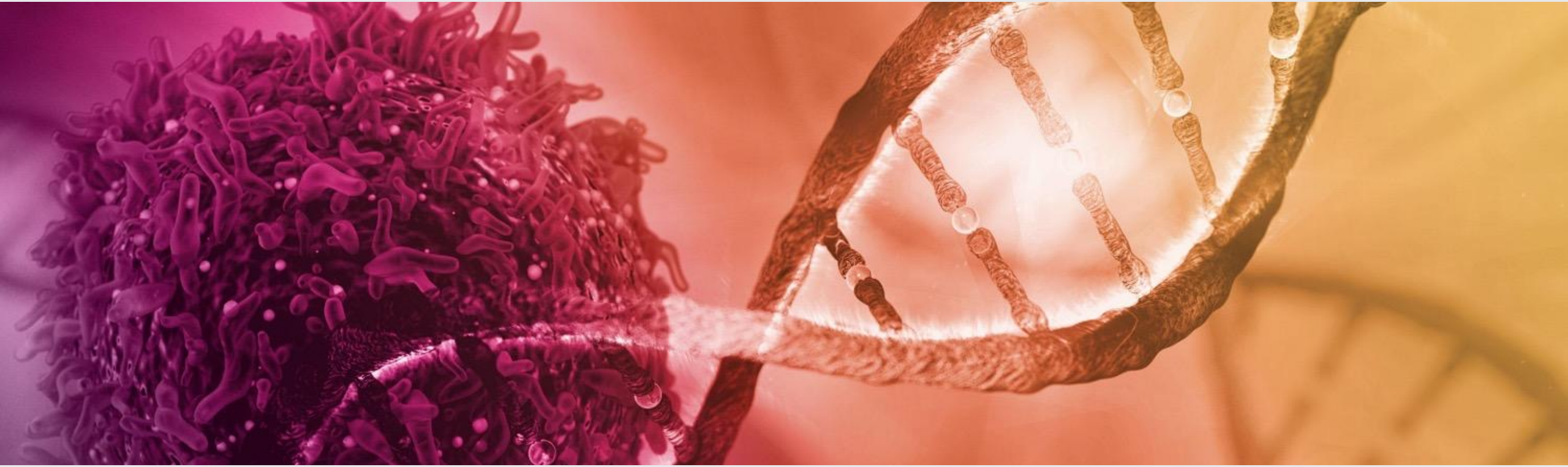
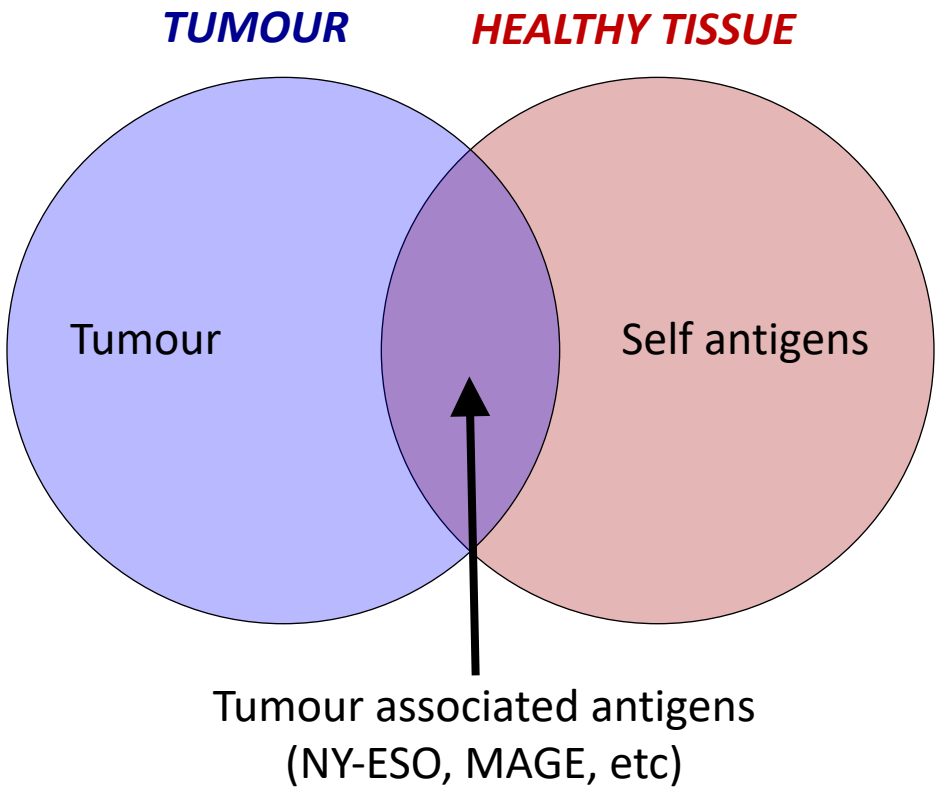


Targeting Clonal Neoantigens in Cancer

SAE Media Group Cell and Gene Therapy - 20 June 2023

Sergio Quezada, Chief Scientific Officer, Achilles Therapeutics

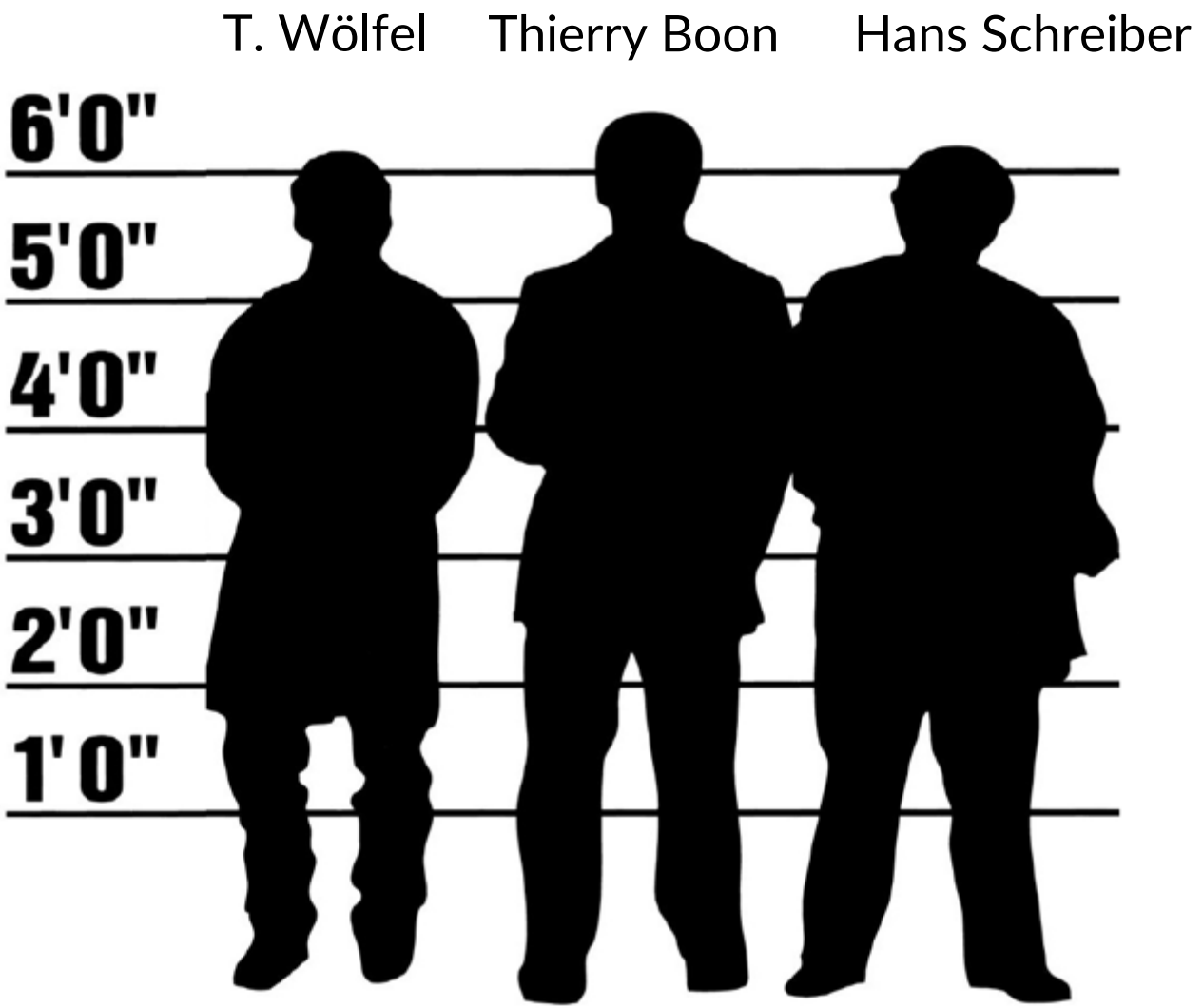
Tumour mutations as substrates for immune recognition



The ideal tumour target should show the following characteristics:

1. Recognised as foreign by the immune system
2. Present **ONLY** on tumour cells (not in healthy tissue)
3. Present in **ALL** tumour cells

Tumour mutations as substrates for immune recognition (90's)

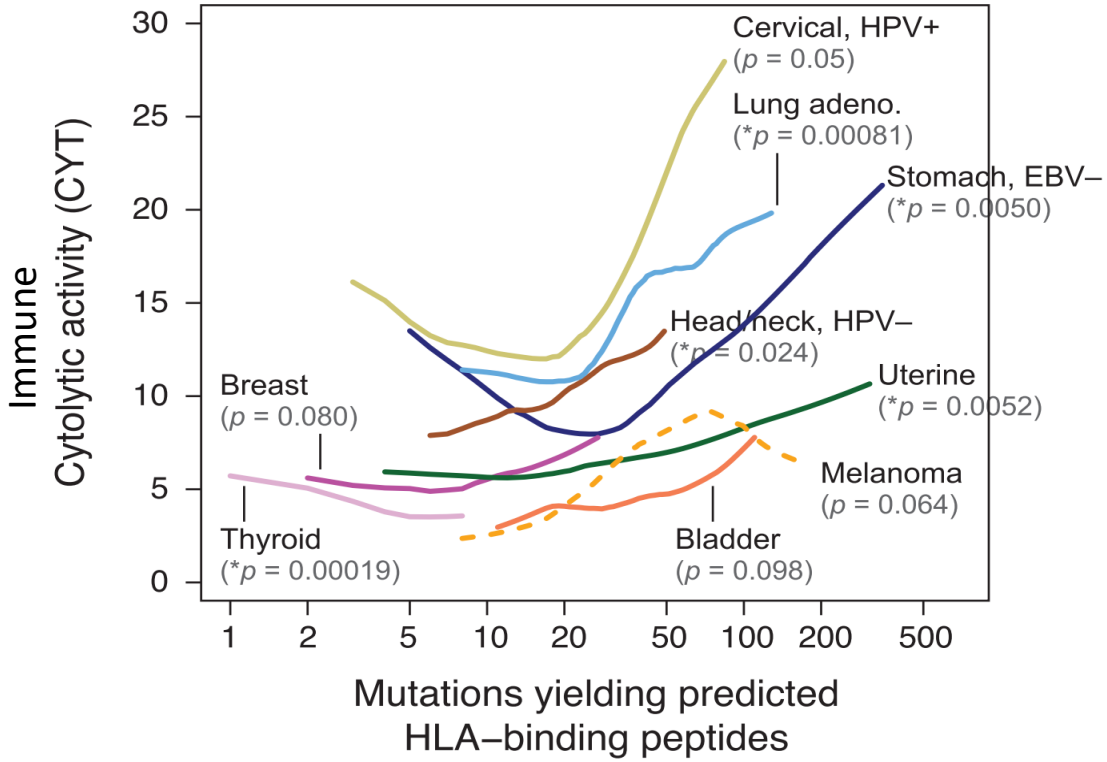


Neo-antigenic burden correlates with immune activation at the tumour site

Cell, 2015

Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity

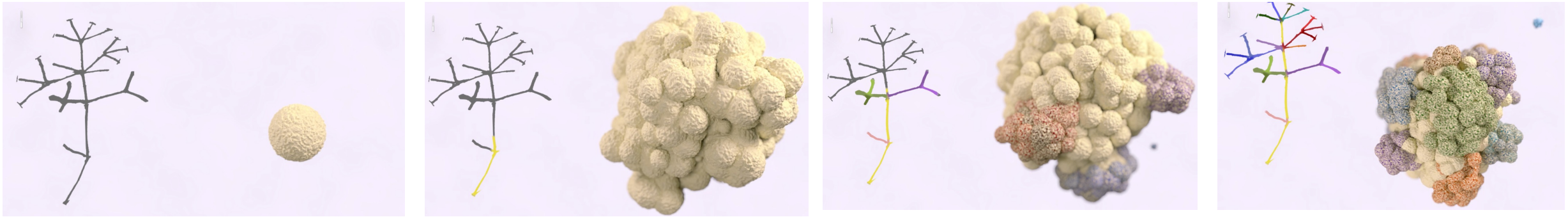
Michael S. Rooney,[1,2] Sachet A. Shukla,[1,3] Catherine J. Wu,[1,3,4] Gad Getz,[1,5] and Nir Hacohen[1,4,6,*]
[1]The Broad Institute, Cambridge, MA 02142, USA
[2]Harvard/MIT Division of Health Sciences and Technology, Cambridge, MA 02141, USA
[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
[4]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA
[5]Massachusetts General Hospital Cancer Center and Department of Pathology, Charlestown, MA 02129, USA
[6]Center for Immunology and Inflammatory Diseases and Department of Medicine, Massachusetts General Hospital, Charlestown, MA 02129, USA



But… are all neoantigens the same?

