SCIENCE MEETS LIFE

Single-Cell Analysis During Checkpoint Immunotherapy Reveals the Tumor Antigen Landscape, Advancing Cancer Vaccine Development

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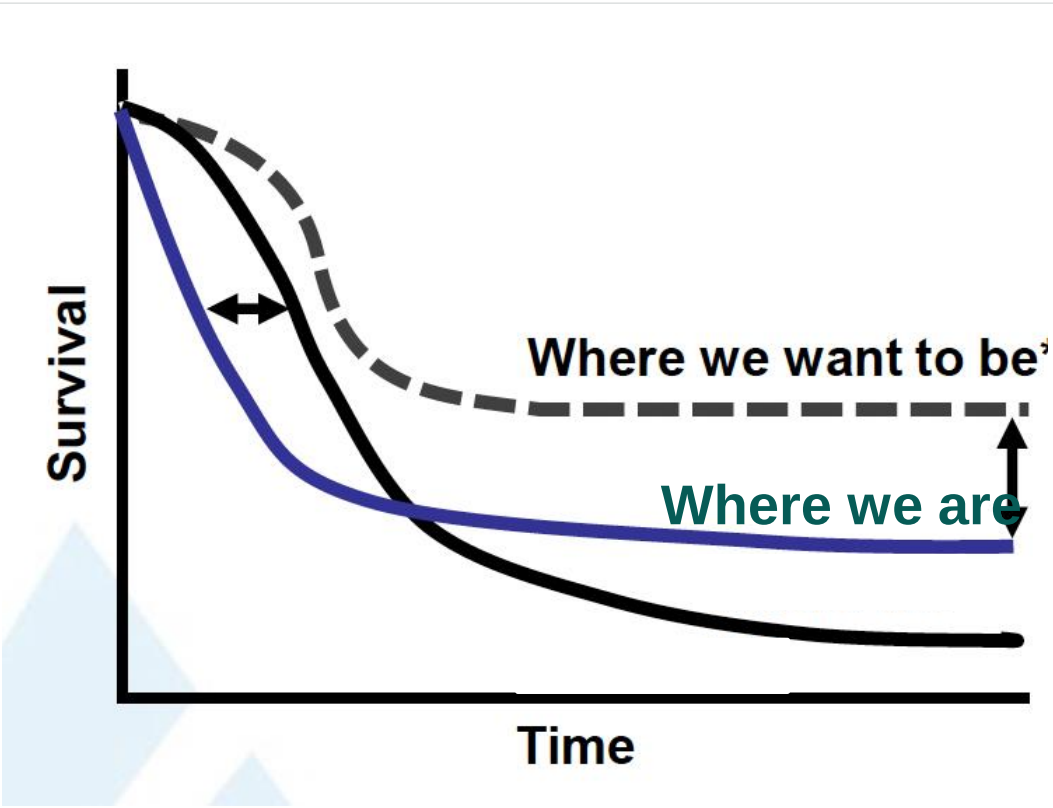
May 22th 2025
Flanders Vaccine, Antwerp, Belgium

VIB KU LEUVEN

The future of cancer immunotherapy is ‘in the combination’



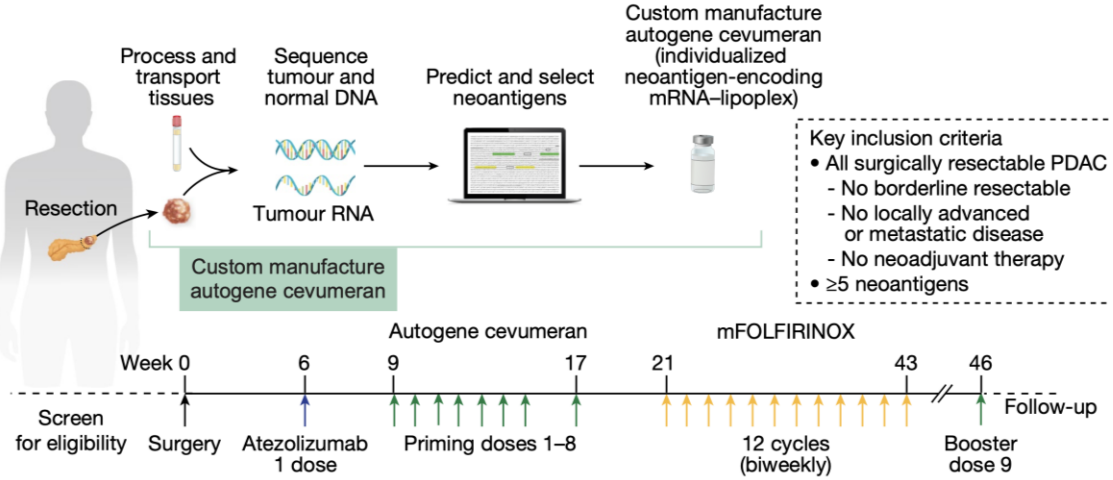
Promising therapeutic targets & combination strategies

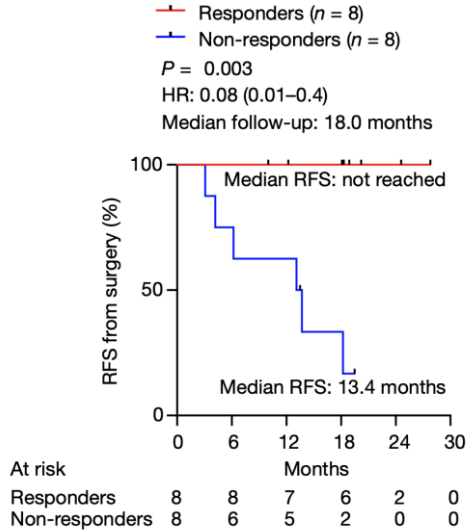
- Immune checkpoint inhibitors (ICI) + conventional treatments
- Next-gen inhibitory receptors
- Next-gen immune agonists
- Bispecific antibodies
- ICI + anti-angiogenic agents
- Immunomodulatory drugs
- Tumor vaccines
- ICI + cellular immunotherapy
- New targets against resistance

**Hypothetical chart illustrating a scientific concept for which few supporting data are available so far. This chart is not intended to predict what may actually be observed in clinical studies.*

Mutanome-driven vaccination improves the outcome of checkpoint immunotherapy: pancreatic cancer

BIONTECH

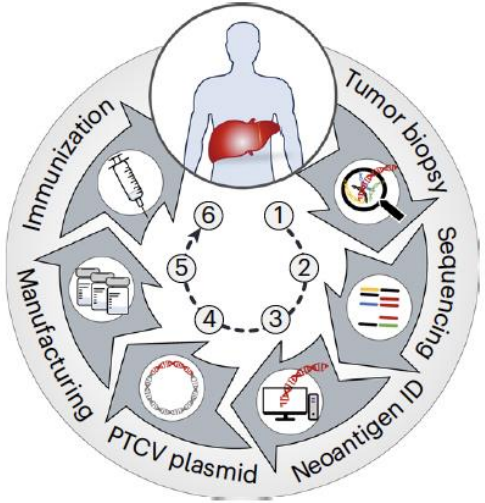


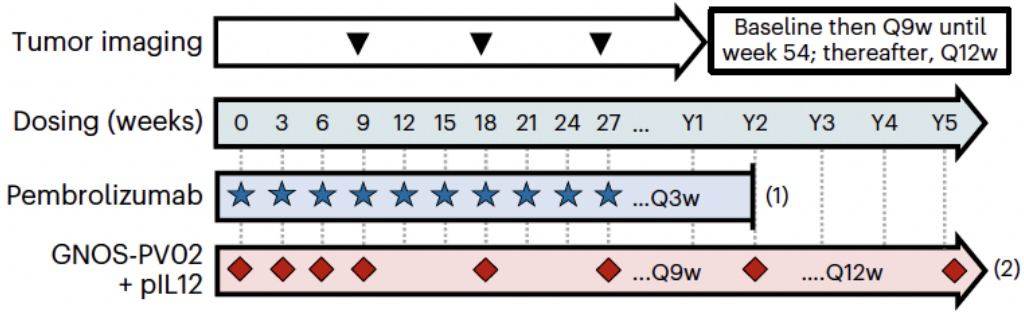


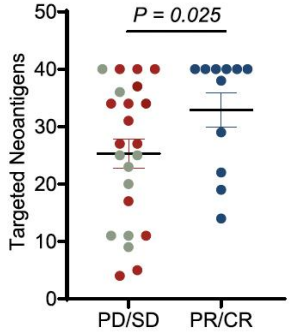
➢ Also described for melanoma, renal cell carcinoma, NSCLC, *etc.*

