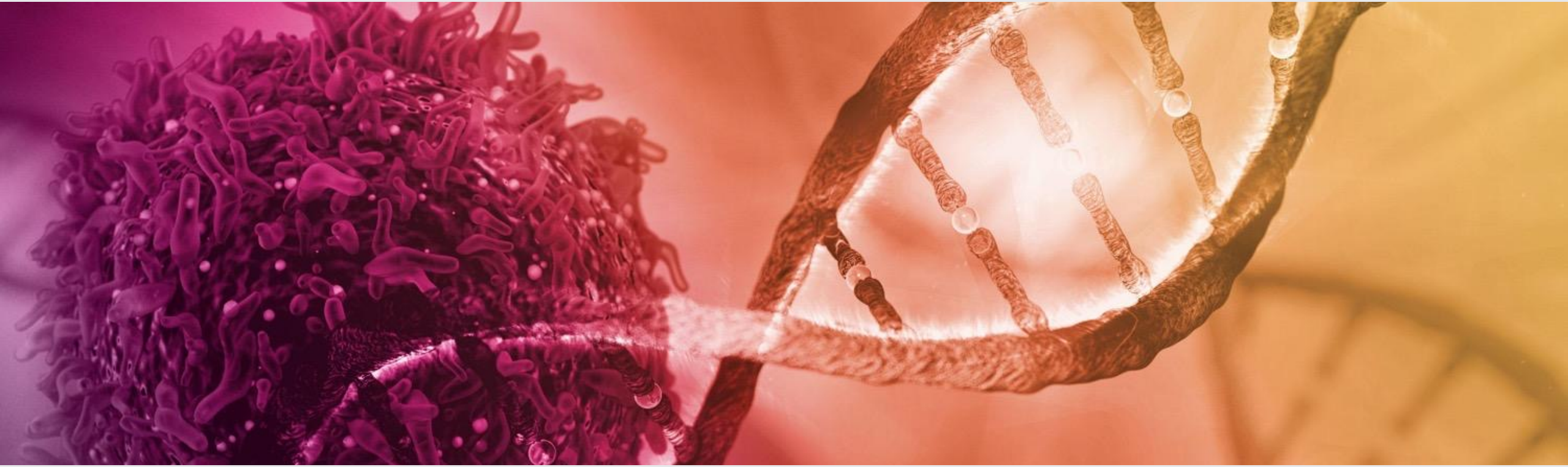


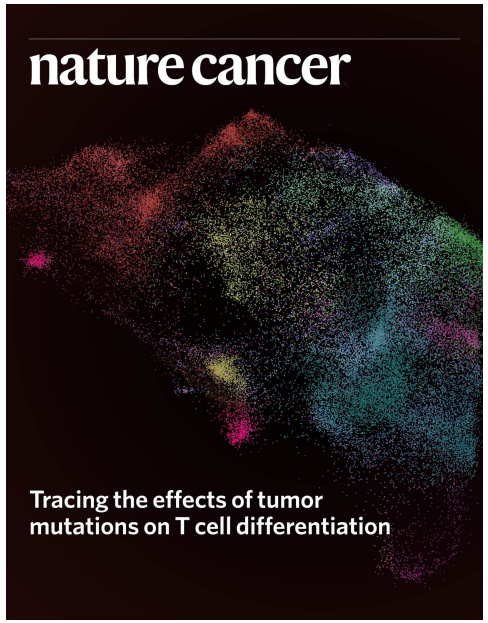
Personalised Neoantigen Therapies: State of the Art Neoantigen Immunogenicity Prediction

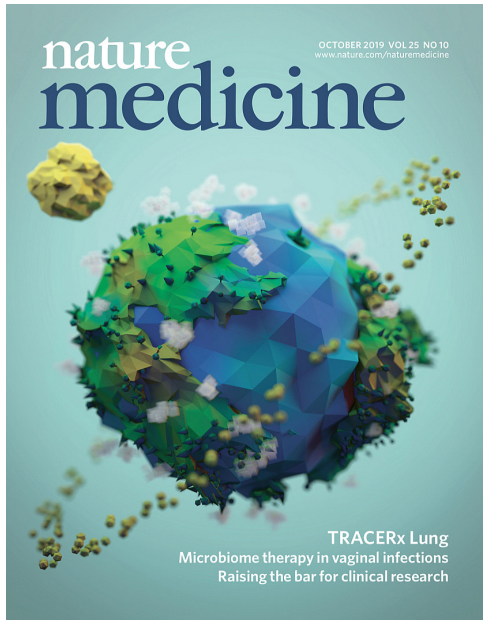
IO Summit EU - 21 June 2023

Andrew Craig, SVP Bioinformatics and Data Science, Achilles Therapeutics

TRACERx: the largest longitudinal real-world NSCLC patient dataset

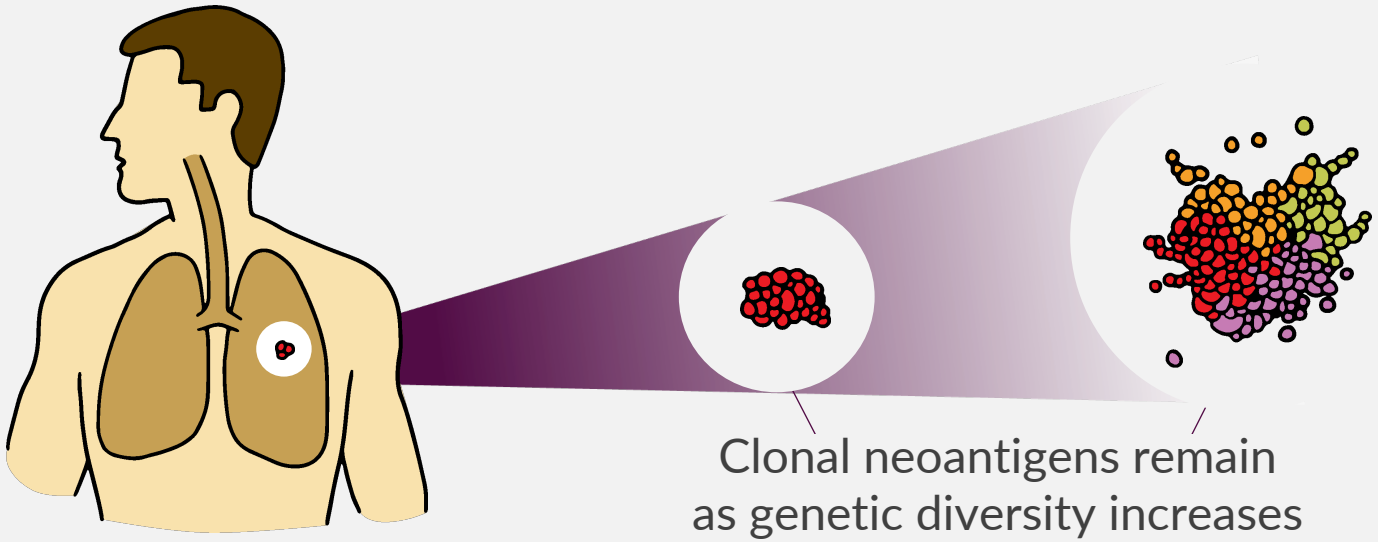
- **Over 9 years, 815 patients enrolled** with adv/ NSCLC
- Extensive **multi-region sequencing of >4,000 biopsy samples**
- **250 investigators** based at **19 hospital sites** in UK
- **25+ publications** with **~6000** citations