Fundamentals of branched tumour evolution

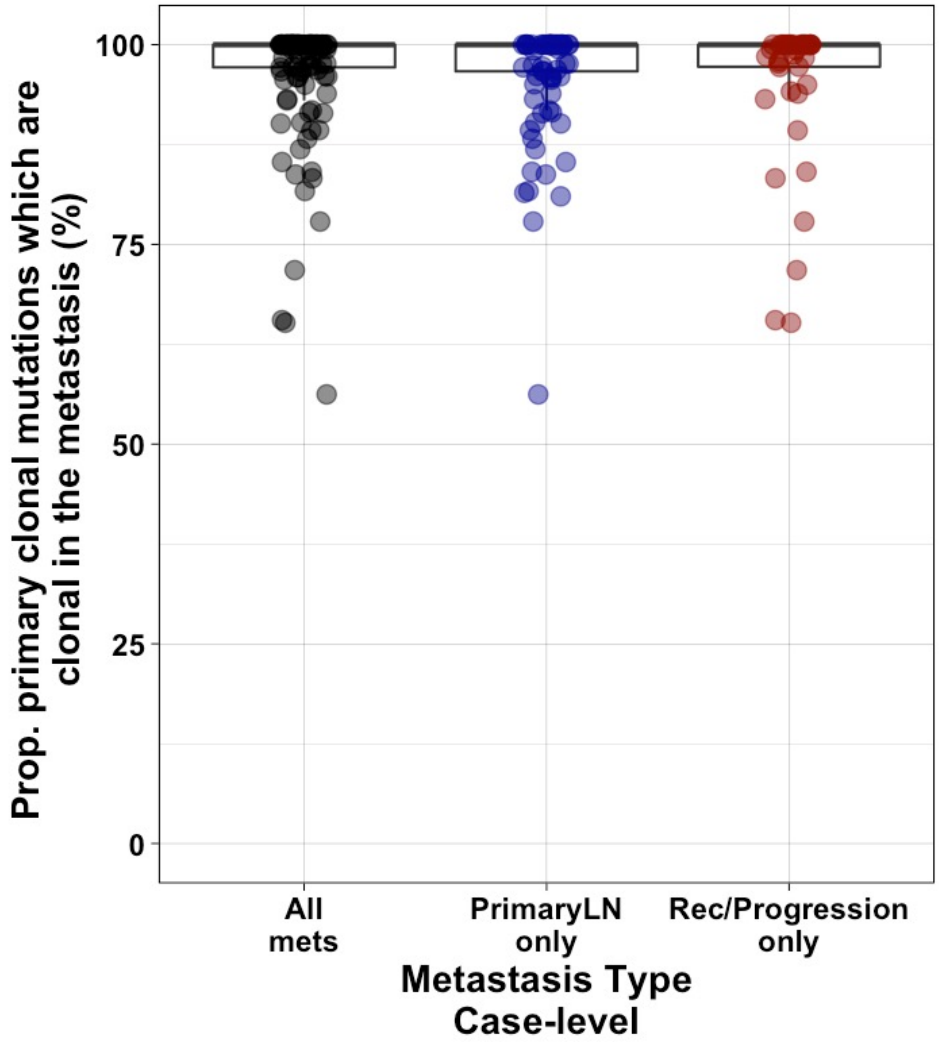
- Clonal mutations occur early in tumour evolution and are present in all tumour cells
- Subclonal mutations occur later in the tumour evolution and are found in a subset of tumour cells
- Clonal neoantigen reactive T cells are found in all tumour regions in NSCLC



Gerlinger et al 2012 N Engl J Med 367(10):976
McGranahan et al 2016 Science 351 (6270):1463-9

Are clonal neoantigens also clonal in metastases?

Persistence of primary clonal mutations in metastases



TRACERx

All metastases:

Median: 100% [IQ range: 97.17-100%]

Primary lymph node (LN) only:

Median: 100% [IQ range: 96.67-100%]

Recurrence/Progression (Rec/Progression) only:

Median: 100% [IQ range: 97.24-100%]

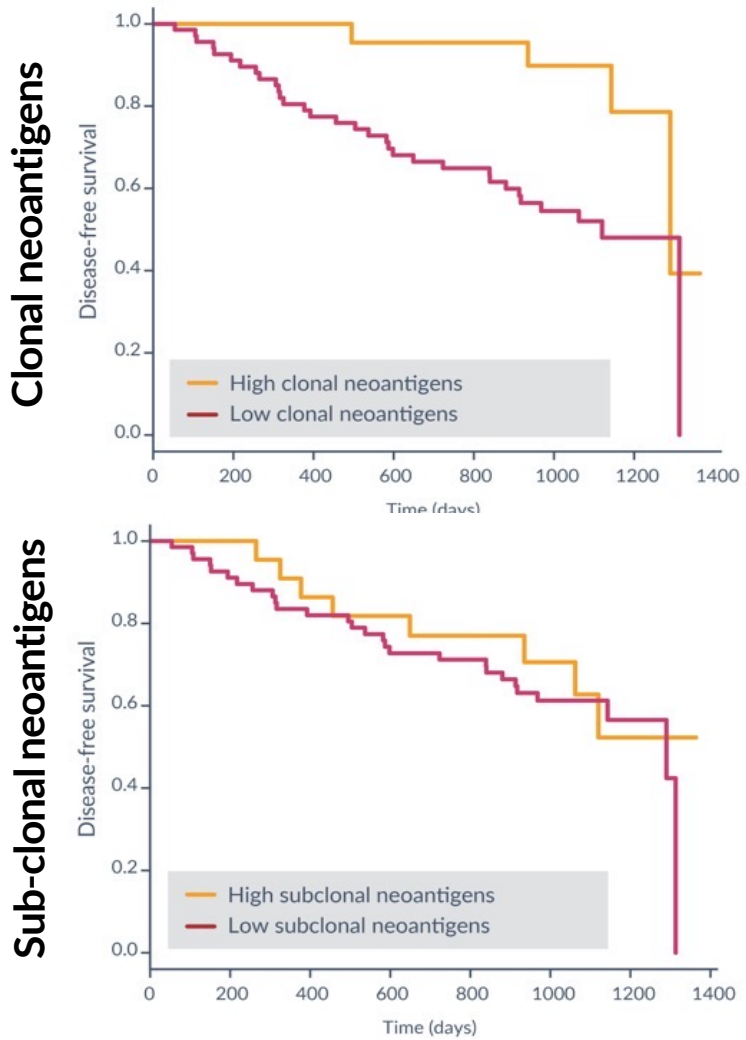
The vast majority of primary clonal mutations persist in the metastases regardless of metastasis type (primary lymph node (PrimaryLN) vs recurrence/progression (Rec/Progression), p=0.65, Wilcoxon rank-sum test)

Inexorable acquisition of subclonal neoantigens that are distinct from one metastatic site to another mandates targeting clonal neoantigens

Does it matter? Is there a difference between clonal and subclonal neoantigens?

Clonal and not subclonal neoantigens predict survival in early stage lung cancer patients

TRACERx



Clonality is the driver of disease-free survival

- Patients with high clonal neoantigen burden have an improved disease-free survival
- This is not seen in patients with high sub-clonal neoantigen burden

The **higher the number of clonal neoantigens**, the greater the chance of immune recognition and **successful elimination of all cancer cells**

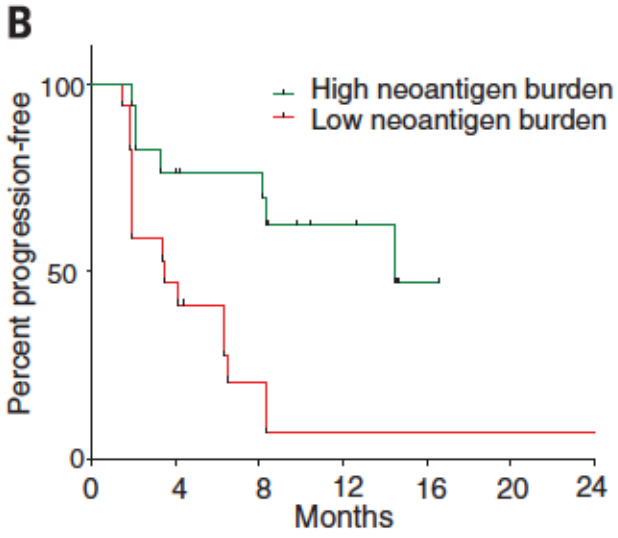
Rosenthal et al 2019 Nature 567: 479–485
McGranahan et al. 2016 Science 351:1463-1469

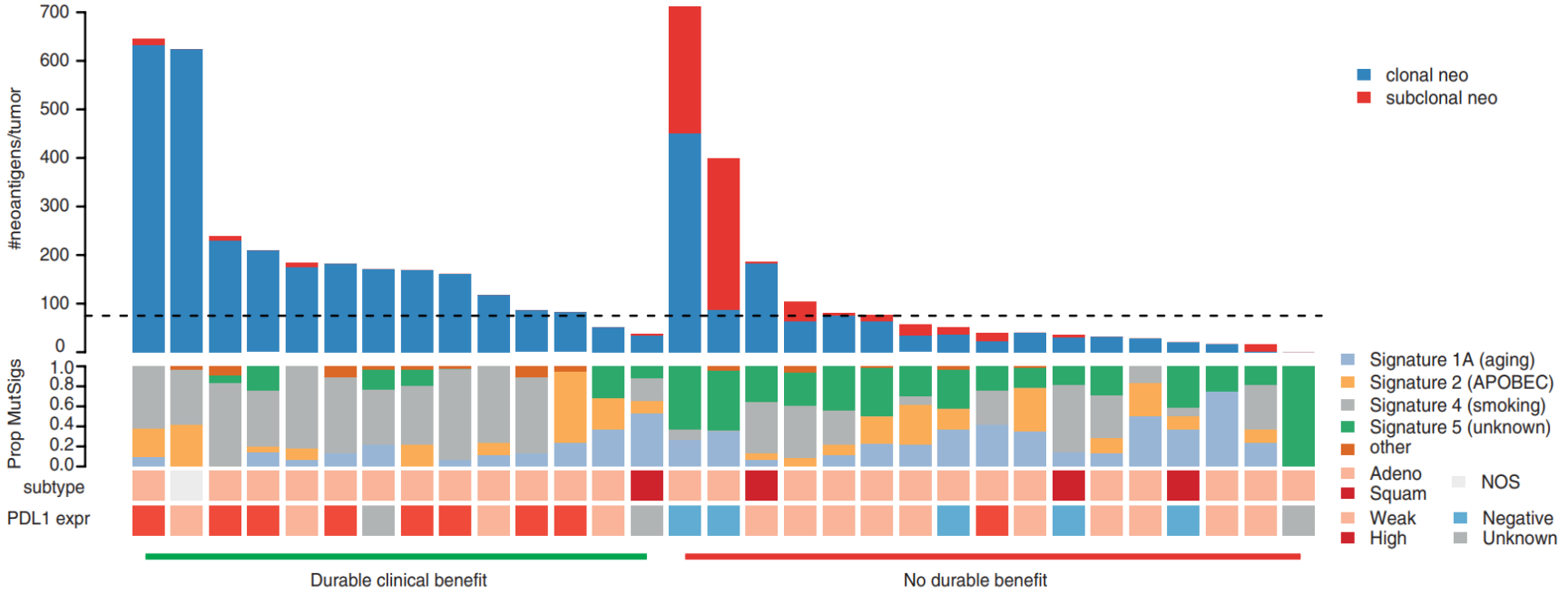
How relevant are clonal neoantigens in the context of immunotherapy?

PD-1 response in NSCLC is correlated with neoantigen burden and clonality

Clonality contributes to survival in patients receiving anti-PD1 treatment

- Patients with a high clonal neoantigen burden have higher likelihood of a durable clinical benefit and improved overall survival relative to patients with low clonal burden
- Patients with high subclonal neoantigen burden do not seem to significantly benefit from anti-PD1



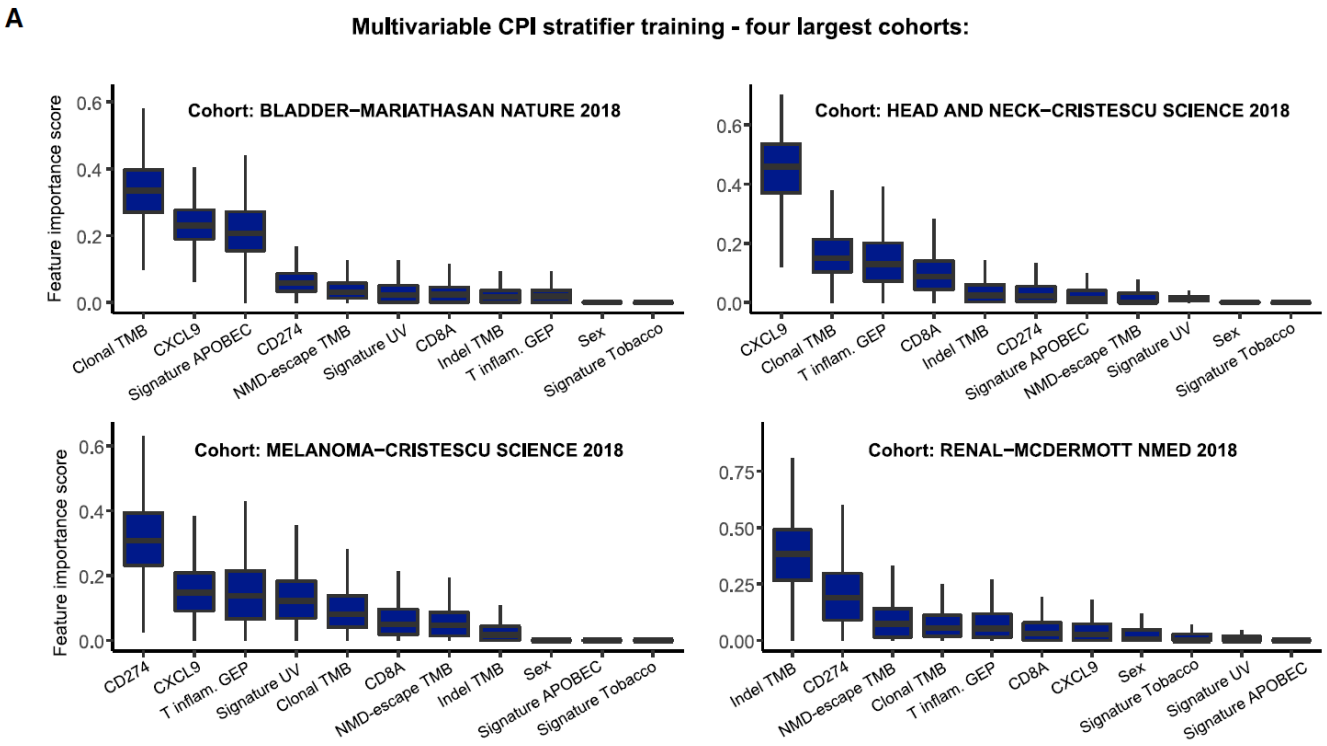


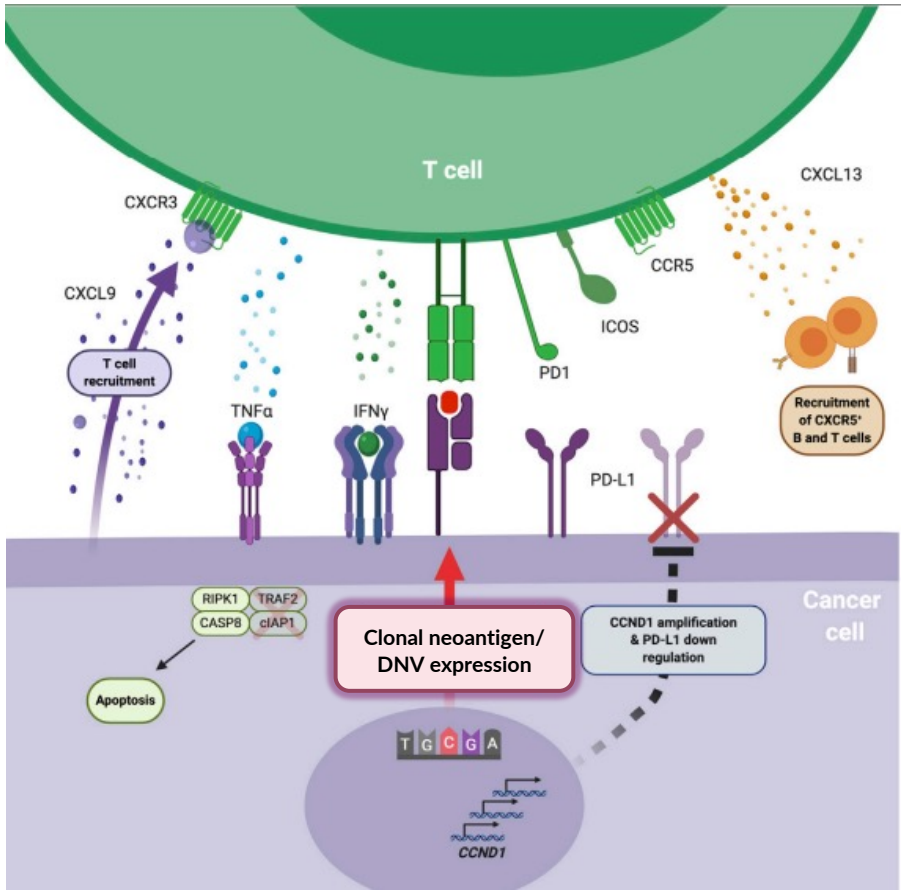
Rizvi et al 2015 Cancer Immuno 348(6230):124-8
McGranahan et al. 2016 Science 351:1463-1469

