

# Immunotherapeutic strategies for countering recurrence in patients with primary resectable PDA

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*German Cancer Research Center*

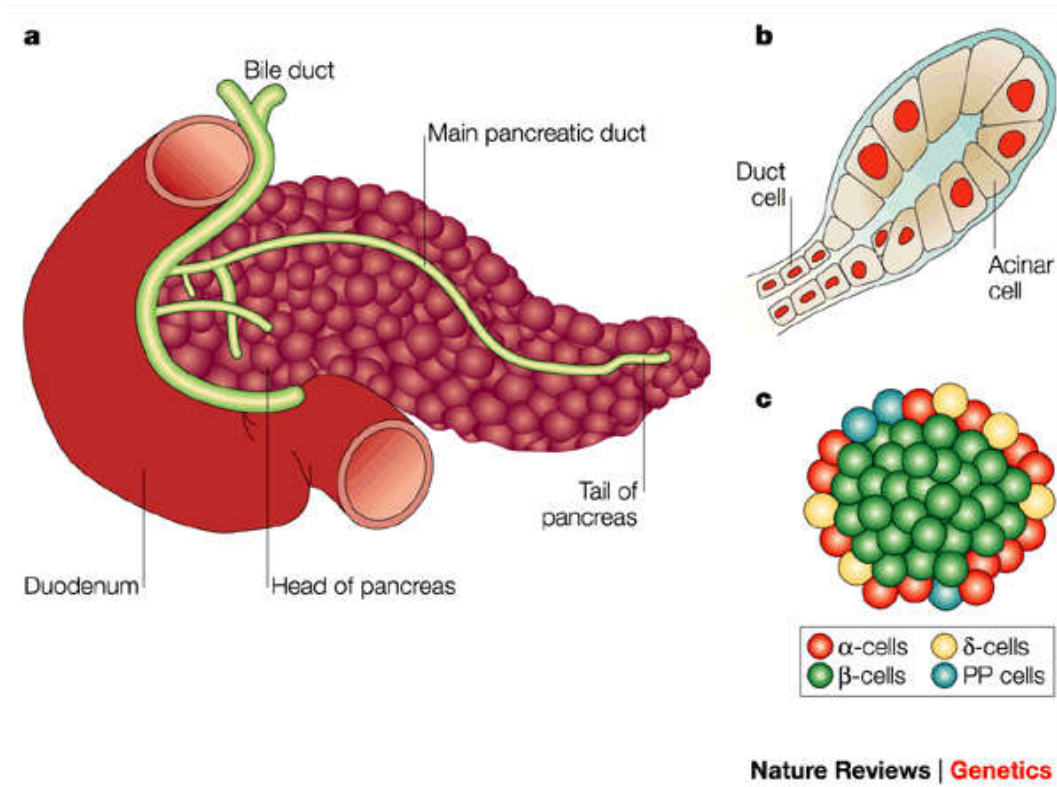
*Heidelberg*



UniversitätsKlinikum Heidelberg



# Pancreatic Ductal Adenocarcinoma (PDA)



Ductal = Exocrine

- enzymes released in gut
- digestion

Endocrine

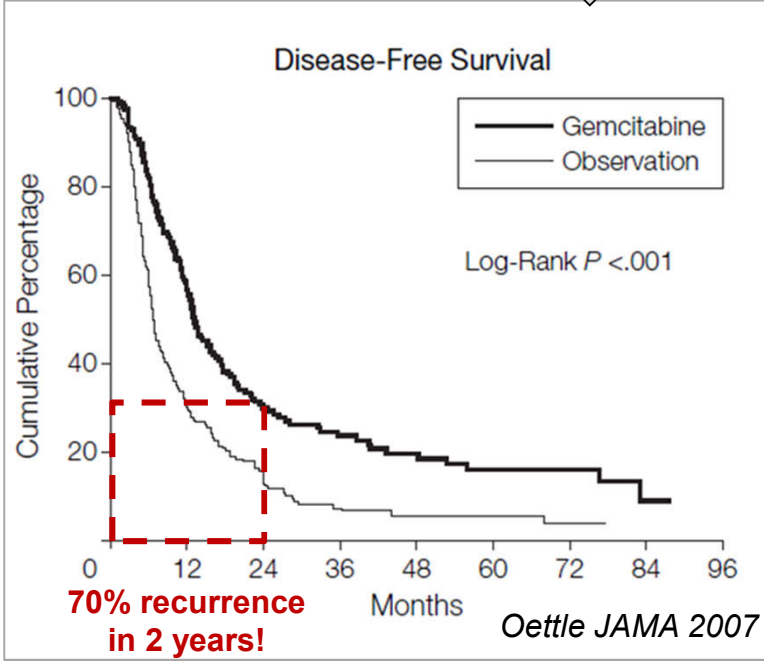
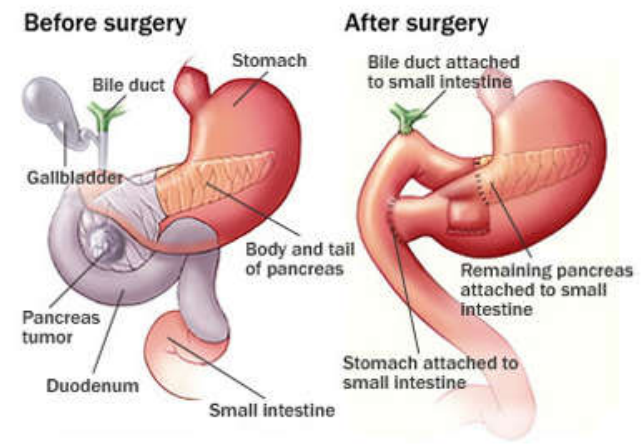
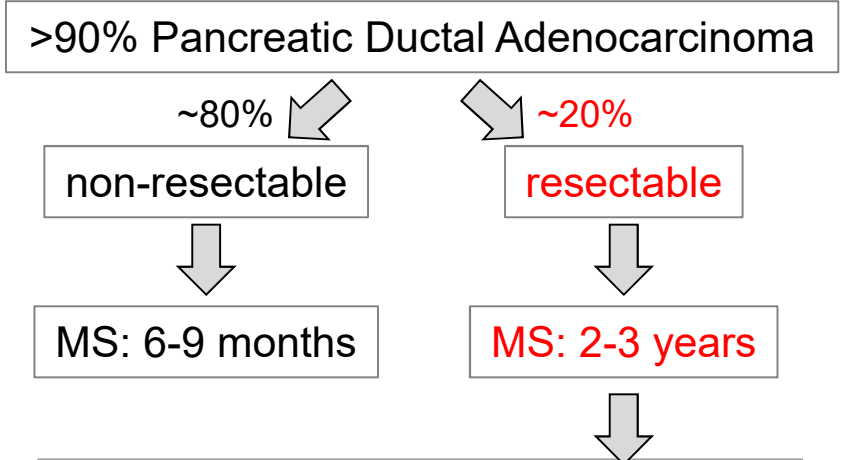
- hormones (e.g. insulin)
- metabolism

PDA:

- Most common type of pancreatic cancer (>90% of cases)
- Most deadly type of pancreatic cancer

# Pancreatic cancer management to date

## *Surgery is still only effective treatment*



	Incidence*	Death*
GER	11.5	11.1
UK	9.6	9.0
USA	10.9	10.9

\* yearly cases/100.000

# Pancreatic cancer management to date

>90% Pancreatic Ductal Adenocarcinoma

~80%

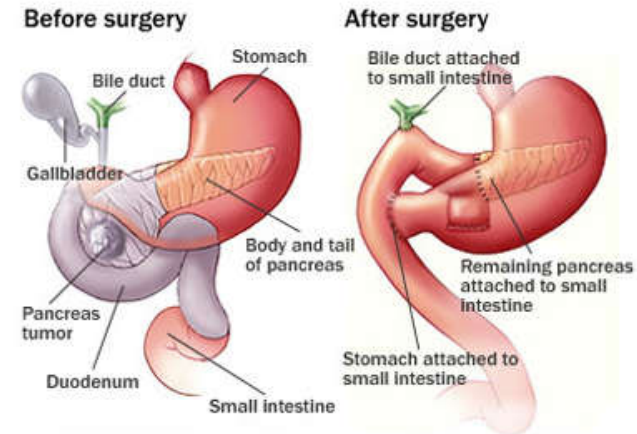
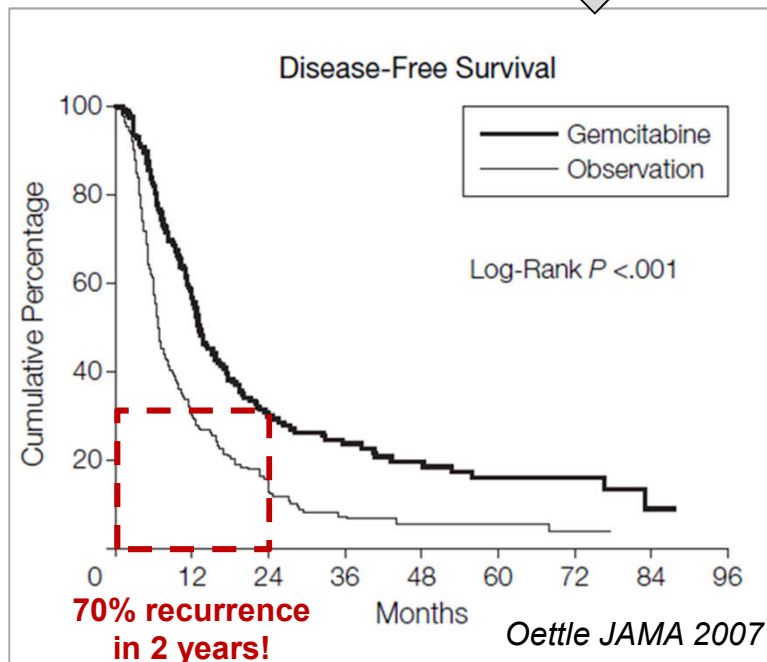
non-resectable

MS: 6-9 months

~20%

resectable

MS: 2-3 years



## My objective:

- Counter devastating recurrence rate by means of immunotherapy

## Starting point:

- European Pancreas Center, Heidelberg
- Large patient cohort
- Tumor biopsies primary resectable PDA



Markus Büchler



**PDA is NOT a 'cold' tumor !!!**

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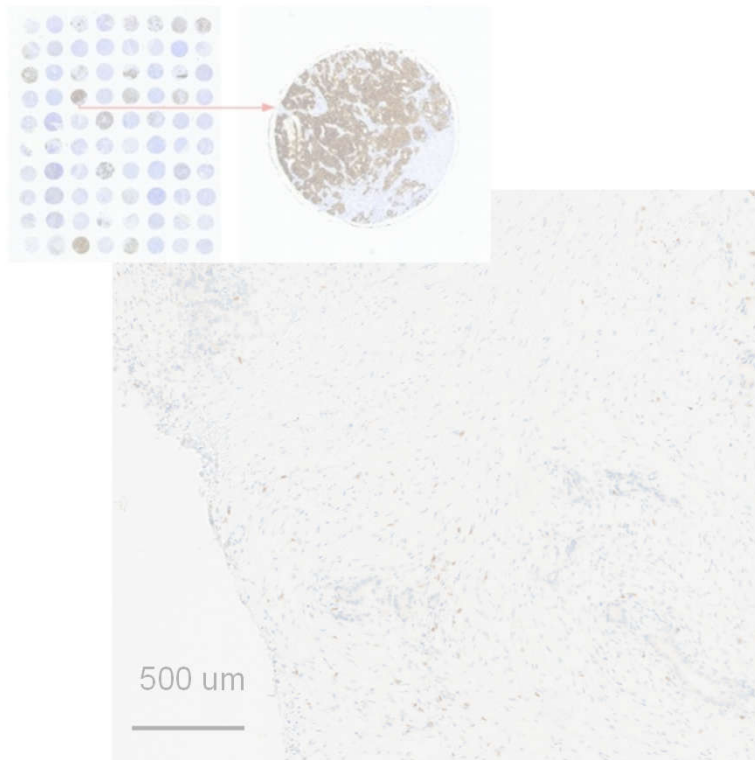
**CD3 IHC**



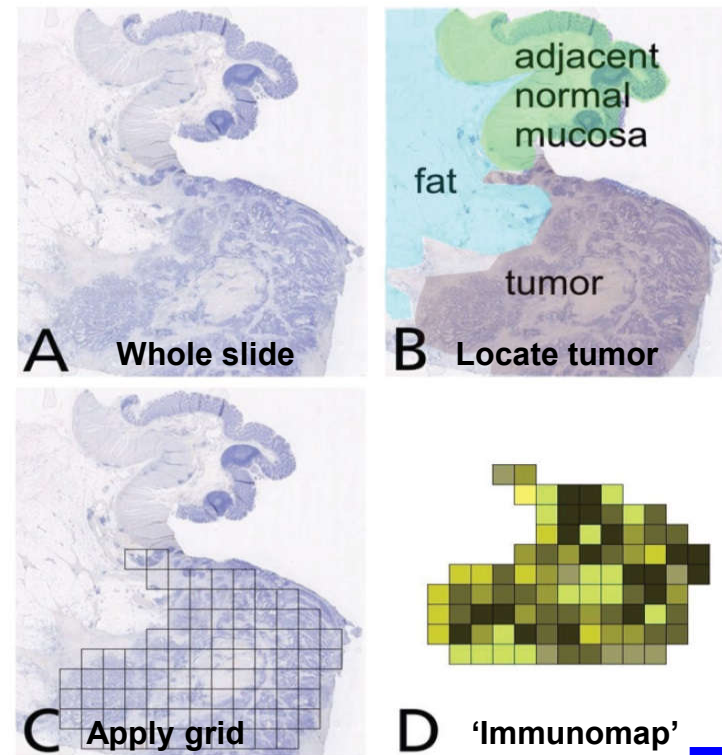


# Vast desmoplastic tumor areas scarcely infiltrated

Tissue micro-arrays



Whole slide imaging



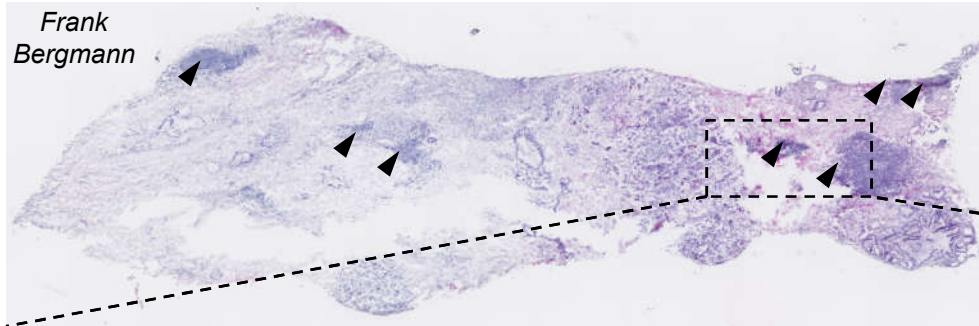
*Visiomorph™*



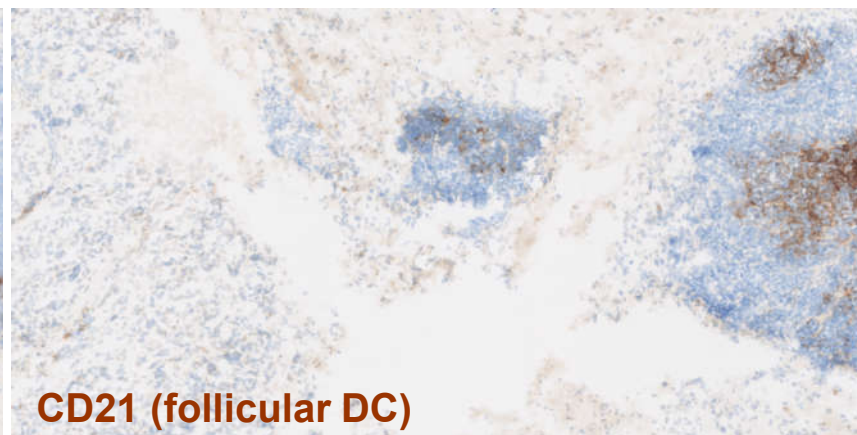
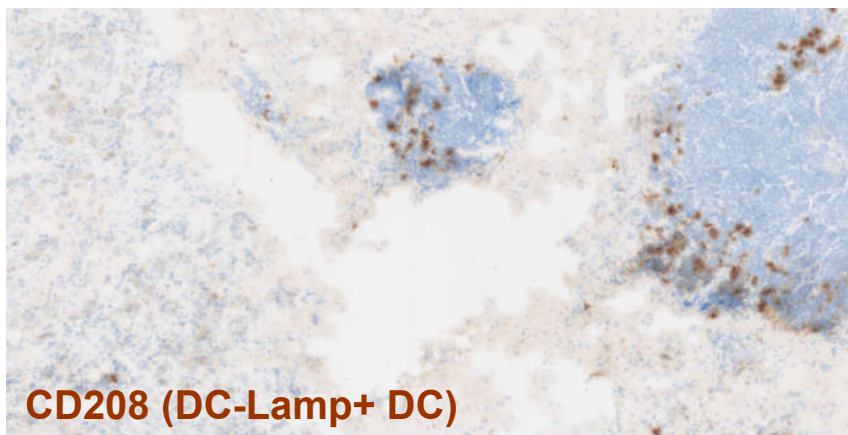
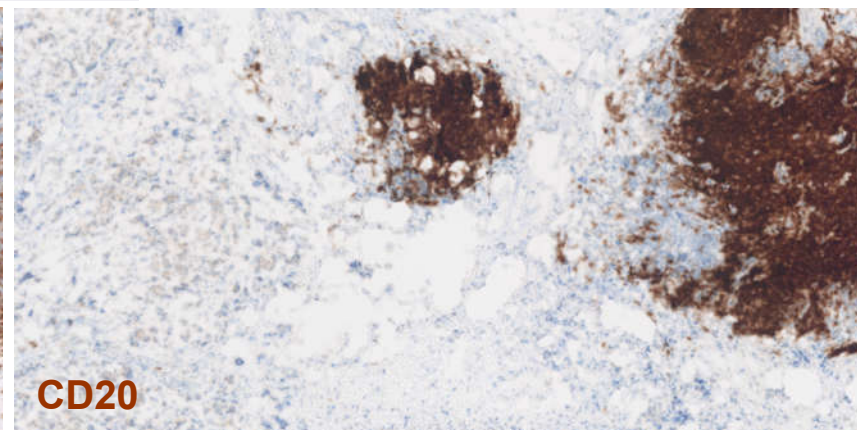
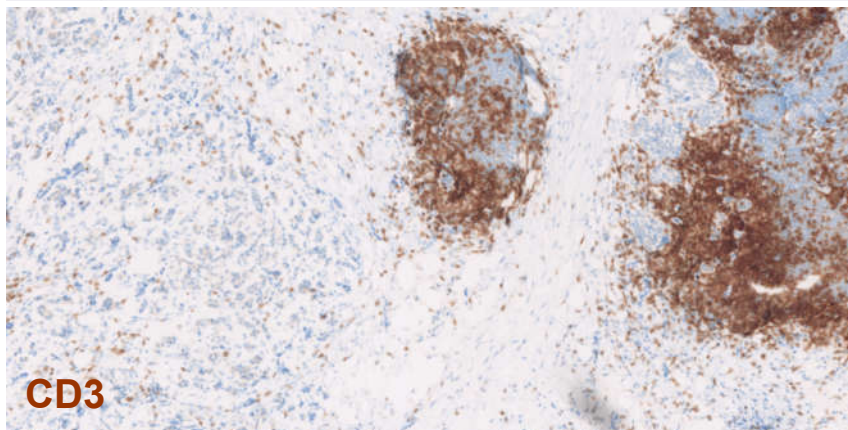
Niels Halama



