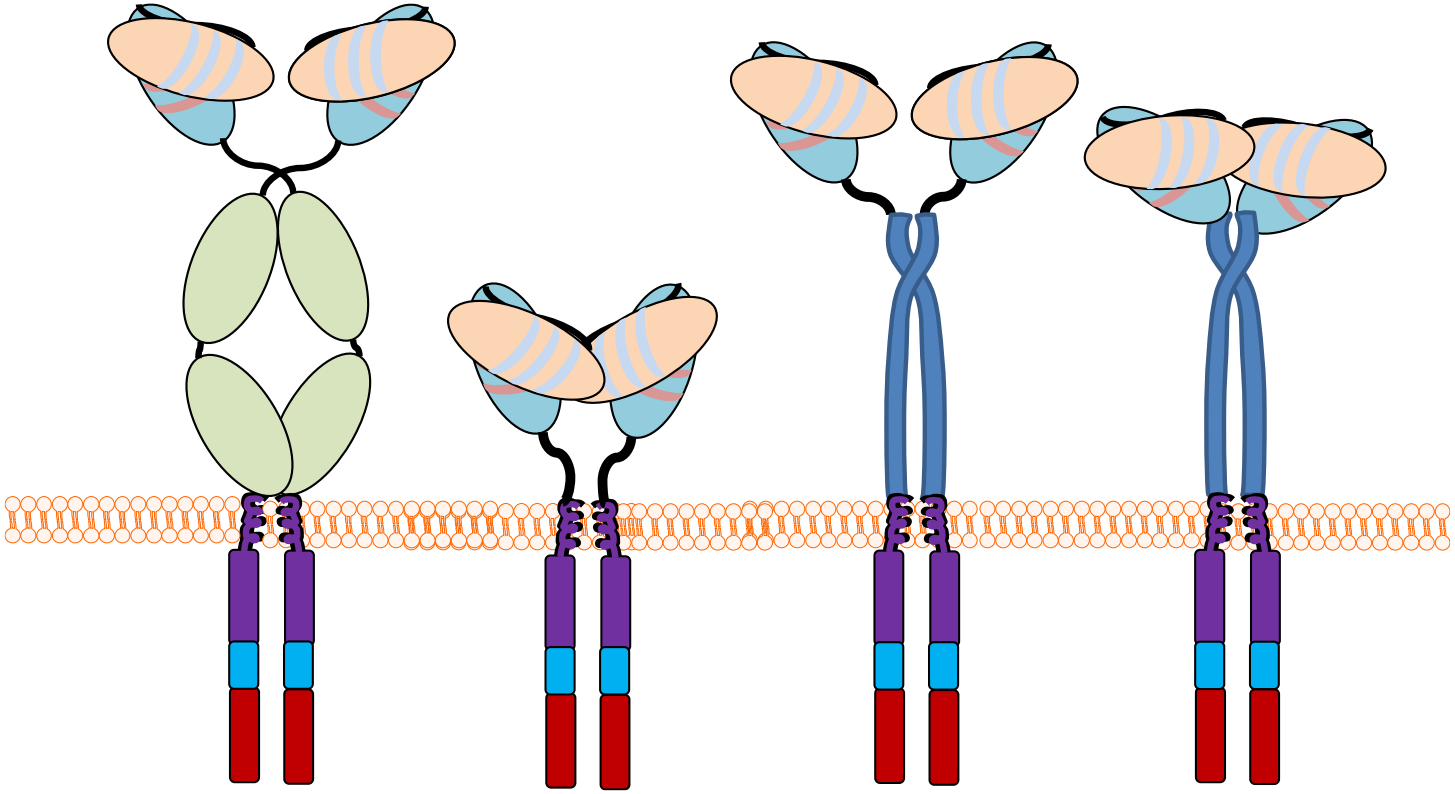
CAR **T** approach for **T** cell Lymphomas



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Disclosures

- Share of Royalties Autolus/Cellectis/Allogene/BBB
- Stock and salary from Autolus Therapeutics

Peripheral T-Cell Lymphoma: No Standard of Care After First Relapse

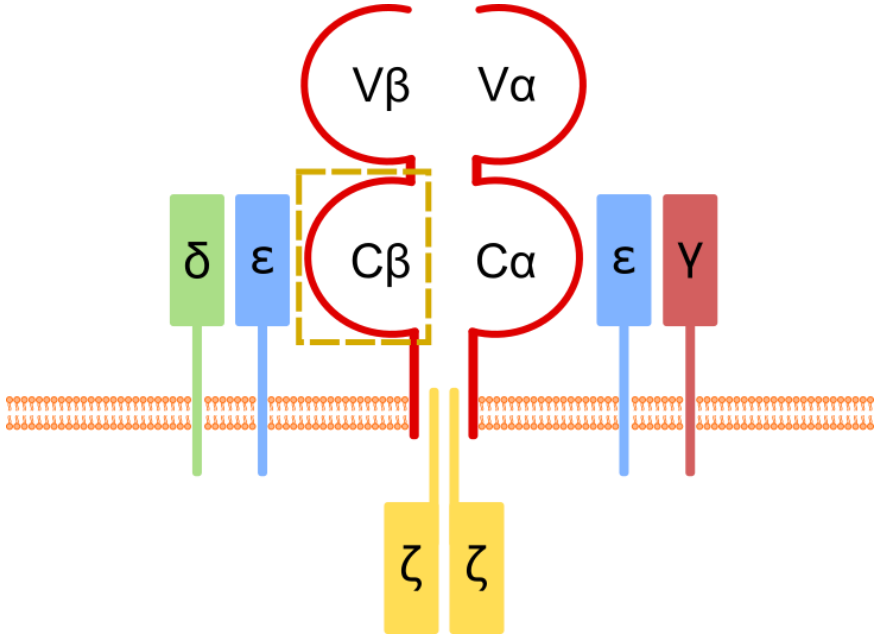
- T cell lymphoma is an aggressive disease with a very poor prognosis
- Many T cell lymphoma patients are refractory/relapse following first-line treatment (68%)[3]
- Standard of care variable, often based on high-dose chemotherapy and stem cell transplants
  - Median 5 yrs OS: 32% [1]
- Relapsed/refractory patients have a worse prognosis
  - Median PFS approximately 3 months/ Median OS < 6 months [2,3]
- T cell lymphoma has not benefited from advances in immunotherapy to date
  - Pan T-cell depletion highly toxic; Few/no tumour-specific antigen targets

[1] Weisenburger et al, Blood 2011; [2]Mak et al, JCO 2013; [3]Bellei M et al, Haematologica 2018

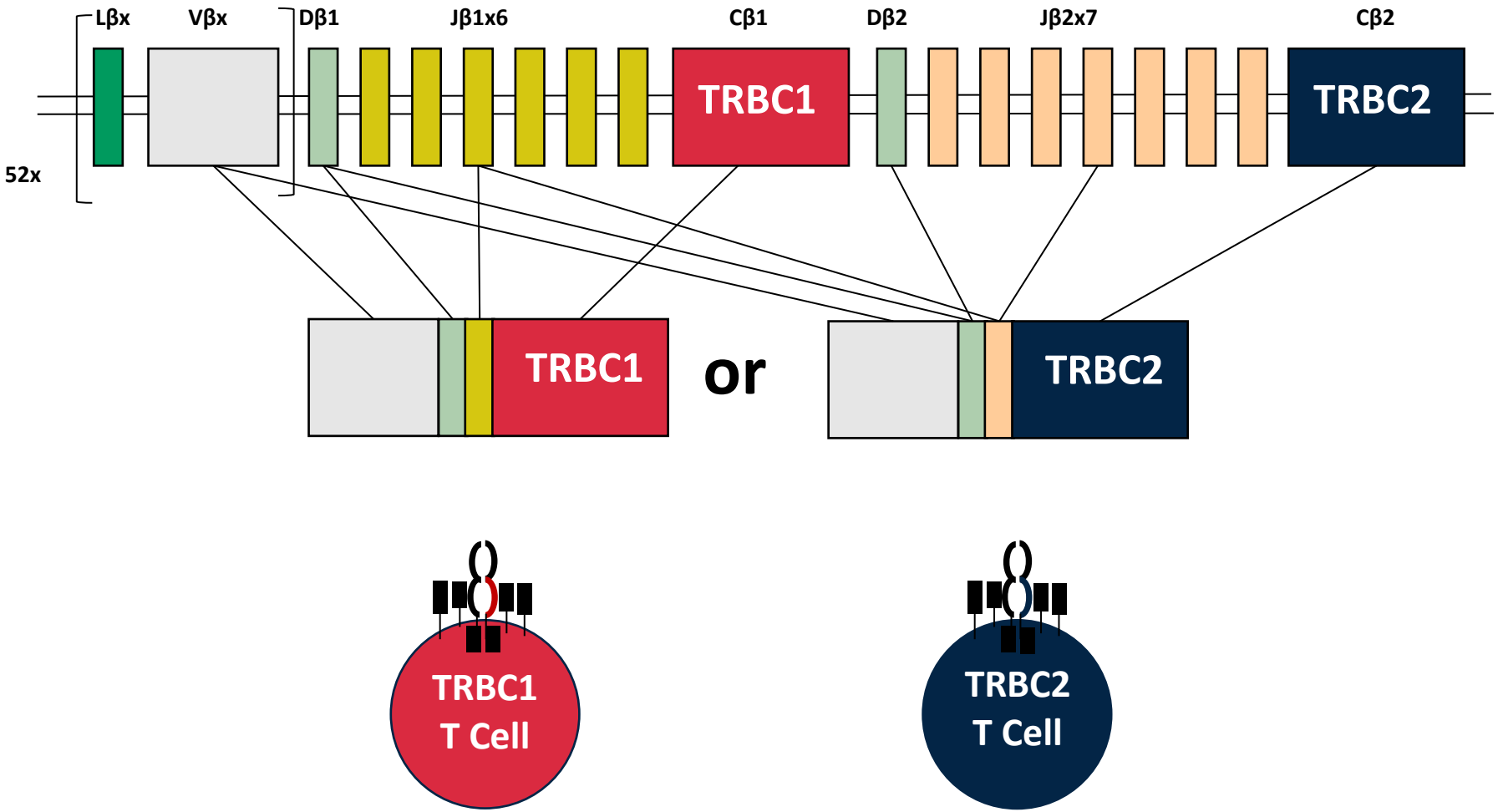
What is a Good CAR Target in PTCL?

