Heidelberg Workshop

Colorectal Cancer

June 5th 2014

Peptide based immunotherapy of cancer

Hans-Georg Rammensee
Antigen presentation by HLA molecule

Antigen processing

This can be recognized by T cells

This is true for all proteins inside a cell, also for tumor associated changes, including mutations
Figure 8-31 Immunobiology, 6/e. (© Garland Science 2005)
Cancer cells differ from normal cells eg., in gene expression

This can be sensed by T cells - no matter which cellular compartment is affected by the change
Each tumor will generally contain a different set of genetic lesions. 

(APC): adenomatous polyposis coli
The prevalence of somatic mutations across human cancer types.

3 classes of tumor antigens
Limited to few cancer entities
Well established for antibodies (e.g., Rituximab)
Popular targets for CARs

We know plenty of such antigens; immune responses tend to be weak but stronger immune responses may lead to autoimmunity

3 categories of tumor antigens:

- **tissue specific** (“differentiation antigen“)
  
  *e.g. Provenge*

- **overexpressed self**
  
  *e.g. IMA901*

- **mutated or viral**

  *for mutated antigens only possible in individualized approach*

This is our vision
Tumor associated/specific antigens should be good targets for molecularly defined cancer immunotherapies of low toxicity.

Important strategies:

- Immunotherapy with antibodies
- Vaccination with proteins
- Vaccination with virus constructs
- Vaccination with peptides
- Vaccination with mRNA
- Adoptive T cell transfer
our approach to find cancer associated antigens
Isolation of Naturally Presented HLA-Ligands

Tissue Sample malignant/ benign

Tissue Preparation

Tissue Lysate

Purification

Solubilized Proteins

Affinity Chromatography

Bound MHC

Acidic Elution

Free MHC, $\beta_2m$, Peptides

Ultrafiltration 10 kDa

Isolated Peptides

Daniel Kowalewski

07.07.2011
Peptide preparation
General survey

- Protein A affinity chromatography using W6/32-antibody
- elution with citrate buffer, pH 3
### Comparative HLA ligandome analysis

**RCC370: Ligand Frequencies**

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<td>PDLIM4 PDZ and LIM domain 4</td>
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<td>SPAGVRTAF</td>
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Off-the-shelf vaccines: Allele-specific target identification (A*02)

6000+ peptides exclusively identified in A*02+ tumor ligandomes

362 peptides identified in ≥ 50% of A*02+ tumor ligandomes
Clinical studies
immatics Pipeline

<table>
<thead>
<tr>
<th>Product</th>
<th>Discovery/Pre-clinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
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<td>IMA901 RCC</td>
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**Disease**

- 1.2 million incidences p.a. worldwide\(^1\)
- 150,000 incidences p.a. in US\(^1\)
- Large unmet medical need
  → 5-year survival 6% in advanced CRC\(^2\)

**Product Candidate**

- 13 tumor-associated peptides
- Best shared peptides selected from the cancer immunopeptidome of colorectal cancer
- Phase I/II trial (92 patients in Europe)
  - IMA910 is safe and well tolerated
  - IMA910 induces immune response in 90% of all patients
  - Multi-TUMAP responses associate with enhanced overall survival

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\(^1\) Globocan 2008
\(^2\) American Cancer Society
## IMA910 composition

13 tumor-associated peptides

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<td>Well-established TAA in CRC, cell adhesion, metastasis;</td>
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<td>MET-001</td>
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<td>NADPH oxidase 1</td>
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<td>Ornithine decarboxylase 1</td>
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<td>Transforming growth factor beta-induced</td>
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<tr>
<td>TGFBI-004</td>
<td>Transforming growth factor beta-induced</td>
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Assays

- Class I responses (CD8⁺ T cells) by MHC multimer and Intracellular Cytokine Staining (ICS)
- Class II responses (CD4⁺ T cells) by ICS

Time points

- Standard analyses on pre-defined time points before, during and after vaccination with IMA910
- Routine Immunomonitoring was performed with time point pools VC/V1; V4/V5; V6/V7 and V8/V9
IMA910 Colorectal Cancer Phase I/II Study
Cohort 1 (- imiquimod) vs. Cohort 2 (+ imiquimod)

**Class I response**

- High rates of T-cell responses in both cohorts
  - Trend to more class I multi TUMAP responder in cohort 2 (p=0.118 overall)
  - Comparable Class II immune response rates between the cohorts
  - Comparable Class I and Class II immune response rates between the cohorts

*All figures: Per Protocol population
P-values from two tailed Fisher’s exact test;
Class II: all p-values >0.6 / Class I + II: all p-values >0.38
**IMA910 Colorectal Cancer Phase I/II Study**

**Overall survival of multi-TUMAP responders**

### OS vs. Class I response

- HR = 0.59
- p = 0.083

### OS vs. Class II response

- HR = 0.60
- p = 0.12

**OS vs. Class I and Class II resp.**

- HR = 0.53
- p = 0.088

Overall survival relative to Visit VC (Follow up 1). Per Protocol population

p-values from Log Rank statistics; HR from Cox prop. hazards model

**Trend for increased OS in multi-TUMAP responders**

- Trend for increased OS observed in Class I or Class II Multi-TUMAP responders

- Consistent finding: effect most pronounced in patients responding to multiple Class I and Class II TUMAPs
MDSC4 and MDSC5 levels are negative prognostic markers for overall survival.