## Tertiary Lymphoid Structures (TLS)

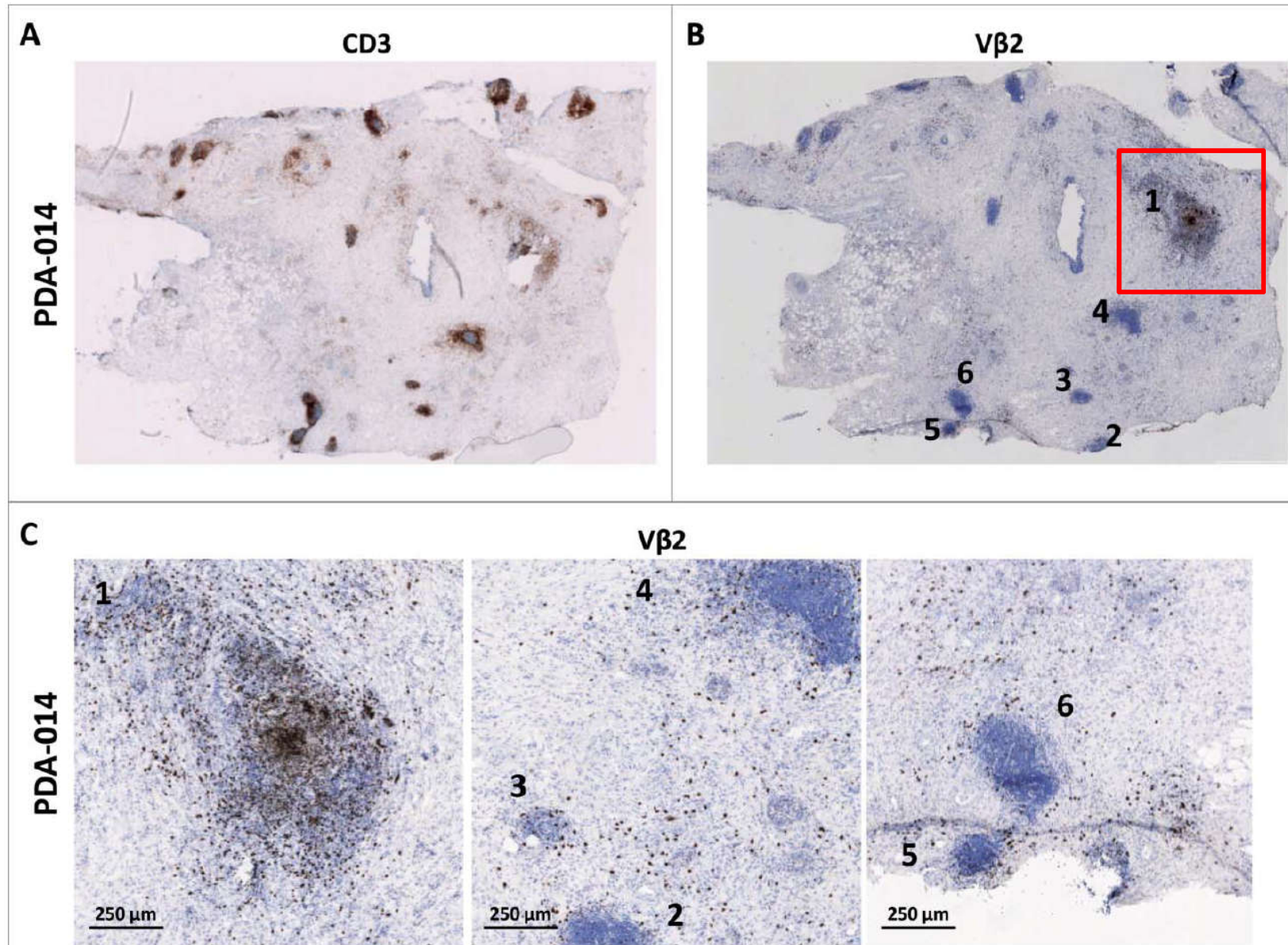


- Definition: B and T cell areas with appropriate DC subsets; HEV
- Associated with good prognosis in lung, breast and colorectal cancer



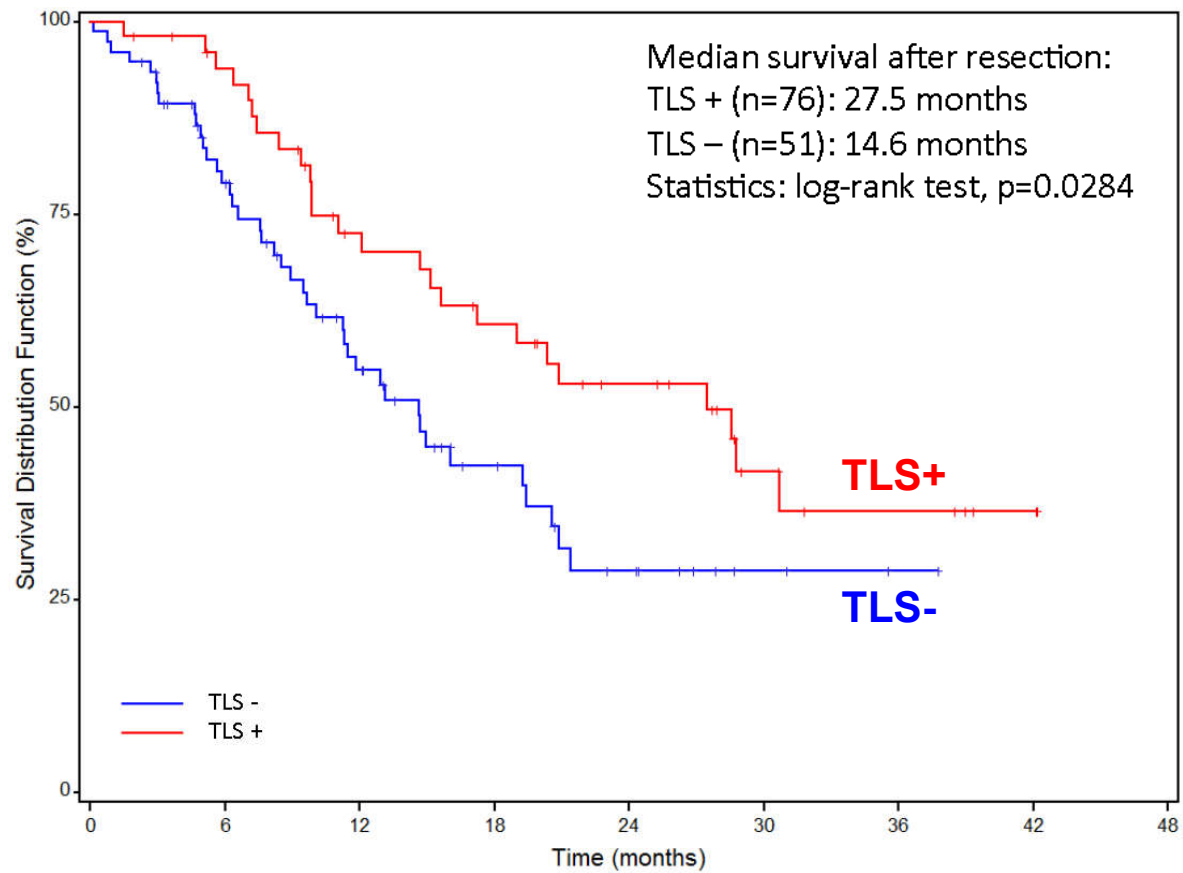


## PDA TLS: evidence for clonal T-cell expansion





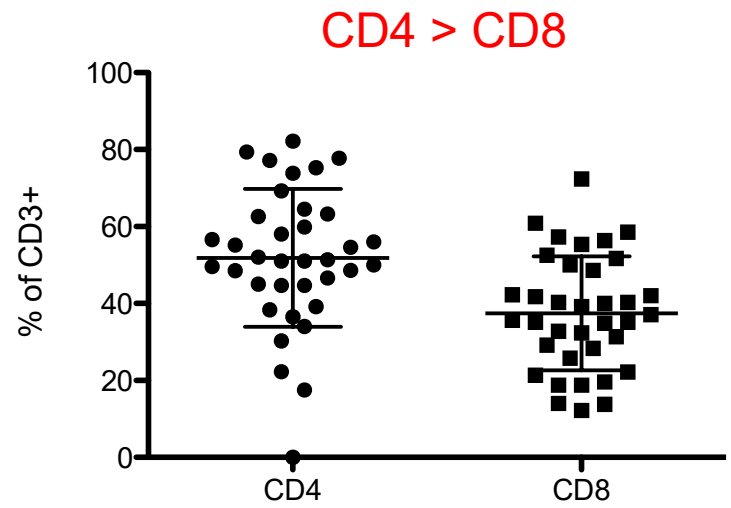
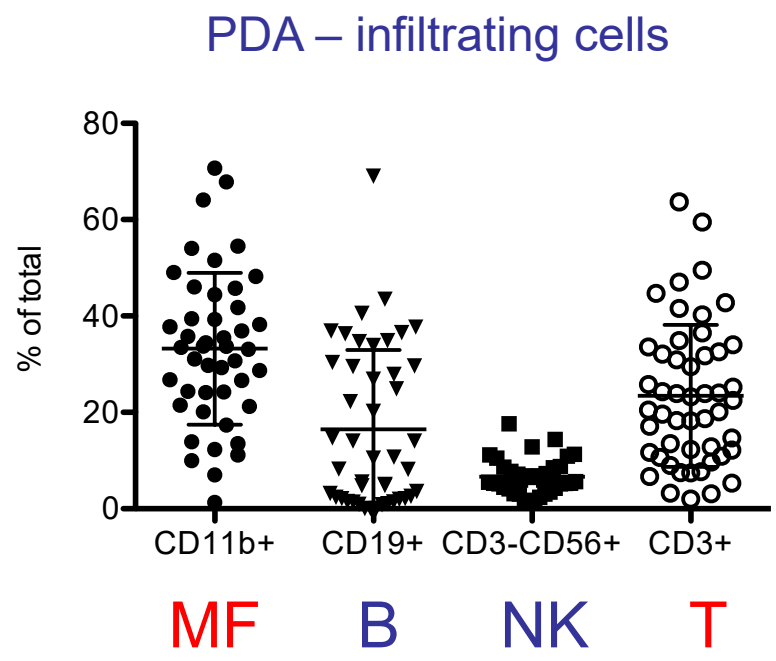
# Prognostic relevance of intra-tumoral TLS



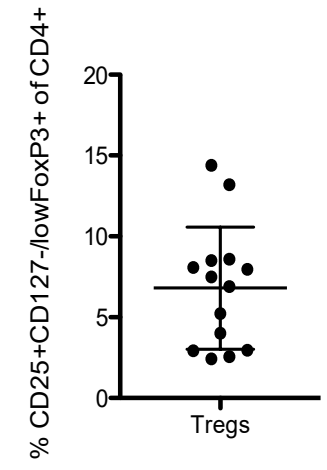


Isabel Poschke

# PDA TILs very similar to melanoma TILs



CD4+ T-regulatory cells (overall not dominating)

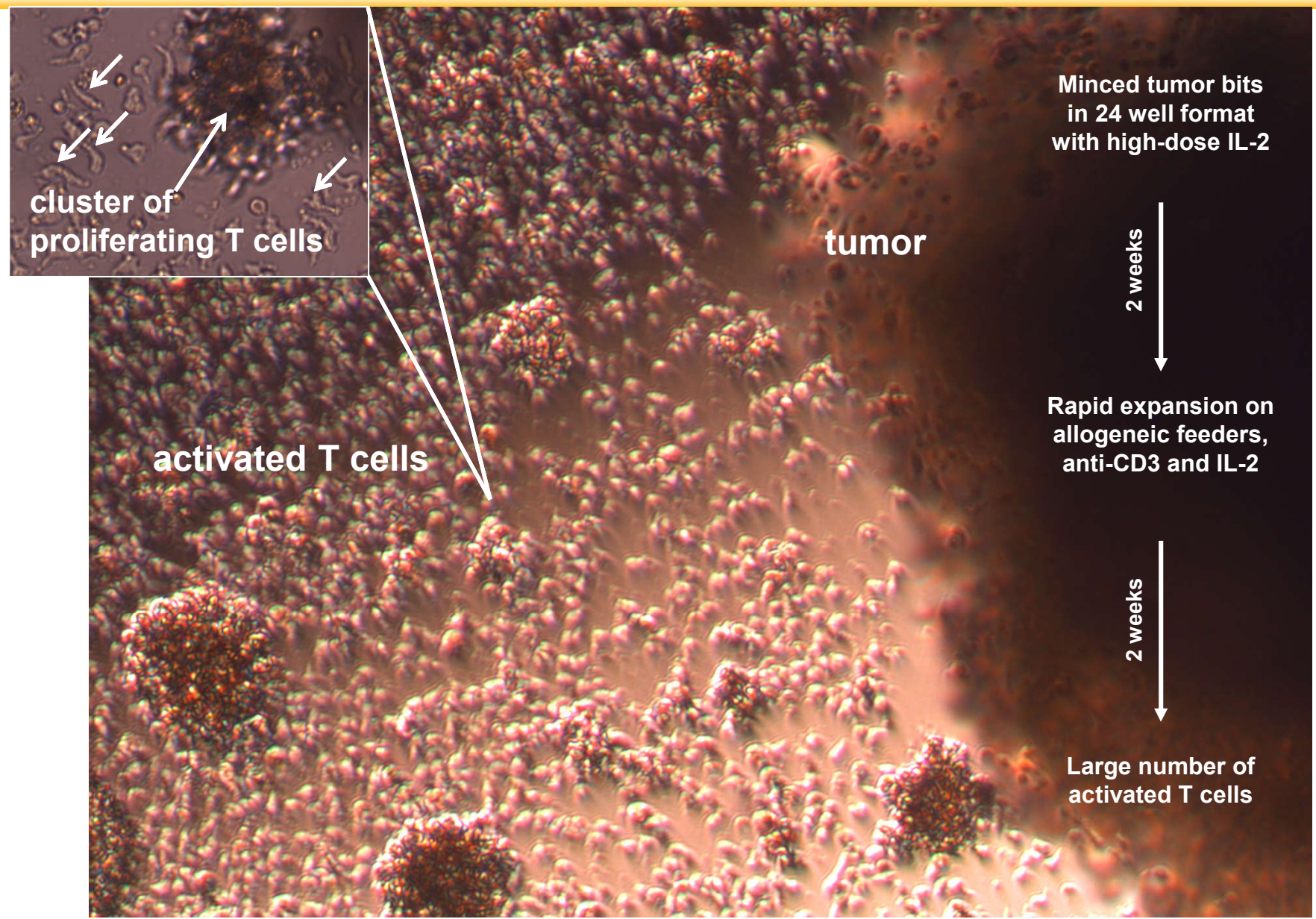


TIL = Tumor-Infiltrating Lymphocyte



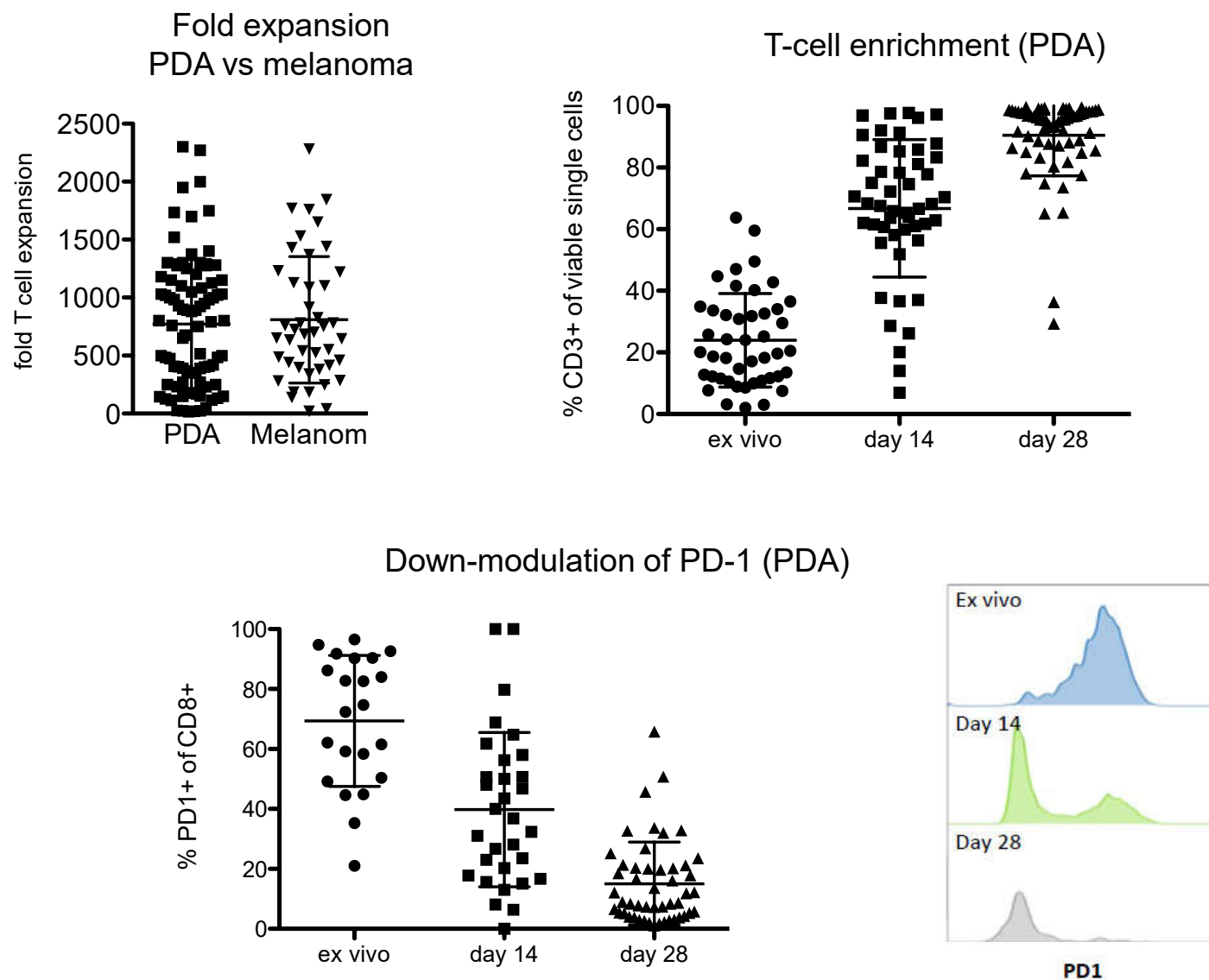
# Ex vivo expansion of Tumor-Infiltrating Lymphocytes

the melanoma 'young TIL' protocol



# Ex vivo expansion of Tumor-Infiltrating Lymphocytes

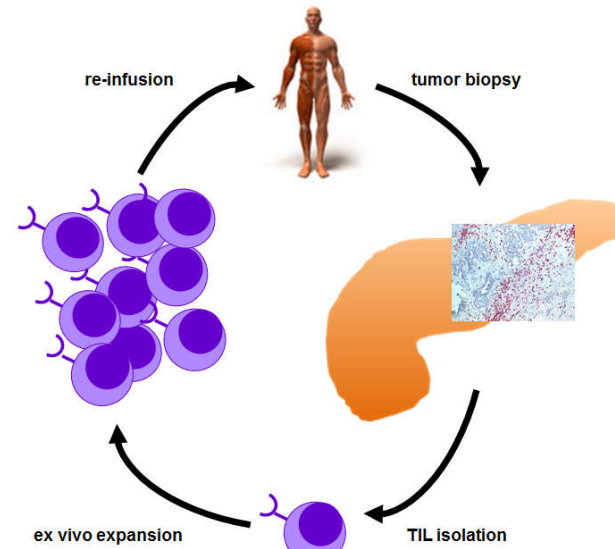
## the melanoma 'young TIL' protocol



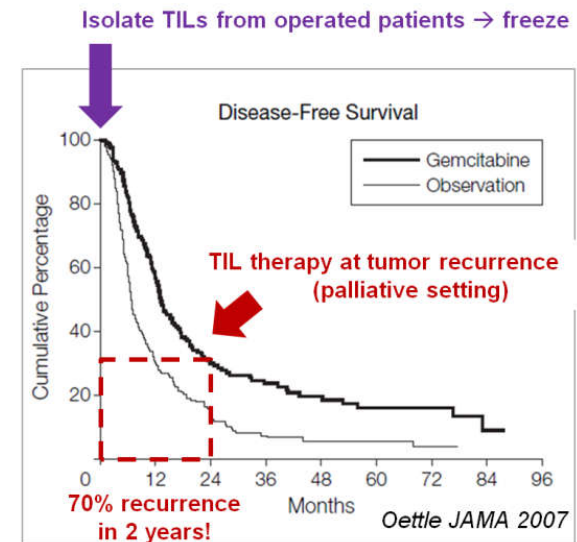
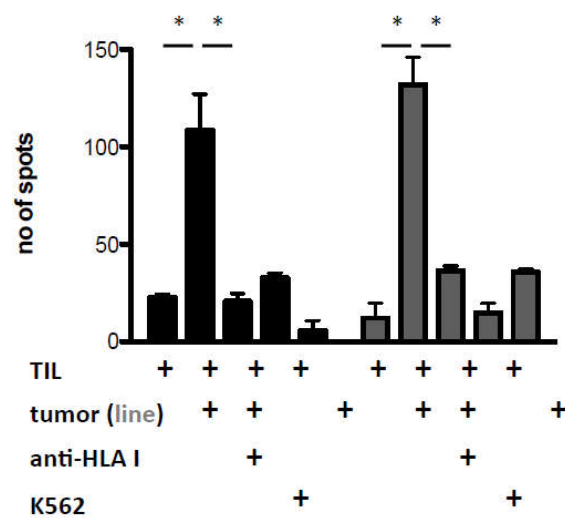
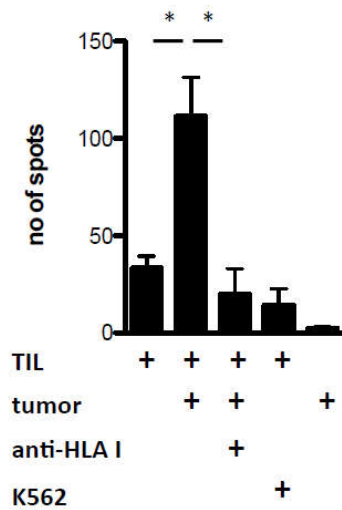