Antigen	PTCL No.	PTCL Positive (%)	AILD No.	AILD Positive (%)
Human TCR βF1	133	97	30	94
CD2	136	70	41	100
CD3	144	86	40	95
CD4	135	46	38	42
CD8	129	15	34	32
CD5	137	20	36	19
CD7	141	19	41	24
CD10	143	1	43	39
CD15	140	4	43	2
CD30	145	3	42	0
CD56	140	6	40	3
CD57	143	10	42	5
TIA-1	138	27	41	34
GB	140	2	40	0
ALK-C	143	0	41	0
EBER	132	5	39	3
Mib-1 high	138	11	40	5
CD20	141	1	42	0
CD79a	142	0	36	0

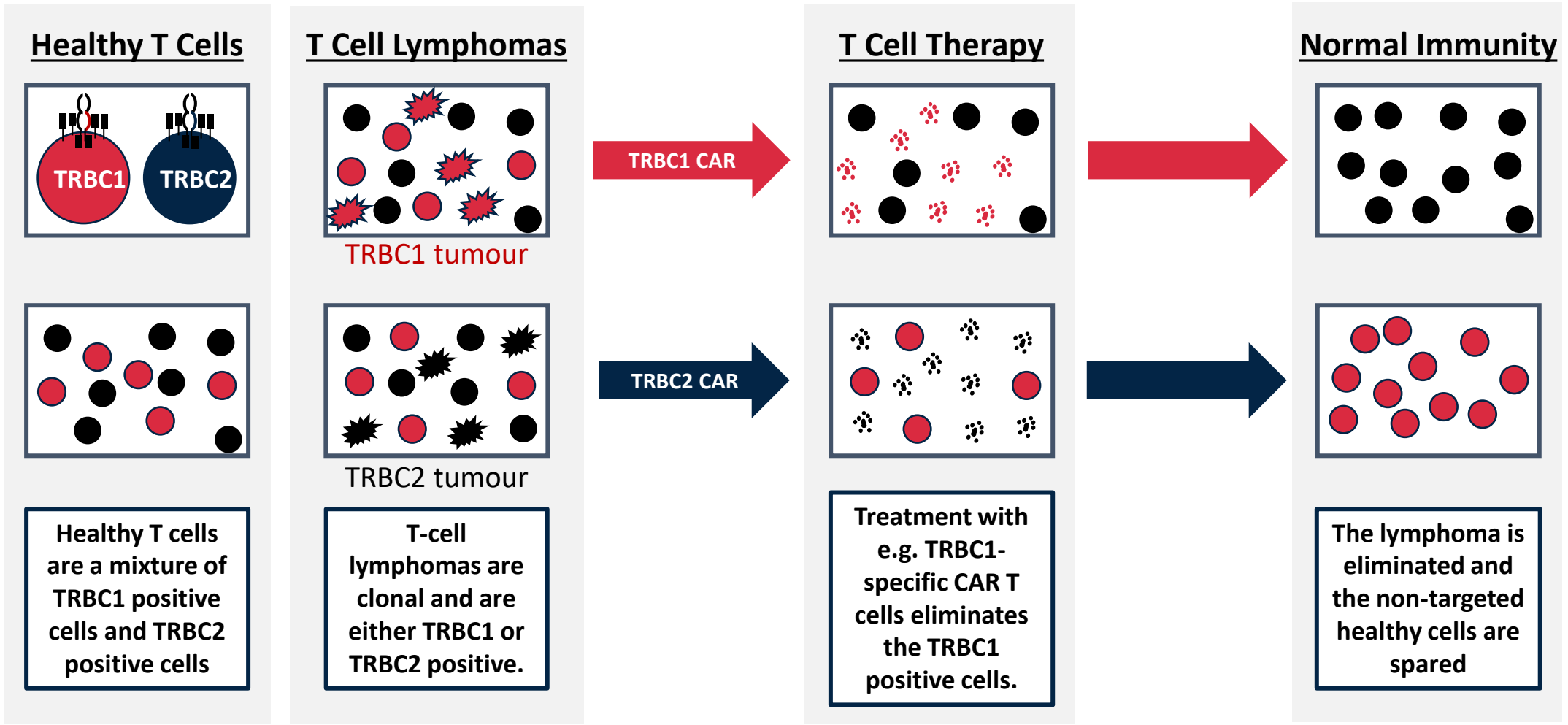
Went *et al*. *J Clin Oncol* (2006); **24**:2472-2479



The T cell Receptor Beta Constant Region is Duplicated

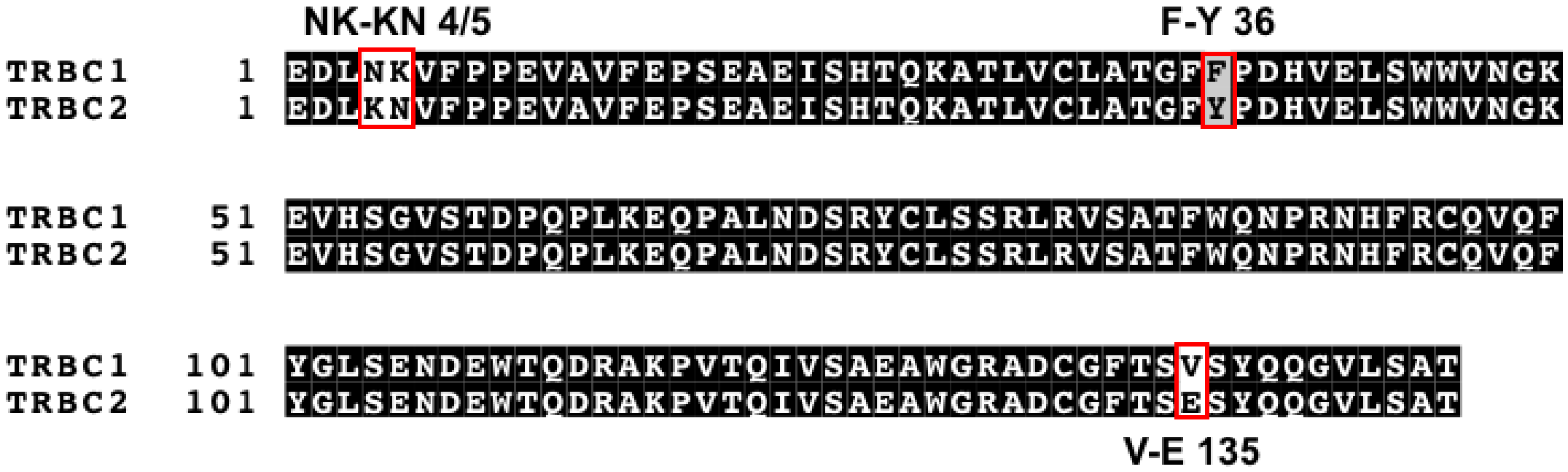


Targeting Strategy for T cell Lymphomas



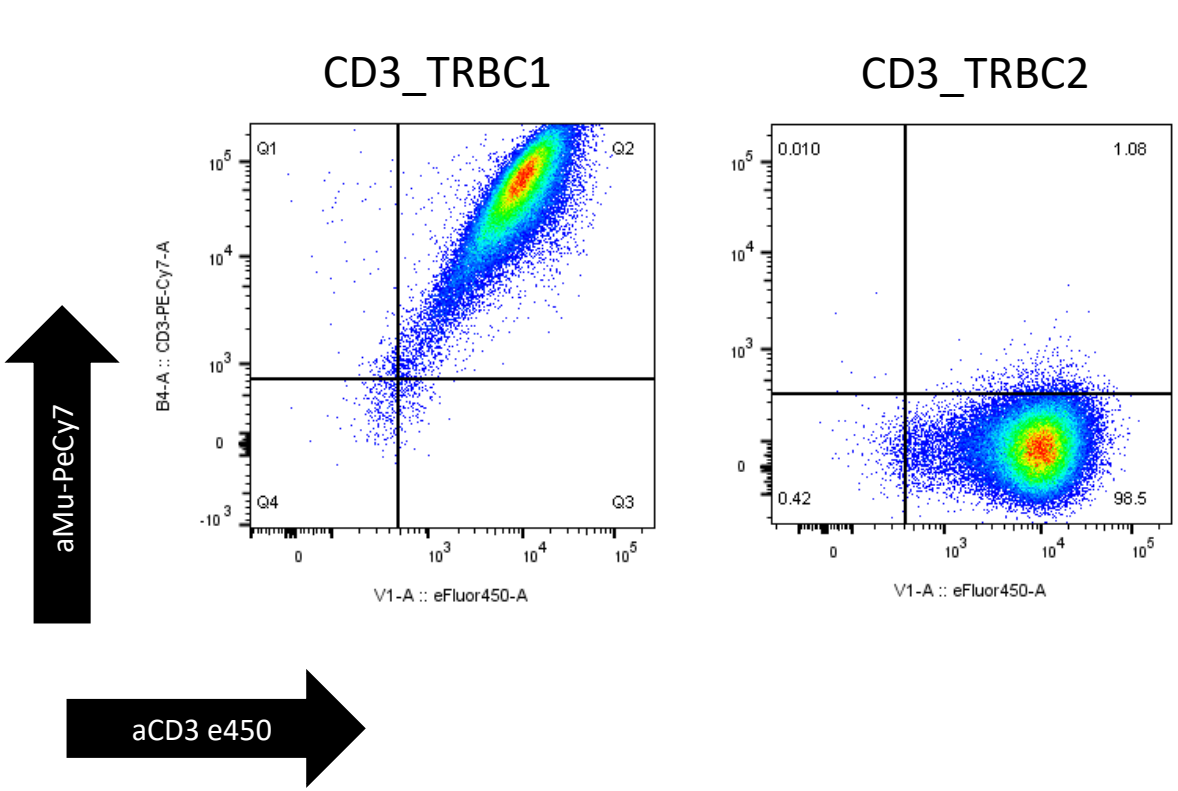
Challenges Targeting TCRβ

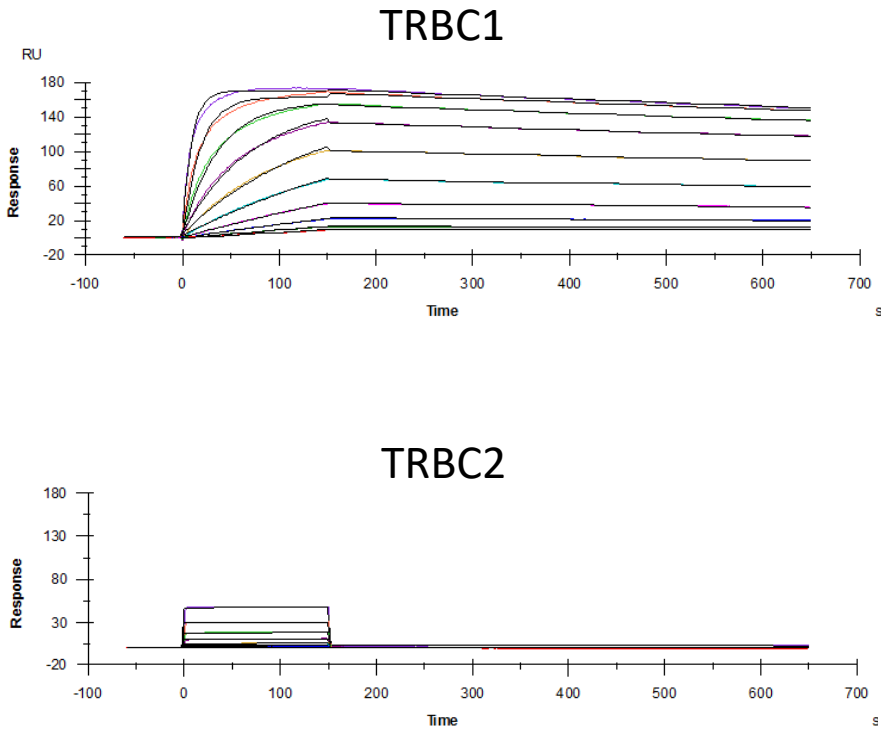
Differences between TRBC1 and TRBC2 are small