*p-values are calculated by Log Rank and HR from Cox Hazard Model*

Low levels of two MDSC phenotypes (MDSC4 & 5) were significantly associated with longer survival. Consistent with observation in renal cell carcinoma.
Active immunotherapy can work, even with self antigens
Lessons:

1. One should aim to have more multipeptide responders.

2. Single low dose of Cyclophosphamide appears to be good.

3. Immunotherapy of this kind may have no impact on progression free survival but on overall survival.
vaccination with mRNA is another option

mRNA-Vaccination in prostate carcinoma patients
A phase I/IIa study with the RNActive® vaccine CV9103 in castration resistant prostate cancer with rising PSA

Patient Population
- Castration-resistant prostate adenocarcinoma patients with rising PSA, 82% had metastatic disease (bone 64%, lymph nodes 59%, 11% visceral)

Study Design
- Phase I: 12 patients – dose finding (256 µg, 640 µg, 1280 µg total dose of mRNA)
- Phase IIa: 32 patients treated at highest dose to test immunogenicity

Study End points
- Primary endpoint: safety and tolerability
- Secondary endpoints: antigen specific immunogenicity

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4 RNActive® compounds targeting different antigens

- PSA
- Steap1
- PSCA
- PSMA

Intradermal application
CV9103 is highly immunogenic in a phase I/IIa trial

Patients responding in different assays, performed *ex vivo*

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<th>Assay</th>
<th>% Responders</th>
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<td>ELISA*</td>
<td>4/33 (12%)</td>
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<td>ELISpot</td>
<td>9/31 (29%)</td>
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<td>ICS</td>
<td>12/26 (46%)</td>
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<tr>
<td>Tetramer</td>
<td>7/12 (58%)</td>
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<tr>
<td>Overall</td>
<td>26/33 (79%)</td>
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Subjects received 5 vaccinations

33/44 patients were immunologically evaluable per protocol definition

*Most of the patients show high pre-existing PSA antibody levels which could be increased in 4 patients

Patients responding/Patients analyzed

CV9103 induced immunological responses in **79%** of patients
Design of the randomized phase IIb trial (CV9104)

Phase IIb trial started in November 2012
- Phase IIb - double blind placebo controlled, n= 189
- Patients: asymptomatic/ minimal symptomatic metastatic CRPC, chemonaive
- Multiple vaccinations even over the progression
- Primary endpoint: Overall survival
- 8 European countries, 50 sites

CV 9104 + subsequent systemic therapy (docetaxel, aberaterone)
Placebo + subsequent systemic therapy
**RNActive® Immunotherapy targets 6 antigens**

**STEAP-1**
- Six-transmembrane epithelial antigen
- Overexpressed in prostate cancers and other urogenital tumors
- Role in regulation of tumor growth

**Muc 1**
- Mucinous glycoprotein
- Aberrantly glycosylated and expressed in many human malignancies including prostate cancer
- Associated with more aggressive disease

**PSA**
- Secreted Serine protease
- Expressed in normal prostate and prostate tumours

**Target antigen of Prostvac-VF**

**PAP**
- Secreted Phosphatase
- Expressed in normal prostate and prostate carcinomas

**Target antigen of Provenge®**

**PSCA**
- Cell surface antigen expressed primarily on basal cells of the prostate
- Overexpressed in various carcinomas (not only prostate cancer)

**PSMA**
- Expressed in normal prostate, prostate carcinomas and tumor vasculature
- Expression correlates with increased aggressiveness
- Upregulated after hormone ablation
Why mutated antigens should be the better targets
1. T cells having left the thymus before tumor occurrence should not be tolerant of tumor specific mutations

indeed, patients develop efficient T cell responses against mutations (Thomas Wölfel)
<table>
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<th>Process</th>
<th>Stage in development</th>
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<td>Proliferation and differentiation to double-positive CD3+ thymocytes</td>
<td>Double-negative CD3+ thymocytes in the subcapsular zone</td>
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</table>

### Positive selection

Recognition of self HLA molecules

### Negative selection

Elimination of self reactive T cells by peptide recognition on bone marrow derived APCs and on medullary epithelium cells (ectopic gene expression)

### Education of self specific Tregs
2. T cells specific for tumor specific mutations should not react to self peptides, since those with crossreactivity to non-mutated peptides should have undergone negative thymic selection.