Rojas et al., Nature 2023

In HCC, clinical response to anti-PD1 + tumor vaccination correlates with the number of epitopes in the vaccine







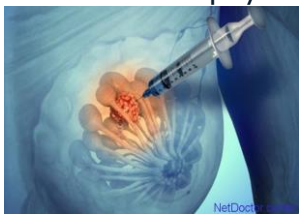
	PD/SD	PR/CR
Average # of neos	25	33
Median # of neos	27	40

- Not all low TMB tumors contain a lot of neoantigens
- Can we identify additional tumor antigens for vaccination

Yarchoan M et al., Nat Med 2024

Work in my lab has focused on TME dynamics during checkpoint immunotherapy using single-cell analyses

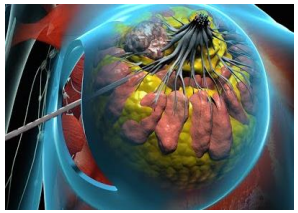
Needle biopsy



1 dose of anti PD-1

T-cell expansion

Resected tumor



NO in 70% of patients

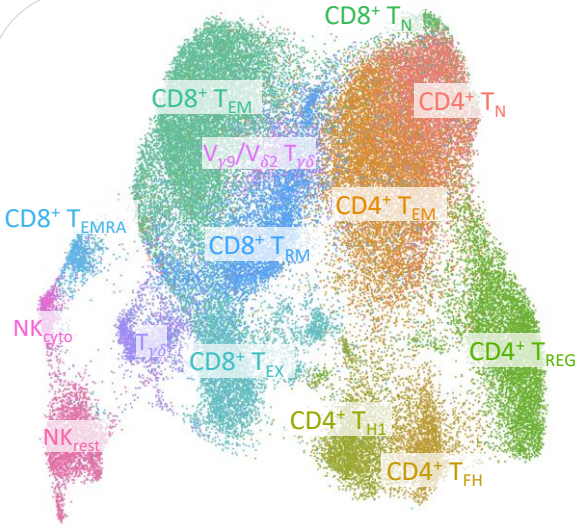
YES in 30% of patients

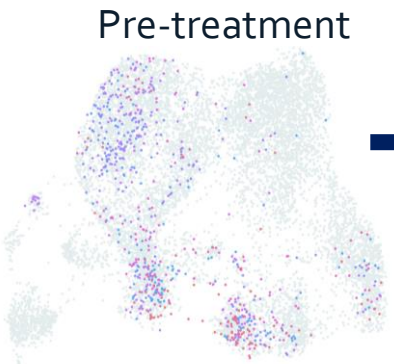
8% → 32%
of T-cells are clonotypes

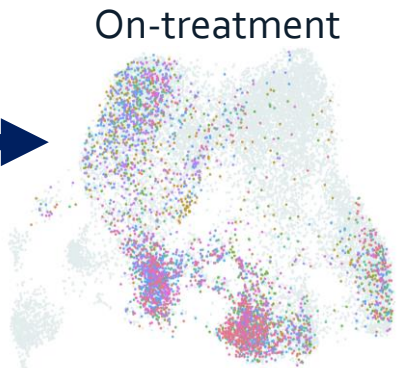
Single-cell TCR-sequencing identifies **T-cell expansion** as an early response marker to checkpoint immunotherapy

Bassez A. et al., ***Nature Med 2021***

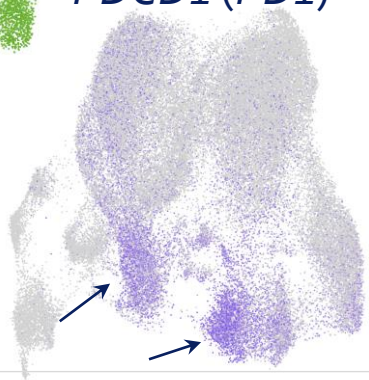
Expanded T-cells mainly comprised re-activated $CD4^+$ T_{H1}- and $CD8^+$ T_{EX}-cells



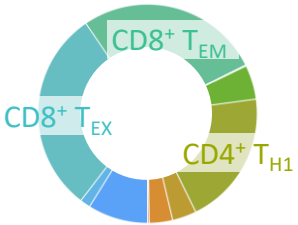




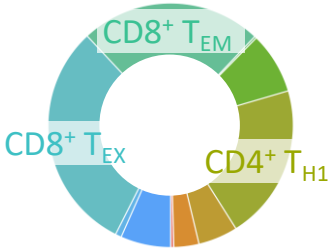
PDCD1 (PD1)