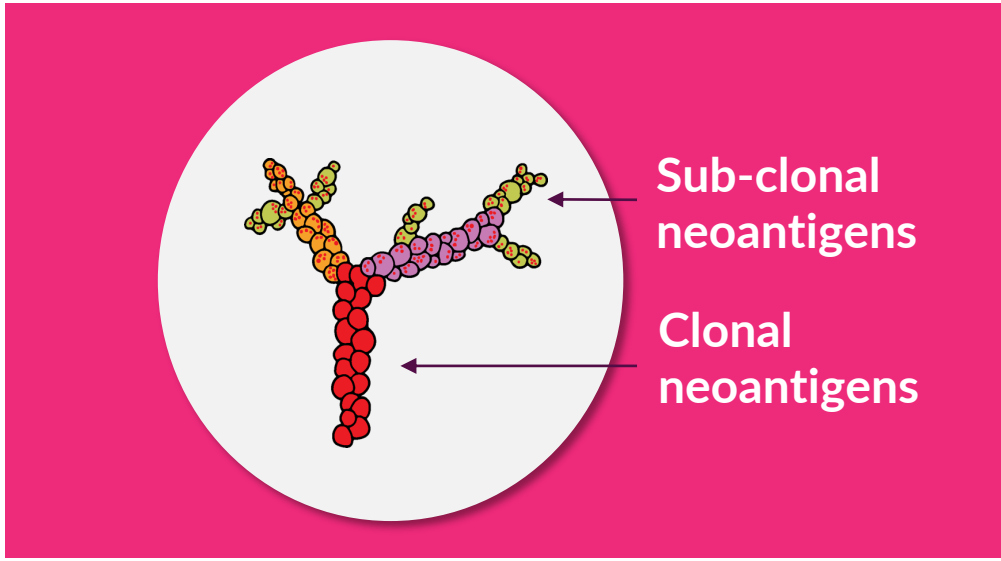




1. Rosenthal et al Nature, 2019
2. Ghorani et al Nat. Cancer, 2020
3. Chemi et al Nat. Med, 2019; Joshi et al Nat. Med 2019
4. Abbosh et al Nature, 2017

The landmark TRACERx study demonstrated that clonal neoantigens are on all tumour cells





Tumours constantly evolve and acquire new mutations

Original, clonal mutations passed down and remain in all tumour cells[1-4]

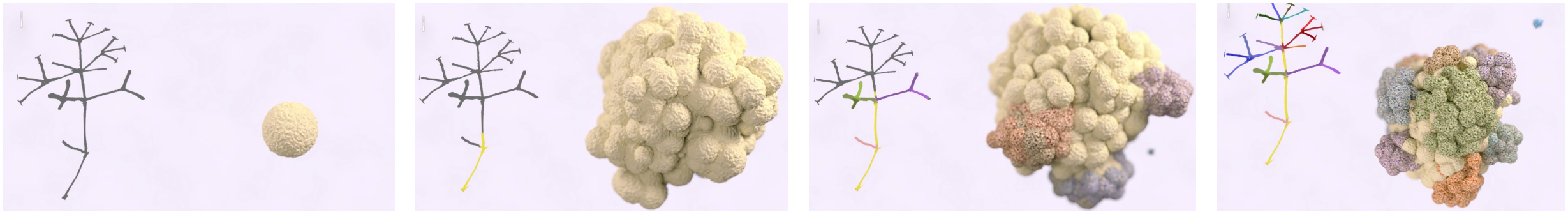
Achilles can identify clonal mutations for each patient & target multiple antigens only on tumour cells[2-4]

1. Gerlinger et al., NEJM, 2012
2. Jamal-Hanjani et al., Plos Biol, 2014
3. Jamal-Hanjani et al. NEJM,
4. Abbosh et al., Nature, 2017

Fundamentals of branched tumour evolution

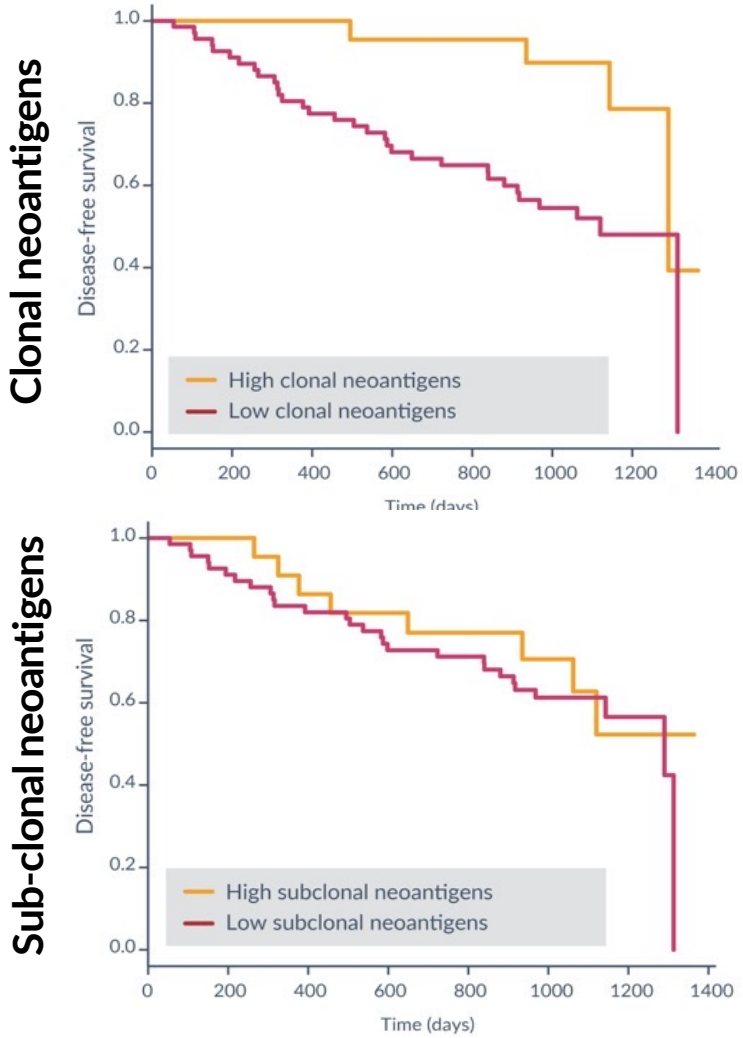
TRACERx played a significant role in furthering our understanding of tumour evolution and heterogeneity, revealing the importance of clonal neoantigens as targets for treating solid tumours

- Clonal mutations occur early in tumour evolution and are present in all tumour cells including metastases[1]
  - Clonal neoantigen reactive T cells are found in all tumour regions in NSCLC[2]
- Subclonal mutations occur later in the tumour evolution and so are found only in a subset of tumour cells
  - Subclonal neoantigens can be detrimental to immune response through subclonal distraction[3]



1. Al Bakir et al. 2023 Nature
2. McGranahan et al. 2016 Science
3. Wolf et al., 2019, Cell

Critical relevance of clonal neoantigens demonstrated clinically



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**

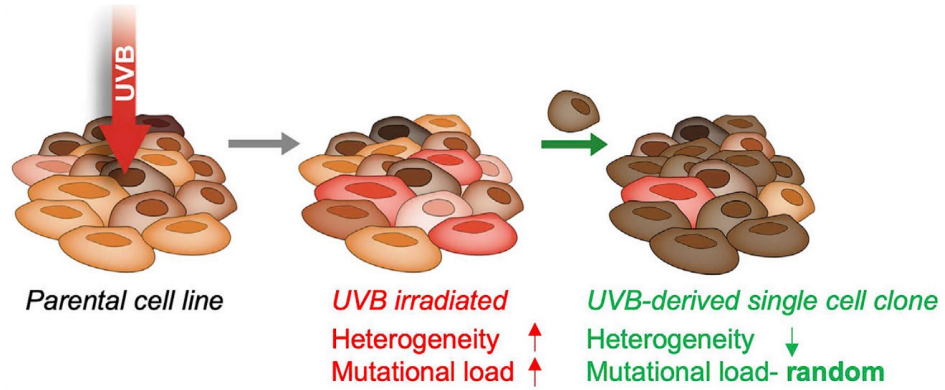
Rosenthal et al 2019 Nature
McGranahan et al. 2016 Science

