TRON Translational Oncology at the University Medical Center of the Johannes Gutenberg University Mainz

BIONTECH RNA Pharmaceuticals iTECH

JG|U UNIVERSITÄTSmedizin. MAINZ

# Exploiting the mutanome for cancer immunotherapy

Ugur Sahin

SITC Washingto 5th November 2015

# Disclosure Information SITC Washington

*Ugur Sahin, M.D.*

I have the following financial relationships to disclose:

I am

- Co-founder and CEO BioNTech AG, Mainz
- Inventor of Licensed Patents related to Cancer Immunotherapy
- Head of the Scientific Advisory Board of Ganymed Pharmaceuticals

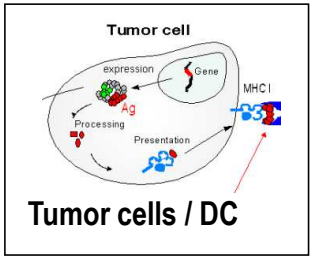
*- and -*

I will not discuss off label use and/or investigational use in my presentation.

# Vaccine Approaches

BIONTECH

## Cellular Vaccines

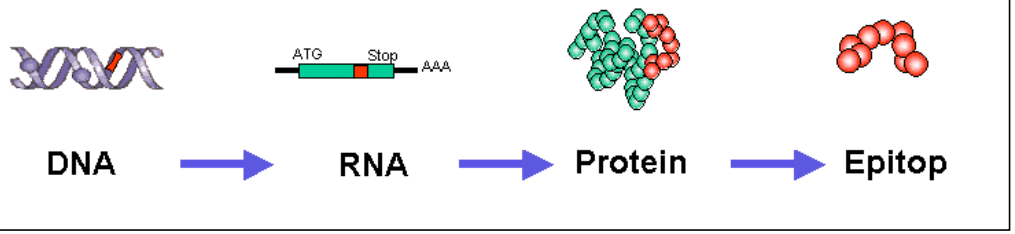



Tumor cells / DC

Autologous
Allogenous
Transduced

Tumorcomponents
•Extracted Lysates
•Exracted mRNA
• Extracted HSPs
• Exosomes & Others

## Molecularly defined vaccines




Plasmids
Rec. Viruses
• Adeno /Poxviruses
• Vaccinia (MVA)
• Canary (ALVAC)
• Fowlpox (TROVAC)
• Retrovirus
Bacteria
•Engineered Salmonella
•Engineered Listeria

Clonal RNA
Rec. Viruses
- Influenza
- Alpha
Sindbis
Semliki Forest

Recombinant proteins

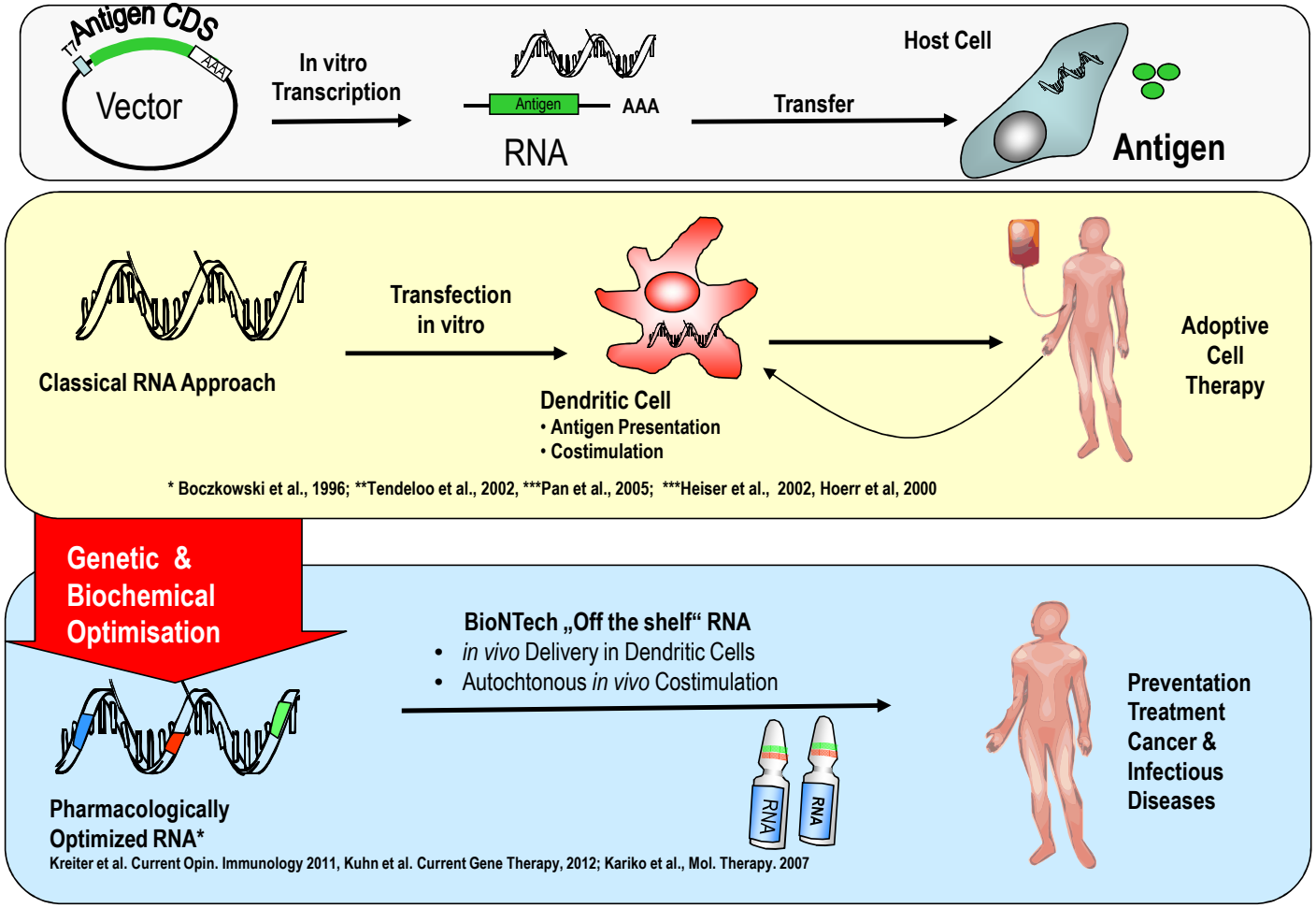
Virus Like Particles
- HBV
- HPV

Synthetic Peptides
- CD8-Epitopes
- CD4-Epitopes
- Mixes

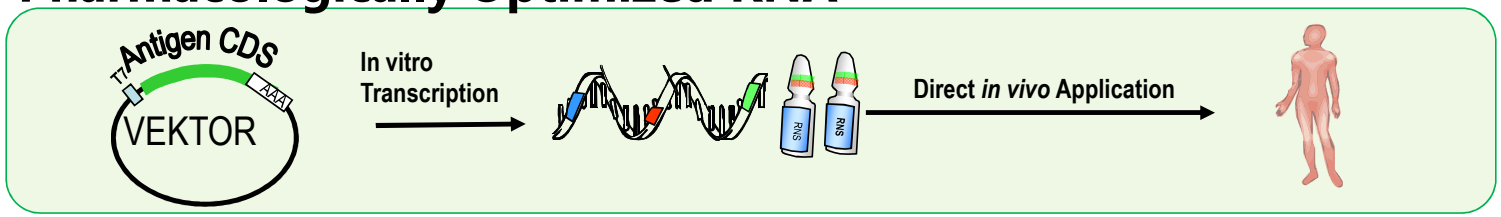
**> 25 antigens tested as targets (out of >> 100 candidate targets)**
**> 50 vaccine approaches under development**
**+ increasing number of adjuvants, various protocols**

**Provenge® Sipuleucel T approved**
**Some Phase III studies failed, several phase III studies ongoing**

# mRNA Immunotherapies



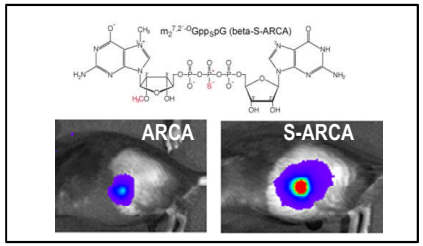
# Potency matters – Pharmacologically Optimized RNA




Example: Molecular improvements

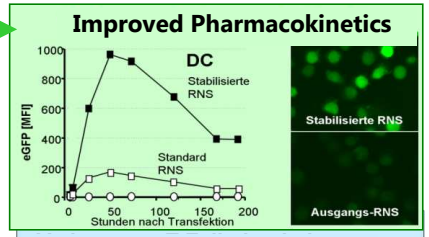
**Modified Cap-analogues to increase stability**

Kuhn et al, GenTher, 2010



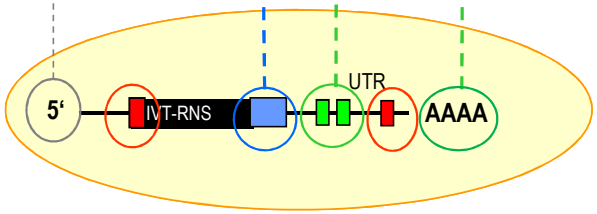

**Regulatory sequences for stabilisation and increased translational efficacy**

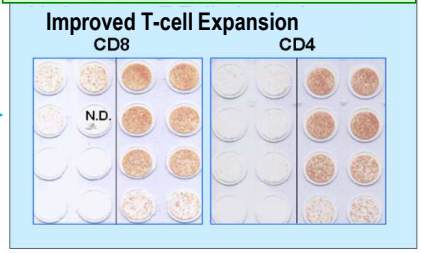
Holtkamp et al, Blood, 2006




**HLA-signal sequences for improved antigen presentation**

Kreiter et al, JI, 2008



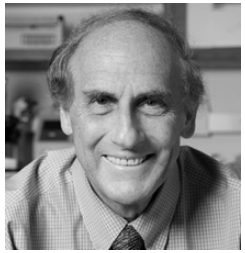




Net Effect:
x 5000
potency* ↑

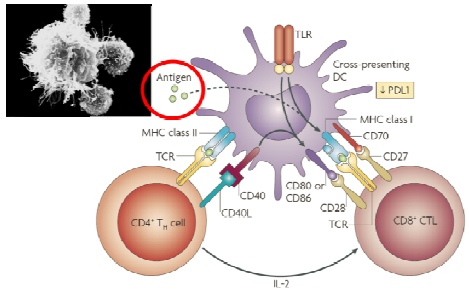
Independent improvements potentiate biological effects

* Compared to Boczkowski et al. Vector pGEM-A64 Vector (Promega)

# Scientific Foundation for Vaccine Development



Dendritic Cells specialized and regulable antigen presentation machines
**I Mellman, RM Steinman - Cell**, 2001




The immune system evolved to discriminate infectious nonself from noninfectious self

Charles A. Janeway, Jr

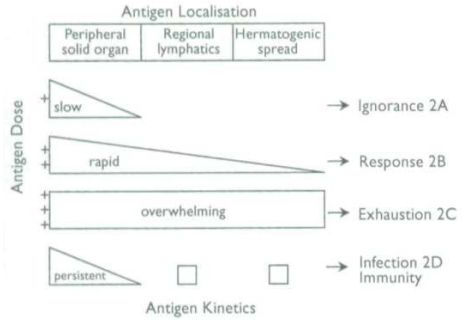
| Characterization of the entity | | | Theories | | |
|---|---|---|---|---|---|
| **Self/Non-self** | **Danger** | **Pathogen associated molecular patterns (PAMPs)** | **Self–non-self theory** | **Infectious non-self theory** | **Danger theory** |
| Self | Not dangerous | | No response | No response | No response |
| | Dangerous | | No response | No response | Response |
| Non-self | Not dangerous | No PAMPs | Response | No response | No response |
| | | With PAMPS | Response | Response | No response |
| | Dangerous | No PAMPs | Response | No response | Response |
| | | With PAMPS | Response | Response | Response |

*According to the classic self–non-self theory (e.g., Burnet, 1959), only non-self entities trigger an immune response. The infectious non-self theory (Janeway, 1989) states that only infectious non-self, i.e., entities that express pathogen-associated molecular patterns (PAMPs) trigger an immune response, through the activation of antigen-presenting cells (APCs). The danger theory offers different predictions by saying that what triggers an immune response is not "foreignness," but the release of "alarm signals" by damaged tissues (Matzinger, 2002).*

Rolf M. Zinkernagel
Stephan Ehl
Peter Aichele
Stephan Oehen
Thomas Kündig
Hans Hengartner

*Immunological Reviews 1997*
*Vol. 156: 199–209*

Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity

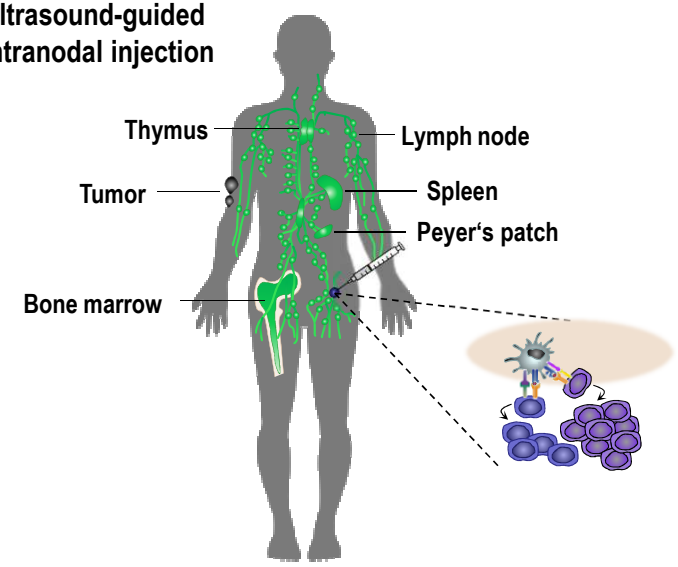



JG|U UNIVERSITÄTSmedizin. MAINZ

TRON

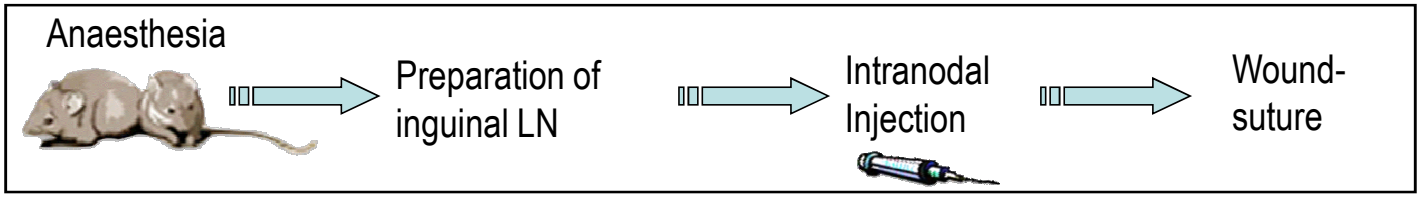
## Local DC targeting

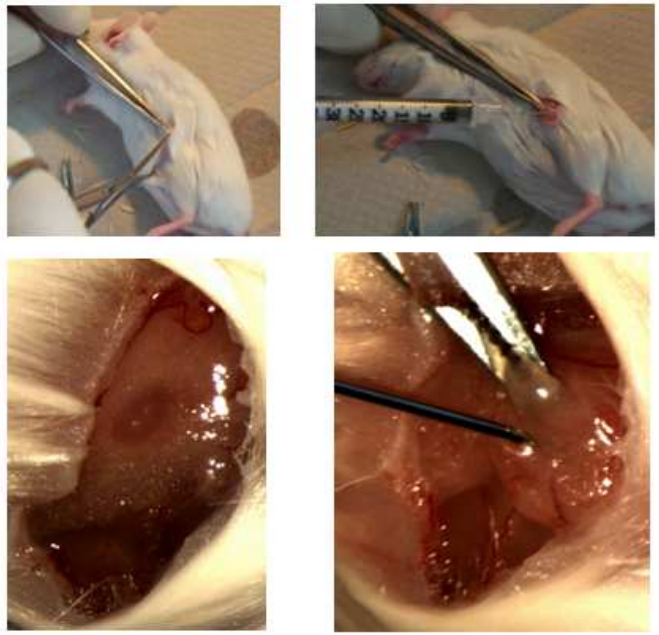
Ultrasound-guided intranodal injection




**mRNA and DCs meet directly at injection site**

# Intranodal Immunisation



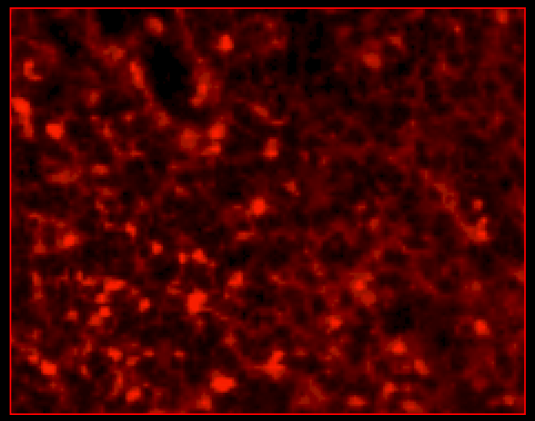



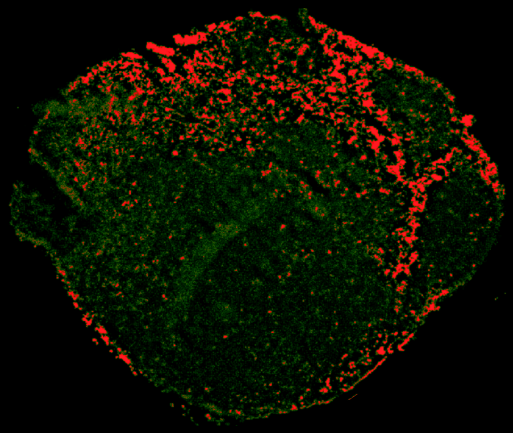
**Intralymphatic immunization enhances DNA vaccination.** Kevin J. Maloy*, Iris Erdmann*, Veronique Basch*, Sophie Sierro†, Thomas A. Kramps†, Rolf M. Zinkernagel†, Stefan Oehen†, and Thomas M. Kündig. Proc. Natl. Acad. Sci. 2001

