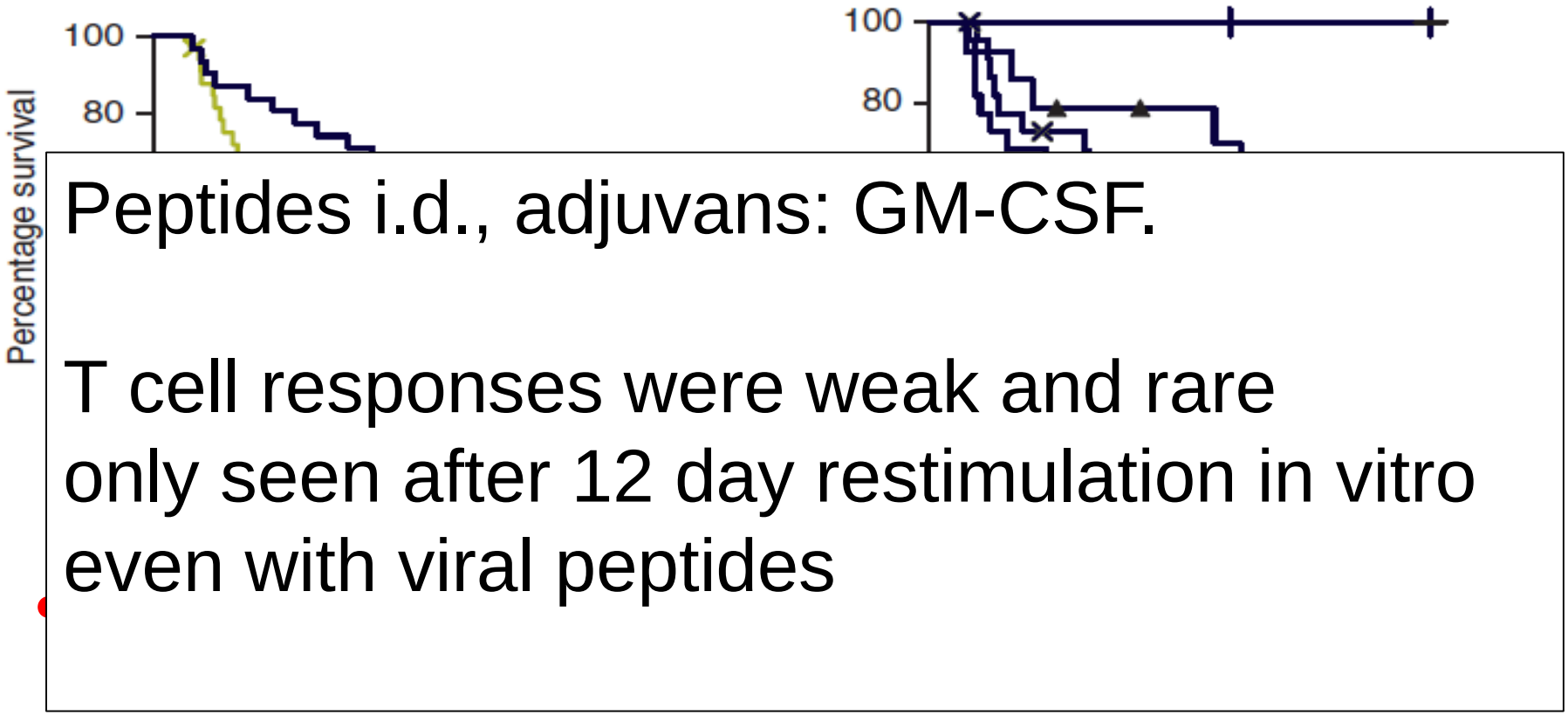


IMA901 phase 2 results, multipeptide vaccination in RCC



Peptides i.d., adjuvans: GM-CSF.

T cell responses were weak and rare
only seen after 12 day restimulation in vitro
even with viral peptides

- Patients who have received Cy and had T cell responses to IMA901 showed longer survival

*Walter et al., Nature Med. 2012

An open-label, randomized, phase 3 study investigating IMA901 multipeptide cancer vaccine in combination with sunitinib vs. sunitinib alone as first-line therapy for metastatic RCC

B. Rini[1], A. Stenzl[2], R. Zdrojowy[3], M. Kogan[4], M. Shkolnik[5], S. Oudard[6], S. Weikert[7], S. Bracarda[8], S. Crabb[9], J. Bedke[2], J. Ludwig[10], D. Maurer[10], R. Mendrzyk[10], A. Mahr[10], J. Fritsche[10], T. Weinschenk[10], H. Singh[10], A. Kirner[10], C. Reinhardt[10], T. Eisen[11]

[1]Cleveland Clinic Taussig Cancer Center, Cleveland, USA; [2]University of Tuebingen, Tuebingen, Germany; [3]Wroclaw Medical University, Wroclaw, Poland; [4]Rostov State Medical University of Roszdrav, Rostov-on-Don, Russia; [5]Russian Scientific Center of Radiology and Surgery Technologies, St. Petersburg, Russia; [6]Hopital Europeen Georges Pompidou, Paris, France; [7]Vivantes Humboldt Clinic, Berlin, Germany; [8]Ospedale San Donato, Arezzo, Italy; [9]Southampton General Hospital, Southampton, UK; [10]immatics biotechnologies GmbH, Tuebingen, Germany; [11]University of Cambridge, Cambridge, UK

published in: Lancet Oncol. 2016 Nov;17(11):1599-1611

General problem with most attempts of therapeutic vaccinations, in particular with multipeptide vaccines:

Weak immune responses,
unless combined with strong adjuvants

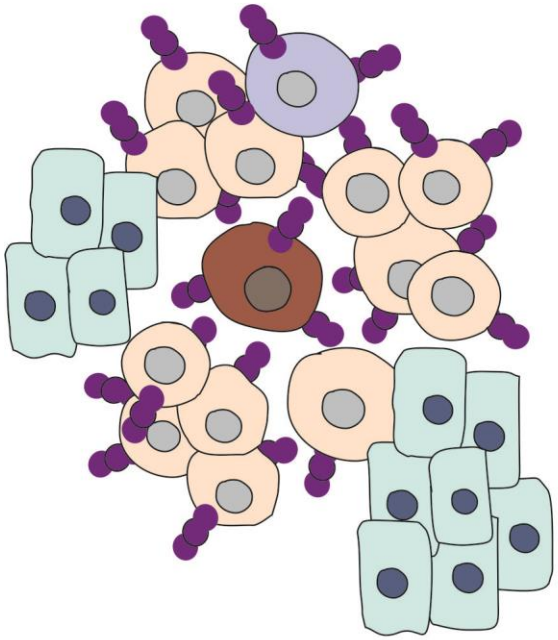
Strategy:

1. Identification of relevant tumor antigens
2. Efficient immune response by strongly adjuvanted vaccination

Identification of relevant tumor antigens

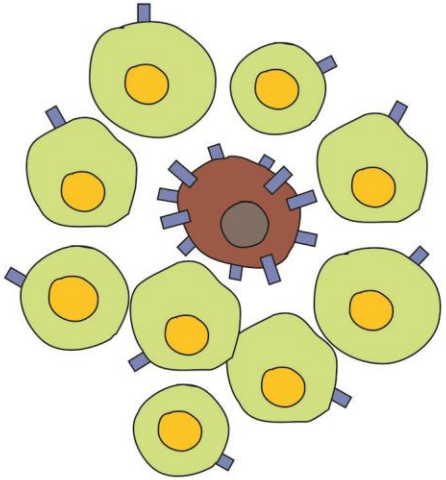
3 categories of tumor antigens:

tissue specific
("differentiation antigen")



eg., PSA, PSMA, CD19.
Limited to few cancer entities
Well established for antibodies (eg., CD19)
Popular targets for CARs

tumor associated
germline antigens



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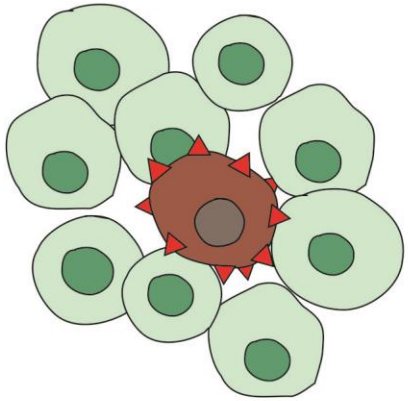
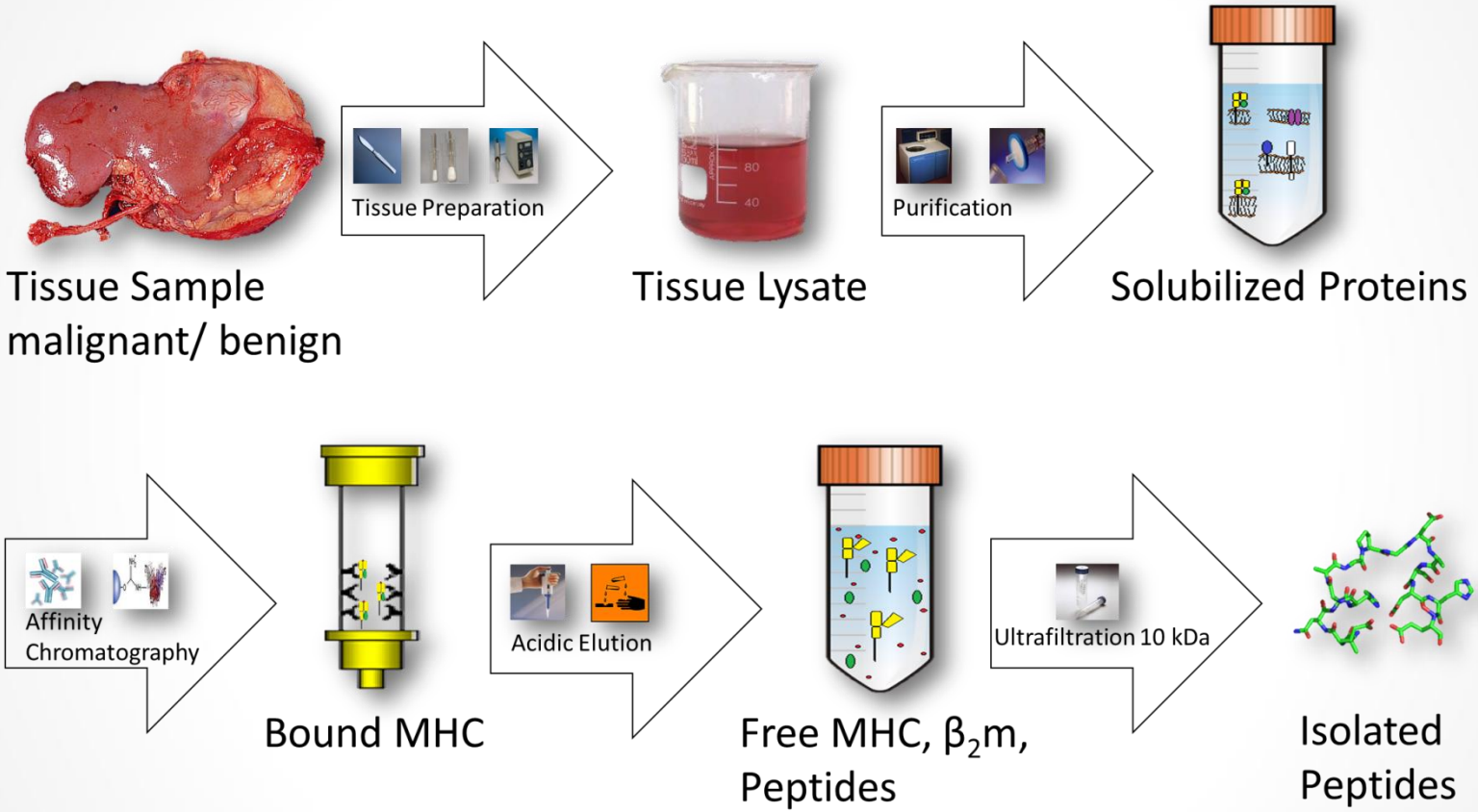


for mutated antigens only possible in individualized approach

Are mutated peptides presented in all human cancer types?

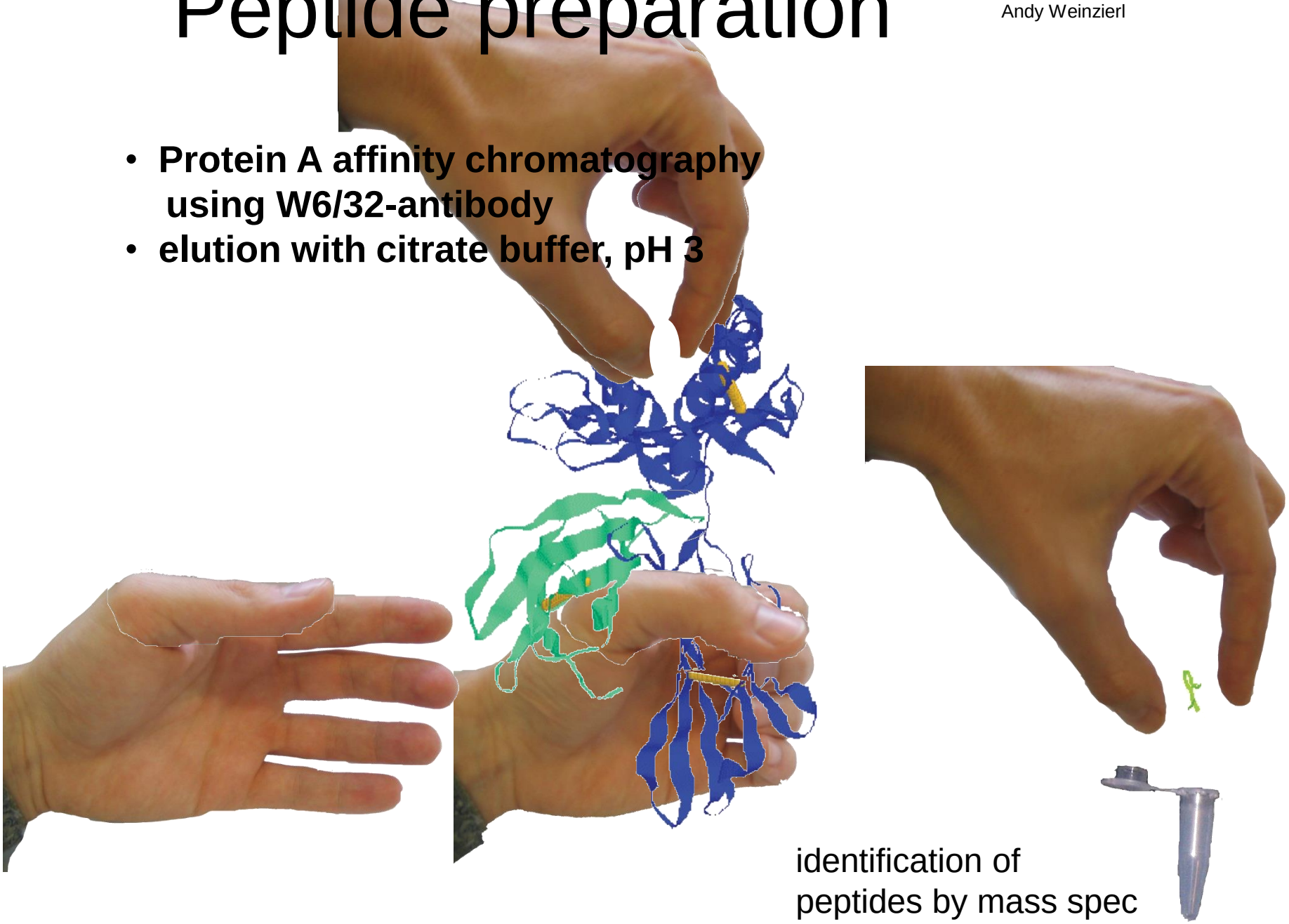
Isolation of Naturally Presented HLA-Ligands



Peptide preparation

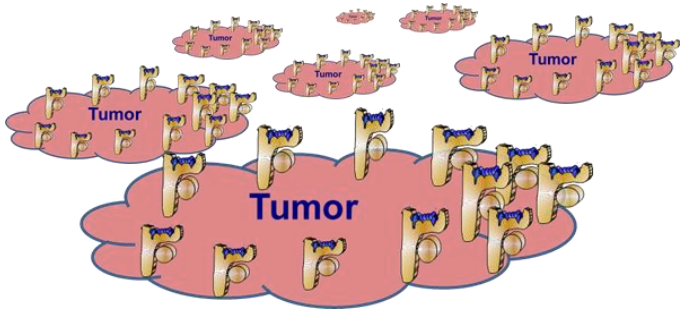
Andy Weinzierl

- **Protein A affinity chromatography using W6/32-antibody**
- **elution with citrate buffer, pH 3**



identification of
peptides by mass spec

HLA ligandome landscape discovery



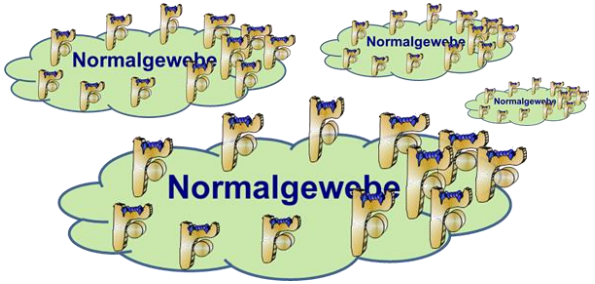
UNIVERSITÄTS KLINIKUM TÜBINGEN

Oncology
Hematology
Immunology
Rheumatology

Klinik für Urologie Tübingen

Transfusionsmedizin Tübingen

SFB 685
Immuntherapie



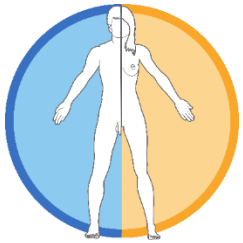
n tumor samples of different entities
(AML, CLL, CML, RCC, HCC, OvCa, Colon Ca,...

n normal tissue samples (kidney, liver, bone marrow, blood, colon, ...