Meta-analysis of tumour- and T cell-intrinsic mechanism of sensitization to CPI

TRACERx

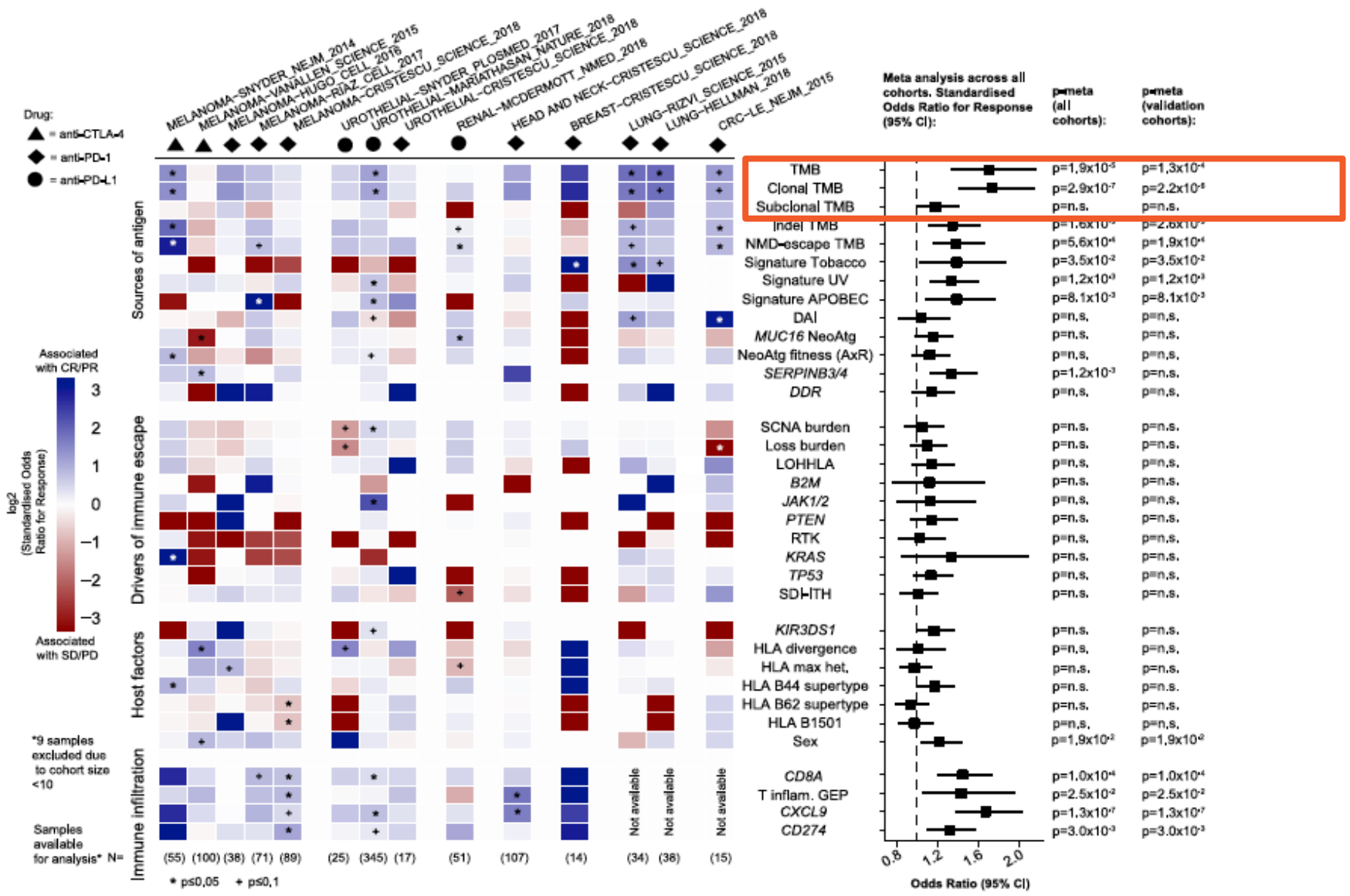
A meta-analysis of >1000 patients across 7 indications treated with CPI underscores the importance of clonal neoantigens in checkpoint inhibitor response





Lichfield, Reading, Puttick et al Cell 2021

Clonal neoantigens drive response to CPI

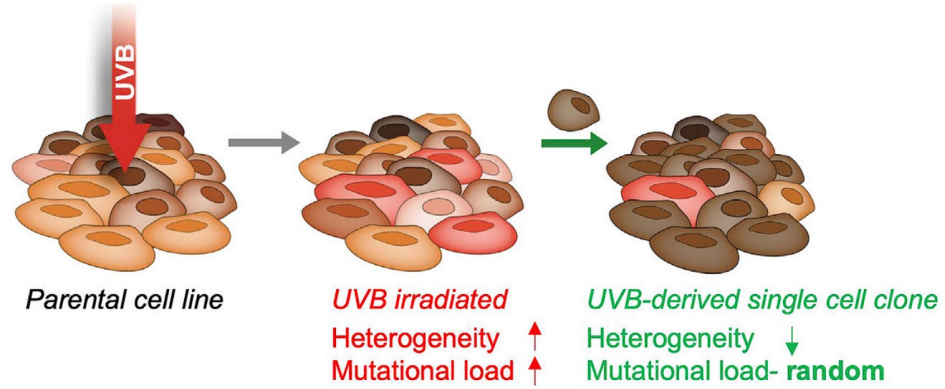


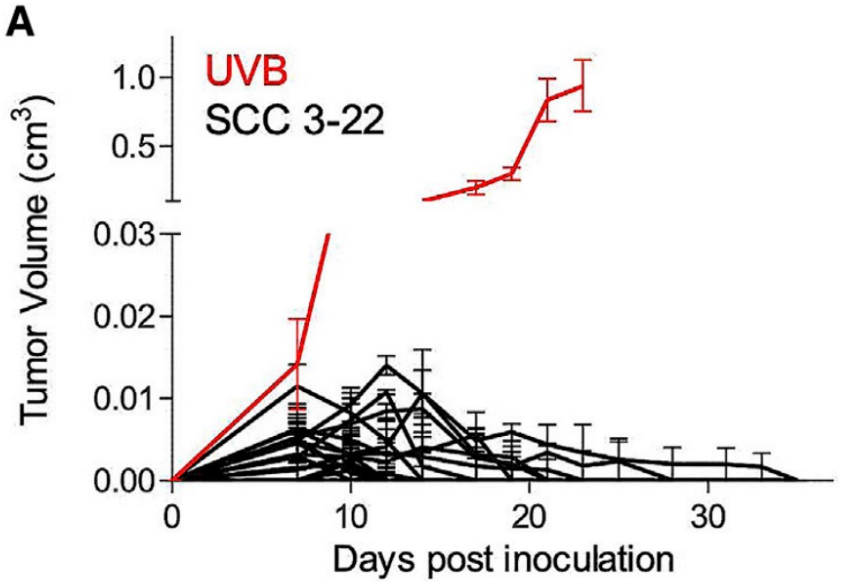
Subclonal neoantigen burden and ITH can reduce the immune response to tumours

UVB-Induced Tumor Heterogeneity Diminishes Immune Response in Melanoma

Cell

Yochai Wolf,[1,13] Osnat Bartok,[1,13] Sushant Patkar,[2,14] Gitit Bar Eli,[1,14] Sapir Cohen,[1,14] Kevin Litchfield,[3,11,14] Ronen Levy,[1] Alejandro Jiménez-Sánchez,[4] Sophie Trabish,[1] Joo Sang Lee,[2] Hiren Karathia,[2] Eilon Barnea,[5] Chi-Ping Day,[6] Einat Cinnamon,[7] Ilan Stein,[7] Adam Solomon,[8] Lital Bitton,[1] Eva Pérez-Guijarro,[6] Tania Dubovik,[9] Shai S. Shen-Orr,[9] Martin L. Miller,[4] Glenn Merlino,[6] Yishai Levin,[10] Eli Pikarsky,[7] Lea Eisenbach,[8] Arie Admon,[5] Charles Swanton,[3,11,12] Eytan Ruppin,[2,15,*] and Yardena Samuels[1,15,16,*]

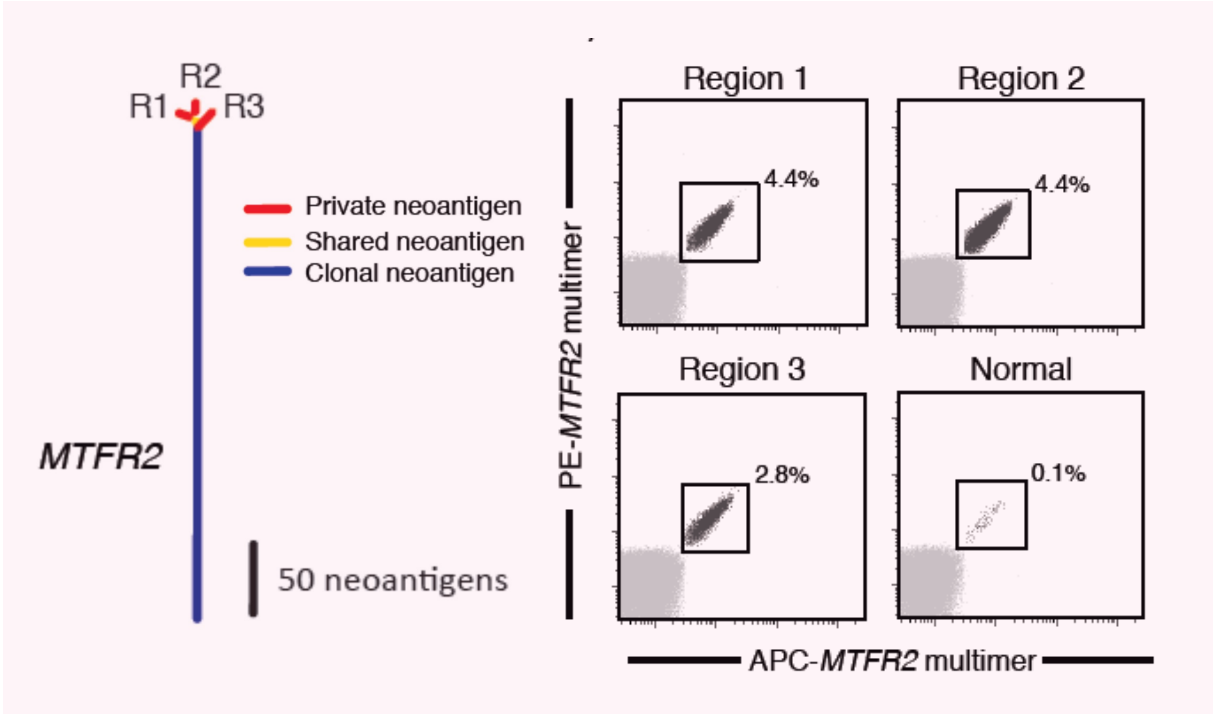


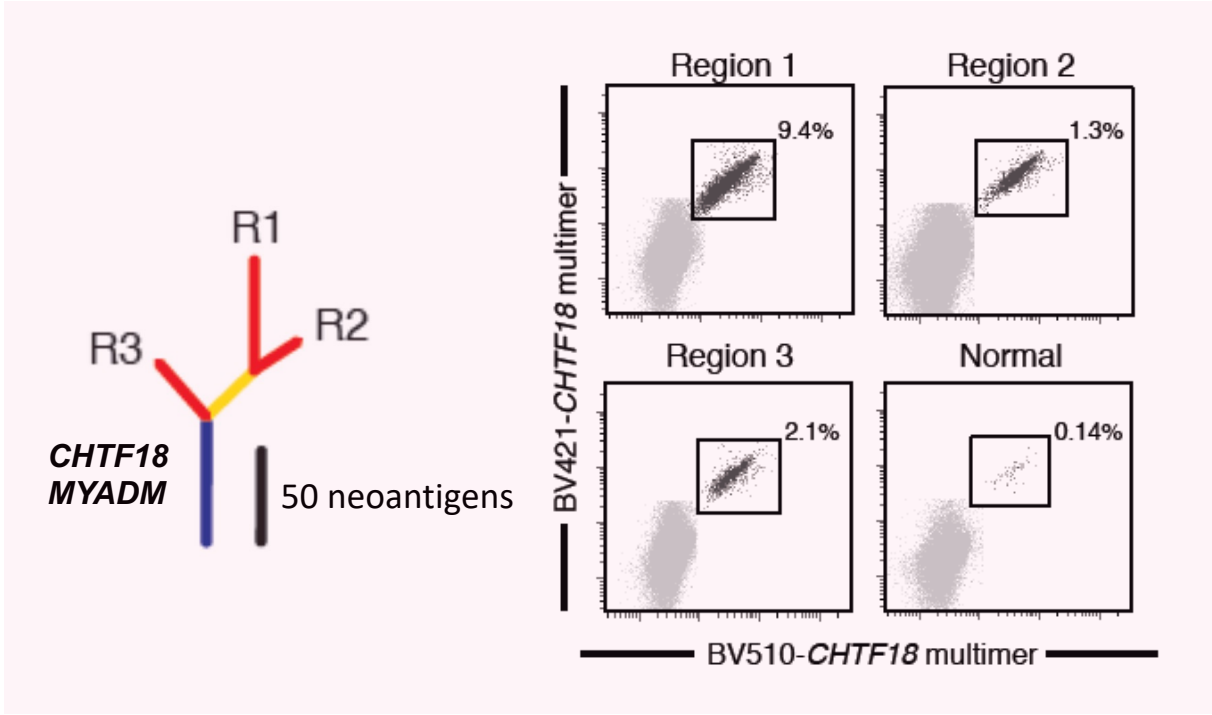


Can we identify clonal-neoantigen specific T cells (cNeTs) in human tumours?