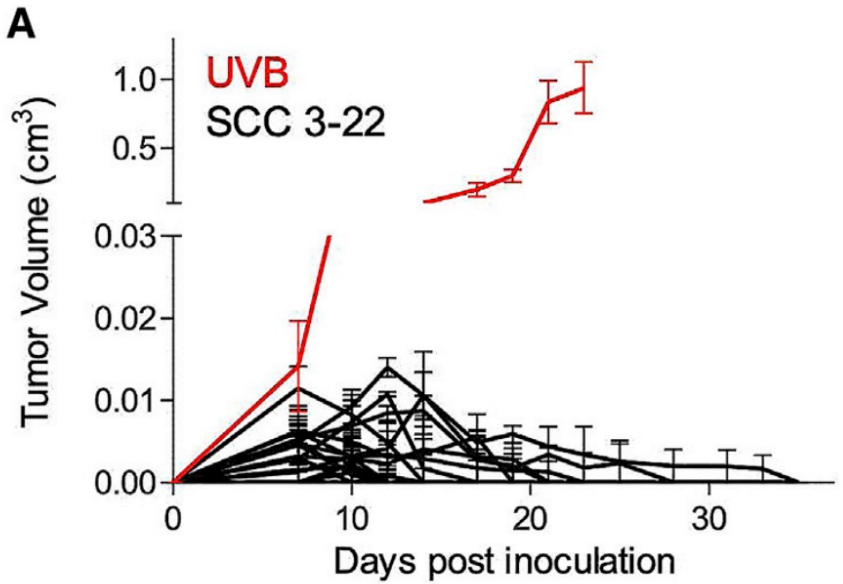
Subclonal neoantigen burden and ITH can reduce the immune response to tumours

UVB-Induced Tumor Heterogeneity Diminishes Immune Response in Melanoma

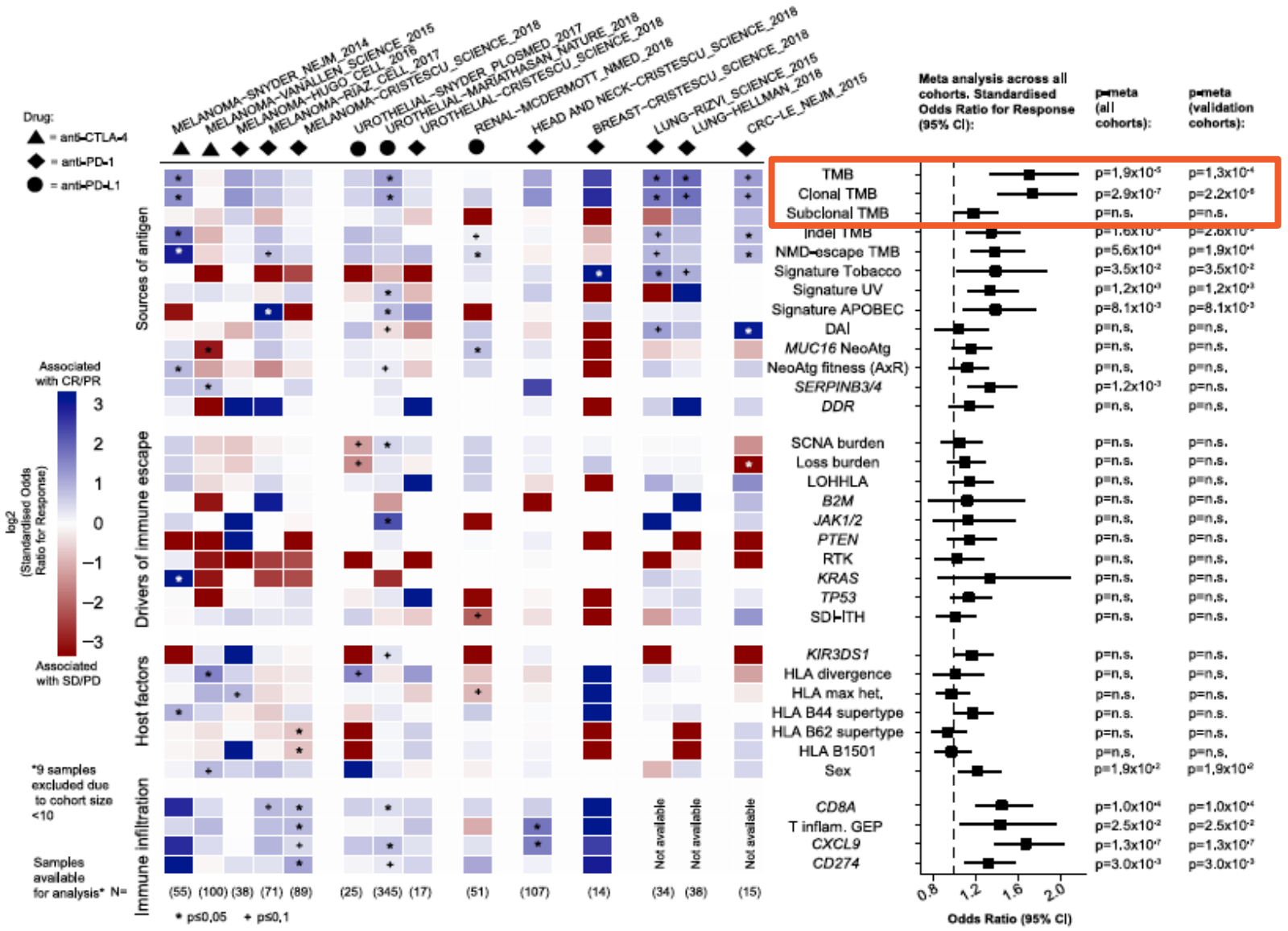
Cell

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,*]





Clonal but not subclonal mutational burden predicts CPI response
Meta-analysis of CPI response in >1,000 patients across 7 indications