TRBC1 Specific Antibody

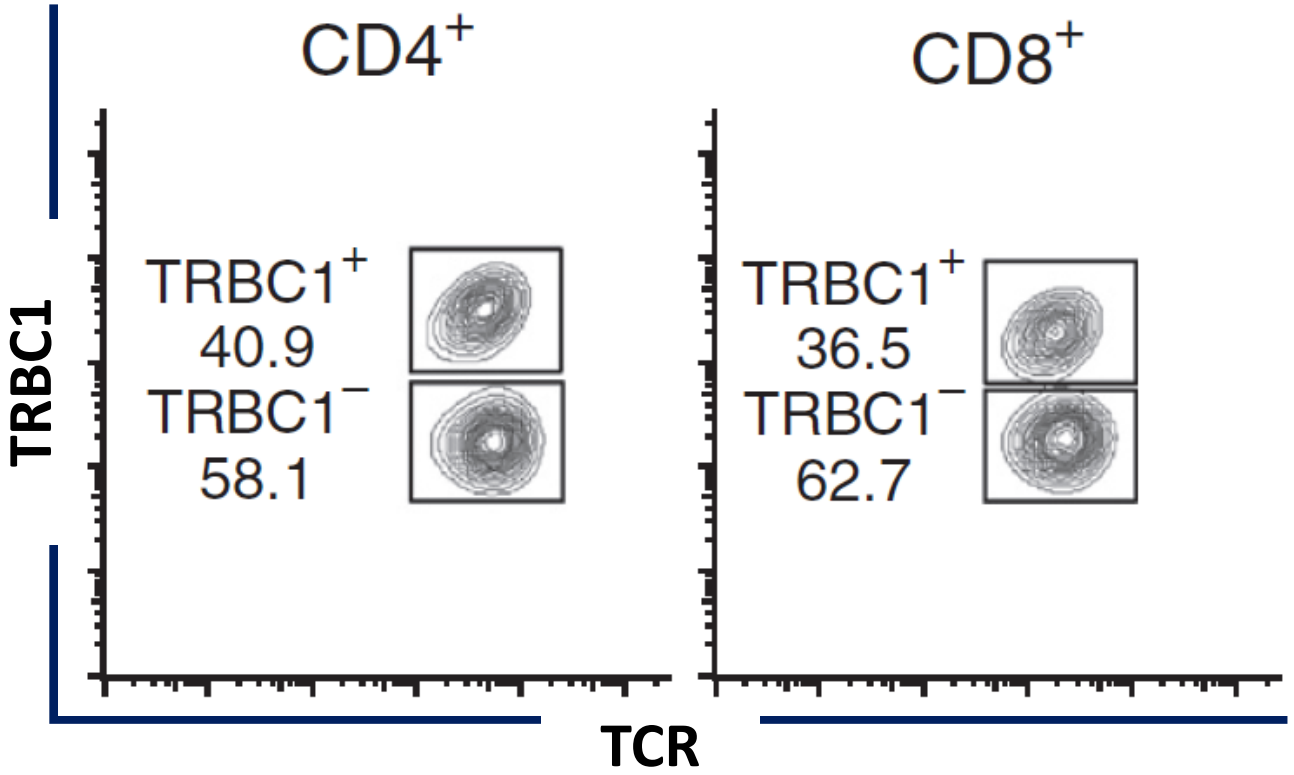
Identification of a TRBC1 specific antibody

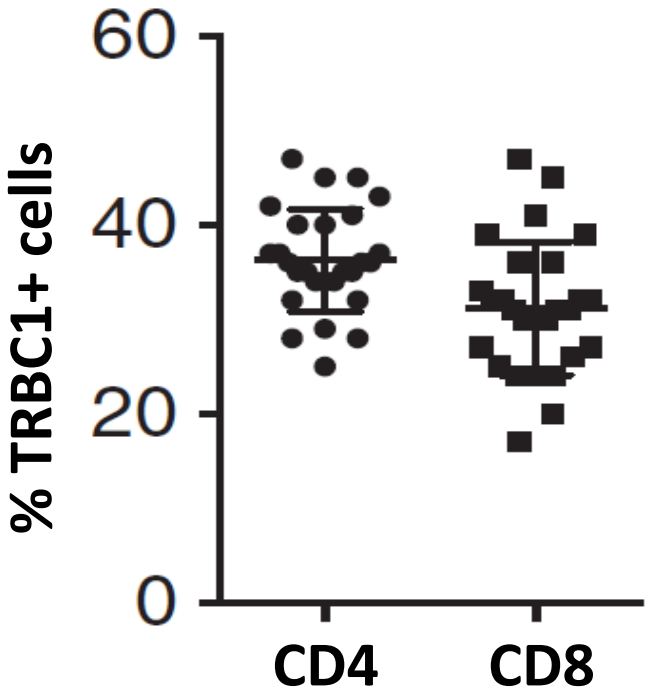




TRBC1 Expression in Normal T Cells

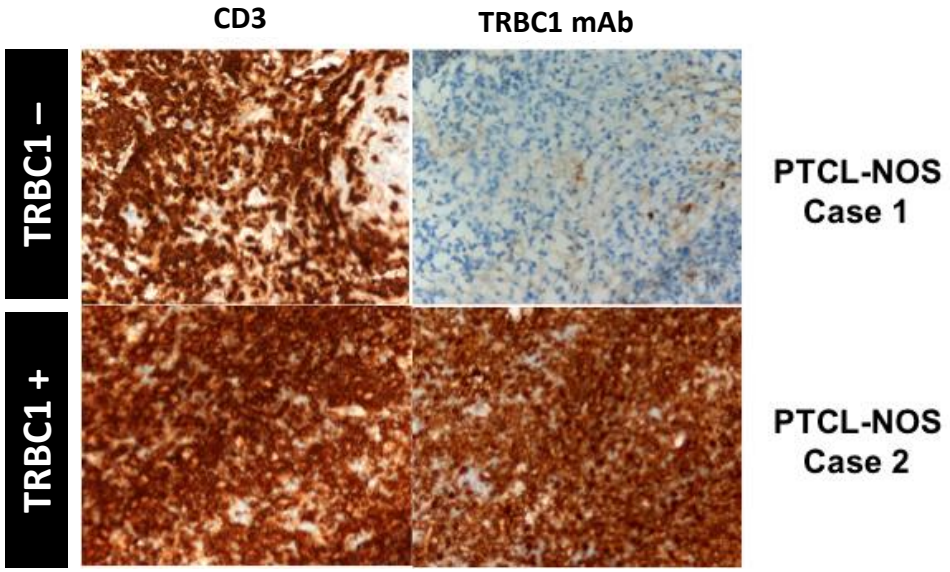
Peripheral blood T-cells contain a mix of TRBC1 and TRBC2 cells





TRBC1 Expression in Primary T cell Cancers

Screening of patient samples with aTRBC1 antibody



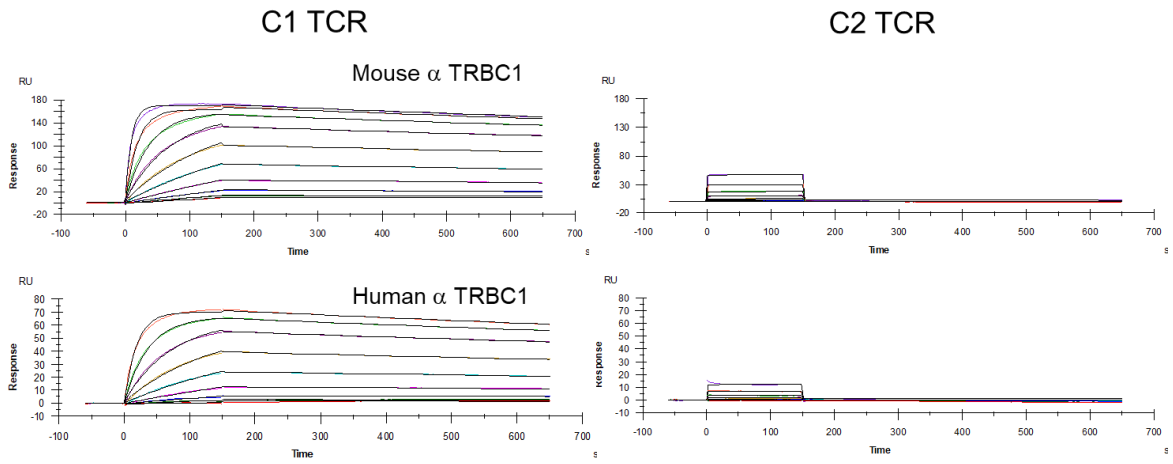
Diagnosis	TRBC1+ (%)	TRBC1-	Total
Anaplastic large cell lymphoma	5 (42)	7	12
Angio-immunoblastic T-cell lymphoma	2 (40)	3	5
Peripheral T-cell lymphoma, NOS	8 (44)	10	18
NK/T-cell Lymphoma	0 (0)	1	1
Sézary syndrome	1 (33)	2	3
T-acute lymphoblastic leukaemia/ lymphoma	2 (25)	6	8
Adult T-cell leukaemia/ lymphoma	2 (100)	0	2
T-prolymphocytic leukaemia	1 (33)	2	2
T-large granular leukaemia	1 (25)	3	4
OVERALL	22 (38)	34	56

Maciocia et al., Nature Medicine 2017 Dec;23(12):1416-1423.