# Ex vivo expanded PDA TILs are tumor-reactive

→ TIL therapy



17/20





Stefan Eichmüller

# Strategy towards T-cell therapy in PDA



Isabel  
Poschke

## TIL study in melanoma

✓ GMP test runs

➤ Trial

- MEL-TIL Study
- Protocols J. Haanen, Amsterdam
- EudraCT No. 2016-004644-11
- Stage 3-4 melanoma
- 8 patients
- Endpoints:
  - Safety
  - RECIST
  - PD biomarkers



## Further characterization of TIL repertoire

✓ TCR repertoire by deep sequencing

✓ TCR cloning

➤ Antigen-specificity

➤ Tumor recognition





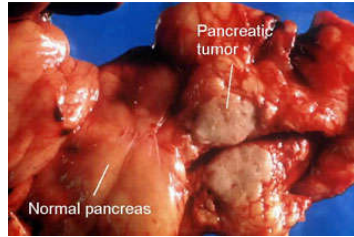
# Analysis of TIL repertoire by TCR deep sequencing



Ugur Sahin

TCR repertoire

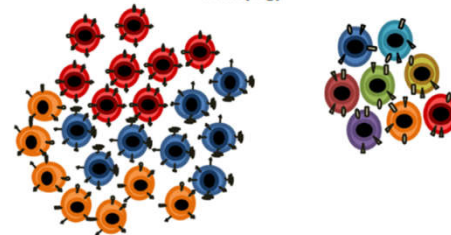
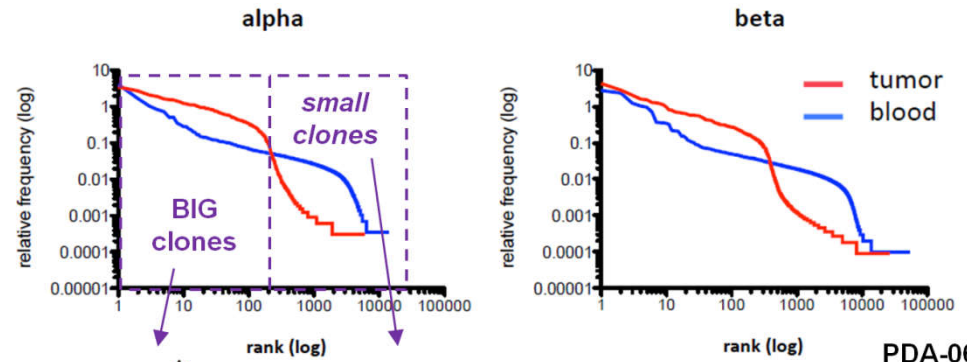
Tumor biopsy



Patient blood



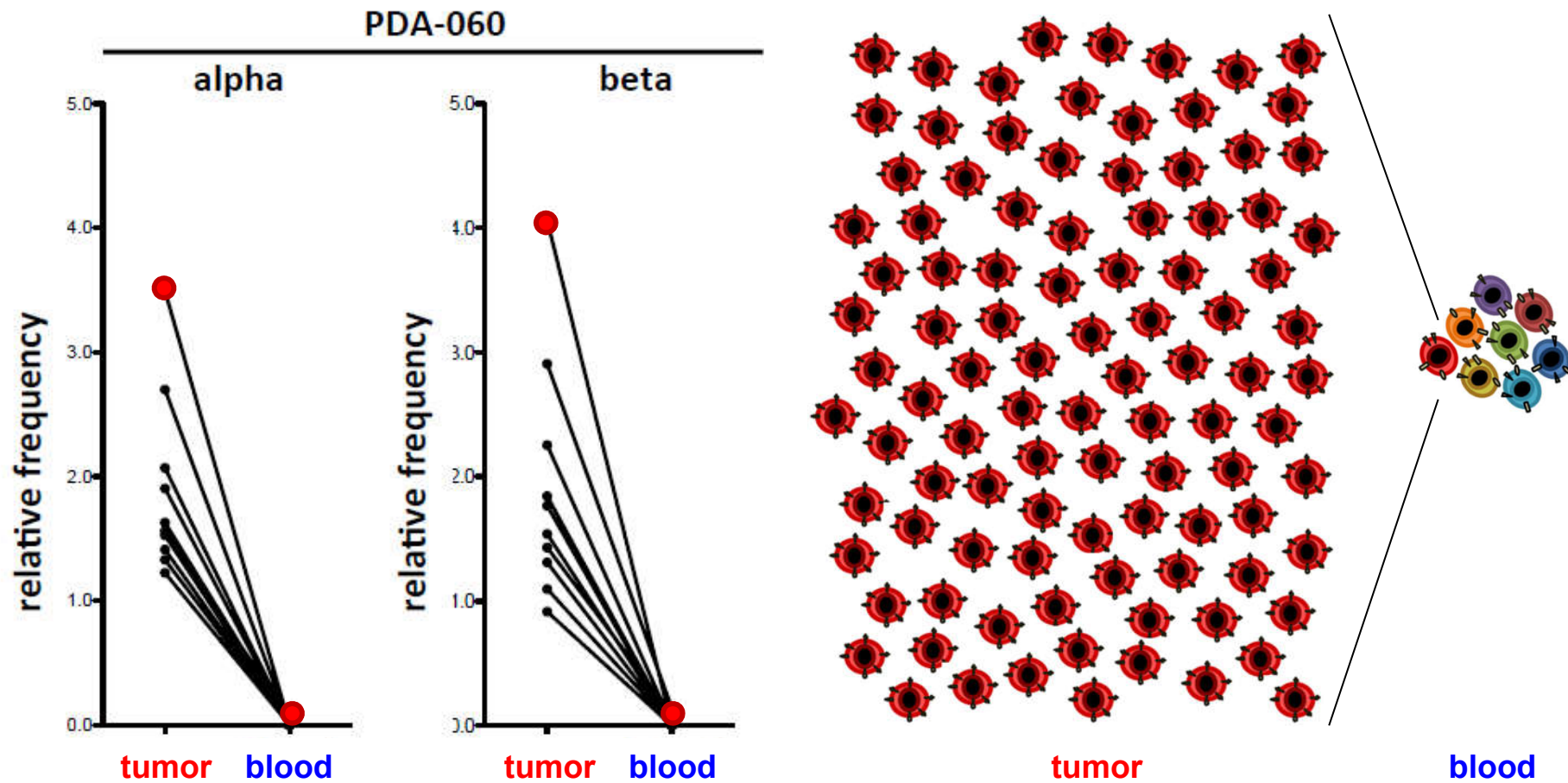
Clone size **tumor** > **blood**



PDA-060

# TCR sequences greatly enriched in tumor

## Top-10 CDR3 sequences in tumor sample



Up to 10.000 – fold more frequent in tumor than in blood

# Tumor-enriched TCR repertoire in PDA and in melanoma



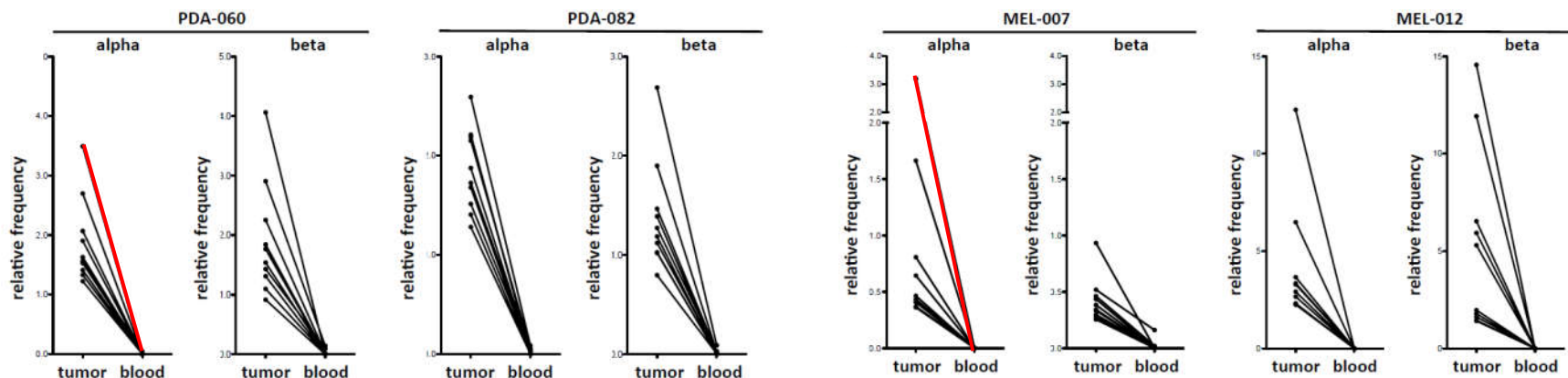
Pancreatic cancer

Melanoma

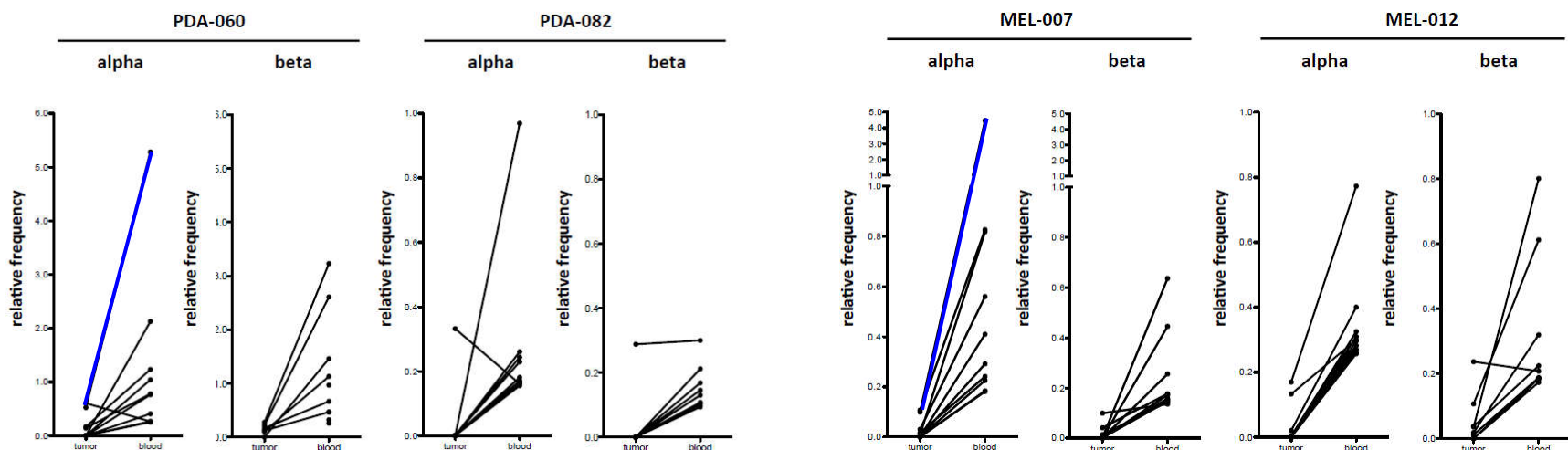
Strobel

Hassel

Top-10 TCR tumor



Top-10 TCR blood





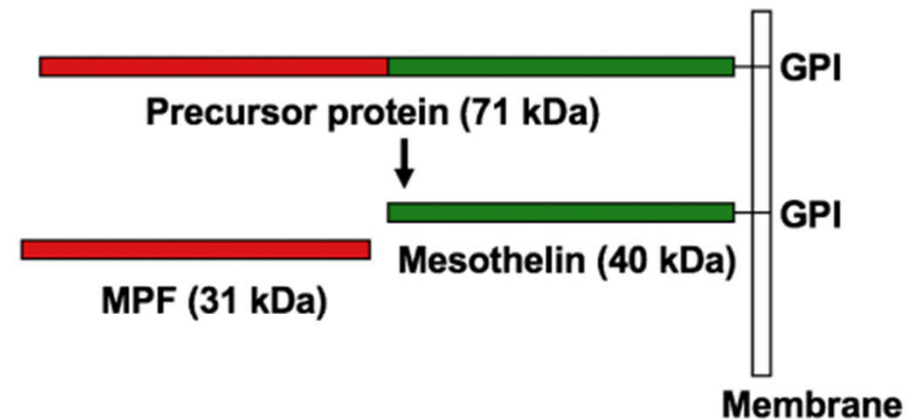
# Cumulative evidence for T-cell tumor interplay in PDA

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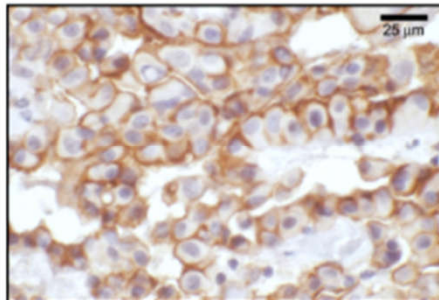
1. Immune cell infiltration, including T-cells
2. 'Antigen experienced', activated phenotype
3. T-cells can be readily expanded ex vivo
4. Findings pointing at antigen encounter and clonal expansion in the tumor:
  - Tumor-enriched TCR-sequences
  - Tertiary lymphoid structures
  - Tumor-reactivity of expanded TILs

# Mesothelin

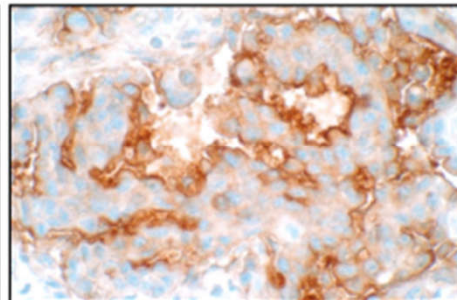
- Cell surface glycoprotein
- Differentiation antigen expressed only on mesothelial cells of pleura, peritoneum & pericardium



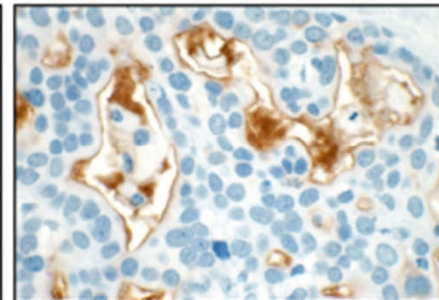
- Mesothelin is highly expressed in many cancers



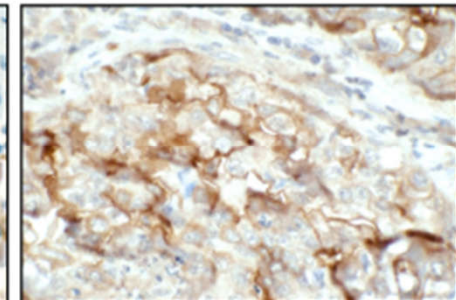
Mesothelioma



Ovarian Cancer



Pancreatic Cancer

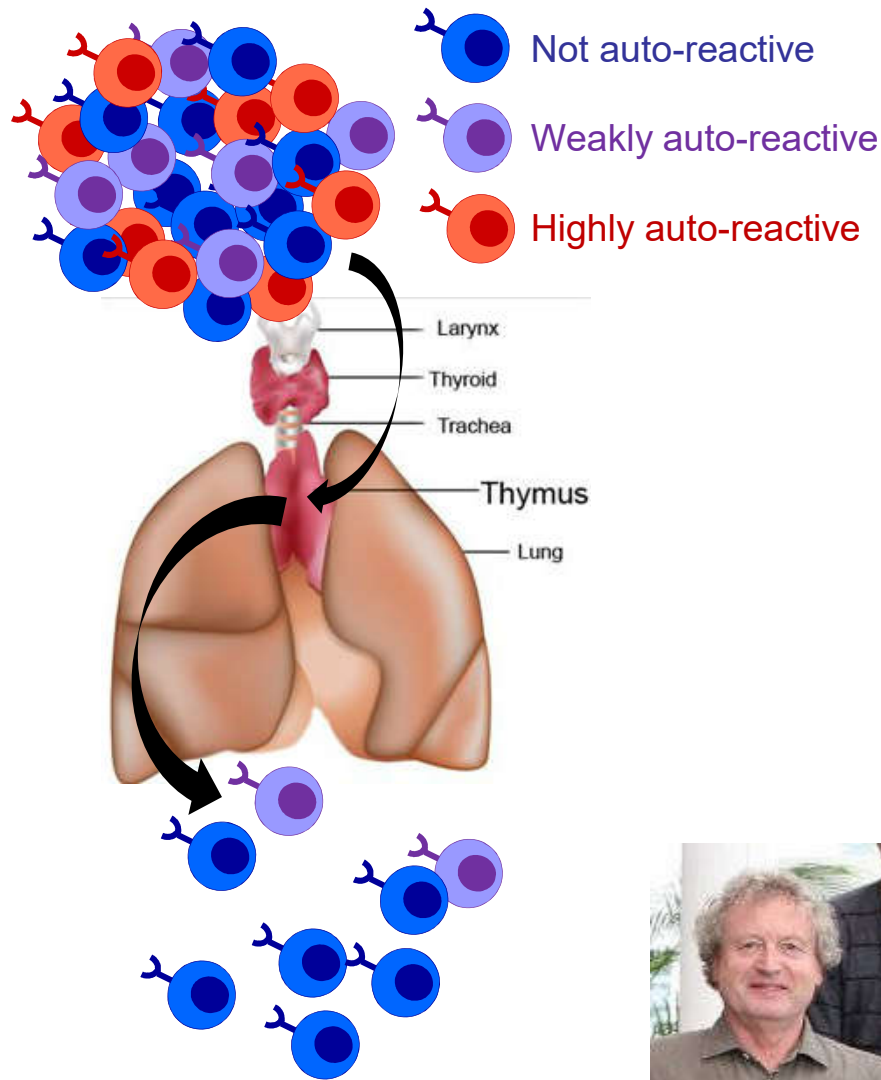


Lung Cancer

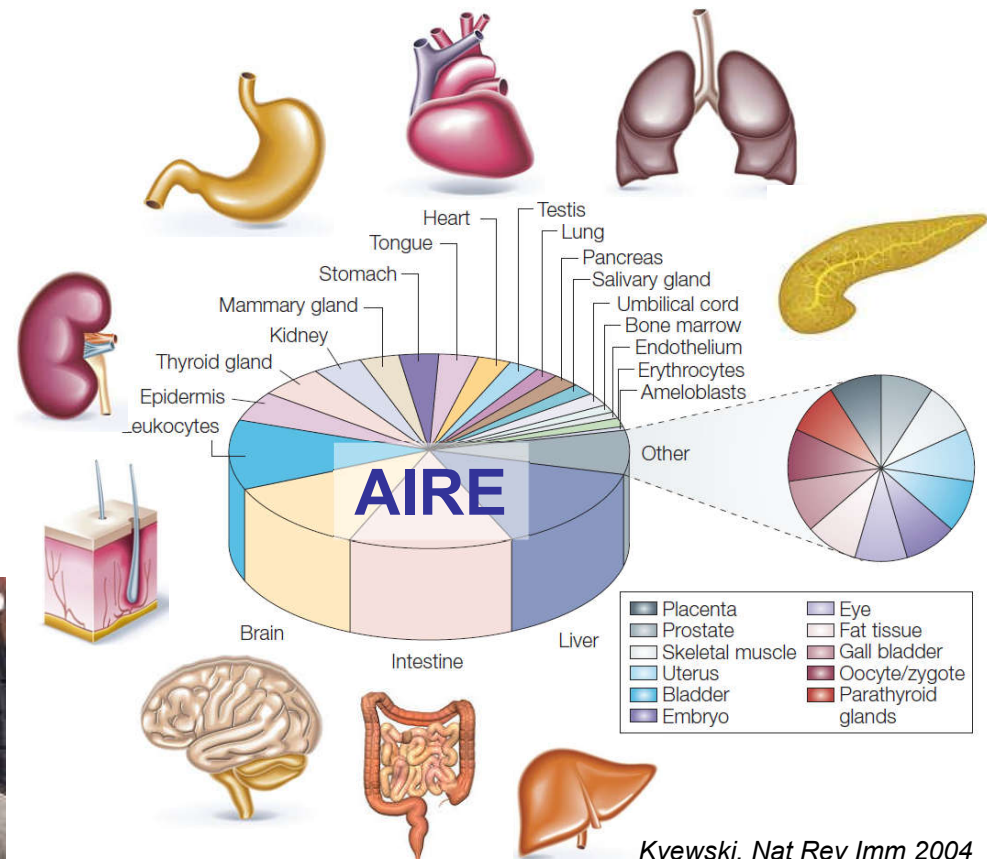
Hassan et al. Clin. Cancer Res., 2004

# Thymic tolerance

## T-cells targeting 'self' antigens are rare



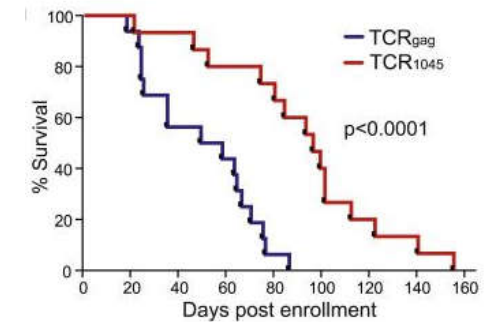
Medullary thymic epithelial cells (mTEC) express of most tissue-specific antigens