> no toxicity expected
Limited to few cancer entities

We know plenty of such antigens; immune responses tend to be weak but stronger immune responses may lead to autoimmunity

This is our vision
Our new strategy for the identification of tumor specific peptides
candidate peptides for immunotherapy
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**CCC IND-01, Genome sequencing and prediction of mutated HLA-ligands by SYFPEITHI**

**KCNJ12** 239E/K QLIKPRVTK, HLA-A*03, score 33

**HGC6.3** 128M/V VVTPTASSF, HLA-A*03, score 25

(Bold: anchor; underlined: auxiliary anchor AA)
88 unique mutated peptide sequences in tumor tissue fitting to the patient's HLA-Type:

- 40 fit to HLA*A03
- 26 fit to HLA*B14
- 22 fit to HLA*B44

67 stem from SNV and 21 from InDel Mutations
Search for mutated peptides by mass spectrometry
Mutated peptides from human tumors could not yet be identified by mass spectrometry
Collaboration

Bob Schreiber lab and Rammensee/Stevanovic lab

Heiko Schuster

Identification of a mutated mouse tumor peptide
First time identification and successful validation of a mutated MHC ligand

**mLama4(G1254V)**

VGFNFRTL

Validation with an isotope labeled synthetic peptide

**VGFNFRTL**

(13C6, 15N1)
We found mutated MHC presented peptides in a mouse tumor cell line.

We failed so far to find mutated HLA ligands in human tumor tissue.
Why is it so hard to find mutated peptides on human tumor tissue by mass spec?
Why is it so hard to find mutated peptides on human tumor tissue by mass spec?

Is it just insufficient sensitivity of our mass spec equipment? (We usually identify >3000 peptides in a 0.5 g tissue sample)
Why is it so hard to find mutated peptides on human tumor tissue by mass spec?

Is it just insufficient sensitivity of our mass spec equipment? (We usually identify >3000 peptides in a 0.5 g tissue sample)

Or is it a consequence of negative selection of tumor cells expressing immunogenic mutated peptides as HLA ligands?
Two step strategy for individualized immunotherapy:

1st step, as fast as possible (eg., right after surgery): vaccination with off the shelf peptides according to HLA-expression

2nd step, after tumor mutation analysis: vaccination with mutated peptides

Since it is harder than we expected to find mutated tumor HLA ligands, we amended our strategy:
Problem:

Patient individualized peptides have to be produced according to GMP conditions, like all other drugs.
GMP center for individualized substances
Bau: Finanziert durch MWK und UKT
Einweihung 2010
Erster Antrag auf Arzneimittelherstellung für Peptide zur Vakzinierung 2008

Erteilung des Zertifikats für Wirkstoffpeptidherstellung 7. März 2012

Antrag auf Arzneimittelherstellung aus diesen Wirkstoffen Mai 2012

Dritter Mängelbeseitigungsbericht geht am 19.7. 2013 ans Regierungspräsidium


Erteilung der Herstellungserlaubnis am 14.3.2014

Erste Inspektion des Herstellungsprozesses durch RP und PEI am 20.5.2014 (verlief positiv)
HERSTELLUNGSERLAUBNIS

1. Nummer der Erlaubnis/Aktenzeichen
DE_BW_01_MIA_2012_0109/DE_BW_01_Uni
Tübingen_Wirkstoffpeptidlabor
Eberhard Karls Universität Tübingen

2. Name des Erlaubnisinhabers
Universität Tübingen, Interfakultäres Institut für
Zellbiologie, Abteilung Immunologie,
Wirkstoffpeptidlabor
Auf der Morgenstelle 15
72076 Tübingen

3. Anschrift/en der Betriebsstätte/n des
Herstellers / des Einführers
Universität Tübingen, Interfakultäres Institut für
Zellbiologie, Abteilung Immunologie,
Wirkstoffpeptidlabor - Reinraumbereich ZKT
Ottfried-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

Stefan Stevanovic
Patricia Hrstic
Monika Stieglbauer
Katharina Graf
Stefan Laufer
Problem:

What will the regulatory authorities say to an individualized study design?
Levels of personalization – concept by CIMT RRG

(A) Passive personalization

(B) Passive personalization

(C) Active personalization („AP“)

Patient

Tumor

Theranostic

Drug Product(s)