Generation of an Anti TRBC1 Chimeric Antigen Receptor

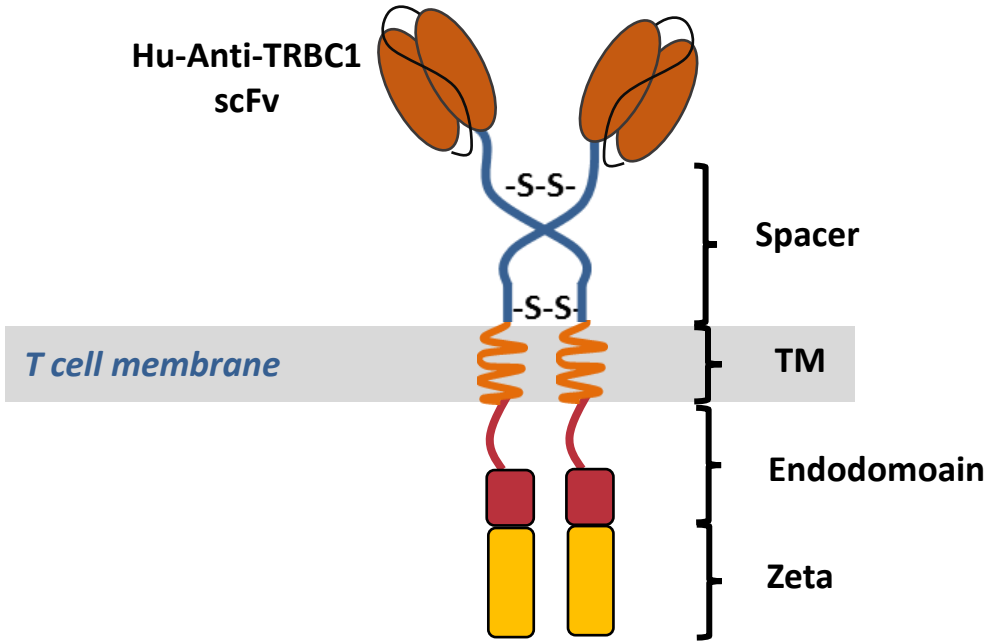
Humanization of aTRBC1 binder and CAR construction

Humanization of aTRBC1 mAb



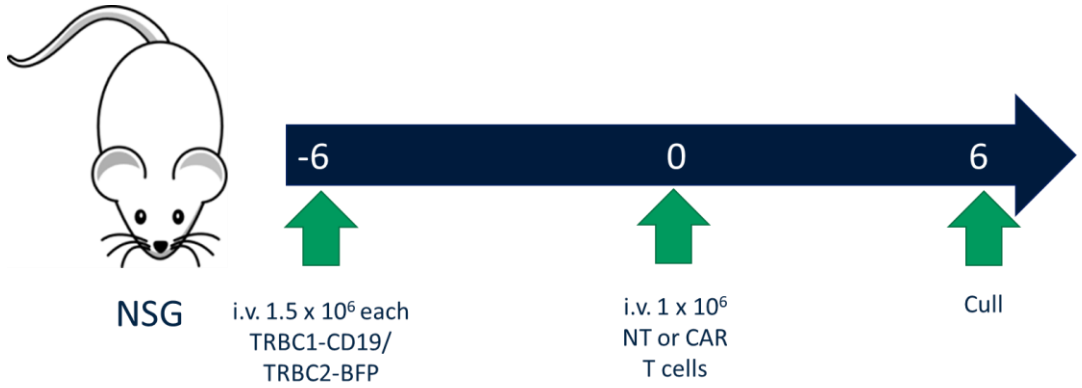
Binder	K_D
Mouse αTRBC1	2.41 ± 0.5
Human αTRBC1	2.96 ± 0.8

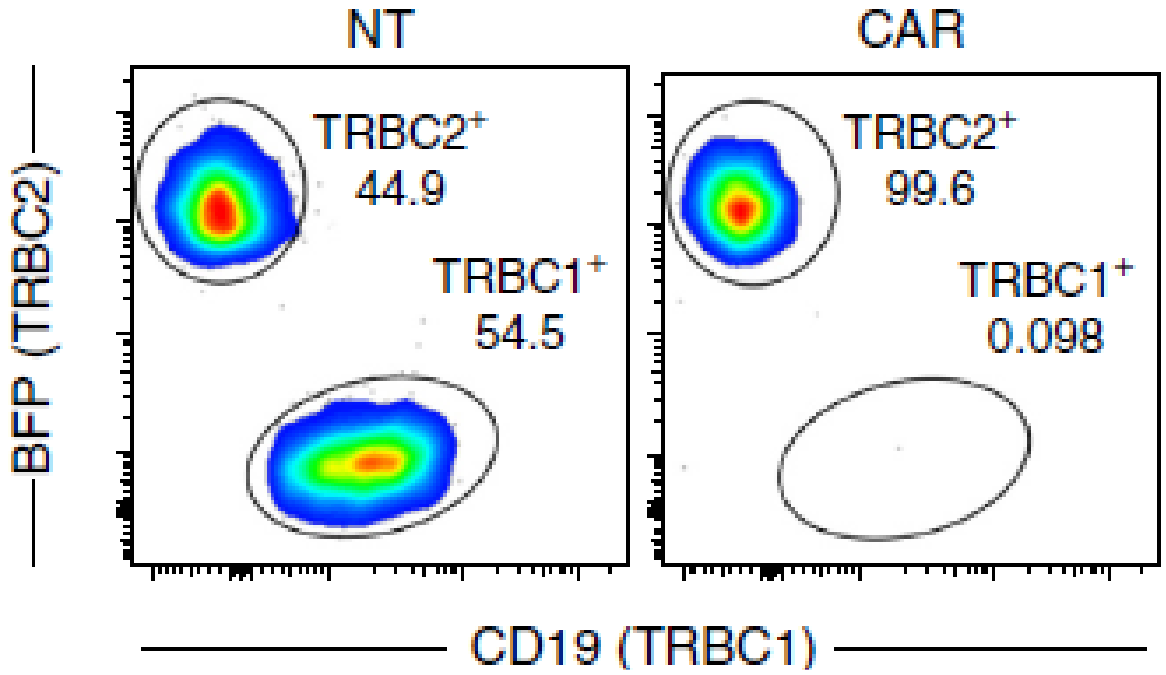
Construction of aTRBC1 Chimeric Antigen Receptor

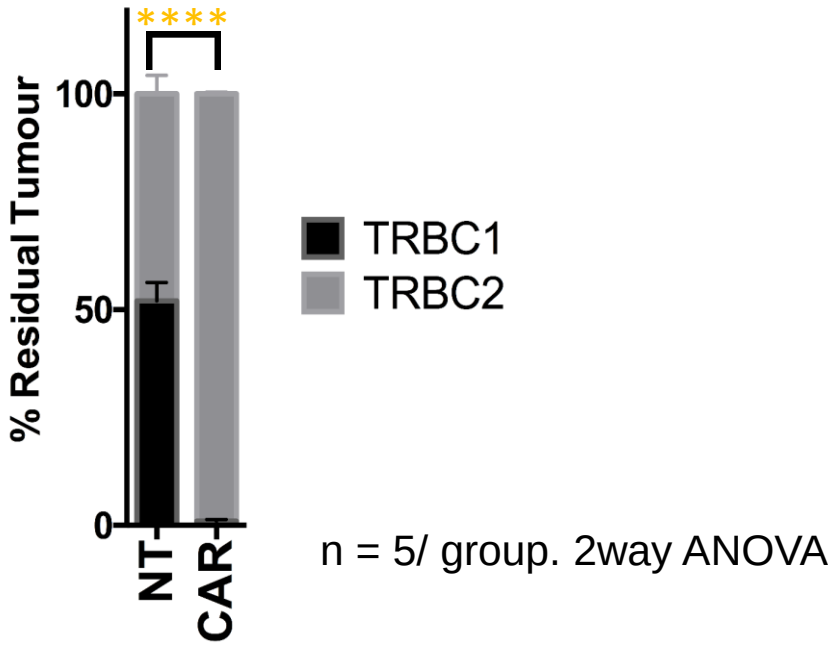


TRBC1 in-vivo CAR Activity

aTRBC1 CARs selectively target TRBC1 cells in the presence of TRBC2 cells







n = 5/ group. 2way ANOVA

SAFETY AND PRELIMINARY EFFICACY FINDINGS OF AUTO4, A TRBC1-TARGETTING CAR, IN RELAPSED/REFRACTORY TRBC1 POSITIVE SELECTED T-CELL NON-HODGKIN LYMPHOMA

NCT03590574

LibraT1

Kate Cwynarski[1], Gloria Iacoboni[2], Eleni Tholouli[3], Tobias Menne[4], David Irvine[5], Leigh Wood[6], Nivetha Balasubramaniam[6], Justin Shang[7], Michael Zhang[7], Silvia Basilico[7], Min Liu[7], Kevin Duffy[7], Birgit Huber[7], Mary Vinson[7], Wolfram Brugger[7], Martin Pule[1,7]

[1]University College Hospital, UK [2]VHIO Vall d'Hebron Hospital, Spain [3]Manchester Royal Infirmary, UK [4]Freeman Hospital Newcastle, UK [5]University of Glasgow, UK [6]Cancer Clinical Trials Unit, UK [7]Autolus Ltd, UK

Phase I/II Study Evaluating AUTO4 in Patients With TRBC1 Positive PTCL: LibraT1

Key Inclusion

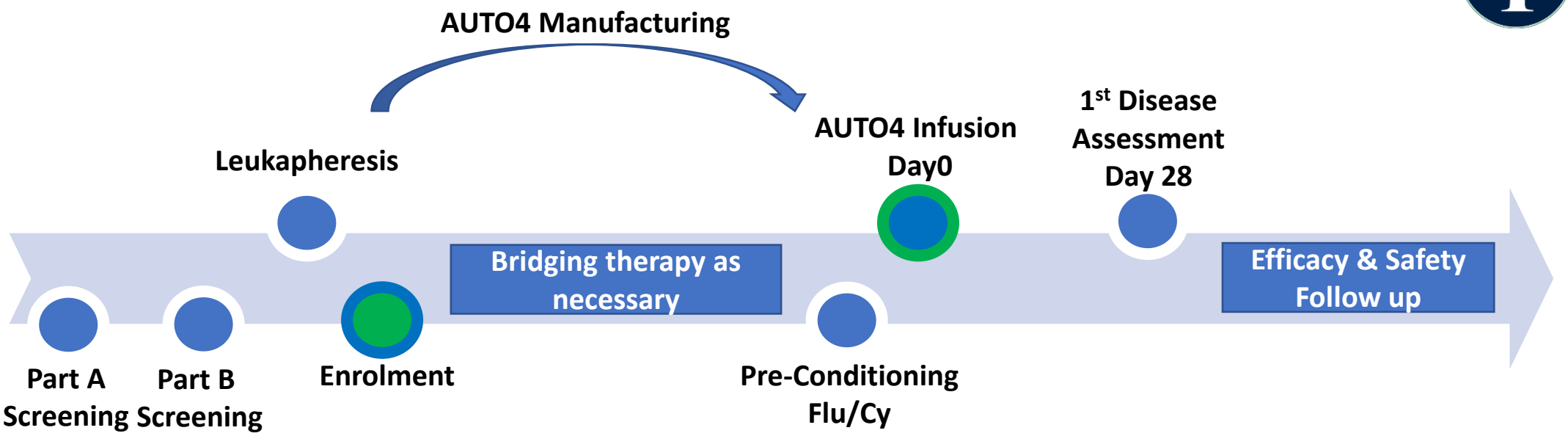
- ≥18 years of age
- ECOG 0-1
- Confirmed diagnosis of PTCL-NOS, AITL, or ALCL
- Confirmed TRBC1+ tumour – confirmed using a NGS assay
- Relapsed/refractory disease following at least 1 line of therapy