# Intranodal in situ Targeting of mRNA into DC

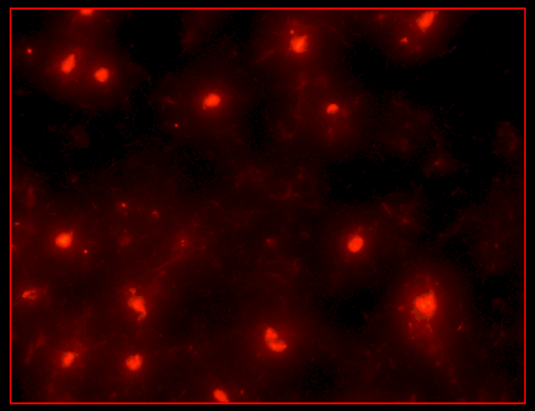
Analysis of Biodistribution after Injection of RNA-CY3

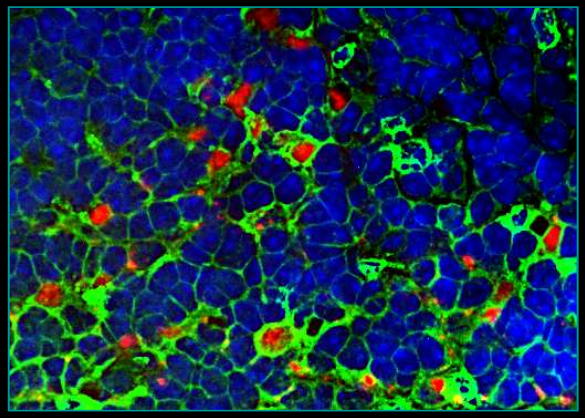
5min





30min

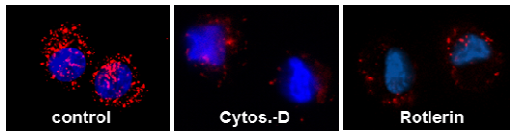




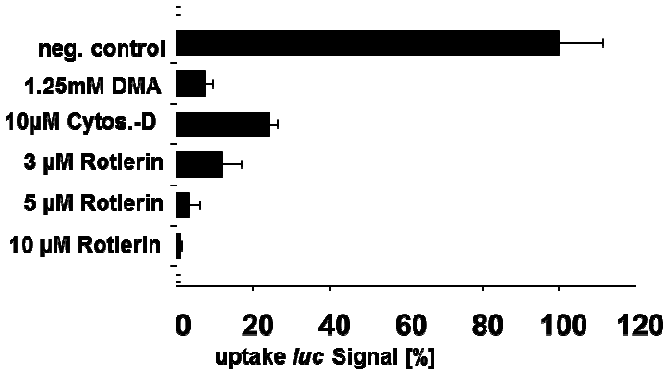
DAPI
RNA-CY3
CD11b

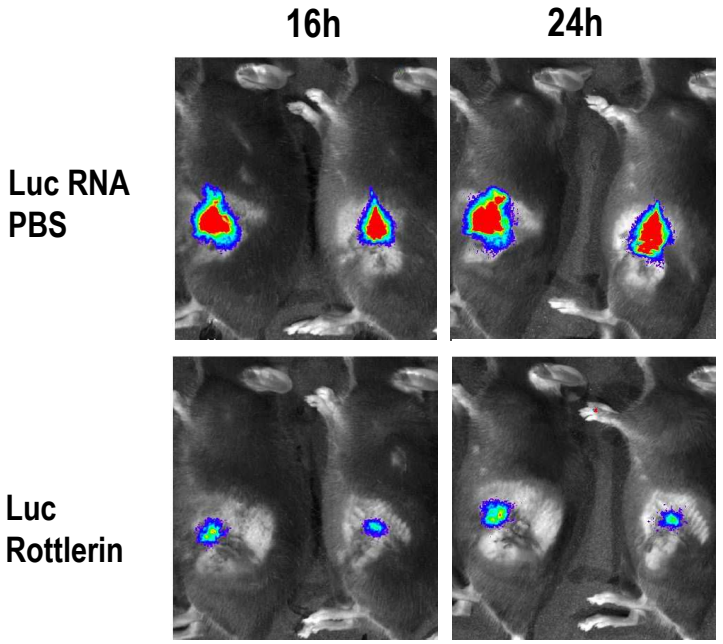
# Macropinocytosis mediated RNA uptake into DC

**Influence of macropinocytosis inhibitors (fluorescence imaging)**



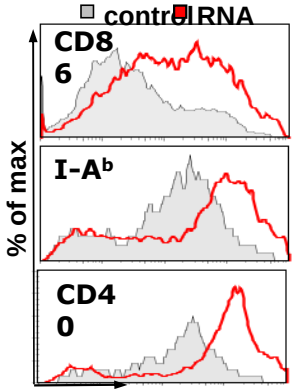

**Influence of macropinocytosis inhibitors (luziferase assay)**

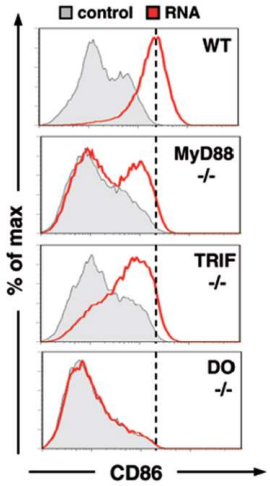


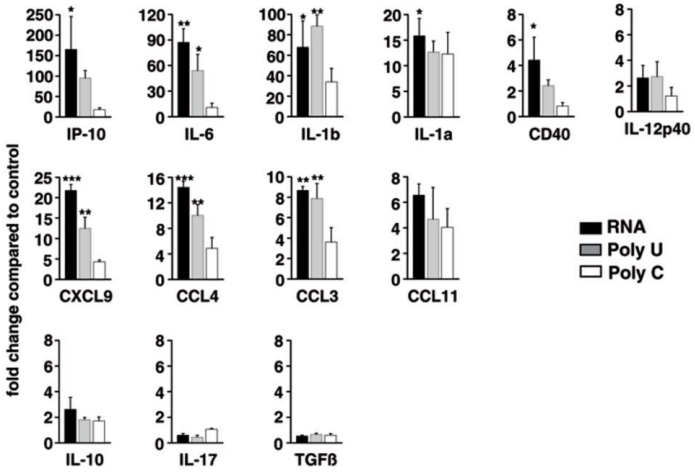




Diken et al., Gene Therapy, 2011

# Effects on the lymph node microenvironment

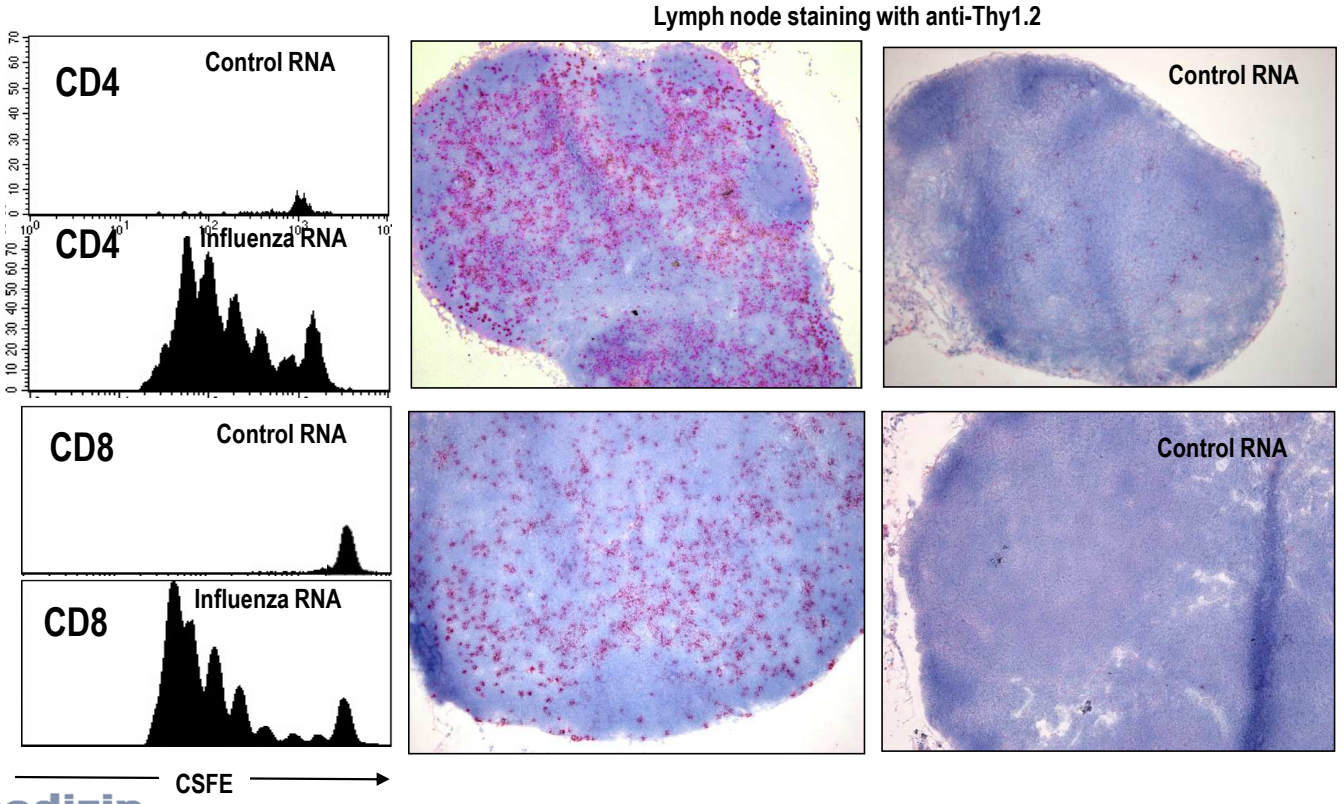




Kreiter *et. al.*, Cancer Research, 2011

# Stimulation of CD8+ and CD4+T cells

Adoptive Transfer of 5 x $10^5$ Influenza HA specific CD8+ or CD4+ T cells followed by 1x RNA Immunisation

## Potent Expansion of Antigen Specific T Cells

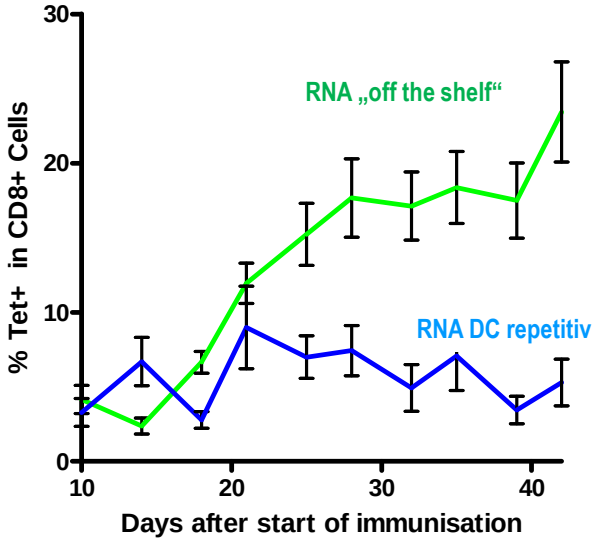



UNIVERSITÄTSmedizin. MAINZ

TRON

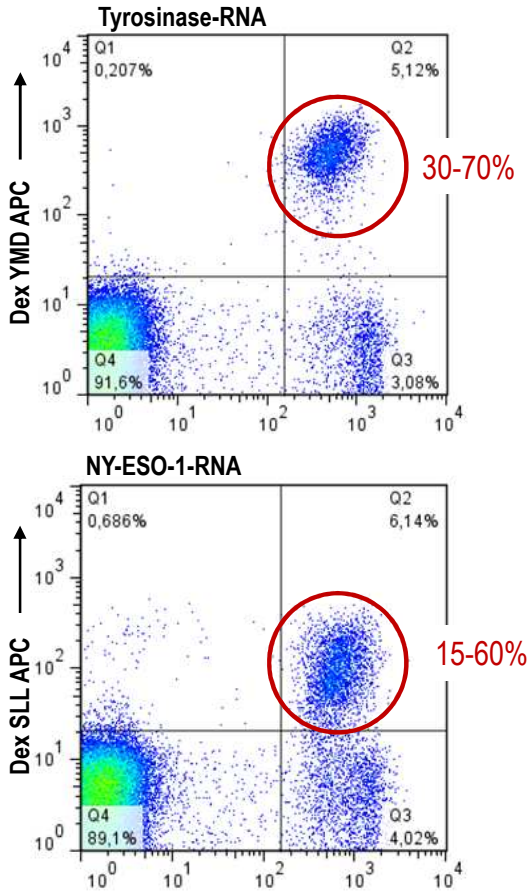
# Robust Expansion of immunodominant CTLs

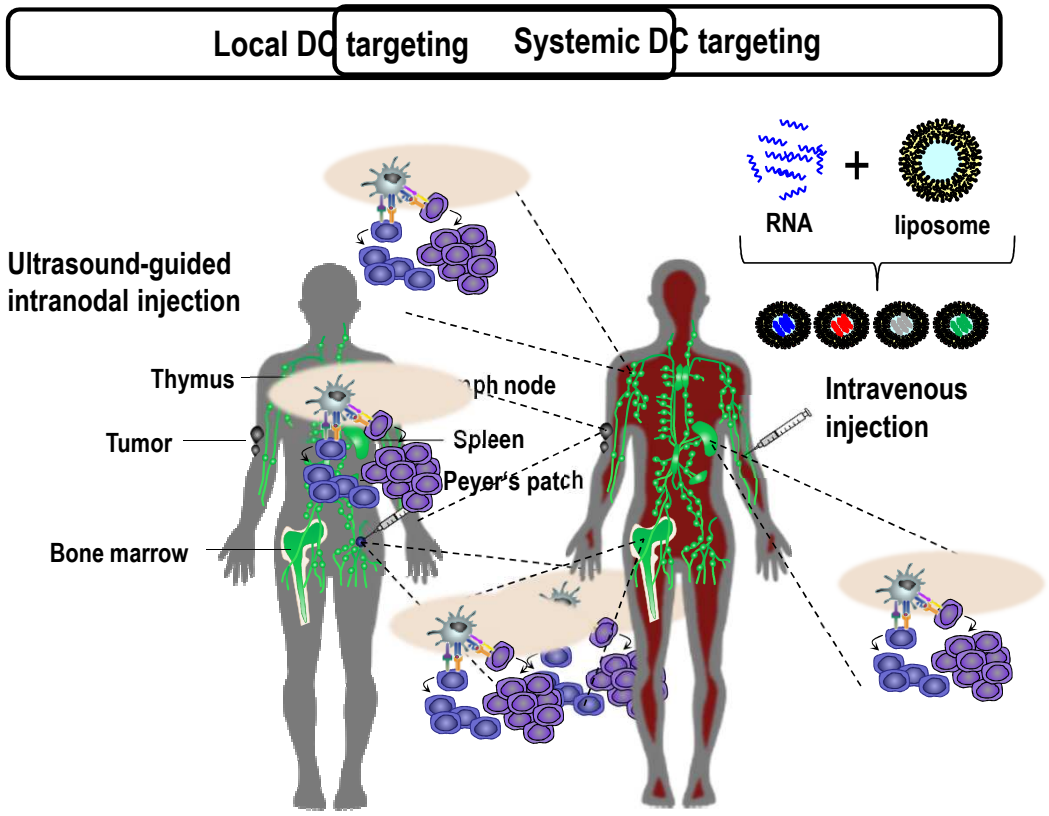

## Repetitive Immunisation in B6/C57L




…Translation into first in human testing*

## Immune Response in A2/DR1 Mice






mRNA and DCs meet directly at injection site

Challenges:
- mRNA integrity
- DC-specific uptake
- Antitumor T cell response

Lena Kranz, Mustafa Diken, Heinrich Haas et al.