Stratification

Invariant DP

Variant DPs

Variant DPs

Britten, Singh-Jasuja et al. (2013), *Nature Biotechnology*

CIMT Regulatory Research Group (RRG)
THE REGULATORY LANDSCAPE FOR ACTIVELY PERSONALIZED CANCER IMMUNOTHERAPIES

Cedrik M. Britten*1,2, Harpreet Singh-Jasuja*3, Bruno Flamion4, Axel Hoos5, Christoph Huber6, Karl-Josef Kallen7, Samir N. Khleif8, Sebastian Kreiter1, Michaela Nielsen9, Hans-Georg Rammensee10, Ugur Sahin1,11, Thomas Hinz#12, and Ulrich Kalinke#13

on behalf of the Association of Cancer Immunotherapy (CIMT) Regulatory Research Group (RRG)

ABSTRACT

Tumors carry multiple somatic mutations, the majority of which are unique to individual patients. Recent data imply that immunogenic tumor mutations can be exploited for the treatment of cancer patients. Here we propose a development strategy for actively personalized vaccines (APVACs) targeting multiple tumor mutations. This strategy is based on the existing regulatory framework thus facilitating the way towards first clinical testing.

Britten, Singh-Jasuja et al. (2013), Nature Biotechnology
GAPVAC

Establish an actively personalized vaccination (APVAC) approach for treatment of glioblastoma patients

Consortium with 14 partners funded by EU with 6 mn EUR

Led by Immatics (Coordinator) and BioNTech (Vice Coordinator)

Clinical study planned to start in 2014

Up to 30 glioblastoma patients will receive APVACs composed of warehouse-selected and mutanome-derived peptides.
Tumor-associated peptides – shared vs. individual

Tumor 1:
- HVNDLFLQY
- TQMPDPKTF
- SHAILEALA

Tumor 2:
- ALRDVRQQY
- FAEGFVRAL
- HVIDVKFLY
- GQFPGHN Ef
- HQITVLHVY
- GLATDVQTV
- GLNDETYGY
- IAMATVTAL

Tumor 3:
- KLHGVNINV
- LEEDSAREI
- LLAERDLYL
- MEDIKILIA
- QEQSFVIRA
- RLASYLDKV
- MQKEITAL

TUMAPs potentially suitable for personalized therapy

Off-the-shelf Multi-TUMAP Vaccine
Glioma Actively Personalized Vaccine Consortium

GAPVAC design

Off the shelf

Peptide Warehouse

Biomarker Tests

Expression Profile
Mutantome
Peptidome
Immunogenicity

d de novo synthesis

Mutated Peptides

APVAC 1

APVAC 2

Patient 1

Patient 2

Patient 3
APVAC analyses and selection process

1. Surgery
2. Enrollment
3. Screening completed
4. Tumor shipment and central pathology review
5. Isolation of peptides, DNA, mRNA
6. Tumor HLA ligandome - mRNA transcriptome
7. TUMAPs selected
8. TUMAP selection
9. in vitro immunogenicity
10. QP release of warehouse APVAC
11. Medication shipment
12. DNA shipment
13. NGS
14. Tumor HLA ligandome - mutations
15. non-GMP synthesis and verification
16. TUMAP selection
17. GMP synthesis peptide 1
18. GMP synthesis peptide 2
19. QP release of mutated APVAC
20. Formulation, release
21. Medication shipment
22. APVAC 1
23. Warehouse-based
24. Vaccination start
25. Warehouse APVAC
26. Vaccination start
27. Fallback
28. TMZ1
29. TMZ2
30. TMZ3
31. TMZ4
32. TMZ5
33. Vaccination start
34. Fallback
To do for each patient:

1. start vaccination with off-the-shelf peptides

2. select 5 - 15 peptides representing mutated **AND** patient-specific wild type HLA class I and class II ligands, synthesize and formulate to a cocktail, start vaccination
Co-workers and Collaborators

IMA901 Chief Investigators
US: Brian Rini, Cleveland
EU: Tim Eisen, London
GER: Arnulf Stenzl, Tübingen

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Jörg Ludwig

IMA910 Chief Investigator
Frank Mayer, Tübingen

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Norbert Hilf (NCI study)

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Howard Fine and Joohee Sul, Bethesda (NCI study)

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Chief Scientific Officer
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**Dept. Immunology**

*Molecular immunology*
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- Christoph Grabenbauer
- Thomas Feger
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- Lea Prokop
- Stefanie Souczek
- Claudia Berlin
- Rita Pfeifer

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- Melanie Widenmayer
- Heinrich Griesemann

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- Philipp Horvath

*Pathology*
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- Susanne Rittig
- Helmut Salih
- Sebastian Haen
- Julia Stickel

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- Peter Bauer
- Michael Bonin
- Christopher Schröder

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- Oliver Kohlbacher
- Mathias Walzer

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