Targeting Clonal Neoantigens with Precision Cell Therapies: Key Mechanistic Insights From cNeT Clinical Trials

TIL Summit – Boston – October 2024

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Forward-Looking Statements

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

Cell

Cell, 2015

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

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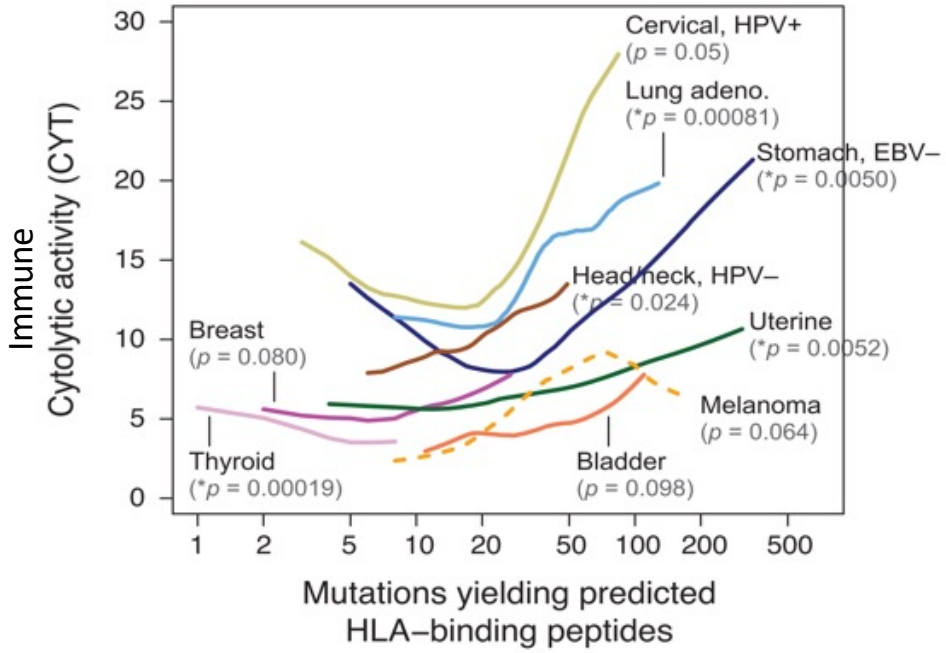
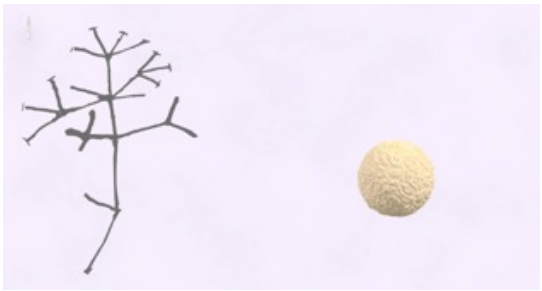
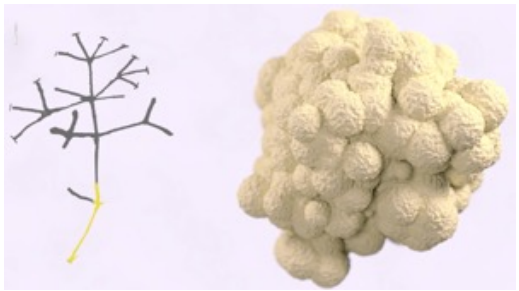
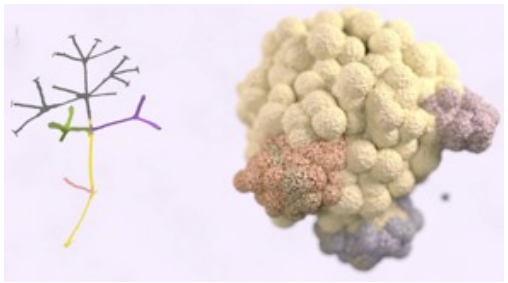
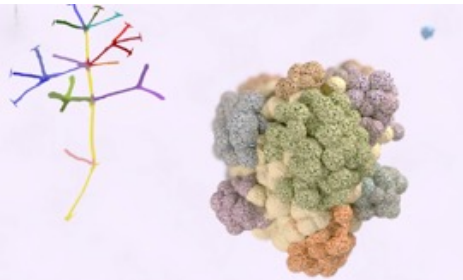


But… are all neoantigens the same?

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 NEJM
McGranahan et al 2016 Science
Jamal-Hanjani et al 2017 NEJM

How relevant are clonal neoantigens in the context of immunotherapy?

Clonal (and not subclonal) neoantigens drive response to CPI across multiple indications

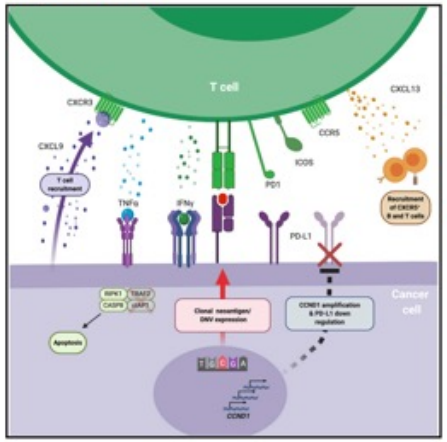
A meta-analysis of >1000 patients across 7 indications treated with CPI underscores the importance of clonal neoantigens in checkpoint inhibitor response

Cell

Article

Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition

Graphical Abstract



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Kevin Litchfield, James L. Reading, Clare Puttick, ..., Sergio A. Quezada, Nicholas McGranahan, Charles Swanton

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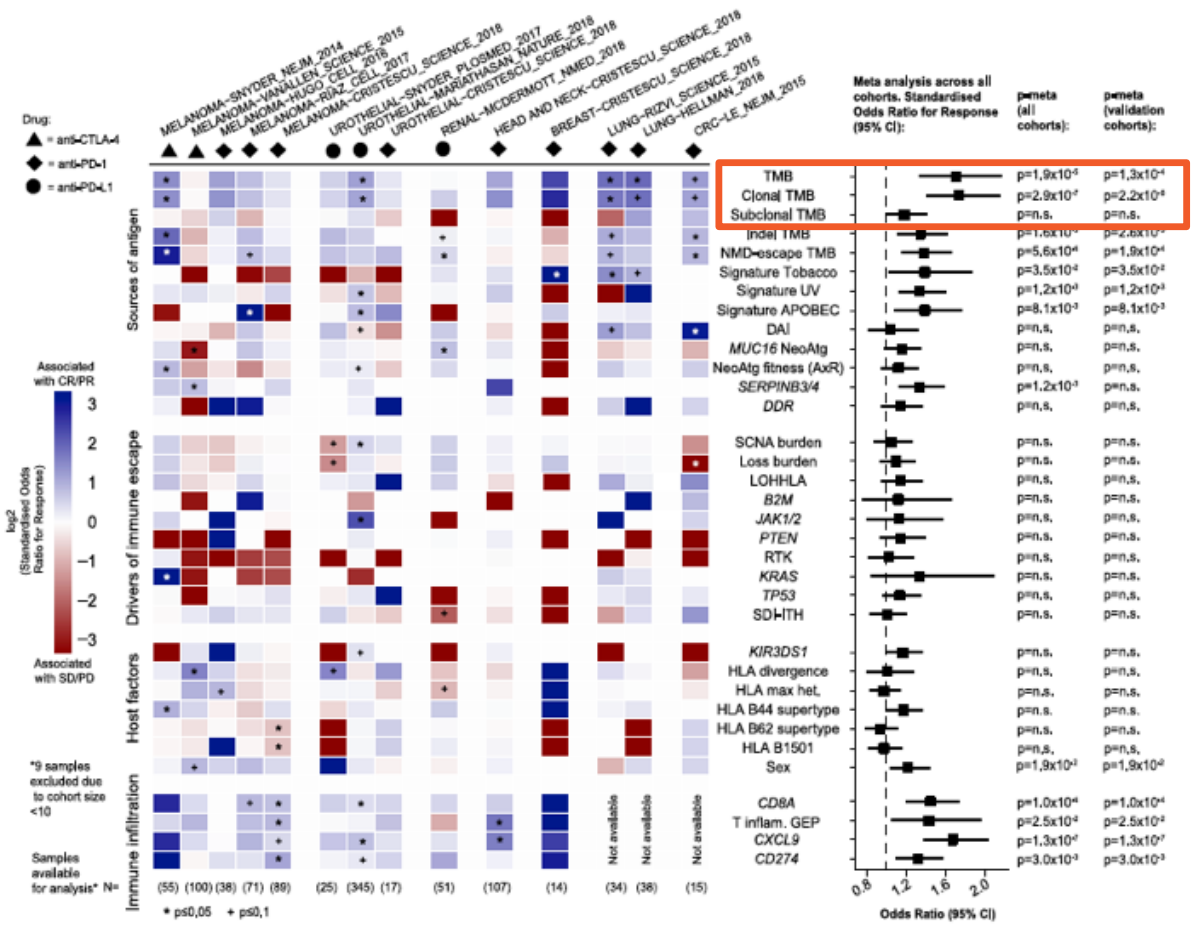
In Brief

A whole-exome and transcriptome meta-analysis of over 1,000 patients treated with immune checkpoint blockade across seven tumor types highlights the potential of multivariable prediction models that consider both tumor- and T-cell-intrinsic mechanisms of response.