Department of Immunology

HLA-ligandome analysis

- 1.5 Mio HLA class I ligands
- 0.6 Mio HLA class II ligands
- ≈ 15.000 source proteins



Determination of the HLA ligandome of normal tissues (from autopsies)

Current sample overview

Marian Neidert

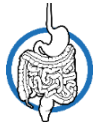
- Sample acquisition in Zürich
- 14 donors
- up to 22 organs per donor

- Samples analyzed so far:

⇒ 810 runs

⇒ 68,845 unique class I peptides

⇒ 103,600 unique class II peptides

	Bladder	4		Aorta	3		Spleen	3
				Heart	4		Lymph Node 3	
	Mamma	1		Trachea	1		Adrenal Gland	1
				Lung	5		Kidney	4
	Skin	2		Liver	4		Cerebellum	5
							Brain	5
	Pancreas	1		Testis	4		Tongue	2
							Esophagus	2
	Thyroid	6		Muscle	3		Small Intestine	2
							Colon	3
							Bone Marrow	3

We found mutated MHC I presented peptides in mouse and human tumor cell lines, and in melanoma samples.

We rarely find mutated HLA I ligands in human tumor tissue of intermediate mutation rates (HCC, RCC, OvCa, Leukemias, Glioma)

Neoantigens

from Toni Weinschenk, immatics

	Expressed Somatic non-synonymous mutations	Peptides with mutation	Peptides %
Own MHC ligandomics data			
B16F10 melanoma	563 [CASTLE 2012]	1	0.18%
GL261 glioblastoma	2003 [OKADA, pers. Comm.]	1	0.05%
CT26 colorectal carcinoma	1172 [CASTLE 2014]	1	0.09%
MC-38 colon carcinoma [YADAV 2014]	1290	7	0.54%
TRAMP-C1 [YADAV 2014]	67	0	0.00%
Published Proteomics studies			
SW480* colorectal line [WANG 2012]	3501	33	0.94%
RKO* colorectal line [WANG 2012]	3995	43	1.08%
TCGA** Breast cancer (n=105) [MERTINS 2014]	91.4	1.1	1.20%

Yadav et al. 2014

0.1%

1%

immatics

ARTICLE

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DOI: 10.1038/ncomms13404 OPEN

Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry

Michal Bassani-Sternberg[1,†,*], Eva Bräunlein[2,*], Richard Klar[2], Thomas Engleitner[3,4], Pavel Sinitcyn[1], Stefan Audehm[2], Melanie Straub[5], Julia Weber[3,4], Julia Slotta-Huspenina[5,6], Katja Specht[5], Marc E. Martignoni[7], Angelika Werner[7], Rüdiger Hein[8], Dirk H. Busch[9], Christian Peschel[2,4], Roland Rad[3,4], Jürgen Cox[1], Matthias Mann[1,**] & Angela M. Krackhardt[2,4,**]

5 patients

>1.000 nonsynonymous mutations per tumor

11 mutated neoantigens found by MS

8, 2, or 1 per patient

4 out of 11 immunogenic

LETTER

doi:10.1038/nature22991

An immunogenic personal neoantigen vaccine for patients with melanoma

Patrick A. Ott[1,2,3]*, Zhuting Hu[1]*, Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4]†, Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]

Effective anti-tumour immunity in humans has been associated with the presence of T cells directed at cancer neoantigens[1], a class of HLA-bound peptides that arise from tumour-specific mutations. They are highly immunogenic because they are not present in normal tissues and hence bypass central thymic tolerance. Although neoantigens were long-envisioned as optimal targets for an anti-tumour immune response[2], their systematic discovery and evaluation only became feasible with the recent availability of massively parallel sequencing for detection of

97 unique neoantigens used across patients, respectively. These T cells discriminated mutated from wild-type antigens, and in some cases directly recognized autologous tumour. Of six vaccinated

and immunogenicity of a vaccine that targets up to 20 predicted personal tumour neoantigens. Vaccine-induced polyfunctional CD4$^+$ and CD8$^+$ T cells targeted 58 (60%) and 15 (16%) of the 97 unique neoantigens used across patients, respectively. These T cells discriminated mutated from wild-type antigens, and in some cases directly recognized autologous tumour. Of six va ated patients, four had no recurrence at 25 months after va on, while two with recurrent disease were subsequently tre with anti-PD-1 (anti-programmed cell death-1) therapy and experienced complete tumour regression, with expansion of the repertoire of neoantigen-specific T cells. These data provide a strong rationale for further development of this approach, alone and in combination with checkpoint blockade or other immunotherapies.

To generate a vaccine that targets personal neoantigens, we conducted

agonist poly-ICLC[4] (Hiltonol) (Fig. 1a and Supplementary Information 1–3). We evaluated this vaccine in a phase I study in patients with previously untreated high-risk melanoma (stage IIIB/C and IVM1a/b) after surgical resection with curative intent (Extended Data Table 1 and Supplementary Information 4a).

Of the ten patients enrolled, eight demonstrated the high mutation rate expected for melanoma, carried expected melanoma-associated mutations (that is, in *BRAF*, *NRAS*, and others) and predominantly C→T transitions (consistent with ultraviolet exposure), and expressed

rash, and fatigue (Supplementary Information 4b).

At a median follow-up of 25 months (range 20–32) after vaccination, four patients who entered the study with stage IIIB/C disease remained without disease recurrence. Two patients entered with previously untreated stage IVM1b disease (lung metastases); both had disease recurrence evident on restaging scans obtained after the last vaccination. Subsequently, both patients underwent treatment with the anti-PD-1 antibody pembrolizumab and, after four doses, both achieved complete radiographic responses that are ongoing (note that complete radiographic response rate of pembrolizumab as first-line treatment for metastatic melanoma was reported as 6.1% (ref. 5)) (Fig. 1b and Extended Data Fig. 1d).

Overlapping 15- to 16-mer assay peptides (ASP) spanning the entirety of each IMP and 9- to 10-mer peptides corresponding to each

LETTER

doi:10.1038/nature23003

Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

Ugur Sahin[1,2,3], Evelyna Derhovanessian[1], Matthias Miller[1], Björn-Philipp Kloke[1], Petra Simon[1], Martin Löwer[2], Valesca Bukur[1,2], Arbel D. Tadmor[2], Ulrich Luxemburger[1], Barbara Schrörs[2], Tana Omokoko[1], Mathias Vormehr[1,3], Christian Albrecht[2], Anna Paruzynski[1], Andreas N. Kuhn[1], Janina Buck[1], Sandra Heesch[1], Katharina H. Schreeb[1], Felicitas Müller[1], Inga Ortseifer[1], Isabel Vogler[1], Eva Godehardt[1], Sebastian Attig[2,3], Richard Rae[2], Andrea Breitkreuz[1], Claudia Tolliver[1], Martin Suchan[2], Goran Martic[2], Alexander Hohberger[3], Patrick Sorn[2], Jan Diekmann[1], Janko Ciesla[4], Olga Waksmann[4], Alexandra-Kemmer Brück[1], Meike Witt[1], Martina Zillgen[1], Andree Rothermel[2], Barbara Kasemann[2], David Langer[1], Stefanie Bolte[1], Mustafa Diken[1,2], Sebastian Kreiter[1,2], Romina Nemecek[5], Christoffer Gebhardt[6,7], Stephan Grabbe[3], Christoph Höller[5], Jochen Utikal[6,7], Christoph Huber[1,2,3], Carmen Loquai[3]* & Özlem Türeci[8]*

T cells directed against mutant neo-epitopes drive cancer immunity. However, spontaneous immune recognition of mutations is inefficient. We recently introduced the concept of individualized mutanome vaccines and implemented an RNA-based poly-neo-

Vaccine-induced T cell infiltration and neo-epitope-specific killing of autologous tumour cells were shown in post-vaccination resected metastases from two patients. The cumulative rate of metastatic

multiple vaccine neo-epitopes at up to high single-digit percentages. Vaccine-induced T cell infiltration and neo-epitope-specific killing of autol tumour cells were shown in post-vaccination resected metasta om two patients. The cumulative rate of metastatic events w highly significantly reduced after the start of vaccination, resulting in a sustained progression-free survival. Two of the five patients with metastatic disease experienced vaccine-related objective responses. One of these patients had a late relapse owing to outgrowth of β2-microglobulin-deficient melanoma cells as an acquired resistance mechanism. A third patient developed a complete response to vaccination in combination with PD-1 blockade therapy. Our study demonstrates that individual mutations can be exploited, thereby opening a path to personalized immunotherapy for patients with cancer.

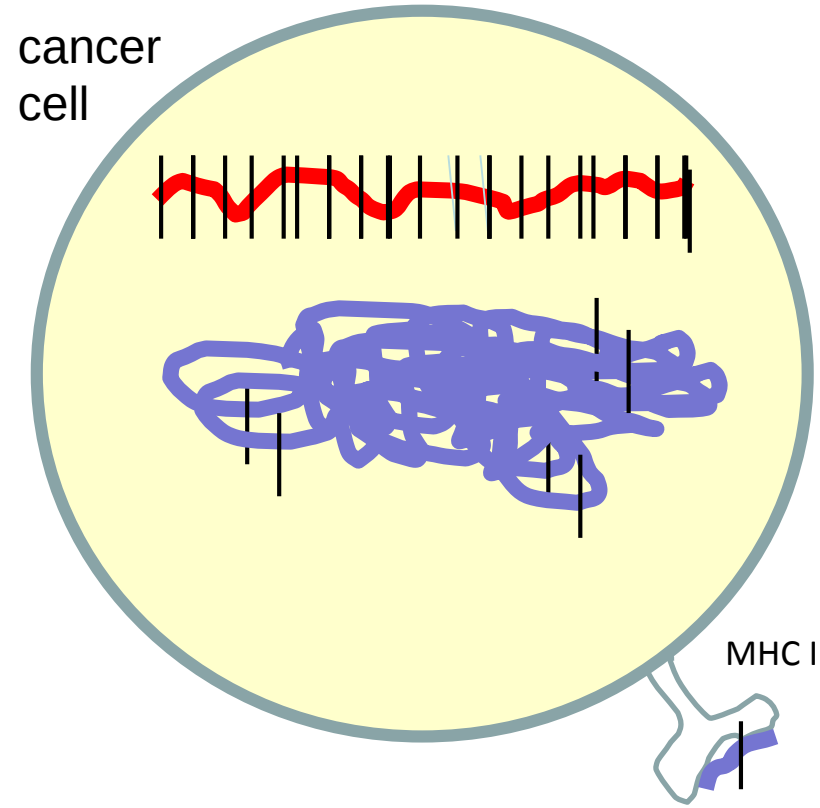
Cancer mutations can form neo-epitopes recognized by T cells

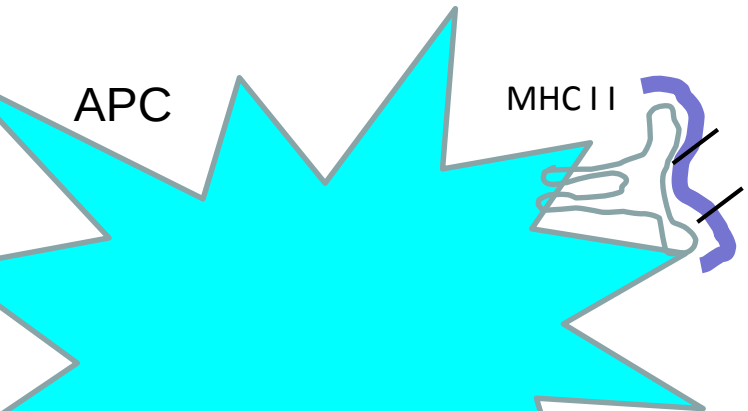
and RNA sequencing of routine tumour biopsies and healthy blood cells. Mutations were ranked according to: (1) predicted high-affinity binding to autologous HLA class II and high expression of the mutation-encoding RNA[2], and (2) predicted HLA class I binding.

median time from selection of mutations to vaccine release to 105 days (range 89 to 160 days). Patients with NY-ESO-1- and/or tyrosinase-positive melanoma received an RNA vaccine encoding these shared tumour-associated self-antigens until release of their neo-epitope vaccine. At least eight doses of the neo-epitope vaccine were injected percutaneously into inguinal lymph nodes under ultrasound control (Fig. 1d). Previously, in mouse models, we showed efficient uptake, translation of RNA-encoded antigens by lymph-node-resident dendritic cells (DCs), and intrinsic adjuvant activity[15].

All patients completed treatment with a maximum of 20 neo-epitope vaccine doses (Extended Data Table 1), which they tolerated well without related serious adverse events. The immunogenicity of each of the 125 mutations administered in this study was analysed by IFNγ ELISpot in $CD4^+$ and $CD8^+$ T cells in pre- and post-vaccination blood samples (Supplementary Table 2). Responses were detected against 60%

Frequency of presented neoantigens on MHC I vs crosspresented MHC II?





1000	Mutations at DNA sequence level
10	Protein
1	HLA class I ligandome
x?	crosspresented HLA class II ligandome

Neoantigens appear to be rare if not absent in many tumors.

But:

There are many **germline** sequence tumor associated HLA ligands,
and T cell responses against such antigens correlate with overall survival or other clinical benefit.