Key Exclusion

- Patients with T-cell leukaemia
- Active or past history of CNS involvement by malignancy

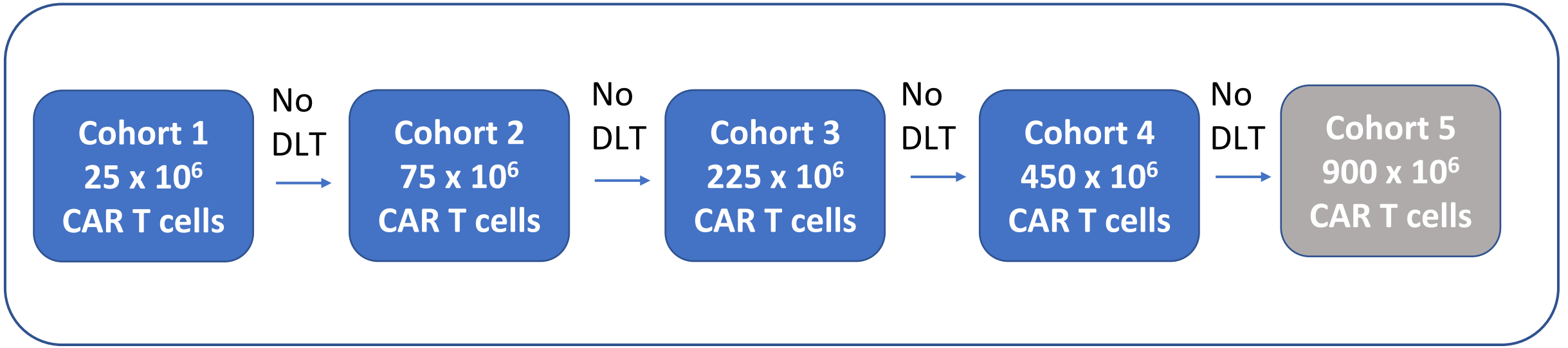
PTCL-NOS, Peripheral T-cell lymphoma, not otherwise specified; AITL, Angioimmunoblastic T-cell lymphoma; ALCL, Anaplastic large cell lymphoma; CNS, central nerve system; NGS, next-generation sequencing.

Study Design



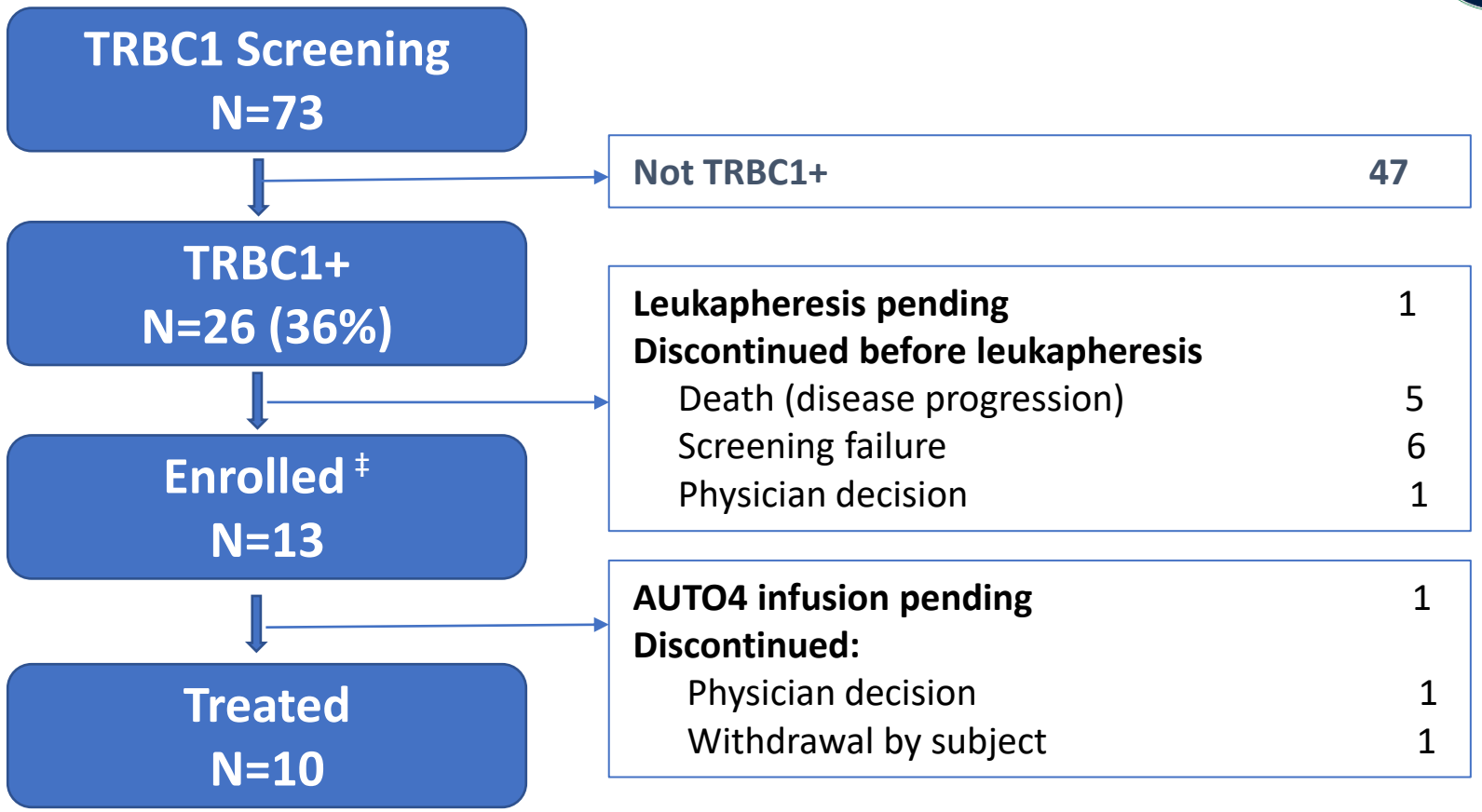
- Part A: Lymphoma tissue screening for TRBC1 or TRBC2 expression using NGS
- Part B: Study screening for patients determined to have TRBC1+ lymphoma

Phase I Dose Escalation of AUTO4



- Pre-conditioning: FLU 30 mg/m^2 IV (Days -6, -5, -4, -3) & CY 500 mg/m^2 IV (Days -6, -5)
- Accelerated escalation: Cohort 2 and 3 may dose <3 patients if there are no DLTs and no CAR T expansion.

Patient Disposition



Safety set: all infused, n=10
Efficacy set: ≥1mth follow up, n=9

Cwynarski et al, EHA'22
Data Cutoff: 26APR2022

Baseline Characteristics

	Total (N=10)
Age, median (range)	55 (34 – 63)
Median prior lines of treatment (range)	3 (1 – 5)
Stage of Lymphoma at screening • I/II • III/IV	 2 (20%) 8 (80%)
Lymphoma Subtype, n (%) • Peripheral T-cell lymphoma NOS • Anaplastic large cell lymphoma, ALK-negative • Angioimmunoblastic T cell lymphoma (AITL)	 5 (50%) 1 (10%) 4 (40%)
Prior Autologous Stem Cell Transplant, n (%)	3 (30%)
ECOG 0/1, n (%)	3 (30%), 7 (70%)
Bridging therapy YES, n (%)	7 (70%)

Cwynarski et al, EHA'22
Data Cutoff: 26APR2022

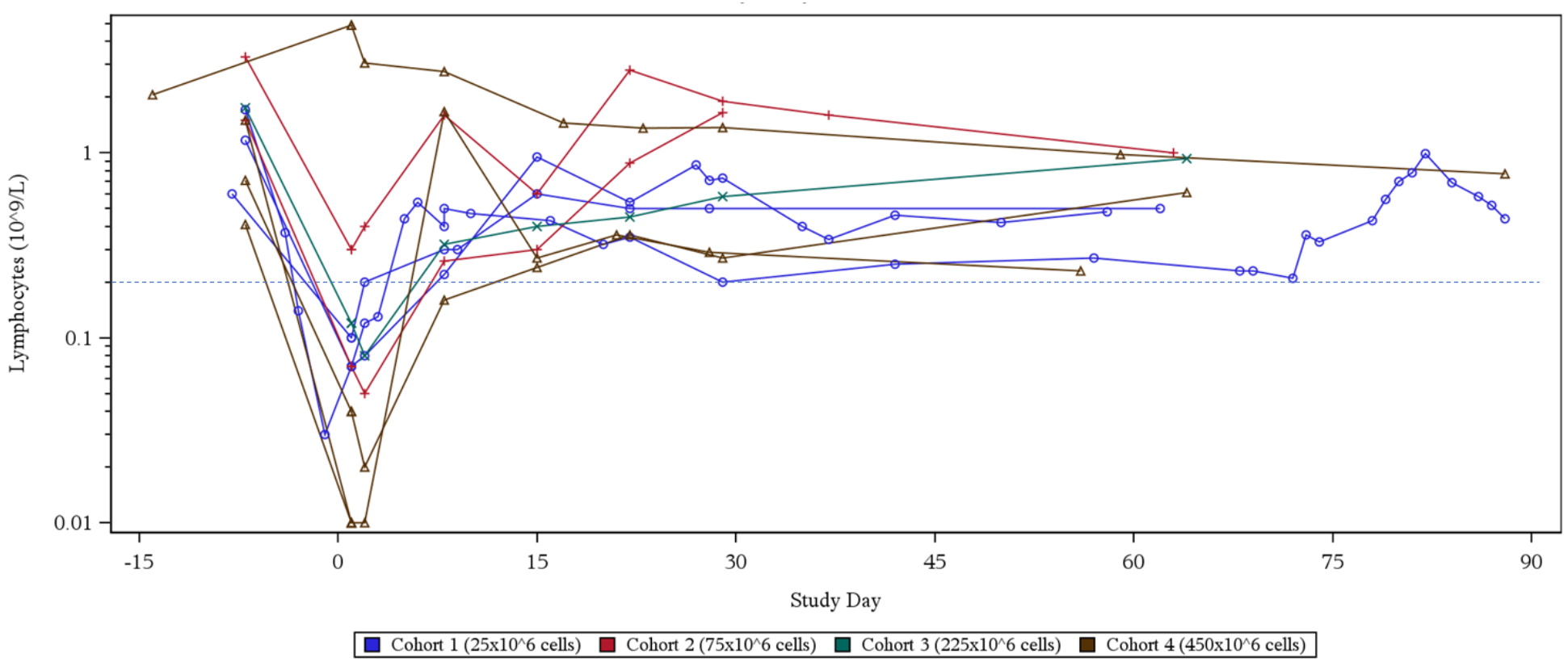
Key Safety Data

	Cohort 1 $25x10^6$ cells (N = 3)	Cohort 2 $75x10^6$ cells (N = 2)	Cohort 3 $225x10^6$ cells (N = 1)	Cohort 4 $450x10^6$ cells (N = 4)	Total (N = 10)
Dose Limiting Toxicity (DLT)	0	0	0	0	0
Grade 3 or 4 TEAE within 60 days	3 (100%)	2 (100%)	1 (100%)	4 (100%)	10 (100%)
Neutropenia	3 (100%)	2 (100%)	0	3 (75%)	8 (80%)
Infections and Infestations	0	0	0	0	0
Serious TEAE	2 (67%)	0	0	2 (50%)	4 (40%)
Any grade CRS	0	0	0	4 (100%)	4 (40%)
Grade 3 CRS	0	0	0	1 (25%)	1 (10%)
Any grade ICANS	0	0	0	0	0