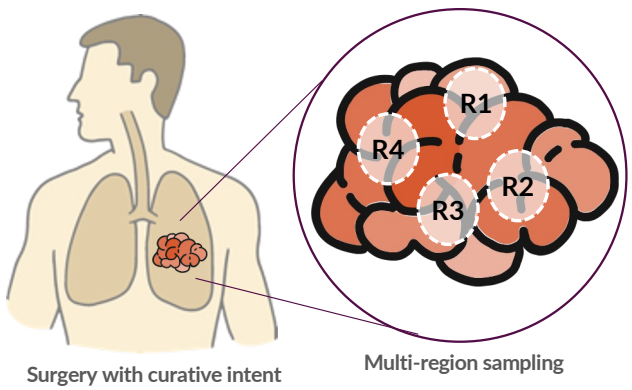


Developing tools for automated, objective and scalable identification of clonal and immunogenic neoantigens

PELEUS™ AI is trained to prioritise the most immunogenic clonal neoantigens

Clonality

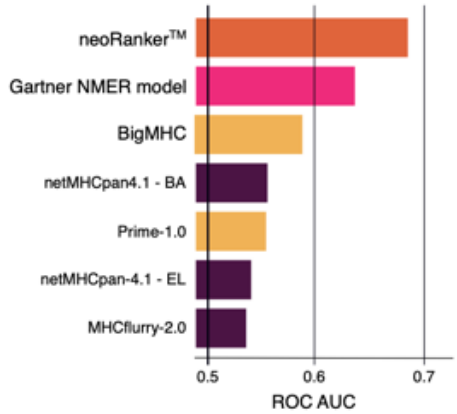
Selecting neoantigen targets shared by all tumour cells



- PELEUS algorithm ClonalX™ uses multi-region analysis from a single biopsy
- Compared to traditional VAF-based methods: VAF makes 11 errors for every 1 error made by PELEUS

Immunogenicity

Increasing odds of potent T cell responses



- neoRanker™ AI is trained on proprietary immunogenicity data and >50% better than BigMHC and netMHC
- 72% of all patient reactivities are found in the top 30 ranked peptides

Immune escape

Anticipating evolution of treatment resistance

- PELEUS can filter peptide lists for clonal neoantigens presented on retained HLAs
- Can be leveraged to double down on the best targets and mitigate impact of immune escape

VAF: variant allele frequency

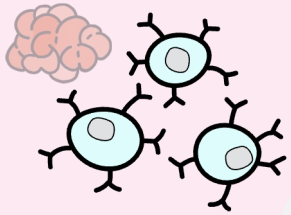
Choosing the right platforms to mobilise the immune system against clonal neoantigens

Achilles Platform enables targeting clonal neoantigens across multiple therapeutic modalities

Advanced development

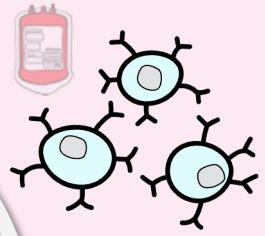
TIL-based cNeT (clonal neoantigen-reactive T cells)

Ph I/IIa clinical trials, developing cNeT from TIL – a clinically validated approach



Blood-based cNeT

IND-enabling studies developing cNeT from peripheral blood, without the need for surgery

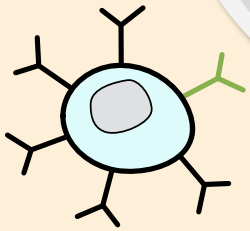


PELEUS

Early development

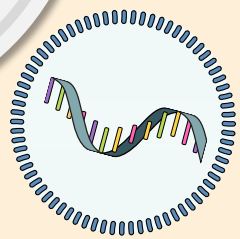
TCR-therapy for shared neoantigens

Engineered TCR therapy equips activated T cells with specific receptors that can target shared neoantigens

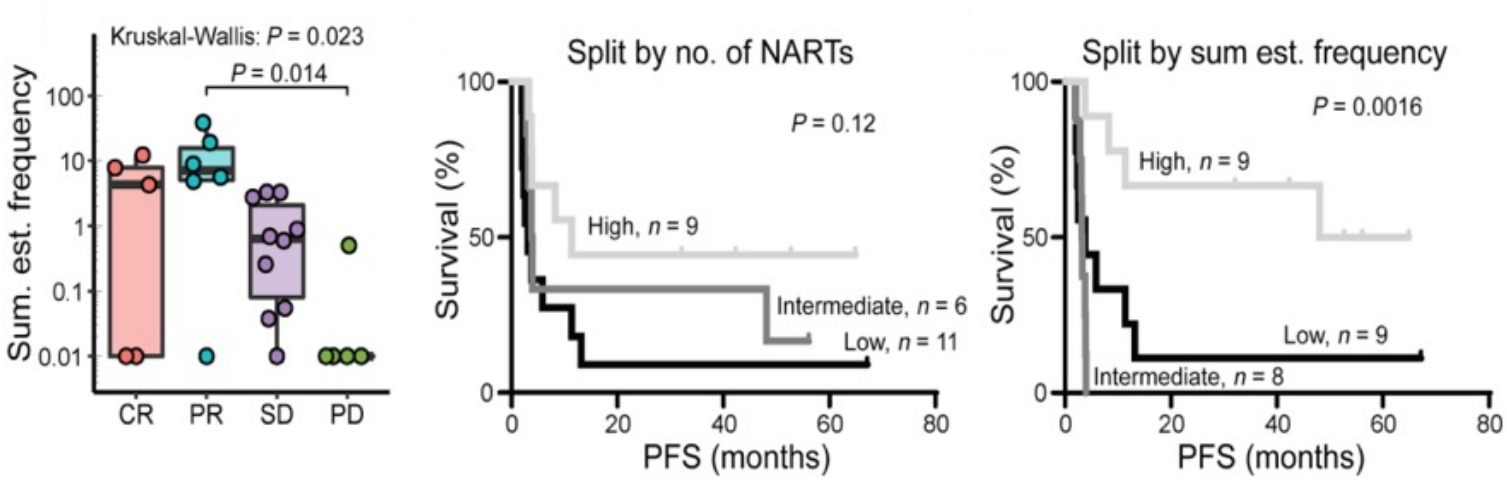


Clonal neoantigen vaccines (mRNA)

Instruct cells in the body to translate RNA to make clonal neoantigens, which activate an immune response