% of expanded T-cells pre-treatment

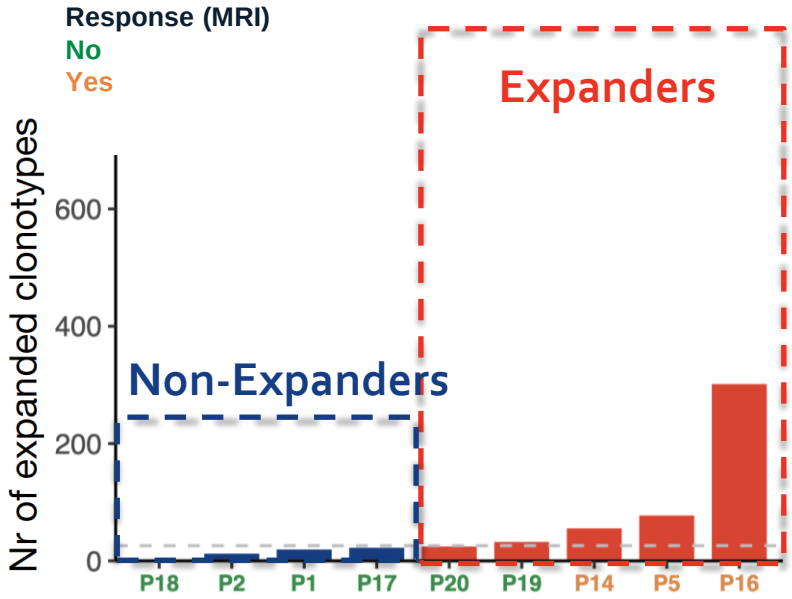


% of expanded T-cells on-treatment



Bassez A. et al., ***Nature Med 2021***

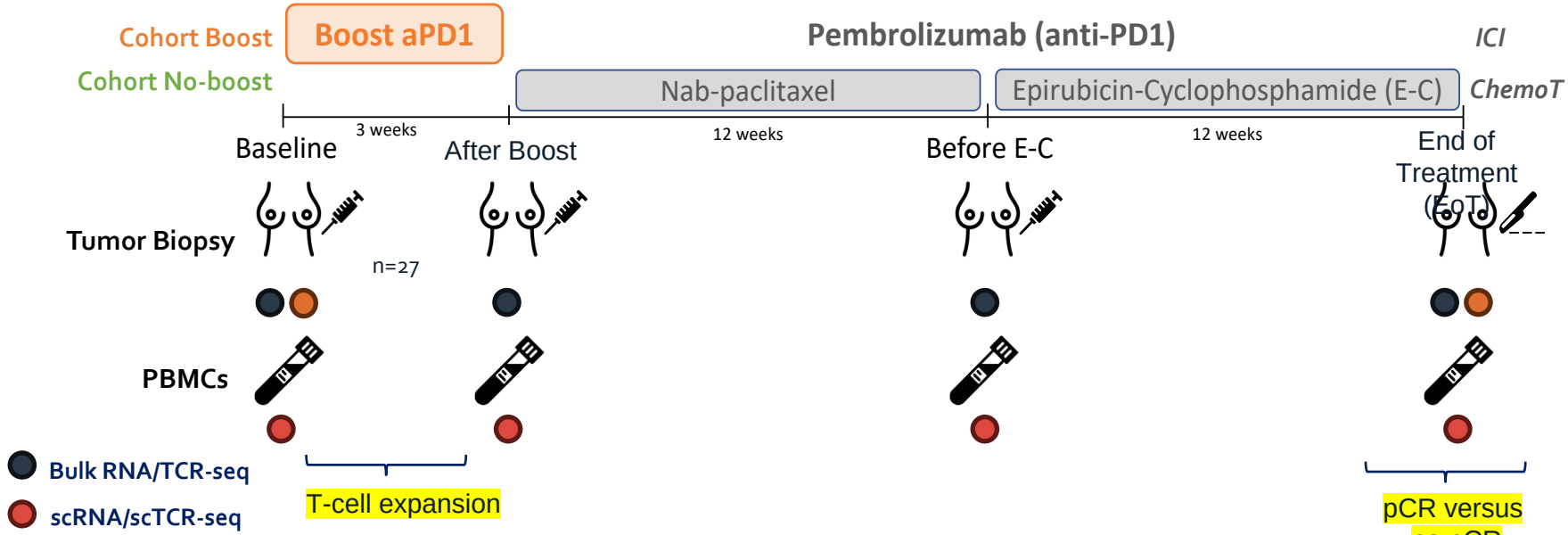
Clinical response to aPD1 is characterized by the extent to which $CD8^+$ T_{EX} AND $CD4^+$ T_{H1}-cells expand



- T-cell expansion translates into clinical response (MRI)

Nederlof I, …, Lambrechts D, Kok M.
Nature Med 2024

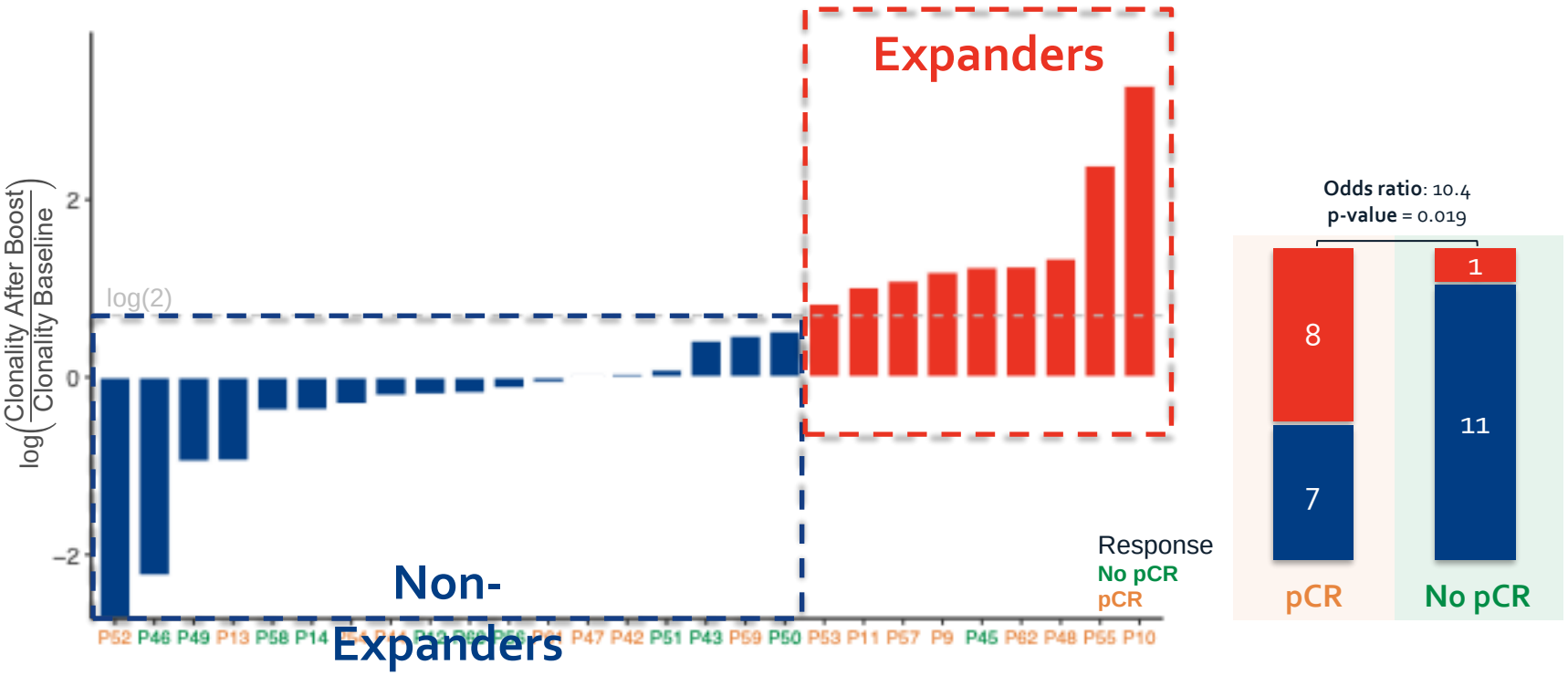
NeoImmunoBoost: neo-adjuvant chemo-immunotherapy trial in early TNBC (KeyNote regimen)



➢ Anti-PD1 boost to explore effect of T-cell expansion at end-of-treatment (EoT)

Collaborative w P Fasching, H Hubner, Erlangen, Germany

Expanders develop a pathological complete response (pCR)

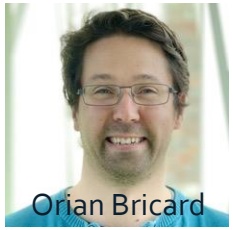


➢ T-cell expansion translates into pCR

Expanding T-cells are tumor-reactive, driving the clinical response to checkpoint immunotherapy

What are the tumor antigens recognized by expanding T-cells during checkpoint immunotherapy ???