# Development of a liposomal RNA vaccine

BIONTECH

**Formulation Development Discovery, R&D**

- Application tailored mRNA formulation development.
  Cancer Immunotherapy
  Infect. Disease vaccines
  Organ specific delivery
  (lung, liver, DC & others)
- Optimized formulations for various routes
  (i.v., s.c., i.d, i.m, i.n.)
- Focus on well tolerated excipients

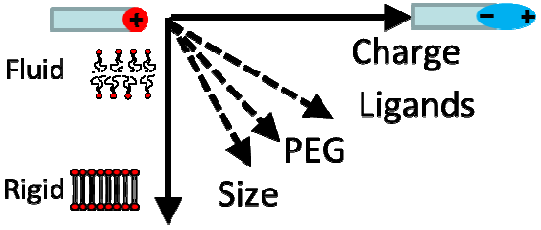
**Analytics Assay Development**

- HPLC Analytics
- Bioanalytics (LC/MS)
- Particle Analytics
- Particle Engineering
- Broad scope of pharma-ceutically relevant methods
- Extended biophysical characterization inhouse or with external partners

**Process Development**

- Upscaling and GMP process development
- GMP manufacturing of liposomes with partners
- Assembly of IMP KIT for administration to pations in own facilities
- Development of market-compliant methods for manufacturing

Formulation discovery




Process development

GMP production

QC & Release

JG|U UNIVERSITÄTSmedizin. MAINZ

Lena Kranz, Mustafa Diken, Heinrich Haas et al.

TRON

# Liposomal mRNA delivery into DCs

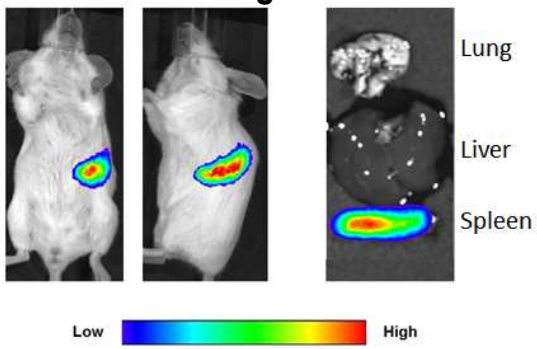
BIONTECH

**Intravenous Delivery**

Liposomal Luc-RNA

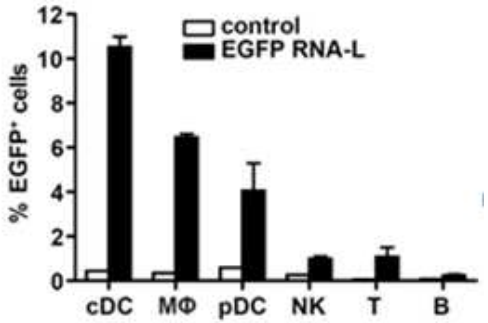
*C57BL/6*

**Luciferase Expression in vivo**



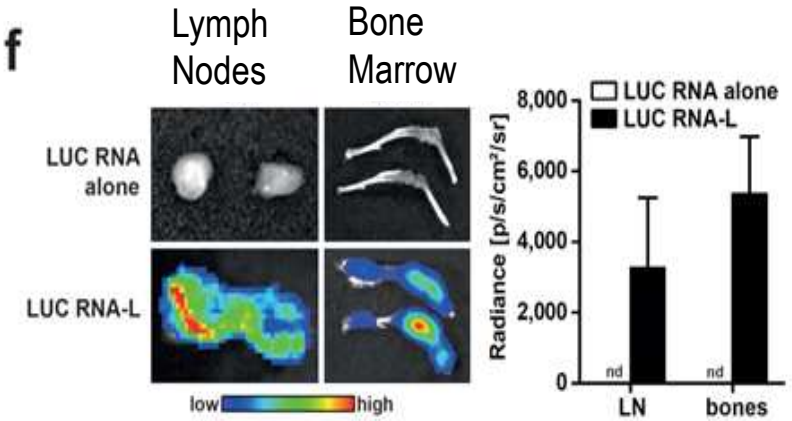

**Organs**

**Transfected Cell Populations**

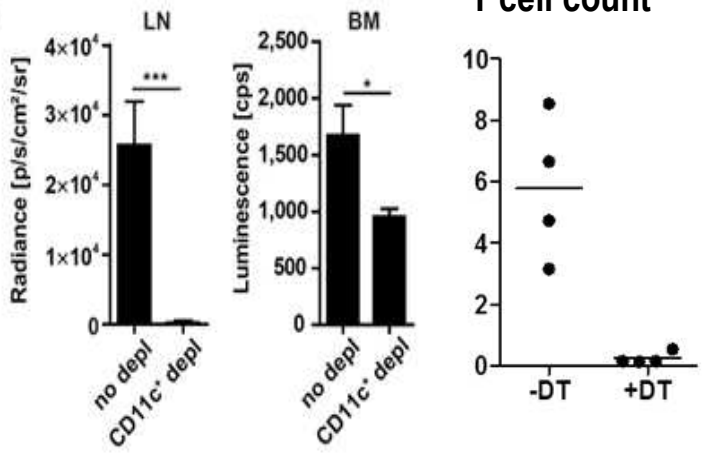



## DC Depletion in CD11x-DTR mice abrogotas expression and Immune Response

**f**




**g**



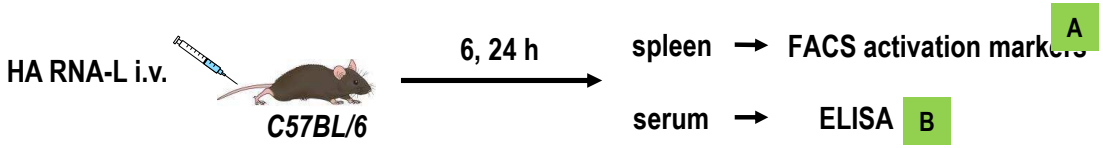

**T cell count**

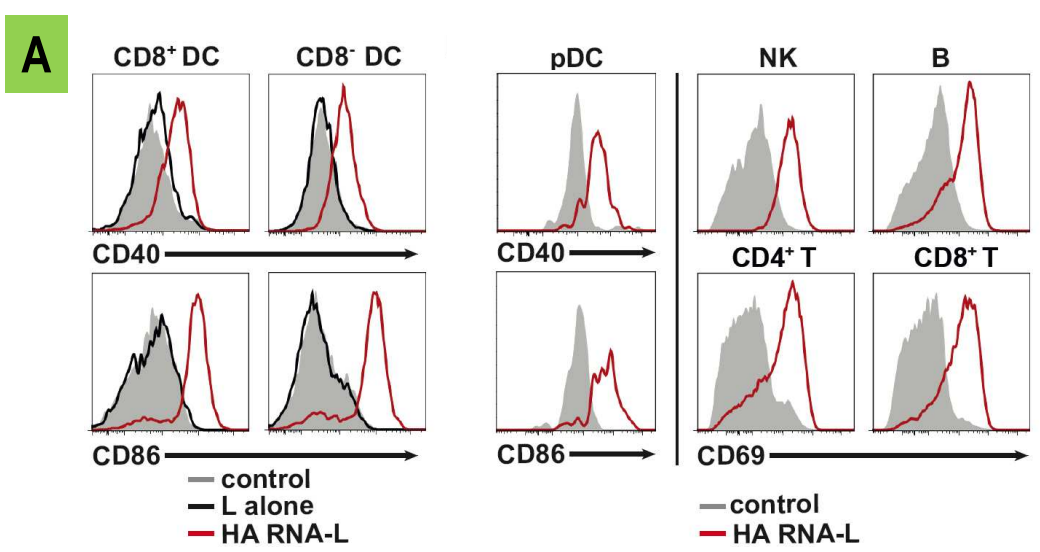


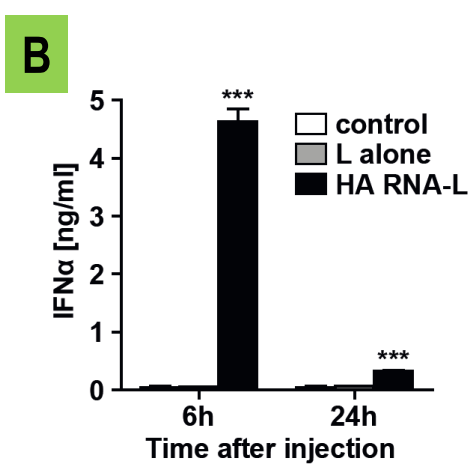

JG|U UNIVERSITÄ

TRON

# Secondary pharmacodynamics of Liposomal RNA

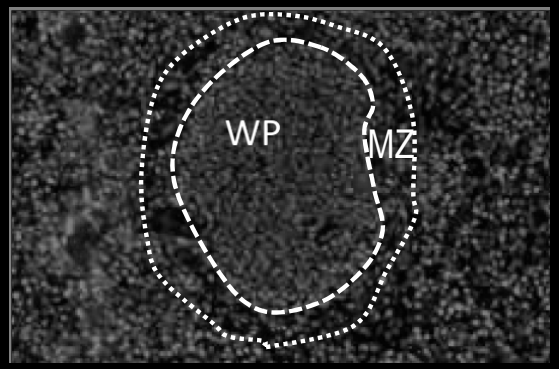


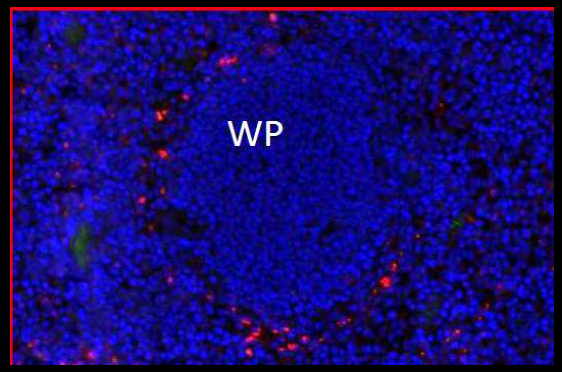



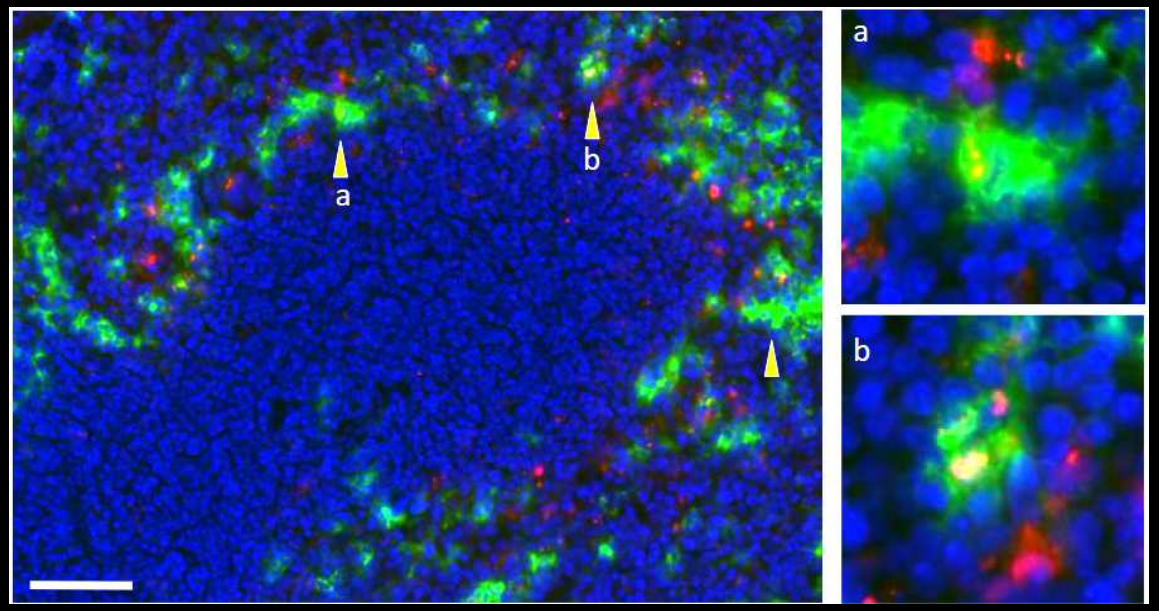

- RNA-L induce APC maturation and activation of tissue resident cells
- RNA-L induce systemic IFNa

# Liposomal RNA targets to CD11c DCs in the Marginal Zone



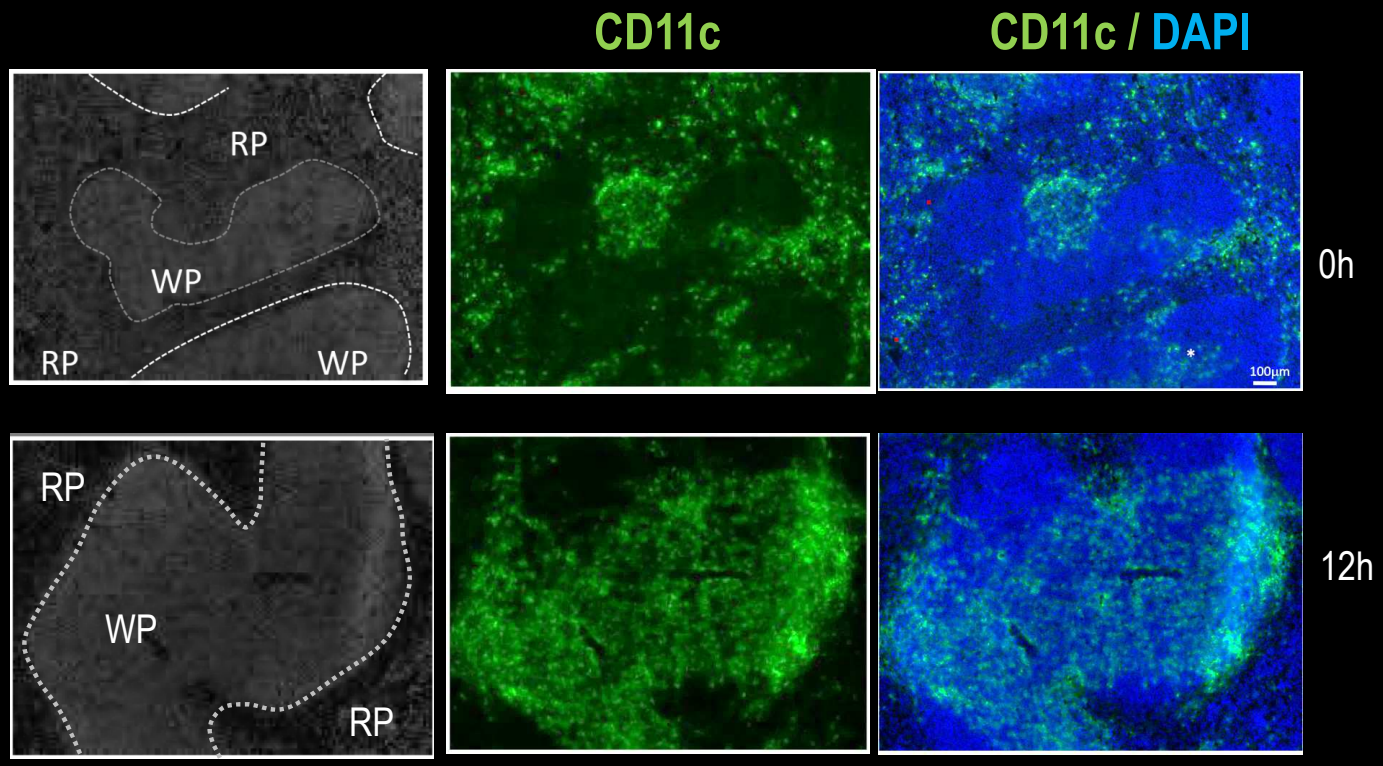

RNA



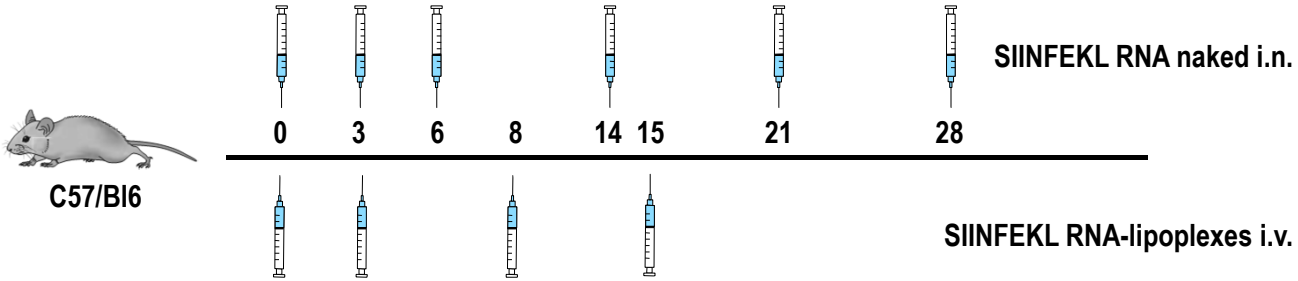

RNA

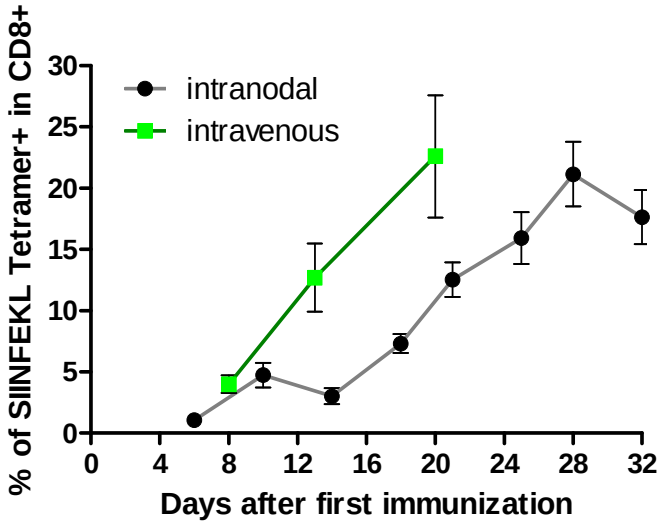
CD11c

# CD11c+ DC enter White Pulp upon Liposomal RNA Transfection



# Rapid induction of T cells via RNA lipoplexes



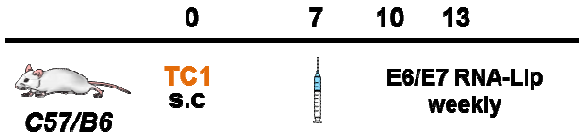

**Liposomal RNA vaccination induces rapid expansion of antigen-specific T cells compared to intranodal (i.n.) naked RNA vaccination**