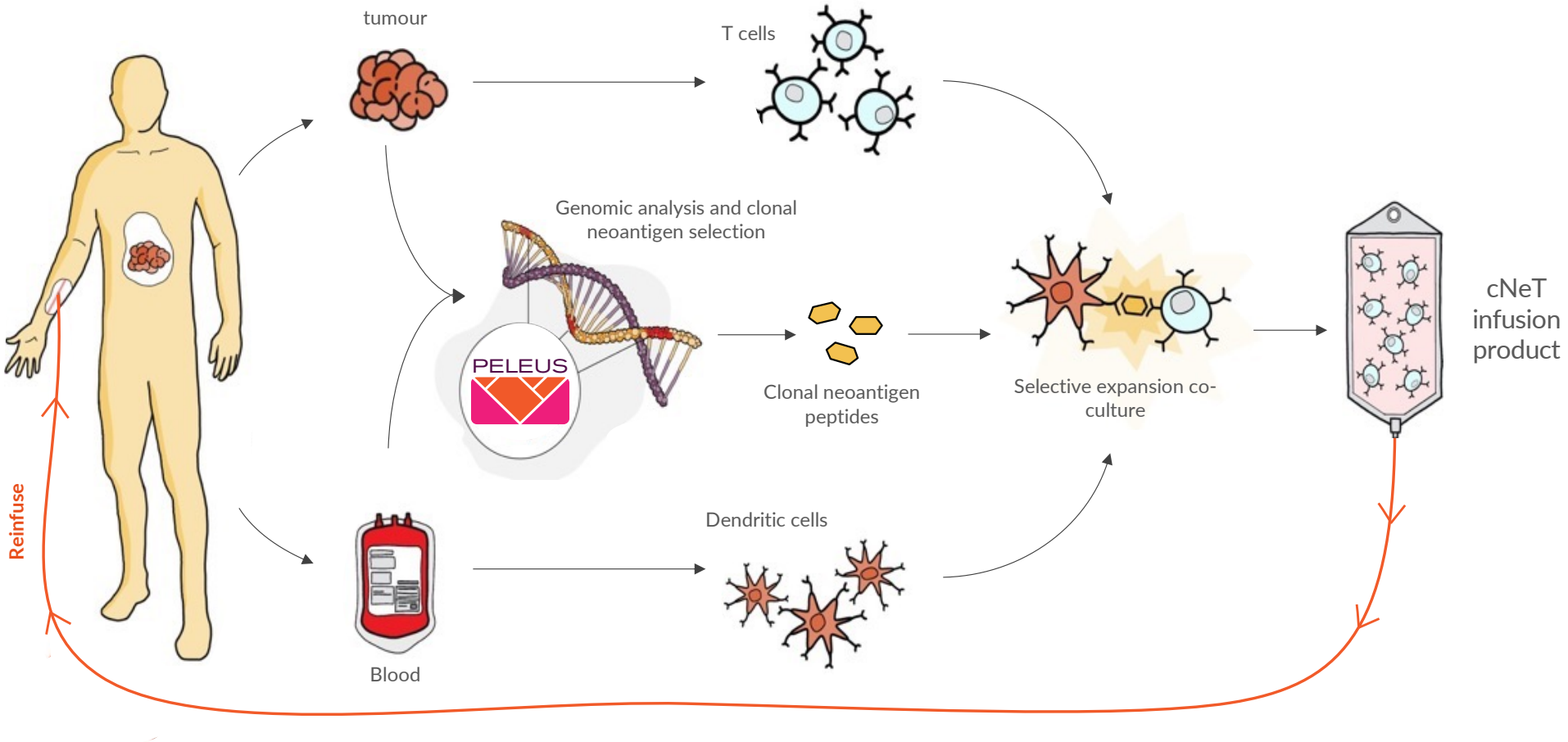


CD8+ Neoantigen-reactive T cells correlate with clinical outcome following TIL therapy



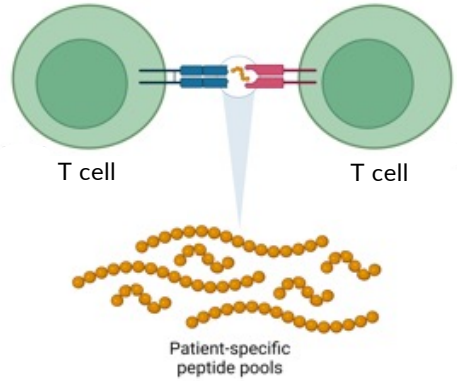
- Data suggest a correlation between Neoantigen-reactive T cell (NART) dose and clinical response
- Neoantigen-reactive T cells could be the active component of TIL
- Optimising both NART number and frequency could help improve clinical response to TIL

Enriching for Clonal Neoantigen Reactive T Cells (cNeT)

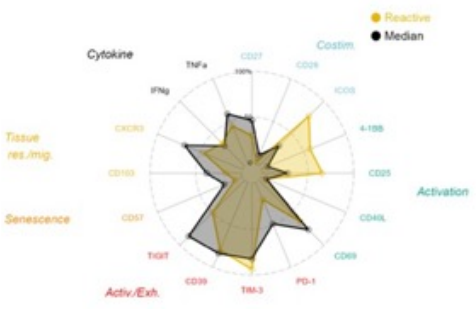


Suite of established assays for in-depth characterisation of personalised cell therapy products

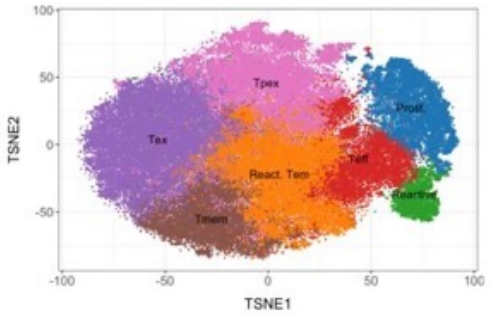
Proprietary T-T stimulation assay



High dimensional cytometry



Single cell RNA and TCR sequencing

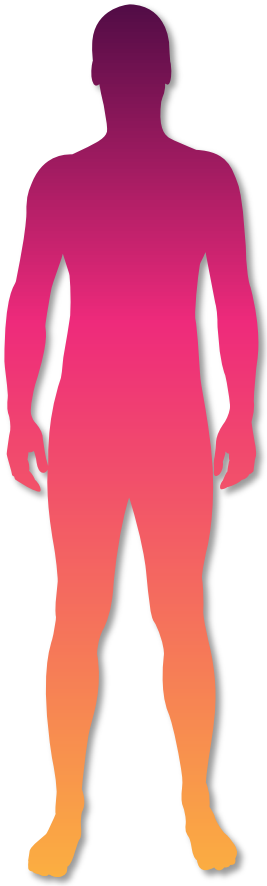


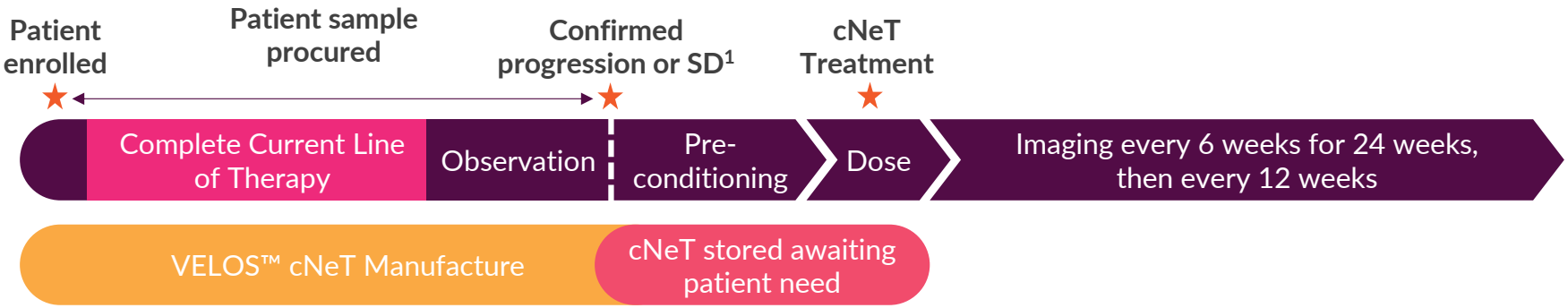
- Proprietary potency assay for identifying neoantigen-reactive component of products*
- Extended phenotyping flow panel to assess stimulated and unstimulated product functionality
- Multiplex soluble analyte assays for biomarker ID
- ELISpot for single peptide reactivity
- Sequencing platform for ID of reactive TCRs
- Single cell characterisation of function for reactive cells and total product
- Functional assays such as proliferation, REP expansion and cell killing

Data platform for aggregating and analysing data from various sources for visualisation and reporting

*PCT published, entered Europe and USA. Examination ongoing

cNeT therapies can be readily delivered within standard treatment pathways





cNeT Doses

	N (CHIRON + THETIS)	cNeT Dose
Group 1	16	<50M
Group 2	13	>50M

Low IL-2 and lymphodepletion (LD)