3 examples

of attempts

to identify relevant tumor antigens

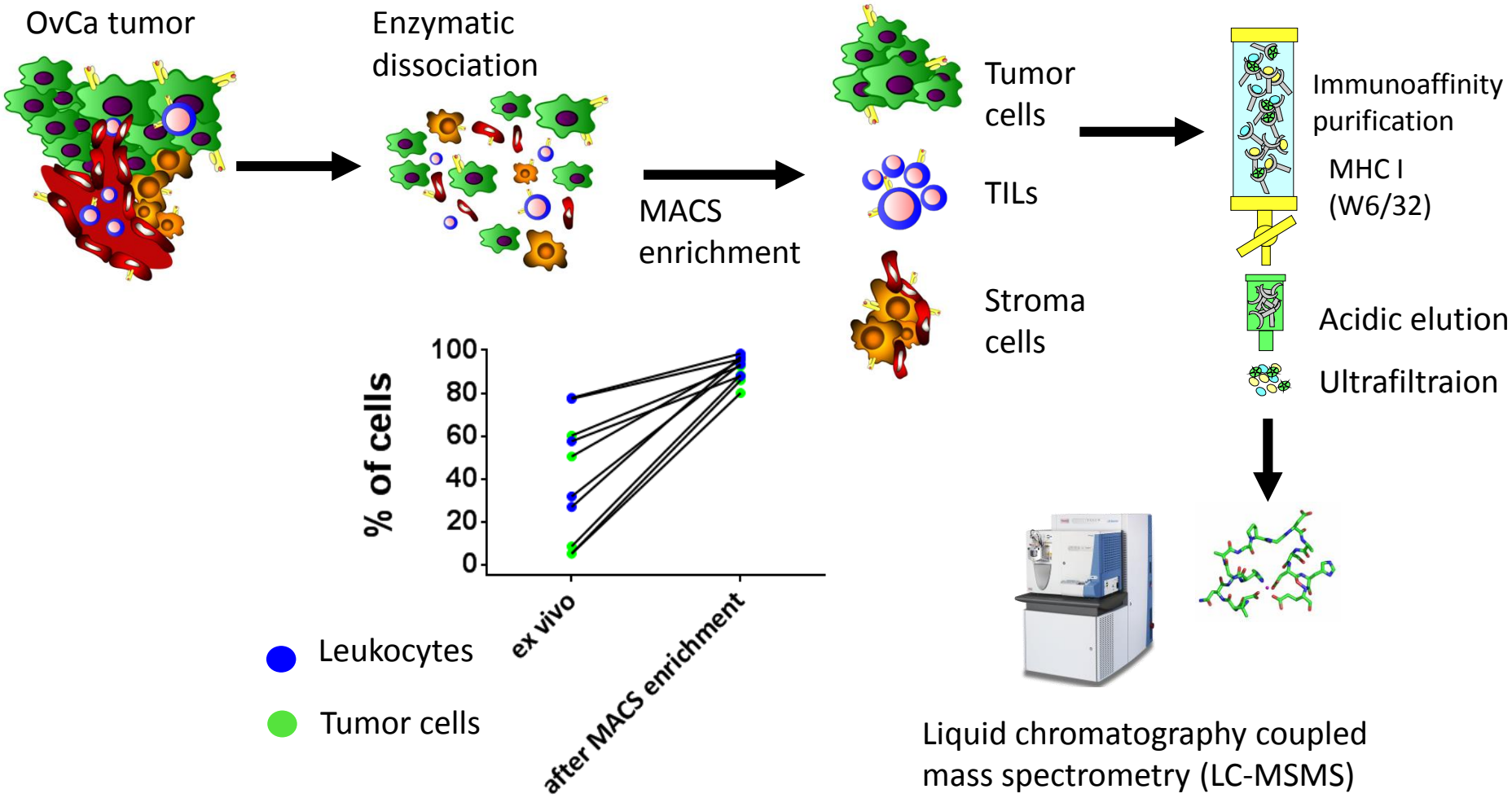
The immunopeptidomic landscape of ovarian carcinomas

Heiko Schuster[1], Janet Kerstin Peper[1], Hans-Christian Bösmüller[2], Kevin Röhle[1], Linus Backert[4], Britta Ney[2], Markus Löffler[1,5], Daniel Johannes Kowalewski[1], Nico Trautwein[1], Armin Rabsteyn[1,6], Tobias Engler[3], Sabine Braun[3], Barbara Schmid-Horch[7], Diethelm Wallwiener[3],Oliver Kohlbacher[4], Falko Fend[2,6], Hans-Georg Rammensee[1,6], Stefan Stevanović[1,6], Annette Staebler[2] and Philipp Wagner[1,3]

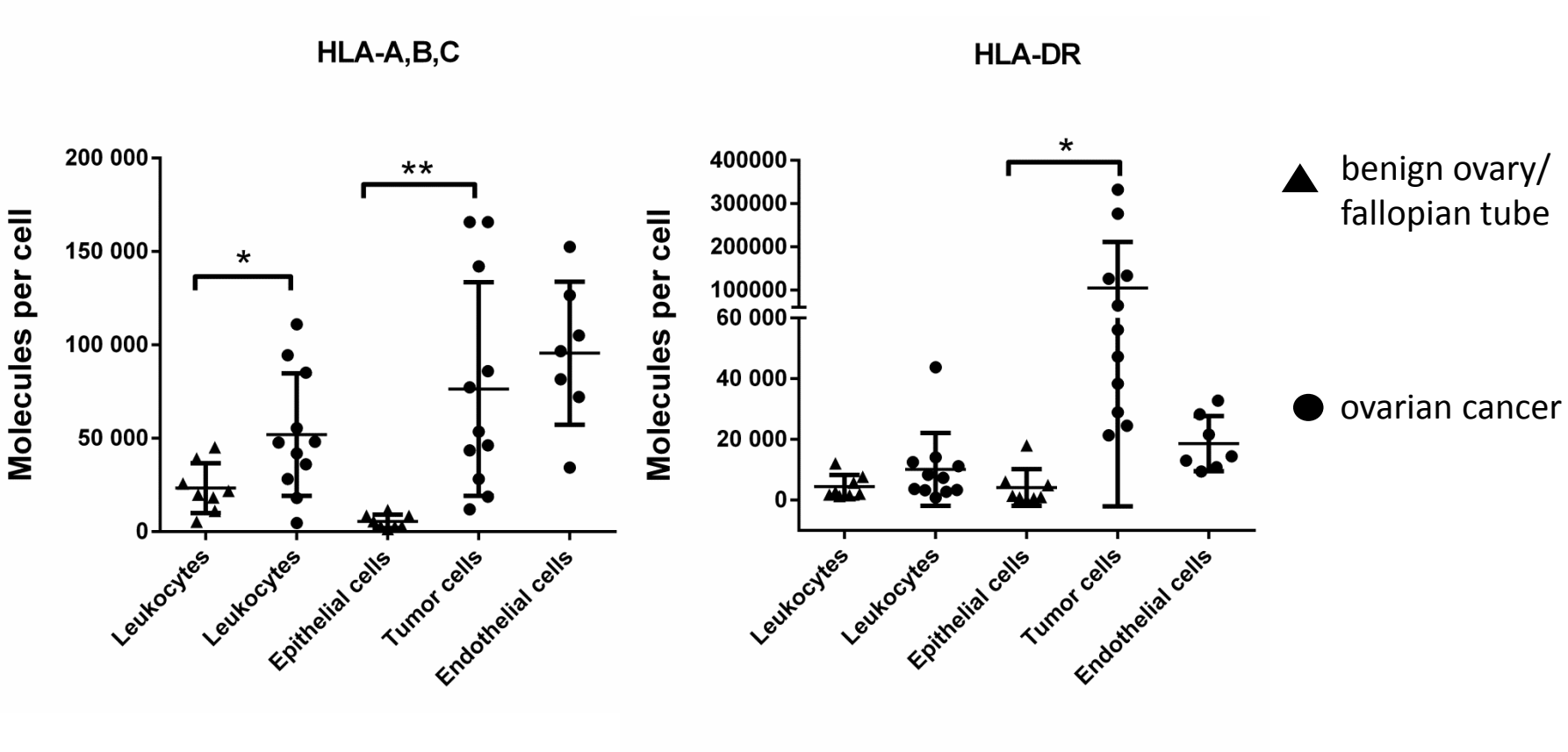
Heiko Schuster and Janet Peper et al., manuscript provisionally accepted (PNAS)

Stevanović Lab

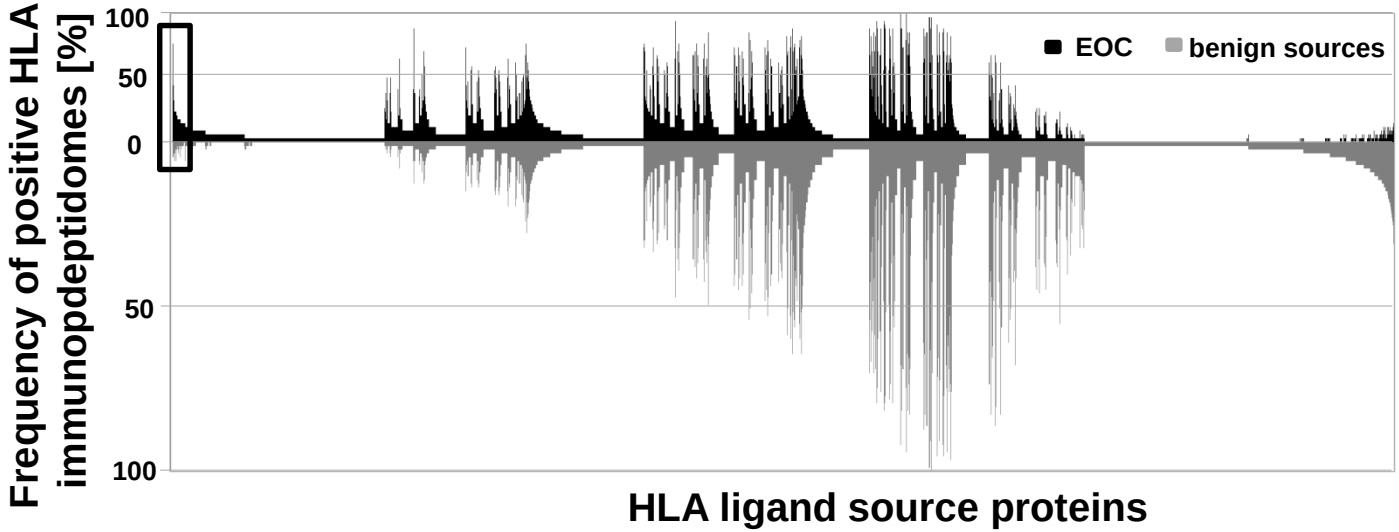
Enrichment and label free quantification



HLA expression within OvCa tissue



The immunopeptidomic landscape of OvCa – MHC class I

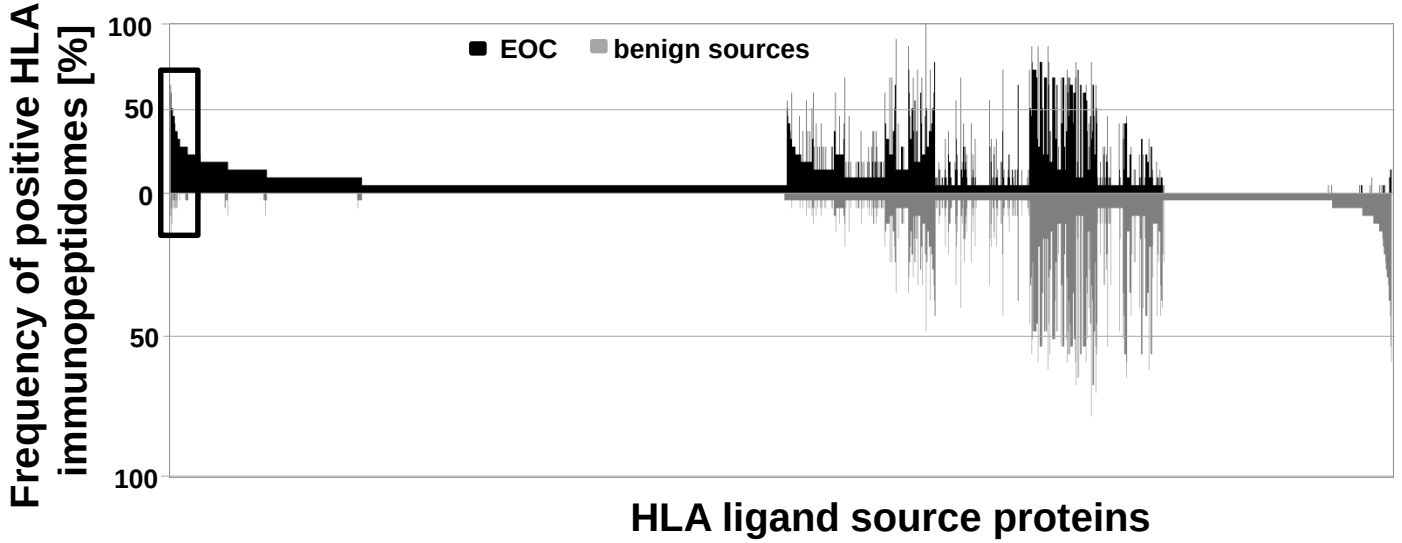


- 34 OvCa samples
- 30 PBMCs, 10 bone marrow, 15 liver,

  12 colon, 4 ovary,

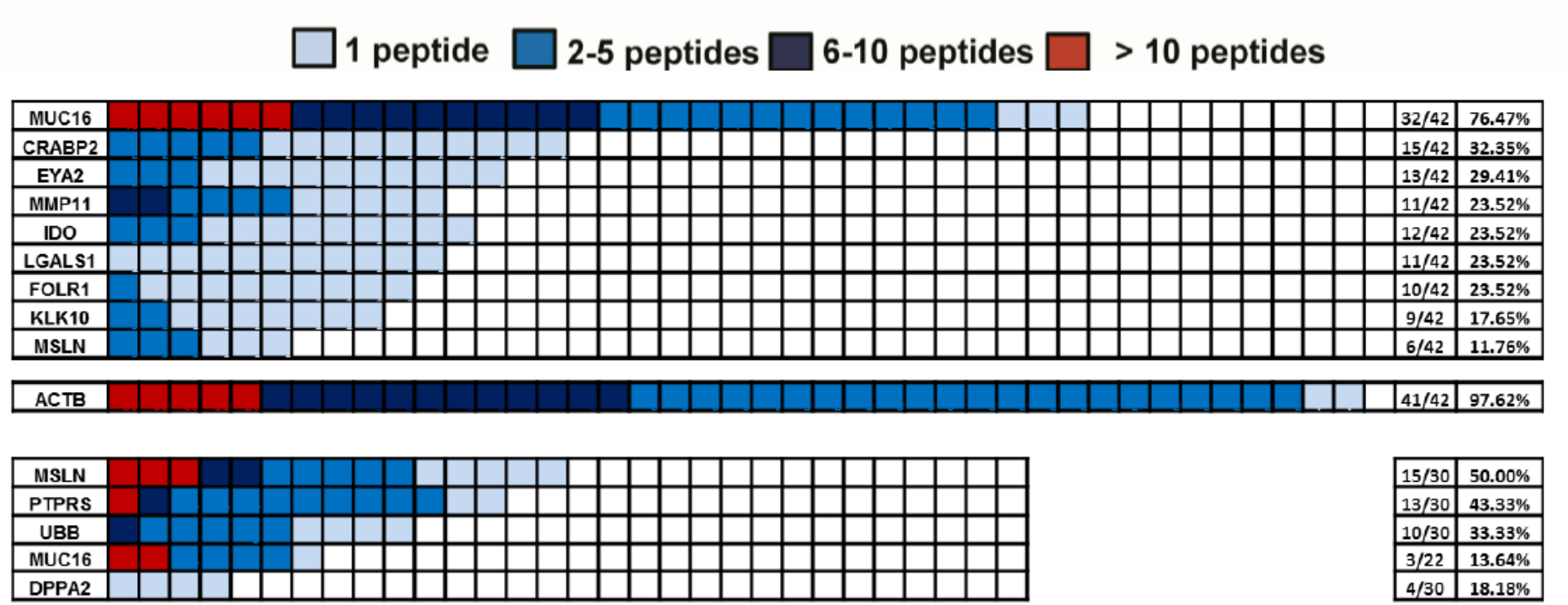
  13 kidney samples

The immunopeptidomic landscape of OvCa – MHC class II



- 22 OvCa samples
- 13 PBMCs, 5 bone marrow, 7 liver, 2 colon, 17 kidney samples

Frequency and number of HLA ligands among OvCa samples



Immunogenicity analysis of EOC presented HLA ligands from MUC16 and further EOC exclusively presented antigens

HLA	Sequence	Protein	positive/tested donors
A*01	STETSTVLY	MUC16	0 / 3
A*02	IITEVITRL	MUC16	3 / 10
A*02	KMISAIPTL	MUC16	4 / 6
A*03	SVLADLVTTK	MUC16	0 / 1
A*11	STSQEIHSATK	MUC16	2 / 6
A*11	GTSGTPVSK	MUC16	0 / 5
A*24	TYSEKTTLF	MUC16	3 / 3
A*24	QFITSTNTF	MUC16	1 / 2
A*24	AVTNVRTSI	MUC16	1 / 3
A*25	EVITSSRTTI	MUC16	1 / 1
A*25	EVISSRGTSM	MUC16	1 / 3
A*25	EVTSSGRTSI	MUC16	2 / 3
A*25	ETILTFHAF	MUC16	2 / 2
B*07	SPHPVTALL	MUC16	1 / 1
B*07	SPQNLRNTL	MUC16	2 / 3
B*07	TPGNRAISL	MUC16	5 / 5
B*07	SPLFQRSSL	MUC16	1 / 3
B*07	SPHPVTALL	MUC16	1 / 2
B*07	LPHSEITTL	MUC16	1 / 3
B*07	TPGGTRQSL	MUC16	0 / 3
B*07	SPSKAFASL	MUC16	3 / 3
B*07	VPRSAATTL	MUC16	2 / 3
B*15	SQGFSHSQM	MUC16	4 / 5
B*15	FQRQGQTAL	MUC16	1 / 6
B*18	TETEAIHVF	MUC16	1 / 1
B*27	ERSPVIQTL	MUC16	1 / 2
B*51	DPYKATSAV	MUC16	3 / 3
B*51	DALVLKTV	MUC16	1 / 3
A*03	RSYHLQIVTK	IDO1	1 / 1
A*11	RSYHLQIVTK	IDO1	1 / 1
A*24	RYMPPAHRNF	IDO1	4 / 4
B*07	NPKAFFSVL	IDO1	3 / 5
A*25	EVAPDAKSF	LGALS1	1 / 1
A*02	RTTEINFKV	CRABP1/2	1 / 2
A*02	RALAKLLPL	KLK10	2 / 2

Individualized peptide cocktail according to HLA ligandome and Exomseq analysis, ovarian carcinoma patient