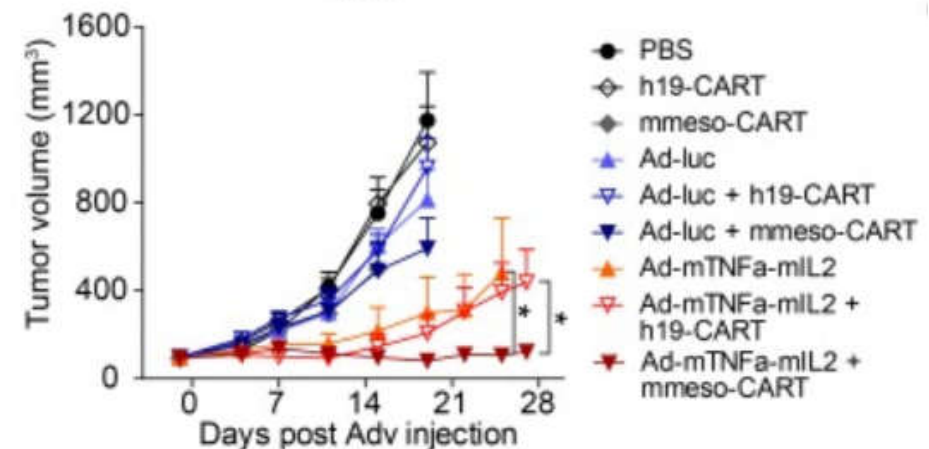


## Mesothelin-specific CART strategy

- Thus far limited efficacy in pre-clinical models and clinic
- RNA-transduced CART-meso seem well tolerated
- SAE with Lenti-transduced CART-meso (single case)
- Attraction to tumor by immunogenic event may create therapeutic window

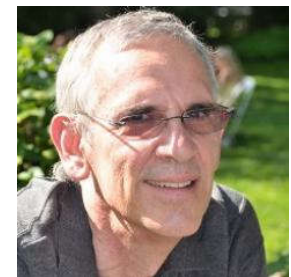
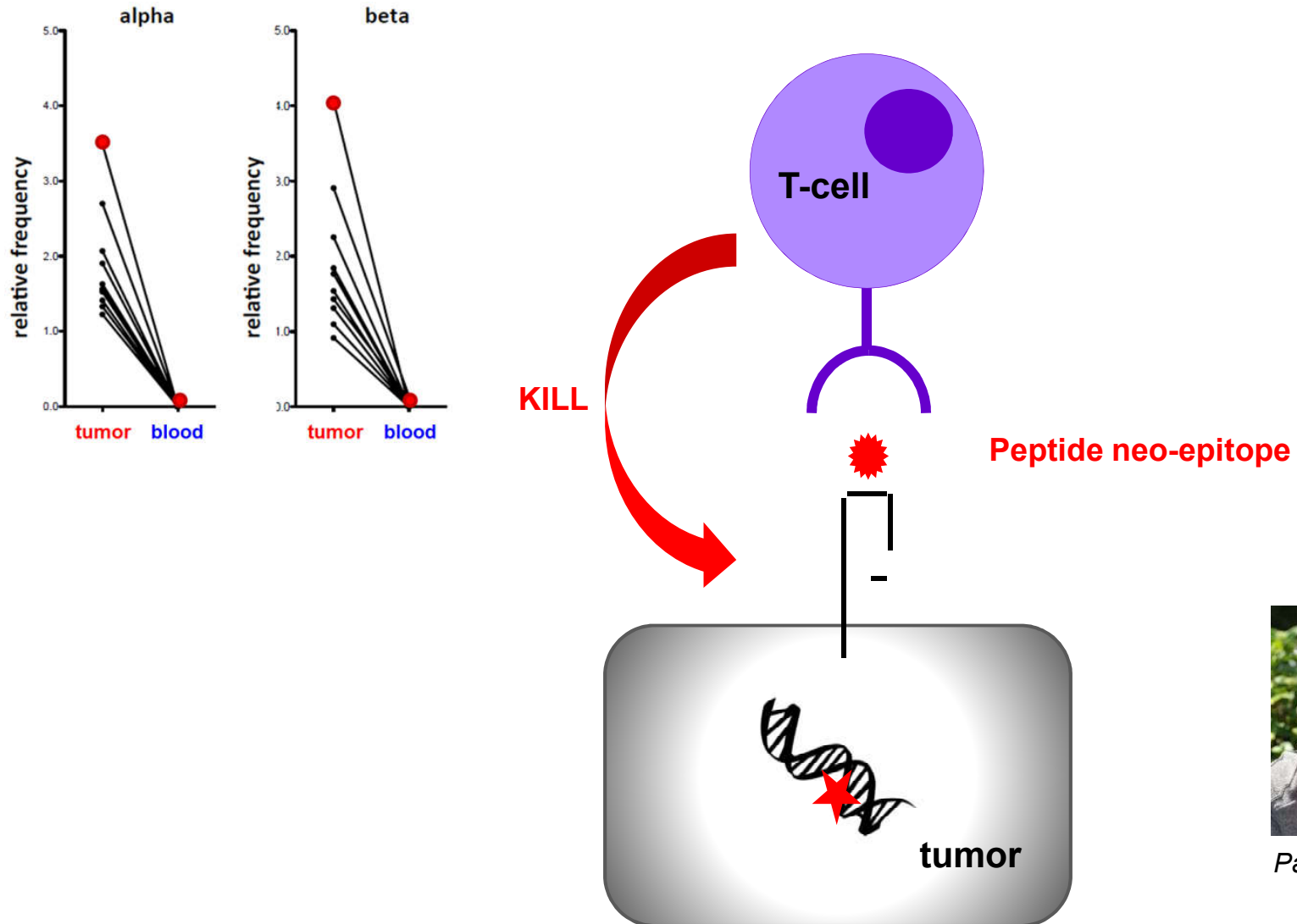


normal function of the T cells and it is expected that they accumulate at locations with target antigen expression. Pleura, pericardium, and peritoneum express a low level of mesothelin, so the migration of the T cells to these places are expected. So, far we did not see the signs of pericarditis, pleuritis or peritonitis in this patient, however, these toxicities are expected in future studies. The continuous proliferation of the T cells in the pleural cavity after repeated negative tumor cell cytology can be explained by either exposure to mesothelin on the normal pleural lining or pleural tumor sites, or by bystander proliferation in a milieu



1. Hingorani et al. Cell 2015
2. June et al. J. Immunother. 2017
3. June et al. Gastroenterology 2018
4. June et al. JCI 2018

# Hypothesis: PDA TILs primarily target tumor mutanome



Paul Robbins, NCI

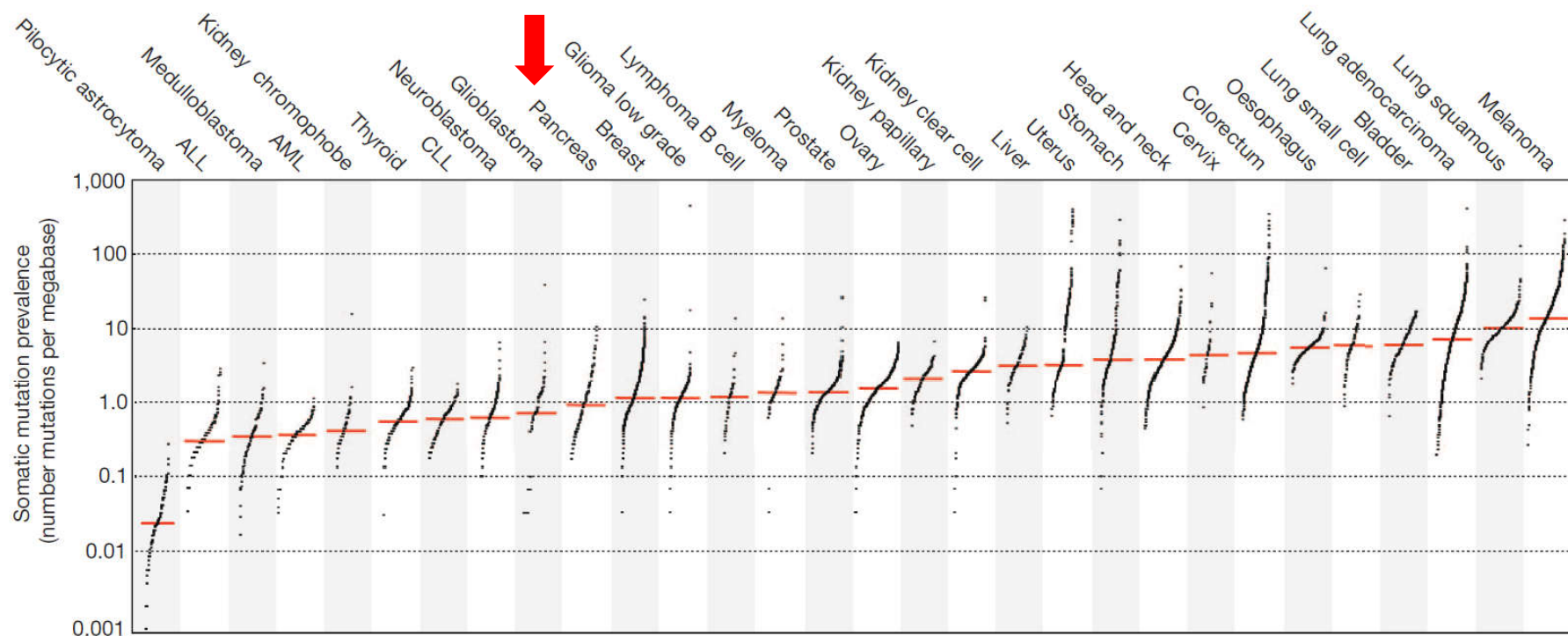
# Number of somatic mutations in PDA more limited than in melanoma

## ARTICLE

doi:10.1038/nature12477

### Signatures of mutational processes in human cancer

primary resectable PDA: ~50-100 non-synonymous mutations + several indels



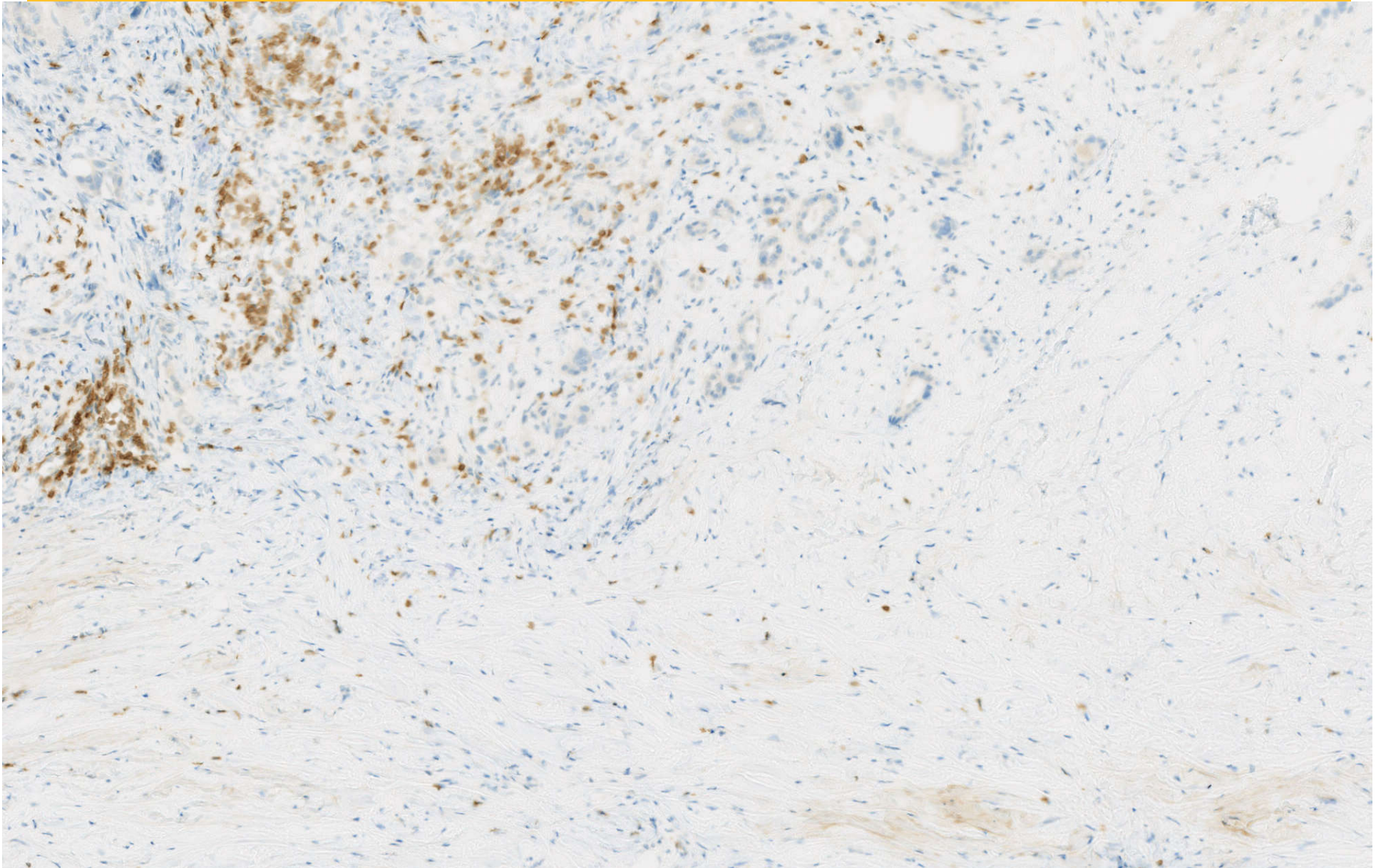
**Figure 1 | The prevalence of somatic mutations across human cancer types.** Every dot represents a sample whereas the red horizontal lines are the median numbers of mutations in the respective cancer types. The vertical axis (log scaled) shows the number of mutations per megabase whereas the different

cancer types are ordered on the horizontal axis based on their median numbers of somatic mutations. We thank G. Getz and colleagues for the design of this figure<sup>26</sup>. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia.