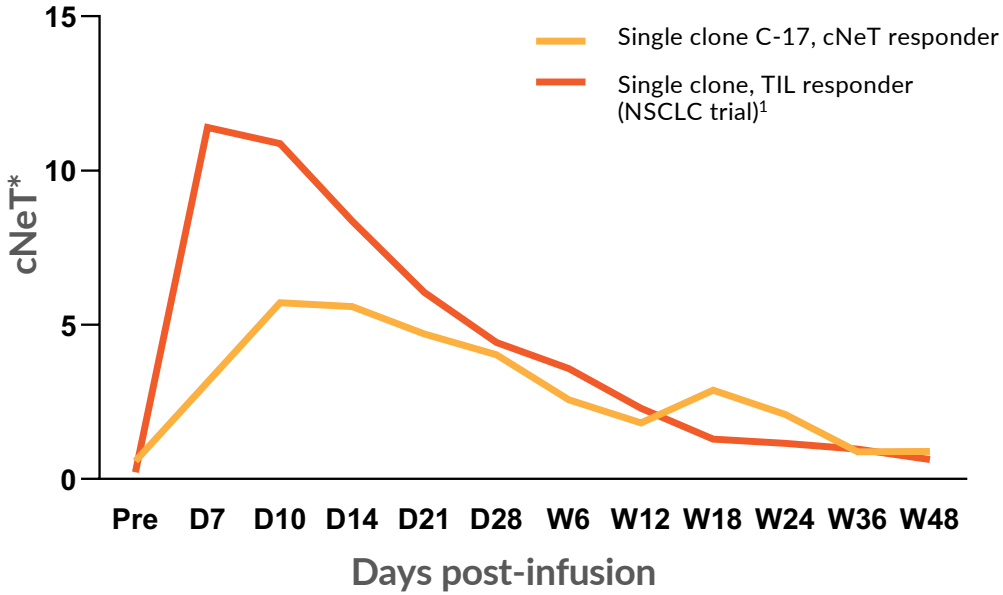
Lower dose IL-2 and low host-conditioning vs existing TIL therapies

1. Begin treatment process with confirmed stable disease or progression per site protocol

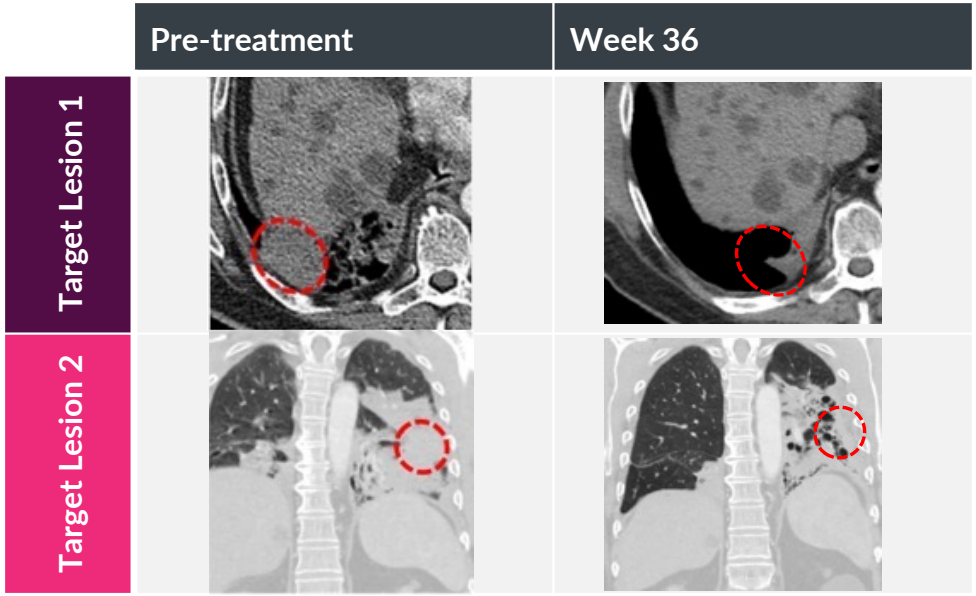
Sustained persistence of reactive TCR clones associated with partial response seen in patient C-17 and standard TIL studies

C-17: Detection of T cell receptors

Sum of Neo-Ag reactive TCRs, TCR/mL
Low intensity conditioning



cNeTs persisted beyond 6 months - longer than any other cNeT patient and similar to kinetics seen in standard TIL studies



Total target lesion **reduction of 56%** at week 36

Immune evasion not detected: No HLA allele loss with even distribution of peptides across alleles

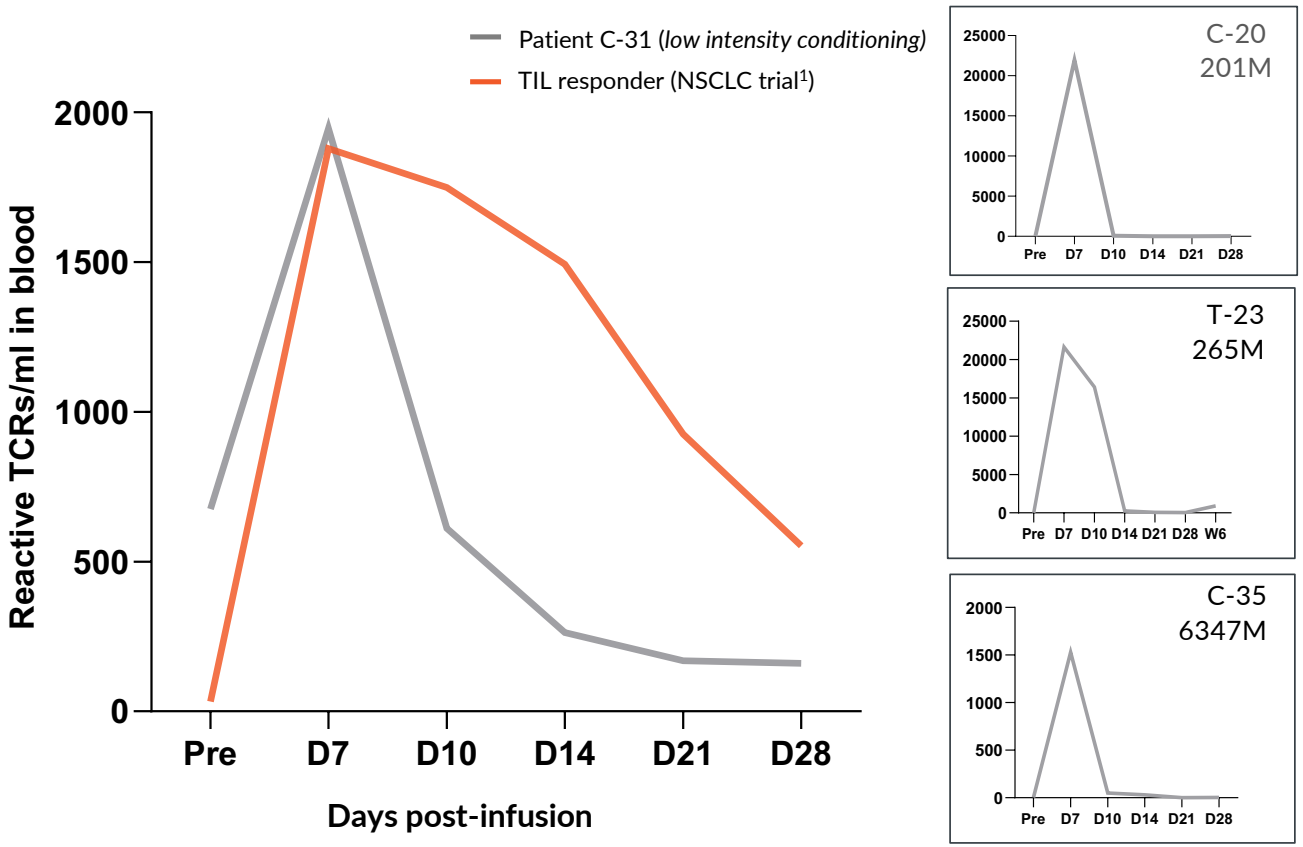
*Sum of NeoAg-specific TCR/mL ($x10^3$)

1. Creelan et al. Nat Med vol27, 1410–1418 (2021)

General lack of cNeT persistence in the majority of treated patients in Group 1 and Group 2

Persistence of neoantigen reactive T cells post-dosing

Patient C31, 5.4 B cNeT, *Single reactive TCR clone*



- **Early peaks** of cNeTs detected post dosing at levels comparable to those observed in standard TIL therapy where objective responses have been observed[1,2]
- Numbers of the cNeT TCR in the blood post-dosing **decay rapidly compared to other TIL** trials with low levels at D21 compared to TIL trials using high dose IL-2 and lymphodepletion[1,2]
- Engraftment could be **potentially improved through optimisation of host conditioning** (increasing IL-2 and/or lymphodepletion)