Personalized multipeptide vaccine for patient OvCA 100		
	class I	
MUC16	KMISAIPTL	A*02
MUC16	SPHPVTALL	B*07
MUC16	SPSKAFASL	B*07
MUC16	TPGNRAISL	B*07
IDO1	NPKAFFSVL	B*07
DDR1	FLAEDALNTV	A*02
	class II	
	Longest variant	
MSLN	DLPGRFVAESAEVLLPR	
MUC16	ELGPYTLDRNSLYVNG	SB DRB3*0101
Predicted neoantigens from Exomseq		
MUC16	S**SLTHELSSRV**TPIP	**A*02**
PLEKHG2	PGGG**APASSRGSW**SS	**B*07**

To do for each patient:

1. start first ro
shelf“)

2. select addit
and class II lig
modifications), s
round of vacci

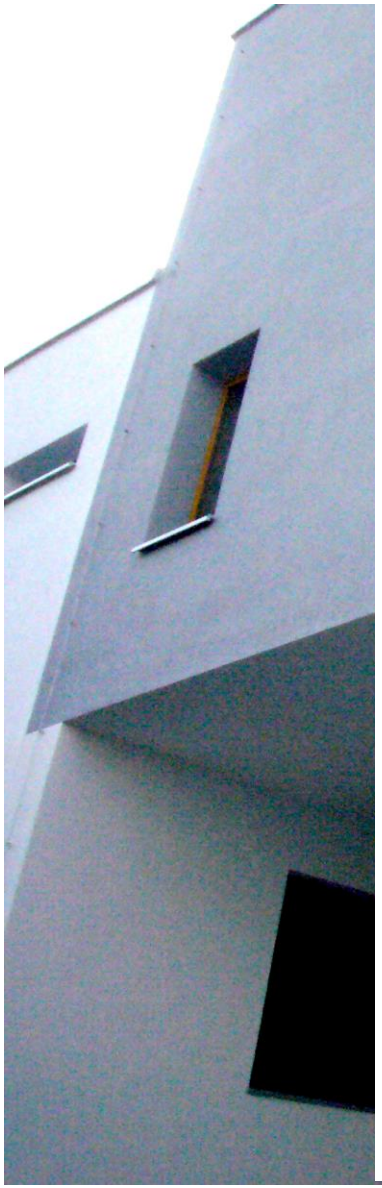
combine a
efficient im

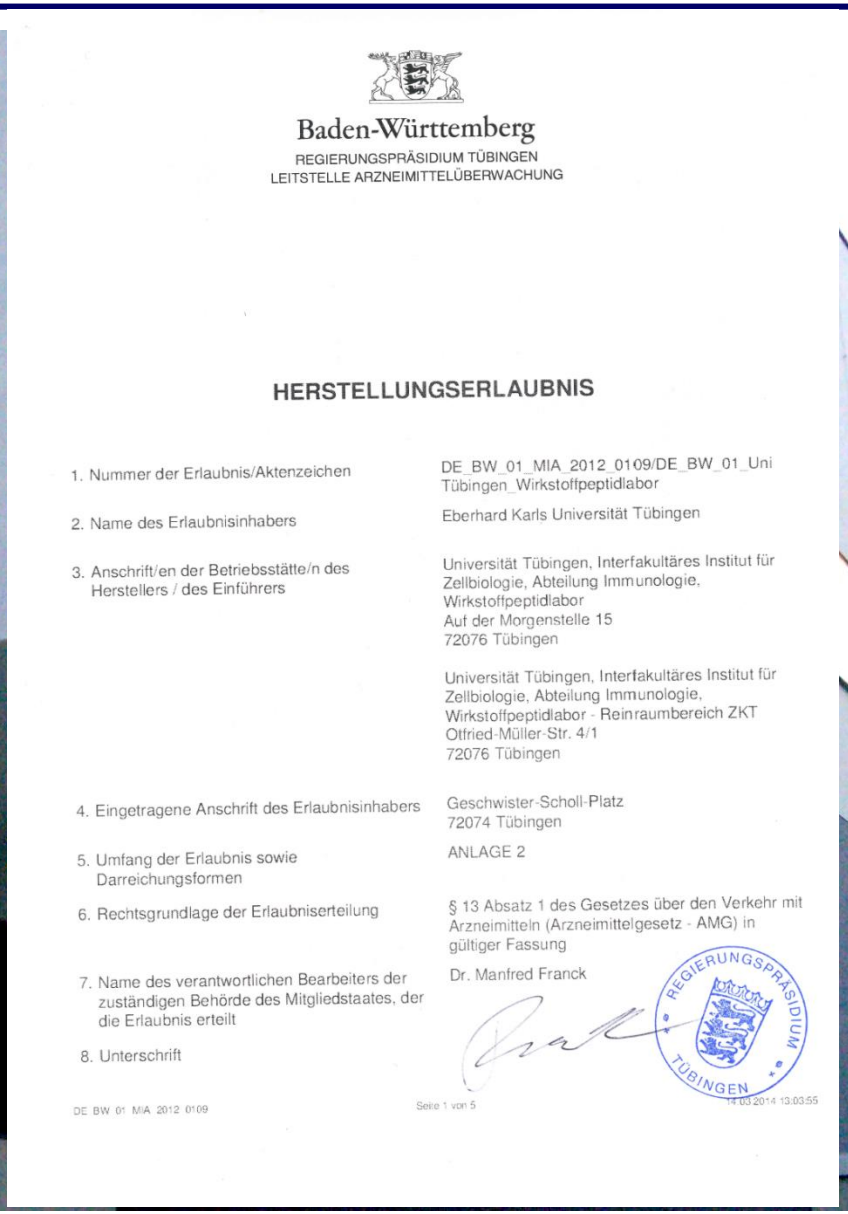
EU FP7 projects:

Glioblastoma
www.GAPVAC.eu

Hepatocellular carcinoma
www.HEPAVAC.eu

GMP center for individualized substances





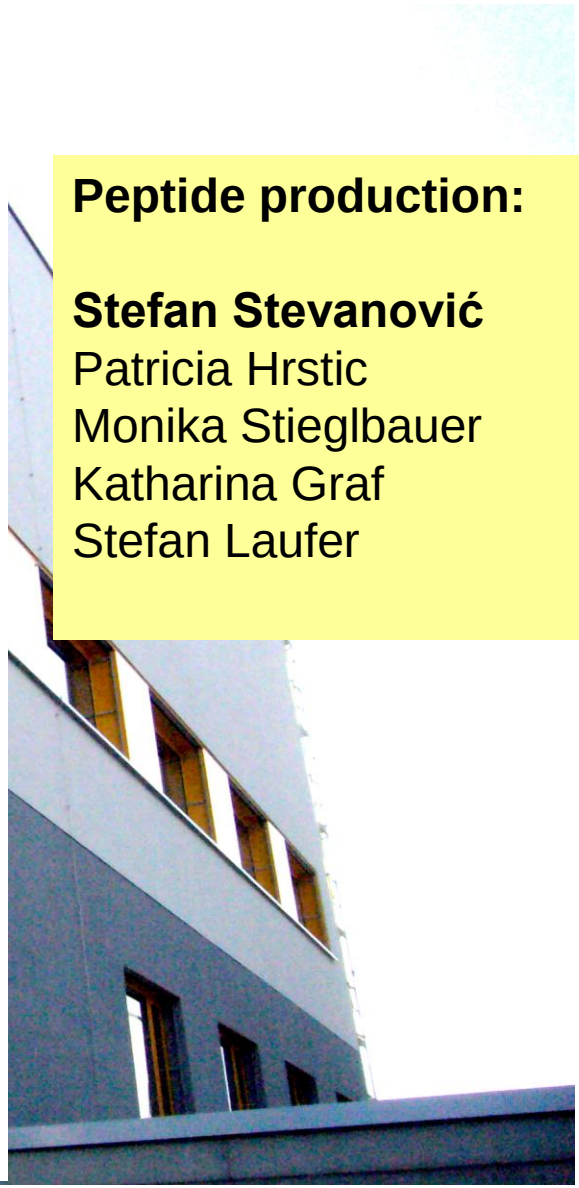
Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN
LEITSTELLE ARZNEIMITTELÜBERWACHUNG

HERSTELLUNGSERLAUBNIS

1. Nummer der Erlaubnis/Aktenzeichen	DE_BW_01_MIA_2012_0109/DE_BW_01_Uni Tübingen_Wirkstoffpeptidlabor
2. Name des Erlaubnisinhabers	Eberhard Karls Universität Tübingen
3. Anschrift/en der Betriebsstätte/n des Herstellers / des Einführers	Universität Tübingen, Interfakultäres Institut für Zellbiologie, Abteilung Immunologie, Wirkstoffpeptidlabor Auf der Morgenstelle 15 72076 Tübingen Universität Tübingen, Interfakultäres Institut für Zellbiologie, Abteilung Immunologie, Wirkstoffpeptidlabor - Reinraumbereich ZKT Otfried-Müller-Str. 4/1 72076 Tübingen
4. Eingetragene Anschrift des Erlaubnisinhabers	Geschwister-Scholl-Platz 72074 Tübingen
5. Umfang der Erlaubnis sowie Darreichungsformen	ANLAGE 2
6. Rechtsgrundlage der Erlaubniserteilung	§ 13 Absatz 1 des Gesetzes über den Verkehr mit Arzneimitteln (Arzneimittelgesetz - AMG) in gültiger Fassung
7. Name des verantwortlichen Bearbeiters der zuständigen Behörde des Mitgliedstaates, der die Erlaubnis erteilt	Dr. Manfred Franck
8. Unterschrift	

REGIERUNGSPRÄSIDIUM TÜBINGEN

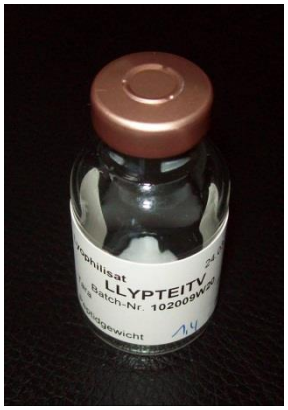
DE_BW_01_MIA_2012_0109 Seite 1 von 5 14.03.2014 13:03:55



Peptide production:

Stefan Stevanović
Patricia Hrstic
Monika Stieglbauer
Katharina Graf
Stefan Laufer

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN



J. Hepatology 2016

Personalized multi-peptide vaccination induces immune responses associated with long term survival in a patient with metastatic intrahepatic cholangiocarcinoma

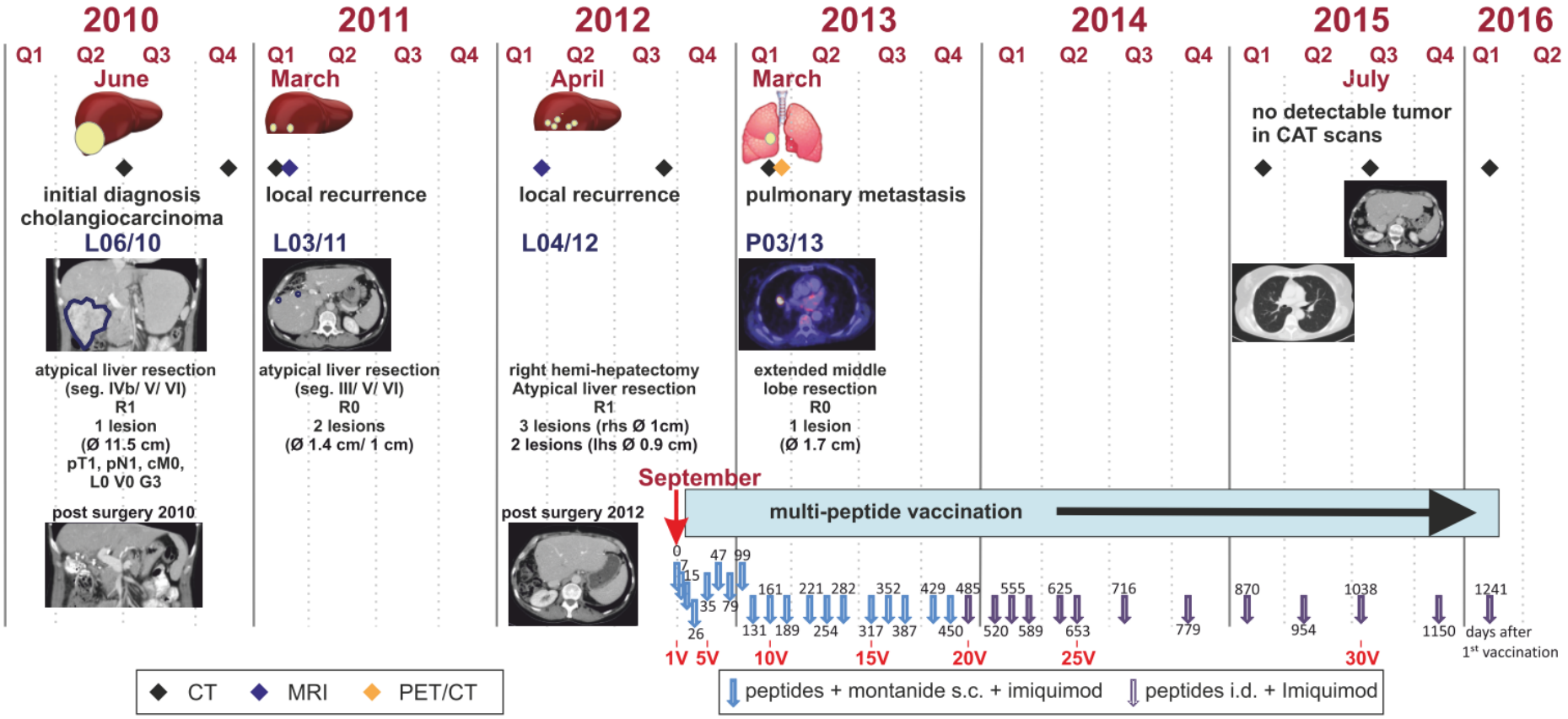
Markus Löffler[1,2]*, Anoop Chandran P[1]*, Karoline Laske[1], Christopher Schroeder[3], Irina Bonzheim[4], Mathias Walzer[1,5], Franz J. Hilke[3], Nico Trautwein[1], Daniel J. Kowalewski[1], Heiko Schuster[1], Marc Günder[1], Christopher Mohr[5], Marc Sturm[3], Huu-Phuc Nguyen[3], Olaf Riess[3], Peter Bauer[3], Sven Nahnsen[5], Silvio Nadalin[2], Derek Zieker[2], Jörg Glatzle[2], Karolin Thiel[2], Stephan Clasen[6], Hans Bösmüller[4], Falko Fend[4], Oliver Kohlbacher[5], Cécile Gouttefangeas[1], Stefan Stevanovic[1,7], Alfred Königsrainer[2], Hans-Georg Rammensee[1,7]

Composition of the multi-peptide vaccine used

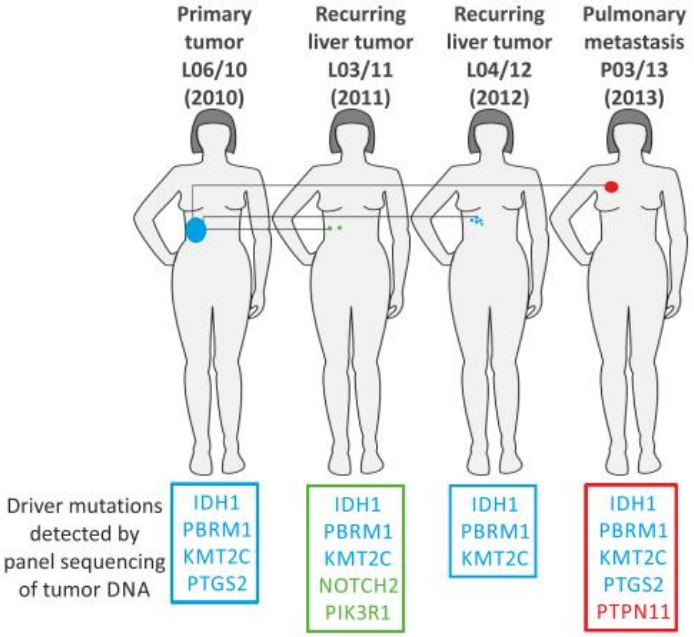
Samples analyzed							L06/10	P03/12	L06/10
Vaccine Peptides					References	Tumor characterization			
Peptide Sequence	Identifier	Mass (Da)	AA Position	HLA	Epitope	Transcript ID	RNA (FPKM)		MS^2
GLASFKSFLK	RGS5	1096.6	74-83	A*03	(14)	NM_003617	32	81	+
SLLTSSKGQLQK	ADFP-2	1288.7	369-380	A*03	(13)	NM_001122	525	53	+
TSALPIIQK	ADFP-3	969.6	63-71	A*03	(13)	NM_001122	525	53	+
SLFPNSPKWTSK	MMP7-(1)	1390.7	79-90	A*03	(16)	NM_002423	141	1113	-
NPPSMVAAGSVVAAV	CCND1	1368.7	198-212	DR	(15, 17)	NM_053056	70	30	n.d.
HSKIIIIKKGHAKDSQ	IGFBP3	1802.1	142-157	DR	(17)	NM_001013398	1	0	n.d.
SQDDIKGIQKLYGKRS	MMP7-(2)	1835.0	153-168	DR	(9, 17)	NM_002423	141	1113	n.d.