## Tumor antigens generated by cancer associated alterations

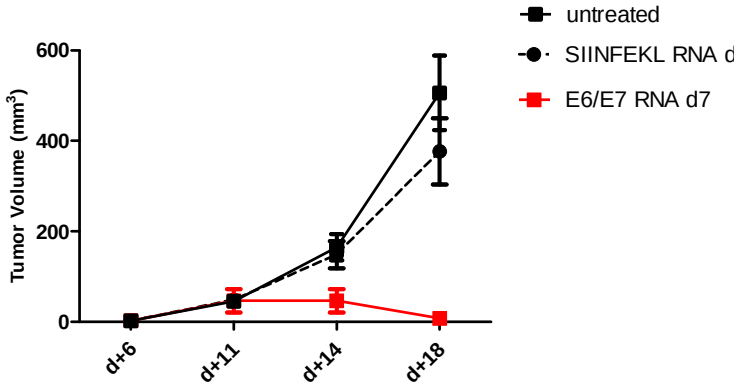
- Viral Neoantigens
  (viral neoantigens , HPV, EBV)
- Gene Mutations
  (point mutations, translocations ➔ mutant neoepitopes
- Epigenetic Mechanisms & Aberrant Transcription
  (Activation of silenced genes, intronic & antisense transcription,..)
- Aberrant Translation
  (alternate ORF, UTR translation, .)
- Posttranslational Modifications
  (amino acid changes, phosphorylation, glycosylation, protein-splicing, ..)
- Aberrant Subcellular Localisation
  (amino acid changes, glycosylation, protein-splicing, ..)

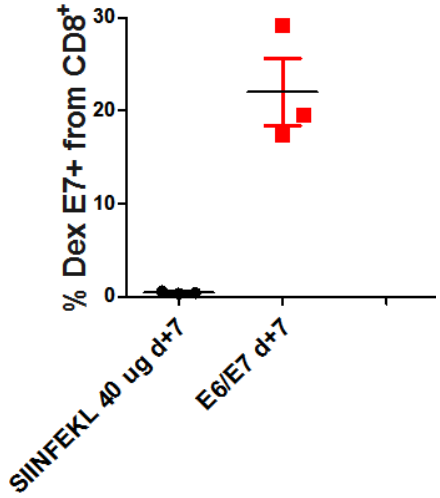
**What are the best vaccine targets for Cancer Immunotherapy ?**

# HPV E6/E7 transformed TC1 tumor model *




Christian Grunwitz
Fulvia Vascotto
Sebastian Kreiter




TC-1, derived from primary epithelial cells of C57BL/6 mice cotransformed with HPV-16 E6 and E7 and c-Ha-*ras* oncogenes

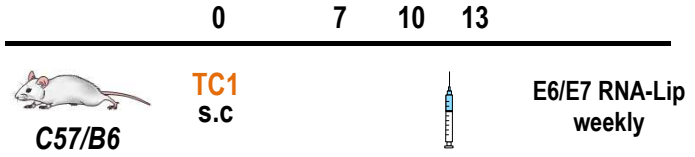
**Treatment of Established Tumors with a Novel Vaccine That Enhances Major Histocompatibility Class II Presentation of Tumor Antigen[1]**

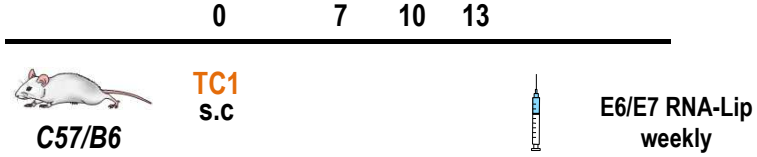
Ken-Yu Lin, Frank G. Guarnieri, Kevin F. Staveley-O'Carroll, Hyam I. Levitsky, J. Thomas August, Drew M. Pardoll, and Tzyy-Choou Wu[2]

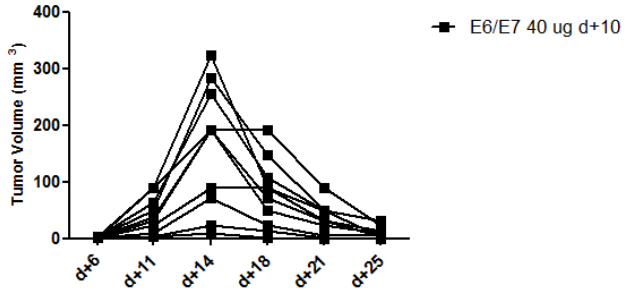
*Departments of Pathology [K-Y. L., T-C. W.], Pharmacology and Molecular Science [F. G. G., J. T. A.], Surgery [K. F. S-O.], and Oncology [H. I. L., J. T. A., D. M. P.], The Johns Hopkins Medical Institutions, Baltimore, Maryland 21287-6417*

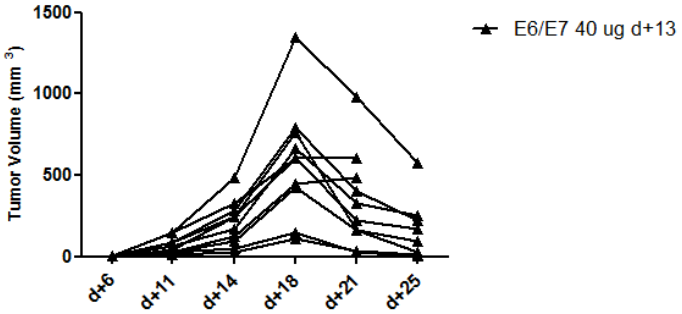
# Rejection of advanced tumors

TC1 HPV E7 transformed tumor model

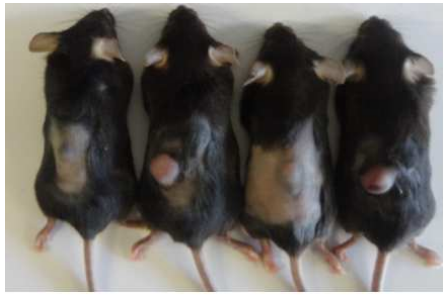


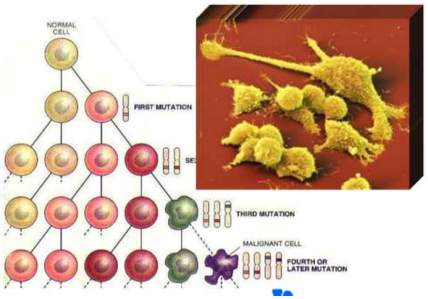

E6/E7 RNA-Lip
weekly



**Clinical Phase I/II Study in HPVpos Head Neck Cancer**
in collaboration **Christian Ottensmeier** (Univ. Soupthampton)
I-ACT consortium **(Coordinator - Rienk Offringa)**

# Targeting Mutant Neopitopes in Cancer

## Cancer




**Genetic / Epigenetic Mutations**

> 100 Mutations/Cancer,> 95% Individual Mutations

> Individual Spectrum of SharedTumor antigens

➔ Tumor Growth, Resistance, Metastasis, Recurrence

➔ Immunosuppression

## Immune Recognition of Cancer Mutations

**A p16$^{INK4a}$-Insensitive CDK4 Mutant Targeted by Cytolytic T Lymphocytes in a Human Melanoma**

Thomas Wölfel,* Martina Hauer, Jörg Schneider, Manuel Serrano, Catherine Wölfel, Eva Klehmann-Hieb, Etienne De Plaen, Thomas Hankeln, Karl-Hermann Meyer zum Büschenfelde, David Beach

SCIENCE • VOL. 269 • 1 SEPTEMBER 1995

**Cloning Genes Encoding MHC Class II–Restricted Antigens: Mutated CDC27 as a Tumor Antigen**

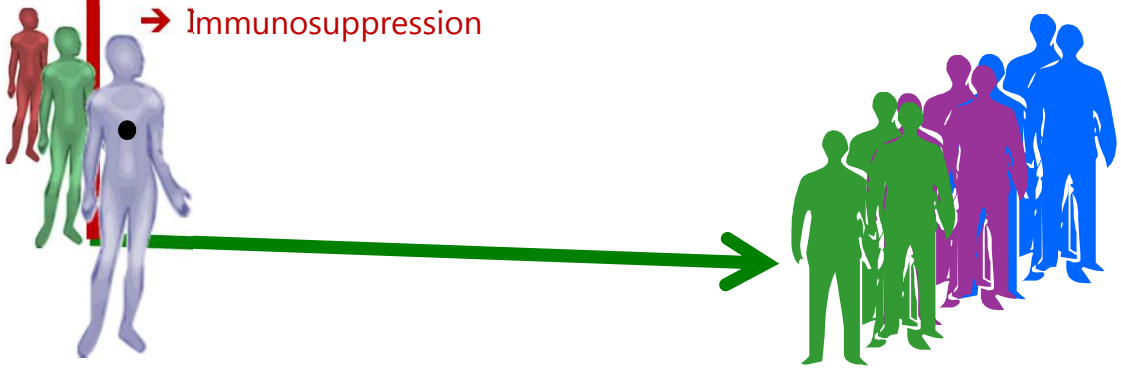
Rong-Fu Wang,* Xiang Wang, Alicia C. Atwood, Suzanne L. Topalian, Steven A. Rosenberg

SCIENCE VOL 284 21 MAY 1999

**The response of autologous T cells to a human melanoma is dominated by mutated neoantigens**

Volker Lennerz[†], Martina Fatho[†], Chiara Gentilini[‡], Roy A. Frye[§], Alexander Lifke[†], Dorothea Ferel[†], Catherine Wölfel[†], Christoph Huber[†], and Thomas Wölfel[†¶]

PNAS | November 1, 2005 |



## Germline Variation

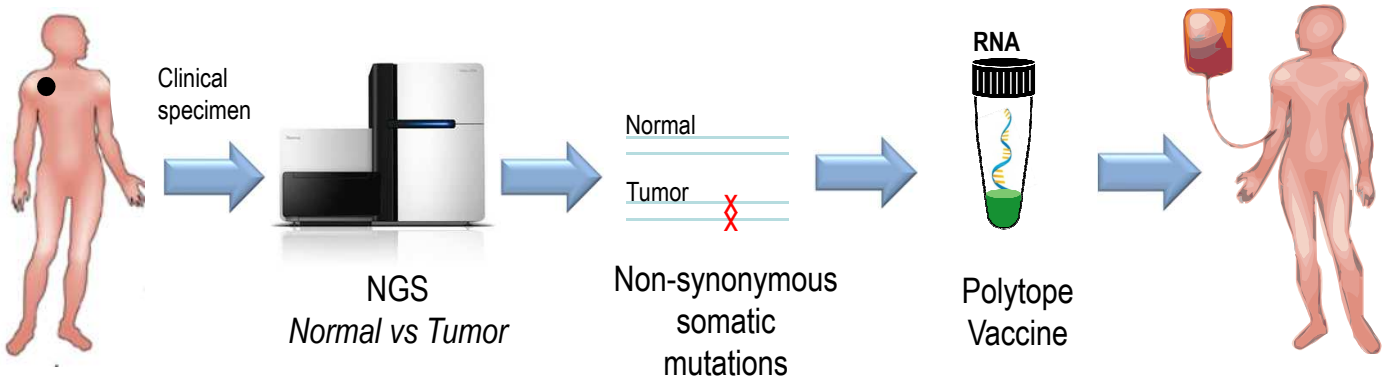
- HLA-Antigens
- Genetic Polymorphisms

## Individual Patients

**IVAC:** A collaborative Project for Development and Testing of an Individualized Vaccine Concept

BIONTECH

## From Cancer Mutations to Vaccines



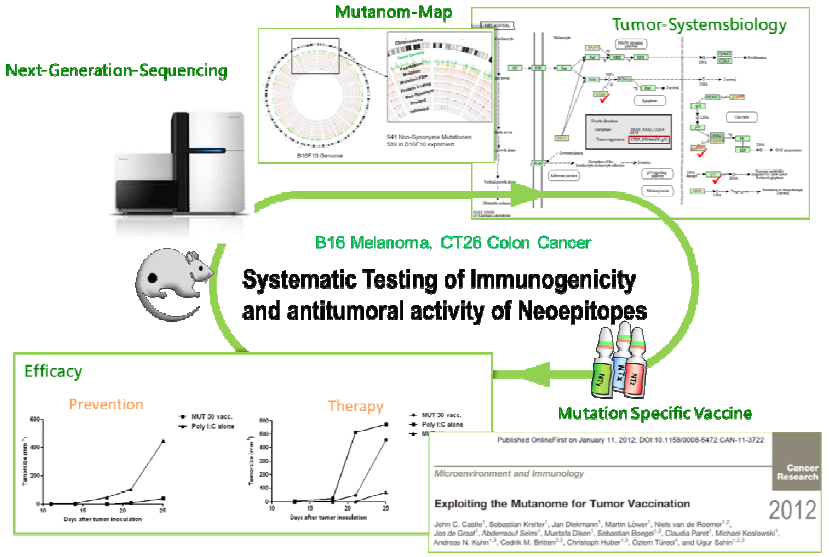

- All somatic mutations in the tumor of the patient are determined by NGS ( Next Generation Sequencing).
- A poly-neo-epitopic coding RNA based vaccine featuring the unique mutation signature of this patient is to be engineered and administered as an individualized treatment.

JG|U UNIVERSITÄTSmedizin. MAINZ

TRON

# NGS discovered mutations as tumor antigens

**Which Individual neoantigens useful as targets for antitumoral vaccines ?**




**Are neoantigens targets for cancer immunoediting ?**

Nature

## Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting

Hirokazu Matsushita[1]†*, Matthew D. Vesely[1]*, Daniel C. Koboldt[2], Charles G. Rickert[1], Ravindra Uppaluri[3], Vincent J. Magrini[2,4], Cora D. Arthur[1], J. Michael White[1], Yee-Shiuan Chen[1], Lauren K. Shea[1], Jasreet Hundal[2], Michael C. Wendl[2,4], Ryan Demeter[2], Todd Wylie[2], James P. Allison[5,6], Mark J. Smyth[7,8], Lloyd J. Old[9], Elaine R. Mardis[2,4] & Robert D. Schreiber[1]

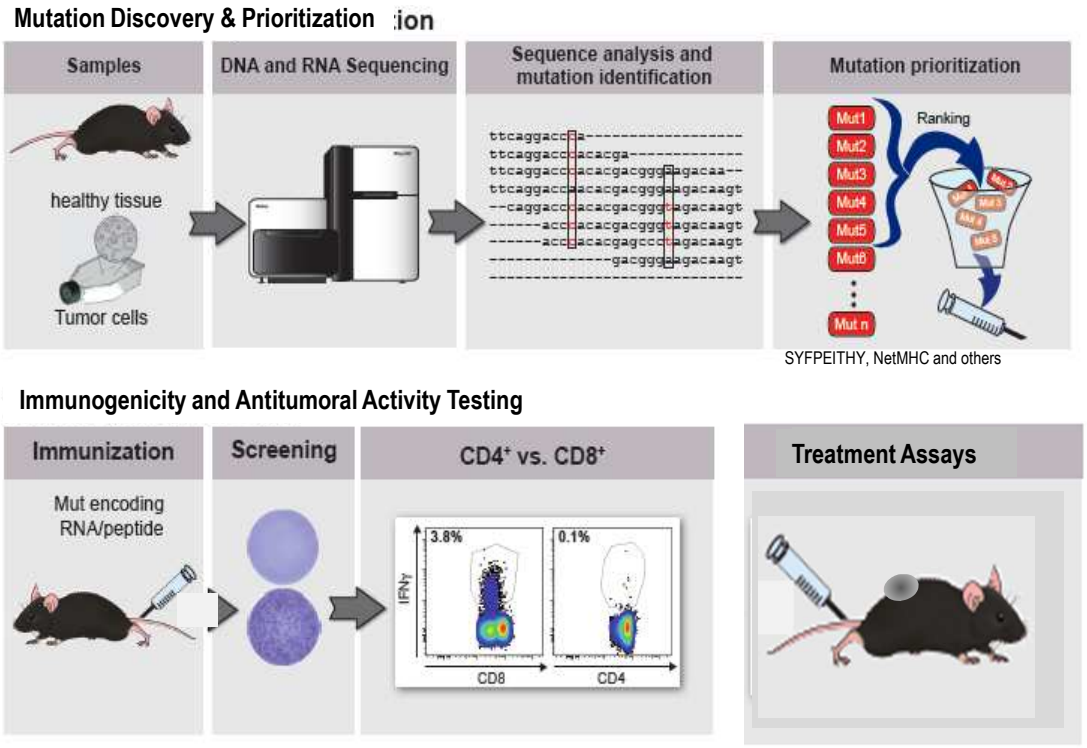
Nature

## Expression of tumour-specific antigens underlies cancer immunoediting

Michel DuPage[1], Claire Mazumdar[1], Leah M. Schmidt[1], Ann F. Cheung[1] & Tyler Jacks[1,2]