TEAE, Treatment-emergent adverse events; CRS, cytokine release syndrome; ICANS, Immune Effect Cell-Associated Neurotoxicity Syndrome

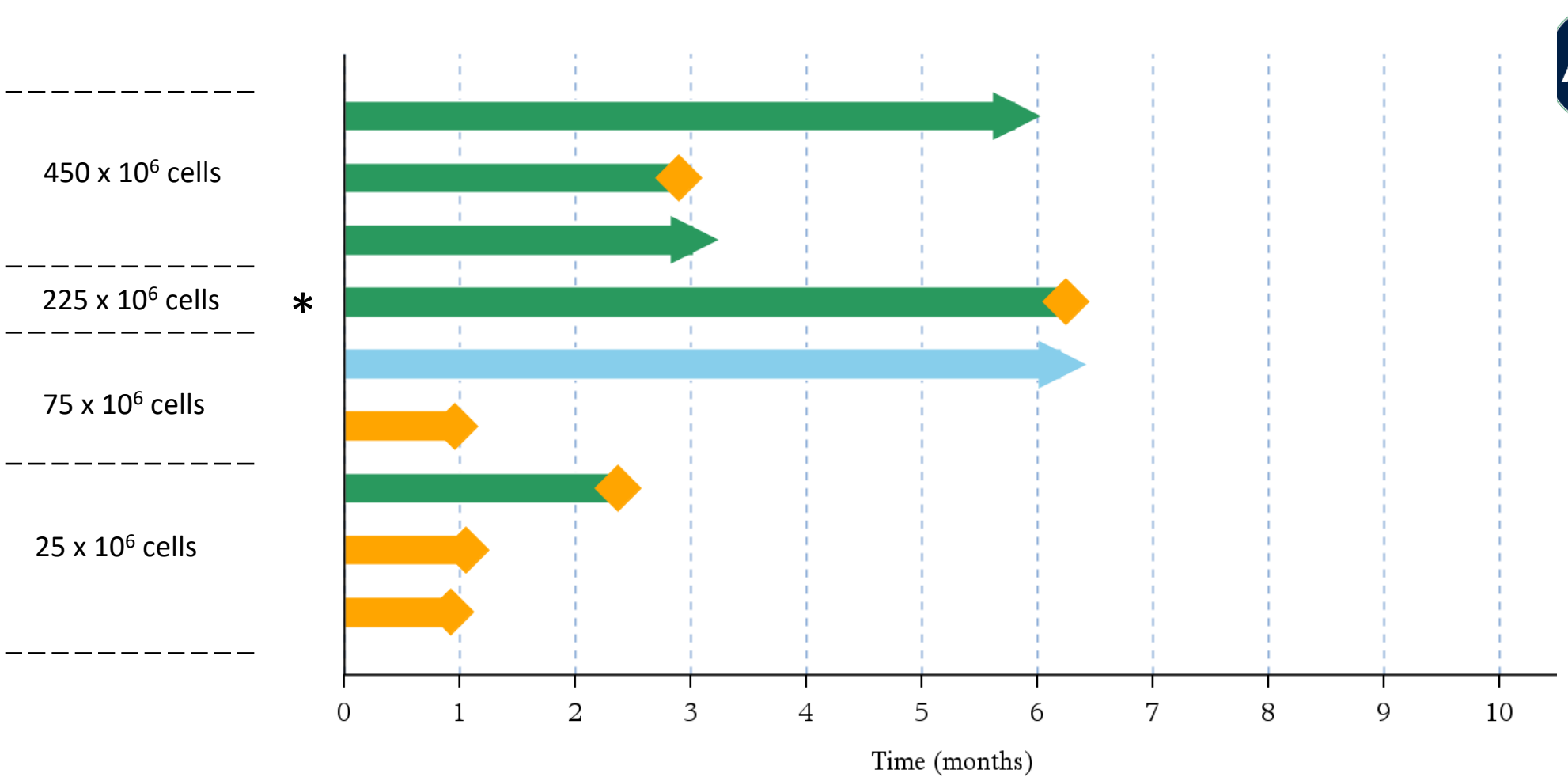
Cwynarski et al, EHA'22
Data Cutoff: 26APR2022

Recovery following transient lymphopaenia after Flu/Cy and AUTO4



Cwynarski et al, EHA'22
Data Cutoff: 26APR2022

Efficacy

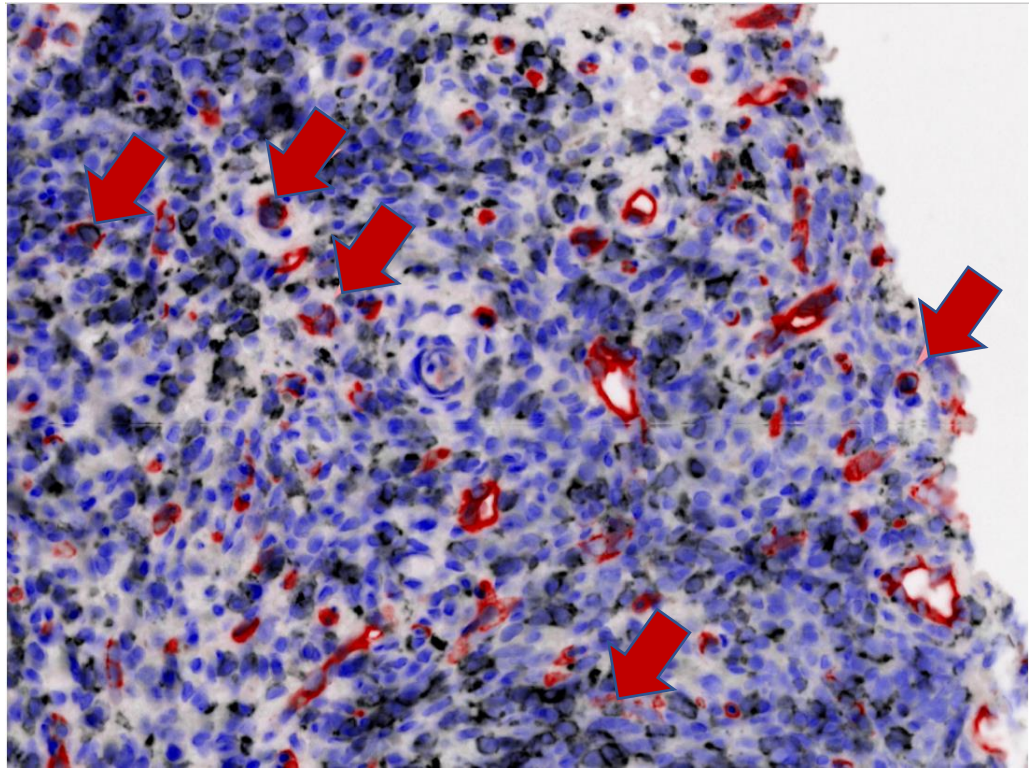


Efficacy assessments were performed by the Investigators according to the Lugano Classification.
Evaluable Set consists of patients who have received an infusion of AUTO4 treatment and completed the Day 28 evaluation.
All patients had relapsed/refractory disease at time of Part B screening and enrolment
* Patient was in PET-negative CMR at the start of pre-conditioning after bridging therapy.

Cwynarski et al, EHA'22
Data Cutoff: 26APR2022

CAR T cells detected in lymph node but not in peripheral blood

- CAR T cells detected in a lymph node biopsy of a patient who achieved complete remission.
  - Approx. 2% nucleated cells in lymph node are CAR T cells (n=1)[1]
- No CAR T expansion detected by PCR or flow in peripheral blood



Double staining for CAR T cell (red) and CD3 (black).
x40 IHC view (deconvoluted)[1]

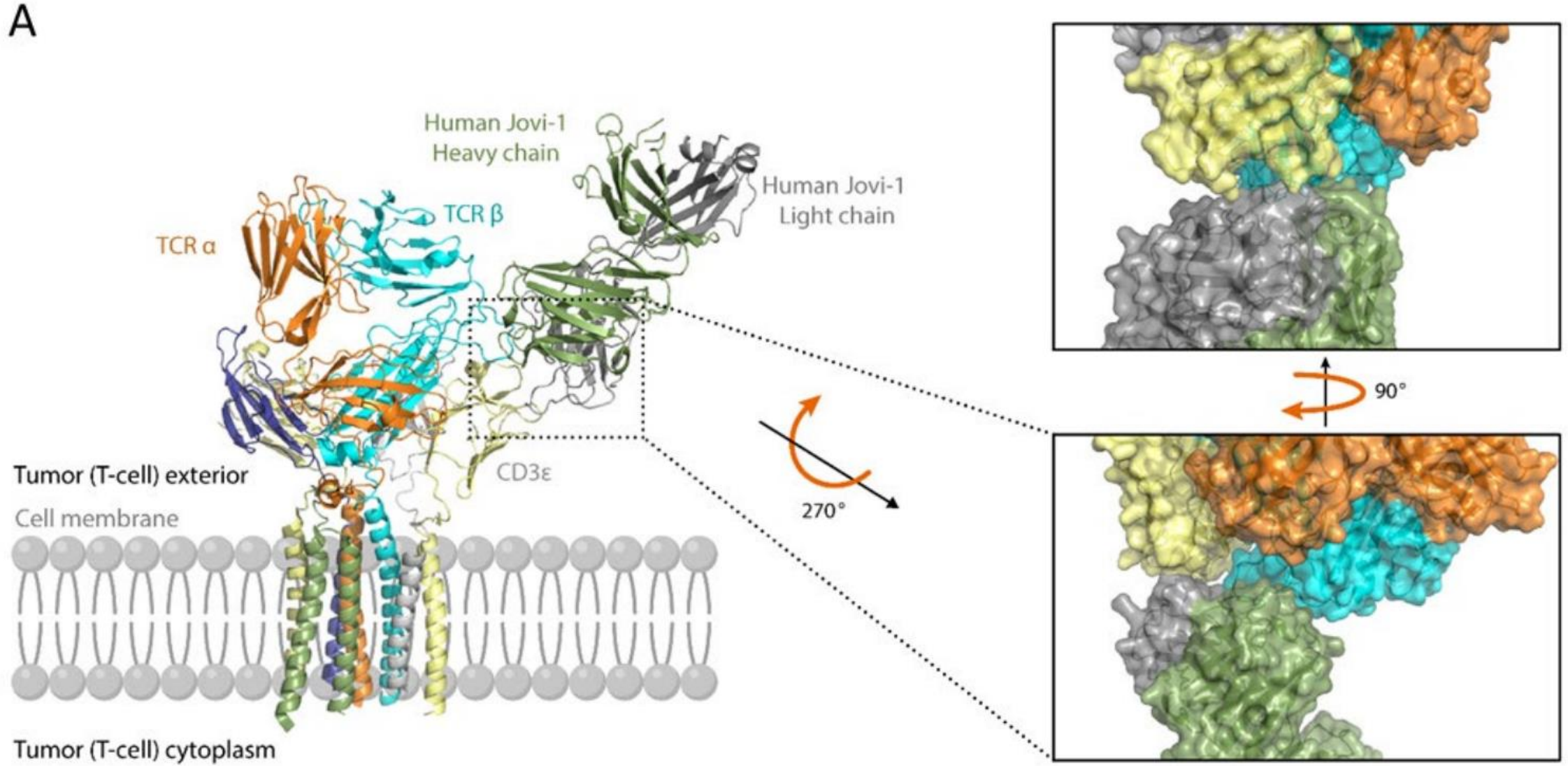
CAR T cell – double stained for CAR and CD3