Induction of peptide-vaccine specific T cell responses correlates with long term survival in a patient with metastatic cholangiocarcinoma

Clinical Course



Tumor Characterization



Leukemias

HLA ligandome analysis in CLL identifies non-mutant epitopes associated with improved patient survival

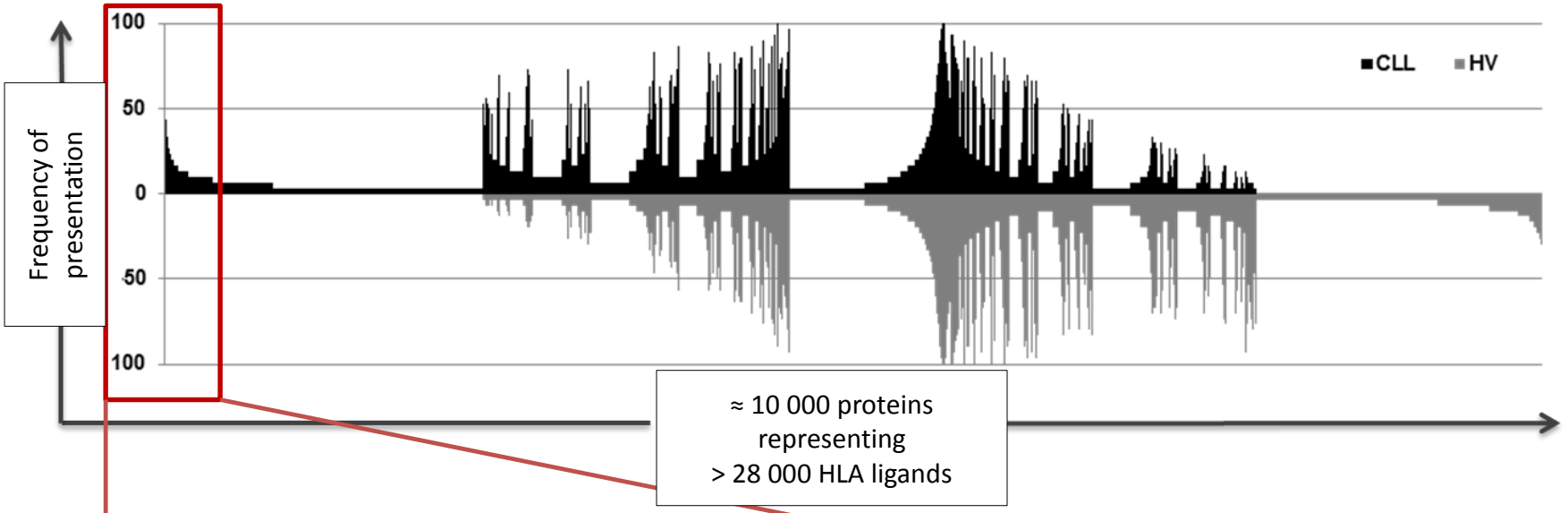
Kowalewski et al., PNAS 2015

Daniel J. Kowalewski

Juliane Stickel

HLA ligandome analysis in CLL identifies non-mutant epitopes associated with improved patient survival Kowalewski ... Stickel et al., PNAS 2015

Antigen presentation in the HLA ligandomes of 30 CLL patients vs. 30 normal individuals



LiTAAs (**Li**gandom-derived **T**umor**A**ssociated **A**ntigens) and corresponding **LiTAPs** (peptides)

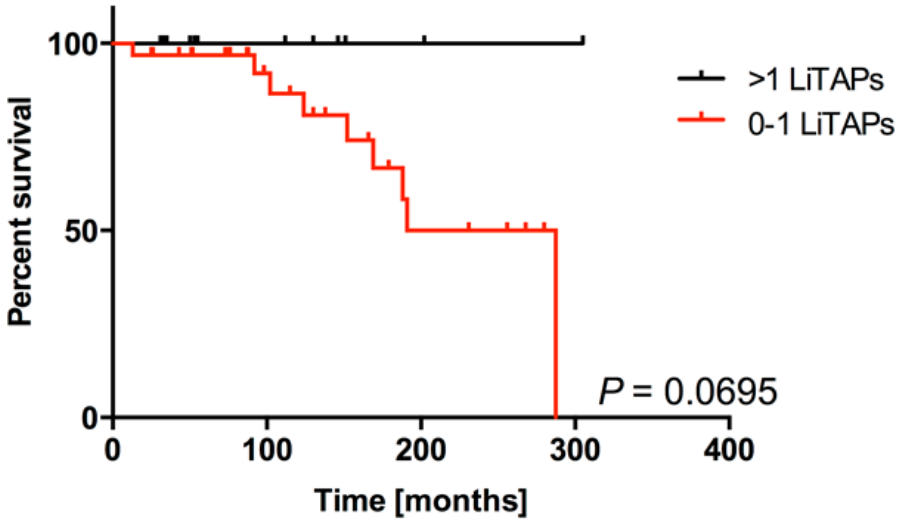
➔ 49 LiTAAs (225 LiTAPs) with tumor exclusive presentation in ≥ 20% of CLL patients

Association of spontaneous anti-LiTAA T cell responses with patient survival

Peptide	Sequence	Source protein	CD8+ T-cell response in CLL
P_{A*03}^{1}	YGYDNVKEY	CDCA7L	3/13 (23.1%)
P_{A*03}^{2}	AVFDGAQVTSK	TP53I11	4/13 (30.8%)
P_{A*03}^{3}	SSSGLHPPK	DMXL1	5/13 (38.5%)
P_{A*02}^{1}	ILDEKPVII	ABCA6	2/12 (16.7%)
P_{A*02}^{2}	YLNKEIEEA	CTDP1	3/12 (25,0%)
P_{A*02}^{3}	SILEDPPSI	ASUN	3/12 (25.0%)
P_{A*02}^{4}	DLDVKKMPL	PARP3	2/12 (16.7%)
P_{A*02}^{5}	QLLDQVEQI	TMED4	3/12 (25,0%)
P_{A*02}^{6}	AAANIIRTL	RASGRF1	1/12 (8.3%)
P_{B*07}^{1}	SPRPPLGSSL	KDM2B	4/6 (66.7%)
P_{B*07}^{2}	APLQRSQSL	TBC1D22A	4/8 (50.0%)
P_{B*07}^{3}	SPTSSRTSSL	CELSR1	3/6 (50.0%)
P_{B*07}^{4}	KPRQSSPQL	DNMBP	3/6 (50.0%)
P_{B*07}^{5}	SASVQRADTSL	ZFAND5	4/14 (28.6%)
P_{B*07}^{6}	APGSVLPRAL	TAGAP	0/9 (0%)

Peptide	Seuquence	Source protein	CD4+ T-cell response in CLL
P_{II}^{1}	LPSQAFEYILYNKG	CTSH	2/15 (13.3%)
P_{II}^{2}	RVEYHFLSPYVSPK	TFRC	1/15 (6.7%)
P_{II}^{3}	NSVIIVDKNGRLV	TFRC	2/15 (13.3%)
P_{II}^{4}	DIMRVNVDKVLERDQKL	VAMP2	2/15 (13.3%)
P_{II}^{5}	YKAFSSLLASSAVSPE	IL4R	3/15 (20.0%)
P_{II}^{6}	VDKVLERDQKLSELDDR	VAMP2	2/15 (13.3%)
P_{II}^{7}	DAGSYKAQINQRNFE	LY9	0/15 (0.0%)

Retrospective overall survival in 45 CLL patients (from time of diagnosis)



→ Trend to improved OS in patients with >1 T cell responses against CLL antigens identified by mass spec

iVAC-L-CLL01: Patient-individualized peptide vaccination in combination with lenalidomide after first line therapy of CLL

NCT02802943

Juliane Walz, Helmut Salih

Present and future developments for (individualized) active antigen-specific cancer immunotherapy:

Use better adjuvants

Improve immune responses by immunomodulators

Combine with checkpoint inhibition after first induction of immune response

Problem adjuvant:

Peptide vaccination generallly results in weak immune responses, especially after i.d. injection with GM-CSF.

Vaccination with Montanide gives stronger responses, but is described to be problematic (T cell sink, T cell dysfunction described)

Adjuvants shown to induce CD8/Th1CD4 responses in humans:

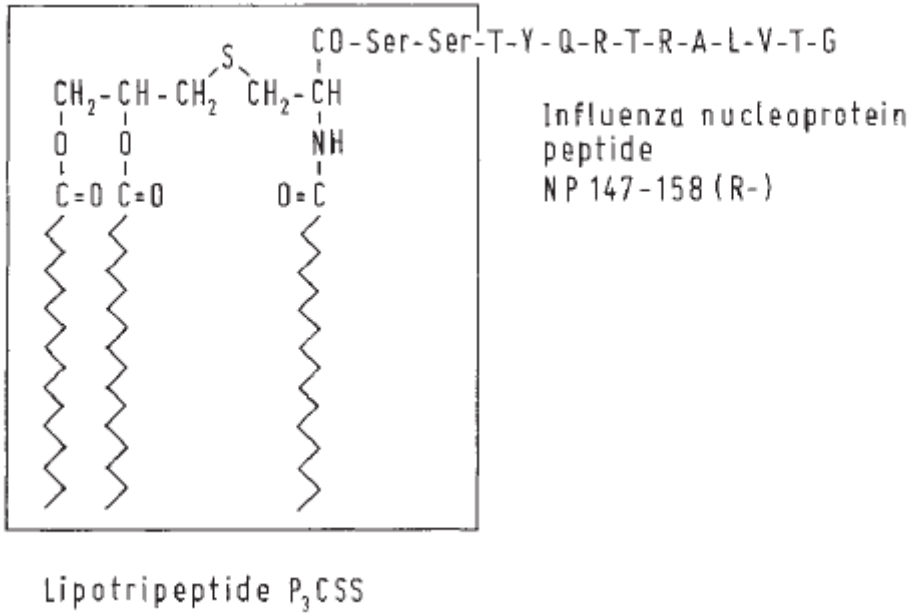
CPG (TLR9-L): probably best one so far, but not GMP-available anymore
GM-CSF: weak, may induce MDSCs
Imiquimod, TLR7-L:, topically: efficient, GMP-substance not easily available for adding to vaccine
Poly-IC, TLR3-L - supply limited, GMP-problems
RNAdjuvant (TLR7-L), developed by CureVac: probably very efficient, but limited supply

Efficient immune response by strongly adjuvanted vaccination

In vivo priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine

Karl Deres*, Hansjörg Schild†, Karl-Heinz Wiesmüller*, Günther Jung* & Hans-Georg Rammensee†‡

Nature 1989

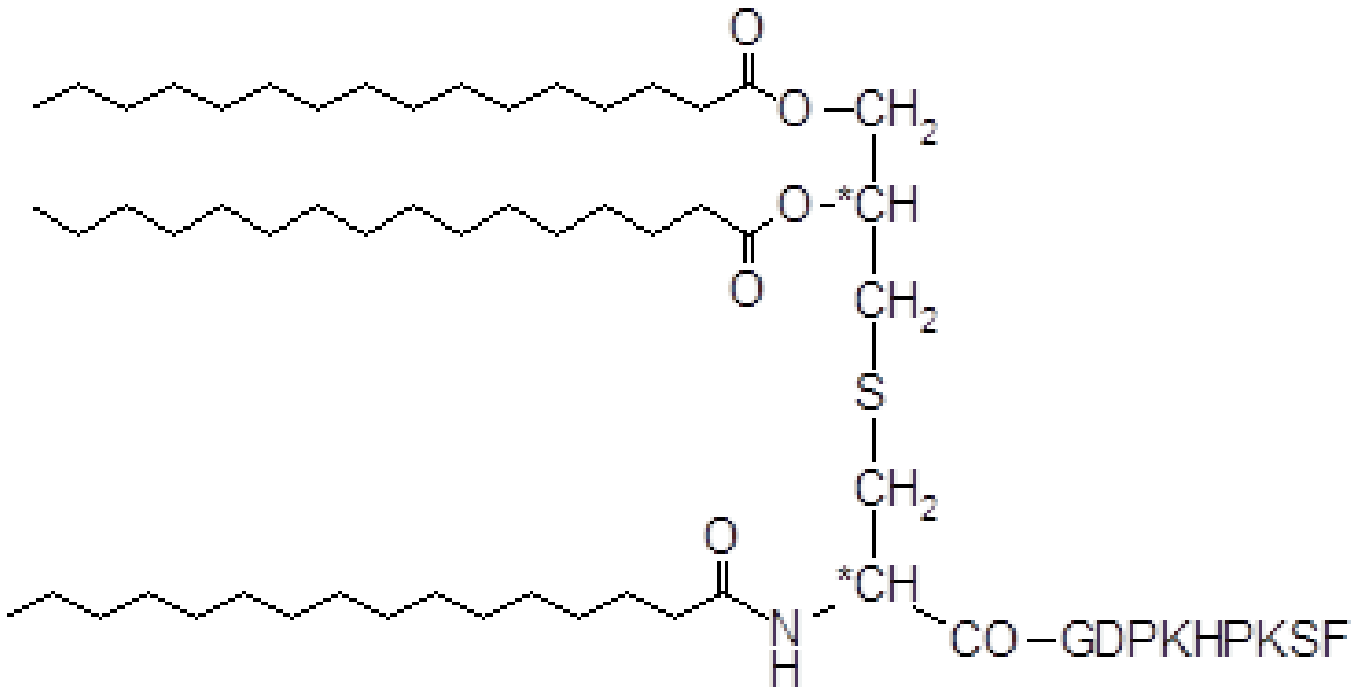


long peptide!

TLR ligand!

TLR2

New synthetic lipopeptide Pam_3Cys-GDPKHPKSF (XS15)



Karl-Heinz Wiesmüller, EMC microcollections, 2015:

new lipopeptide, **XS15,** (Pam3Cys compound), watersoluble, easy to purify by HPLC: GMP-friendly

Test of XS15 with short synthetic viral peptides and Montanide in a healthy volunteer.

Peptides are not coupled to the lipopeptide, they are just included in the emulsion!

PRPVSRFLGNNSILY	(HLA-DR, EBV)	Personalized
LTDLGQNLLY	(HLA-A01, Adenovirus)	Multi-Peptid-
ELRSRYWAI	(HLA-B08, Influenza)	Vaccine

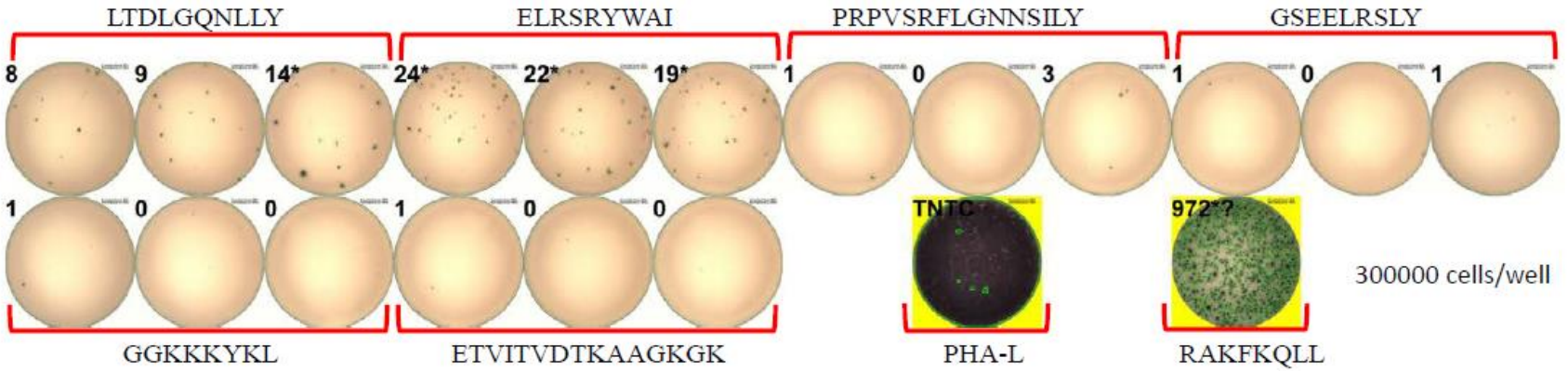
80 µg XS15
240 µg per Peptide
200 µl Montanide ISA51
injected s.c. abdominally, total volume 400 µl