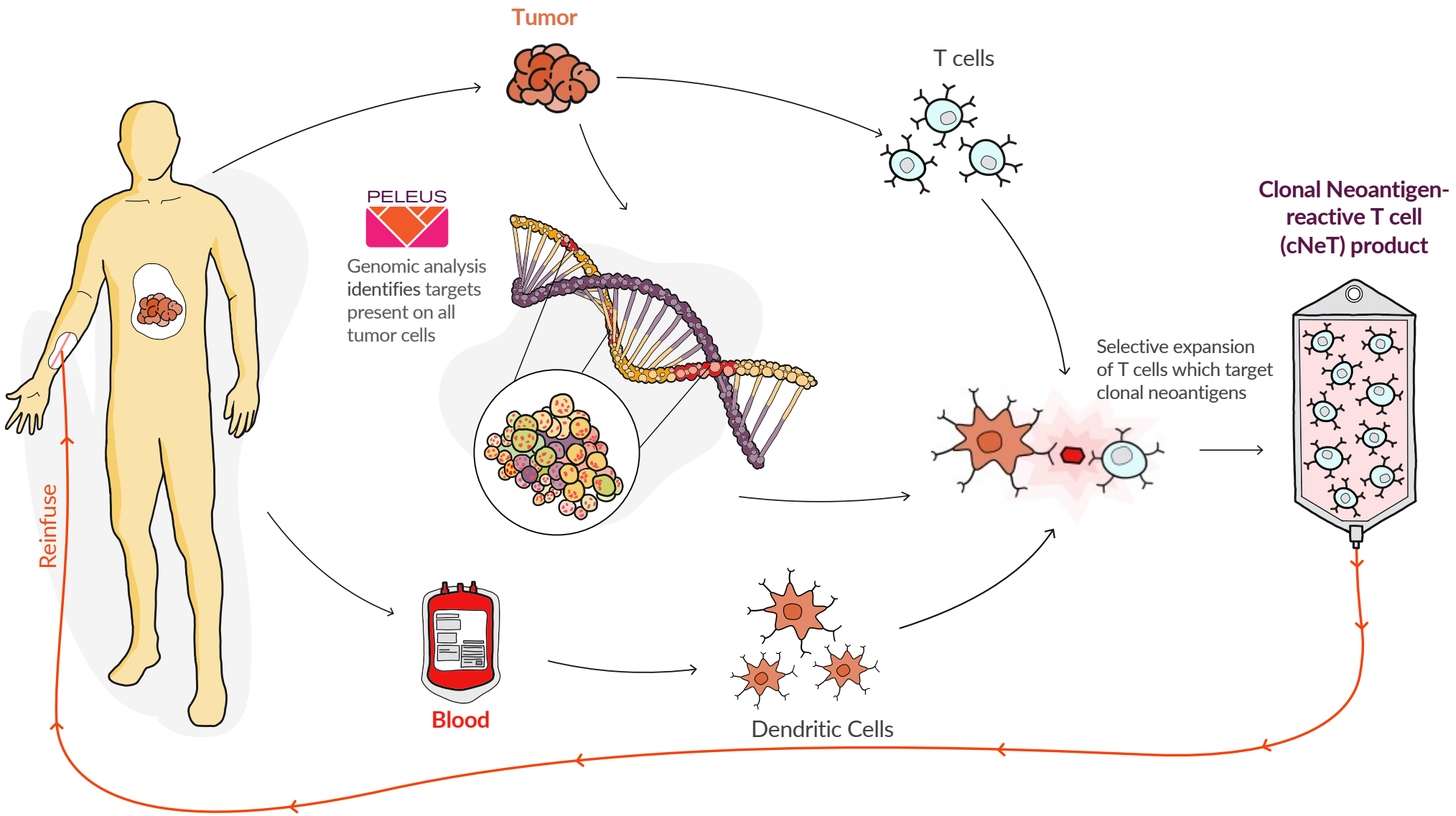


A meta-analysis performed by our scientific founding team (Swanton & Quezada) demonstrated the importance of clonal neoantigens in checkpoint inhibitor (CPI) response across indications:

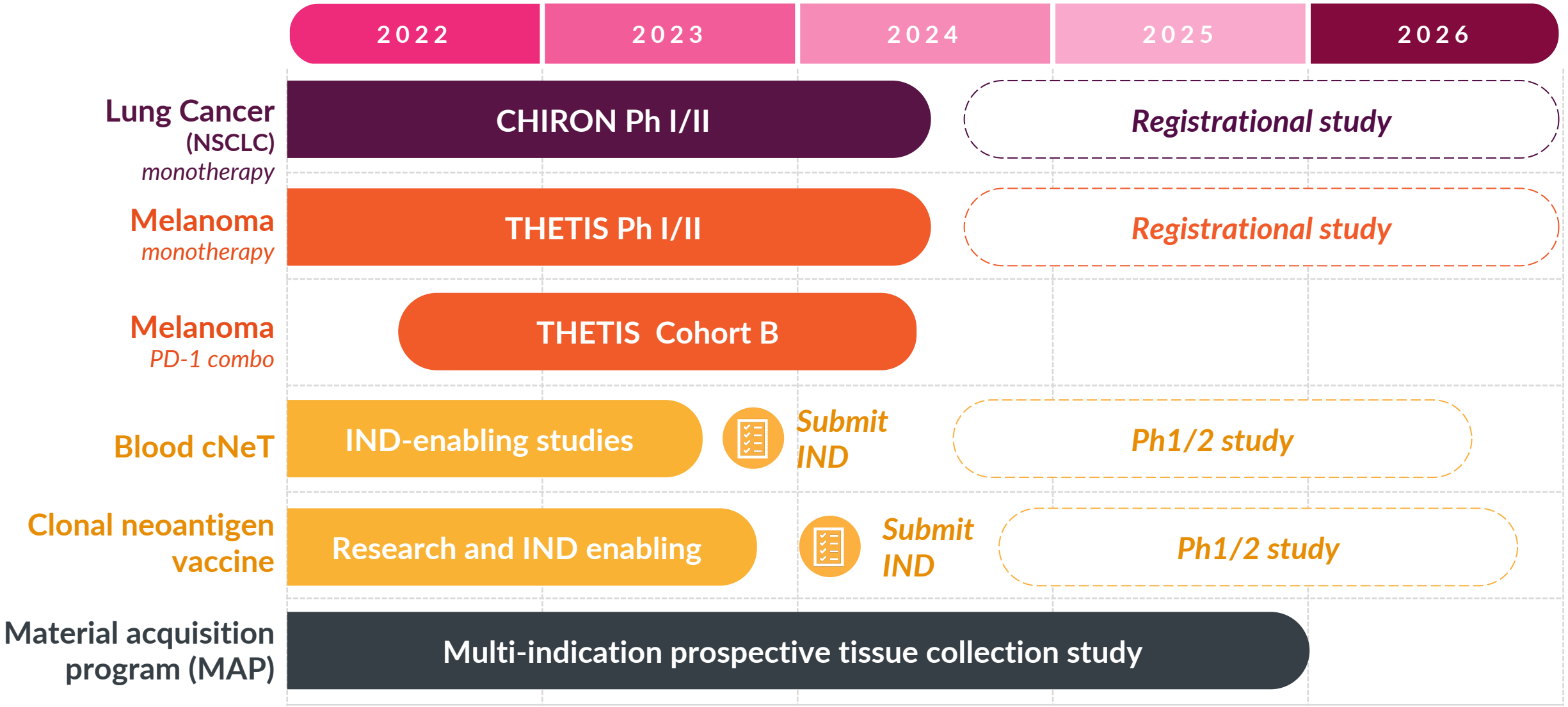
- Melanoma
- Lung
- Renal
- Head & neck
- Breast
- Bladder
- Colorectal

Achilles process delivers precision clonal neoantigen targeting T cell therapy (cNeT)

Cutting edge personalized genomics and machine learning enable targeting of all cancer cells

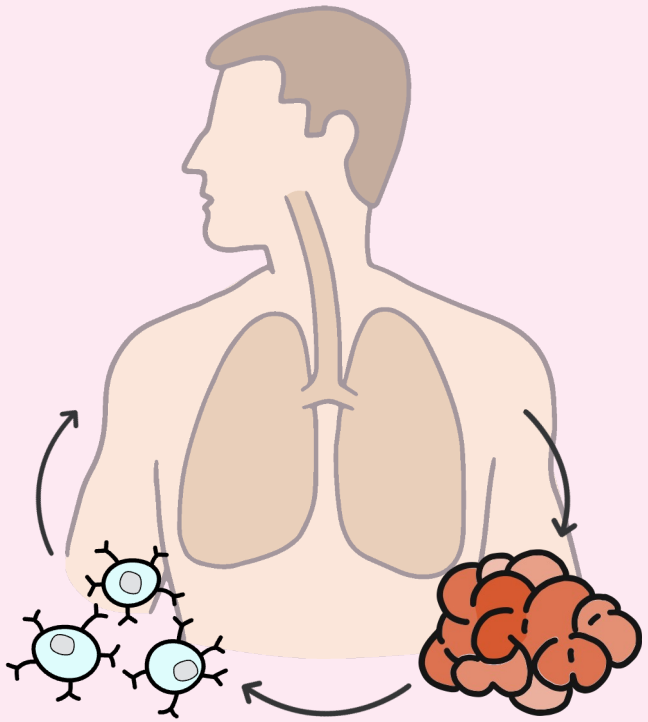


Differentiated pipeline of precision T cell therapies across multiple solid tumours