# Low tumor cell content in PDA tumors hampers NGS-based mutation scoring (and other applications)

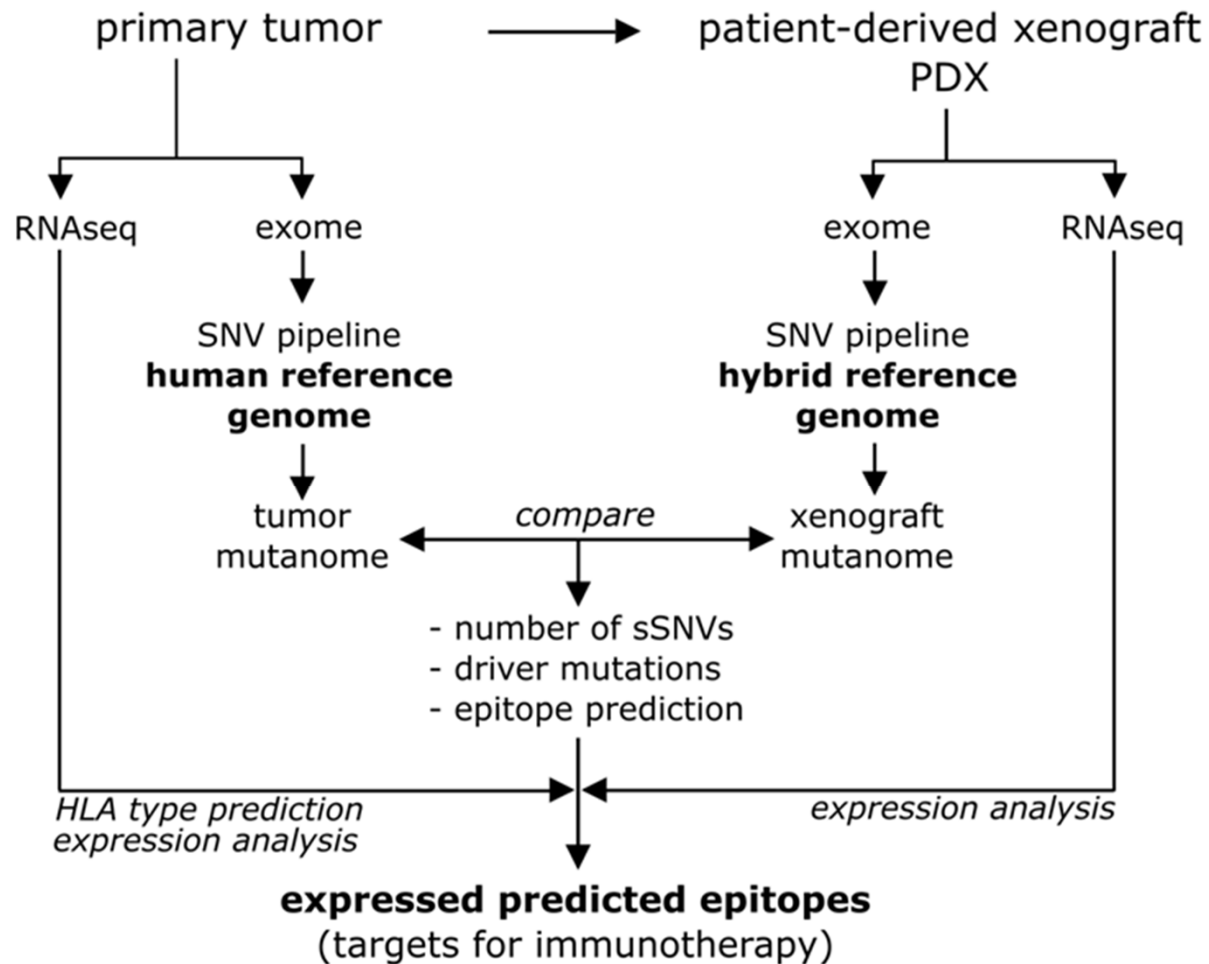
24



# Parallel mutation scoring in primary tumor and PDX model



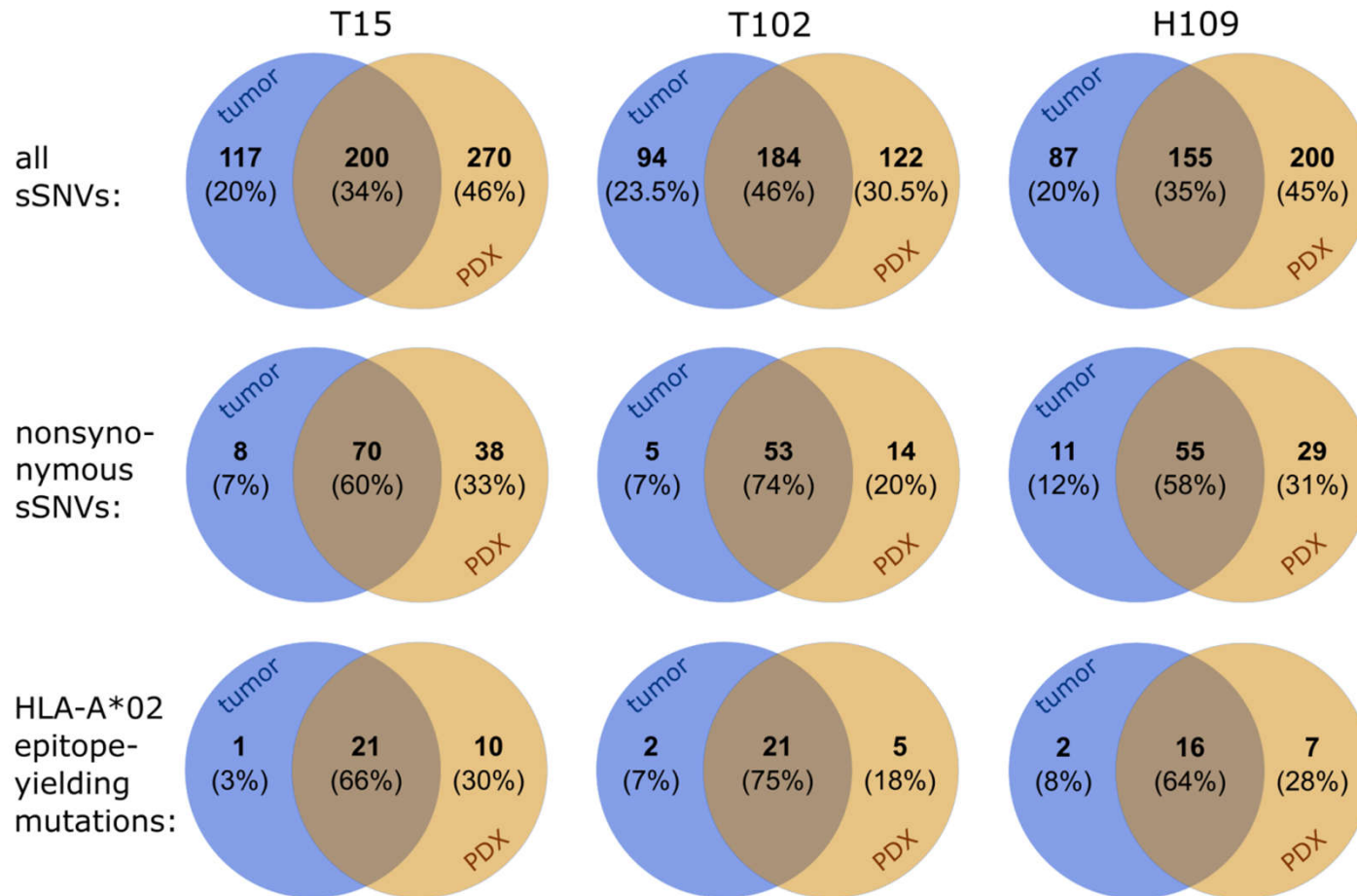
Michael Volkmar





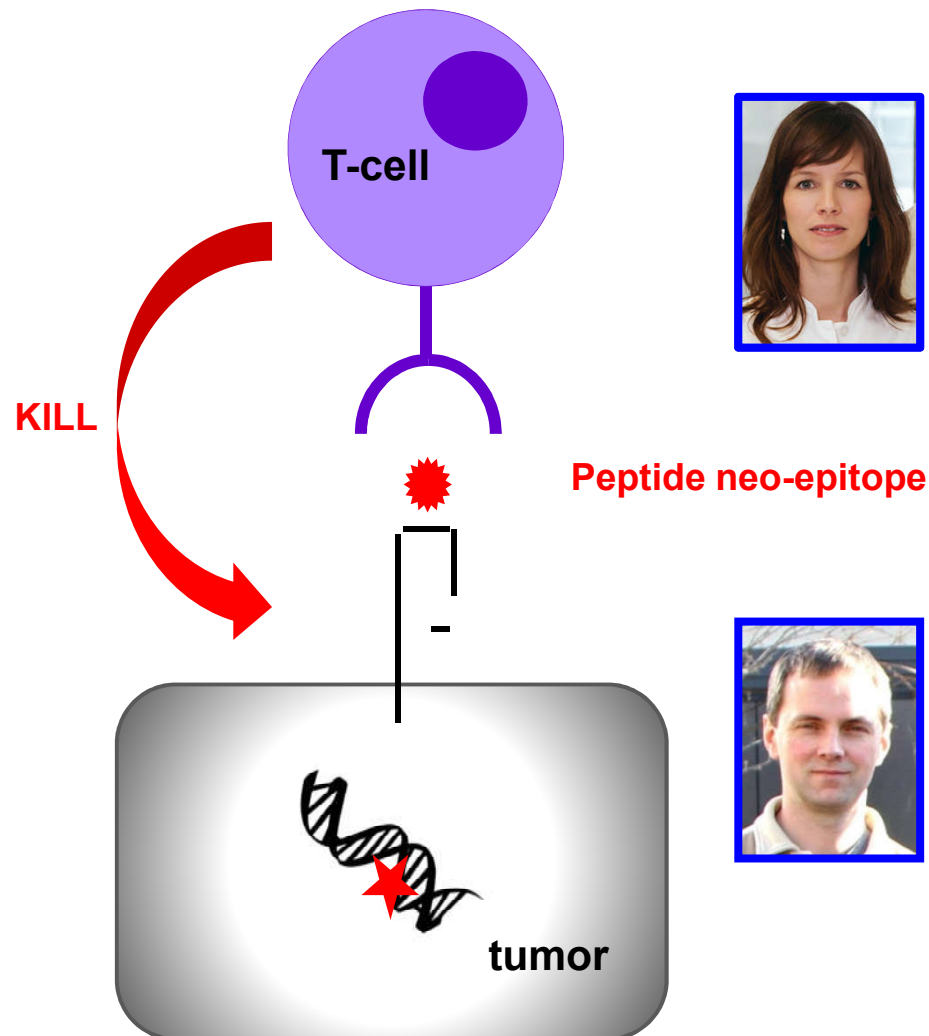


# Improved neo-epitope calling in PDX exomes using hybrid reference genome



**Tumor biopsy: 20-30% of mutations/potential epitopes missed**

# Complementary strategies for POC identification mutanome-encoded neo-epitopes in PDA



## FWD Immunology

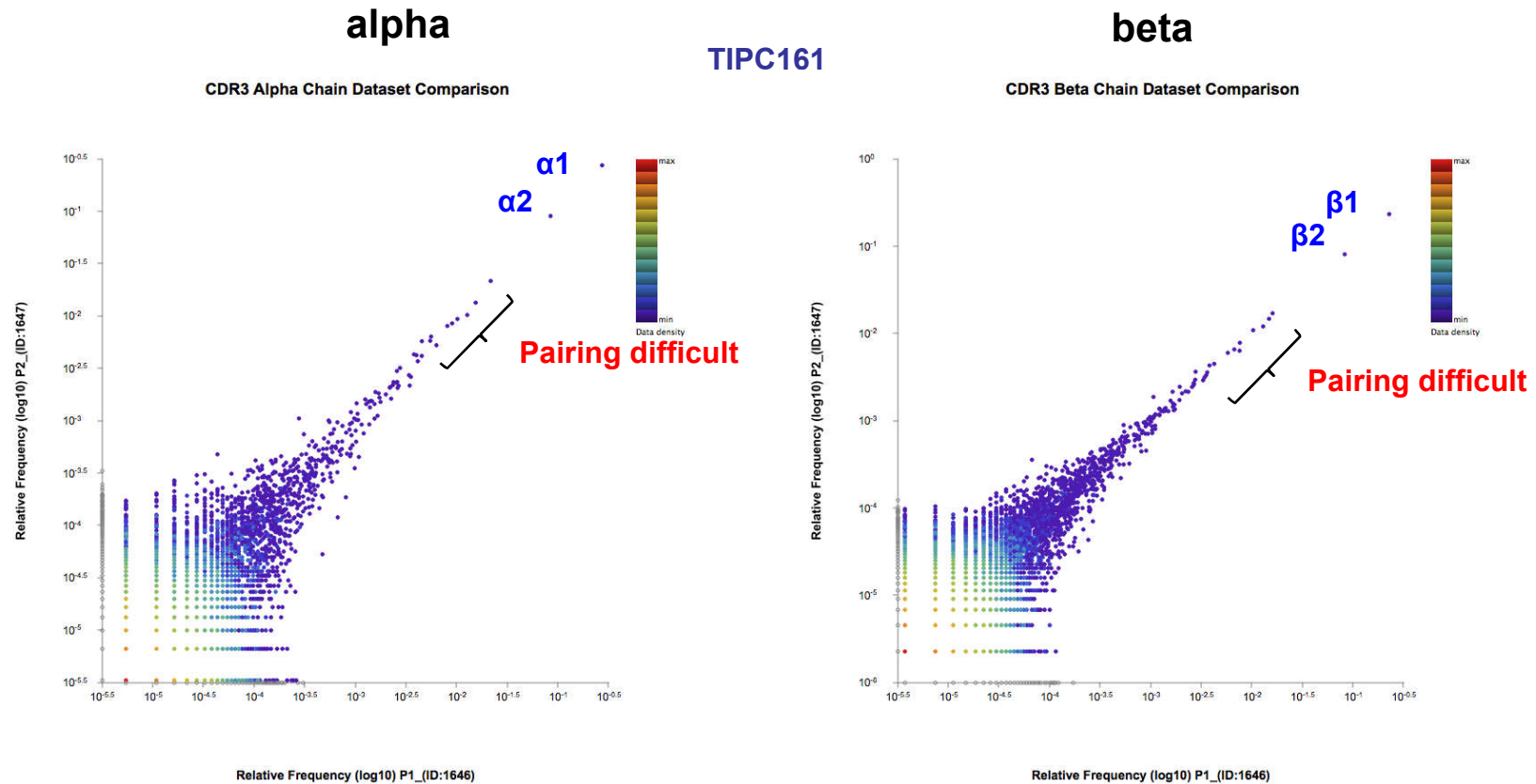
- Starting point: TILs/TCRs
- Clone TCRs
- Identify epitopes



## REV Immunology

- Starting point: mutanome
- Predict neo-epitopes
- Generate TCRs

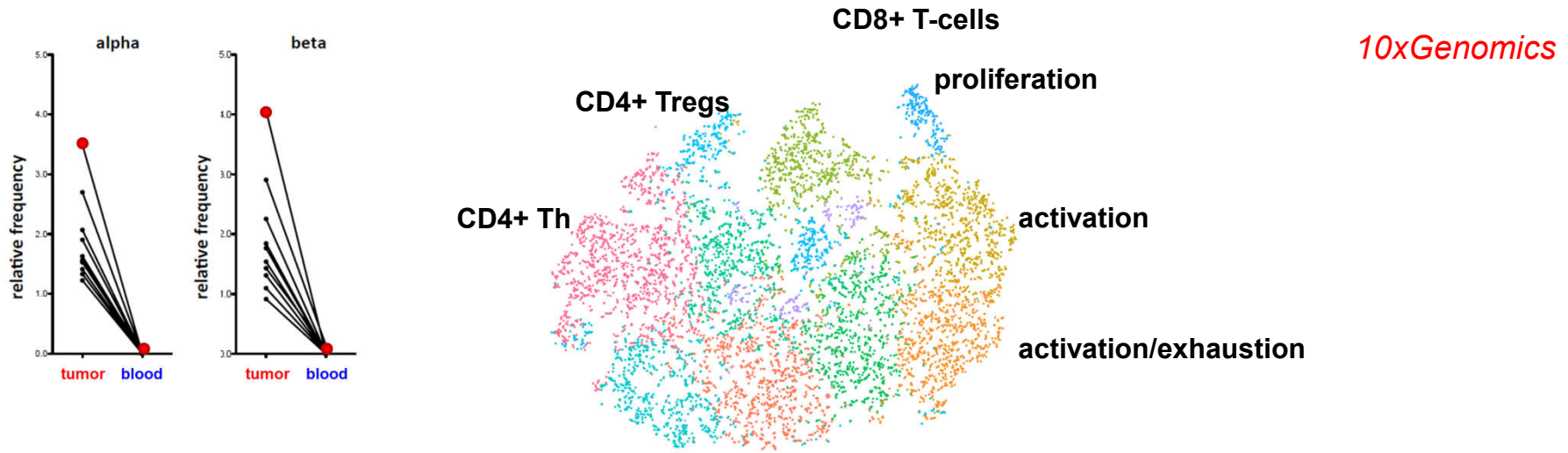
# Cloning of TCR alpha/beta pairs on basis of TCR-seq



→ Single cell TCR cloning

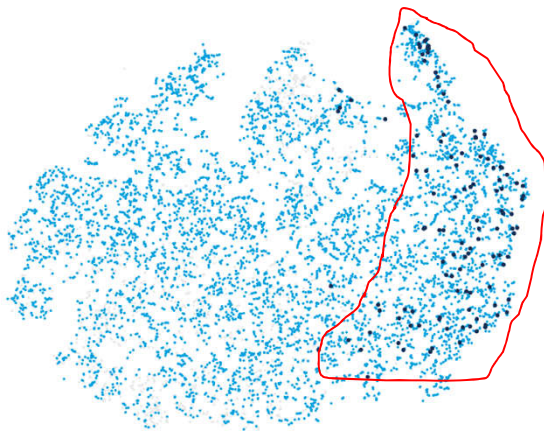


# Single cell TCR cloning in context of frequency and phenotype

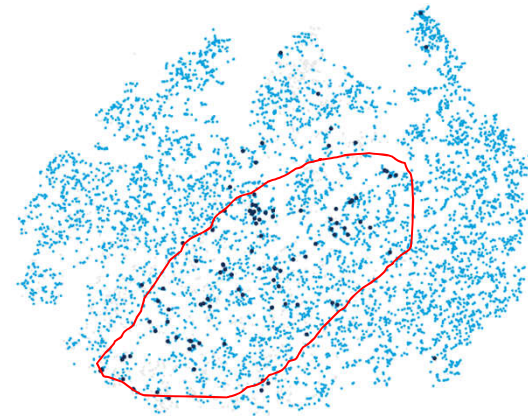


5/10 top-10 clones, incl. #1 and 2

3/10 top-10 clones, incl. #3

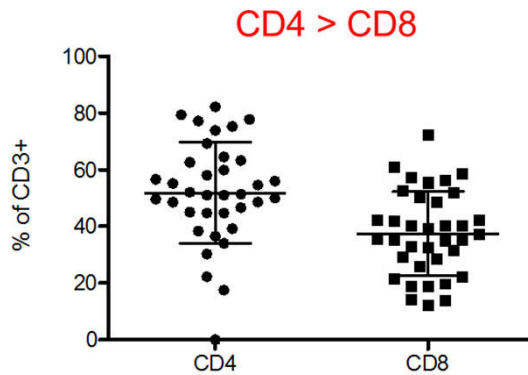


*activated, proliferating CD8+*

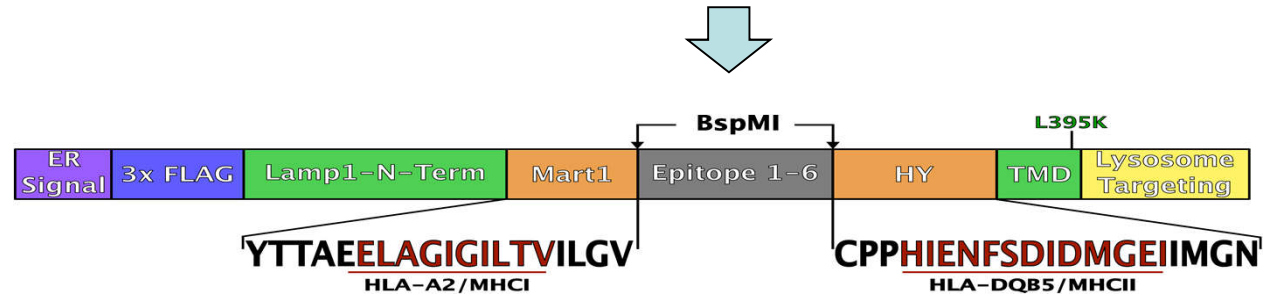


*other CD8+ sub-type*

# Unbiased neo-epitope screening by means of LAMP1-based expression cassette

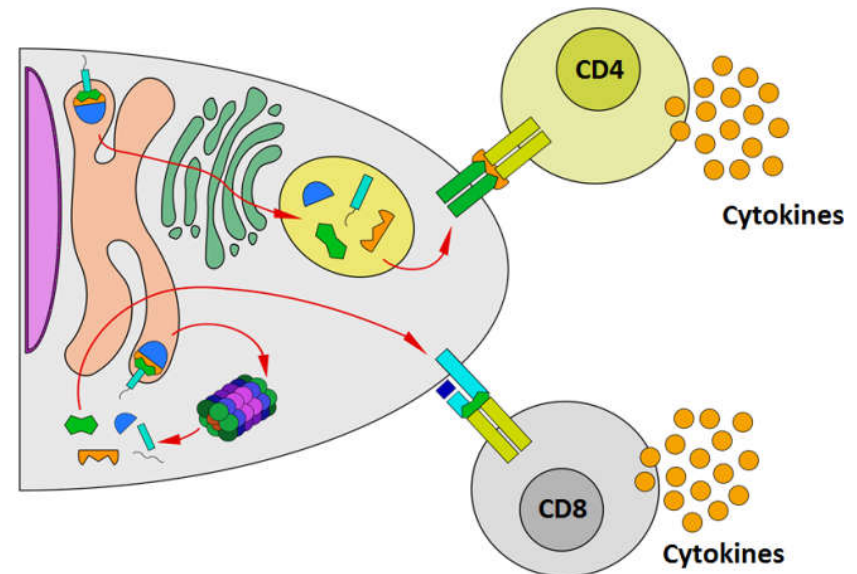


30-mer peptides encompassing non-syn mutations in expressed genes



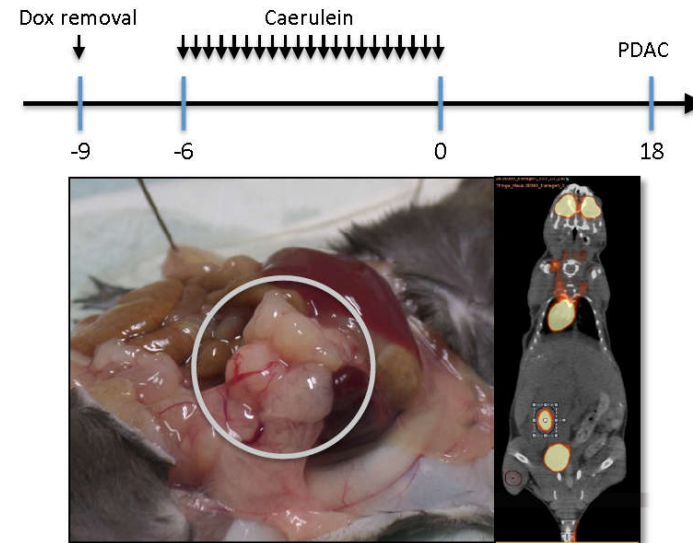
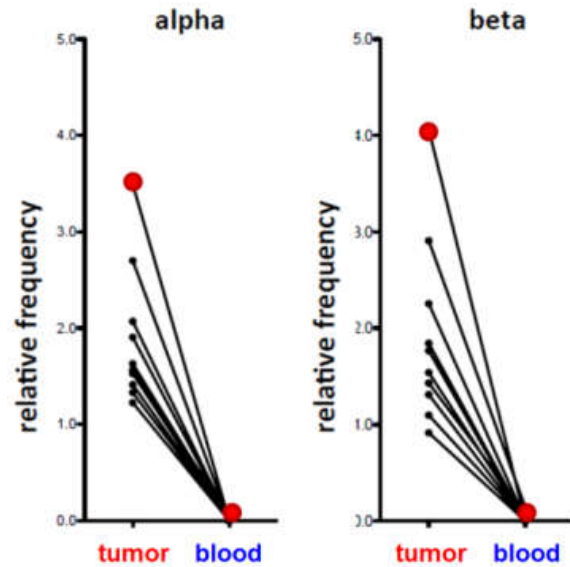
Transduced into autologous APC