Castle et al., Cancer Research 2012

# Systematic Analyses of Mutations as Targets




Kreiter et al., 2015 Nature

## Summary

# Mutant MHC class II epitopes drive therapeutic immune responses to cancer *

Sebastian Kreiter[1], Mathias Vormehr[2]*, Niels van de Roemer[2]*, Mustafa Diken[1], Martin Löwer[1], Jan Diekmann[1,3], Sebastian Boegel[1], Barbara Schrörs[1], Fulvia Vascotto[1], John C. Castle[1], Arbel D. Tadmor[1], Stephen P. Schoenberger[4], Christoph Huber[2], Özlem Türeci[1]§ & Ugur Sahin[1,2,3]§

- **Frequent immune recognition** of mutant neoepitopes
- Nonsynonymous mutations are **mostly recognized by CD4 T cells**
- Neoepitope specific **CD4 T cells are antitumoral** and induce favorable changes in the tumor microenvironment
- CD4 mediated tumor control may involve CD8 T cells and CD40L signaling and may induce **antigen spread**
- Potent mutated vaccine targets can be identified via **computational priorization** utilizing MHCII binding prediction and RNA expression level
- **Blueprint process** for Just in time design and production of neoepitope vaccines
- Antitumoral Activity 1 in 15 Mutated Targets

# Clinical translation: Regulation matters

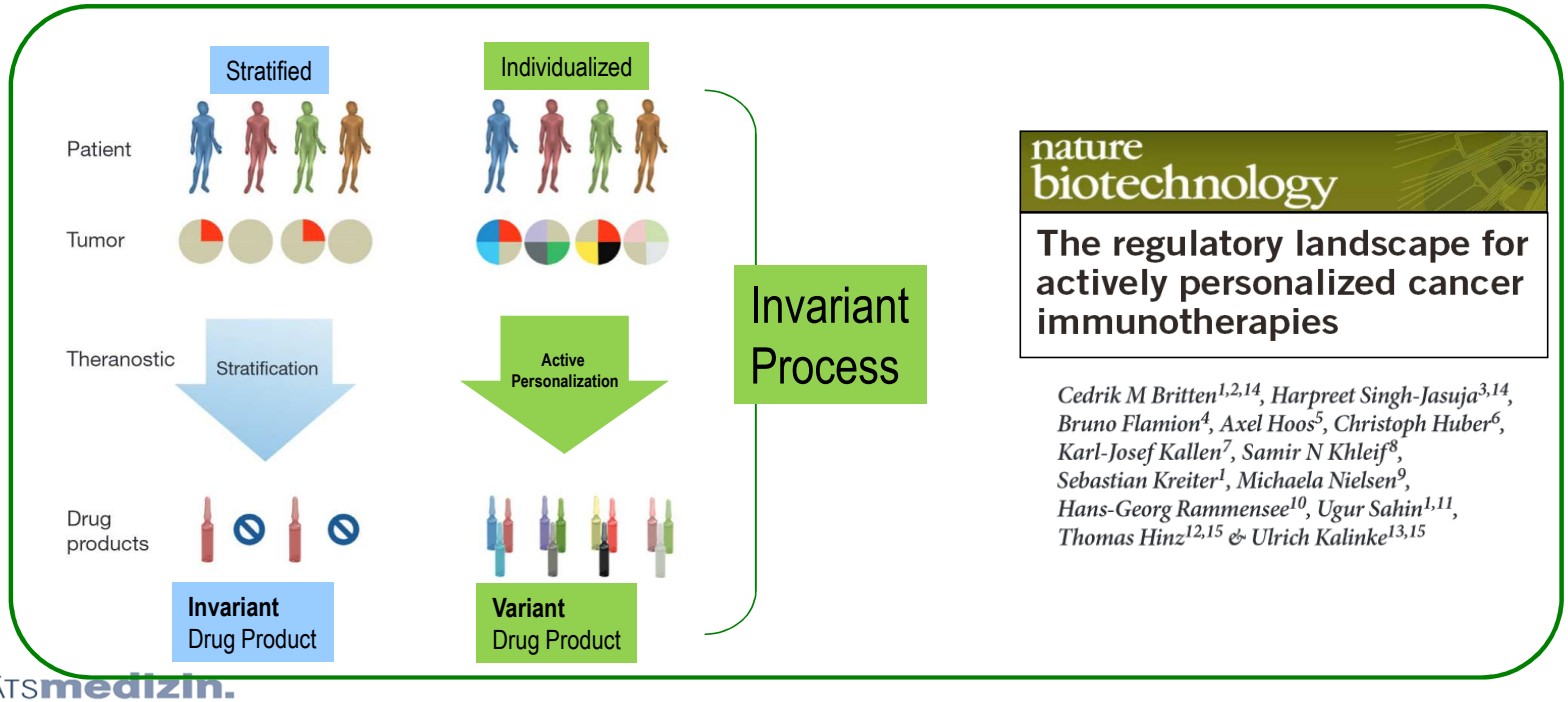
## CIMT Regulatory Research Group

**Regulatory Research Group**
**(founded 2008)**

Ulrich Kalinke, Harpreet Singh, Cedrik Britten, Thomas Hinz, Kajo Kallen, Axel Hoos, Sebastian Kreiter, Ulrich Granzer, Samir Khleif, Bruno Flamion & Christoph Huber

- Scientific Sessions
- Comments to Guidelines
- Co-operations
- Research Projects

CIMT Chair Ch. Huber




nature biotechnology

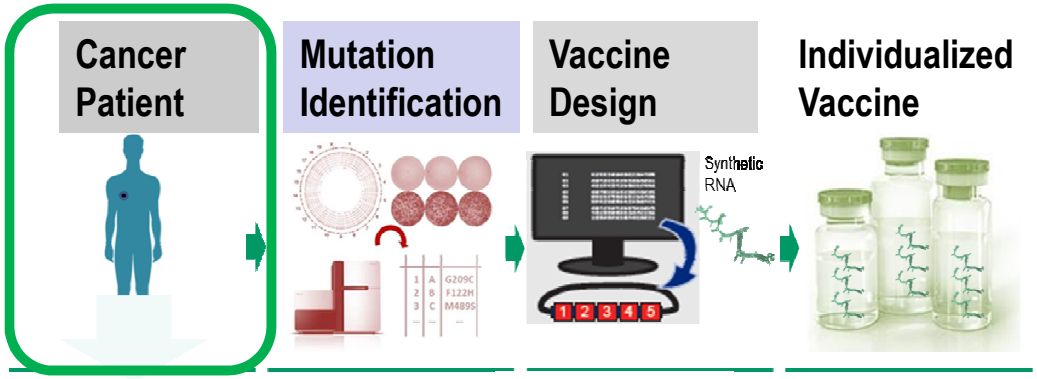
**The regulatory landscape for actively personalized cancer immunotherapies**

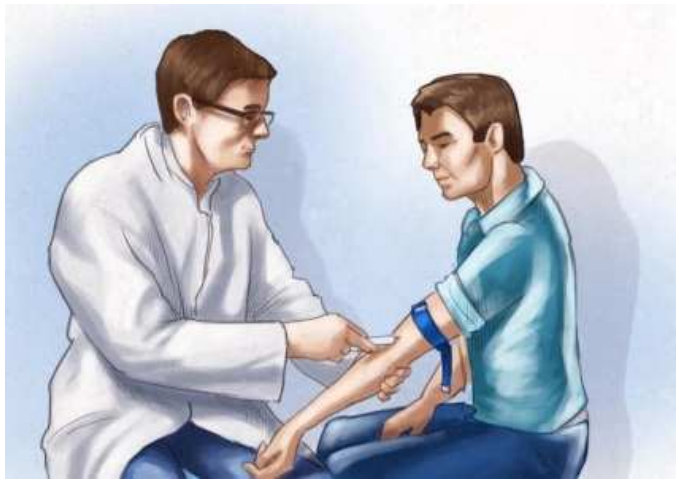
*Cedrik M Britten[1,2,14], Harpreet Singh-Jasuja[3,14], Bruno Flamion[4], Axel Hoos[5], Christoph Huber[6], Karl-Josef Kallen[7], Samir N Khleif[8], Sebastian Kreiter[1], Michaela Nielsen[9], Hans-Georg Rammensee[10], Ugur Sahin[1,11], Thomas Hinz[12,15] & Ulrich Kalinke[13,15]*

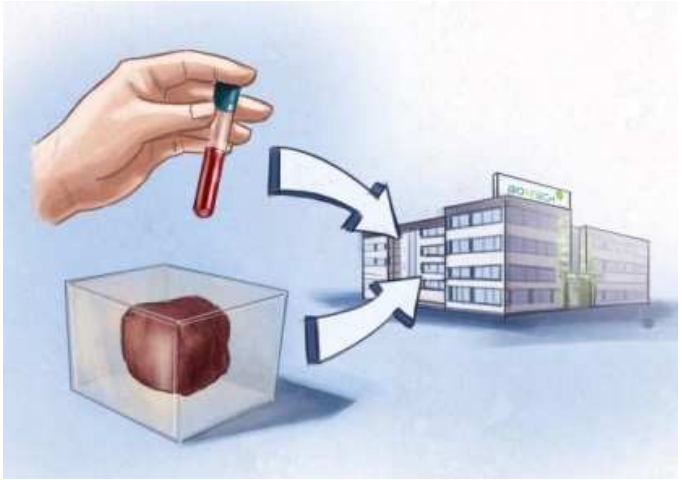
# IVAC Mutanome Trial

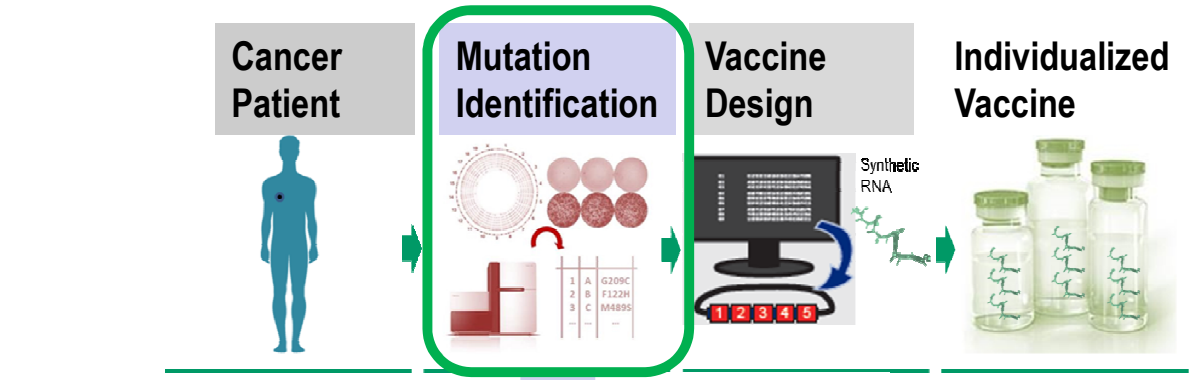
## DESIGN AND OUTLINE (NCT02035956)

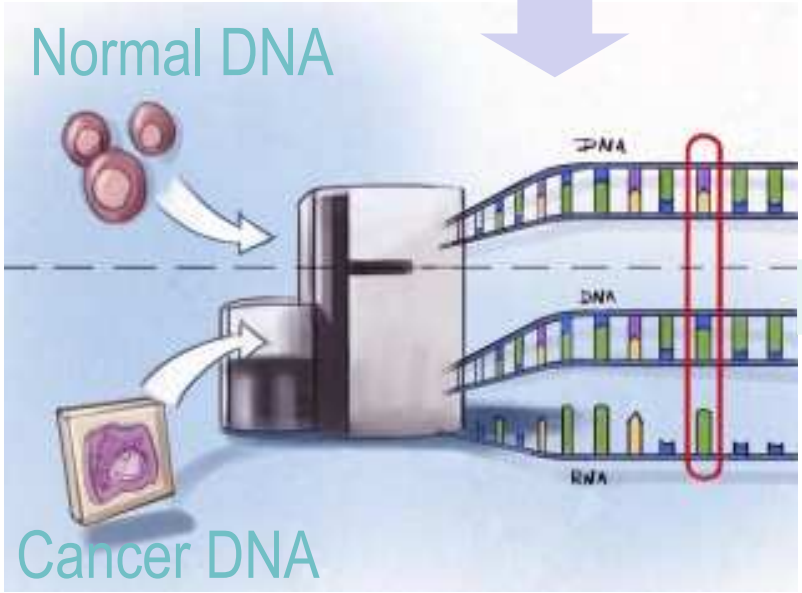
| | |
|---|---|
| **Indication** | Malignant Melanoma, stage IIIA-C |
| **Number of Patients** | Total number of patients is 15 |
| **Study design** | • Phase I, International – Multicenter, Open-label, Interventional<br>The study design includes an optional continued treatment with IVAC MUTAMONE for patients that show continued clinical benefit. |
| **Endpoints** | **Primary:** Safety, adverse reactions, tolerability of repetitive doses of IVAC MUTANOME vaccine after initial treatment with RBL001/RBL002<br>**Secondary:**<br>Determine the vaccine induced antigen-specific immune response.<br>Determine antitumor activity of IVAC MUTANOME vaccine after initial treatment with RBL001/RBL002. |
| **Investigators** | UMM – Universitätsmedizin Mainz<br>**Carmen Loquai, Dr (PI)**., Stephan Grabbe, Prof. Dr.<br><br>UMM - Universitätsmedizin Mannheim:<br>Jochen Utikal, Christoffer Gebhardt<br><br>Medizinische Universität Wien<br>Christoph Höller, Prof. Dr. |

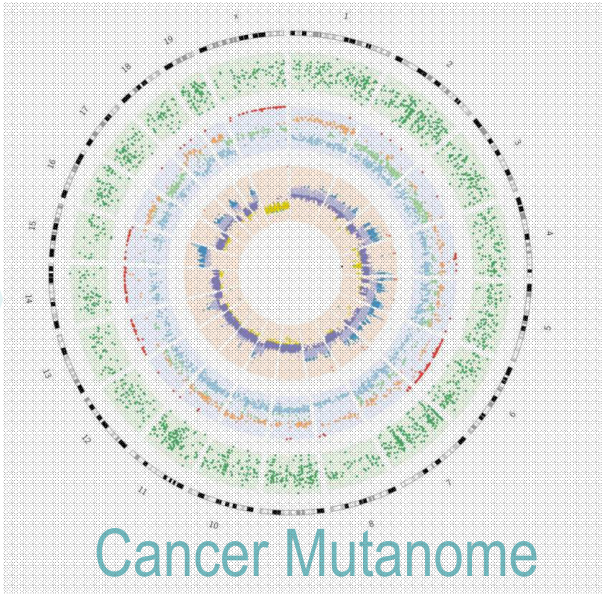

Cancer Patient
Mutation Identification
Vaccine Design
Individualized Vaccine
Synthetic RNA
1 2 3 4 5