Delivered emerging proof of concept for cNeT therapy showing durable clinical benefit and encouraging safety and tolerability data [1]

Ph I/II interim clinical data in advanced NSCLC patients
Potential for deep, durable clinical responses with reduced lymphodepletion and IL-2



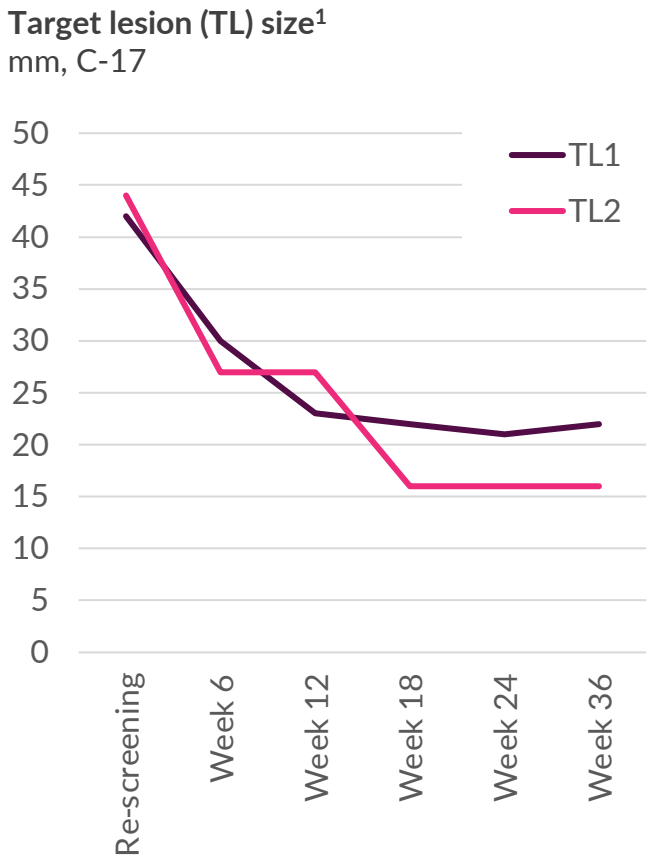
- **Lower dose lymphodepletion and IL-2** and 95% of IL-2 doses well tolerated
- Supports potential for wider applicability of cNeT, including in an ambulatory setting

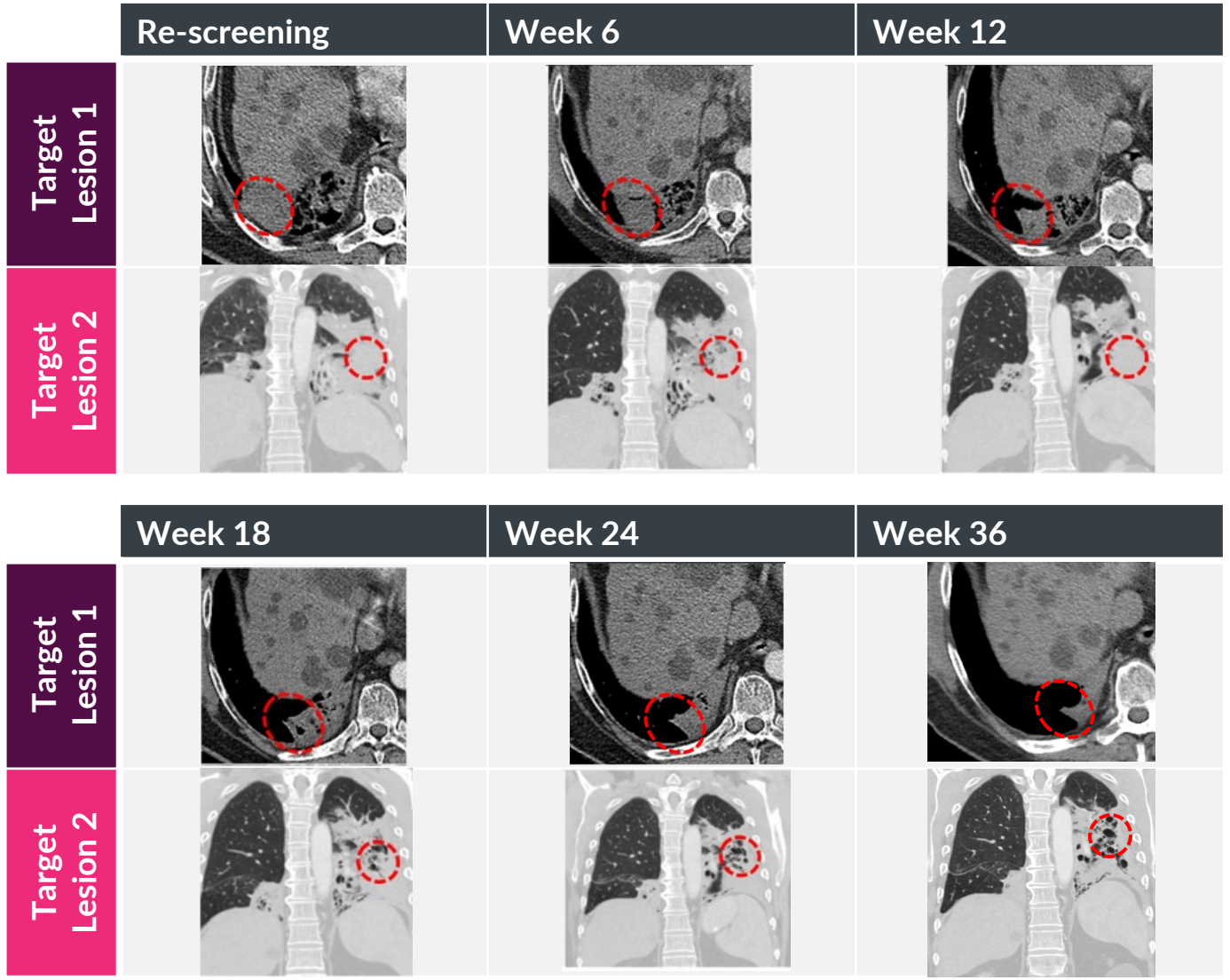
- **Disease control >12 weeks in 71% patients**, including **one PR (>36 weeks)**

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

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

Only Achilles can accurately 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]

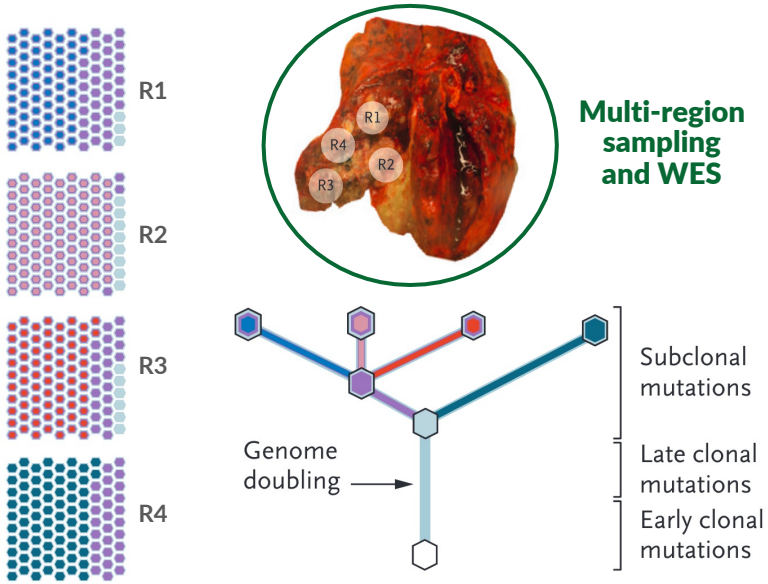
1. Jamal-Hanjani *et al* 2017
2. McGranahan *et al* 2017

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 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