- expanded TILs
- BCL-6/BCL-XL B-cells)

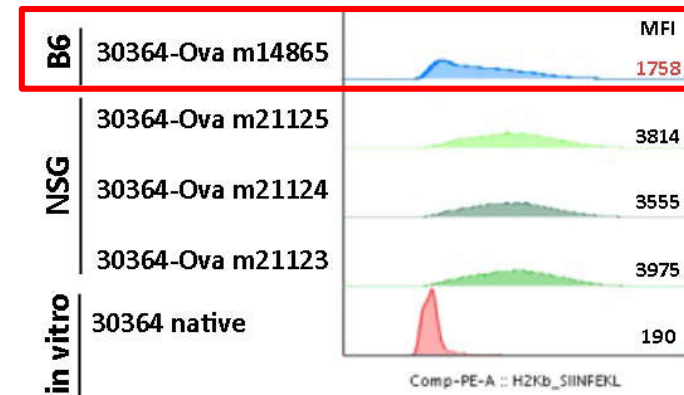


# Are top TCRs found in TIL repertoire tumor-reactive?

Elas-tTA/tetO-Cre Kras<sup>LSL-G12D/+</sup>  
p53<sup>LSL-R172H/+</sup> GEMM → 30364 cell line

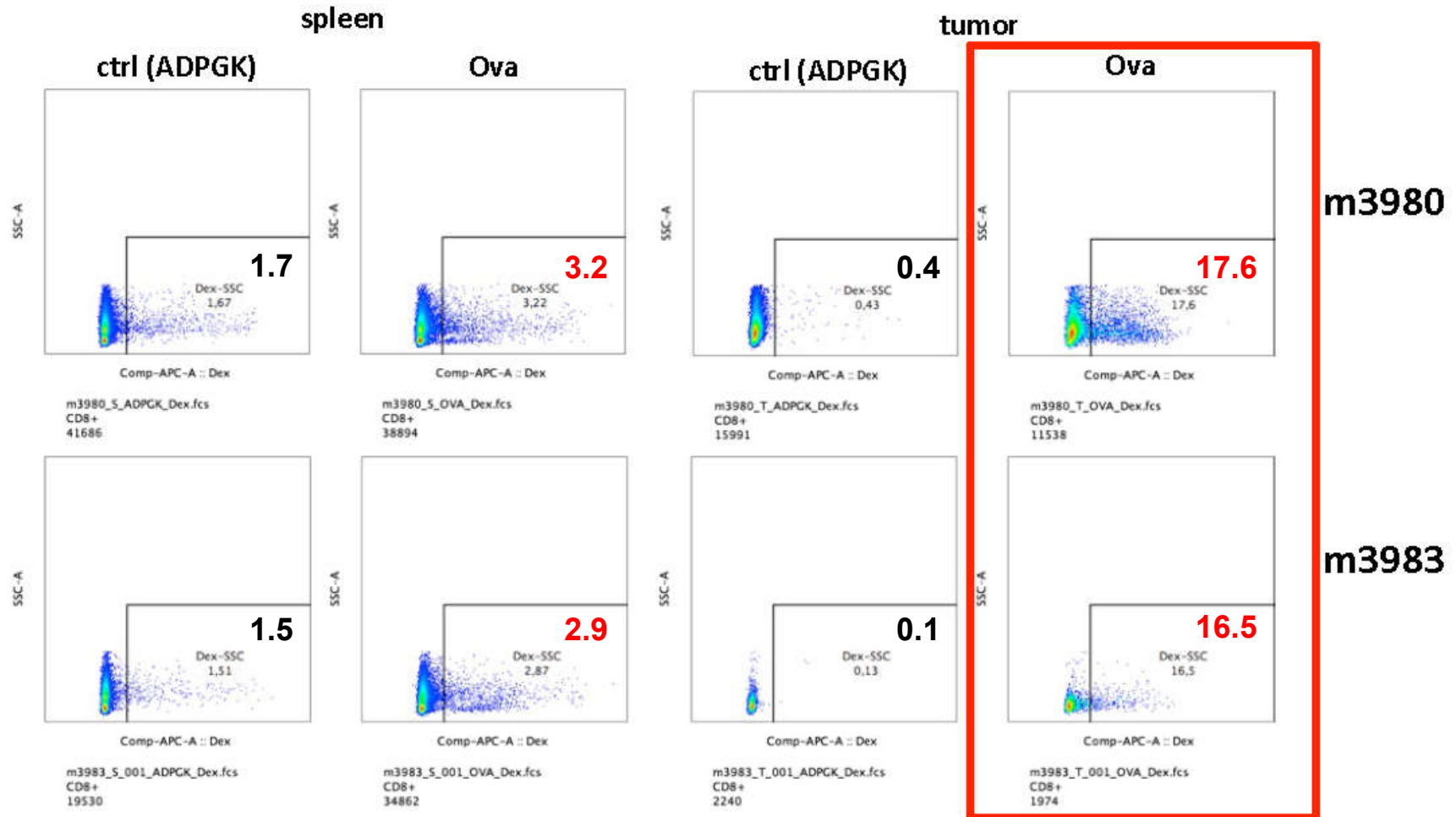


Ova → H2Kb SIINFEKL





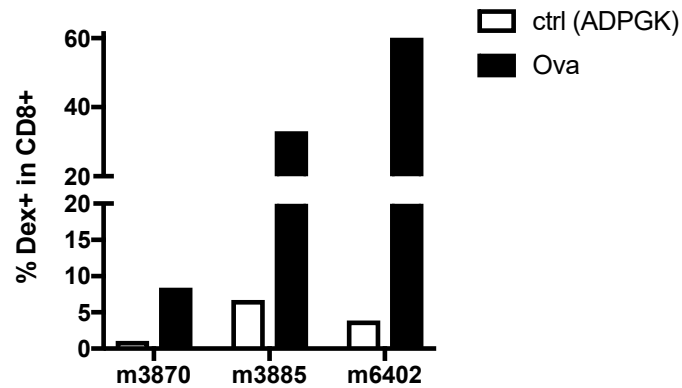
# Spontaneous OVA-specific CD8+ T-cell response



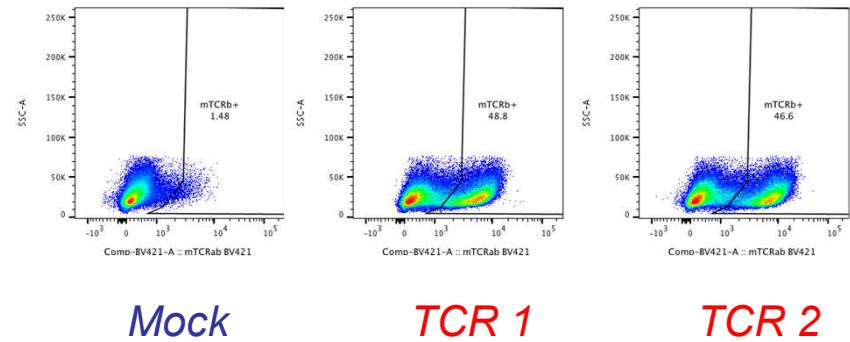
H-2Kb/SIINFEKL dextramer staining →

# Dominant TCRs are OVA tumor antigen reactive

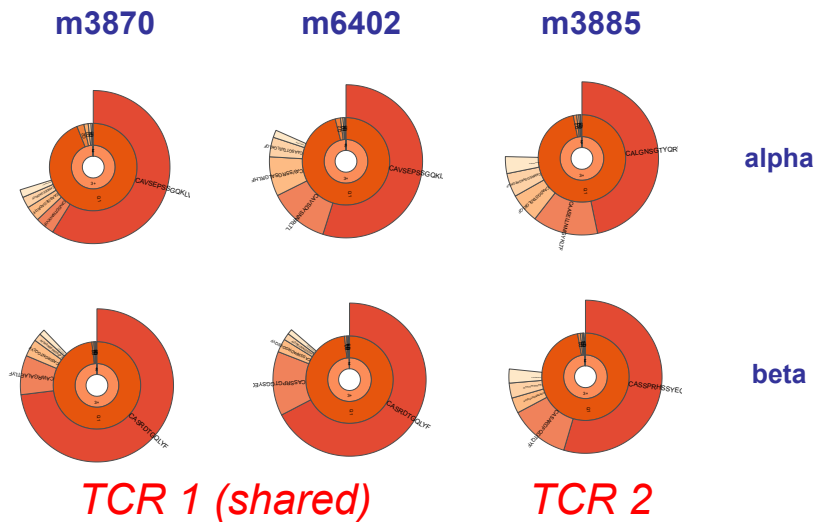
## SIINFEKL-dextramer+ CD8+ T-cells



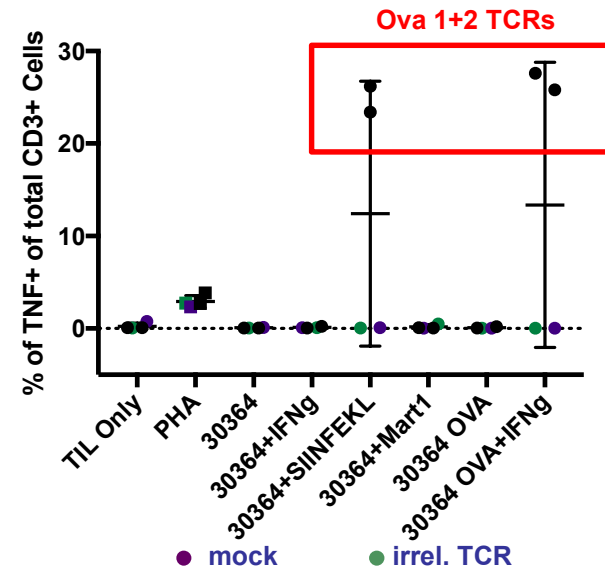
## RNA-electroporated human TILs



## TCR-seq (mouse protocol)

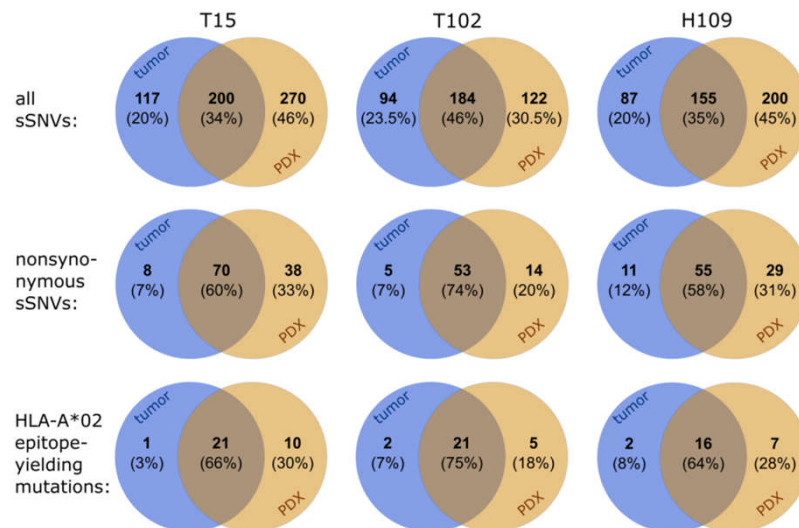


## Functional testing

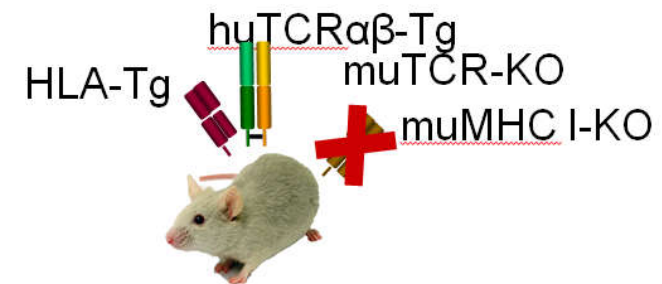


# Reverse immunology strategy

- Identification of potential neo-epitopes based on tumor mutanome data
- Isolation of reactive TCRs from human PBMC and HLA/huTCR-tg mice



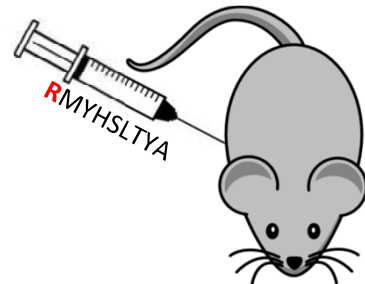
## HLA/human TCR-transgenic mice A2.1; DR4, DR1



Blankenstein & Willimsky



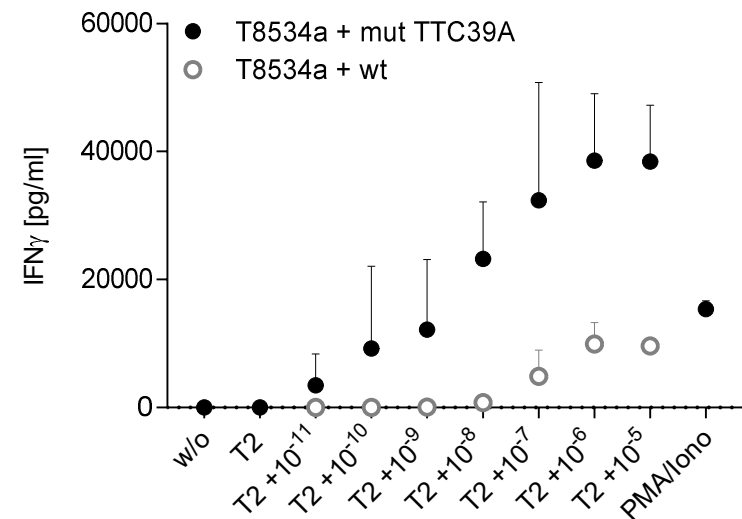
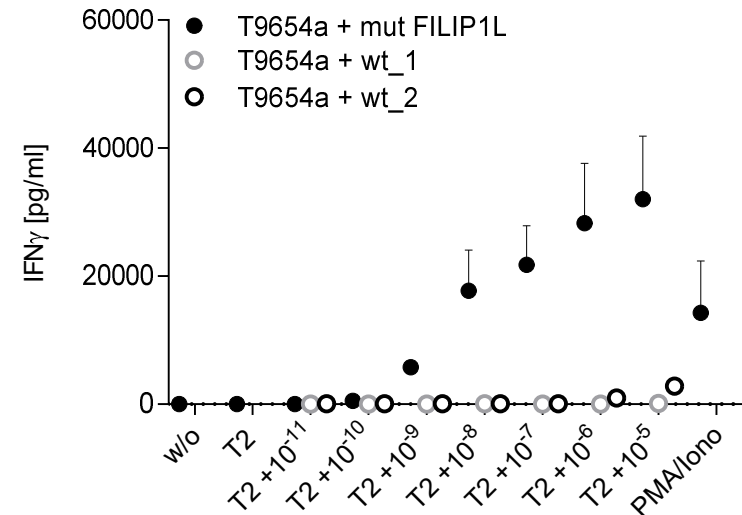
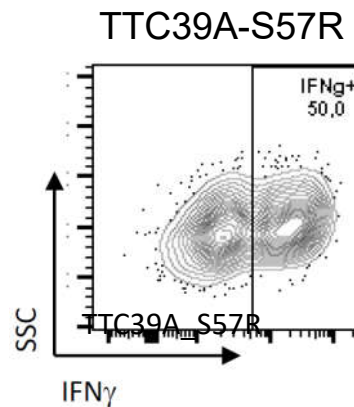
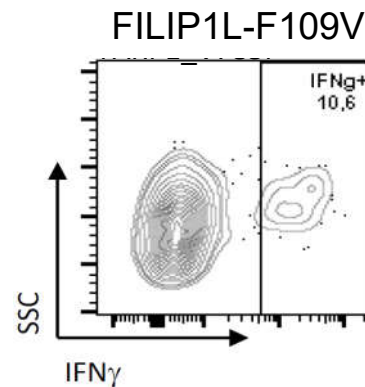
# TCRs selectively recognizing neo-epitopes



human HLA-A\*02:01  
human TCR  $\alpha$ ,  $\beta$

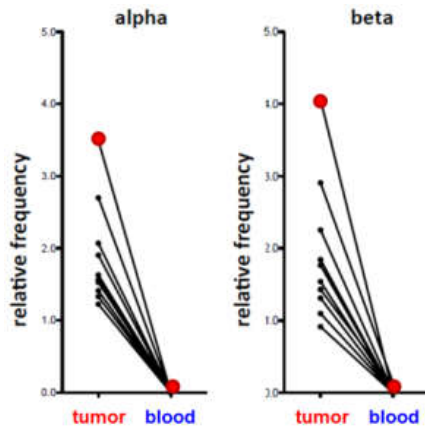
## Workflow:

- ✓ Peptide immunization
- ✓ Isolation IFN $\gamma$ + T-cells
- ✓ Cloning TCRs
- ✓ Expression in human T-cells
- ✓ Testing against peptide
- Testing against PDX
  - in vitro
  - in vivo



# TCR gene therapy instead of TIL therapy

## Tumor-dominant TCRs

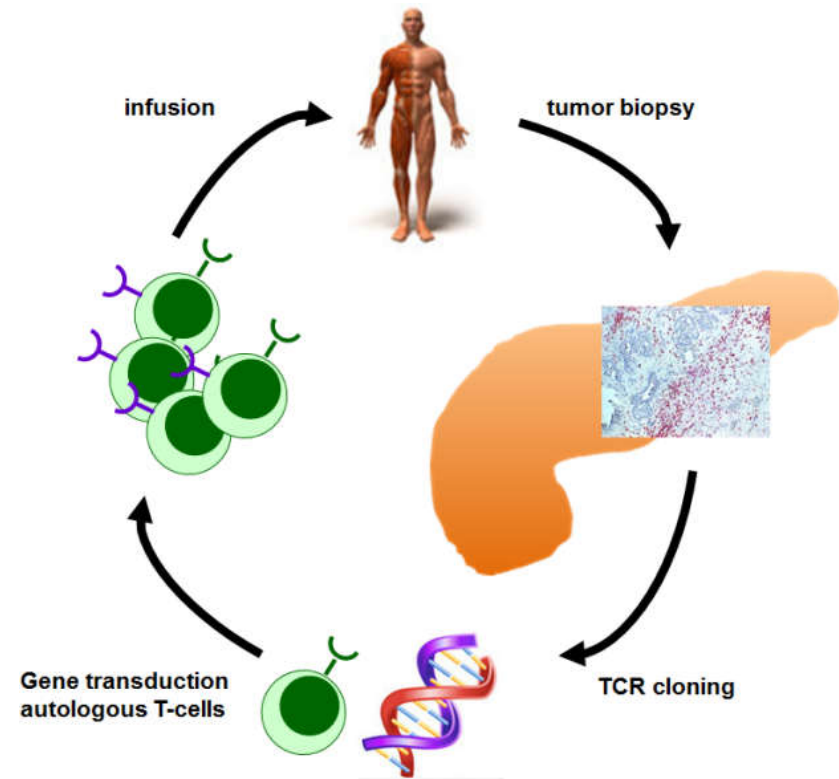


## Shotgun cloning tumor-dominant TCRs

## Tumor DNA/RNA-seq



## Mutanome-based generation TCRs (recurrent antigens > patient-spec. antigens)



# TCR gene therapy of PDA: Stardate 2318?



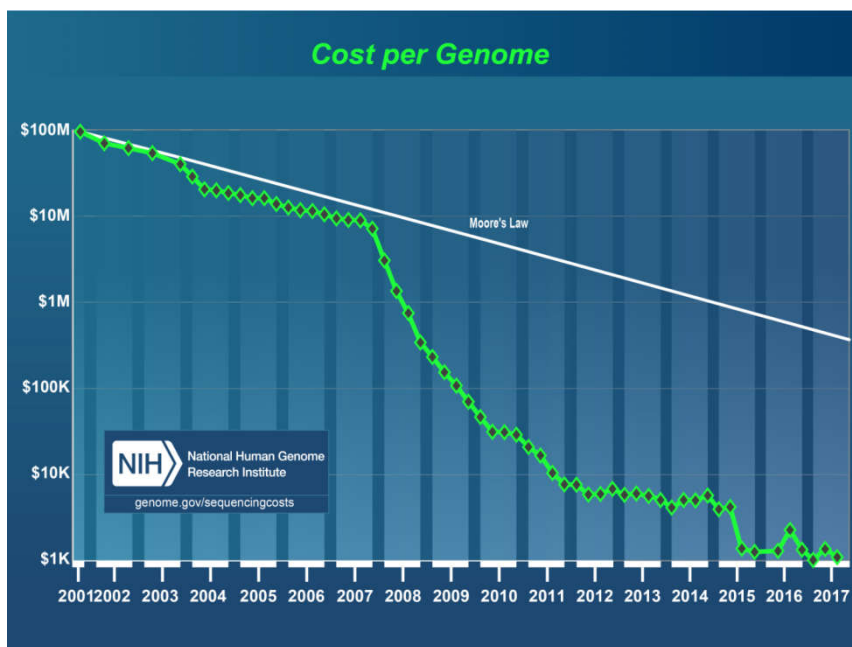


# Personalized cancer therapy is the future!








Tumor mutanome NSG will soon become SOC procedure

Anything scalable can become economically profitable/feasible




*Europe is lagging behind in clinical translation of immuno oncology!*

 <p>Paul H. Alley, MD Medical Director</p>	 <p>Two projects in phase I and one in Phase II.  Autologous T-Cell.  (In the Top 3 Biggest Pharma companies worldwide) Market cap € 21.5Bn</p>	 <p>One Candidate in Phase II, one in Phase I and one at a preclinical stage.  In collaboration with Alpine Immune Science, Amgen (at a preclinical stage).  IPO €12.7M - Market Cap €1.95Bn</p>
	 <p>Registration for Clinical Trial, plus several project at preclinical stage.  Collaboration with Servier &amp; Pfizer  With Allogeneic T-Cell (UCART).  Market cap €1.07Bn</p>	 <p>Two candidates in Phase II, and Four in phase I stage.  Collaboration with Opus Bio (phase I) and Pre-clinical: Editas Medicine, Fate Therapeutics, MabVax Therapeutics.  Signed a €950M upfront deal with Celgene, raised €535M. Market cap €4.92Bn</p>

