**Targeting immune modulatory pathways in cancer**

**Critical hurdles on the way to successful cancer immunotherapy**

- **Select**
  - Target antigens

- **Induce**
  - T cell response

- **Avoid**
  - Counter regulation

- **Support**
  - T cell Infiltration

- **Break**
  - Tumor cell Resistance

**Succesful Cancer Immunotherapy**

**Prerequirements of successful tumor immune surveillance**

- Systemic tumor –specific Teff response
- Immigration of TA specific Teff into tumor tissue
- In situ activity of TA specific T cells

in CRC TNF expression is confined to TCR stimulated TIL

TNF expression indicates TCR activation in TIL

**In situ activity of TIL in CRC**

**TNF is expressed by extremely low numbers of TIL**

0.2% of $CD4^{pos}$ and $CD8^{pos}$ TIL express TNF in situ

TNF in T cells correlates with total TNF

- **CD8 TC**
  - $r^2$: 0.77
  - $p<0.0001$

- **CD4 TC**
  - $r^2$: 0.65
  - $p<0.0001$

*N= 36*
**TNF expression in CRC TILs**

**TNF expression is increased in CRC TILs**

**% TNF pos TIL in CRC patients**

<table>
<thead>
<tr>
<th></th>
<th>CD8 TC</th>
<th>CD4 TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
</tr>
<tr>
<td>Tumor</td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
</tbody>
</table>

**TNF conc. in CRC**

<table>
<thead>
<tr>
<th>tissue</th>
<th>TNF$\alpha$ pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td><img src="image5" alt="Bar graph" /></td>
</tr>
<tr>
<td>Tumor</td>
<td><img src="image6" alt="Bar graph" /></td>
</tr>
</tbody>
</table>

**20x magnification**

*in coop. with N. Halama*

In situ activity of TIL is increased in patients with systemic anti tumor T cell response

detection of tumor specific T cell response

**IFN-γ Elispot-Assay (representative CRC patient)**

**Representative CRC patient 1174 IFNγ Elispot**

% TNF^{pos} TIL

**CD4^{pos} TIL**

**CD8^{pos} TIL**

**systemic T A specific T cell response is required for intratumoral TC activity**

N=26
**In situ activity of TA specific TIL**

**TA-specific TCs are enriched in TIL**

![Flow cytometry dot plots](image)

**TA-spec. TIL**

<table>
<thead>
<tr>
<th>Muc1</th>
<th>Her2/Neu</th>
<th>CEA</th>
<th>EGFR</th>
<th>p53</th>
<th>HPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Met</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% TA spec. of CD8* TC

N=15

**TA-spec. CD8* TC**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>PT</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% TA spec. of CD8* TC

40%

*Reissfelder&Stamova, J. Clin. Invest., 2014*
**In situ activity of TIL is confined to TA-specific TCs**

- **TIL**
  - CD3⁺CD8⁺
  - CD3⁺CD8⁺ pentamer⁺
  - PT: Red circle
  - Met: Blue circle
  - 40%

- **PB**
  - CD3⁺CD8⁺
  - CD3⁺CD8⁺ pentamer⁺
  - *

- **BM**
  - CD3⁺CD8⁺
  - CD3⁺CD8⁺ pentamer⁺
  - *

**N=14**

**No correlation of in situ activity in TA specific TIL and PBTC**

**PB vs BM**

- %TNFα⁺ of pentamer⁺ BMTC vs %TNFα⁺ of pentamer⁺ PBTC

- * p<0.002

**PB/BM vs TIL**

- %TNFα⁺ of pentamer⁺ PB/BMTC vs %TNFα⁺ of pentamer⁺ TIL

**TA specificity is required for intratumoral TC activity**
The impact of the immune system on cancer prognosis

TNF expression is a strong prognostic parameter in UICC stage III CRC

Cohort of 102 CRC patients
- collected >10 years ago
- retrospectively assessed for:
  - TC infiltration
  - TC activity in situ (TNF)
  - Treg
  - mast cells

multivariate analysis

<table>
<thead>
<tr>
<th>Tumor related death</th>
<th>HR*</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 4+</td>
<td>0.95</td>
<td>(0.78, 1.15)</td>
<td>0.60</td>
</tr>
<tr>
<td>CD 8+</td>
<td>1.00</td>
<td>(1.00, 1.01)</td>
<td>0.32</td>
</tr>
<tr>
<td>Treg</td>
<td>0.62</td>
<td>(0.39, 0.99)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mast cell</td>
<td>0.99</td>
<td>(0.96, 1.02)</td>
<td>0.40</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.28</td>
<td>(0.08, 0.95)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

In situ activity of tumor specific T cells determines colorectal cancer prognosis

A. Benner, DKFZ
Critical hurdles for T cell mediated immune surveillance in CRC

- Systemic TC response
- TC infiltration
- TC activity in situ

Successful:
- 40% anergic tumor vasculature

Unsuccessful:
- 40% Local immune suppression/Immune modulatory ligands
- app.6% successful immune protection
The problem of vascular anergy
Local Low Dose Irradiation Increases Effector T Cell Infiltration through INOS+ Macrophages

**Objective:** Modify Tumour Vasculature to Prevent T Cell Recruitment and Improve Teff Immigration

The riptag-5 mouse model of spontaneous insulinoma - a model of vascular anergy

...based on tg rat insulin promotor driven oncogene SV40 large T antigen

RIP-Tag mouse model of spontaneous insulinoma

Local low dose irradiation
Tumor specific TCRtg donor T cells

Treatment protocol:
- Irradiation
- ADI
- Tumor tissue analysis

<6Gy

Ganss et al., Cancer Res., 2002
Low dose irradiation + T cell transfer cause vascular normalization + activation