TWISTAR: Transcriptome-wide screening of T cell antigen research



3. APC cell line with patient-matched HLA

+ transduction of tumor transcriptome with RFP reporter

4. Screen for GFP and RFP

1. scTCRseq

Lentiviral TCR

Stable universal T-cell line

Expanding TCR encoding

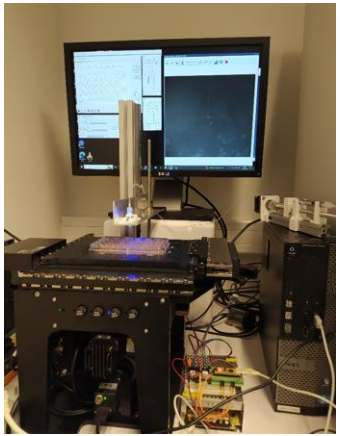
2. NFAT-GFP reporter Jurkat T-cell line

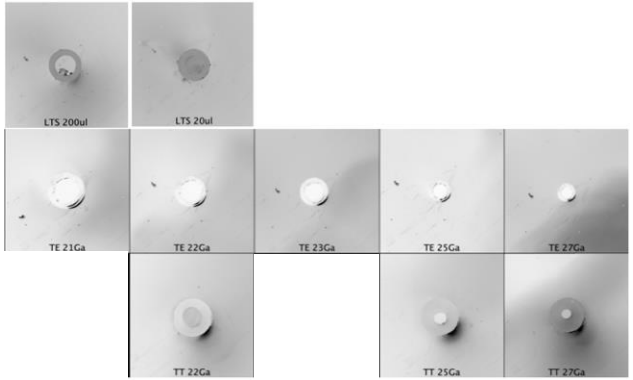
Unique approach: we start from the scTCR data !!

Unpublished

Provirus antigen coding sequence

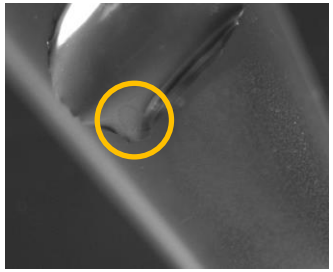
Fast-track cell cluster picking of positive cells (amongst 10M of APCs) and antigen identification





Prototype of syringe pump and needle allowing motorized picking of positive cells

Transfer in 0.2ml tube →



2 days

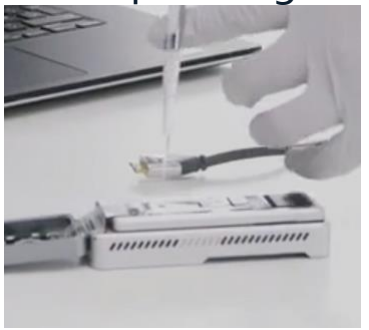
Cell Direct cDNA

↓

provirus PCR

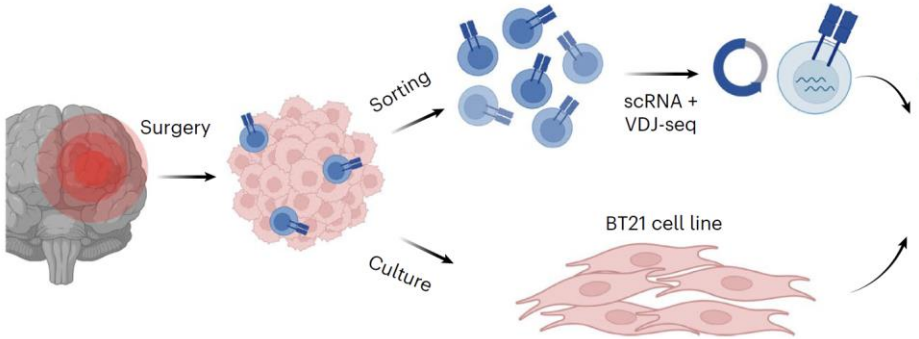
↓

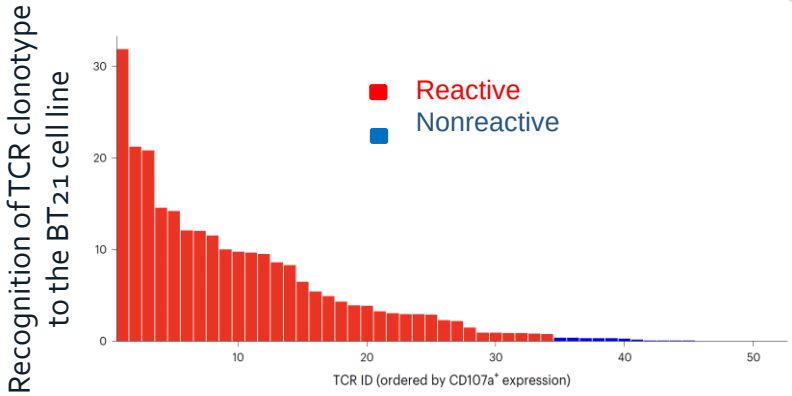
ONT Flongle sequencing



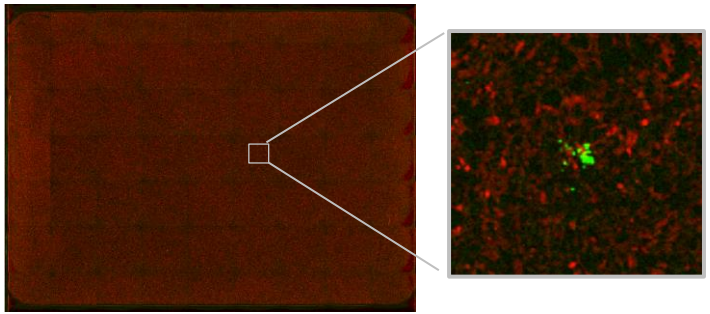
- 200-300 bp sequence identified encoding for the antigenic peptide

TWISTAR on BT21 melanoma brain metastasis cell

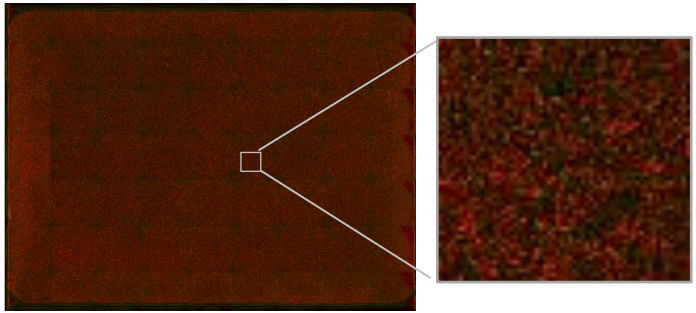




Reactive TCRs



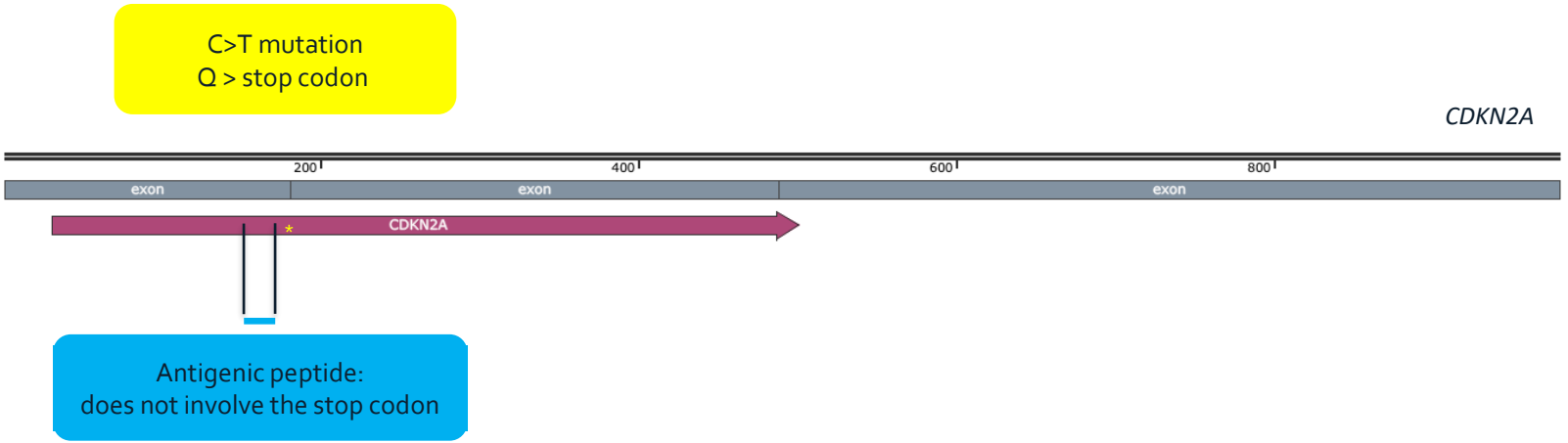
Nonreactive TCRs



- 50 TCRs selected: 30 reactive and 20 non-reactive
- For 21 out of 30 reactive TCRs a hit, 20 non-reactive TCRs were negative