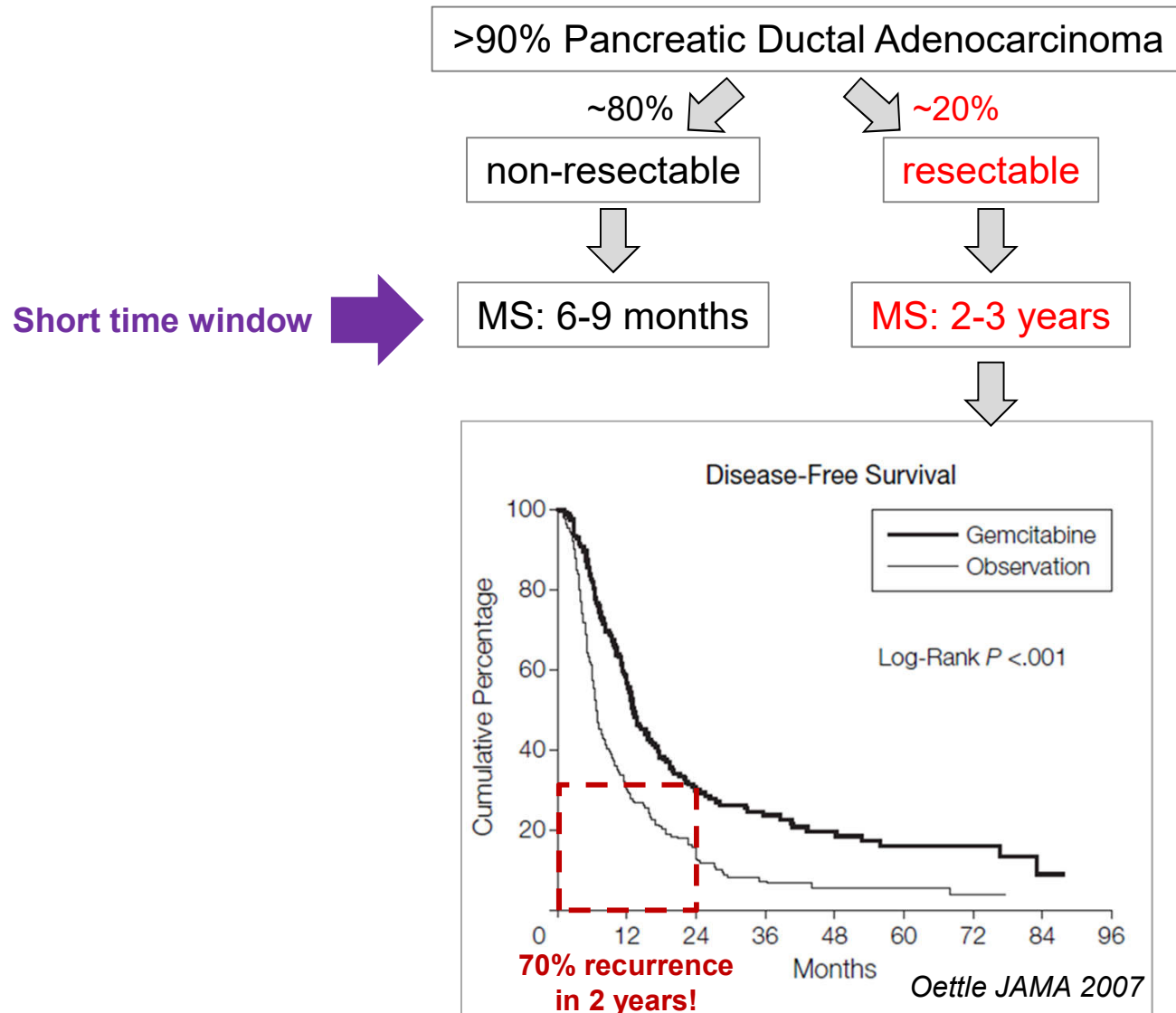


# Towards T-cell therapy of PDA: pick your battles

Non-resectable disease (primary tumor)

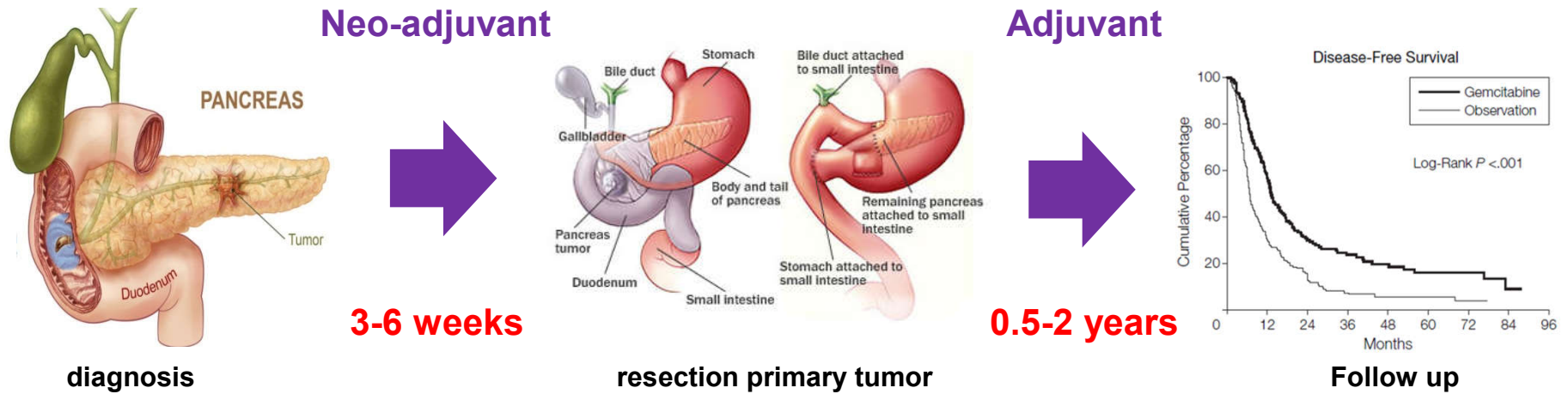


# Focus on primary resectable disease



- Longer time window
- Primary tumor
  - Mutanome
  - TILs
  - TCRs
- Clinical markers
  - Stage
  - LN mets
  - R0/R1
  - CA19-9
  - ctDNA

# Strategies towards harnessing T-cell response in patients with primary resectable PDA



**Agonist CD40 Ab**

**Adoptive T-cell therapy**

**Anti-PD-L1/TGFβ-trap**

**Mutanome-based vaccines**

**PD-biomarker studies!**



# My Pancreatic Cancer Lab @ German Cancer Research Center



*Div. Molecular Oncology of Gastrointestinal Tumors*





# IAPACA Trial : Agonist anti-CD40 Ab in PDA

EudraCT No. 2016-000496-24

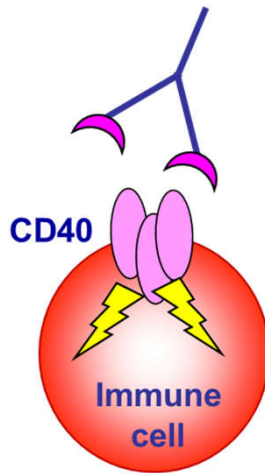


Martin Glennie

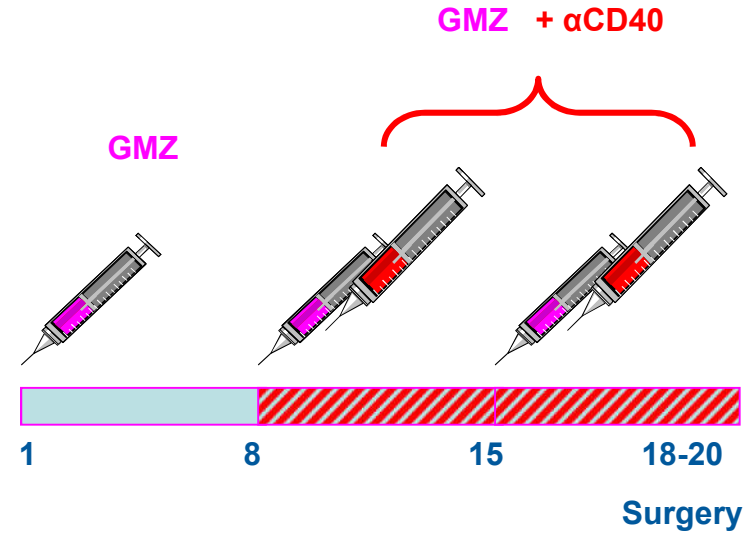


Peter Johnson

ChiLob7/4



Sahin



UniversitätsKlinikum Heidelberg  
UNIVERSITY OF  
**Southampton**



**dkfz.**  
GERMAN  
CANCER RESEARCH CENTER  
IN THE HELMHOLTZ ASSOCIATION

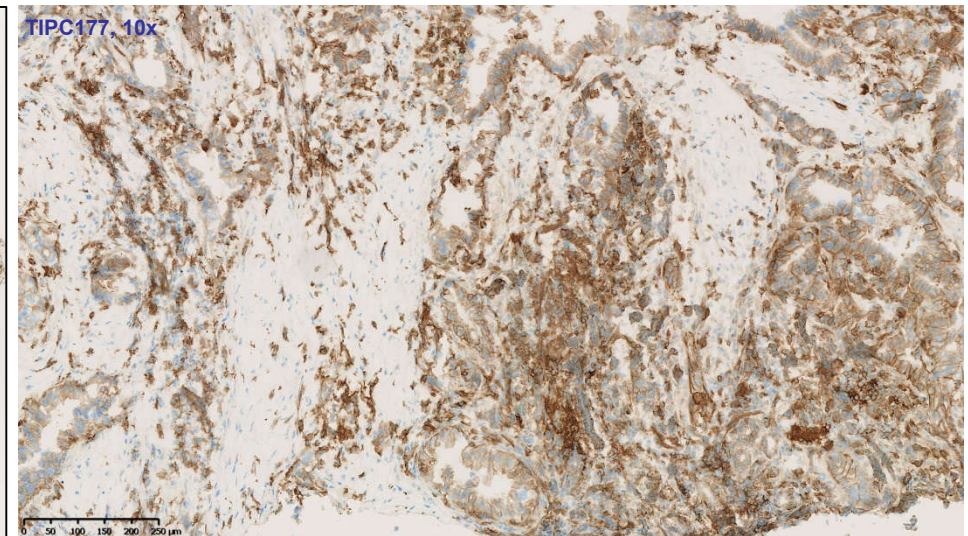
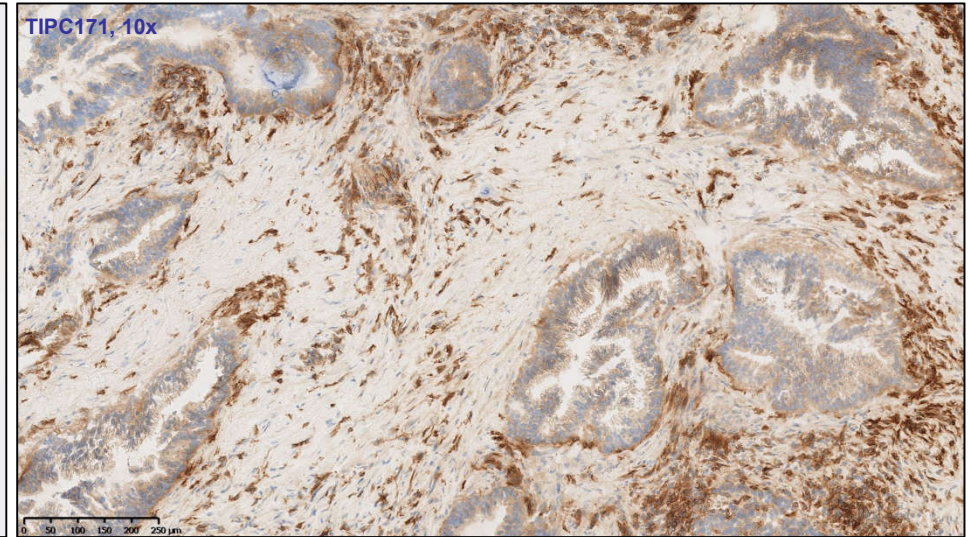
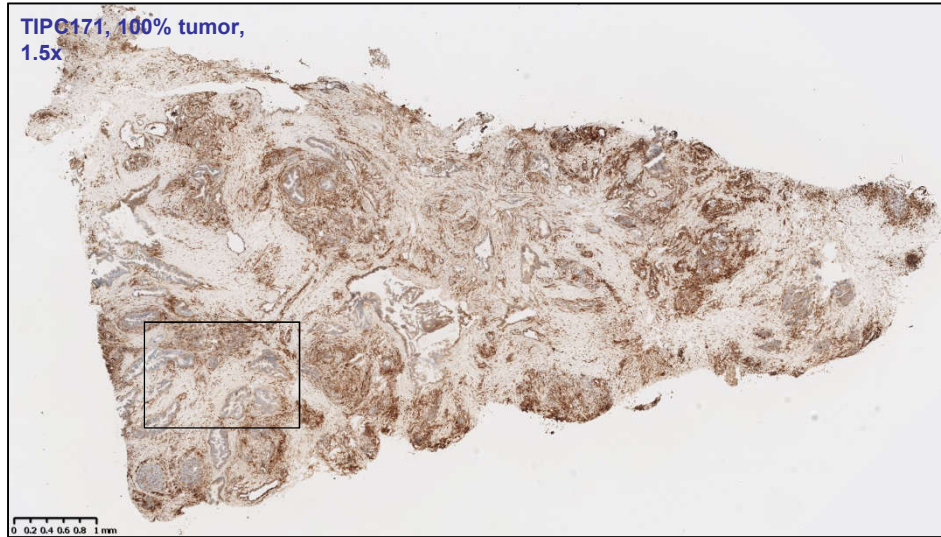
University Hospital Southampton **NHS**  
NHS Foundation Trust

**ChiLob7/4** 20 mg/mL [2.6 mL Monoclonal Antibody  
For i.v. infusion after dilution. For clinical trial use only.  
Study Name / Studienname: IAPACA  
Eudra-CT No. / Nr.: 2016-000496-24  
Keep vial and box together! Store at -20±5 °C  
Batch No./ Ch.-B./ Lote: MAB.16.02a

**Sponsor / Sponsor:**  
Universitätsklinikum Heidelberg,  
Medizinische Fakultät der Ruprecht-Karls-Universität Heidelberg  
Im Neuenheimer Feld 672, D-69120 Heidelberg  
Study Name / Studienname: IAPACA  
Eudra-CT No. / Nr.: 2016-000496-24

**ChiLob7/4** 20 mg/mL [2.6 mL Monoclonal Antibody 1 vial  
For i.v. infusion after dilution according to clinical study protocol. For clinical trial use only.  
Keep vial and box together!  
Study Name: IAPACA Expiry date: see box label  
Patient ID: Store at -20±5 °C

# CD40 expression in PDA

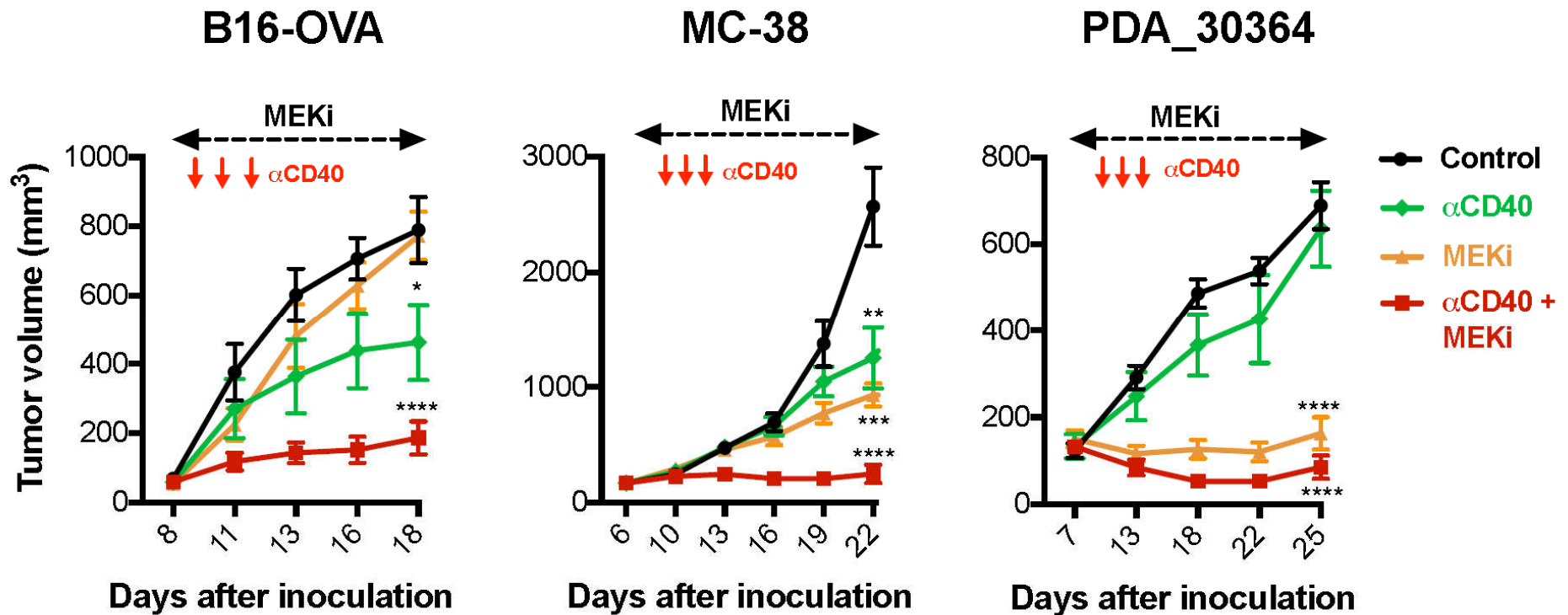




# Synergistic anti-tumor action by MEKi and CD40 Ab



Daniel Baumann



*Therapeutic impact depends on T-cells*

**DO NOT POST**

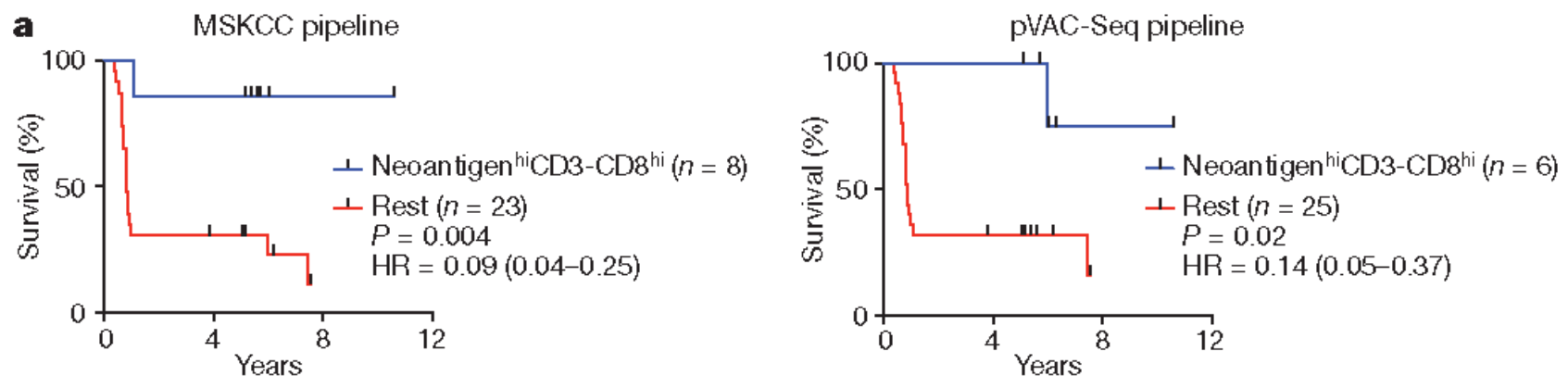
# Interest in T-cell immunity against PDA mutanome is on the rise 47