Neoantigen specific T cells are found in all tumour regions at high frequency in samples from NSCLC patients

- Clonal mutations occur early in tumour evolution and are present in all tumour cells
- Subclonal mutations occur later in the tumour evolution and are found in a subset of tumour cells
- Clonal neoantigen reactive T cells (cNeTs) are found in all tumour regions in NSCLC samples

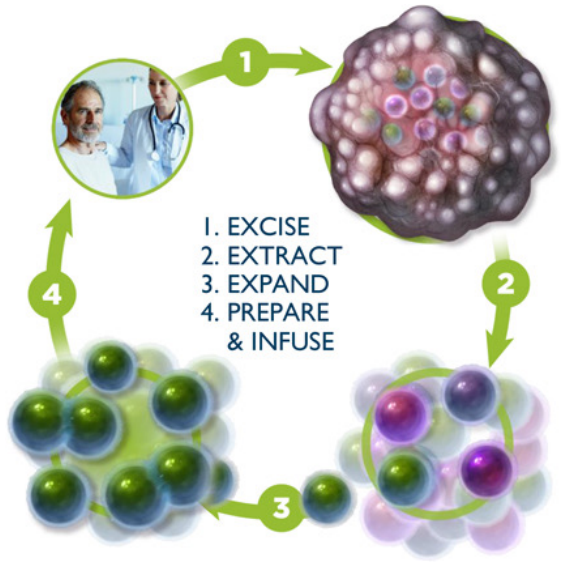


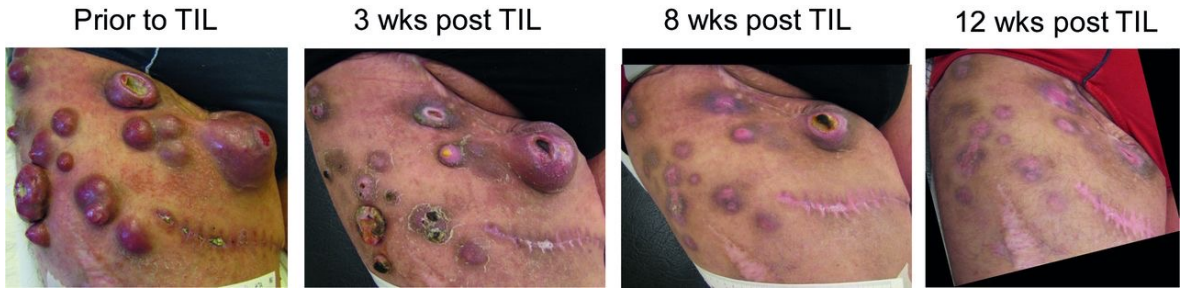


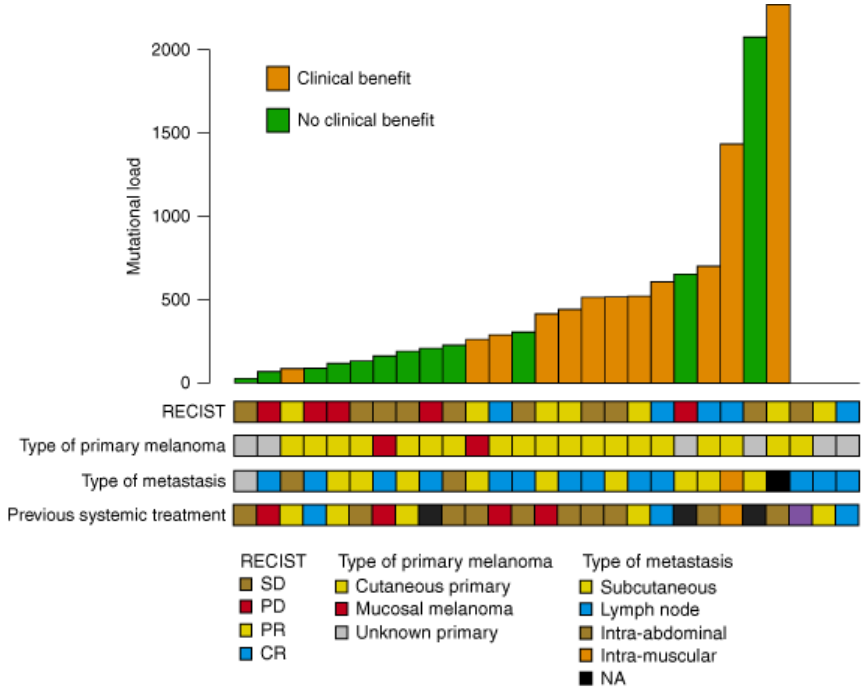
McGranahan et al. 2016 Science 351:1463-1469

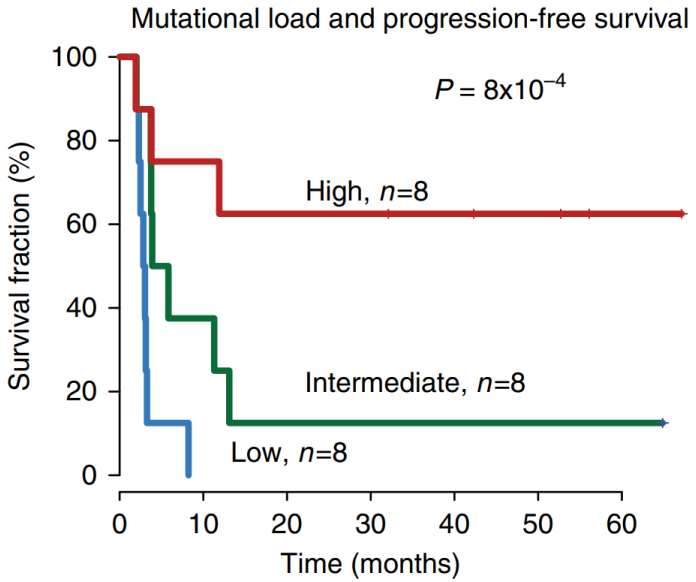
Choosing the right platform to mobilise the immune system against clonal neoantigens

Tumour infiltrating lymphocyte (TIL) therapy – a clinically validated platform in melanoma





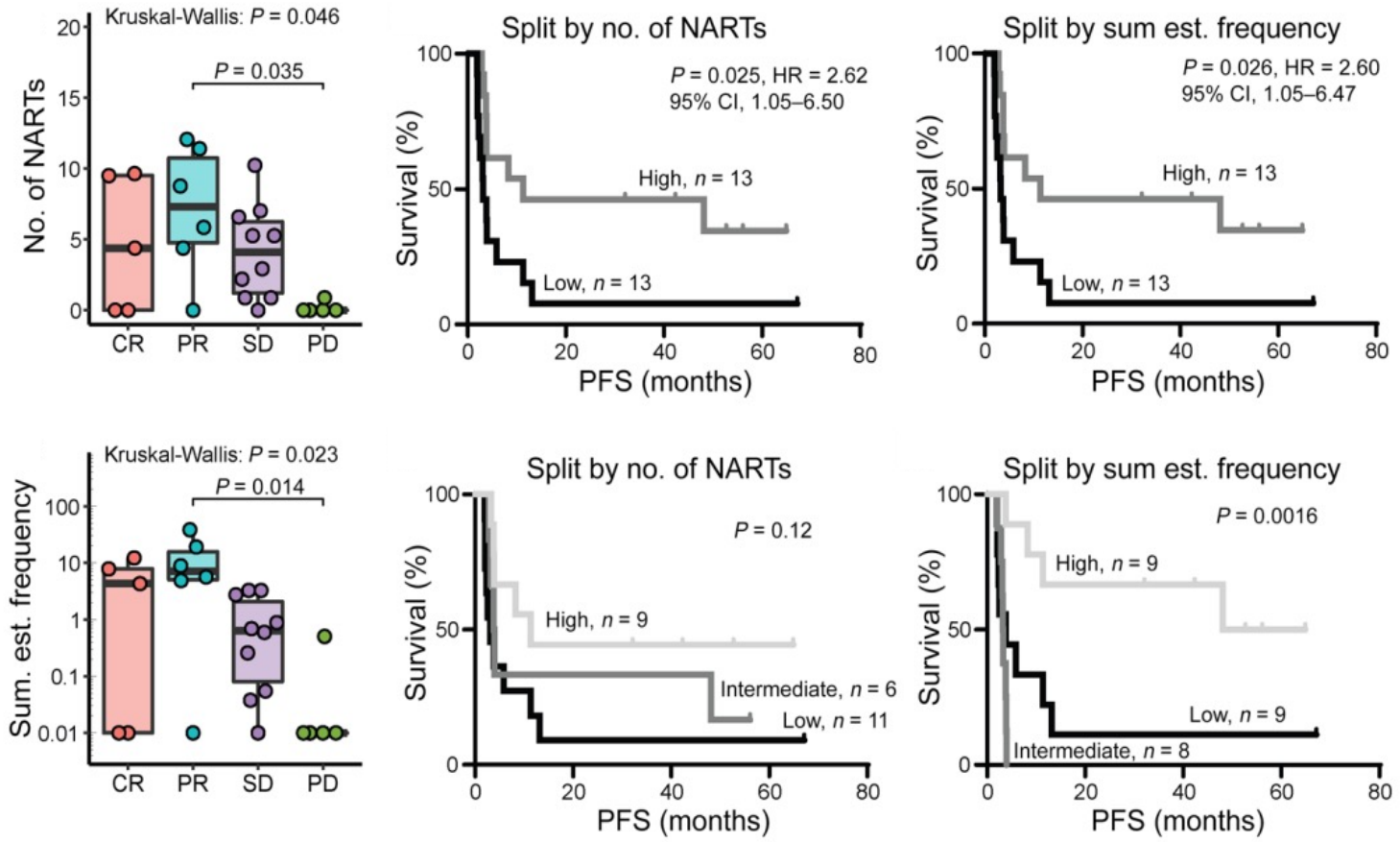




Lauss et al 2017 Nat Commun. 2017 8(1):1738

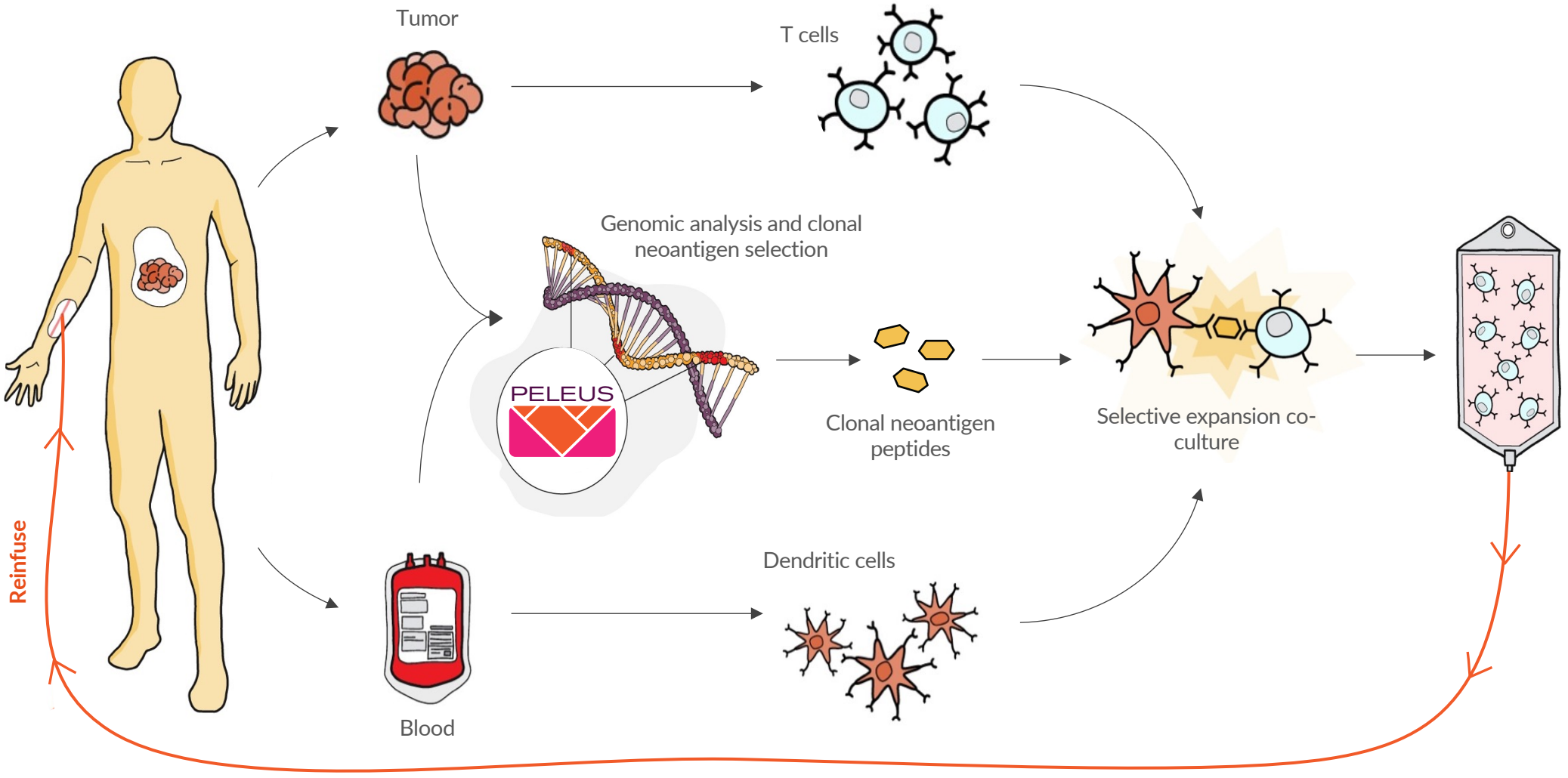
CD8+ Neoantigen-reactive T cells correlate with clinical outcome following TIL therapy

- Data suggest a correlation between Neoantigen-reactive T cell (NART) dose and clinical response
- Neoantigen-reactive T cells could be the active component of TIL
- Optimising both NART number and frequency could help improve clinical response to TIL



Kristensen et al 2022 J Clin Invest 132(2):e150535

Enriching for Clonal Neoantigen Reactive T Cells (cNeT)



Only Achilles can accurate identify clonal neoantigens with PELEUS™ platform

World-leading, bioinformatics platform

Patented clonal neoantigen identification

PELEUS is the only platform using multi-region analysis and the only method to accurately identify clonals[1]

Proprietary AI and machine learning for validated prediction of target immunogenicity

Platform prioritizes antigens for a polyfunctional response to minimize immune evasion[2]