**Single reactive clones with highest TCR levels at D7*

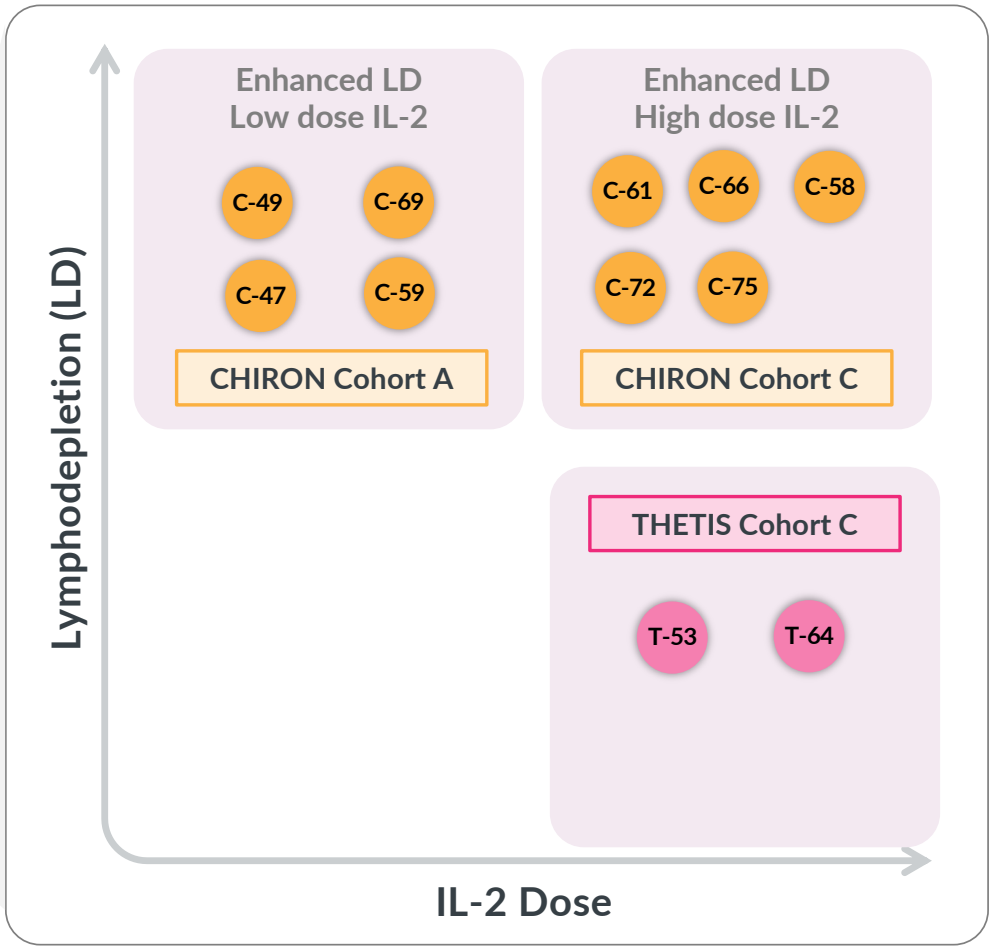
1. *Reconstructed from Creelan et al. Nat Med vol27, 1410–1418 (2021)*
2. *Kristensen et al. J Clin Invest.;132(2):e150535 (2022)*

Enhanced host conditioning to boost cNeT engraftment

Three patient groups considering cNeT dose and host conditioning (CHIRON & THETIS)

	N (CHIRON + THETIS)	cNeT Dose	Host Conditioning
Group 1	16	<50M	Low
Group 2	13	>50M	Low
Group 3	11	>50M	High

Current enrolment group

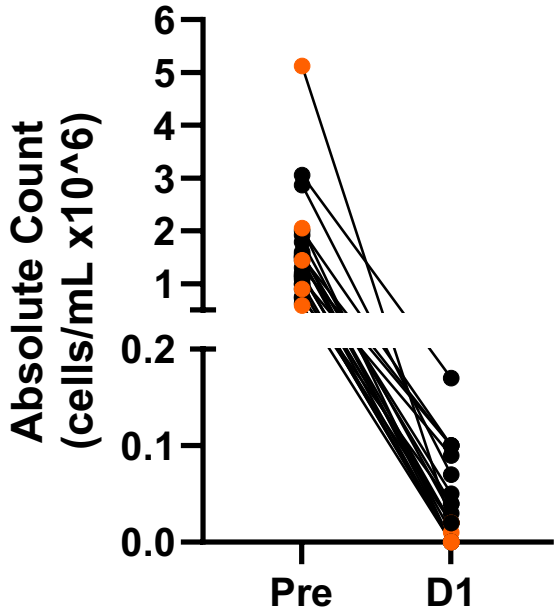


Enhanced host conditioning (EHC) drives deeper lymphodepletion

- Low lymphocyte counts seen for the higher dose of Cy/Flu consistent with better lymphodepletion
- Extent and duration of lymphodepletion from our new cohort is comparable to previously published study comparing high and low lymphodepletion[1]

Lymphodepletion	Median nadir (K cells/μl)
30mg/kg Cy (n=4)	0.01
Low dose LD (n=19)	0.04
Standard TIL study - high LD[1]	**0.02**

Lymphocyte count at baseline and day 1 post-infusion

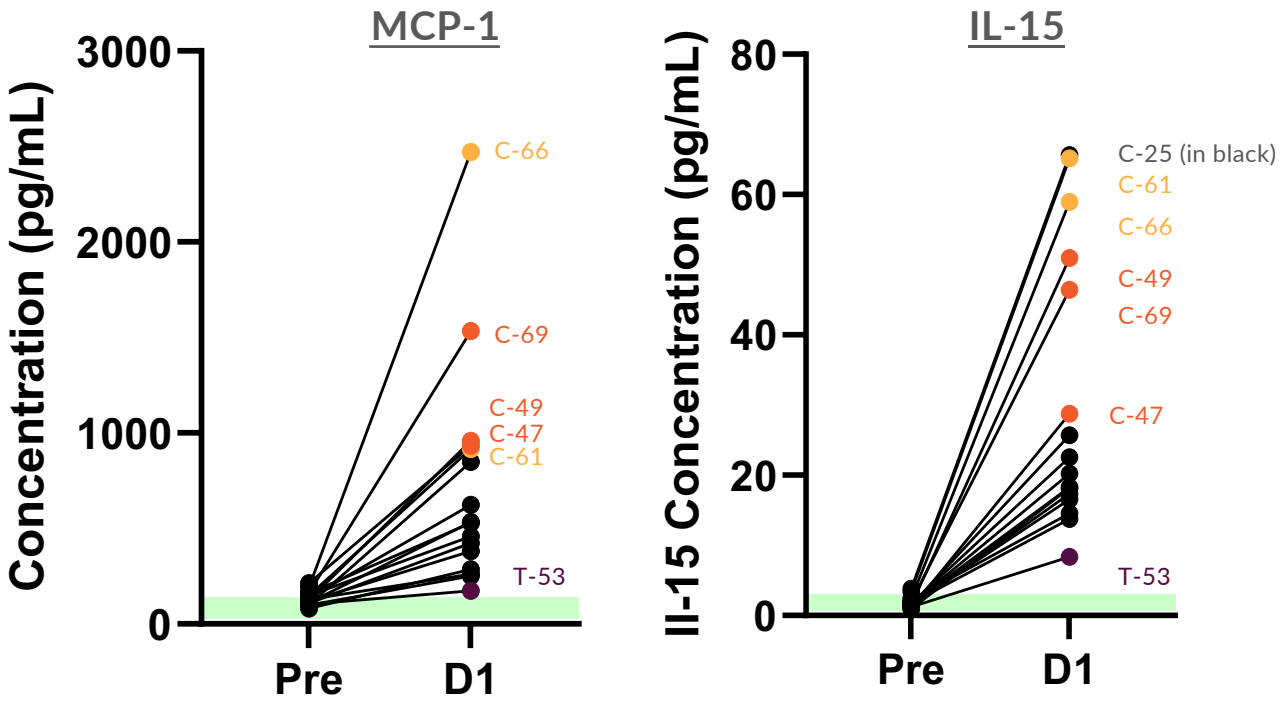


Enhanced conditioning N=5
Chiron Low LD/IL-2 N=19

1. .Nissani *et al* 2021 J. Immunother Cancer Day at Day 0, our data is Day 1

Enhanced host conditioning (EHC) promotes higher levels of cytokines associated with immune reconstitution