Day 31

Ex-vivo IFN-g ELISPOT

22-01-2016

04-12-2015



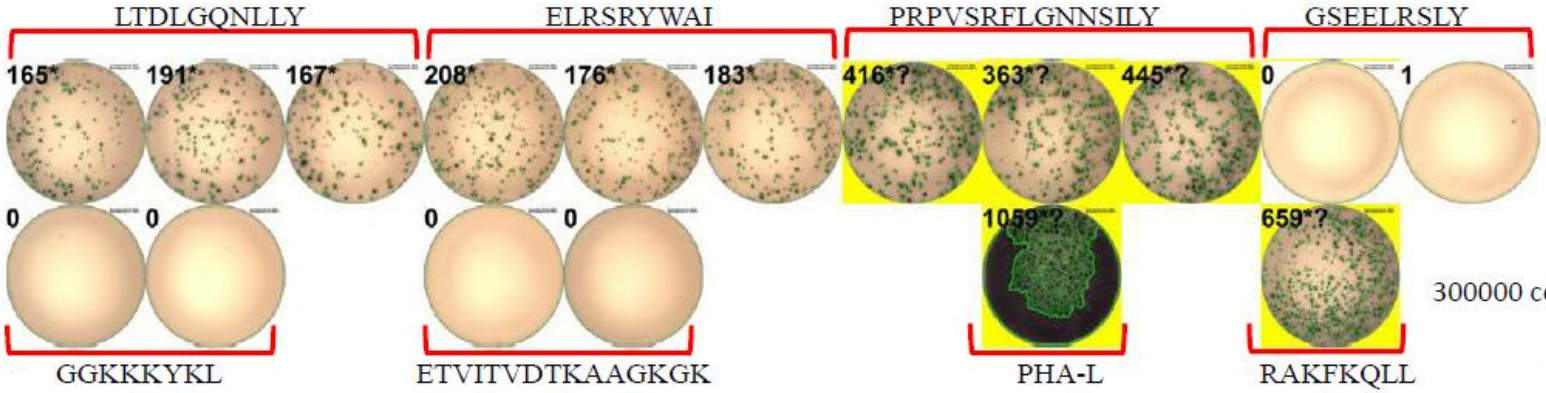
22.12.2015

PRPVSRFLGNNSILY
LTDLGQNLLY
ELRSRYWAI

→ each 3 mg dissolved in 1 ml DMSO/H2O.
→ from this 400 µl + 150 µl I XS15 ; (400 µg) + 450 µl H2O + 1 ml Montanide ISA51 emulgated.
→ from this 400 µl s.c. belly left

	Synth. No.	Protein	Sequence	HLA
Vaccine peptides	121209	Hex-Ade	LTDLGQNLLY	A01
	131288	Inf-NCAP	ELRSRYWAI	B08
	141206	EBV-GP350	PRPVSRFLGNNSILY	DR
Negative controls	121178	HIV	GSEELRSLY	A01
	141345	HIV	GGKKKYKL	B08
	110311	Filamin A	ETVITVDTKAAGKGK	DR
Positive control	91212	BZLF1_EBV	RAKFKQLL	B08

19-01-2016



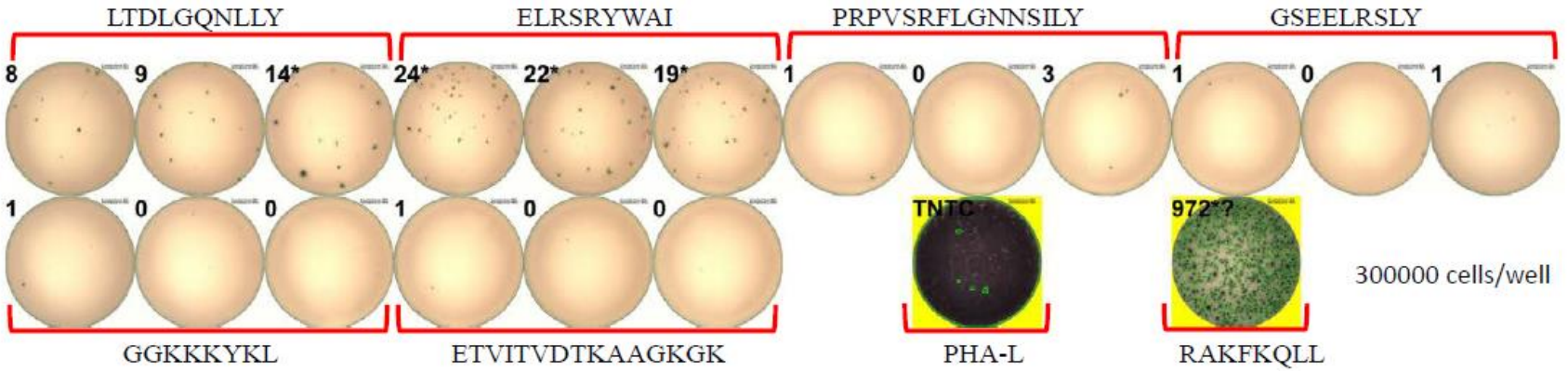
Anoop
23-01-2016

Day 31

Ex-vivo IFN-g ELISPOT

22-01-2016

04-12-2015



22.12.2015

PRPVSRFLGNNSILY
LTDLGQNLLY
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→ each 3 mg dissolved in 1 ml DMSO/H2O.
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Vaccine peptides	121209	Hex-Ade	LTDLGQNLLY	A01
	131288	Inf-NCAP	ELRSRYWAI	B08
	141206	EBV-GP350	PRPVSRFLGNNSILY	DR
Negative controls	121178	HIV	GSEELRSLY	A01
	141345	HIV	GGKKKYKL	B08
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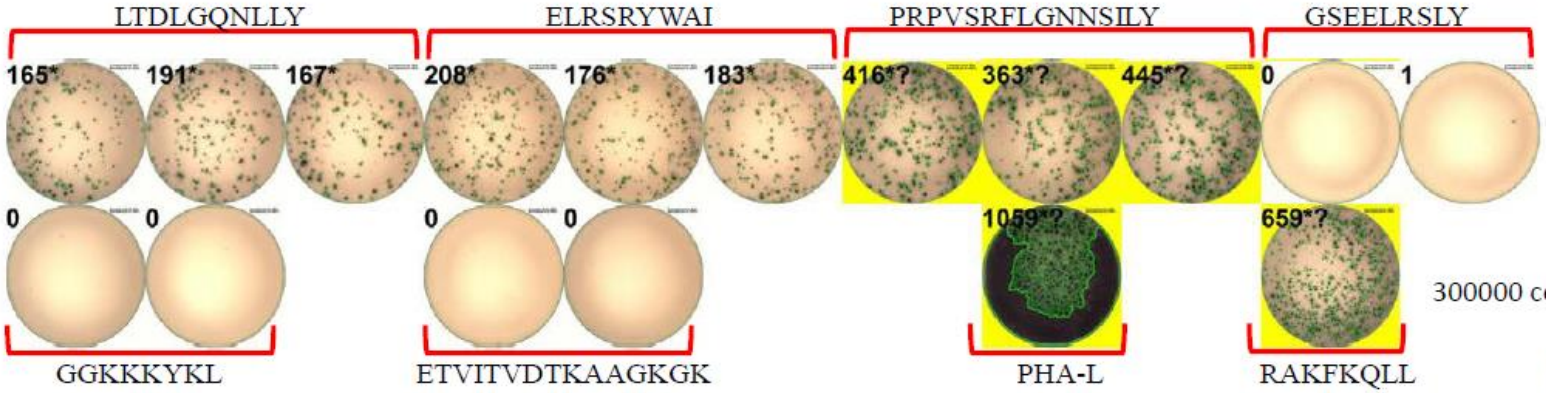
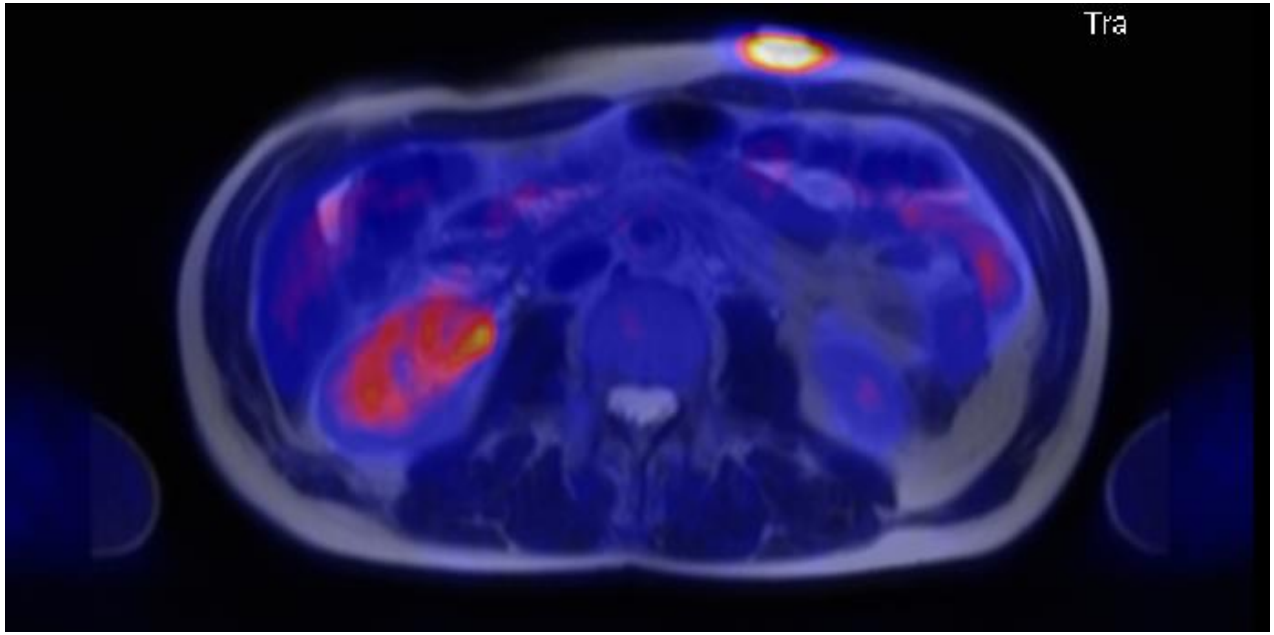
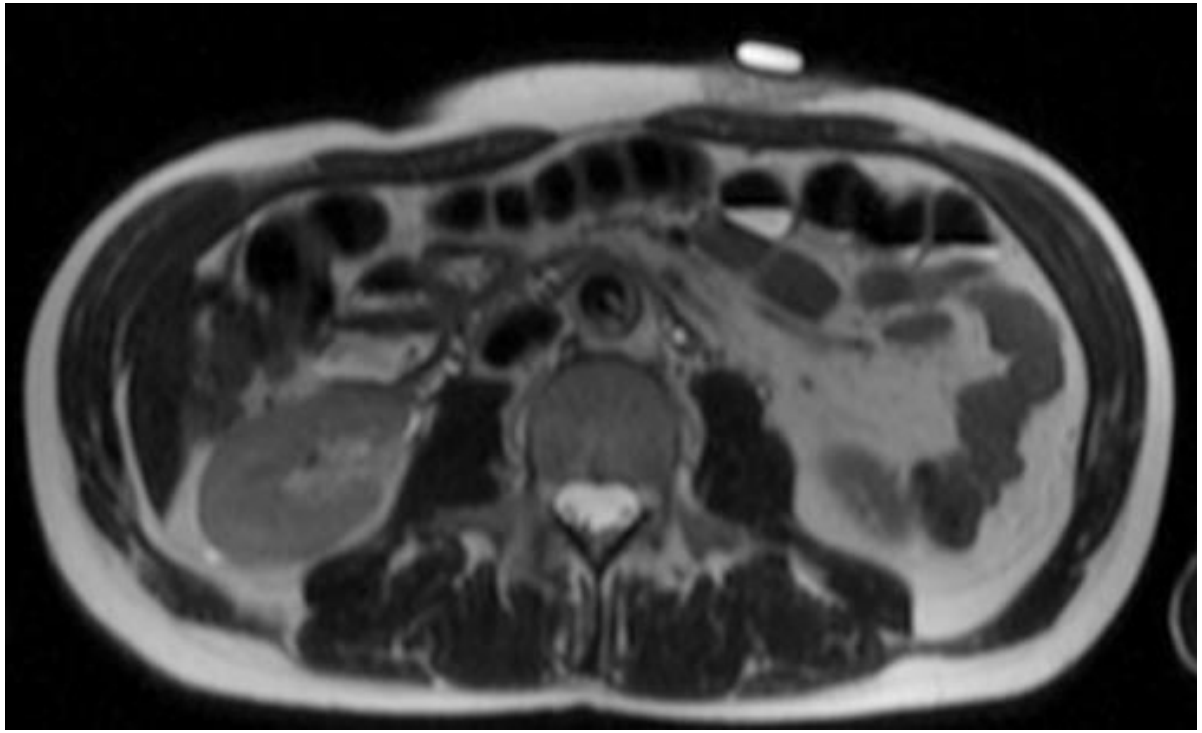
Positive control	91212	BZLF1_EBV	RAKFKQLL	B08