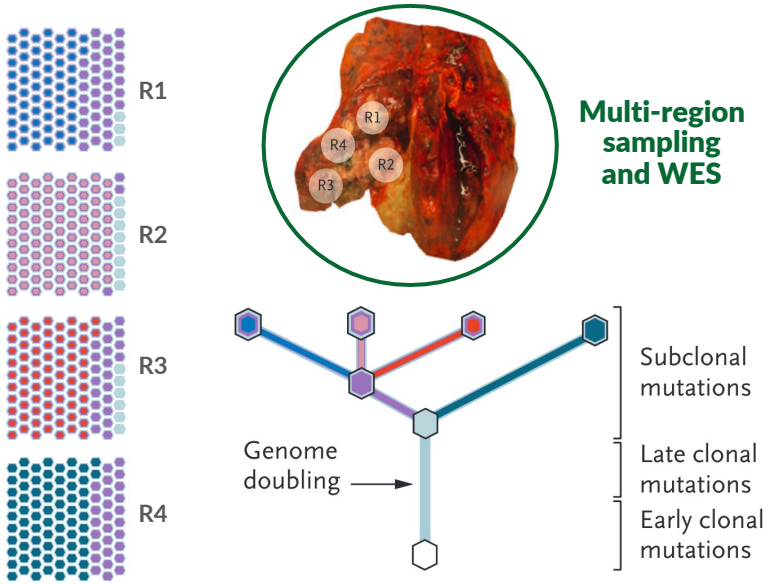
1. Jamal-Hanjani *et al* 2017
2. McGranahan *et al* 2017

Only Achilles has capability to accurately identify clonals from multi-region sequencing

TRACERx

The TRACERx consortium established the **importance of multi-region sampling for identifying *true clonals*** by manually mapping out the evolution of each patient's tumour

"Without multi-region whole-exome sequencing, 76% of subclonal mutations could have appeared to be clonal"



PELEUS™

Achilles Therapeutics has developed an **automated Bayesian model to infer clonality from multiple tumour samples and avoid clonal illusion** without the need for manual curation

Informed and validated against TRACERx

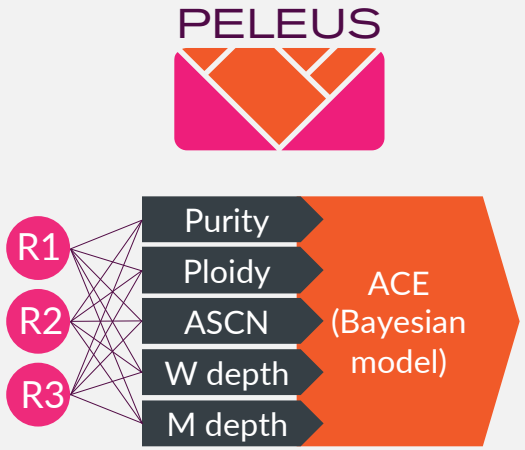
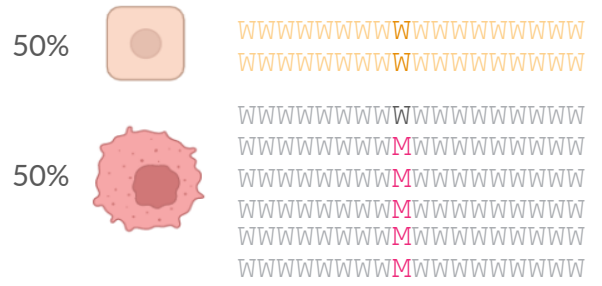


Illustration: Sub-clonal mutation followed by amplification

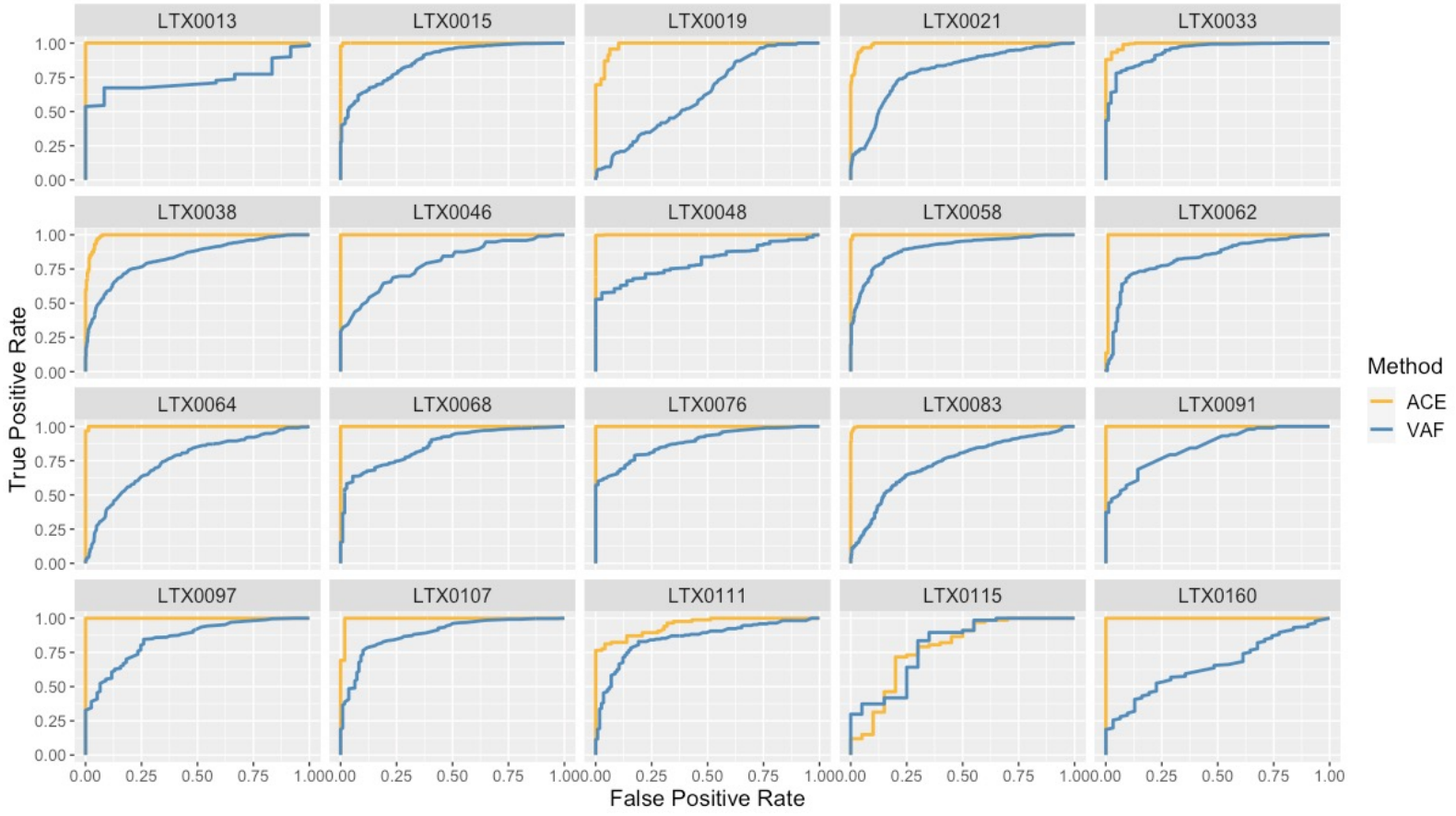


Other methods such as VAF may mistake this for a clonal mutation

Jamal-Hanjani et. al. 2017

Multi region PELEUS™ clonality ranking substantially outperforms single region VAF method

TRACERx clonality ranking benchmark
(n=20 TRACERx patients, 2-7 regions per patient)



PELEUS performance is consistent across varying levels of mutation burden and ITH

ROC AUC

	VAF	PELEUS
Mean	**0.78**	**0.98**
Standard Dev	0.16	0.05

ROC AUC measures skill to rank clonal neoantigens ahead of sub clonal neoantigens

- VAF gets this right 3.5 times for every 1 mistake, on average
- PELEUS gets this right 49 times for every 1 mistake, on average
- PELEUS performance is highly consistent across different patients

PELEUS target lists are consistently highly clonal across different patient tumour clonal architectures

Two studies open in advanced NSCLC and melanoma

CHIRON
Advanced NSCLC

Monotherapy

- Advanced unresectable or metastatic Stage III-Stage IV NSCLC
- Never-smokers and EGFR/ALK/Ros-1 mut excluded
- Open-label
- n = up to 40
- Option to open Cohort B in combination with a PD-1 inhibitor

Evaluating safety, tolerability and activity (RECIST) and biomarkers of clinical activity

Ongoing in UK, Europe and US

THETIS
Melanoma

Cohort A – Monotherapy

- Recurrent or metastatic malignant melanoma (n = up to 40); Open-label
- Acral, uveal and mucosal melanoma excluded

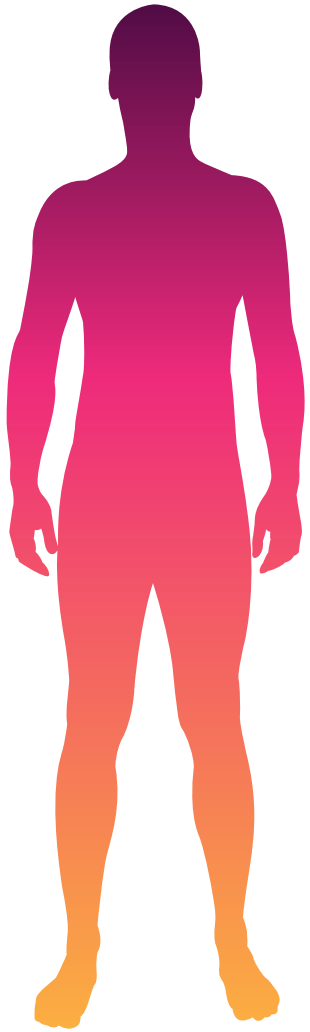
Cohort B – Combination with PD-1 inhibitor (nivolumab)

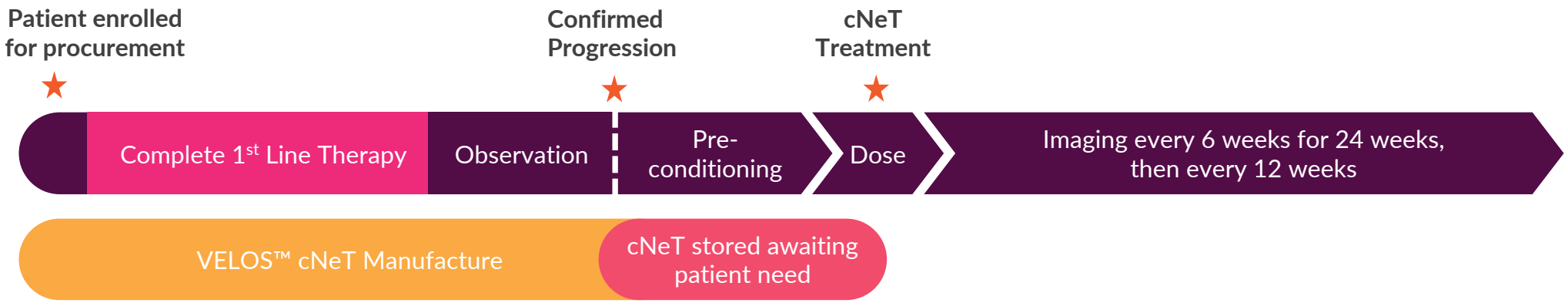
- n = up to 20 checkpoint refractory patients; Open-label
- CPI dosed 7-13 days prior to cNeT and restarted day 14 post-cNeT

Evaluating safety, tolerability and activity (RECIST) and biomarkers of clinical activity

Ongoing in UK and Europe, expanding to US

cNeT therapies can be readily delivered within standard treatment pathways





Manufacturing

Manufactured and cryopreserved for infusion after patient progression

Tolerable pre-conditioning

Lower, more tolerable pre-conditioning (cy/flu)

Low IL-2

Lower dose IL-2 vs existing TIL therapies

cNeT were generally well tolerated in the fourteen patients treated in CHIRON & THETIS

Heavily pretreated patients with advanced cancer

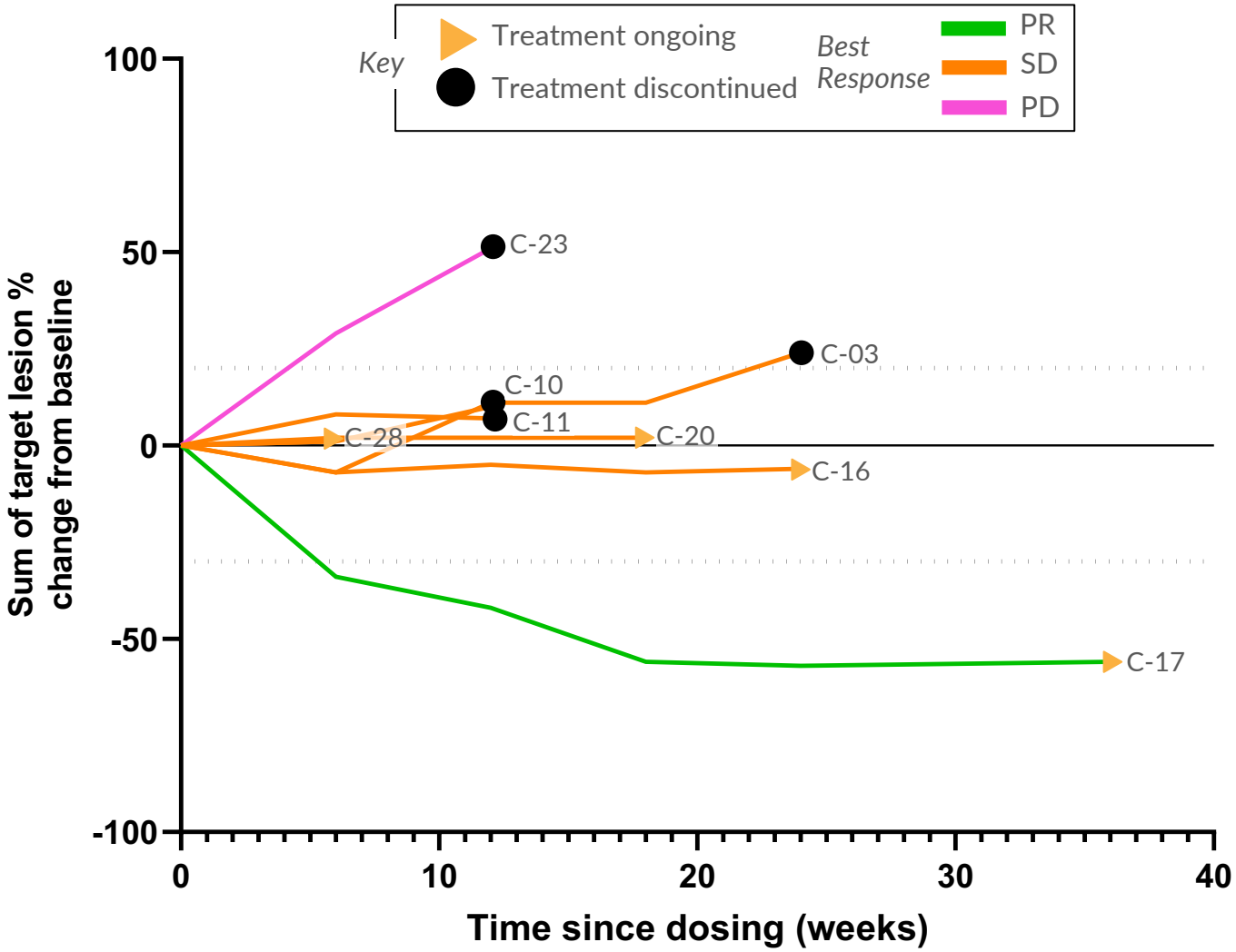
- Eight advanced unresectable or metastatic NSCLC patients (CHIRON)
- Six relapsed/refractory melanoma patients (THETIS)
- Two median lines of prior therapy, all patients refractory to checkpoint inhibitor (CPI)
- All patients had progressive disease at time of lymphodepletion
- Process improvements delivering median cNeT dose of 78M (n=3 dosed patients)

cNeT tolerability profile[1]