- IL-15, IL-7 and MCP-1 are markers of lymphodepletion, increases in homeostatic cytokines supports T cell growth
- **Higher levels of MCP-1 and IL-15 markers for higher LD doses**

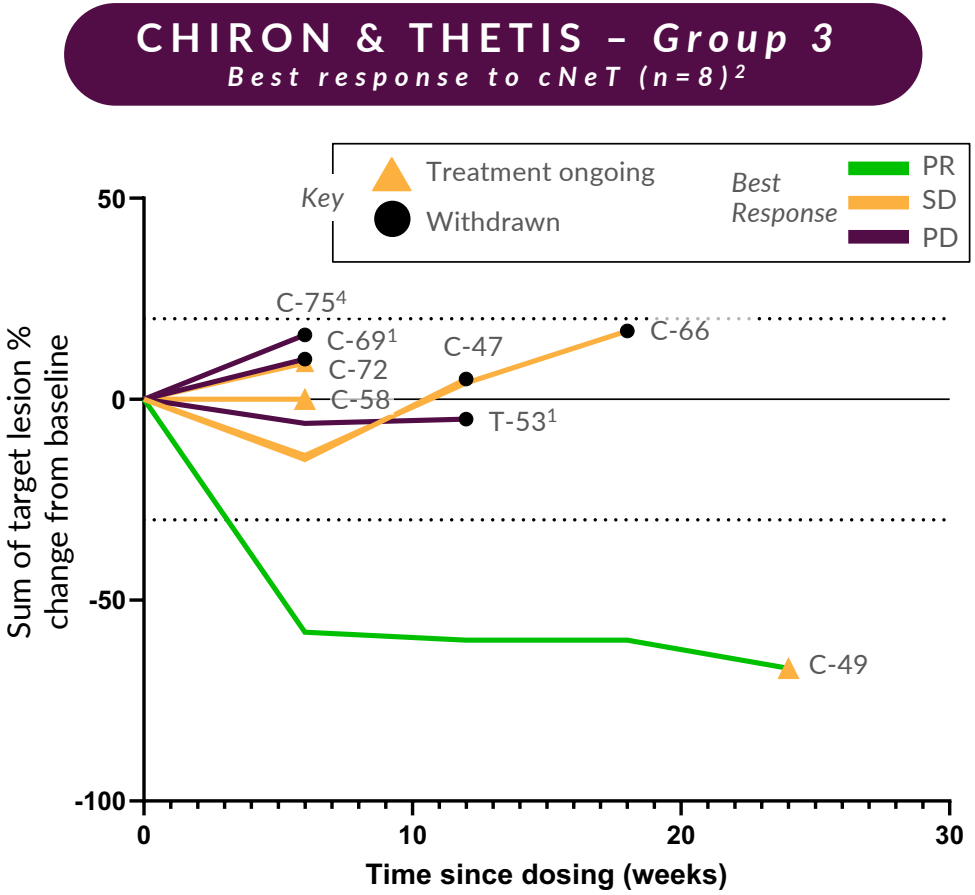


Black circles = Chiron Low LD/IL-2 n=10
Green area show healthy donor range (ATX data)

All Patients in Group 3 – Enhanced Host Conditioning (n=8 with data)

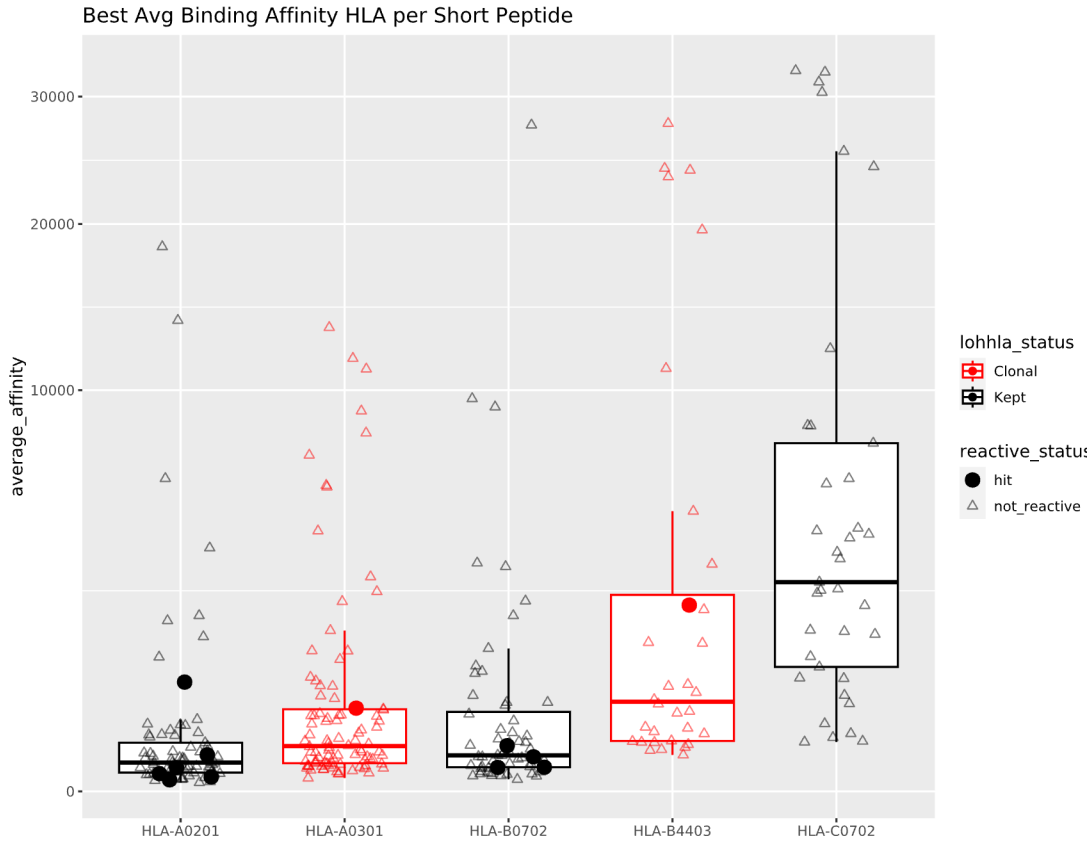
Pt ID	Cohort	cNeT Dose	LD	IL-2	Best Response
T-53	C	216M	Low	High	PD (-6%)[1]
C-61	C	4.6B	High	High	PD[2]
C-66	C	326M	High	High	SD (-16%)
C-69	A	325M	High	Low	PD (+10%)[1]
C-49	A	3.3B	High	Low	PR (-67%)
C-47	A	11.1B	High	Low	SD (-16%)
C-58	C	61M	High	High	SD (0%)
C-72	C	183M	High	High	SD (+9%)
T-64	C	1.5B	Low	High	SD[3]
C-75	C	584M	High	High	PD (+16%)[4]

[1] *T-53 and C-69 presence of new lesions at W6*
[2] *C-61 clinical PD and withdrawal prior to W6 scan*
[3] *PI reported SD*
[4] *PI reported PD in NTL*



Responding patient has multiple retained reactivities in the product despite high immune evasion by the tumour

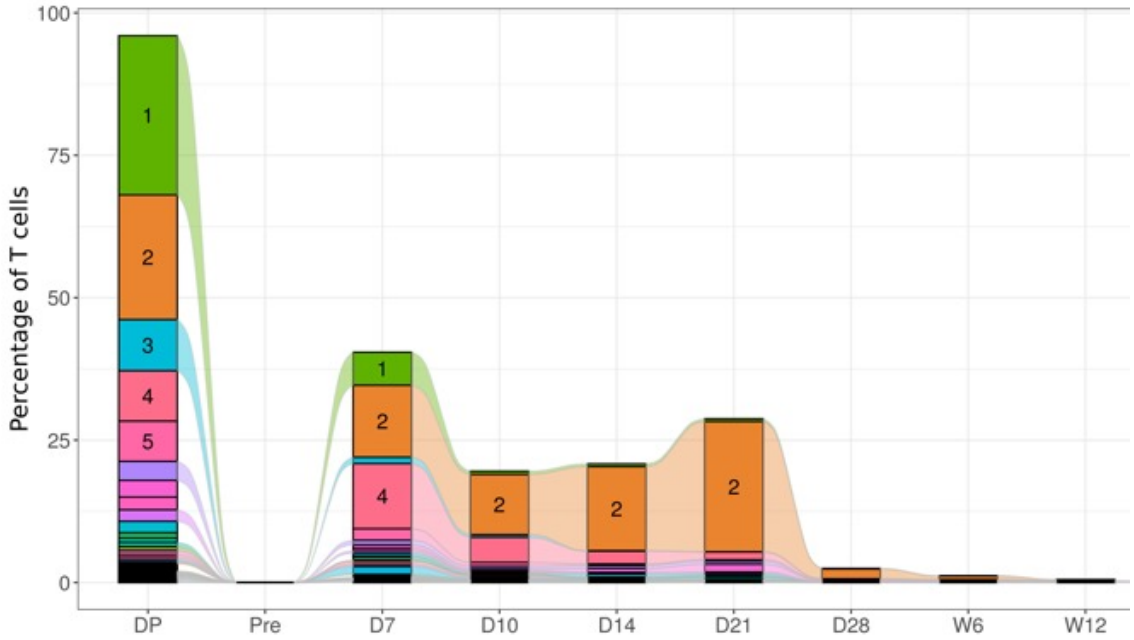
- TMB slightly above average with low ITH – however neither are at the extreme of the cohort distribution
- HLA LOH in HLA-A and HLA-B with reactivities predicted to bind to lost alleles
- Clonal stop-gain mutation disrupts kept HLA B07:02 responsible for presenting **three additional reactive peptides** most strongly.