## LETTER

doi:10.1038/nature24462

### Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer

Vinod P. Balachandran<sup>1,2,3</sup>, Marta Luksza<sup>4</sup>, Julia N. Zhao<sup>1,2,3</sup>, Vladimir Makarov<sup>5,6</sup>, John Alec Moral<sup>1,2,3</sup>, Romain Remark<sup>7</sup>, Brian Herbst<sup>2</sup>, Gokce Askan<sup>2,8</sup>, Umesh Bhanot<sup>8</sup>, Yasin Senbabaoglu<sup>9</sup>, Daniel K. Wells<sup>10</sup>, Charles Ian Ormsby Cary<sup>10</sup>, Olivera Grbovic-Huezo<sup>2</sup>, Marc Attiyeh<sup>1,2</sup>, Benjamin Medina<sup>1</sup>, Jennifer Zhang<sup>1</sup>, Jennifer Loo<sup>1</sup>, Joseph Saglimbeni<sup>2</sup>, Mohsen Abu-Akeel<sup>9</sup>, Roberta Zappasodi<sup>9</sup>, Nadeem Riaz<sup>6,11</sup>, Martin Smoragiewicz<sup>12</sup>, Z. Larkin Kelley<sup>13,14</sup>, Olca Basturk<sup>8</sup>, Australian Pancreatic Cancer Genome Initiative\*, Mithat Gönen<sup>15</sup>, Arnold J. Levine<sup>4</sup>, Peter J. Allen<sup>1,2</sup>, Douglas T. Fearon<sup>13,14</sup>, Miriam Merad<sup>7</sup>, Sacha Gnjatic<sup>7</sup>, Christine A. Iacobuzio-Donahue<sup>2,5,8</sup>, Jedd D. Wolchok<sup>3,9,16,17,18</sup>, Ronald P. DeMatteo<sup>1,2</sup>, Timothy A. Chan<sup>3,5,6,11</sup>, Benjamin D. Greenbaum<sup>19</sup>, Taha Merghoub<sup>3,9,18</sup>§ & Steven D. Leach<sup>1,2,5,20</sup>§

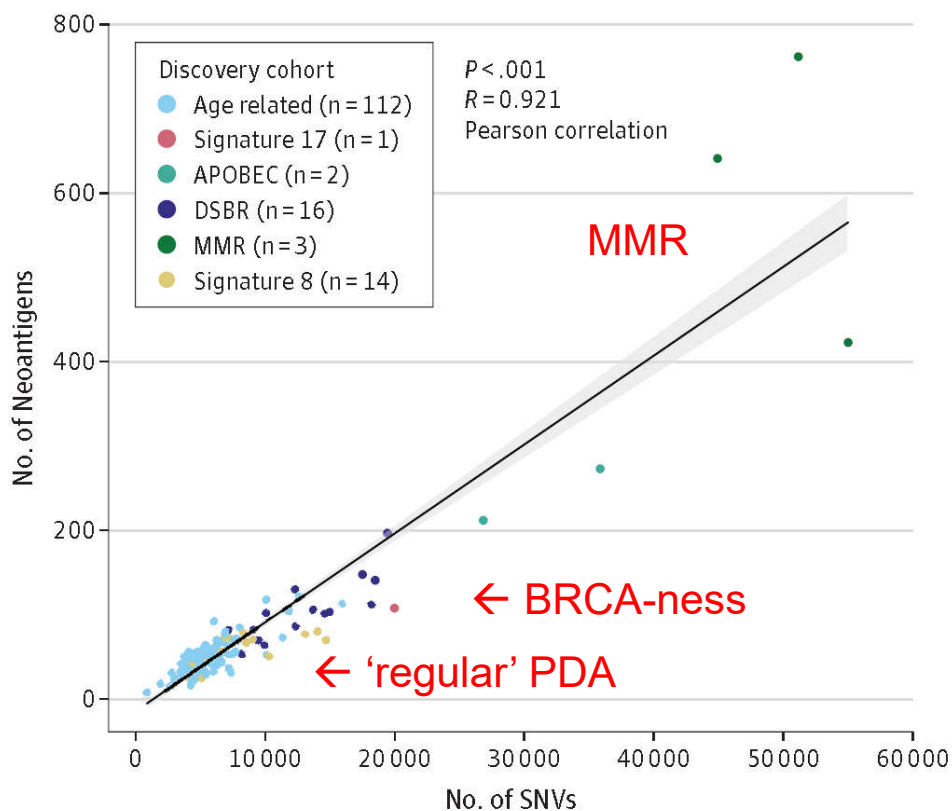




# Interest in T-cell immunity against PDA mutanome is on the rise

JAMA Oncology | Original Investigation

## Association of Distinct Mutational Signatures With Correlates of Increased Immune Activity in Pancreatic Ductal Adenocarcinoma



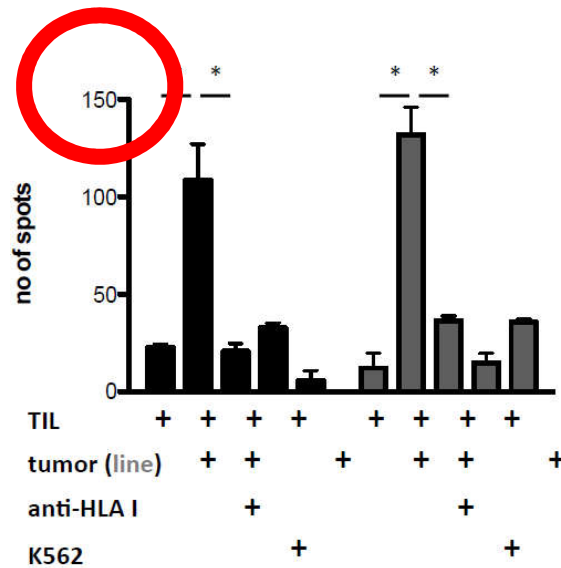
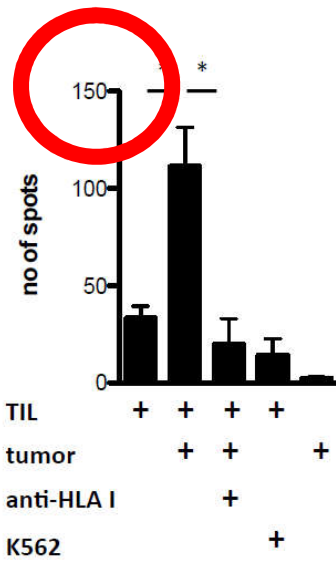
D; Lee Timms, MSc; Sangeetha N. Kalimuthu, MD; Iris Selander, MSc; eng-Yue, BSc; Ivan Borozan, PhD; Vincent Ferretti, PhD; Robert C. Grant, MD; Daniel Palmer, PhD; Paula Ghaneh, PhD; John P. Neoptolemos, MD; Michael A. Hollingsworth, PhD; Alana Sherker, BSc; Daniel Durocher, PhD; ollett, MD; Michael H. A. Roehrl, MD, PhD; Prashant Bavi, MD; 1, PhD; Ludmil B. Alexandrov, PhD; Malcolm Moore, MD; Bradly G. Wouters, PhD; PhD; Steven Gallinger, MD, MSc

# Ex vivo expanded PDA TILs are tumor-reactive

→ TIL therapy

## Low frequency of tumor-reactive T-cells

17/20



Free Survival

Percentage

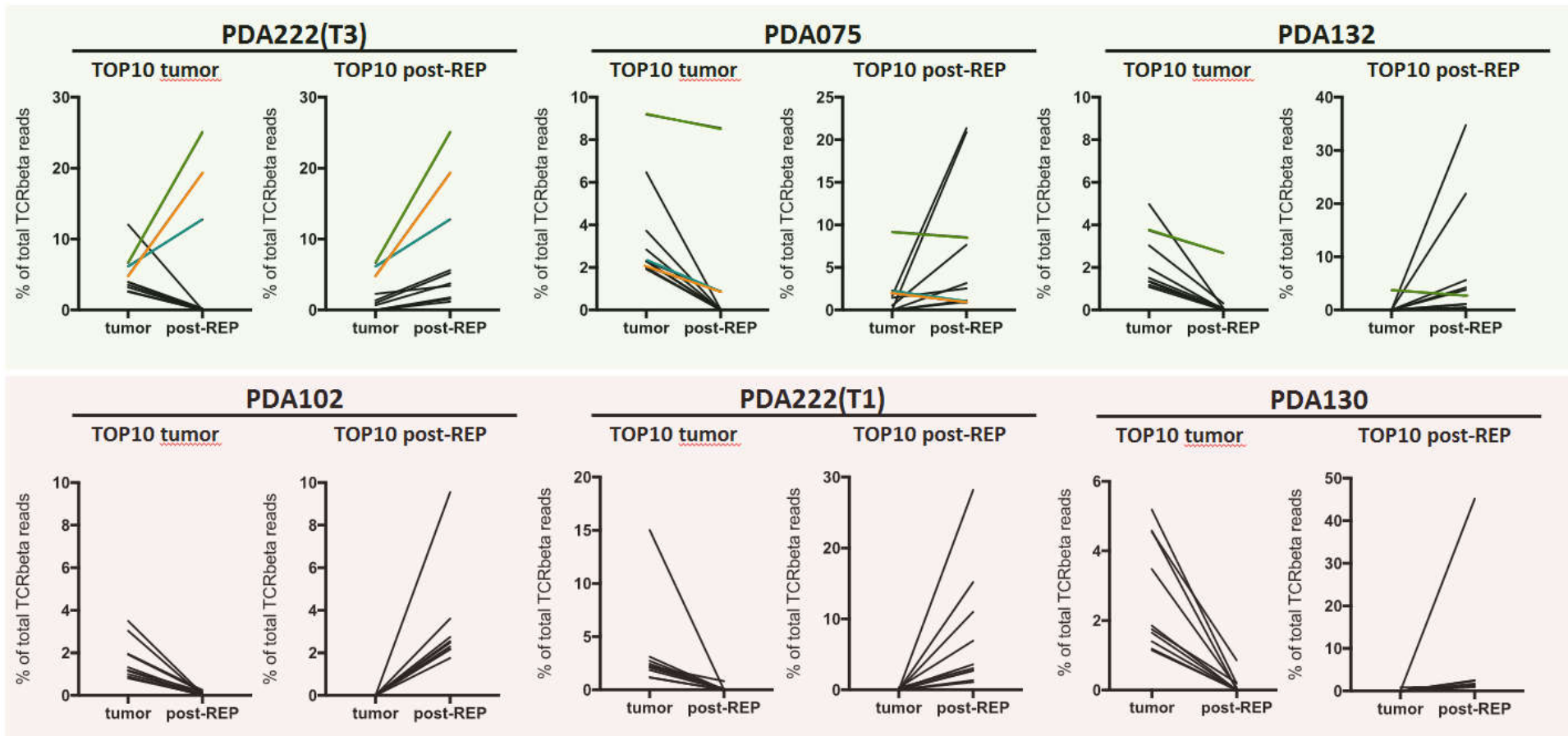
Months

70% recurrence in 2 years!

Oettle JAMA 2007

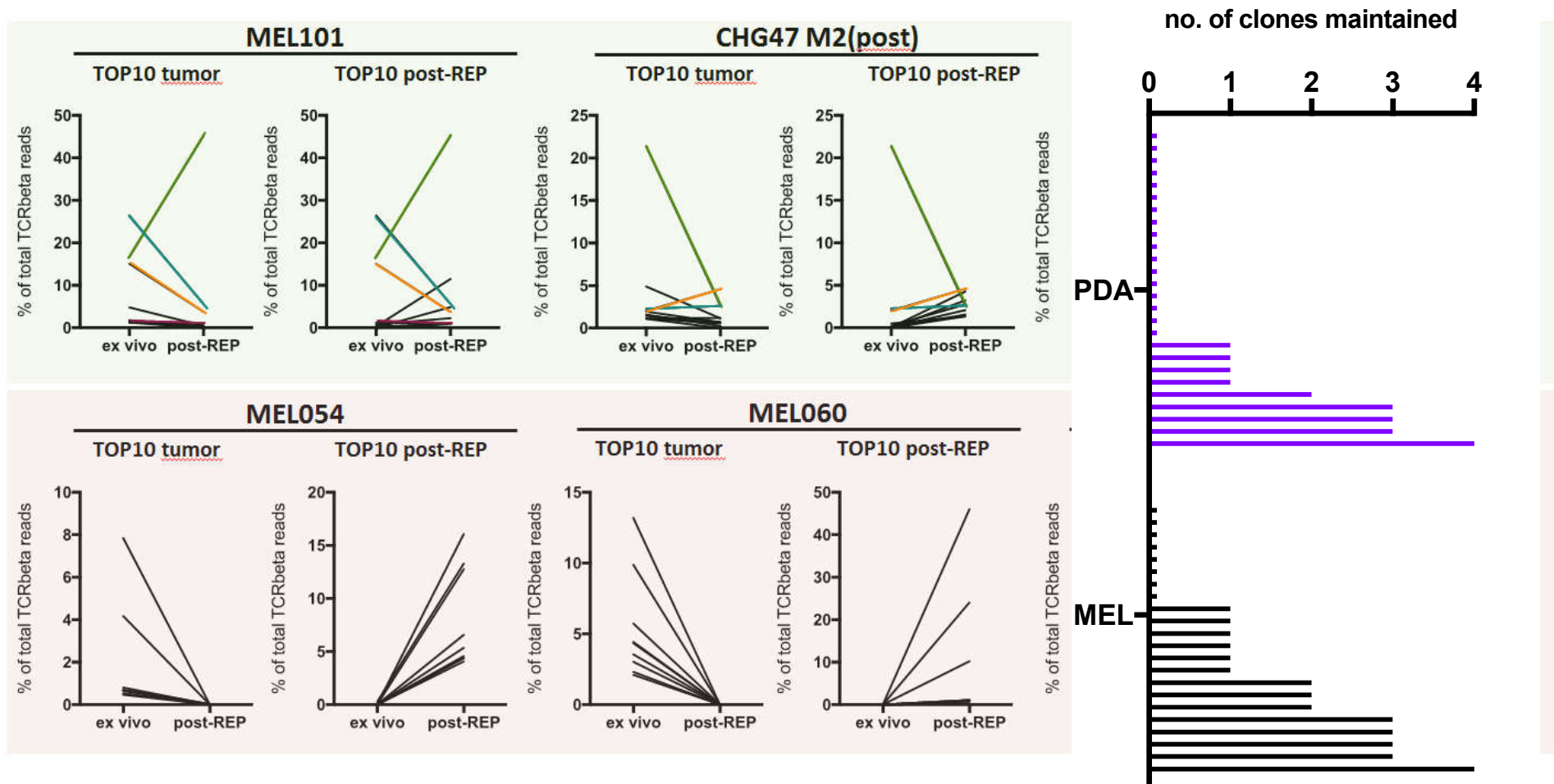
# Ex vivo TIL expansion can result in loss tumor-dominant TCRs

## PDA



# Ex vivo TIL expansion can result in loss tumor-dominant TCRs

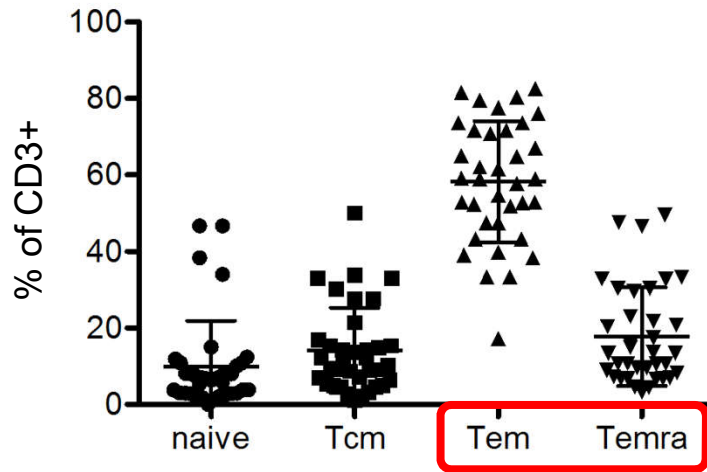
## Melanoma



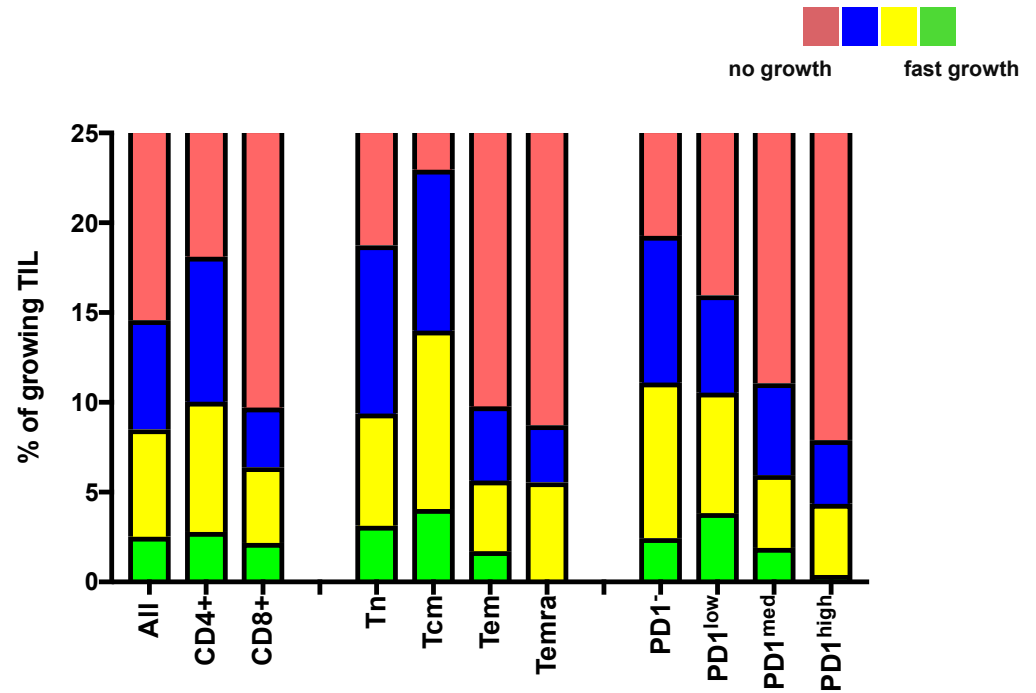
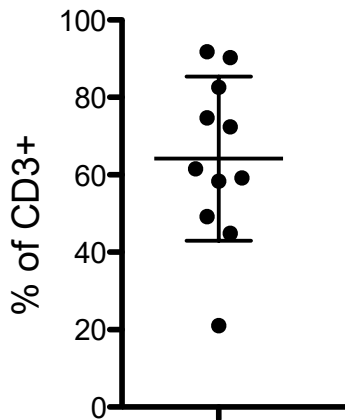


# Poor outgrowth of activated T-cell clones as observed in limiting dilution cultures

## Memory markers



## PD-1 receptor



→ Optimization of TIL expansion needed