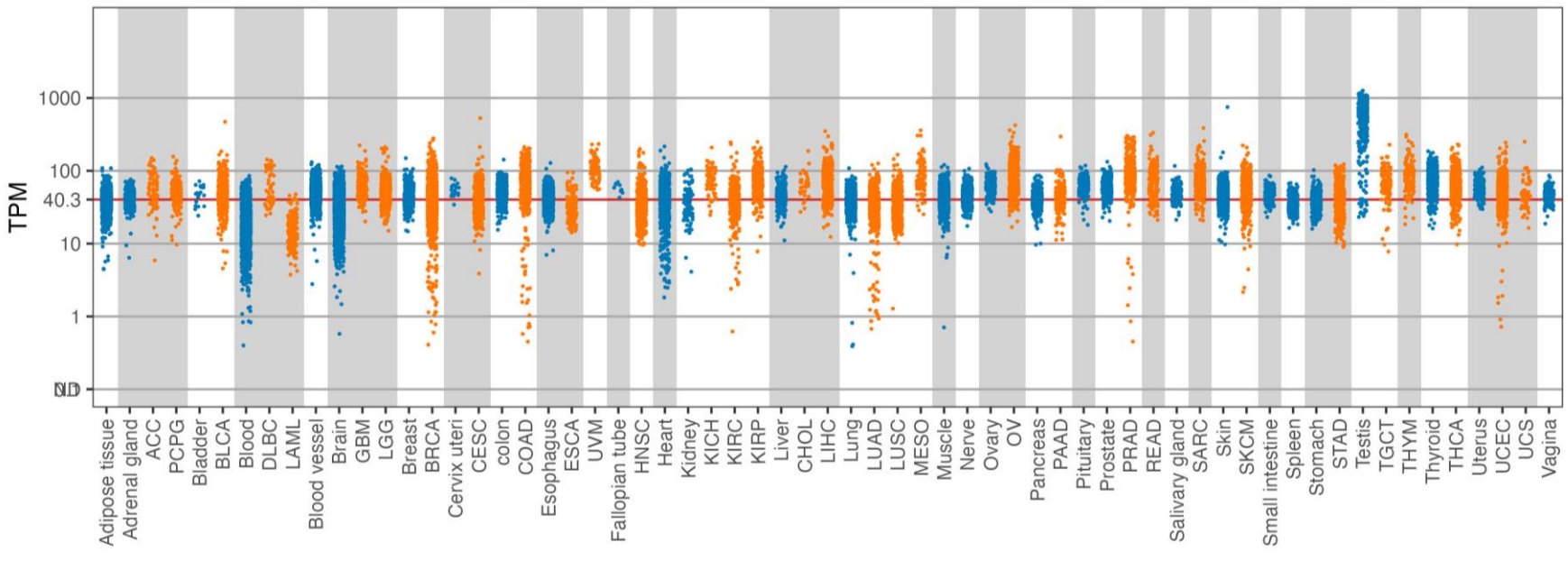
In collaboration with E Green, M Platten, DKFZ

EX1: Some antigens are unique to a patient and are the result of complex gene regulation events



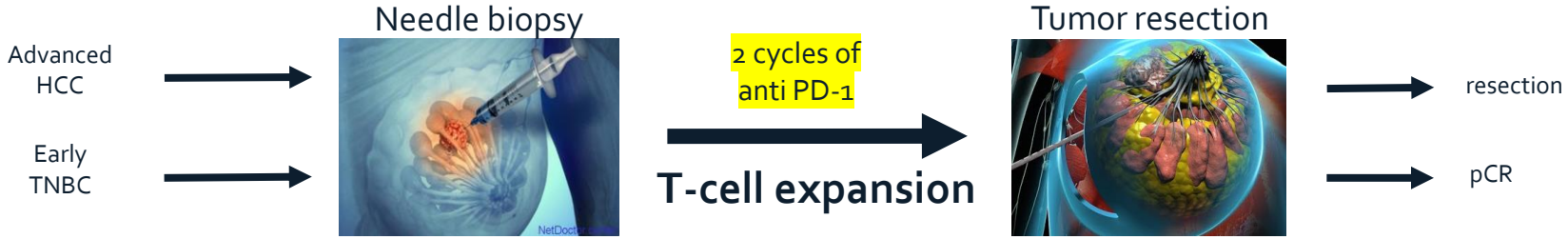
➢ Hypothesis: stop codon mutation changes antigens processed for CDKN2A by proteasome

EX2: A shared tumour antigen upregulated in most tumors, but also widely present in normal tissue



- Expression is high in most tumors from TCGA
- Expression also in normal tissue, albeit at lower levels

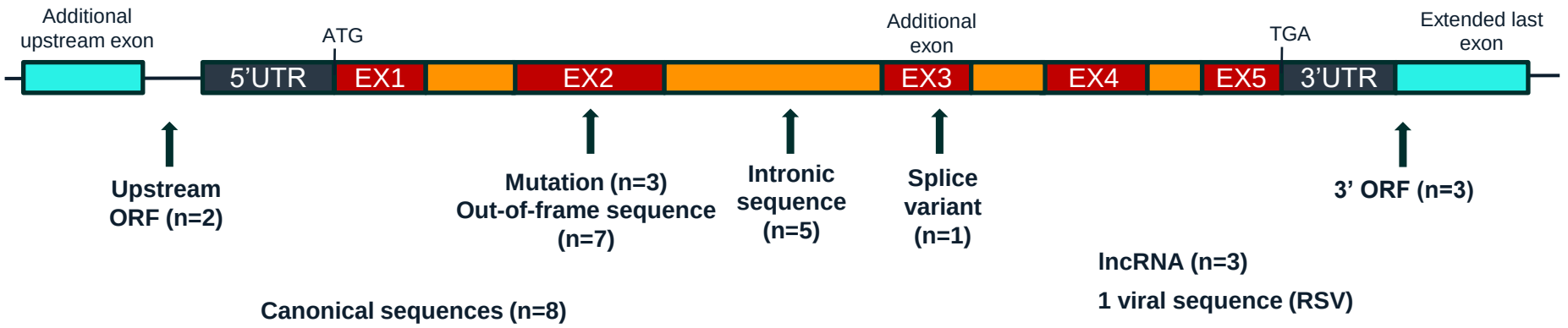
TWISTAR on HCC and BC patients 'hyper'-responding to anti-PD(L)1



We selected the following T-cells based on scTCRseq:

- PD1^{+} T-cells &
- Prominent expansion of CD8^{+} T-cells &
- CD8^{+} T_{EM} or T_{EX} activated phenotype

High diversity of antigenic sequences identified: only a minority (<10%) involves somatic mutations