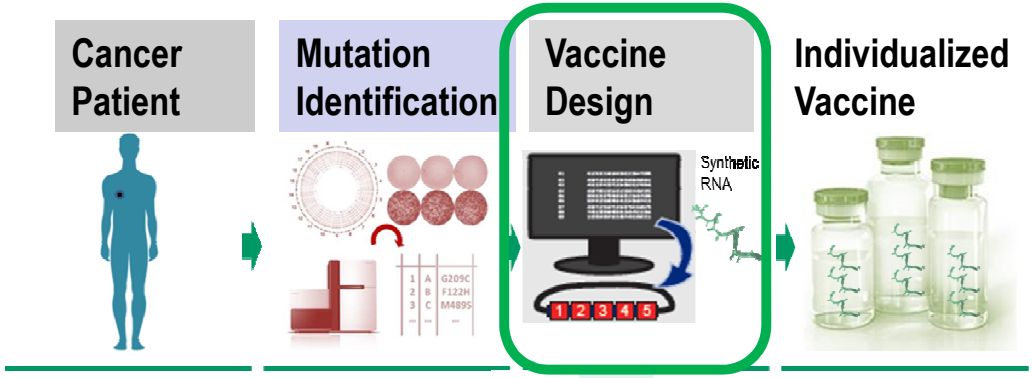


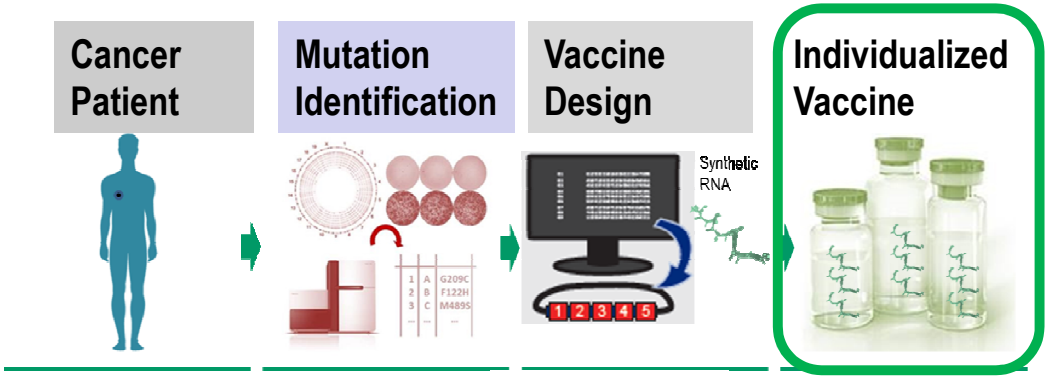


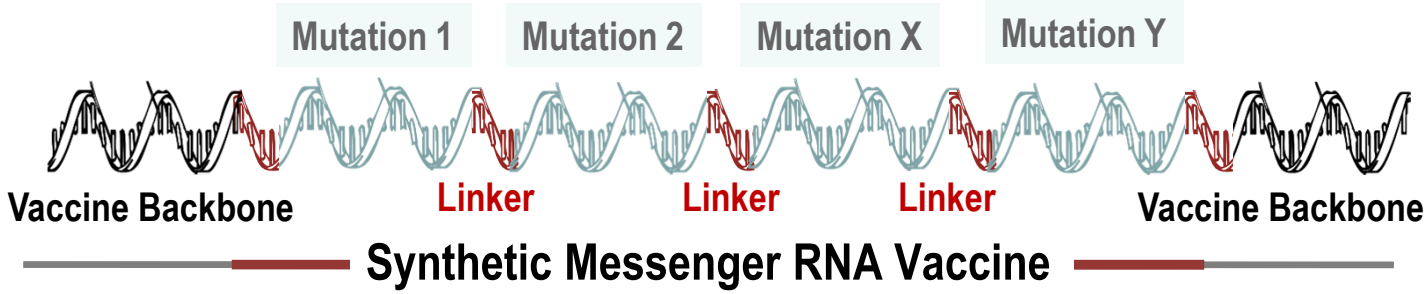

Cancer Patient
Mutation Identification
Vaccine Design
Individualized Vaccine
Synthetic RNA


Normal DNA
DNA
DNA
RNA
Cancer DNA


Cancer Mutanome


Cancer Patient
Mutation Identification
Vaccine Design
Individualized Vaccine
Synthetic RNA

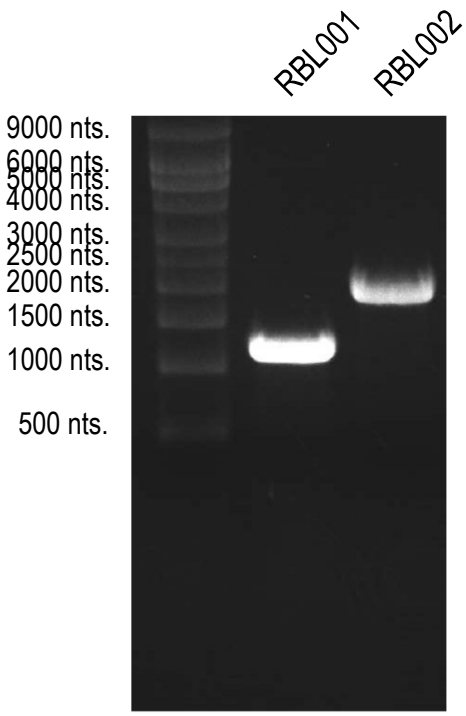


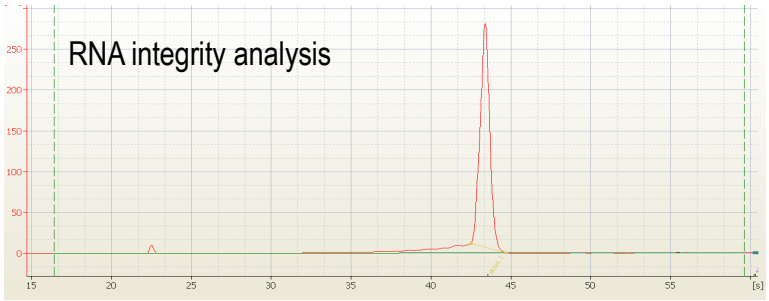

## Synthetic Messenger RNA Vaccine

# RNA manufactured at BioNTech manufacturing facility

**Gel analysis of RBL001 (1270 nts.) and RBL002 (2047 nts.)**



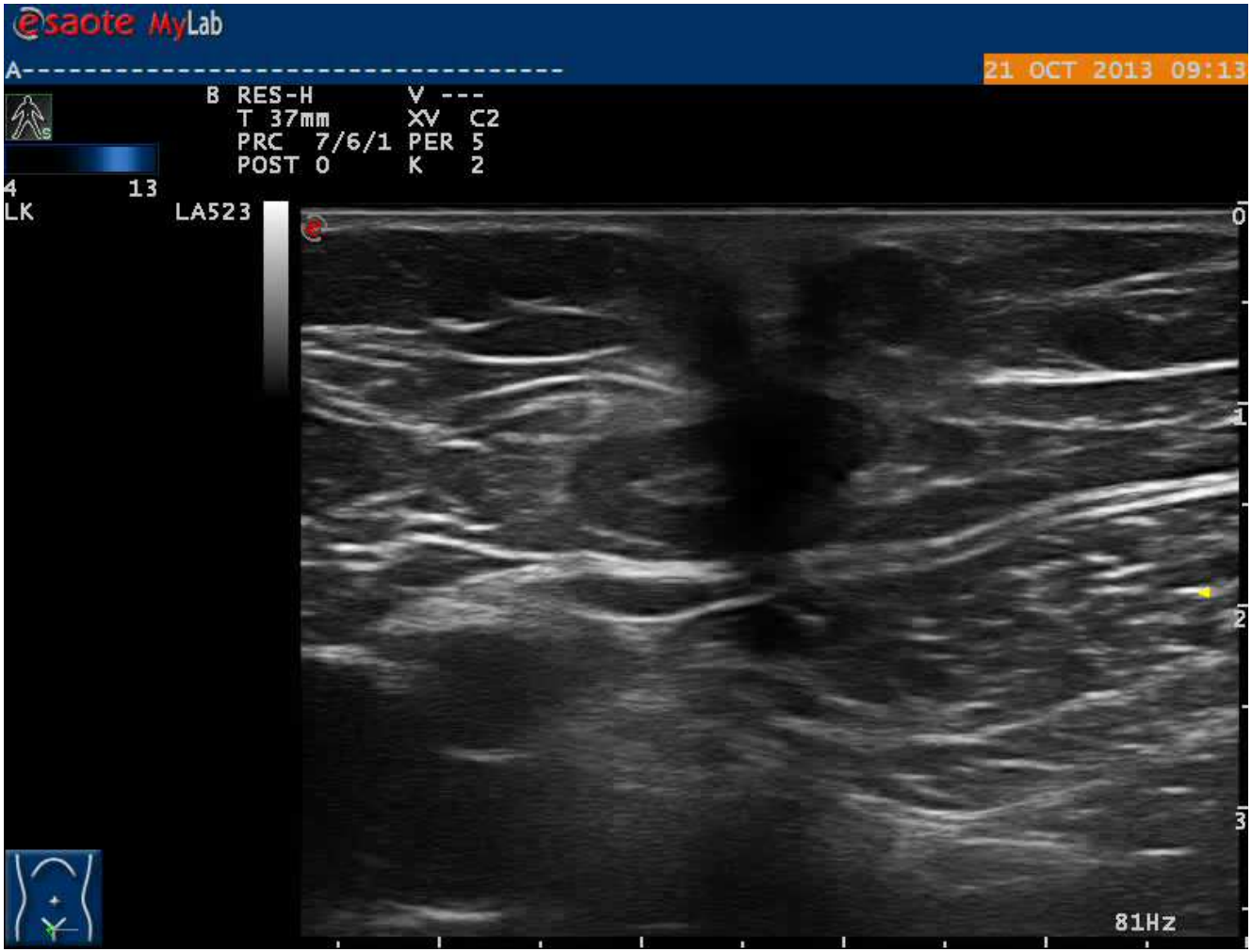

- Optimized manufacturing process
- Inhouse production, release testing, fill & finish.




GMP Certification for both production of pharmacologically optimized RNA for clinical trials

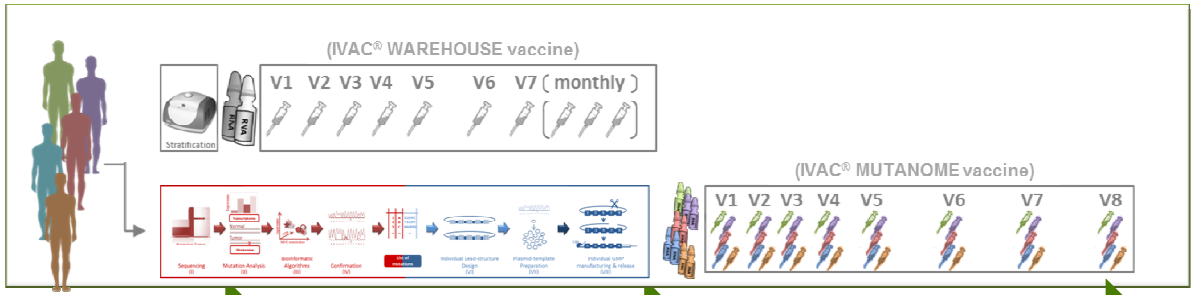
Production scales for clinical trials 1 -1000 patients

# IVAC® MUTANOME intranodal application




Diken et al. Gene Therapy 2011, Kreiter et al. Cancer Res. 2010

# Overview Biomarker Assessment




| Screening | Baseline max. 28 days | Vaccination IVAC WAREHOUSE | Vaccination IVAC MUTANOME | Follow up until day 90 |
|---|---|---|---|---|
| Visit | V1 | V2 | V12 | V20 V21 |
| day | Stratification | 1 4 8 11 15 43 71 | 1 4 8 11 15 22 29 43 | 50 90 |

DTH with naked RNAs i.d.

Leukapheresis

Whole Blood

**Blood sampling for:**

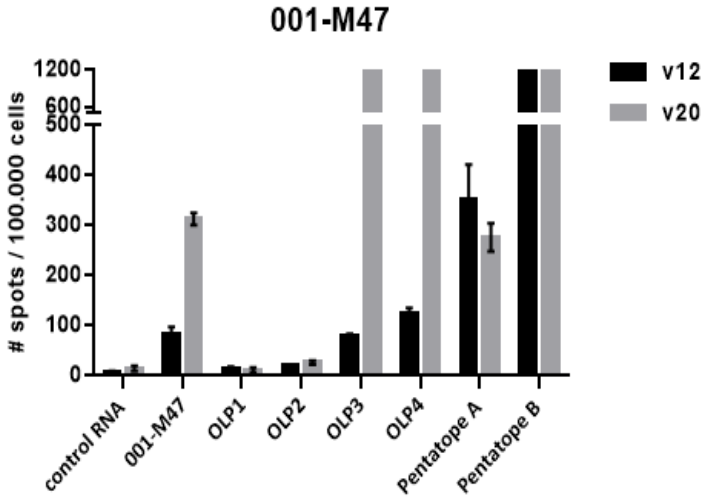
| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MRI / CT | M | | | | | | | M | | | | | | | | | | | | | M |
| Haematology | H | | | | | | | H | | | | | | | | | | | | | H |
| Biochemistry | B | | | | | | | B | | | | | | | | | | | | | B |
| Serology | S | | | | | | | | | | | | | | | | | | | | |
| Coagulation | Co | | | | | | | Co | | | | | | | | | | | | | Co |
| Cellular IR | IR | | | I | | IR | | I | | I | IR | | | | | IR | IR | IR | IR | IR | |
| CNAPS | CN | | | R | | | | R | | R | CN | | | | | | | | | | |
| Serum biomarkers | Se | | | | | | | | | | Se | | | | | Se | | | | | Se |
| HLA typing | HL A | | | | | | | | | | | | | | | | | | | | |

UNIVERSITÄTSmedizin MAINZ

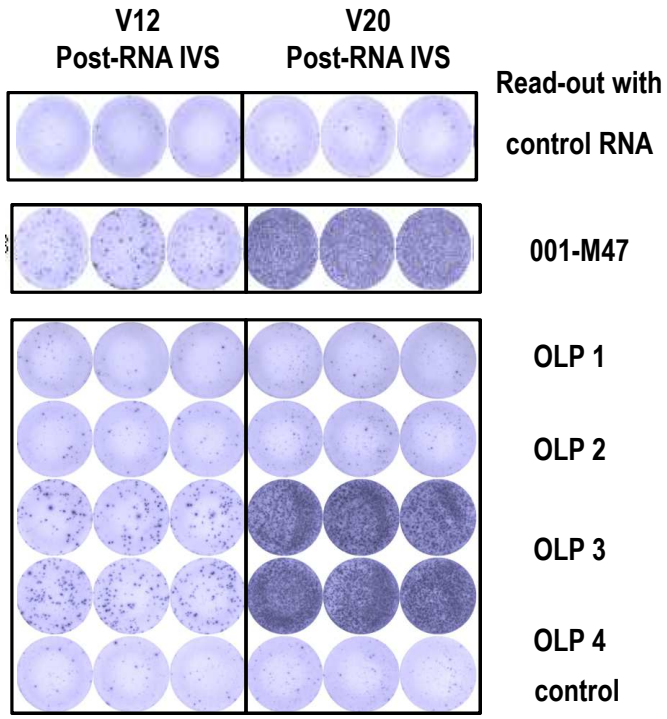
TRON

# Post-IVS ELISPOT data
# 001-M47, Pentatope B, position 1

Unit GxP Analytics



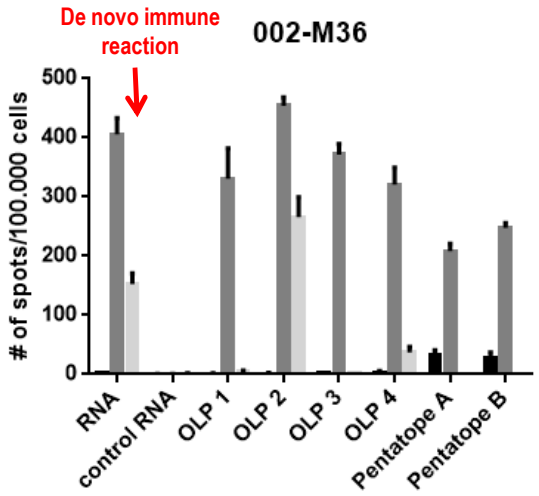

| Peptide | Sequence |
|---|---|
| OLP1 | PQTLGKKGSKNNIFV |
| OLP2 | GKKGSKNNIFVYMTL |
| OLP3 | SKNNIFVYMTLNQKK |
| OLP4 | IFVYMTLNQKKSDSS |

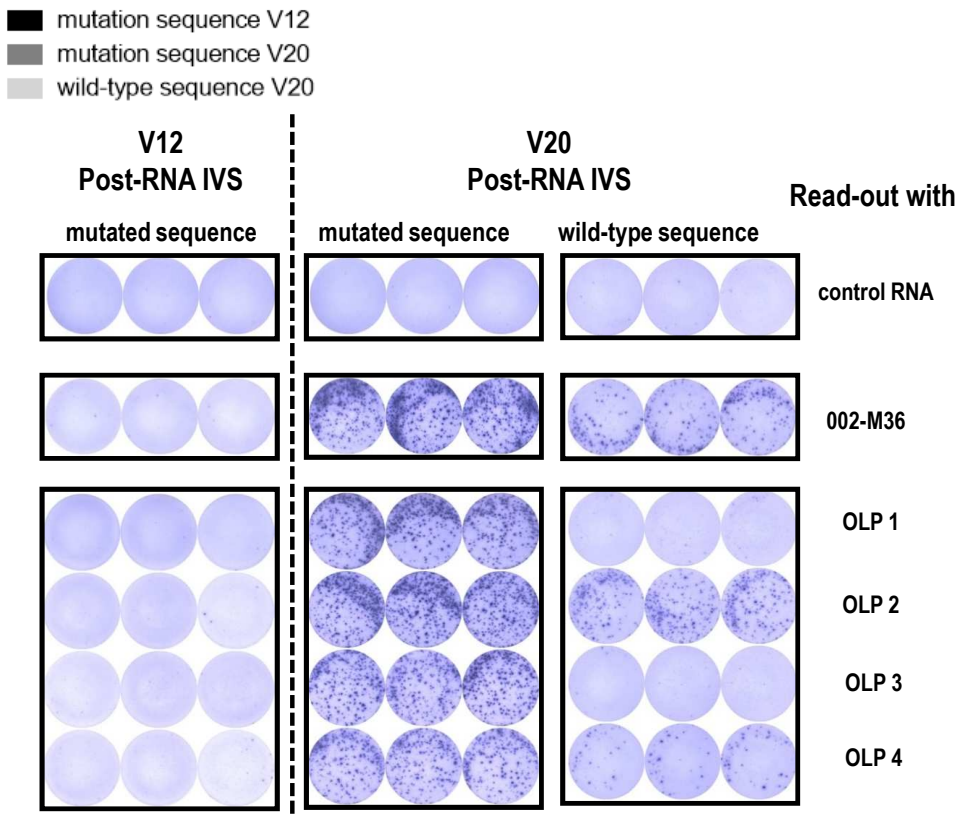



Best-predicted epitope: GSKNNIFVY (HLA A*3002)