CD8 T cell transfer

0 Gy  2 Gy

CD31+ vessel – phenotype

activation (VCAM-1)

T cell survival (%)

CD8 T cell transfer

Irradiation (Gy)

0  0.5  1  2  6

weeks

survival (%)

2GY + CD8
CD8
2 Gy
untreated
Indispensable role of macrophages in T cell recruitment

**CD8 T cell transfer**

- T cells / 0.5 mm²
- Irradiation (Gy) 0, 2, 2
- CLIP, PLIP

**Macrophage depletion by clodronate liposomes**

- 0 Gy
- 2Gy+CD8
- CLIP

**Survival (%)**

- untreated
- 2Gy + CD8 + CLIP
- 2Gy + CD8 + PLIP
- CLIP only

* p<0.01
Local Low Dose Irradiation Increases Effector T Cell Infiltration through INOS+ Macrophages

% iNOS+CD11b+ in RT5 tumors

vascular activation

CD8 T cell transfer

- CD3+
- CD8+
- CD4+
Effector T-cell Recruitment through iNOS+/M1 macrophages

Low dose irradiation

Tumor
- CCL2, CCL-20
- VEGF, IL-6
- Dis Tu C
- VCAM
- RANTES
- iNOS
- IL-
- TAM
- Normalized vasculature
- VEGF
Two clinical trials launched in 2010

2 Randomized controlled phase I/II studies to investigate T cell infiltration after neoadjuvant local low dose radiotherapy in

- Locally advanced operable pancreatic cancer
- Single, operable liver metastases of colorectal cancer


Automated full slide imaging:
Niels Halama & Dirk Jäger, NCT, Heidelberg
**CTL-A4 and PD1/PDL-1 are major targets for cancer immunotherapy**

**B7 family of immune modulatory molecules and their inhibitory receptors on T cells**

**PD-L1**

- **Immune modulatory ligands**
  - Extracellular PD-L1
  - CD80, CD86, B7-H1, B7-DC

- **Inhibitory receptors**
  - CTLA4, PD1

**T cell exhaustion**

**Loss of function**

**T cell Apoptosis**

**Immune suppressive cytokines**

**Regulatory T cell**

**Molecular shield**

**Protected from cytotoxic lysis**

**Are there more immune regulatory ligands?**

**PDL1 not expressed on all tumors treatment failure despite PDL1 expression**

Ligand or receptor inhibition through blocking antibodies restores T cell activity against tumor cells

adapted from Nature Reviews Immunology
Targeting immune modulatory pathways in cancer

A high-throughput siRNA based Screen for novel immune modulators on tumor cells

Test principle

Khandelwal et al., EMBO Mol Med 2015
High-throughput RNAi screen with syngenic TILs and melanoma cells; 1,500 genes

Data Analysis

Replicate 2
Replicate 1
Hit Selection

Candidate Validation:
- Re-tests
- Secondary Assays

Khandelwal et al., EMBO Mol Med 2015
Targeting immune modulatory pathways in cancer

Screen performance

Target Identification

Khandelwal et al., EMBO Mol Med, 2015
• Low z-score: Potential immune-stimulatory molecule
• High z-score: Potential immune-inhibitory molecule

Khandelwal et al., EMBO Mol Med, 2015
CCR9 knock down in tumor cells increases function of TA-specific T cells

CCR9 blocks TNF secretion

CCR9 blocks STAT activation

CCR9 blocks granzyme B secretion

Khandelwal et al., EMBO Mol Med 2015
CCR9 knock down in tumor cells increases function of TA-specific T cells

Tumor lysis

- Control siRNA
- CCR9 siRNA 1

% specific lysis

Survivin T cell : MDA MR 231

- Control vector
- CCR9 vector

% specific lysis

Survivin T cell : MCF7

- CCR9+ M579-A2 + TIL 209
- CCR9- M579-A2 + TIL 209

Tumor volume (mm³)

p = 0.007

days after tumor implantation

- CCR9+ M579-A2
- CCR9- M579-A2

days after tumor cell injection

Khandelwal et al., EMBO Mol Med 2015
The bone marrow: a site for induction of tumor specific T cells

Feuerer & Beckhove et al., Nat. Med. 2003:1151-7
Feuerer & Beckhove et al., Nat. Med. 2001:7:452-8

Schmitz-Winnenthal et al., Gastroenterology, 2010:138:1178-88
Schmitz-Winnenthal et al., Cancer Res., 2006:65:10079-87
Choi et al., Blood, 2005, 105:2132-4

<table>
<thead>
<tr>
<th>Entity</th>
<th>n</th>
<th>TA reactive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Ca.</td>
<td>450</td>
<td>60%</td>
</tr>
<tr>
<td>Pancreatic Ca.</td>
<td>180</td>
<td>80%</td>
</tr>
<tr>
<td>HNSCC</td>
<td>150</td>
<td>40%</td>
</tr>
<tr>
<td>Colorektal Ca.</td>
<td>250</td>
<td>40%</td>
</tr>
<tr>
<td>Malignant Melanoma</td>
<td>80</td>
<td>50%</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>140</td>
<td>42%</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>250</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>50%</td>
</tr>
</tbody>
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