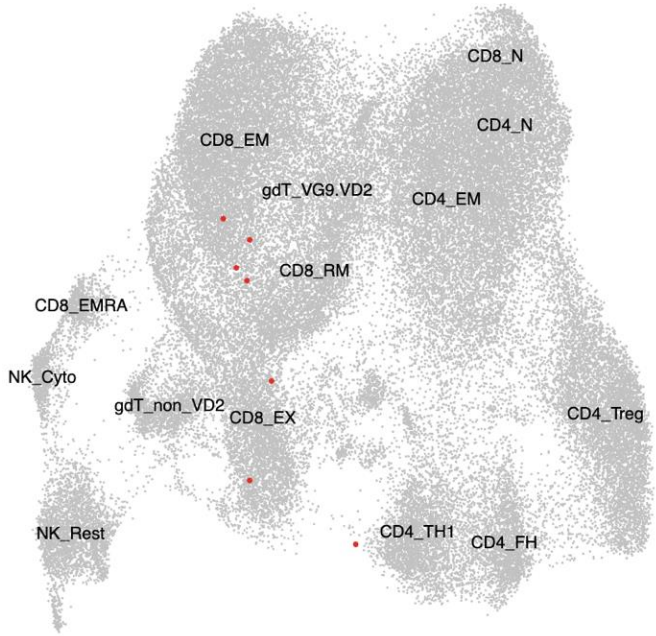


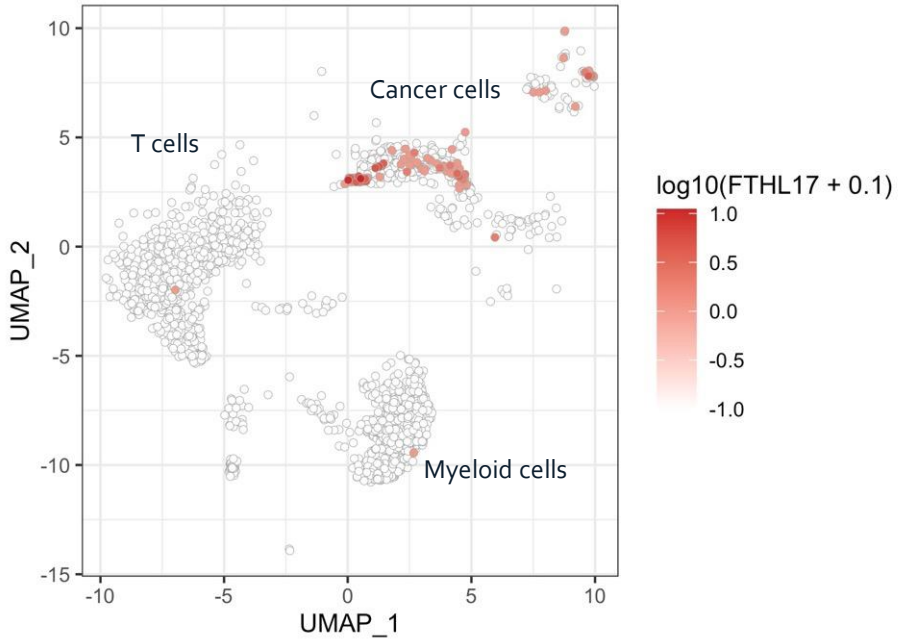
✓The success rate to identify a tumor antigen is ±70%

✓ We identify **'canonical sequences'**, representing cancer testis antigens or differentiation antigens

✓Few **'mutations'**, most are potential **'dark antigens'**

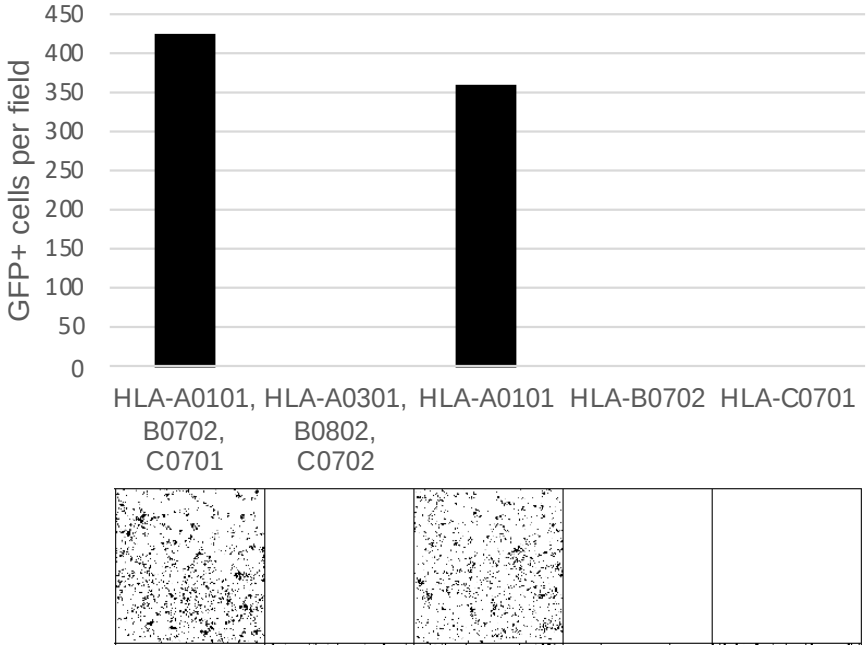
EX3: Around 10% of the tumor antigens that we identify represent shared tumor antigens



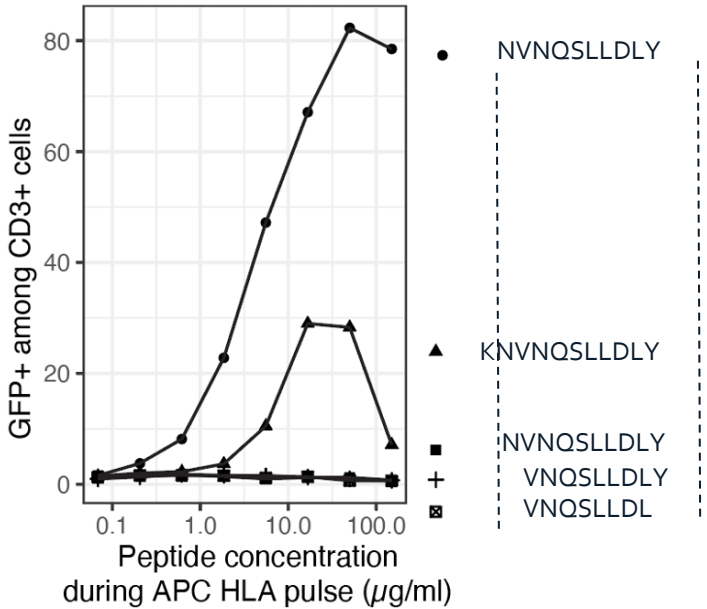


- An expanding TCR (TCR28) from activated T-cells in breast cancer
- Recognizes a cancer testis antigen *FTHL17* expressed in cancer cells