- Tolerability similar to standard TIL
- No new cNeT-related SAEs or dose-limiting toxicities since last report (ESMO 2022)
- Lower dose lymphodepletion and lower dose IL-2 well tolerated
  - 124/130 (95%) scheduled IL-2 doses delivered
- Lymphopenia and neutropenia the most common AEs

1. Safety data cut-off on September 7th 2022, at which point 13 of the 14 pts had been dosed

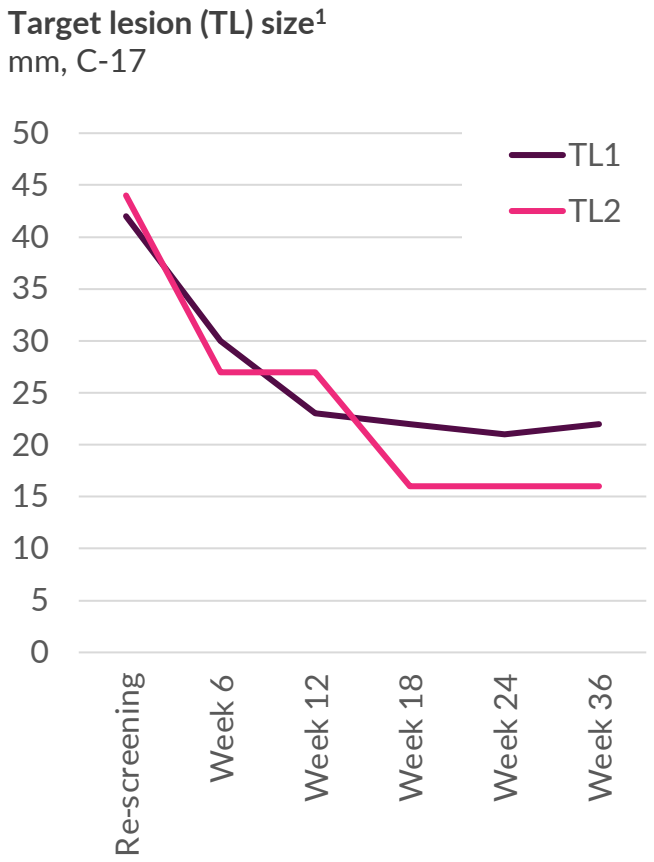
8 CHIRON (NSCLC) patients dosed with Best Response of PR and SD[1]

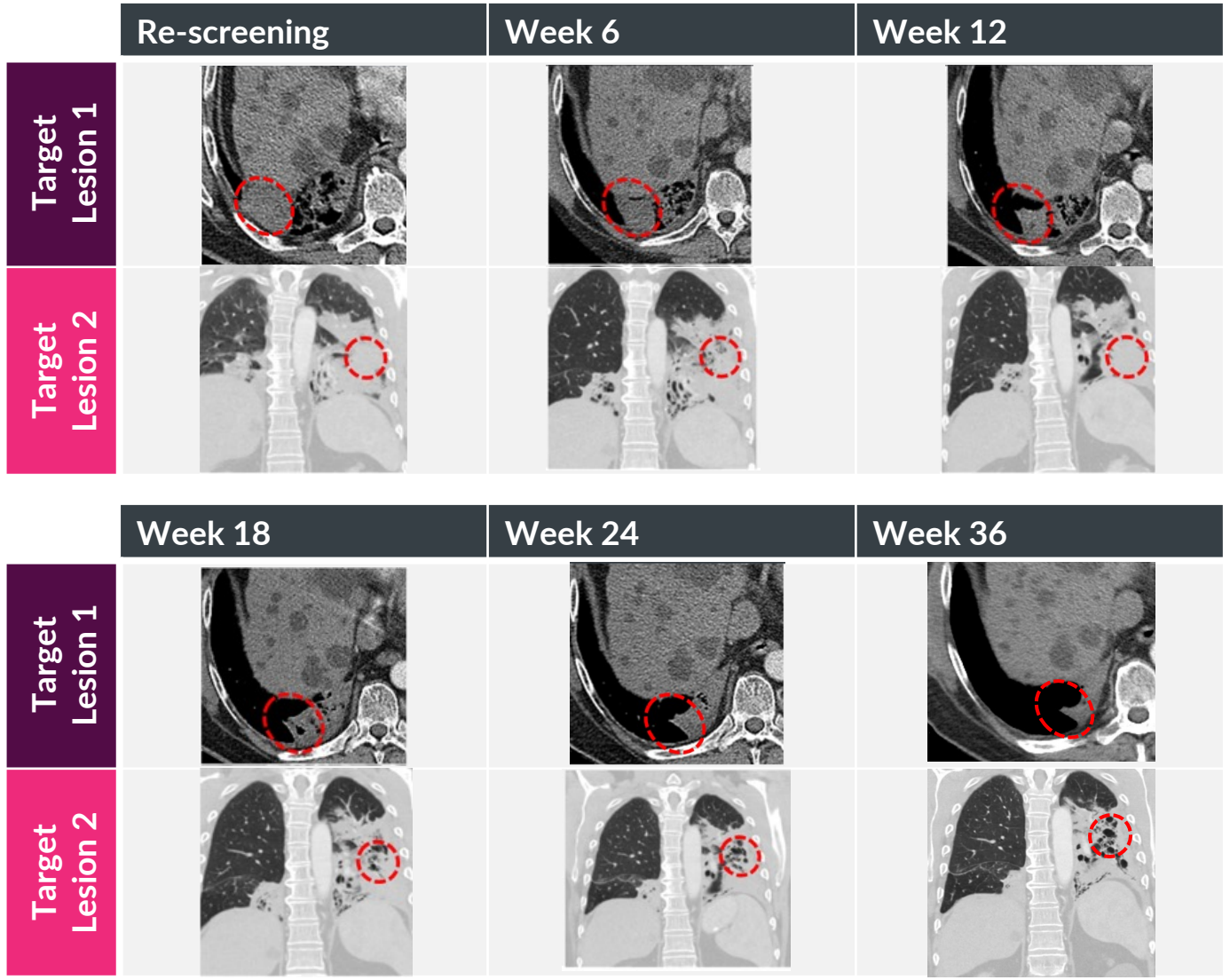


- **Early proof-of-concept demonstrated in NSCLC**
  - Disease control at >12 weeks observed in 5 of 7 evaluable patients (71%), including one PR (>36 weeks)
  - 4 of 7 (57%) out to >18 weeks
- **PR and SD with lower dose lymphodepletion and IL-2**
  - Supports potential for wider applicability of cNeT, including in an ambulatory setting

1. *As reported at ESMO IO 2022*

Patient C-17: 56% reduction in total target lesion size vs. baseline at week 36



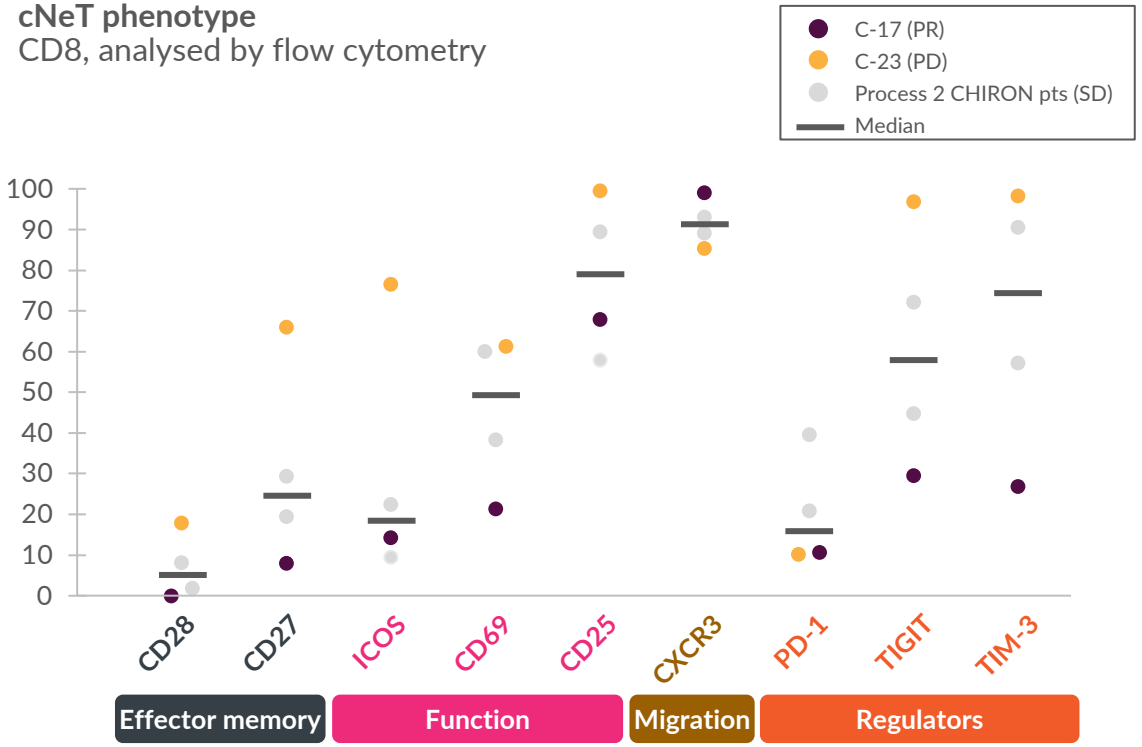


Total target lesion reduction of 56% at week 36, with a 64% reduction in Target Lesion 2

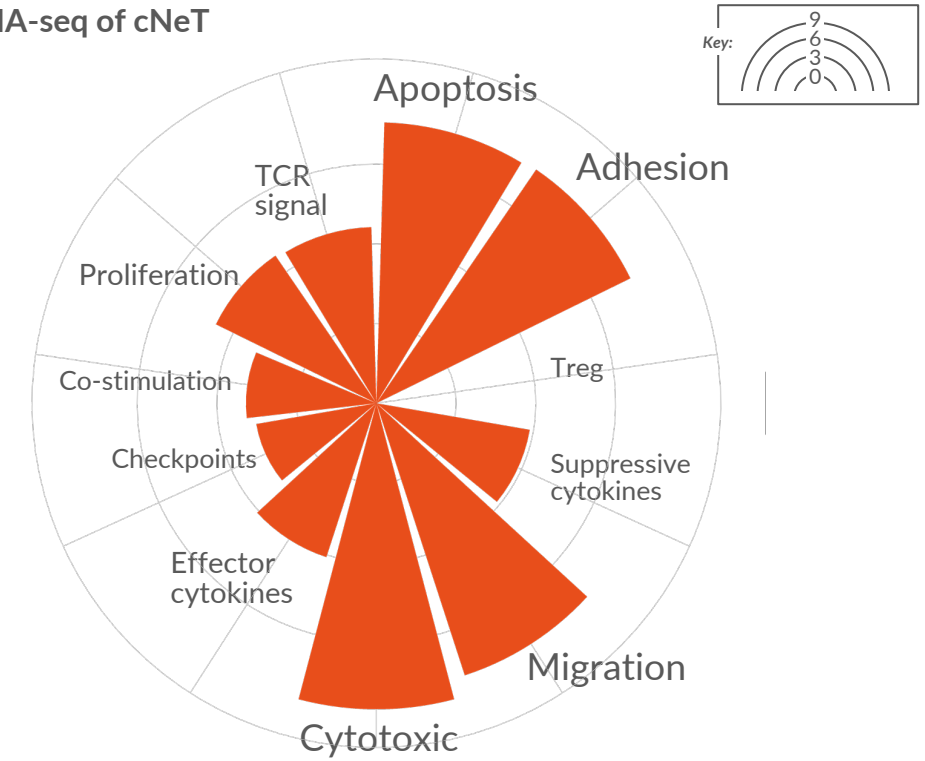
1. Site-reported lesion size
NB C-17 known to have polycystic liver disease

Patient C-17: dosed product shows IL-2 sensitivity, migratory receptors and a polyfunctional transcriptional programme including cytotoxicity, proliferation and effector function

cNeT phenotype
CD8, analysed by flow cytometry



Single cell RNA-seq of cNeT
#cells (log2)

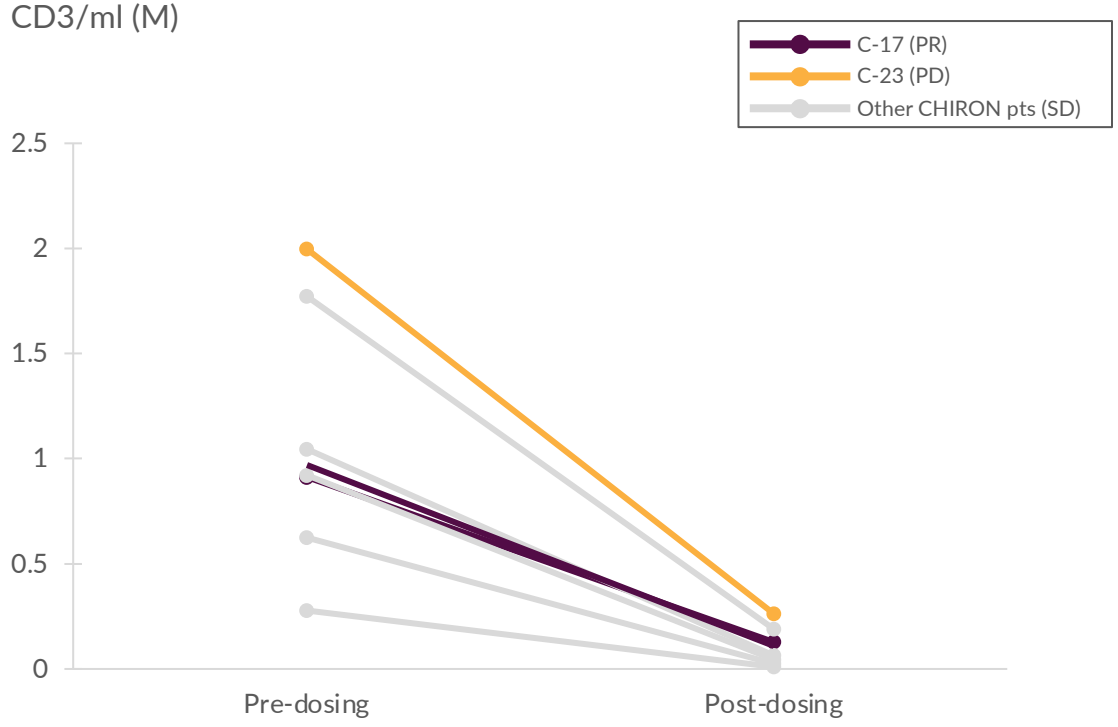


- Product can be restimulated with specific clonal neoantigens and analysed by flow cytometry
- CD8 cNeT are fit, sensitive to IL-2, express receptors for tumour migration, and lower levels of inhibitory checkpoints than other products