Minimal immunogenic sequence: IFVYMTLNQKK

# Post-IVS ELISPOT data summary for 002-M36 Pentatope B Position 3



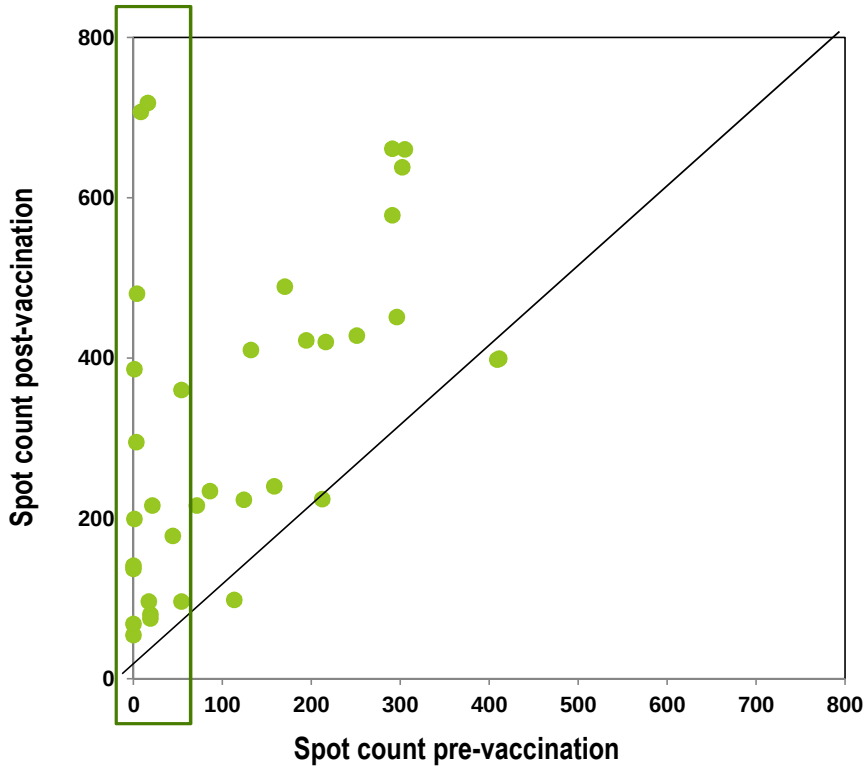




| Peptide | Sequence |
|---|---|
| OLP1 | SADARLMVFDKTERT |
| OLP2 | RLMVFDKTERTWRLL |
| OLP3 | FDKTERTWRLLCSSR |
| OLP4 | ERTWRLLCSSRSNAR |

Best-predicted MHC-I epitope: **VFDKTERTW (HLA A*2402)**

Minimal immunogenic sequence: not possible to define. An oligo-epitope response?

# Correlation between pre- and post-vaccination responses – All reponses




*Each spot represents a post-vaccination positive response (>50 spots/50.000 cells after subtraction of background) by CD4 or CD8 cells against RNA or peptide. The green box defines the area of no or weak pre-vaccination responses (>50 spots/50.000 cells after subtraction of background).*

# Patient IVAC Mutanome uID-004

- 75 female, Stage IIIC BRAF negative
- 10/2013 removal of inguinal LN metastases / Radiatio
- 02/2014 CT no remaining lesions

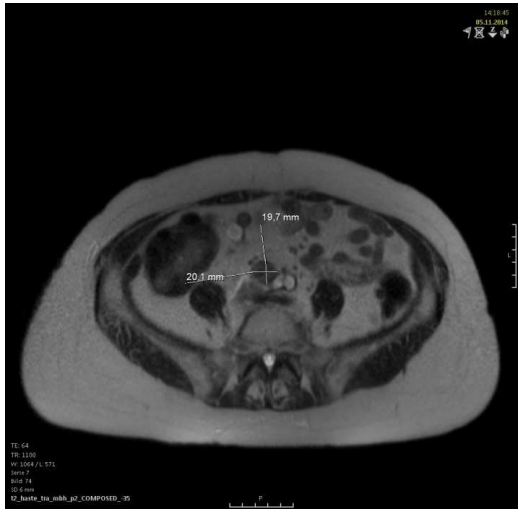
- 04/2014 Recruitment to IVAC Mutanome study
  **246 Non-Synonymous Mutations,**
  **Start of vaccine production**

- **07/2014 CT Disease Progression**
  Novel abdominal LN metastases

- 07/ 2014 – 11/2014 IVAC Mutanome (8 Injections)

- **11/ 2014 CT stable disease,**
- **6 /2015 CT stable disease, necrotic metastases ?**

- 7/ 2015 Surgical removal of metastatic lesions



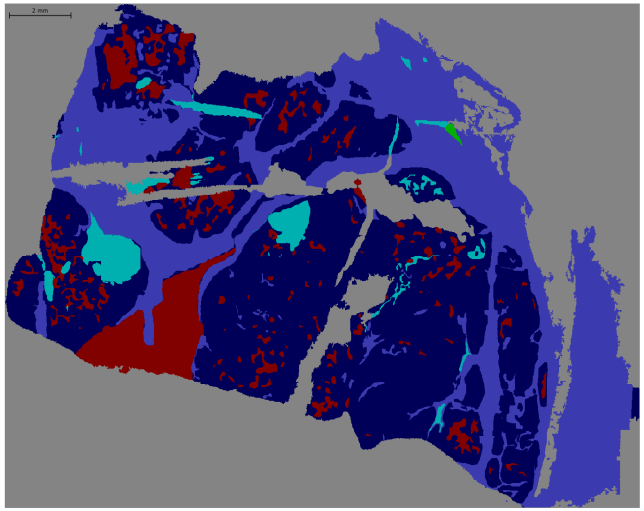

**Removal of the stable metastatic lesion**

- Analyses of TIL Infiltration
- Immunohistological Analyses of
  Tumor content ,
  T cell Infiltration, PDL1 Staining

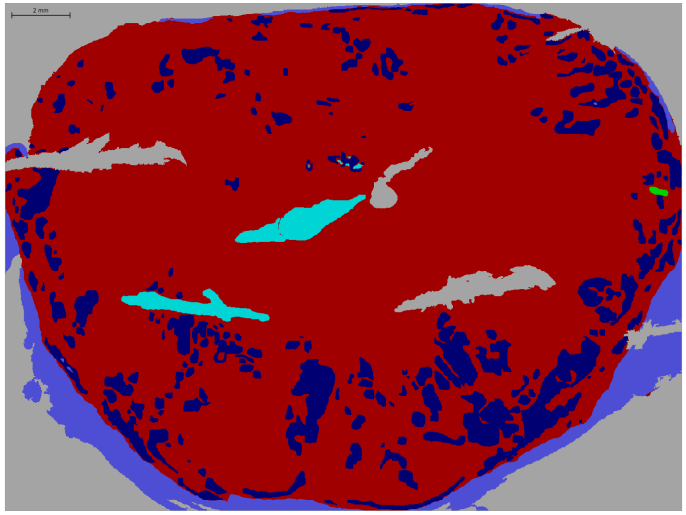
## Patient IVAC Mutanome uID-004

# Computerized Analyses of necrotic tumor area*

Pre-Vaccination Metastasis



Post -Vaccination Metastasis



- Normal tissue
- Tumor tissue
- Necrosis
- Whitespace
- Background
- Artefact

* Definiens Imaging Software

Patient IVAC Mutanome uID-004

BIONTECH

## Immune Response Data

| Mutation ID | Gene | AA exchange | Pre-formed respnse | Post-vaccination response | Type of response | Response to wt sequence | Minimal class I epitope | HLA-restriction |
|---|---|---|---|---|---|---|---|---|
| 004_M32 | C7 | E258K | No | Yes | CD4+, CD8+ | No | nd | nd |
| 004_M05 | CDC37L1 | P186L | Weak, no | Yes | CD4+, CD8+ | No | YES | HLA A*0201 |
| 004_M06 | MLL3 | P1028S | No | Yes | CD4+, CD8+ | No | YES | HLA B*0702 |
| 004e_M02 | HSD17B4 | N397I | No | Yes | CD8+ | No | | |
| 004e_M20 | DSE | R735C | No | No | na | na | na | na |
| 004e_M33 | NRAS | G13D | No | Yes | CD4+, CD8+ | No | na | na |
| 004_M20 | SAMD9L | S412L | No | Yes | CD4+ | No | na | na |
| 004e_M14 | FLNA | P369L | Weak, no | Yes | CD4+, CD8+ | No | YES | HLA A*0201 |
| | | | | | | | YES | HLA B*4402 |
| 004e_M34 | DICER1 | P627S | No | No | na | na | na | na |
| 004_M44 | DOPEY2 | R365C | Yes | Yes | CD8+ | No | YES | HLA B*0702 |

➔ Immune Responses against 8 of 10 Neoepitopes

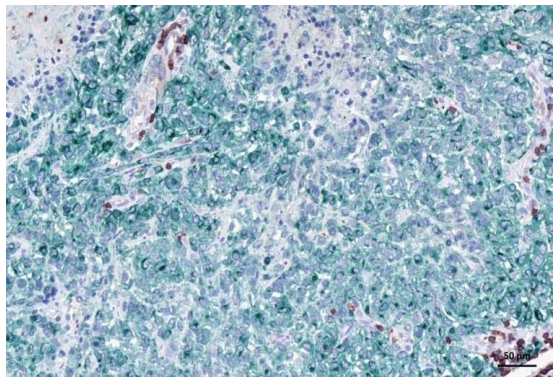
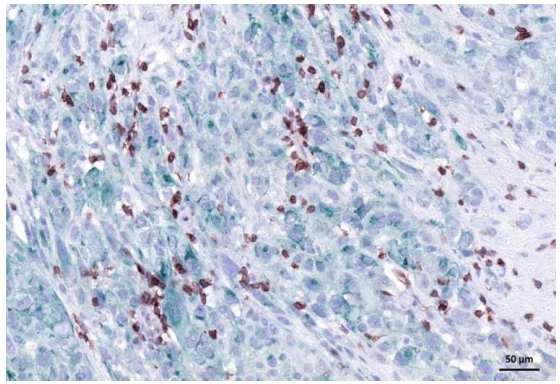
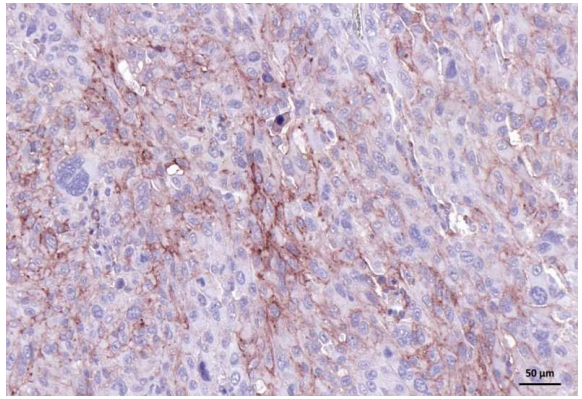
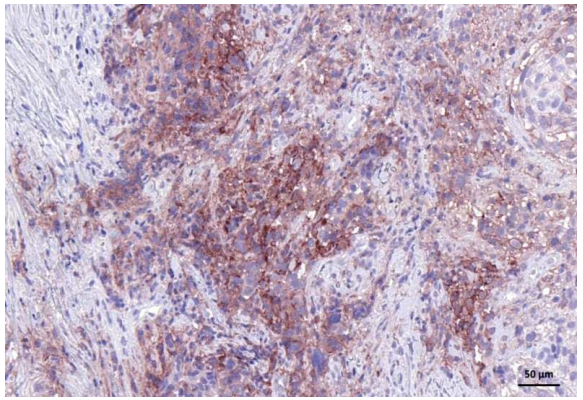
➔ Immune response NRAS G13D (13-25%) of all melanomas

JG|U UNIVERSITÄTSmedizin. MAINZ

TRON

Patient IVAC Mutanome uID-004

# Immune Infiltration and anti-PDL1 Staining

| | Pre-Vaccination Metastatic Lesion | Post -Vaccination Metastatic Lesion |
|---|---|---|
| Anti-CD3 |  |  |
| Anti-PDL1 |  |  |

UNIVERSITÄTSmedizin. MAINZ

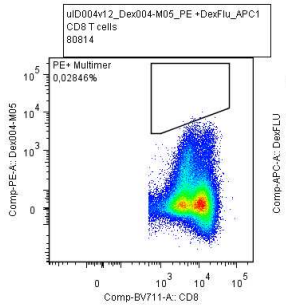
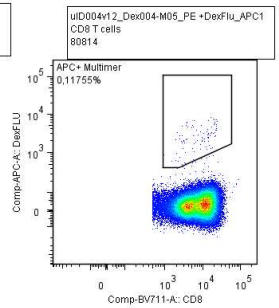
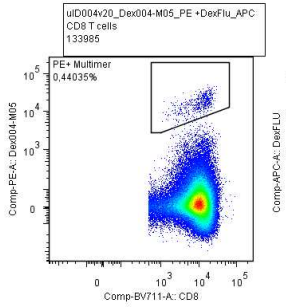
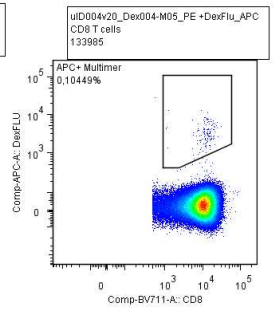
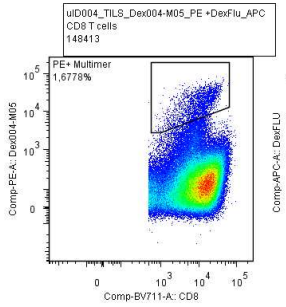
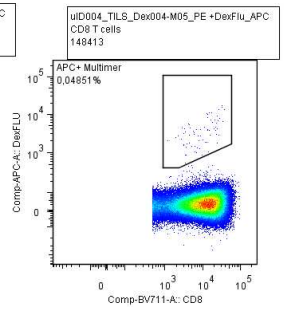
TRON