Responding patient (PR) engraftment data shows persistence for 12 weeks

- Product had **high cNeT dose (3.3b)** and **high frequencies of both CD4 and CD8** reactivity (**95% of the infusion bag were cNeTs**)
- **cNeT TCR engraftment shows high frequency of product in blood and persistence of clones**
- Levels drop below 1% at week 12. Hypothesis that high dose IL-2 would have further increased product frequency in blood
- Data confirmed by multimer staining

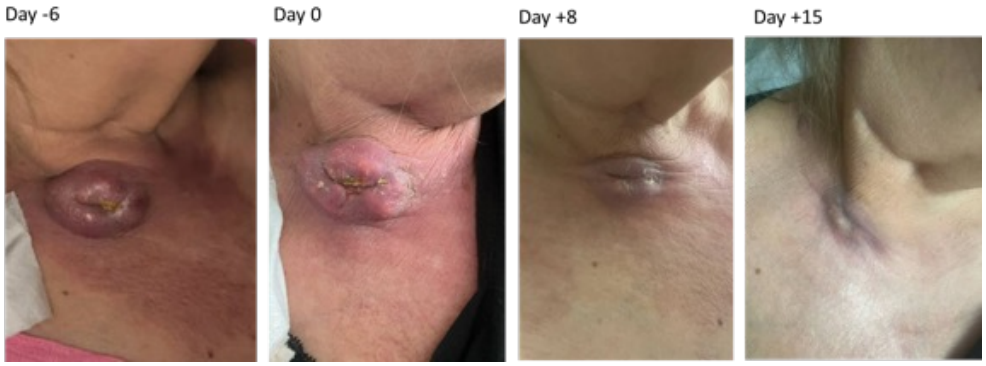
Detection of T cell receptors in C-49
Sum of NeoAg-specific TCR (% of sample)



Clinical summary of partial responder

- Lung adenocarcinoma, metastatic
- Extensive prior therapies with best response SD to CPI
- Visible healing of ulcerated skin lesion post cNeT dosing
- Ongoing partial response

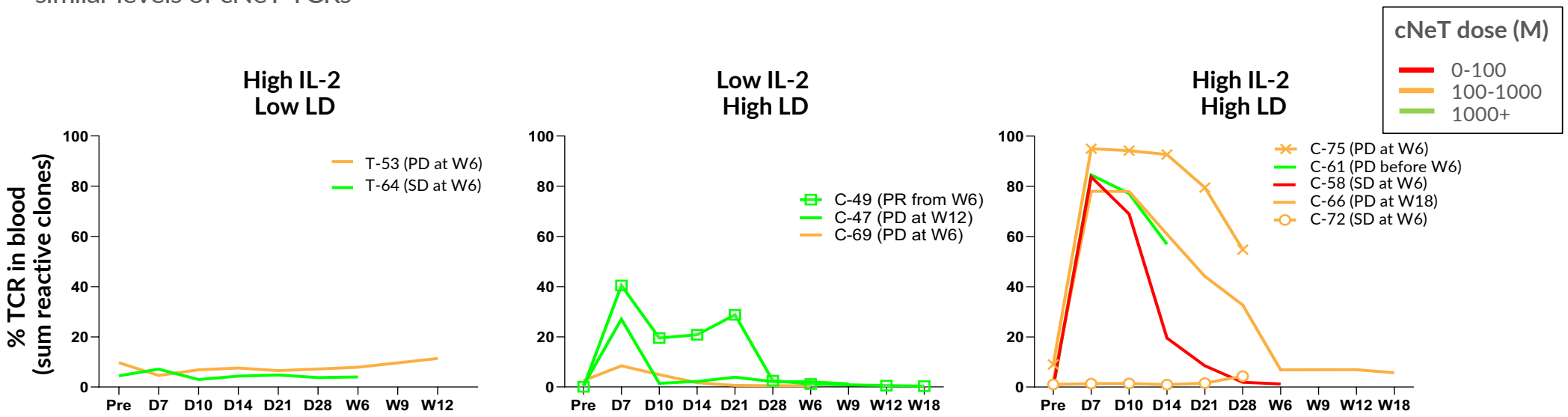
Type of lesion		SOD* (mm) [% change vs baseline]				
		Baseline	Wk 6	Wk 12	Wk 18	Wk 24
TL1	Lymph node	15	3 [CR]	3 [CR]	3 [CR]	** [CR]
TL2	Lymph node	33	17 [-48%]	16 [-52%]	16 [-52%]	16 [-52%]
Total		**48**	**20 [-58%]**	**19 [-60%]**	**19 [-60%]**	**16 [-67%] ****
NTL1	Soft tissue	Present	Present	Present	Present	Present
NTL2	Right hilar nodes	Present	Present	Absent	Absent	Absent
NTL3	Soft tissue	Present	Present	Present	Present	Present
Overall Response *(RECIST1.1)*			**PR**	**PR**	**PR**	**PR**



** Investigator-reported*
*** TL1 not measurable*

High dose IL-2/ enhanced LD drives highest frequency of product in blood

- High IL-2 with low LD shows poorer level of engraftment (T-53, T-64) whilst low IL-2/high LD promotes variable extent of engraftment, potentially impacted by dose (C-49>C-47>C-69). We believe engraftment is critical but not sufficient for clinical efficacy
- High IL-2 and LD synergise to drive highest level of cNeT detection in blood to date (C-61, C-66, C-58)
  - Additional factors such as product quality (i.e. poor *in vitro* proliferation in response to IL-2) may also contribute to poor engraftment even in the context of high dose IL-2 and LD, as shown for C-72 which was unresponsive to IL-2 *in vitro*
- cNeT expansion is likely driven by target expression as viral-reactive TCRs present in infusion bag fail to expand post-dosing to similar levels of cNeT TCRs

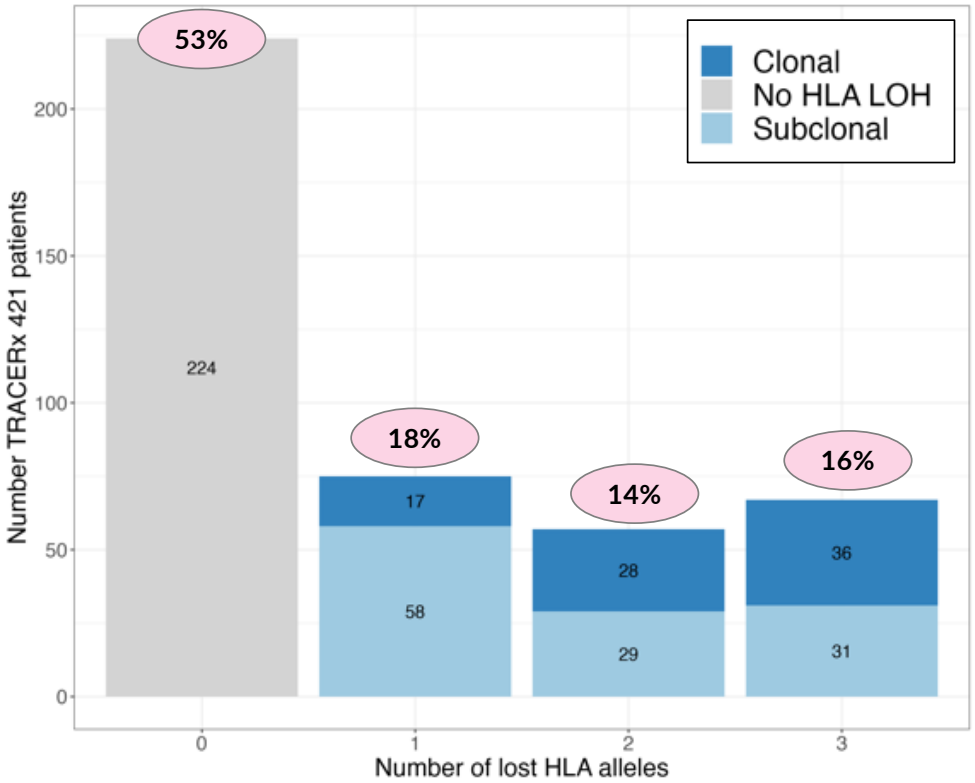


[1]Kristensen et al, JCI 2022

Late-drop data, under review

PELEUS™ tracks immune evasion to inform target prioritisation and patient stratification

