Improved CAR T cell approaches for lymphoid malignancies

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Disclosure & Conflict of Interest

Dr Pule is the founder and Chief Scientific Officer of Autolus Therapeutics plc and a clinical senior lecturer in the Dept. of Hematology at UCL Cancer Institute. The views and opinions expressed in this presentation are of Dr Pule in his personal capacity. The views expressed are his own and do not necessarily represent the views of Autolus Therapeutics.

AUTO1 and AUTO3 are investigational therapies in ongoing trials and no definitive conclusions regarding safety or effectiveness can be drawn from the data presented.

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Dr Pule is on the scientific advisory board of Mana Therapeutics

- Fast off-rate CD19 CAR T-cell therapy in adults with r/r B-ALL

- CD19/22 CAR T-cell therapy for DLBCL

- TRBC1/2 targeting of T-cell Lymphomas

CAR T Cell Therapies In Adult B-ALL

Current Status of CAR T Cell Therapies in Adult ALL

- No approved CAR T therapy for adult B-ALL patients

- Relapsed refractory patients have a significant number of co-morbidities

- Current experience with CAR T therapies in r/r adult ALL is immature and limited

- Highly active, but no clear sense of durability without subsequent allograft

- Toxicity seems to be a particular problem with adults with r/r B-ALL

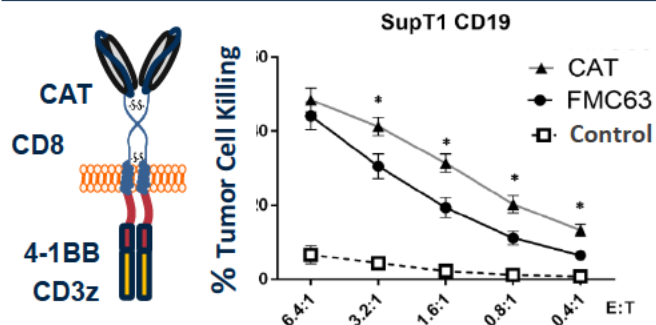# Desired Characteristics of a CAR T Therapy for Adult B-ALL

- Highly active
  - High complete molecular response rate
  - Durability of responses without subsequent allo-transplant
  - Prolonged persistence of CAR, > 2yrs

- Safe
  - Low severe CRS and Neurotoxicity
  - Safe administration to patients with high leukemic burden

- Manufacturing
  - High manufacturing success rate
  - Short vein to vein time
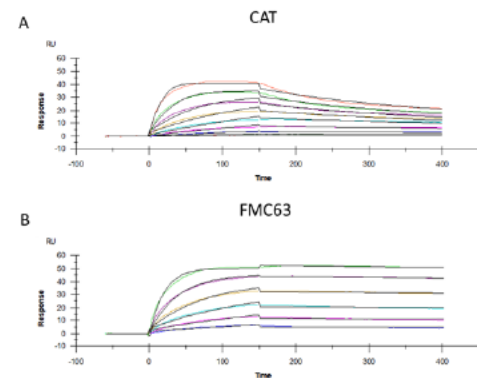
AUTO1 Preclinical Data[1]

Enhanced activity vs. FMC63 CARs

- AUTO1 (CAT) binder with lower affinity for CD19
- Half-life of target interaction very short compared to FMC63 (e.g. Kymriah®)[2] binder:
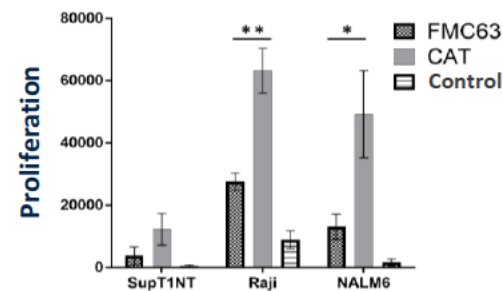
  - AUTO1 = 3.7 minutes
  - Kymriah = 168 minutes