19-01-2016



Anoop
23-01-2016

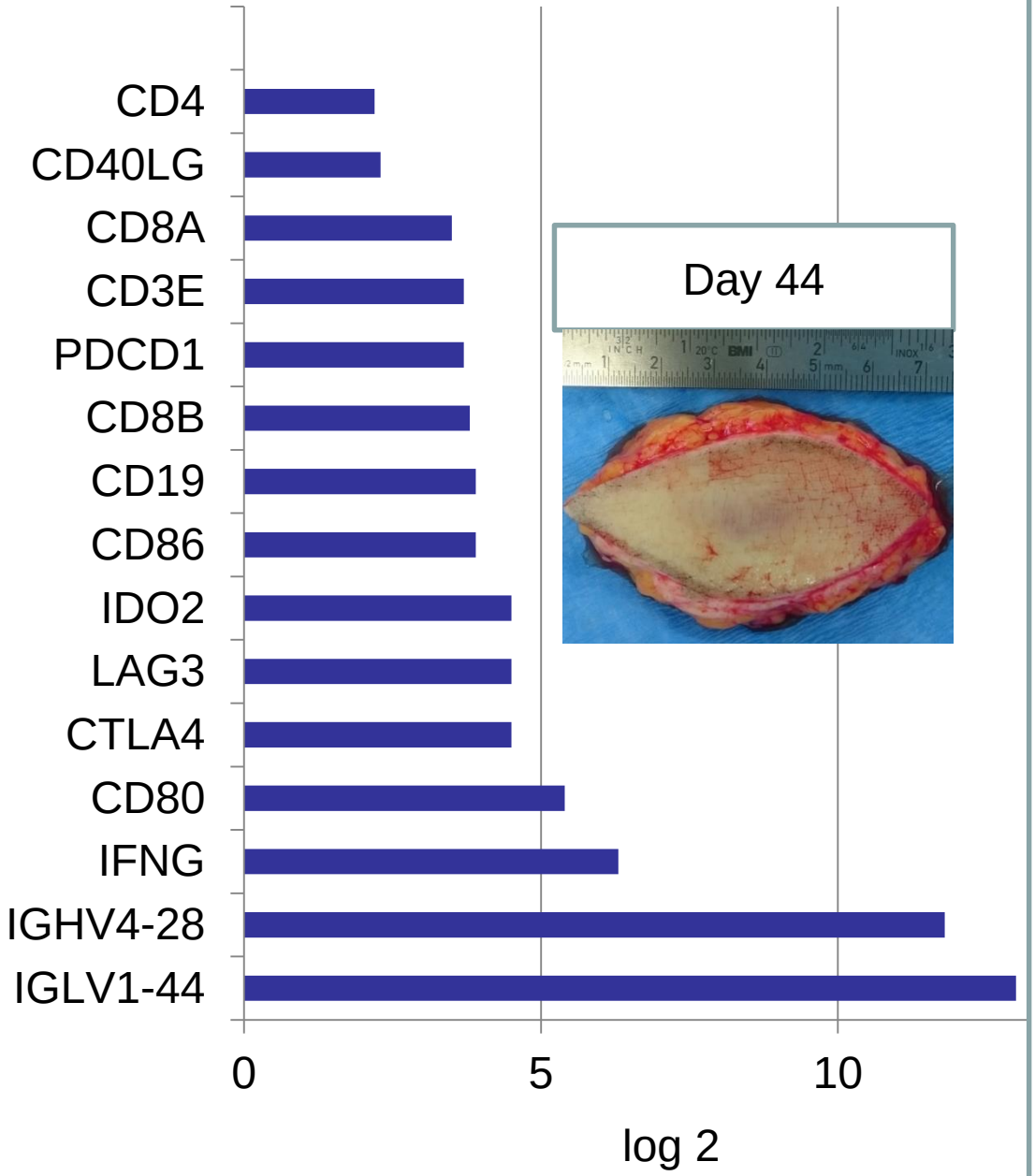


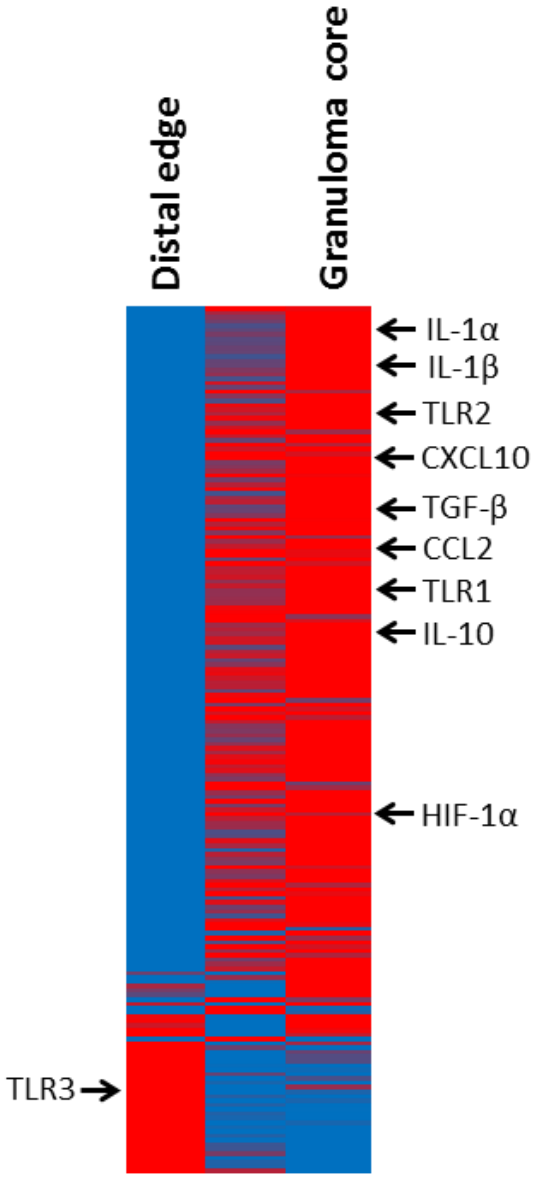


Day 43

^{18}FDG-PET/MRI

Differential gene expression analysis
Granuloma center vs. normal skin





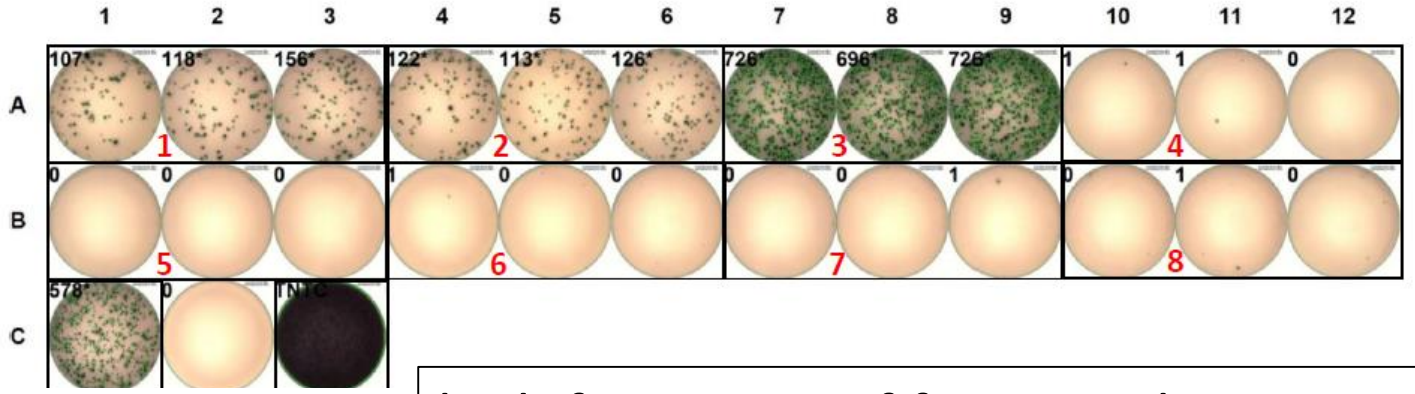
Hallmark „inflammatory response“
GSEA dataset (197 genes)

Day 44

Ex-vivo IFN-γ ELISPOT

Plate 1

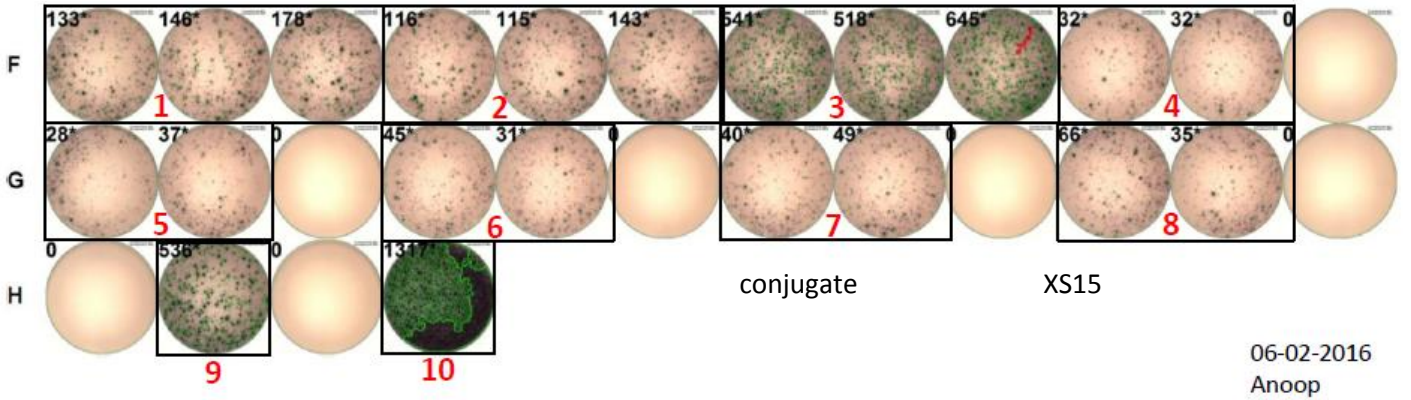
PBMCs
04-02-2016
300.000 cells/well



		syn.no	Protein	Sequence	HLA
vaccinated peptides	1	121209	Hex-Ade	LTDLGQNLLY	A01
	2	131288	Inf-NCAP	ELRSRYWAI	B08
	3	141206	EBV-GP350	PRPVSRFLGNNSILY	DR
Negative controls	4	121178	HIV	GSEELRSLY	A01
	5	141345	HIV	GGKKKYKL	B08
	6	110311	Filamin	ETVITVDTKAAGKGK	DR
GDP...peptide	7	-	-		-
P3C	8	-	-		-
Positive peptide	9	91212	BZLF1_EBV	RAKFKQLL	B08
Positive control	10	-	PHA-L		

high frequency of functional antigen specific T_{EM} CD4 and CD8 T cells in the granuloma infiltrating lymphocytes producing IFNy, IL2, TNF, and CD107 for CD8 cells

GILs
04-02-2016
50.000 cells/well



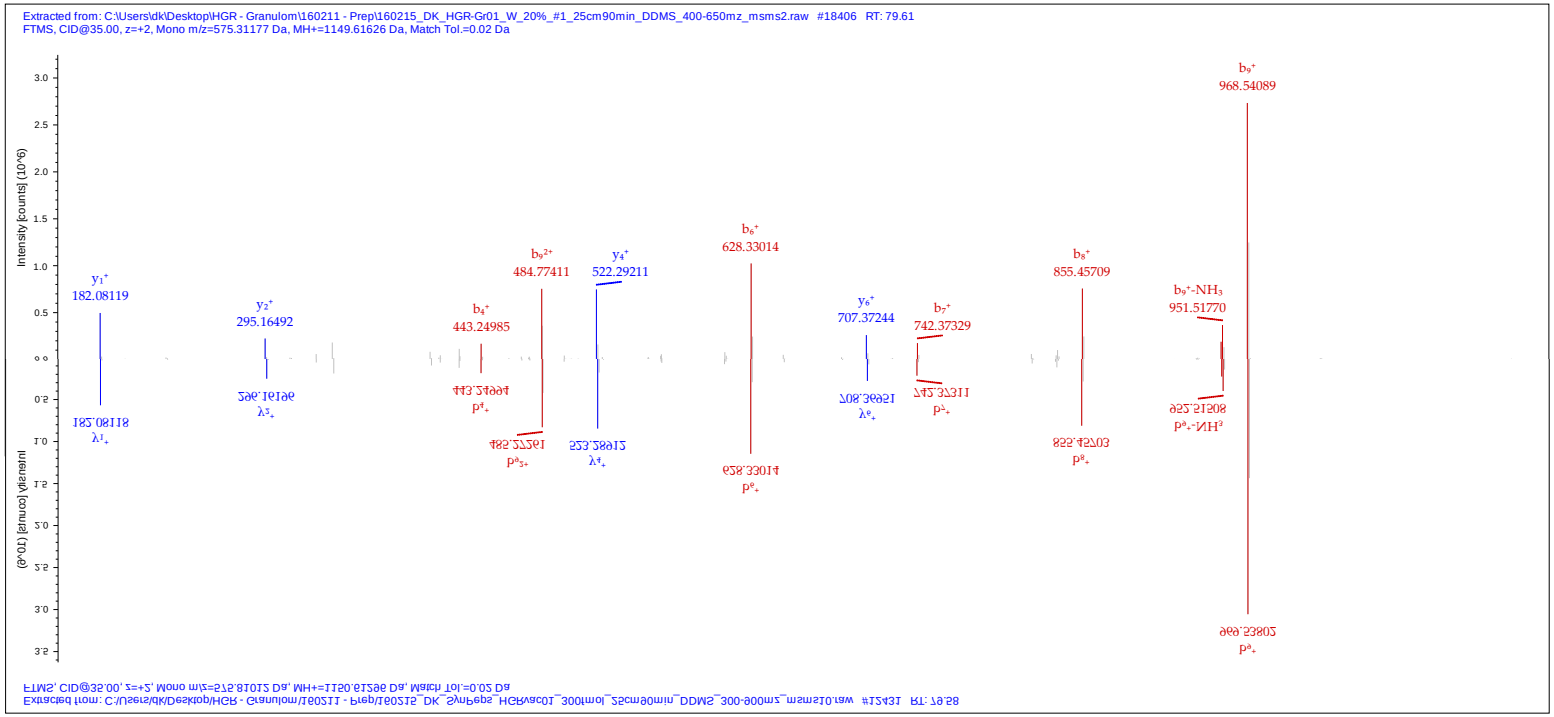
conjugate XS15

06-02-2016
Anoop

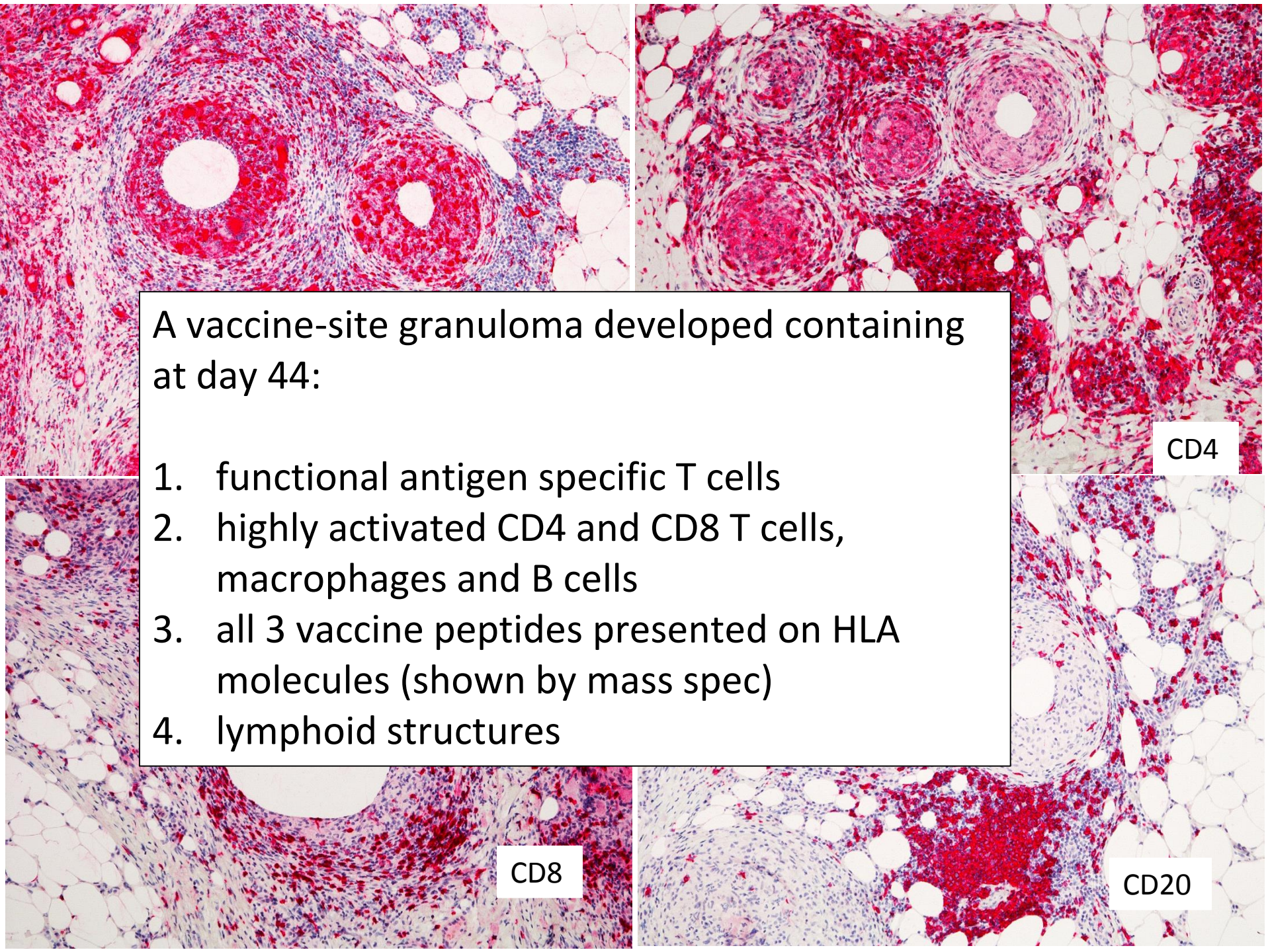
Prep: W6/32 20% DDMS: ADV_Hexon Protein: LTDLGQNLLY (A*01)

Primary Sample
HGR-GR01
W6/32 20% DDMS

SynPep
L9 15N labeled!
(300 fmol w/o matrix)

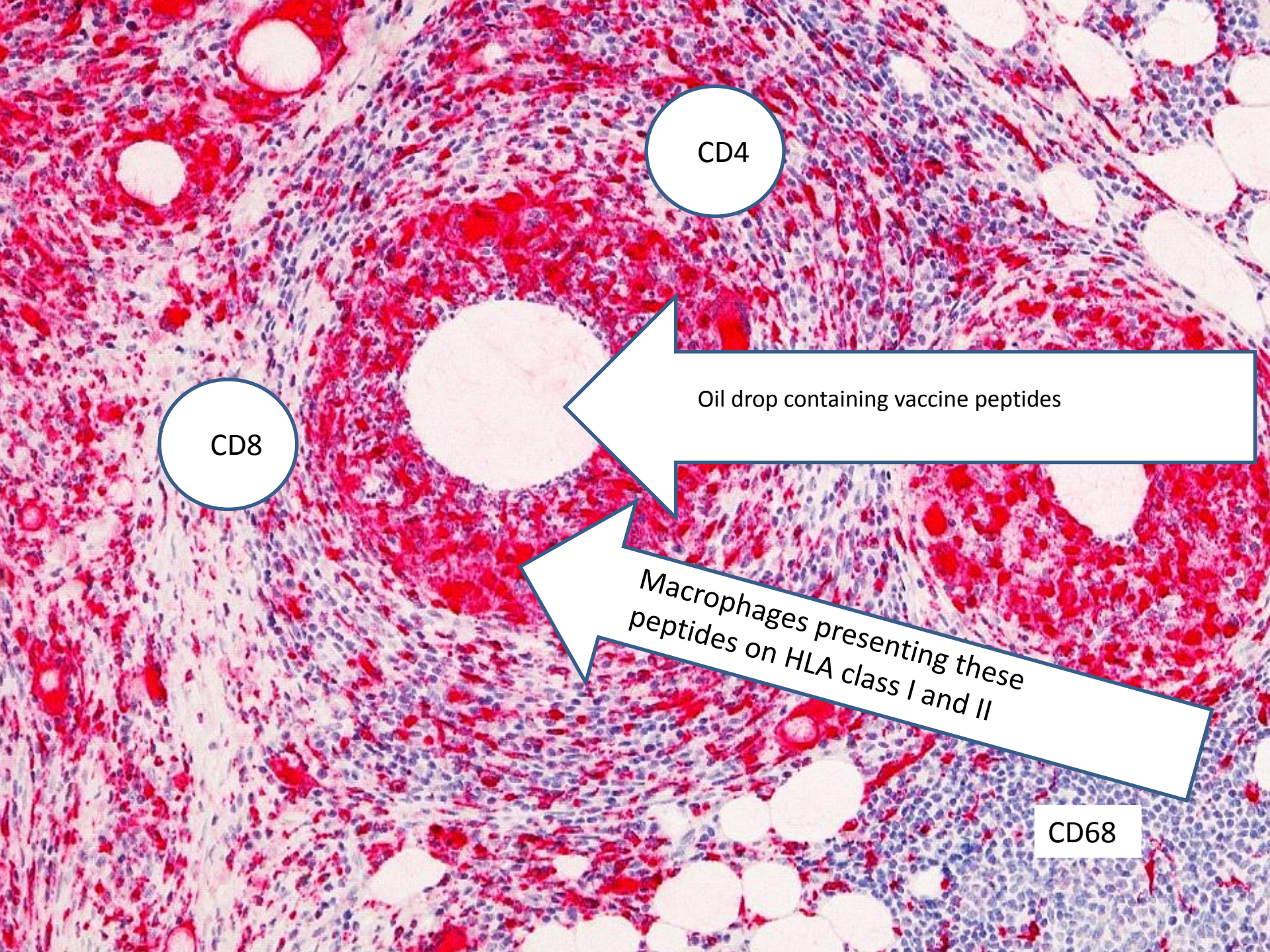


All 3 vaccine peptides are detectable as HLA-bound peptides in the granuloma 44 days after injection.