**Patient IVAC Mutanome uID-004**

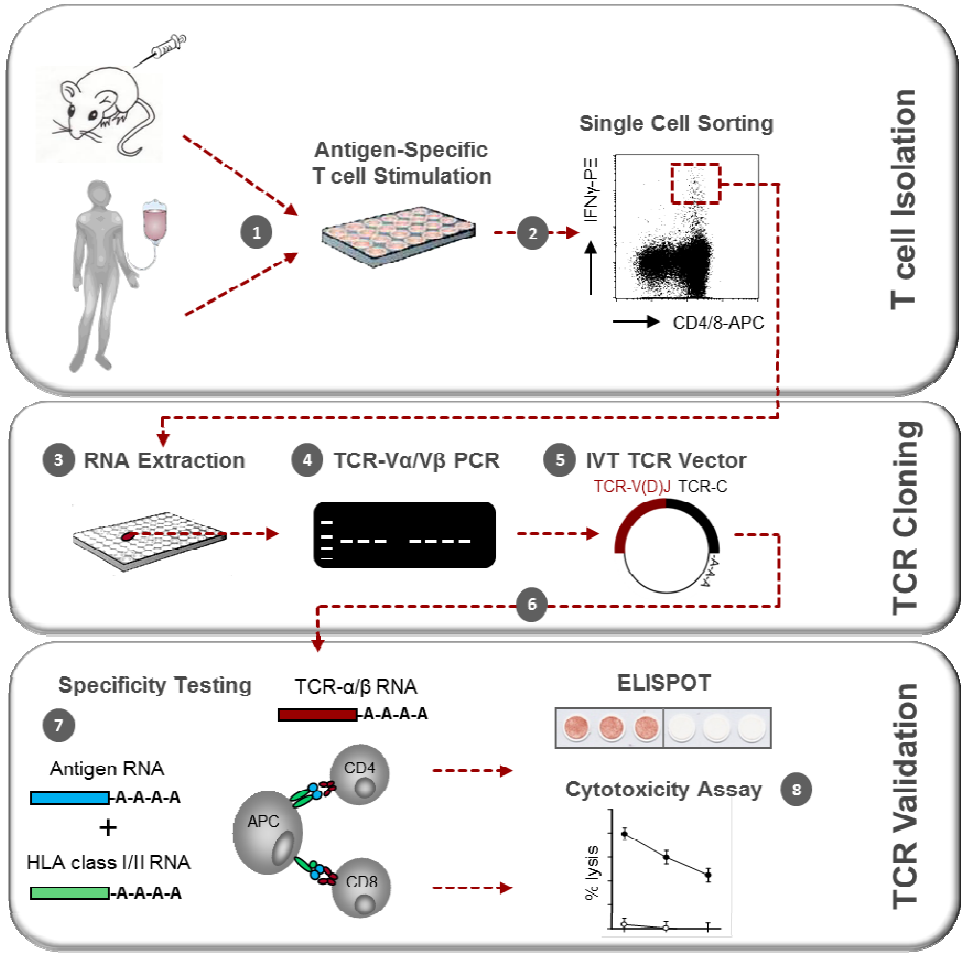
# Dextramer Analyses of PBMC and TILs

Mutation Mut05
**CDC37L1** P186L

Minimal HLA-A201 Epitope
FLSDHLYLV

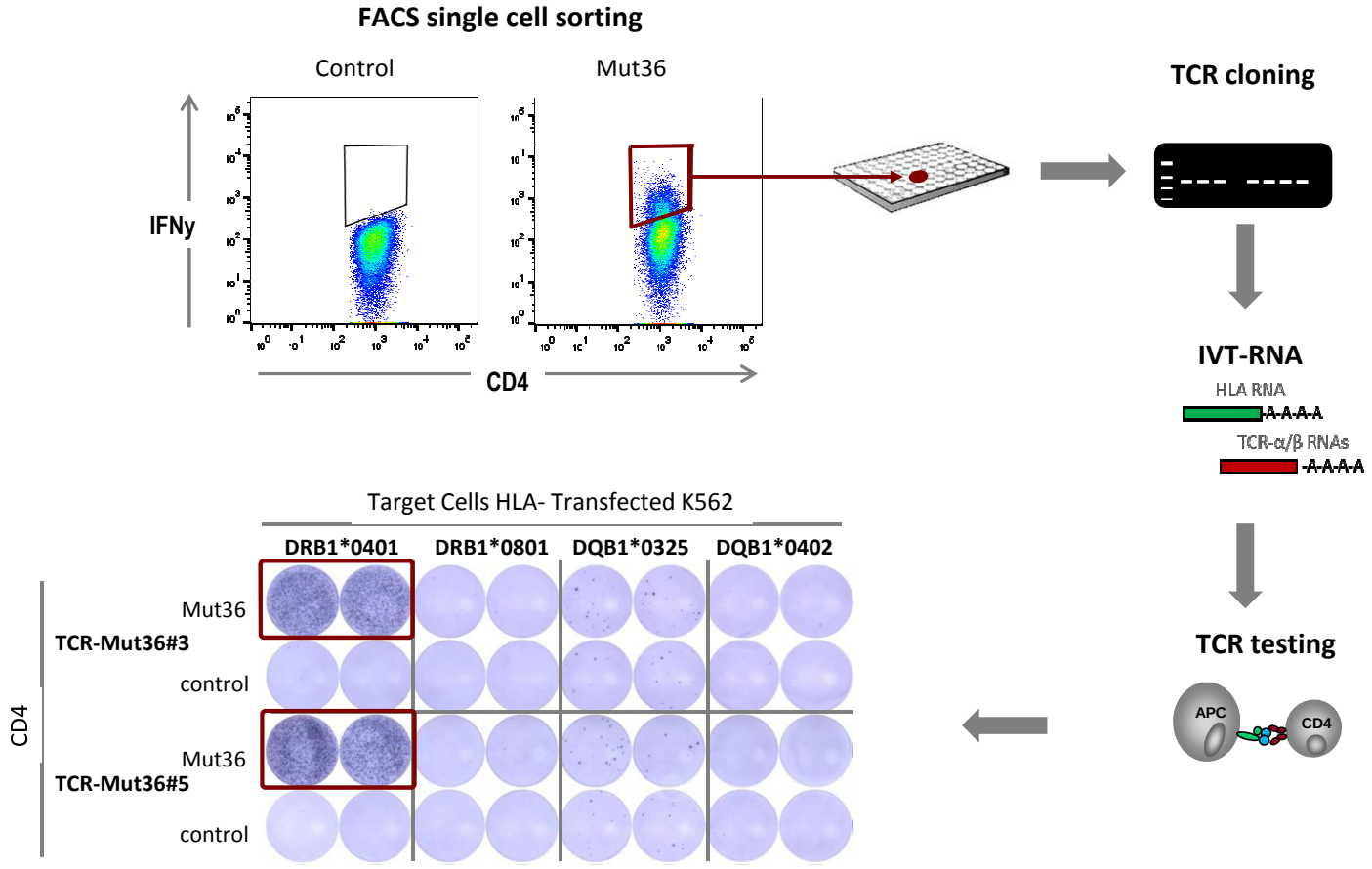
| | FLSDHLYLV **Dextramer** | Influenza **Dextramer** |
|---|---|---|
| V12 PMBC<br>Before Vaccination<br>07/2014 |  |  |
| V20 PMBC<br>After Vaccination<br>11/2014 |  |  |
| TILs<br>After Vaccination<br>7 / 2015 |  |  |

# Single Cell TCR cloning & validation




Simon et al. J Cancer Immunol Resesearch, 2015
Simon, Omokoko, Türeci & Sahin Oncoimmunology, 2015

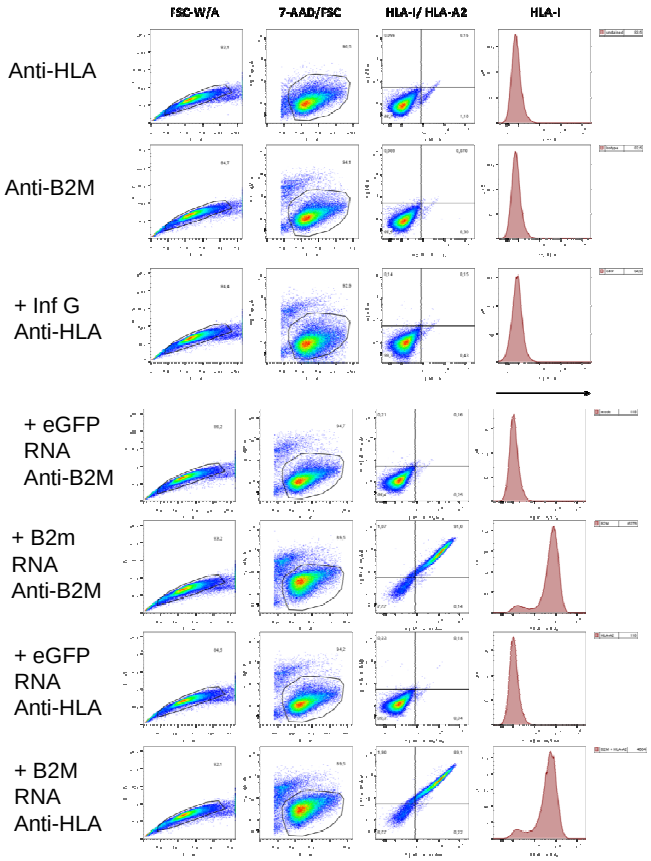
# Isolation of Mut36-specific TCRs from Postvaccination Blood of Patient ulD002



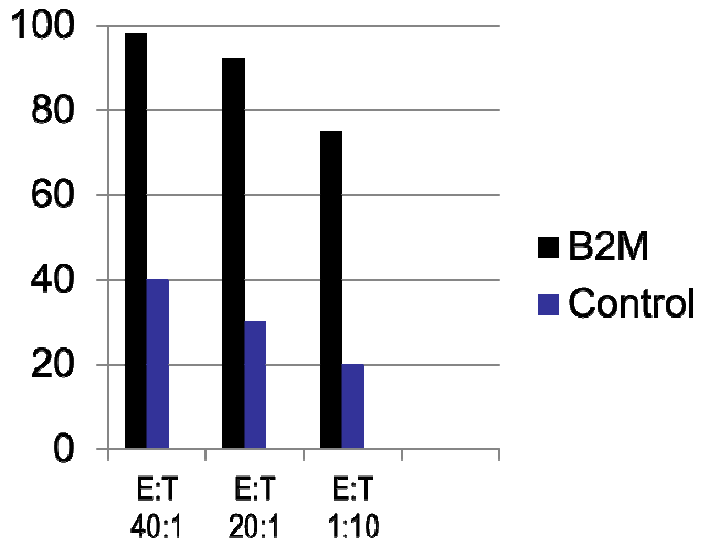
# Immune Recognition of Cancer Cells by Vaccine Induced Mutation Specific T cells

BIONTECH

## HLA Class I Surface Expression




## Cytotoxicity Mutation with Specific TCR transduced T cells on autologous Melanoma Cells

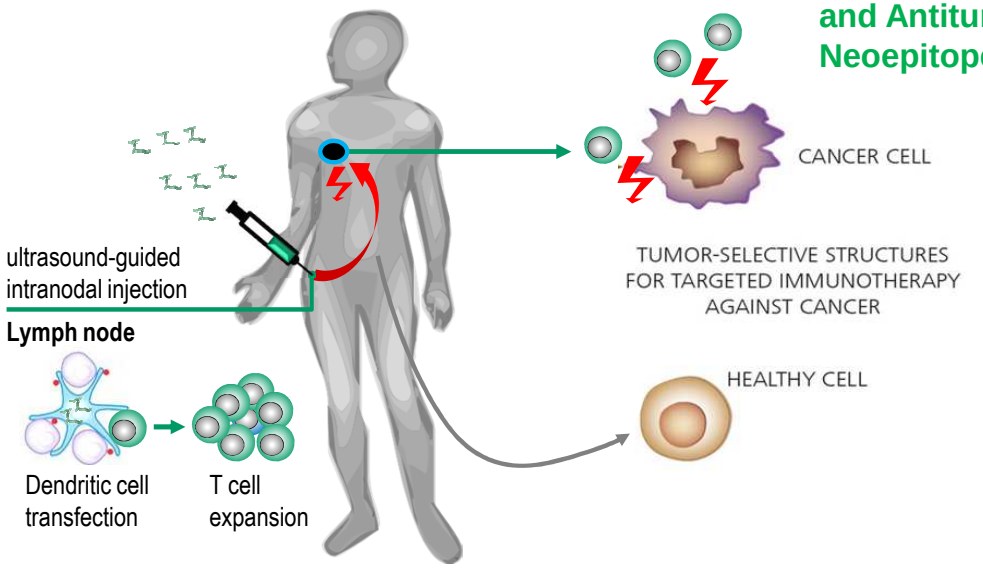



JG|U UNIVERSITÄTSmedizin. MAINZ

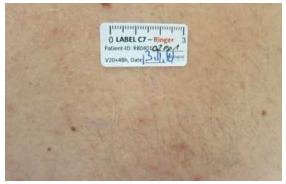
TRON

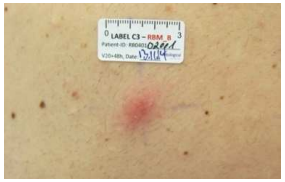
# Mutanome Vaccines Clinical Evidence

## Tumor Cell Recogntion and Antitumoral Activity of Neoepitope Specific T cells

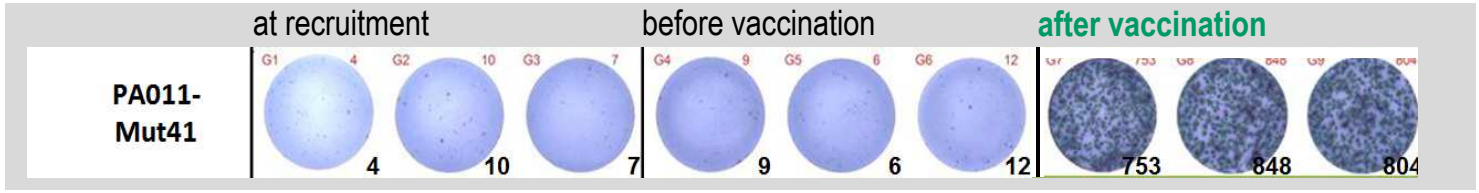



## DTH Response





## De novo induction and amplification of mutation specific T cells



## Summary Clinical Study

- Targeting Neopitopes with Polytopic RNA Mutanome Vaccines is safe
- Induction of neoepitope specific T cell responses in all patients analyzed so far
- Augmentation of pre-exisiting and induction of *de novo* CD4+ and CD8+ T cell responses against 50% of neoepitopes used for vaccination
- Vacine induced neoepitopic specific T cells infiltrate tumor lesions
- Neoepitope vaccination is associated with increase of overall TILs and intensification of anti-PDL1 staining
- Induction of functionally relevant cytotoxic CD8+ T cell responses
- Evidence of medically relevant antitumoral activity in some patients

# Acknowledgments


## Immunology & RNA

- Sebastian Kreiter
- Mustafa Diken
- Niels van der Roemer
- Mathias Vormehr
- Lena Kranz
- Raouf Selmi
- Sebastian Attig
- Richard Rae
- Andreas Kuhn
- Janina Buck
- Burkhard Otte
- Fulvia Vascotto
- Isabel Vogler,
- Nicole Bidmon
- Inga Ortseifer
- Eva Godehardt
- Özlem Yildiz
- Evelyna Derhovasnessian

## Sequencing & Validation

- Valesca Boisguierin
- Jos de Graaf

## Computational Medicine

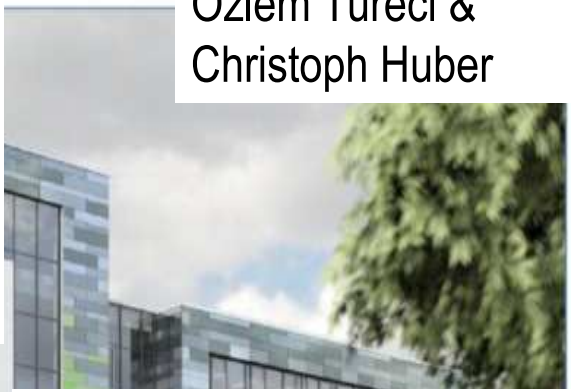
- Martin Löwer
- Tadmor Arbel
- John Castle
- Sebastian Boegel
- Barbara Schrörs

## Development

- Björn Kloke,
- Birgit Pless,
- Sandra Heesch,
- Alexander Kemmer-Brück
- Felicitas Müller
- Ulrich Luxembuger
- Christina Seck
- Cedrik Britten,
- David Langer

## Leadership & Advice

Özlem Türeci &
Christoph Huber


## Collaborators

- Carmen Loquai (UM Mainz)
- Andrea Tütenberg (UM Mainz)
- Tina Müller-Brenne(UM Mainz)
- Stephan Grabbe (UM Mainz)
- Jochen Utikal (UM Mannheim)
- Christoph Höller (Unispital Wien)
- Stephen Schönberger (San Diego)
- Edward Darzynkiewicz, Univ. Warschau
- Jacek Jamiliety, Univ. Warschau

# MERIT Project Consortium

MERIT
Mutanome Engineered RNA Immuno-Therapy

UPPSALA UNIVERSITET

Tobias Sjoeblom
Henrik Lindman
Uppsala University
SWEDEN

Vrije Universiteit Brussel

Kris Thielemans
Jacques de Greve
Vrije Universiteit Brussel
BELGIUM

BIONTECH

Coordinator
Ugur Sahin
BioNTech AG
GERMANY

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Laurence Zitvogel
Fabrice Andre
Institute Gustave Roussy
FRANCE

UniversityHospital Zurich

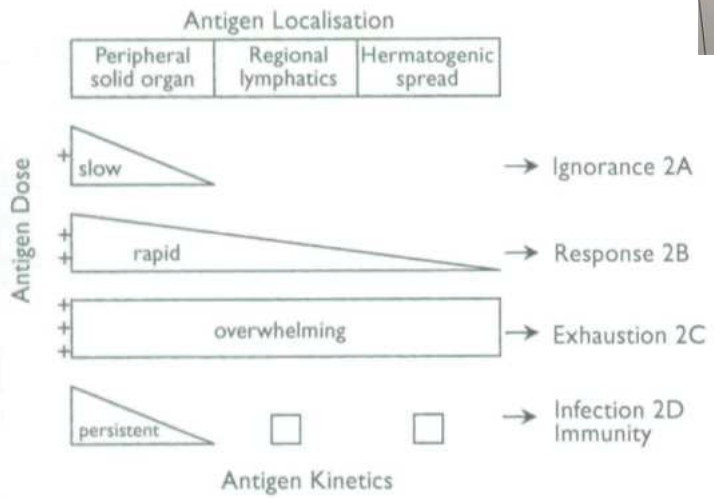
Steve Pascolo
Thomas Kündig
University Hospital of Zurich
SWITZERLAND

Rolf M. Zinkernagel
Stephan Ehl
Peter Aichele
Stephan Oehen
Thomas Kündig
Hans Hengartner

*Immunological Reviews 1997*
*Vol. 156: 199–209*

## Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity

**Rolf Zinkernagel**




*...antigen that ist transported to secondary lymphoid organs in sufficient (but not excessive) amounts and for sufficient time period (but does not persist) induces an effective immune responses*
***Rolf M. Zinkernagel,*** *2000, Seminary in Immunology*