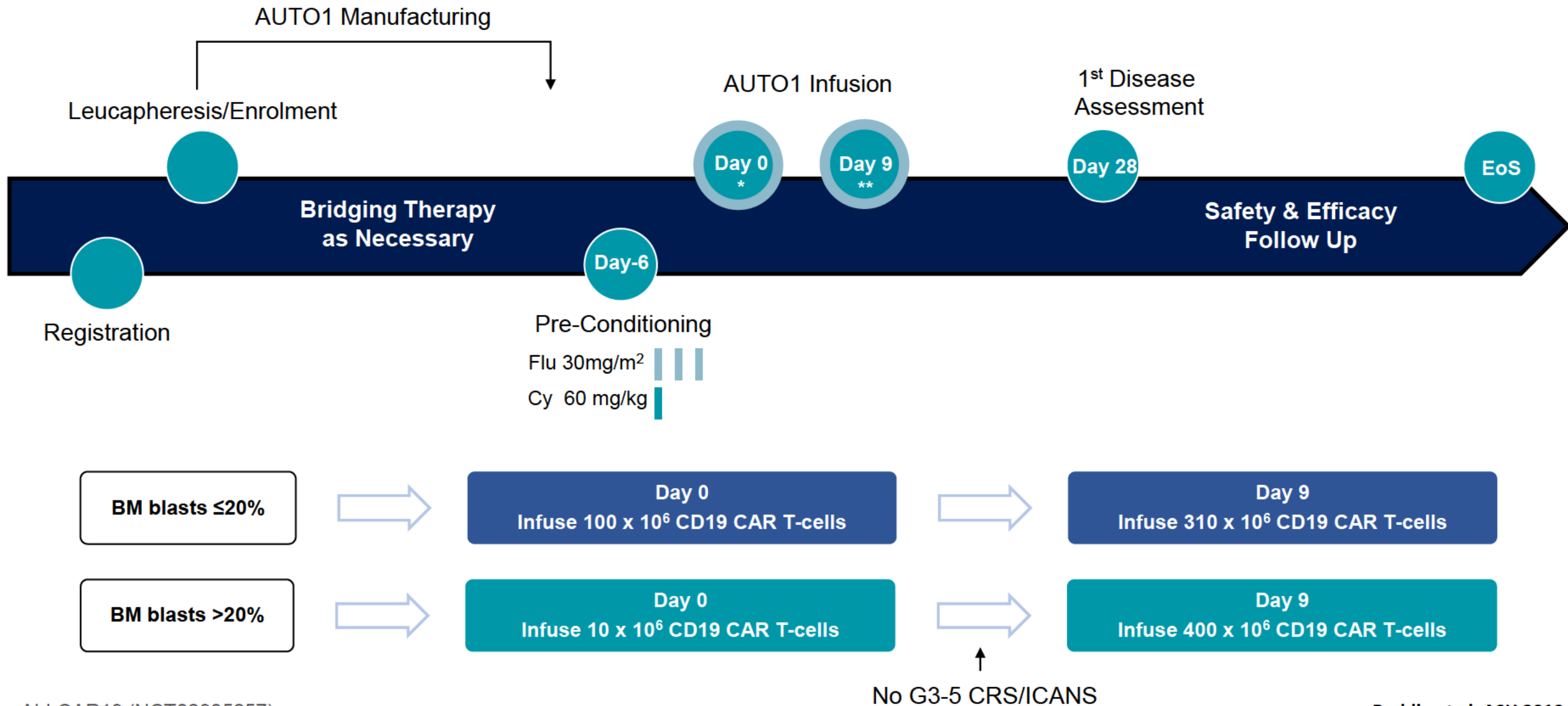**Fast Off-Rate**

Enhanced Cytotoxicity

Enhanced Proliferation

1. Pule at al., Keystone Symposia: Emerging Cellular Therapies 2018
2. Similar binders are used in Yescarta and JCAR-017

ALLCAR19 Phase I Study Design

AUTO1 Manufacturing

AUTO1 Infusion

1st Disease Assessment

Leucapheresis/Enrolment

Day 0
*

Day 9
**

Day 28

EoS

Bridging Therapy as Necessary

Day-6

Safety & Efficacy Follow Up

Registration

Pre-Conditioning

Flu 30mg/m^2

Cy 60 mg/kg

| BM blasts ≤20% | → | Day 0 Infuse 100 x 10^6 CD19 CAR T-cells | → | Day 9 Infuse 310 x 10^6 CD19 CAR T-cells |
| BM blasts >20% | → | Day 0 Infuse 10 x 10^6 CD19 CAR T-cells | → | Day 9 Infuse 400 x 10^6 CD19 CAR T-cells |

No G3-5 CRS/ICANS

ALLCAR19 (NCT02935257)

Roddie et al, ASH 2019

Patient Characteristics: Treated (n=16)

Baseline Characteristics	N=16 (%)
Median age, years (range)	35.5 (18-58)
Gender	10M/6F
Chromosomal/Molecular status	
• Ph+ (bcr-abl)	5 (31%)
• MLL	1 (6%)
• Other	6 (38%)
• Normal	3 (19%)
• Failed	1 (6%)
Prior lines of treatment	
• Median (range)	3 (2-6)
• **Prior Inotuzumab/Blinatumomab**	**10 (63%)**
• **Prior allo-HSCT**	**11 (69%)**
• Sibling/Haplo/VUD	2p/1p/8p

Leukemia Burden Prior to Lymphodepletion	N=16 (%)
Status at LD:	
• Primary refractory	4 (25%)
• 1st Relapse	0 (%)
• 2nd Relapse	8 (50%)
• > 2nd relapse	4 (25%)
Morphological disease	
• ≤ 5% blasts	5 (31%)
• 5 - 49% blasts	4 (25%)
• **≥ 50% blasts**	**7 (44%)**
CNS status at registration	
• CNS 1	0 (0%)
• CNS II – III	0 (0%)
Other extranodal sites	3 (81%)