10-mer antigenic peptide is presented by HLA-A0101 and recognized by TCR028

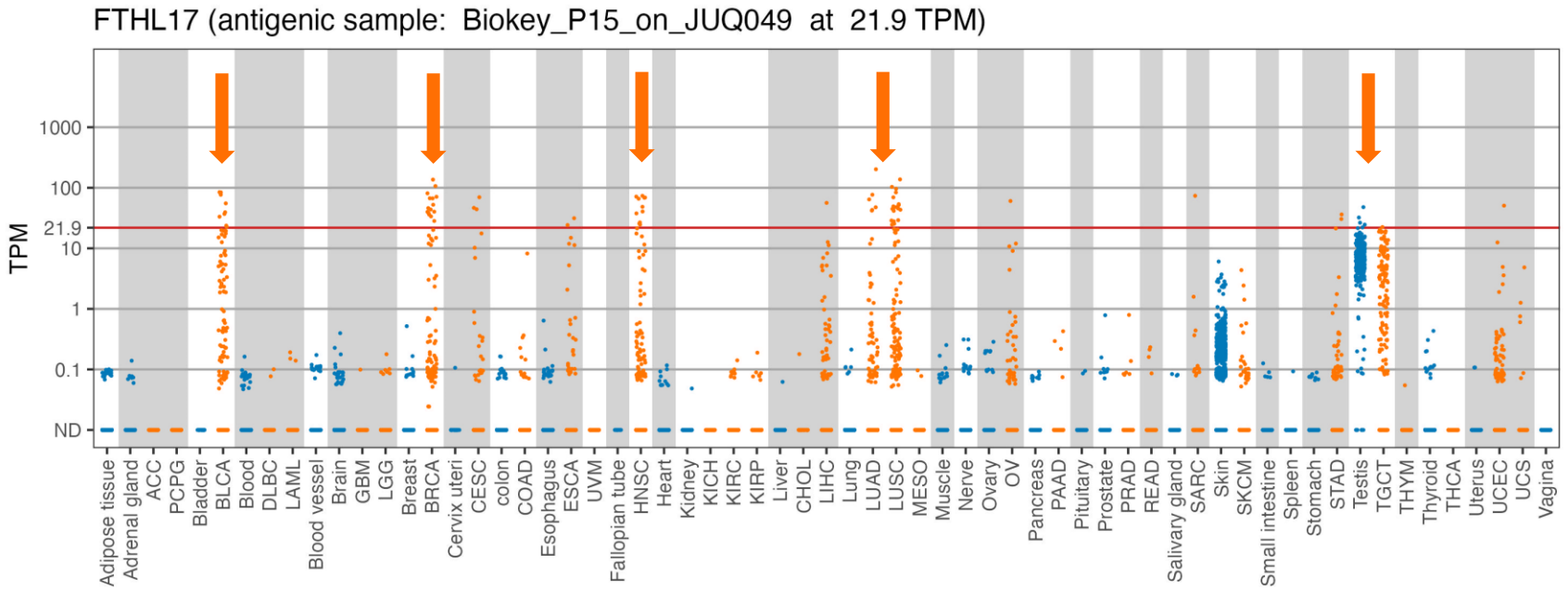


➢ HLA-A0101 presents peptide, is frequent HLA genotype



➢ NVNQSLLDLY is the antigenic peptide

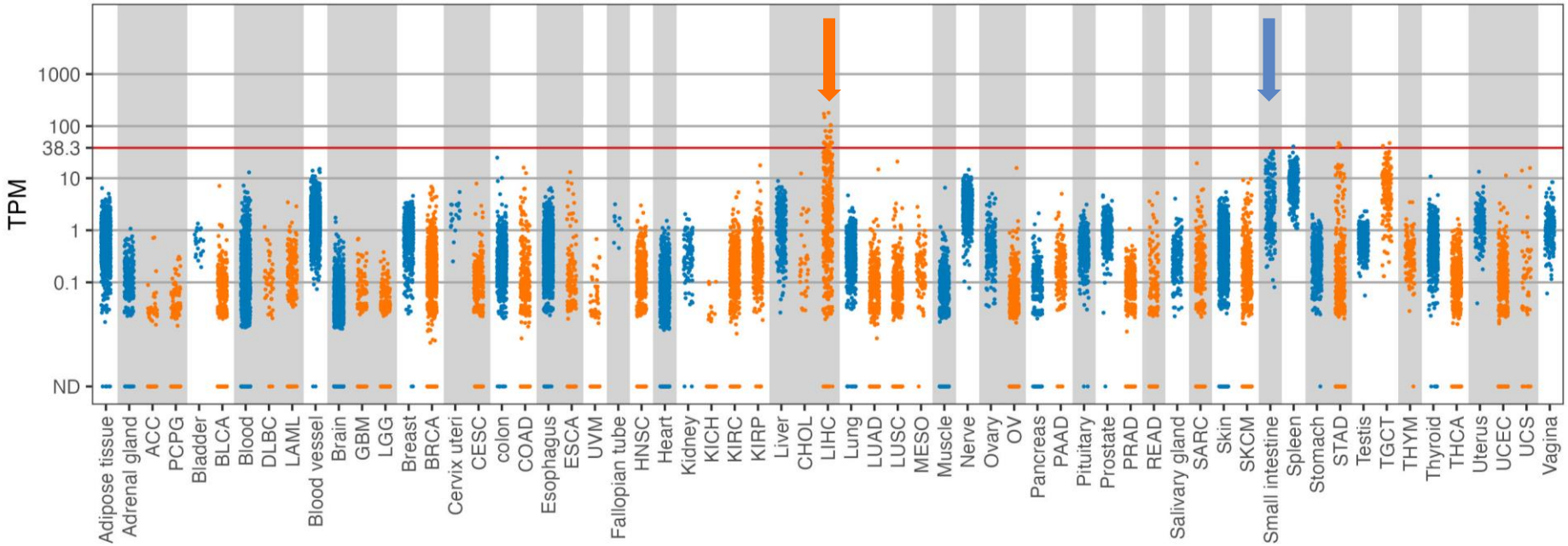
Tumor-specific expression of *FTLH17* in the TCGA database



- Highly specific expression in tumors
- Expression in normal tissue is absent

Normal tissue from GTEX
Tumor from TCGA

EX4: A shared tumour antigen, representing a tumor differentiation antigen specific for HCC



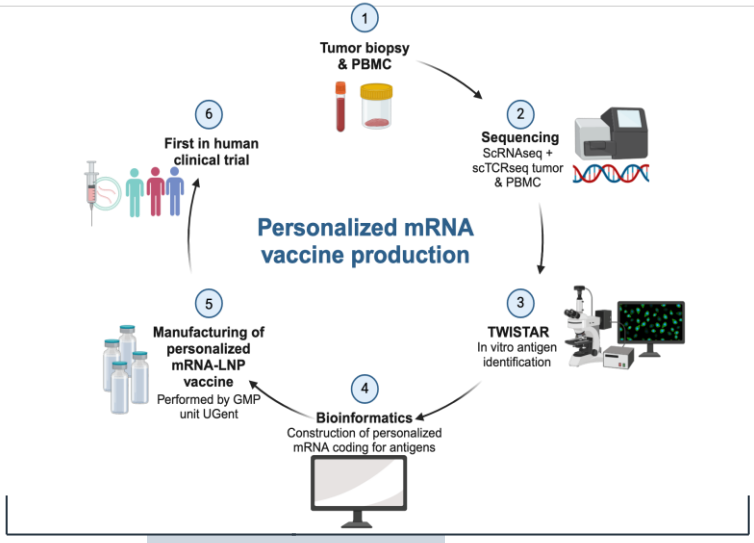
- Normal tissue (GTEX)
- Tumor from TCGA

- High expression in liver cancer (LIHC)
- Intermediate expression in intestine, no expression in other tissues