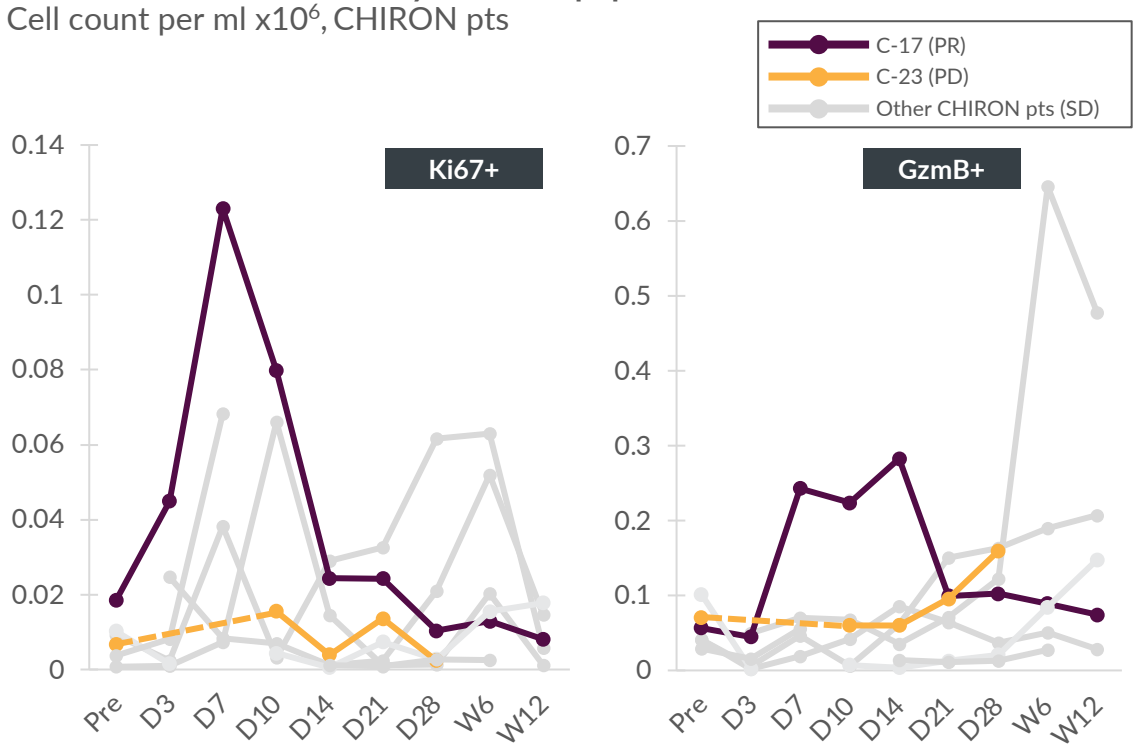
- Single cell RNA-seq of cNeT identify functional modules of cytotoxicity, migration, proliferation and effector function
- Gene signatures identified in response to clonal neoantigen peptides show the active component is polyfunctional

Patient C-17: Efficient lymphodepletion and early reconstitution with functionally active T cells

Lymphodepletion
CD3/ml (M)



CD8+Ki67+ and CD8+GranzymeB+ cell populations
Cell count per ml x10^6, CHIRON pts

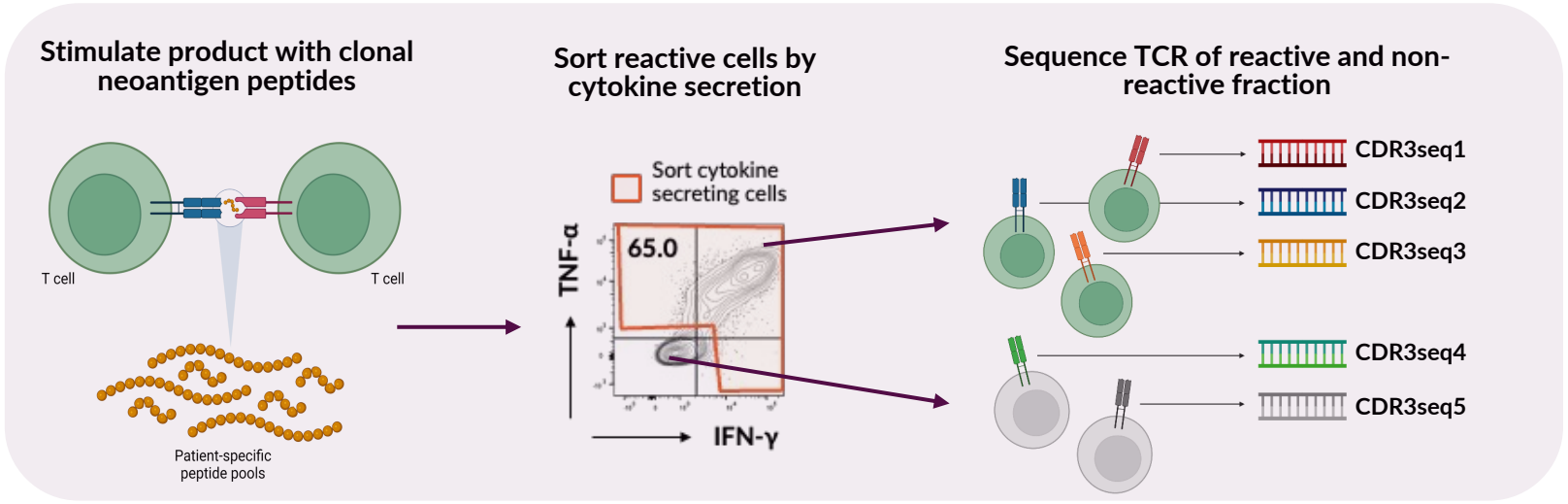


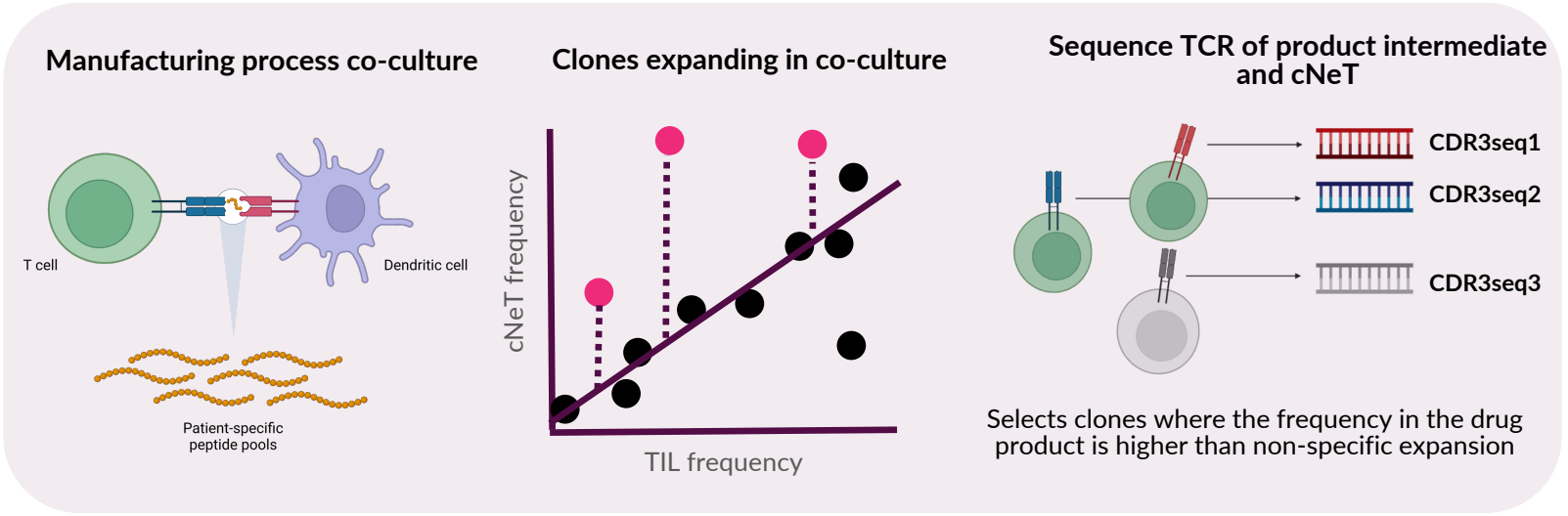
Low intensity conditioning regime is capable of producing effective lymphodepletion of patients' immune cells

- **Phenotypic markers can be analysed to link response with changes in cellular characteristics in blood**
- **Proliferative and cytolytic cells are detected post-dosing in C-17 and such cells have been previously associated with responses to CPI[1]**

[1]*Machiraju et al, Oncoimmunology 2021*

Tracking cNeT post dosing



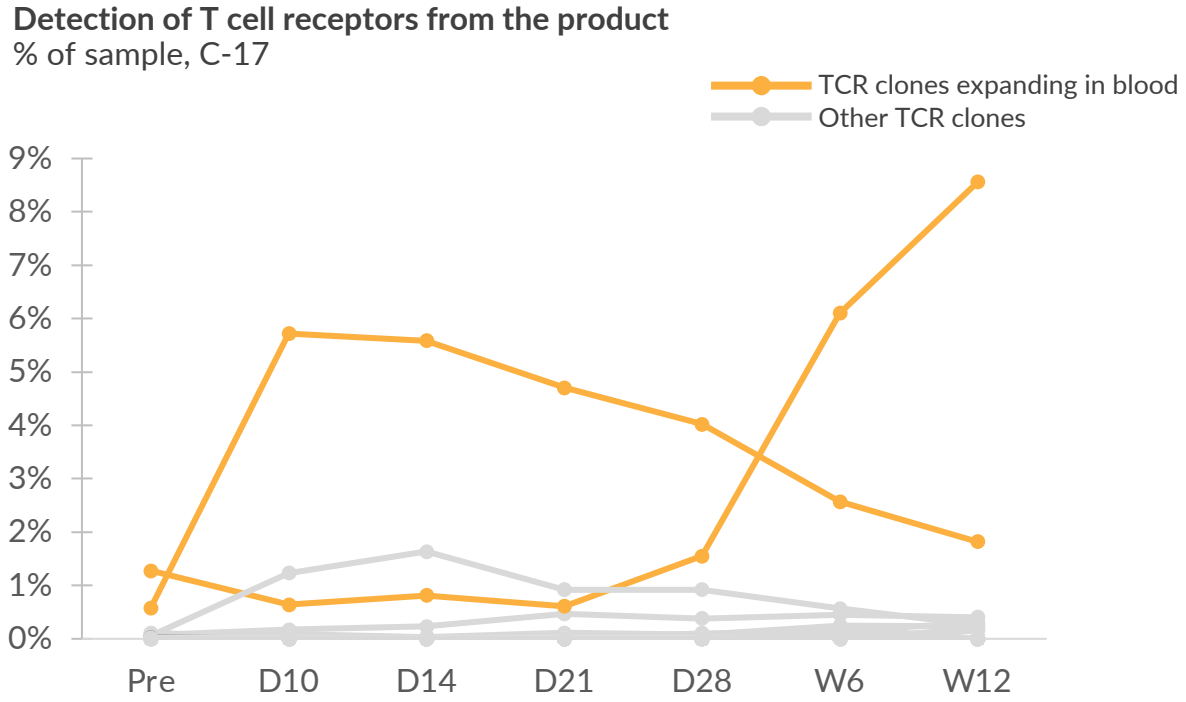


Product characterization

Downstream analysis of T cell engraftment

Patient C-17: cNeTs expand and persist beyond week 12 coincident with tumour regression

T cell clones that are clonal neoantigen-specific are identified expanding in the patient beyond 12 weeks and to a greater extent than other patients



Durable clinical benefit and encouraging safety and tolerability data with cNeT therapy[1]

Lymphodepletion & IL-2 well tolerated

- Lower dose lymphodepletion and IL-2 and 95% of IL-2 doses well tolerated
- Supports potential for wider applicability of cNeT, including in an ambulatory setting

Early PoC in NSCLC

- Disease control >12 weeks in 71% patients, including one PR (>36 weeks)
- Potential for deep, durable clinical responses with reduced lymphodepletion and IL-2

cNeT Driving Anti-tumor Activity

- Engraftment & cytokine profiles supportive of cNeT driving anti-tumor activity
- Active cNeT peak expansion at day 21 coincides with peak in IL-6 (marker of activity)

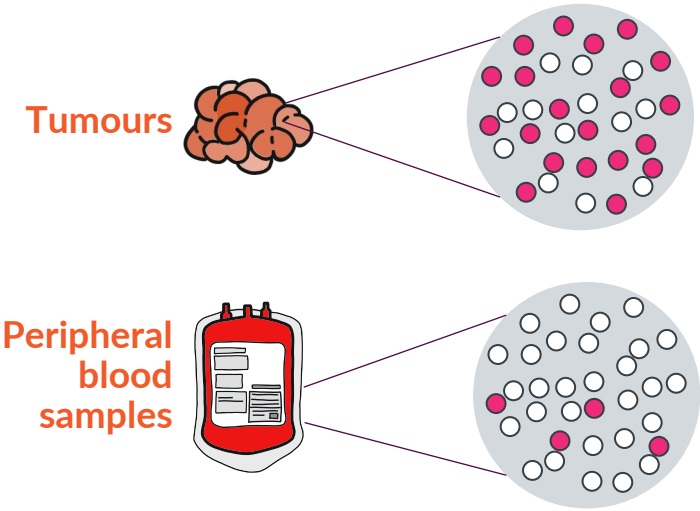
1. *As reported at ESMO IO 2022*

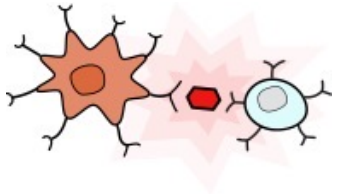
Leveraging clinical data on reactivity & PELEUS™ to develop new tools for predicting immunogenicity: Impact to additional modalities for clonal targeting

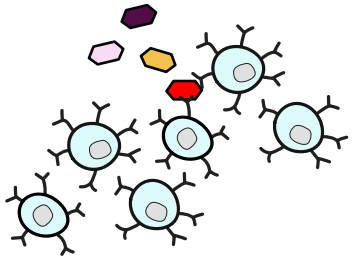
Real-world cancer patient immunogenicity data is the foundation of our model

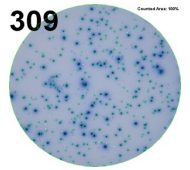
>80 patients	>10,000 clonal neoantigens screened	>500 memory responses

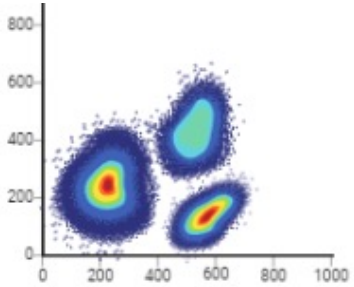
Patient samples 5 indications from material acquisition program & trials → **Culture TIL** or memory cells from blood → Synthesise clonal neoantigen peptides and **enrich the T cells** → **Expand the reactive cells** and re-stimulate → **Validate immunogenicity** → **Characterise T cells**