16 patients have been infused (data cut off 25/11/2019)

AUTO1 Expansion and Persistence by qPCR
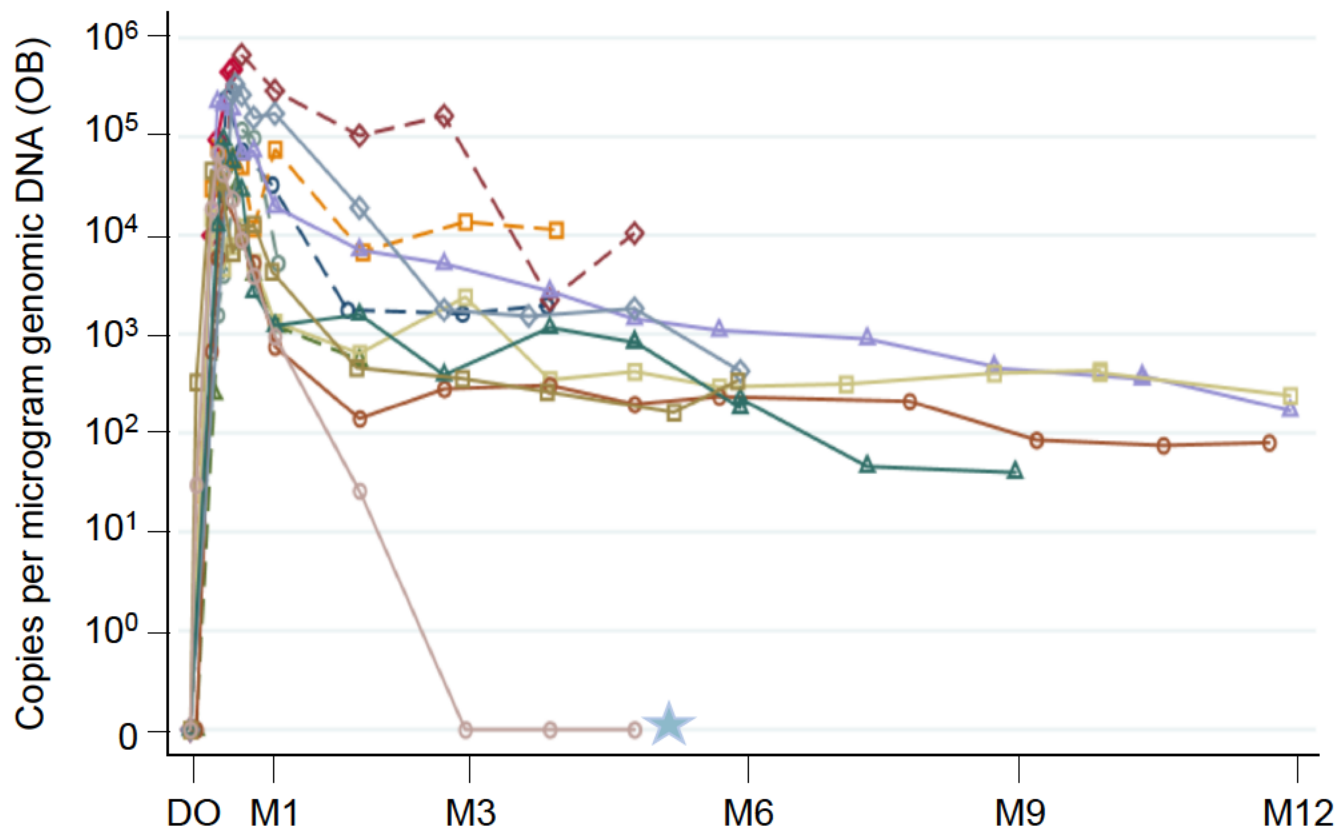
ALLCAR19 qPCR PK all patients (n=13)		*Mueller 2017 (responders)
AUC D0-28 (Geometric Mean) (Copies/µg x days)	**634 719**	**342 732**
Half life (Median Days)	26.3 (Range 21-29)	14.2
Max CAR T level (Geometric Mean) (Copies/µg)	111 239	47 988
T (Cmax) (Days)	11.9 (Range 9-15)	

★ *Developed a HAMA reaction to reject CAR*

> Prolonged CAR T cell persistence was observed, CARs detected in 14 of 16 patients at last follow up

Safety Profile

CRS (Lee Criteria)	Neurotoxicity (CRES#)	≥ Grade 3 Cytopenia	Day -6	At Day 28
• CRS (any) in 7/16 • Grade 2 in 4/16 • ≥ Grade 3 CRS in 0/16	• CRES (any) in 4/16 • Grade 2 in 1/16 • Grade 3 in 3/16	• ≥ Grade 3 Neutropenia	5/16	8/14

 # CAR-T-cell-related encephalopathy syndrome

- **Grade 3 CRS was reported in 0/16 patients:**
  - 7 (44%) patients had ≥ 50% BM blasts prior to LD (CRS 'high risk')
  - Tocilizumab used in 3/16 patients (19%)
  - 0/16 patients required admission to ICU for CRS