[1]Professor Teresa Marafioti, Dept of Pathology, UCL.

Summary

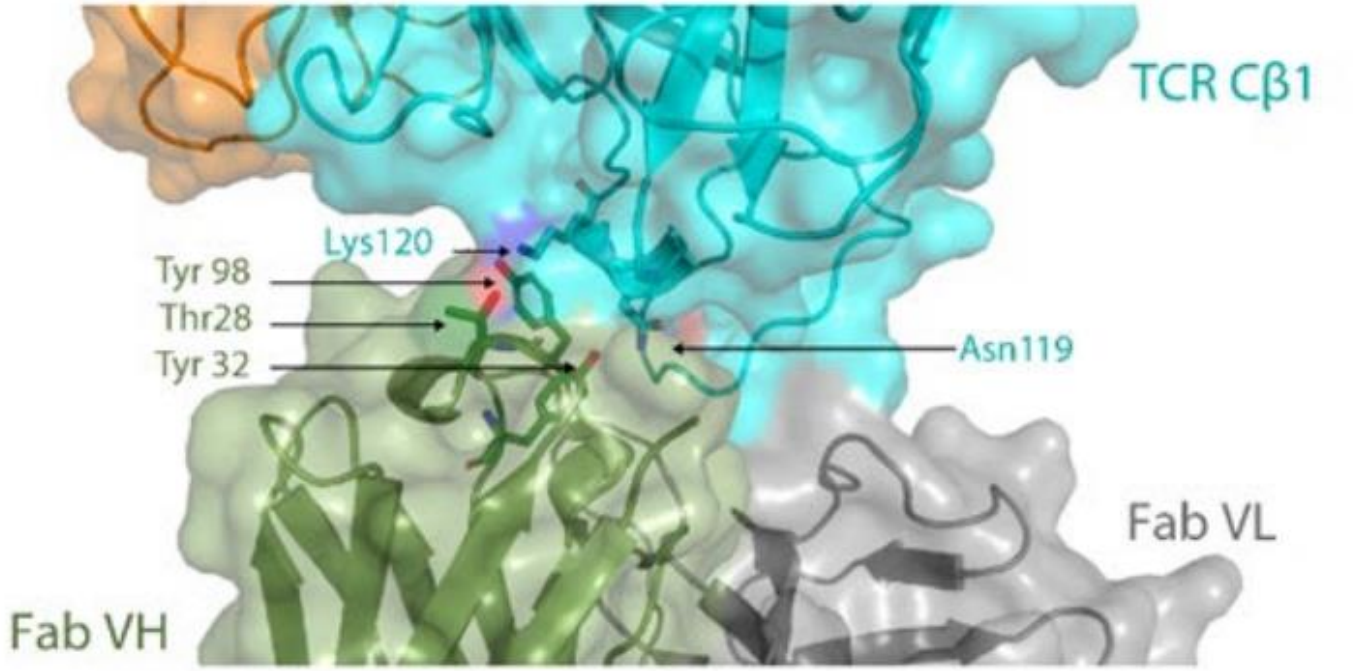
- AUTO4 treatment generally well tolerated
- Early efficacy is encouraging
- Longer follow-up ongoing
- CAR T-cells detected in lymph node but no expansion observed in peripheral blood
- Study ongoing, with additional patients due to be treated to define recommended phase II dose

Structure of TRBC1 antibody binding to the



Ferrari et al, Research Square, https://doi.org/10.21203/rs.3.rs-1475171/v1

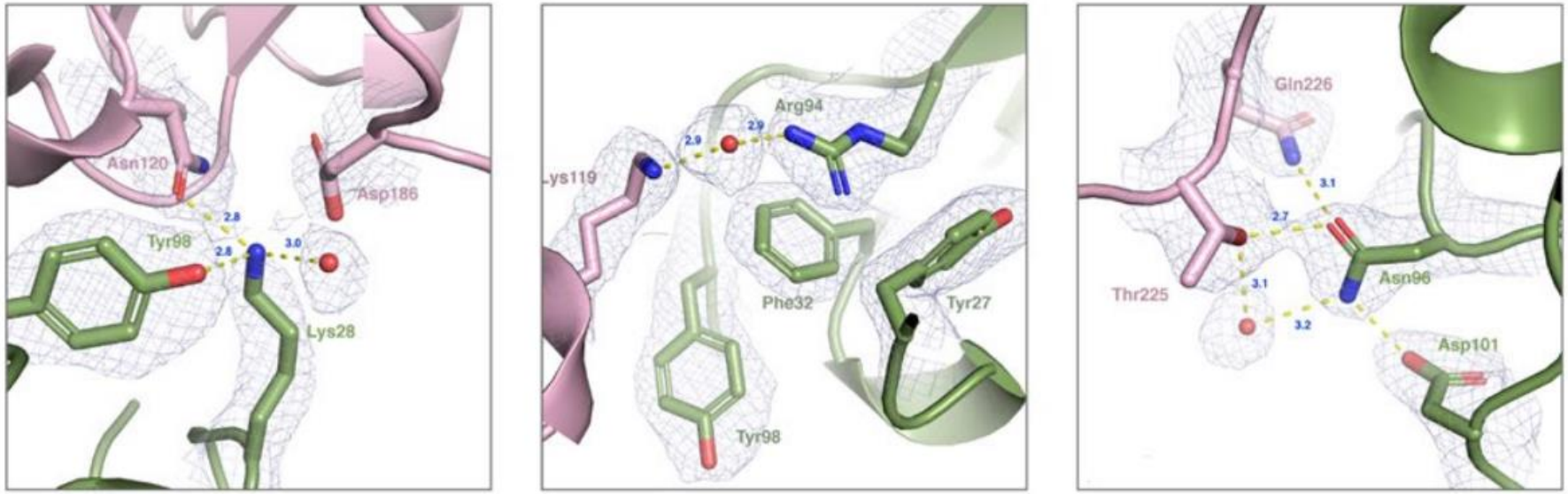
Crystal Structure of a TRBC1 Antibody in Complex with TCR

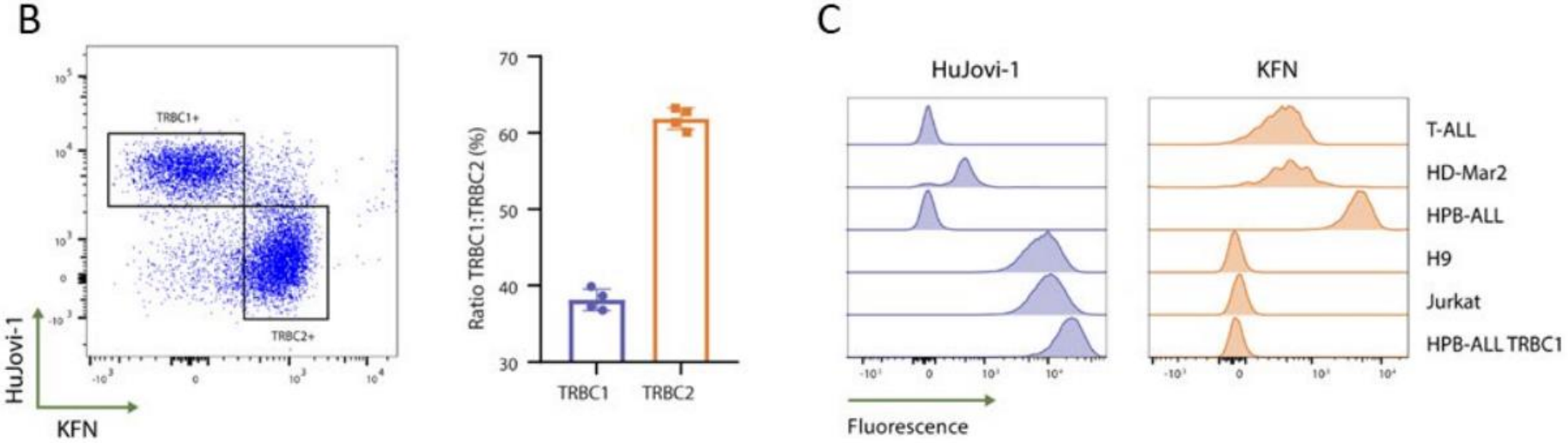


NK-KN 4/5

TRBC1	1	EDLNKVFPPEVAVFE
TRBC2	1	EDLKNVFPPEVAVFE

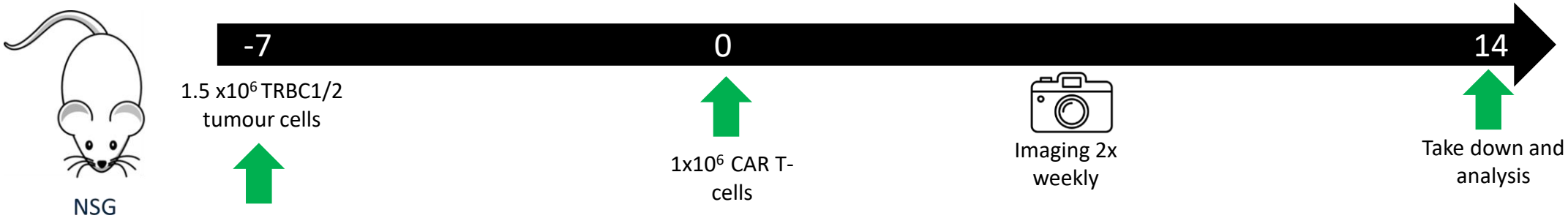
Converting JOVI-1 to a TRBC2 antibody… in silico design + phage display