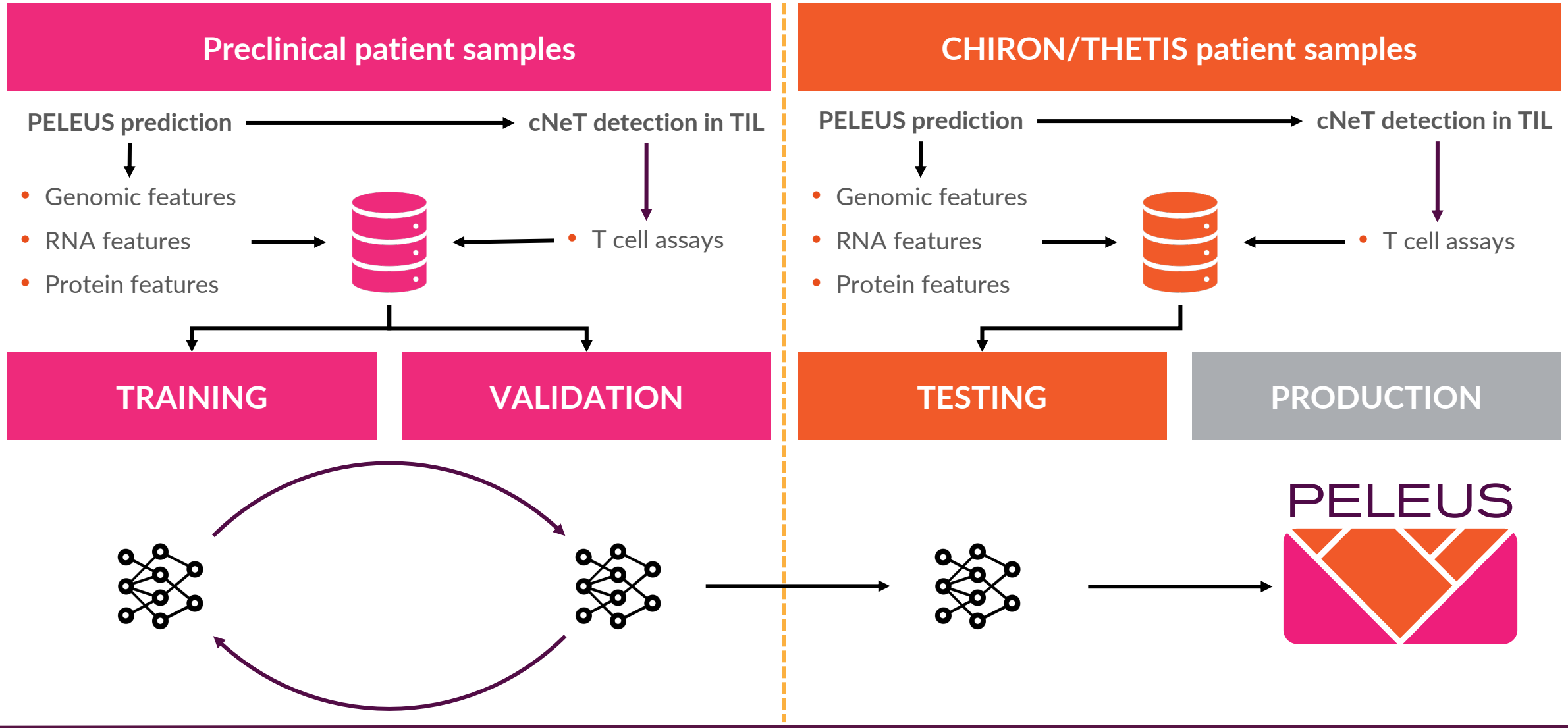






Benefits of our platform	TIL or circulating specific cells are **more relevant than viral datasets**	Up to 200 peptides per patient provides enormous **breadth across the mutations with minimal bias**	Enrichment improves **sensitivity of detection**	ELISpot detects non-immunogenic and **immunogenic neoantigens** to feed into our model	Characterisation of CD4/CD8 using a second assay with flow cytometry provides **confirmation of hits**

Developing an AI tool to enhance PELEUS™ capability to prioritise immunogenic neoantigens

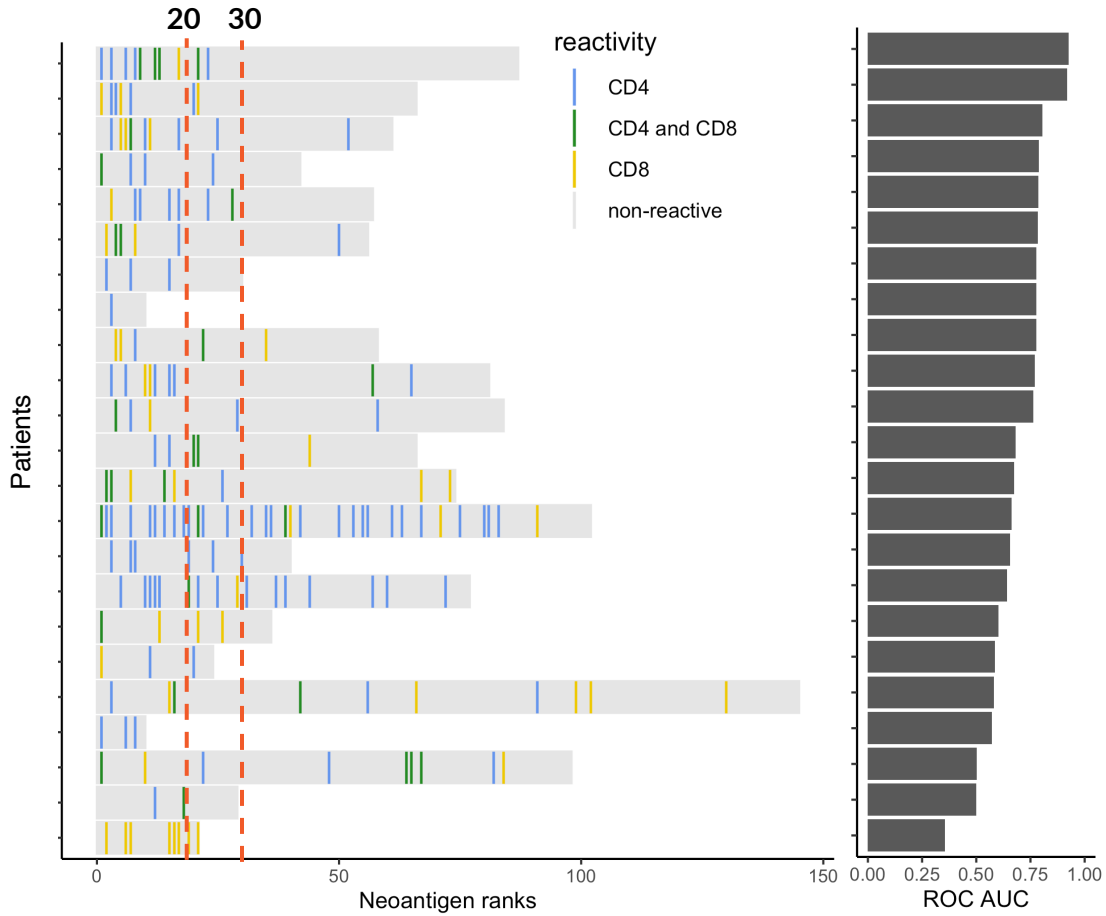


PELEUS™ prioritizes clonal neoantigen targets with reactive CD8 & CD4 T cell responses

- Novel AI tool trained with TIL-derived immunogenicity data predicts both CD8+ and CD4+ responses to clonal neoantigens
- Reactivity screens use up to 200 peptides per patient creating a sizeable and unbiased dataset for training
- **59% of reactivities** are found in the **top 20 ranked peptides** with a mean of 4.3 (range 1-10) per patient
- **72% of reactivities** are found in the **top 30 ranked peptides** with a mean of 5.4 (range 1-13) per patient

Immunogenic peptide prediction

Product reactivities to PELEUS peptides, n=23



Neoantigen rank = immunogenicity ranking prediction (1 is highest ranked)

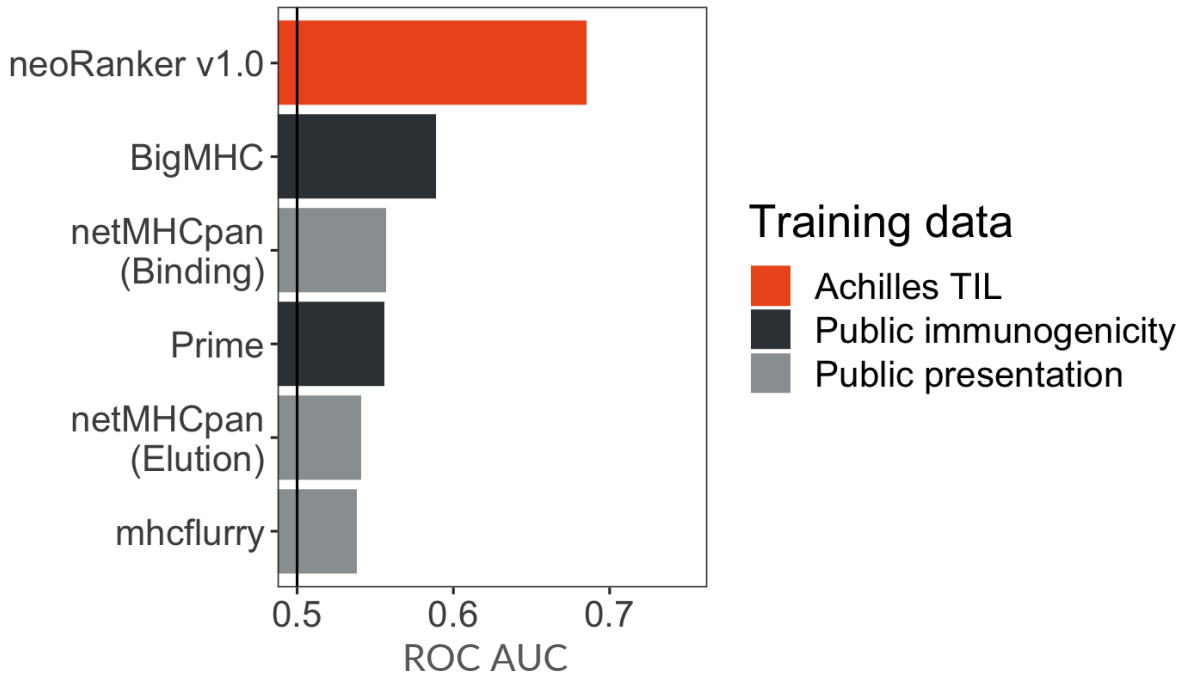
ROC AUC = Receive Operator Characteristic Area Under Curve; measure of ranking ability

Achilles neoRanker immunogenicity predictions outperform existing tools

- Our dataset comprises ~10,000 screened neoantigens across ~80 patients and continues to grow
- Our unique training dataset is proprietary whilst competitor tools rely on limited public databases
- Achilles outperforms competition and is at least 50% better than BigMHC and 73% better than netMHC at predicting immunogenic neoantigens.
- Clonality + immunogenicity: Great relevance to precision cell therapy and personalised cancer vaccines

Immunogenicity ranking benchmark

Validation dataset of TIL derived *bona fide* neoantigen T cell reactivities (patients = 21, true positives = 166)



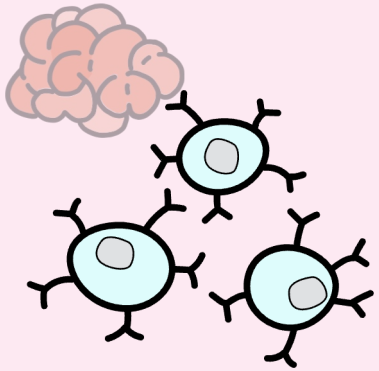
Clonal neoantigens can be targeted with a range of therapeutic modalities

Current Achilles approach

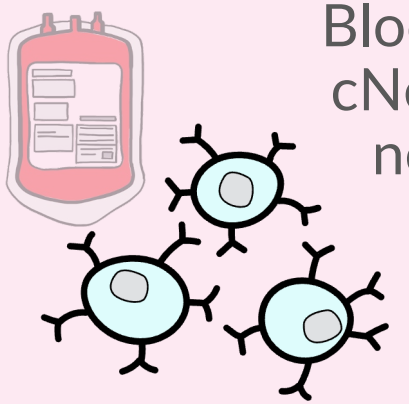
Alternative modalities

TIL-based cNeT

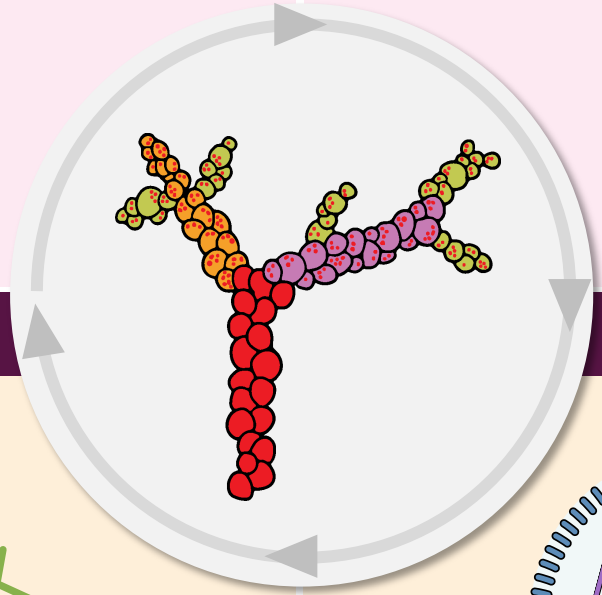
Clinically validated across multiple solid tumour settings



Blood-based cNeT

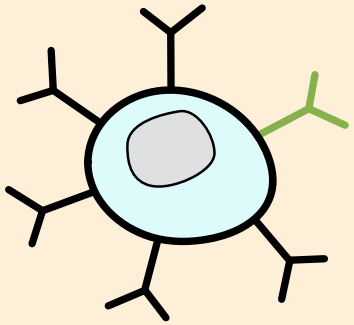


Blood as source of cNeT, without the need for surgery

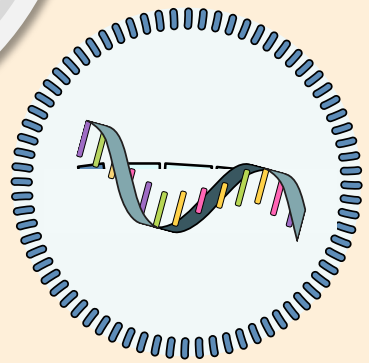


TCR-therapy

T cells engineered with receptors that target shared neoantigens



Clonal neoantigen vaccines



mRNA vaccines using highly immunogenic clonal neoantigens to improve efficacy

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