- **Grade 3 CRES was reported in 3/16 patients,**
  - All 3 patients had ≥ 50% blasts
  - 2/3 cases resolved to G1 in <24h with steroids
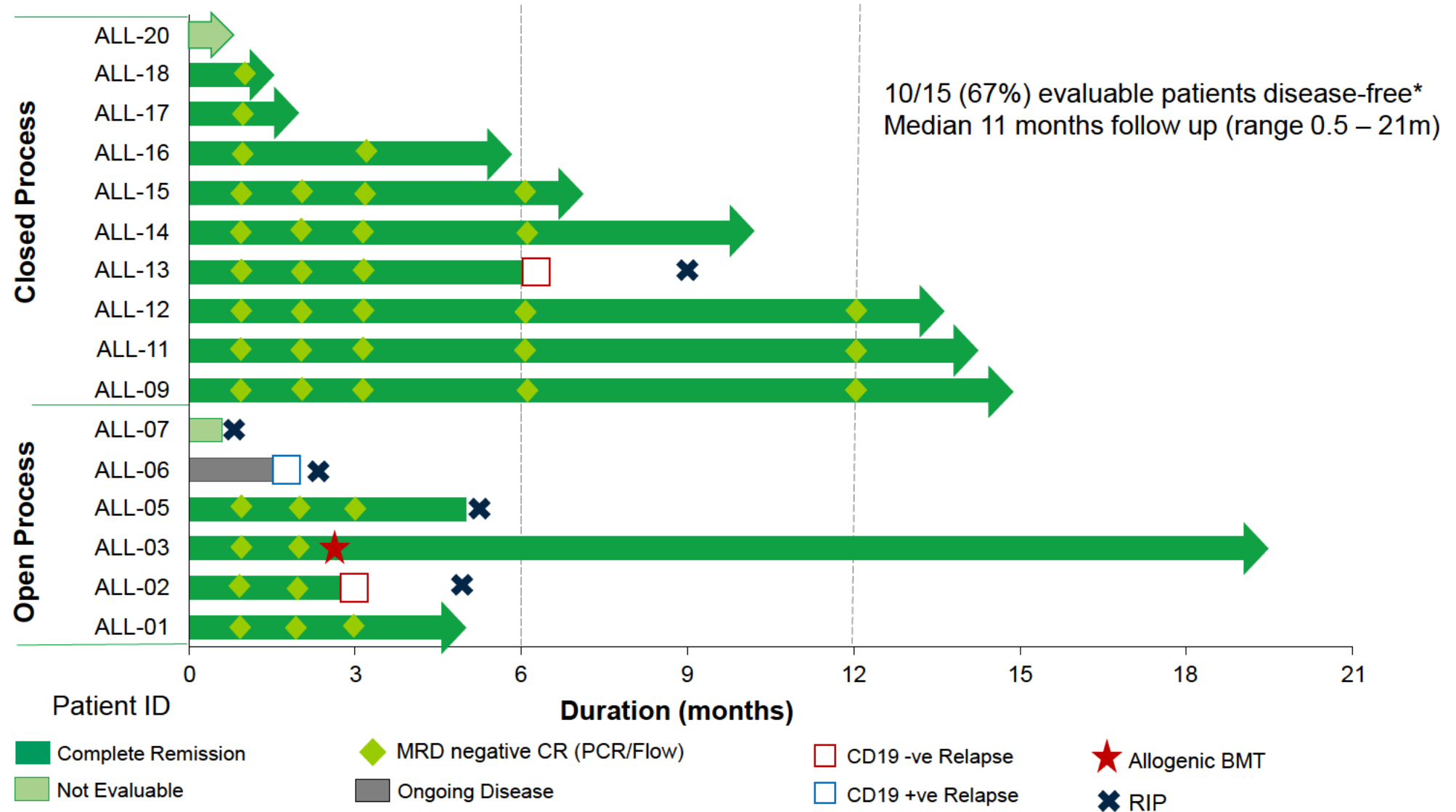 - 1/3 cases resolved to G1 in 72h with steroids *(latter case complicated by hyponatraemia)*

- **≥ Grade 3 neutropenia:**
  - Pre-dated treatment in 5/16 patients
  - At Day 28, 8/14 evaluable patients had ≥ Grade 3 neutropenia with most resolving by Month 2/3

- **5/16 patients died on study:**
  - 2/16 died from progressive B-ALL
  - 1/16 died post-progression from allo transplant-related complications (VOD/sepsis)
  - 2/16 from infection: 1/2 @D17 (2 organisms in blood & toxoplasma in sputum); 1/2 @6M in CR (MDR-pseudomonas in blood)

Roddie et al, ASH 2019

Efficacy & Duration

10/15 (67%) evaluable patients disease-free*
Median 11 months follow up (range 0.5 – 21m)

Legend:
- Complete Remission (dark green)
- Not Evaluable (light green)
- MRD negative CR (PCR/Flow) (green diamond)
- Ongoing Disease (grey)
- CD19 -ve Relapse (red open square)
- CD19 +ve Relapse (blue open square)
- Allogenic BMT (red star)
- RIP (dark blue cross)

MRD < 10^{-4} by PCR or < 5×10^{-4} based on limits of detection of assay
Data cutoff 25-Nov-2019, Evaluable = All patients with at least M1 follow-up or RIP prior to Month 1.