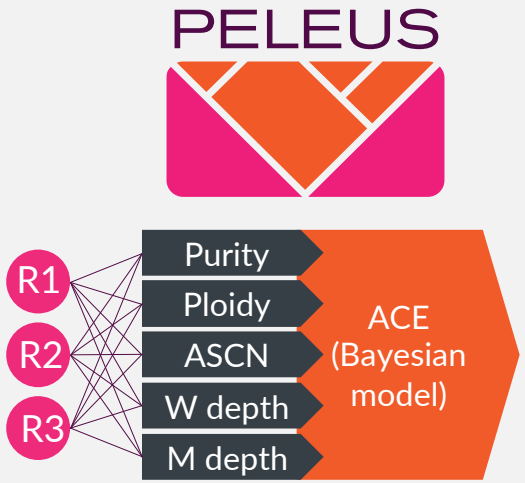
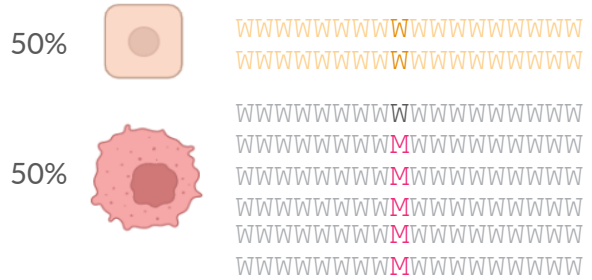


Illustration: Sub-clonal mutation followed by amplification



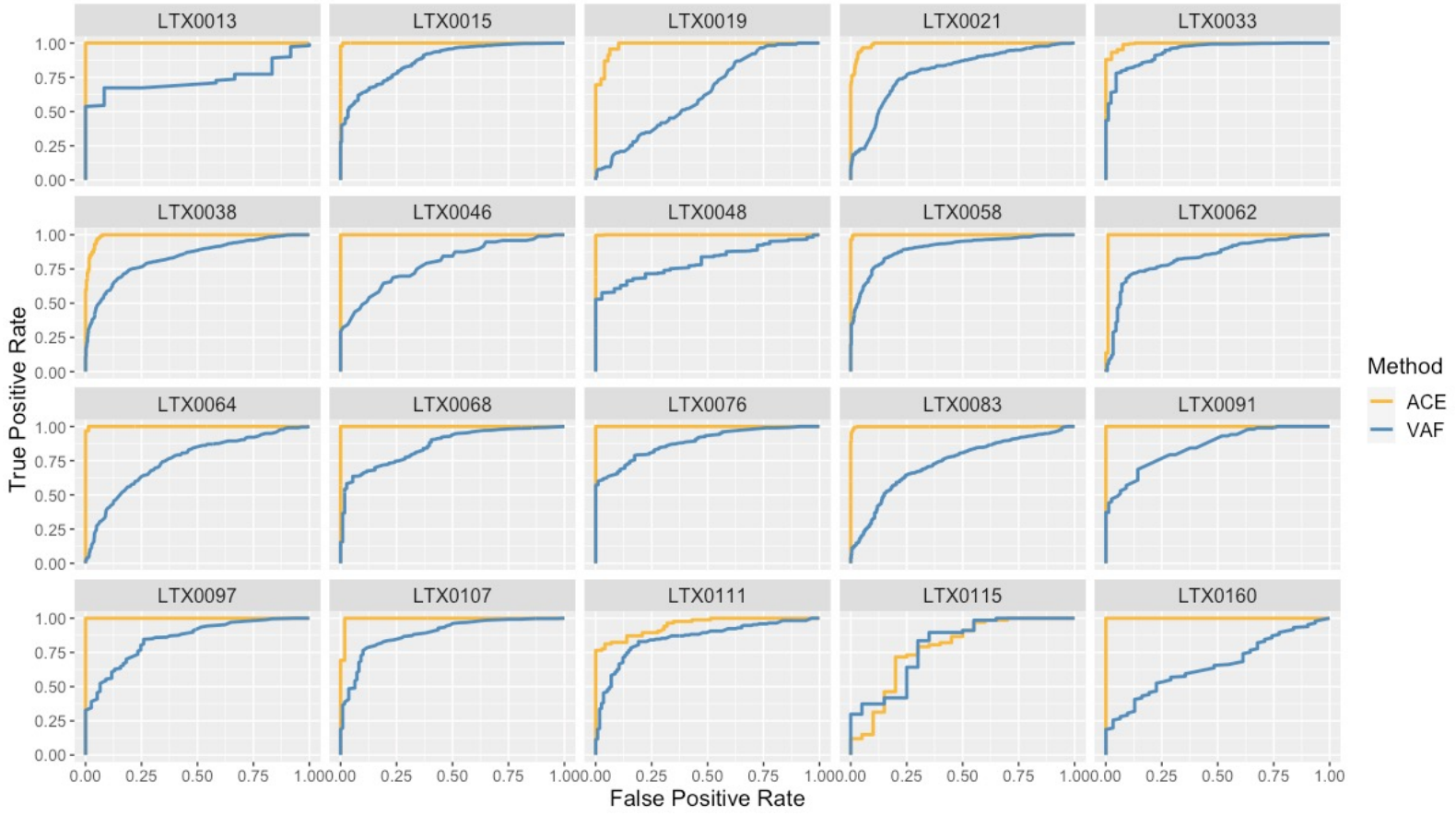
Other methods such as VAF may mistake this for a clonal mutation

Jamal-Hanjani et. al. 2017

Achilles Clonality Engine patent (US Pat. No. 11504398)

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

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

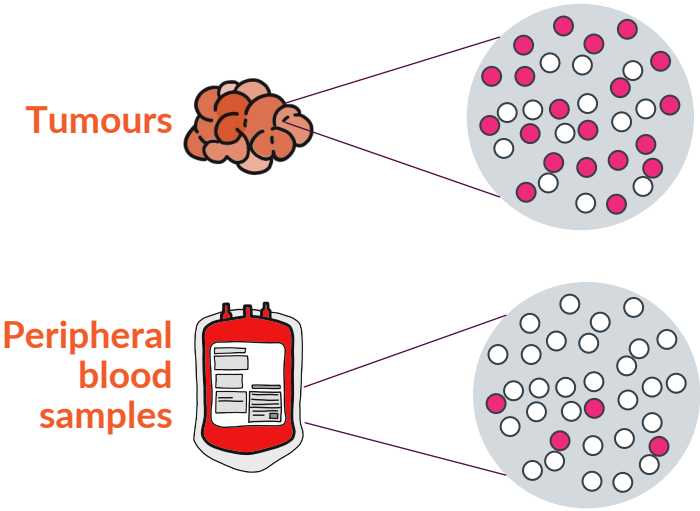
Culture TIL or memory cells from blood

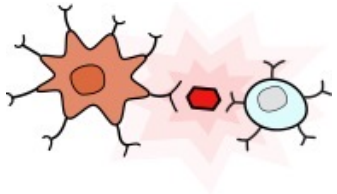
Synthesise clonal neoantigen peptides and **enrich the T cells**

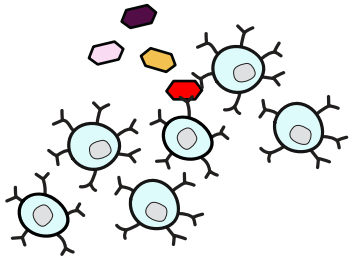
Expand the reactive cells and re-stimulate