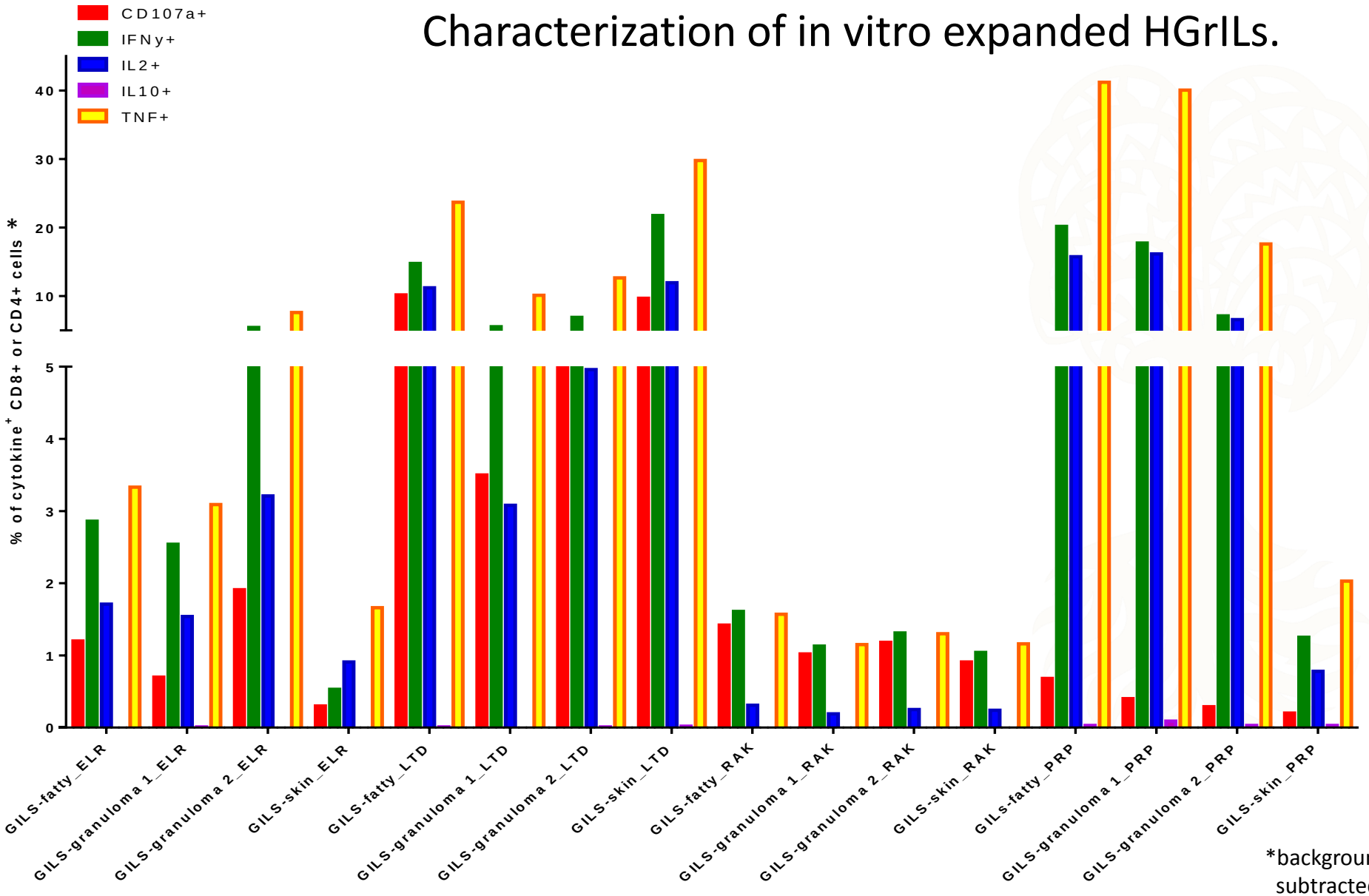
A vaccine-site granuloma developed containing at day 44:

1. functional antigen specific T cells
2. highly activated CD4 and CD8 T cells, macrophages and B cells
3. all 3 vaccine peptides presented on HLA molecules (shown by mass spec)
4. lymphoid structures


CD4
CD8
Oil drop containing vaccine peptides
Macrophages presenting these peptides on HLA class I and II
CD68

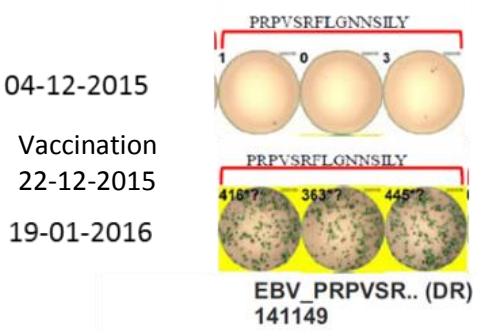
- ICS results -

ICS after cultivation with Anti-CD3 and IL2 of GILs – 19.02.2016



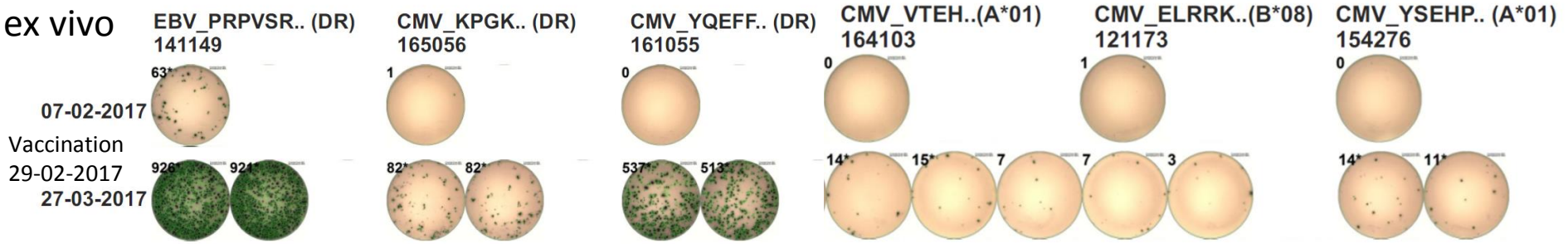
*background subtracted

Single vaccination with CMV-peptides plus XS15 induces strong CMV- specific CD8 and CD4 T cell response in CMV-seronegative indidual within 4 weeks (ex-vivo ELISPOT)

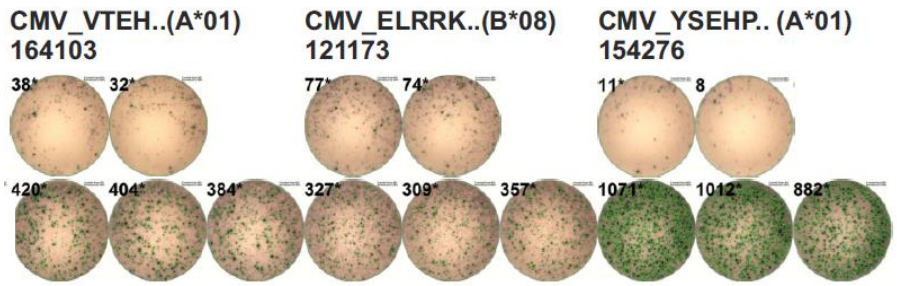


PRPVSRFLGNNSILY	EBV	preexisting response
VTEHDTLLY	CMV, A1	vacc, but negative
ELRRKMMYM	CMV, B8	vacc, but negative
KPGKISHIMLDVAFTSH	CMV, DR	vacc, but weak
YQEFFWDANDiYRIF	CMV, DR	new
YSEHPTFTSQY	CMV, A1	vacc, but negative

One shot vaccination with CMV-peptides plus XS15 induces strong CMV-T-cell response CMV-seronegative individual within 4 weeks (Ex-vivo ELISPOT)



after 12d in vitro restimulation



Patient with MDS (Myelodysplastic syndrome), 14 validated mutations

1st vaccination Dec 16, 2015, peptides i.d. with Aldara topical.
8th vaccination Feb 19, 2016, peptides i.d. with Aldara topical.

LEKFLQNHSHLFFPL	mut GREB1 H1820N DR*01/B*1501
TEVVRRCPHYERCSDSDGL	mut TP53 H179Y elongated A*03

no response

Patient with MDS (Myelodysplastic syndrome), 14 validated mutations

1x peptides, XS15, Montanide

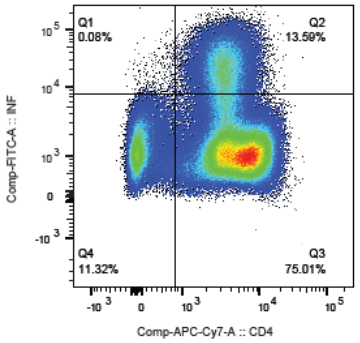
07.05.17

ICS 05.07.17, 12d

Vaccine cocktail

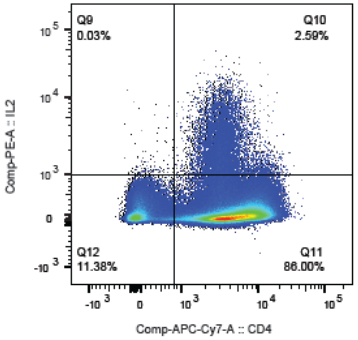
		pre vaccine response	post vaccine response
LEKFLQNHSHLFFPL	mut GREB1 H1820N DR*01/B*1501	-	++
TEVVRRCPHYERCSDSDGL	mut TP53 H179Y elongated A*03	-	-
KLLPENNVLSPLPSQAMDDL	p53 wt	**NT**	++
FRLGFLHSGTAKSVT	p53 wt	**NT**	++
MAIYKQSQHMTEVVRR	p53 wt	**NT**	-
TAKSVTCTYSPALNKMF	p53 wt	**NT**	+
TLGEFLKLDRERAKN	Survivin	**NT**	++
KTSLYNLRRGTALA	EBV EBNA1 class II, no preexisting response	-	+++

INFg



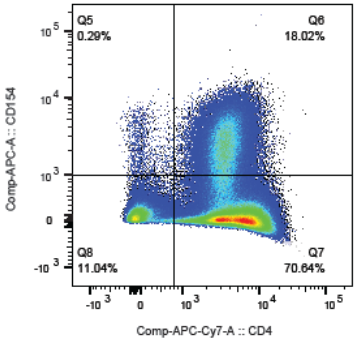
pool2_9.fcs
CD8-
432296.00

IL2



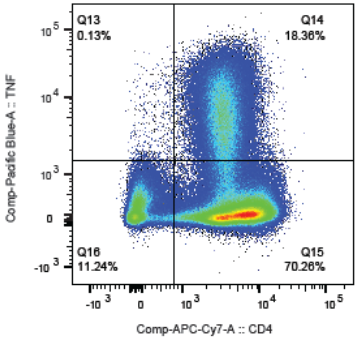
pool2_9.fcs
CD8-
432296.00

CD154



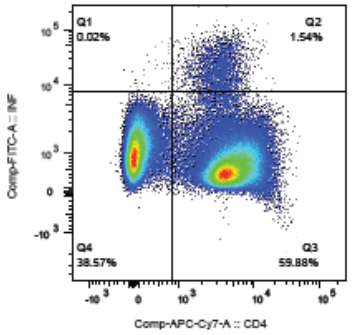
pool2_9.fcs
CD8-
432296.00

TNF



pool2_9.fcs
CD8-
432296.00

EBV
EBNA1
KTS



pool1_1.fcs
CD8-
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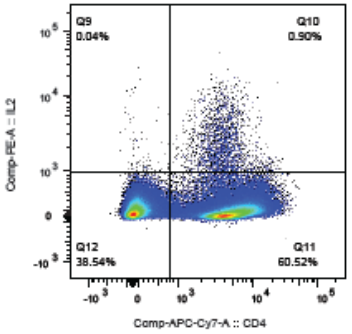

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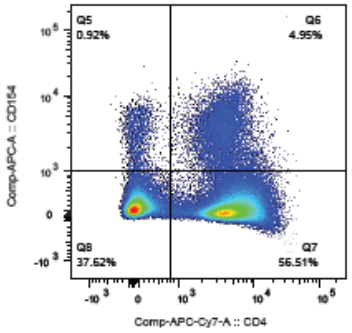

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CD8-
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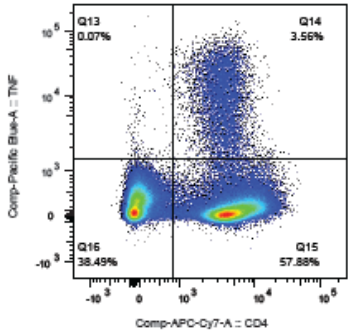
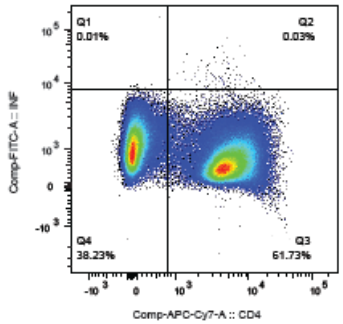
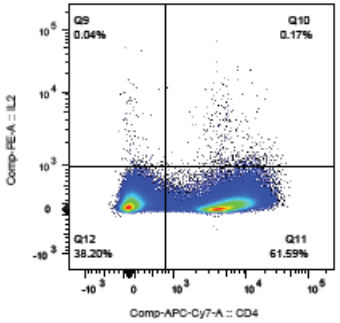


pool1_1.fcs
CD8-
234236.00

GREB1
mut
LEK



pool1_2.fcs
CD8-
299970.00



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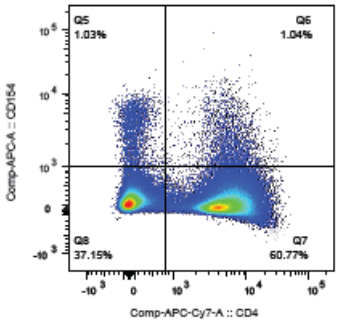

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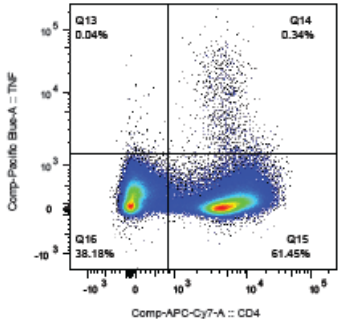

pool1_2.fcs
CD8-
299970.00

TP53mut
TEVV

Conclusions:

1. XS15 is a watersoluble TLR2 ligand inducing a strong CD8 and Th1CD4 response against free short peptides in Montanide ISA51 after a single s.c. injection
2. Vaccine peptides persist at the injection site at least for 7 weeks
3. Vaccine induced response persists for > 1 year
4. Works for viral, mutated, and non-mutated self peptides

A new synthetic lipopeptide is an efficient adjuvant for one shot peptide vaccination

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erc
European Research Council

Future of tumor vaccination

Active immunotherapy with individually selected peptides with strong adjuvant (e.g., the TLR2 ligand XS15) in a water-in-oil or other subcutaneous depot could advance the field, in particular in the MRD setting

In patients with tumor load checkpoint inhibition after induction of vaccine specific T cell responses should be considered

The awesome landscape of tumor antigens beyond mutated neoantigens

mutated neoantigens

shared nonmutated antigens (incl. viral)

private non mutated antigens (incl. posttranslational modifications)