Roddie et al, ASH 2019

AUTO1: Efficacy Overview
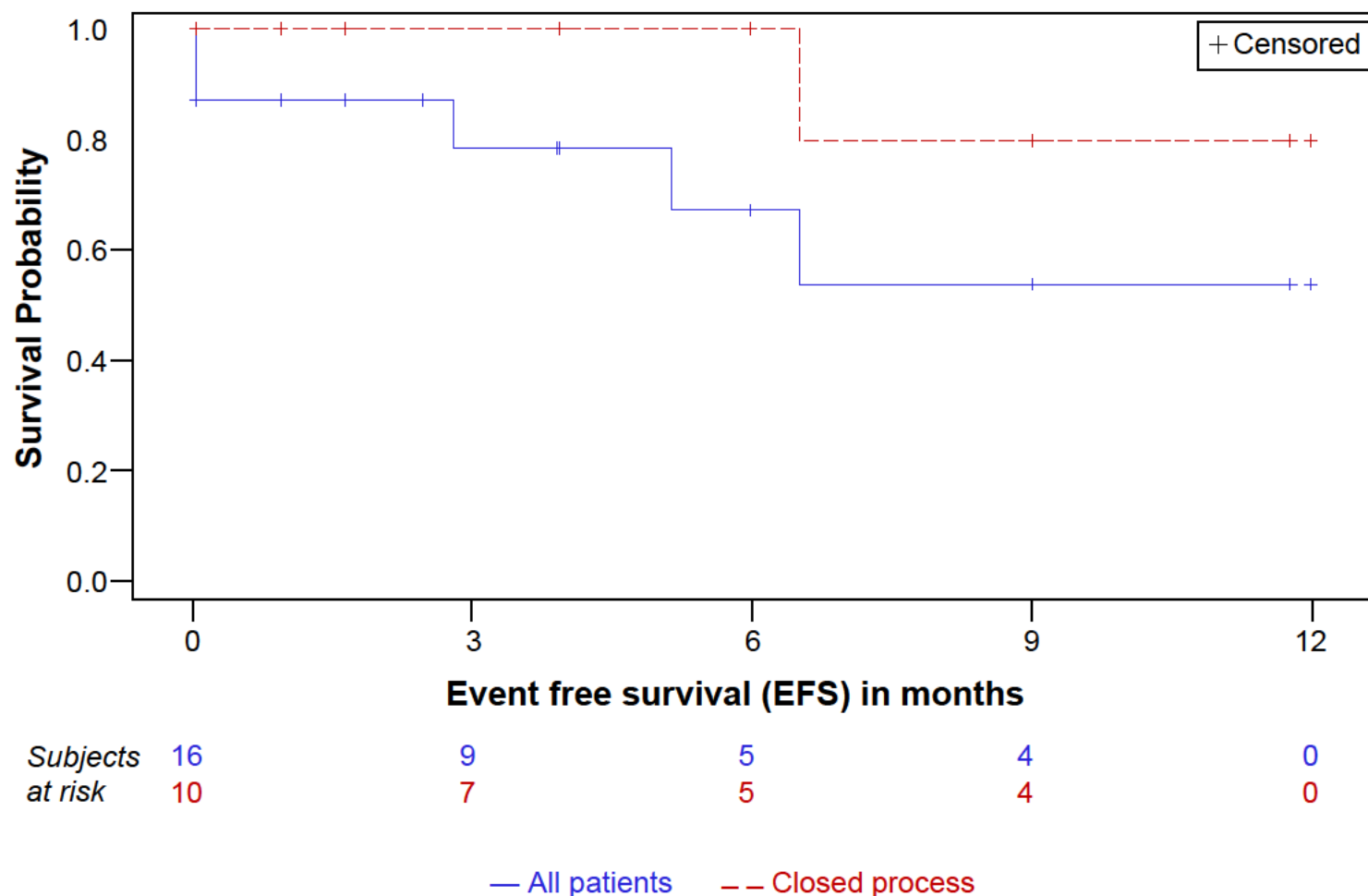
	All patients Est [95% CI]	Closed process Est [95% CI]
N *	15	9
ORR	87%	100%
MRD Neg CR	87%	100%
DOR		
Median	Not reached	Not reached
6 month	72% [24%, 93%]	80% [20%, 97%]
EFS		
Median	Not reached	Not reached
6 months	68% [33%, 87%]	100% [-,-]
OS		
Median	Not reached	Not reached
6 months	66% [33%, 86%]	100% [-,-]