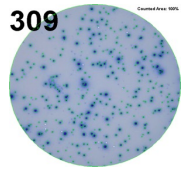
Validate immunogenicity

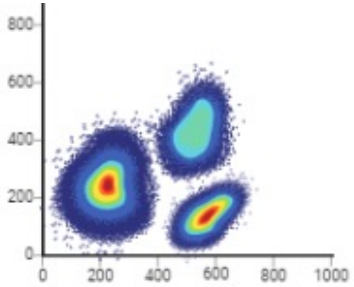
Characterise T cells





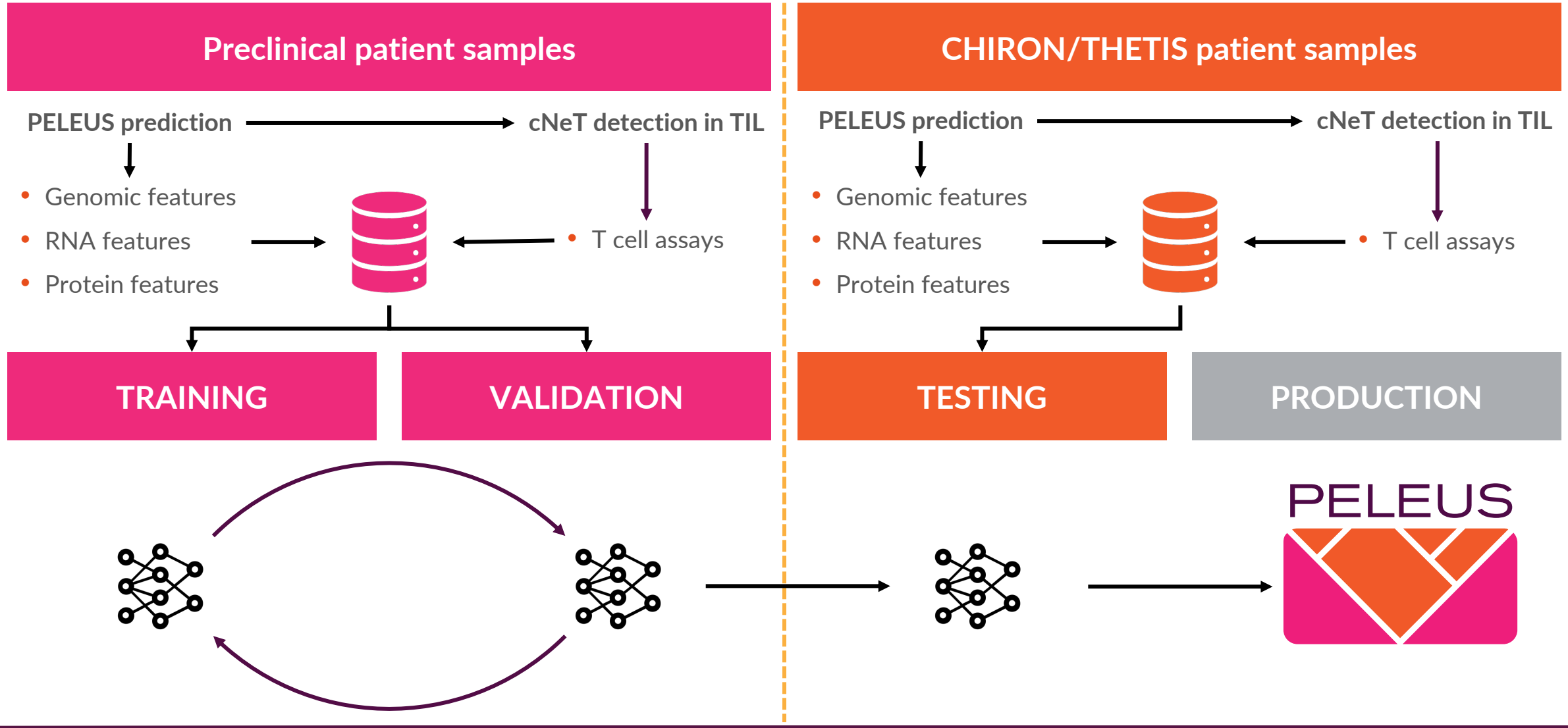






Benefits of our platform	TIL or circulating specific cells are **more relevant than viral datasets**	Up to 200 peptides per patient provides enormous **breadth across the mutations with minimal bias**	Enrichment improves **sensitivity of detection**	ELISpot detects non-immunogenic and **immunogenic neoantigens** 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

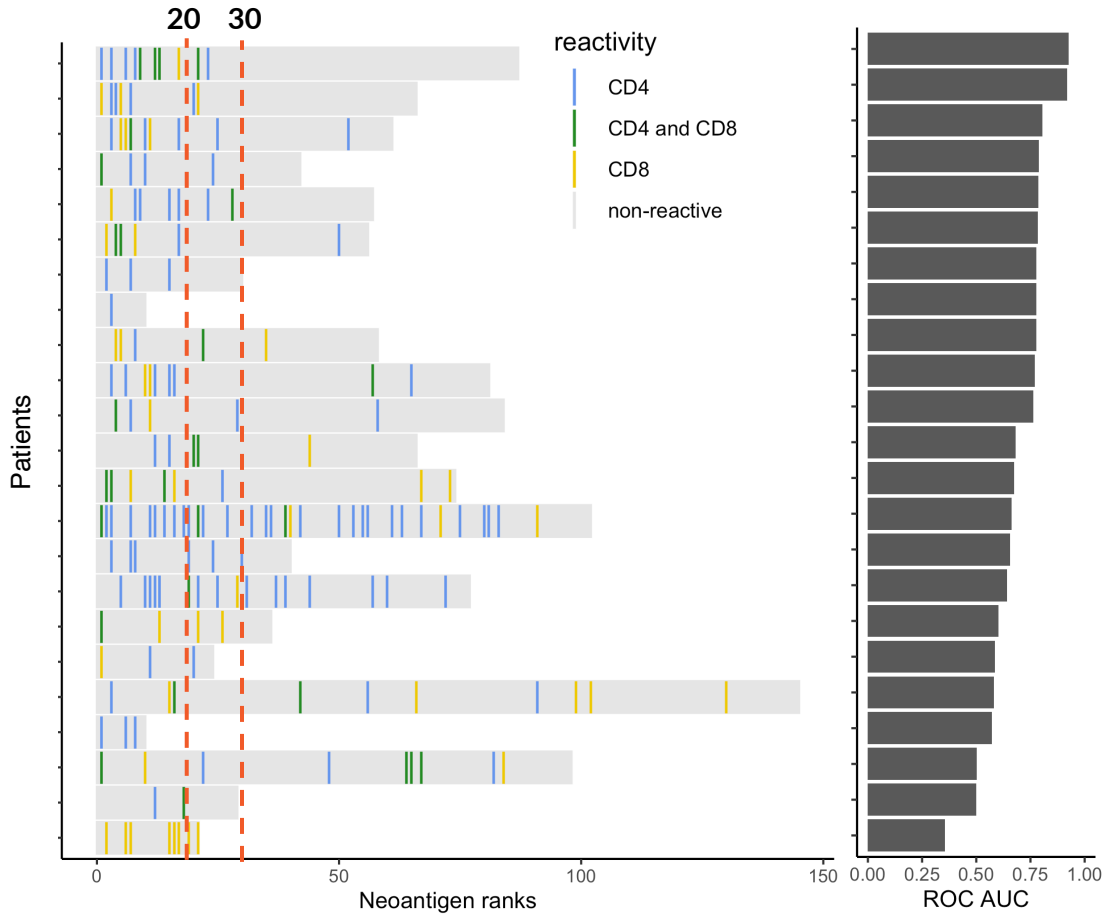


PELEUS™ prioritizes clonal neoantigen targets with reactive CD8 & CD4 T cell responses

- 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

Benchmarking against representative methods used in the field

Methods trained to predict antigen binding and presentation

(Public data HLA – antigen binding affinity / immunopeptidome eluted ligand data, viral antigens, TAA)

Methods employing transfer learning to fine tune a binding/presentation model to predict immunogenicity (TAA and neoepitope immunogenicity)