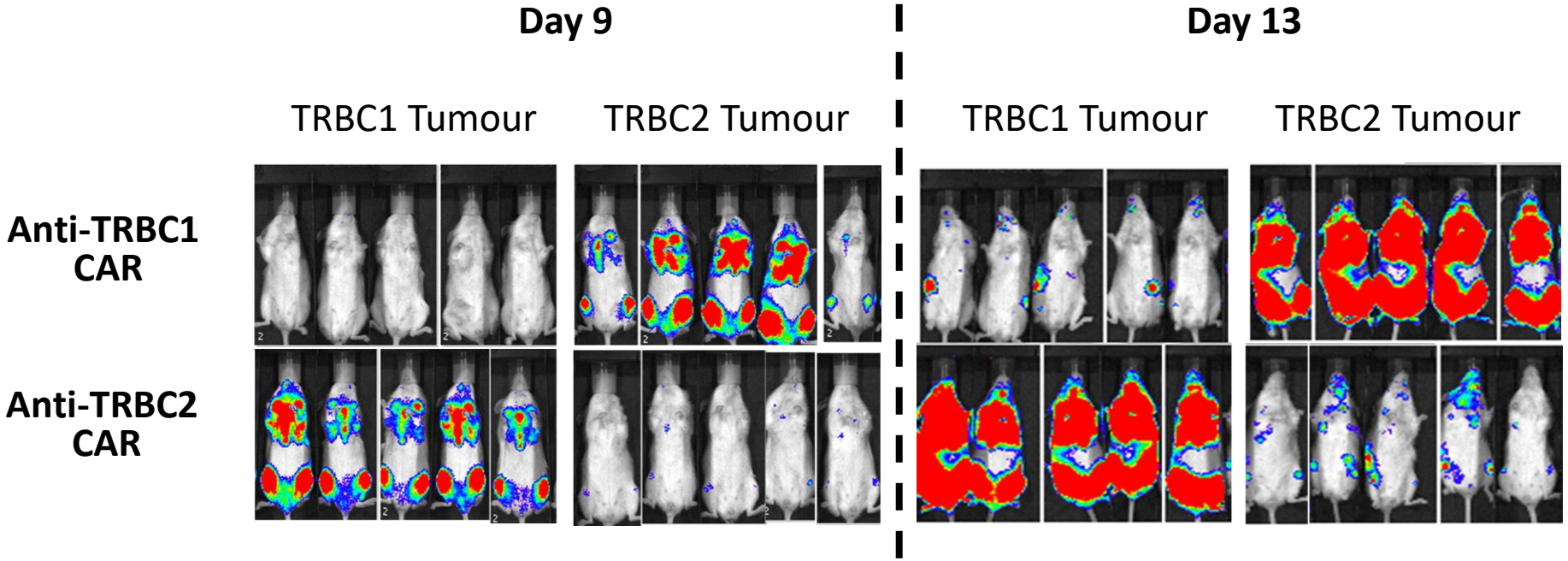




TRBC2 in-vivo CAR Activity

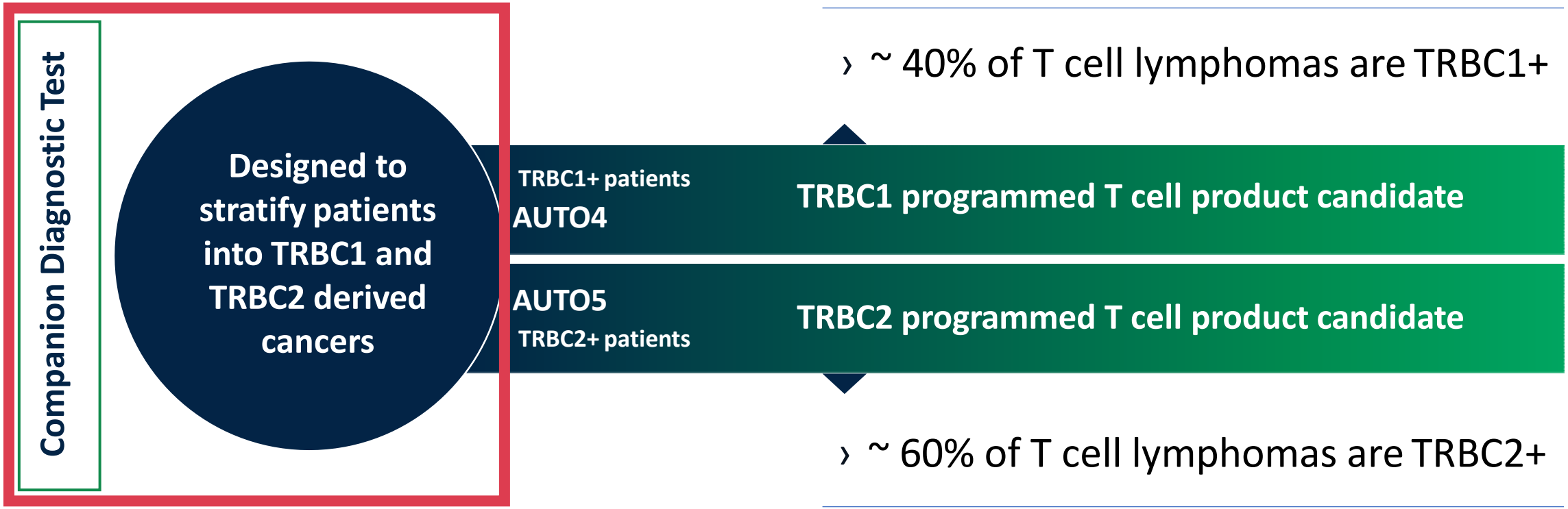
aTRBC2 CARs clear tumour in NSG model





Addressing T cell lymphomas

Three key elements - AUTO4, AUTO5 and a companion diagnostic test



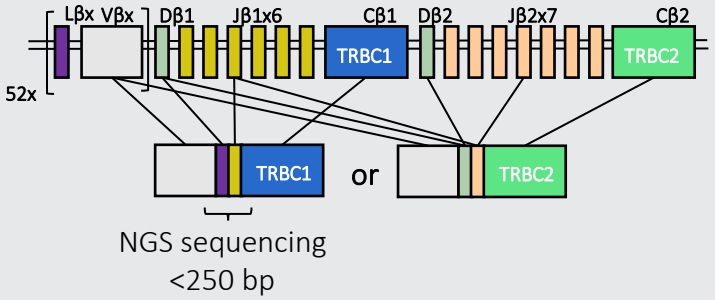
› ~ 40% of T cell lymphomas are TRBC1+

› ~ 60% of T cell lymphomas are TRBC2+

Companion Diagnostic

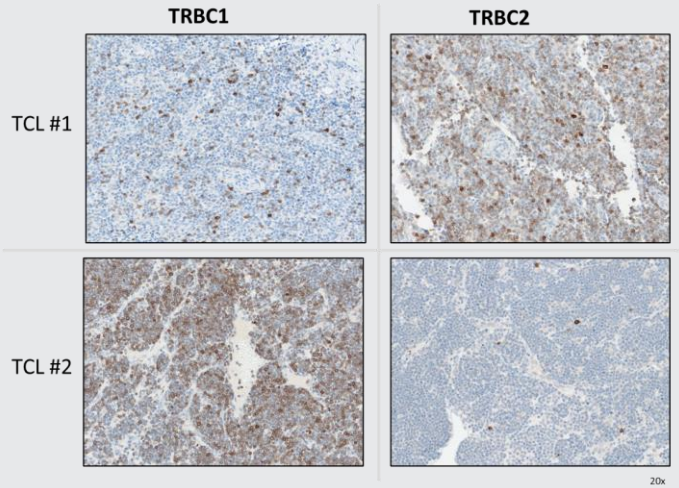
- Multiple approaches de-risked for development

• Next Generation Sequencing



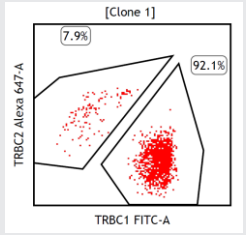
- T cell clonality NGS assay currently used in AUTO4 Phase 1

• Immunohistochemistry

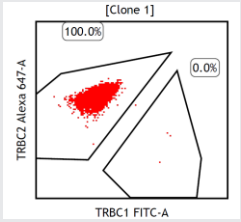


- FFPE specific antibodies can discriminate between TRBC1 and TRBC2 patient tumors

• Flow Cytometry



TRBC1 positive T-cell Prolymphocytic Leukemia



TRBC2 positive small Sezary cell cutaneous T-Cell Lymphoma

- Flow specific antibodies can discriminate between TRBC1 and TRBC2 in patient tumors

Other CAR T cell targets for T cell lymphomas

Antigen	PTCL No.	PTCL Positive (%)	AILD No.	AILD Positive (%)
Human TCR βF1	133	97	30	94
CD2	136	70	41	100
CD3	144	86	40	95
CD4	135	46	38	42
CD8	129	15	34	32
CD5	137	20	36	19
CD7	141	19	41	24
CD10	143	1	43	39
CD15	140	4	43	2
CD30	145	3	42	0
CD56	140	6	40	3
CD57	143	10	42	5
TIA-1	138	27	41	34
GB	140	2	40	0
ALK-C	143	0	41	0
EBER	132	5	39	3
Mib-1 high	138	11	40	5
CD20	141	1	42	0
CD79a	142	0	36	0

Went *et al*. *J Clin Oncol* (2006); **24**:2472-2479

- CD4 – Ablation of CD4 compartment
- CD5
- CD7

} Fratricide
Pan T cell aplasia

- CD30
- CD70

} Limited expression
Expressed on activated T cells

Acknowledgements

UCLH, UK
Kate Cwynarski
UCLH CAR T Trials Team

VHIO Vall d'Hebron, Spain
Gloria Iacoboni
VHIO Clinical trials team

Manchester Royal Infirmary, UK
Eleni Tholouli
MRI Trials team and CAR-T CNSs

Freeman Hospital, UK
Tobias Menne
NCCC CAR-T cell team

University of Glasgow, UK
David Irvine
Glasgow QEUH CAR-T trials team

UCL, UK
Teresa Marafioti
Paul Maciocia

Autolus
Wolfram Brugger
Mary Vinson
Shimobi Onouha
Michael Zhang
Kevin Duffy
Birgit Huber
Silvia Basilico
Meera Raymond

We would like to thank our patients, carers and families

https://www.ucl.ac.uk/cancer/research/ucl-car-t-programme