* N = All patients with at least M1 follow-up or RIP prior to Month 1. DOR, EFS and OS data are preliminary considering the small n

Survival Probability vs Event free survival (EFS) in months

| Subjects at risk | 16 / 10 | 9 / 7 | 5 / 5 | 4 / 4 | 0 / 0 |

— All patients   – – Closed process

Roddie et al, ASH 2019

AUTO1 has Potential for Best-in-class Profile for Efficacy and Safety

AUTO1 compared to competitor CAR T cell therapies in Adult ALL

	[1]AUTO1	[2]Kymriah	[3]KTE-X19		[4]UCART19[$]
Patient Numbers	16	35	41		21
CR Rate	87% (100%[#])	69% (90%[@])	68% (84%[##])		88%
EFS	68% at 6 months	5.6 median (2.2m to 19.4m)	TBD		TBD
Allo-Transplant	8%	38%	19%		78%
CAR T Persistence	+++	+++	[++]		+
Tox Management	Normal	Normal	Normal	Intensive	Normal
CRS all Grade	44%	94%	100%	100%	94%
CRS ≥ Grade 3	0%	71% (17% G4/5)	29%	22%	16%
Neurotoxicity all Grade	25%	40%	93%	78%	33%
Neurotox ≥ Grade 3	19%*	6%	38%	11%	0%

\# Patients treated with closed manufacturing process
* Observed in three patients with > 50% tumor burden
@ Patient received 500 mil dose as a split dose 10%, 30%, 60% over 3 days
CR Rate from 19 evaluable pts at Ph2 dose
$ Pooled pALL and adult ALL data from 18 patients

1. Roddie et al., ASH 2019
2. Frey et al., JCO 2019
3. Shah et al., ASCO 2019
4. Benjamin et al., ASH 2018

AUTO1: Conclusions

- **Manufacturing feasibility was confirmed:**

  - 100% of successful harvests resulted in a successfully QP released product

- **Tolerable Safety Profile was observed:**

  - Despite high disease burden and despite heavily pre-treated patient population on study

  - No ≥ Grade 3 CRS was observed

  - 3/16 cases of Grade 3 CRES (rapid resolution with steroids)

- **Prolonged CAR persistence was observed:**

  - 14/16 patients at last follow up

- **Efficacy:**

  - 87% of patients were MRD negative CR at 1 month

  - 67% were disease-free at a median of 11 months *(range 0.5 – 21m, infusion to cut-off)*

  - EFS at 6 months is 68% in all treated patients *(100% in closed process patients)*

Global Phase II AUTO1 study will commence in Q1 2020

CAR T Cell Therapies In DLBCL

Current Status of CAR T Cell Therapies in DLBCL

- Two approved CAR T therapies for r/r adult DLBCL and one close to approval

- CD19 CARs are highly active in r/r DLBCL

- Unmet need remains with CD19 CAR T cell therapy
  - 70-80% ORR, but only 29-37% durable CRR in DLBCL[1,2]
  - The potential causes for relapse include:
    - PD-L1 upregulation[3] which contributes to CAR T exhaustion
    - CD19 antigen loss[4]

- Rate of severe (grade ≥3) cytokine release syndrome (CRS 13-22%) and neurotoxicity (NT 12-28%)[2,4]

- Relapsed/ refractory DLBCL is common, but currently CAR T therapies require intensive inpatient management

1. Locke F et al Lancet Oncol 2019
2. Schuster S et al NEJM 2019
3. Neelapu S et al ASCO 2018
4. Neelapu S et al NEJM 2017

Desired Characteristics of a CAR T Therapy for DLBCL

- High sustained complete response rate
  - Preventing target negative relapse
  - Preventing checkpoint mediated resistance / exhaustion

- Safety profile suitable for out patient therapy
  - Low severe CRS without intensive management
  - Low neurotoxicity rates

- Manufacturing at scale
  - Vector production at scale
  - Cost effective production process

AUTO3: CD19 and CD22 Targeting Bicistronic CAR

Gamma retroviral-based vector with RD114 Pseudotype
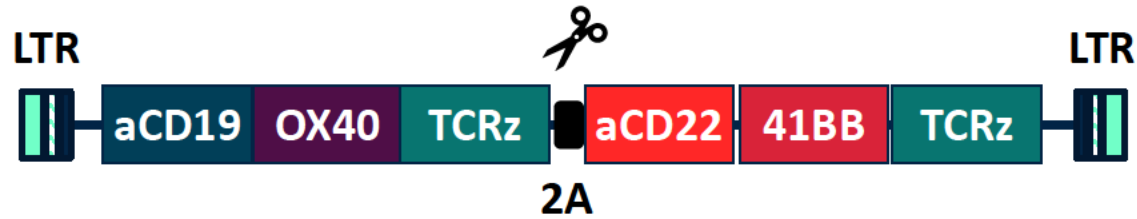
- Dual antigen targeting