NetMHC Pan v4.x

BigMHC

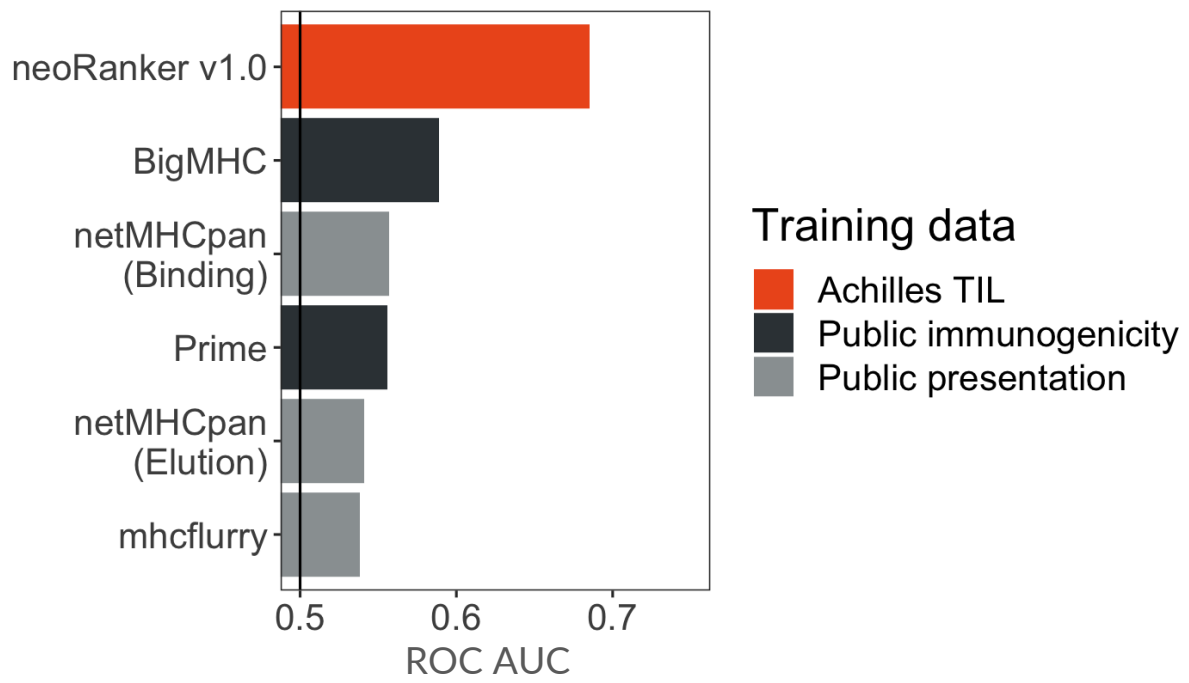
MHCflurry

PRIME

Achilles neoRanker immunogenicity predictions outperform existing tools

Immunogenicity ranking benchmark

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



- 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 as measured by ROC AUC
- Potential to further improve ranking ability

Future directions for immunogenicity modelling

Data

- Collect more data
- Refinement of existing data
- Higher throughput assays
- Alternative data streams

Models

- Error analysis
- New feature engineering
- More flexible models
- Transfer learning

Biology

- Incorporate domain knowledge
- Broadening definition of reactivity
- Clinical validation in clinical trials
- Ability to predict *de novo* priming

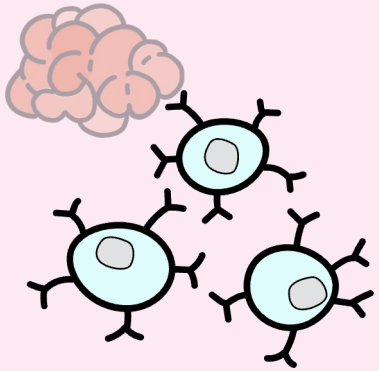
Clonal neoantigens can be targeted with a range of therapeutic modalities

Current Achilles approach

Alternative modalities

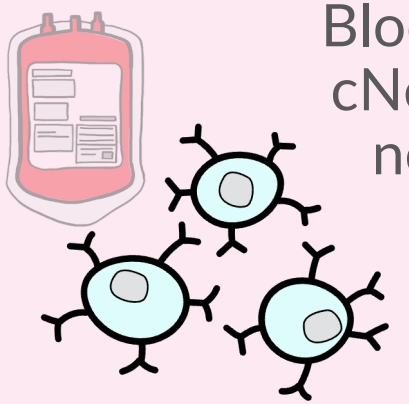
TIL-based cNeT

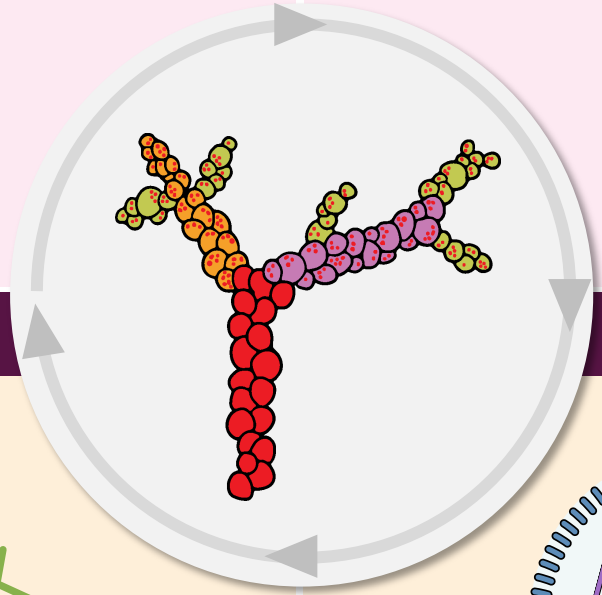
Clinically validated across multiple solid tumour settings



Blood-based cNeT

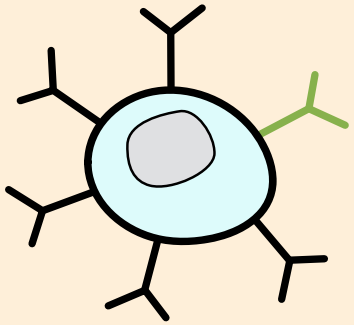
Blood as source of cNeT, without the need for surgery





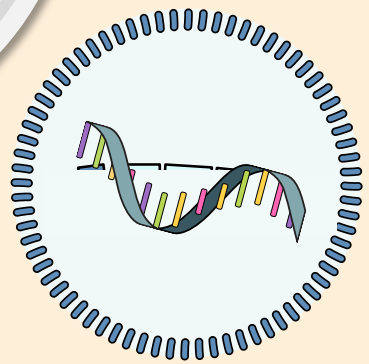
TCR-therapy

T cells engineered with receptors that target shared neoantigens



Clonal neoantigen vaccines

mRNA vaccines using highly immunogenic clonal neoantigens to improve efficacy



Focus for CHIRON and THETIS data readouts in 2023

Clinical	Dose & deliver data from 15-20 additional patients with cNeT monotherapy (lung & melanoma) and CPI combo (melanoma)
	Drive additional confirmed responses in CHIRON and THETIS patients with higher cNeT doses
Translational	Leverage world-class translational science platform to define actionable cNeT features of response
Process	Continue PELEUS™ and process development to optimize dose and identify new sources of clonal neoantigens

Acknowledgments

Achilles Therapeutics

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- Lukas Black
- Theres Oakes
- Jesse Gallagher

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- Gareth Wilson
- Fong Chan
- Lili Cadieux
- Max Salm
- Hugh O'Brien

GMP Manufacturing

- Edward Samuel
- Henrieta Fraser
- Sarah Thirkell
- Asiya Arsad
- Sam Jide-Banwo
- Rebecca Pike
- Michael Pruchniak

Clinical sites and investigators

The patients and their families