47% of TRACERx 421 cohort have some LOHHLA

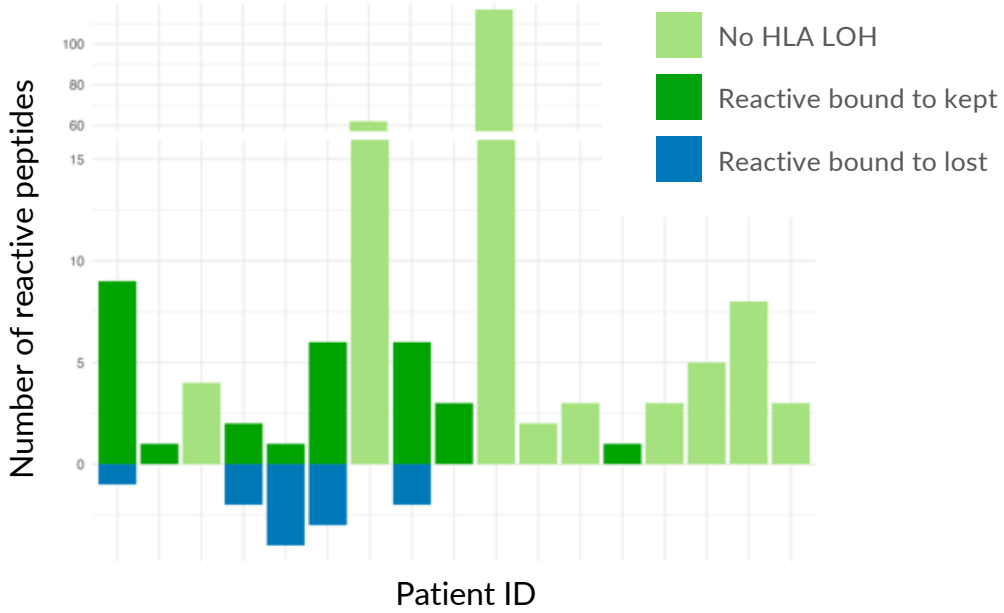


- **53%** of TRACERx patients have **no evidence of loss of HLA alleles** – suggesting limited immune evasion
- **16%** of TRACERx patients show **loss of 3 HLA alleles** - reducing chance of cNeT efficacy if targeting these alleles
  - Population less likely to respond due to clonal loss of 3 alleles (~10% of TRACERx population)
- With PELEUS we are **evaluating alternative peptide lists** skewing **cNeT reactivities towards retained alleles**
- PELEUS has capacity to identify these patients supporting new strategies for **potential patient stratification**

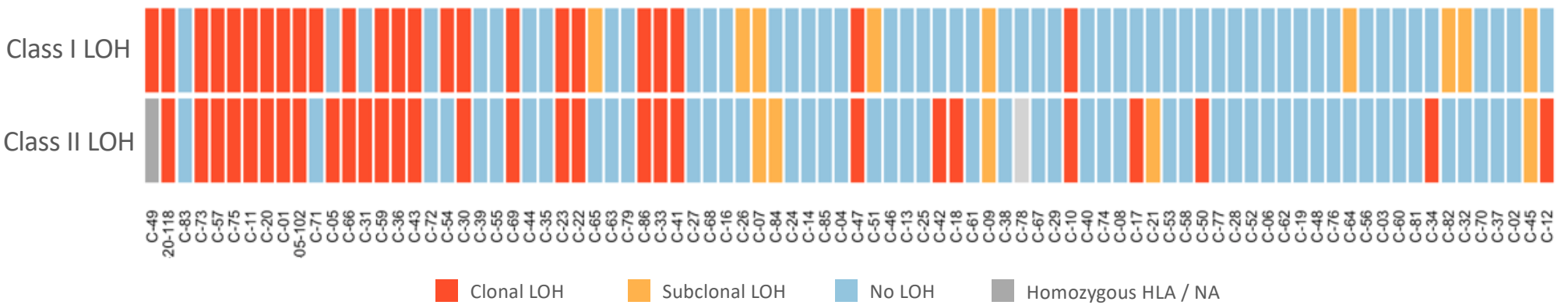
Achilles cNeT platform and PELEUS™ allows in depth understanding of the potential impact of immune evasion in our cell therapy product

- Translational science platform allows characterisation of products at the single reactivity level
- PELEUS predicts immune evasion and identifies reactivities to kept and lost alleles in every single product
- Immune evasion data can be used to inform target selection and prioritise reactivities to kept HLA alleles
- Target prioritisation can be applied across modalities including cNeT, vaccines and TCR-T cell therapies

Assessment of HLA LOH impact (n=17)



Class II HLA LOH detection method developed and run on all patients



There is a highly significant association between HLA class I LOH and HLA class II LOH (OR = 16.6, p = 1.89e-12).

No relationship was observed between HLA class II LOH and any other immune evasion mechanism.

Dose, engraftment and patient status associate with clinical performance of cNeTs

- **Dose and Engraftment**
  - Our data suggest that high dose IL-2 and enhanced LD synergise to drive highest level of cNeT engraftment in blood (C-61, C-66, C-58, C-75). Consistent with the literature, the data suggest that small doses of cNeT may show shorter persistence even in high host conditioning (C-58)
  - Other factors such as product quality (i.e. poor *in vitro* proliferation in response to IL-2) may also contribute to poor engraftment even in the context of high dose IL-2 and LD as shown for C-72 which was unresponsive to IL-2 *in vitro*
  - Data suggest that engraftment requires antigen engagement by cNeTs supporting ability of cNeTs to recognise antigen post-infusion
  - High dose IL-2/enhanced LD is the most effective host conditioning regime for cNeT engraftment
- **Patient status at dosing**
  - Most of our patients have moderate clinical status at dosing. Poor clinical status is typically driven by clinical symptoms (dyspnoea, pain), heavy pre-treatment and high dNLR (derived neutrophil-to-lymphocyte ratio)
  - Good product quality, high dose and engraftment are not sufficient to drive clinical responses in patients with poor clinical status (C-61)
- **CD4/CD8 cNeT balance**
  - There is a potential impact of CD4/ CD8 cNeT balance in engraftment and clinical activity as this may help explain the moderate engraftment and lack of clinical response in C-66 and C-47

Key mechanistic learnings from TIL-cNeT

Efficacy

- Our data support a correlation between cNeT persistence and durable clinical response[1]
- We observe a correlation between cNeTs dose and cNeT persistence and engraftment post dosing
- We observe a negative correlation between immune evasion and clinical activity

Safety

- Tolerability in line with standard TIL therapies with majority of adverse events related to the host conditioning
- No observed impact of cNeT dose on tolerability

Engraftment & persistence

- IL-2 and LD independently contribute to cNeT engraftment and synergise to drive the highest level of engraftment and persistence of cNeTs in our trial
- Engraftment is essential but not sufficient and is impacted by cNeT dose and host conditioning[1]
- We have optimized host conditioning to maximise engraftment without compromising the safety profile of cNeTs

Immune evasion

- ~50% of all NSCLC patients will have some level of loss of HLA expression[1] negatively impacting:
  - The active dose of cNeTs in the drug product (4/10 products have an estimated reduction in dose)
  - The ability of cNeTs to recognise and kill tumour cells
- Implementing PELEUS HLA loss tool will allow targeting of cNeT therapy at antigens for which there are retained HLA molecules

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