- Two independent CARs delivered in single retroviral vector

- Humanized binders

- CD22 CAR with novel pentameric spacer

- OX40/41BB costimulatory domains designed to improve persistence

- Independently target CD19 or CD22
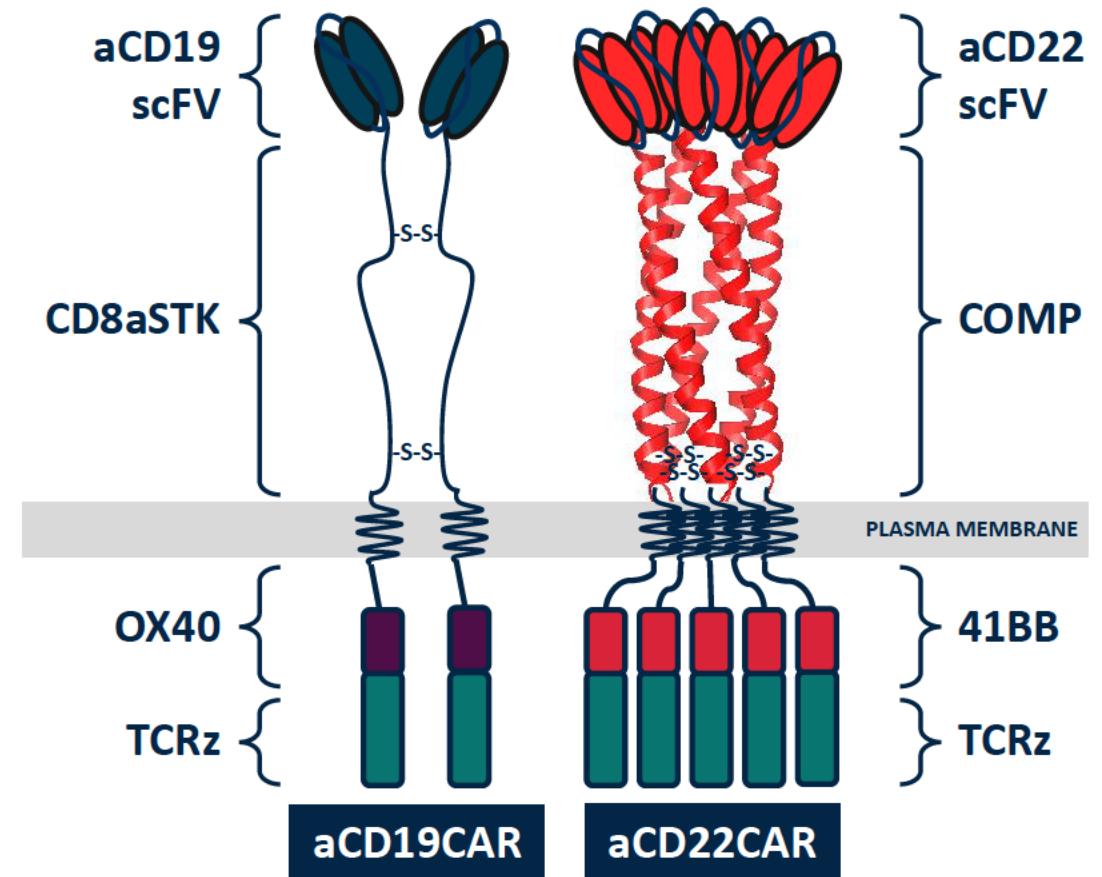
Alexander Study Design

AUTO3-DB1, single-arm, open-label, multi-center, Phase 1/2 Study in r/r DLBCL

Phase I Dose Escalation
(Safety Cohort)

Phase II
(Efficacy Cohort)