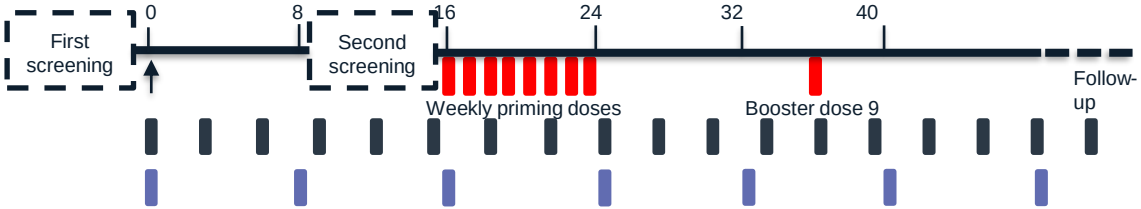
In the pipeline: vaccination trial in aHCC based on TWISTAR-driven, personalized 'dark' antigen vaccines

- Atezolizumab-bevacizumab q3w
- TCR-based individualized Galsome vaccine
- Imaging q8w

➢ Vaccination against 'dark antigens' using TWISTAR



Key inclusion criteria:
- Advanced HCC
- Standard-of-care atezolizumab/bevacizumab
- Good performance status and liver function
- 4-10 tumor antigens



In collaboration with B Vandekerkhove, UZ Gent

TWISTVAX-01: a phase 1 clinical study assessing feasibility and safety

Antigen identification TWISTAR

- Screening of **expanded tumoral antigen repertoire**
- T-cell based **in vitro** assay

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TWISTAR

Vaccine technology

- Source of antigen: mRNA
- Carrier: Lipid nanoparticle
- Adjuvants: α-galactosylceramide

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Galsome-NEO

TWISTVAX-01

- Phase Ia clinical trial
- Advanced HCC, in combination with standard immunotherapy
- 12 tumor antigens in each vaccine

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Stakeholders of TWISTAX-01

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CRIG

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- Full professor KU Leuven

Prof. Dr. Jeroen Dekervel

- Adjunct Head of Clinic UZ Leuven
- Associate professor KU Leuven

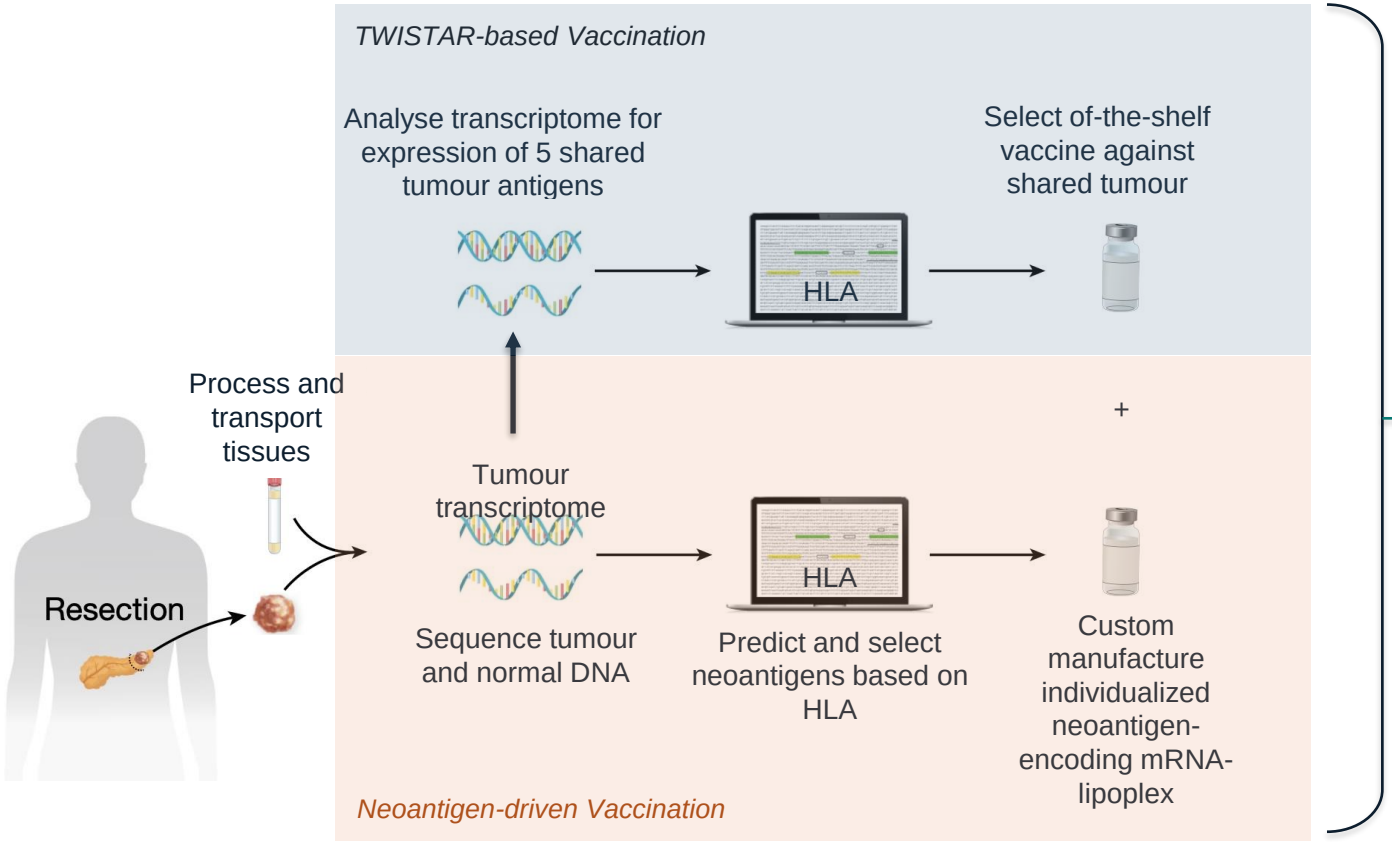
Prof. Dr. Bart Vandekerckhove

- Director of GMP unit Cell Therapy UZ Gent
- Full professor Universiteit Gent

Dr. Ine Lentacker

- Principle investigator of the vaccine delivery Group Universiteit Gent
- Member of mRNA Vac working party (EDQM)

Shared tumour antigens offer potential for off-the-shelf vaccines (± combination with neoantigens)



Combined Vaccination against neoantigens and ‘dark’ antigens

Increased number of antigens against which an immune response is raised

POINTILLISM: Improving immune checkPOINT Inhibitor response by single-ceLL Integrative multi-omicS of the tumor ecosysteM

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Agilent Technologies

erc Advanced Grant

IMMUCAN

RESCUER

Horizon 2020 European Union Funding for Research & Innovation

Laboratory of Translational Genetics: Amelie Franken, Aurelie Mechels, Ayse Bassez, Heesoo Song, Sam Van Massenhove, Ling Wang, Francesca Lodi, Thomas Van Brussel, Rogier Schepers, Evy Vanderheyden, Frederik Peeters, Laura Elst, Kaat Vandermaesen, Elena Donders, Toon Vandepitte, Tom Venken, Gino Philips, Sylvia Ujwari, Pieter Busschaert, Bram Boeckx, Ingrid Arijs, Jieyi Xiong, Razgar Seyed Rahmani, Orian Bricard, Diether Lambrech

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LISCO and Genomics Core Leuven: T Voet, K Vandereycken, S Kint

University Hospital Leuven: *Multidisciplinary Breast Center:* Ann Smeets, Patrick Neven, Hans Wildiers, Giuseppe Floris, Christine Desmedt; *Medical Oncology:* Paul Clement, Michel Bila; *Digestive Oncology:* Jeroen dekervel, Sarah Cappuyns, Chris Verslype, Baki Topal; *Pneumology:* Els Wauters, Birgitte Weynand; *Urology:* Maarten Albersen; *Gyneco-oncology:* Els Van Nieuwenhuyse, Toon Van Gorp, Frederic Amant, Wout De Wispelaere, and the patients consenting …

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