900

900

450

450

150

50 50

Cohort 1

Cohort 2

Dose in x10^6 CD19/CD22 CAR T Cells

☐ Preconditioning Flu/Cy, No Pembro

■ Preconditioning Flu/Cy + Pembro day +14 x 3 doses

■ Preconditioning Flu/ Cy + Pembro day -1 x 1 doses

Cohort 1: DLBCL NOS, high grade B cell lymphoma, tDLBCL from FL, > 2prior lines

Cohort 2: Primary mediastinal, tDLBCL from other iNHL, > 2prior lines

- Phase 1 – Rolling 6 design

- Phase 2 – Simon's 2-Stage optimal design

Economical & Scalable Product Delivery Platform

Semi-automated and parallel processing

Day 0
- Load apheresis
- Activate with transact

- Harvest CAR T-cells
- Cryopreserve cells

- Transduce with single vector
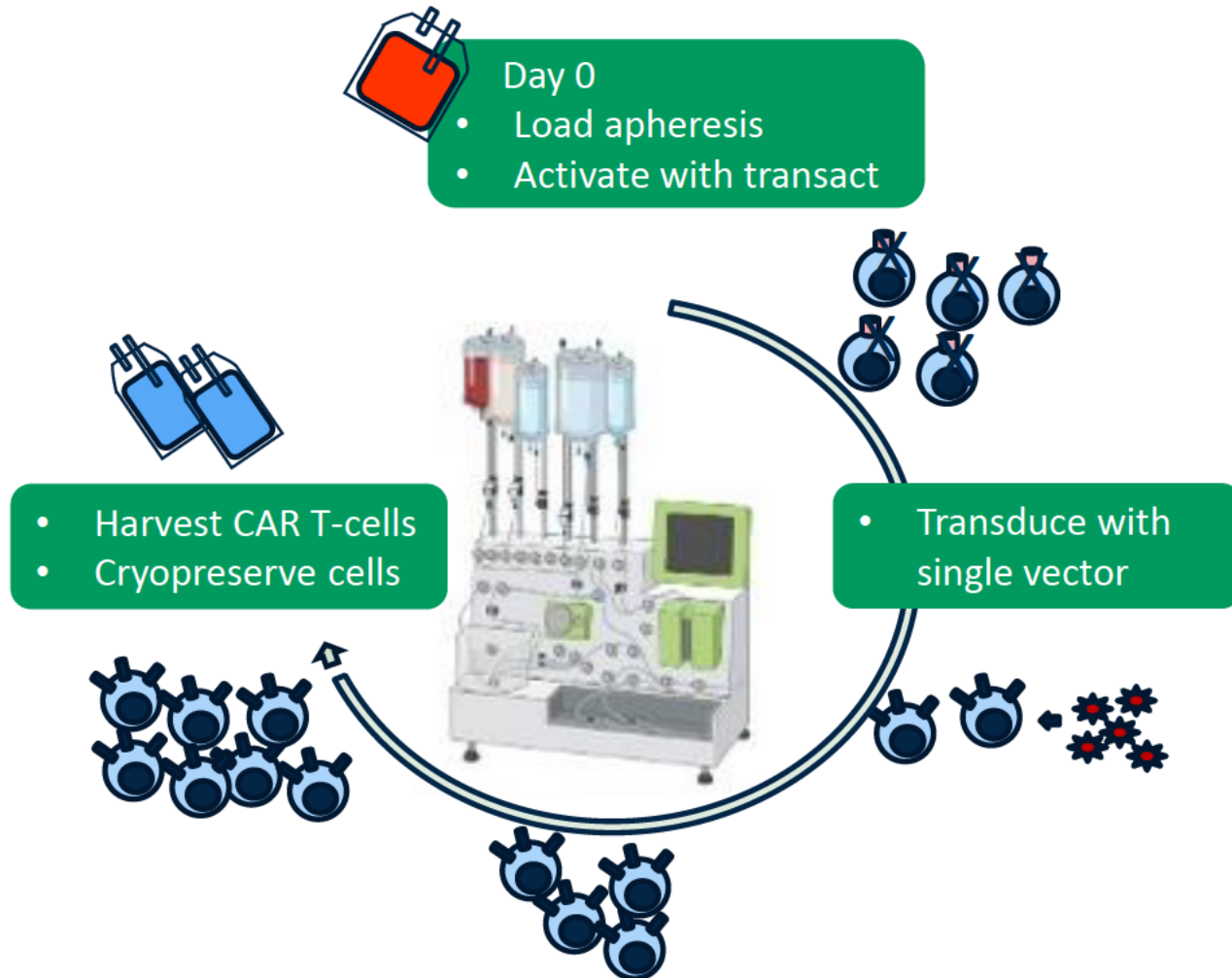
Clinical supply & commercial launch

- Multiple samples to be processed within the same environment
- CGT Catapult (UK)
- Global clinical supply since Q3 2019

Planned US commercial supply

- Planned capacity of 5,000 patients p.a.

Adverse Events of Special Interest

	50 x10^6 AUTO3 no pem (n=4)	50 x10^6 AUTO3 D14 pem (n=3)	150 x10^6 AUTO3 D14 pem (n=4)	450 x10^6 AUTO3 D14 pem (n=4)	450 x10^6 AUTO3 D -1 pem (n=3)	Total (n=18)
All grades CRS*	1	0	2	2	2	7 (38.9%)
≥ G3 CRS	0	0$^&$	0	0	0	0
All grades NT*	1	0	0	0	0	1 (5.6%)
≥ G3 NT	1	0	0	0	0	1 (5.6%)

*CRS and NT grading changed to ASTCT after amendment 8 in September 2019; Only patients with 28 day follow-up are included

- With primary infusion:
  - 2 patients received tociluzumab for CRS
  - 1 patient received steroids for NT

- No prophylactic measures of any kind

- Predominant SAEs were related to hematological event, 1 patient experienced a Grade 4 lung infection caused by para influenza virus-induced pneumonia

&1 patient who had no CRS with primary infusion, developed Grade 3 CRS (severe hypoxia) with re-treatment 1 year later, which happened in a setting of no CAR T expansion and significant disease burden in lung that had been treated with radiation

21 January 2020 data cut-off

Serum Cytokine Profile

IL-6

IL-15

IL-10

TNF-α
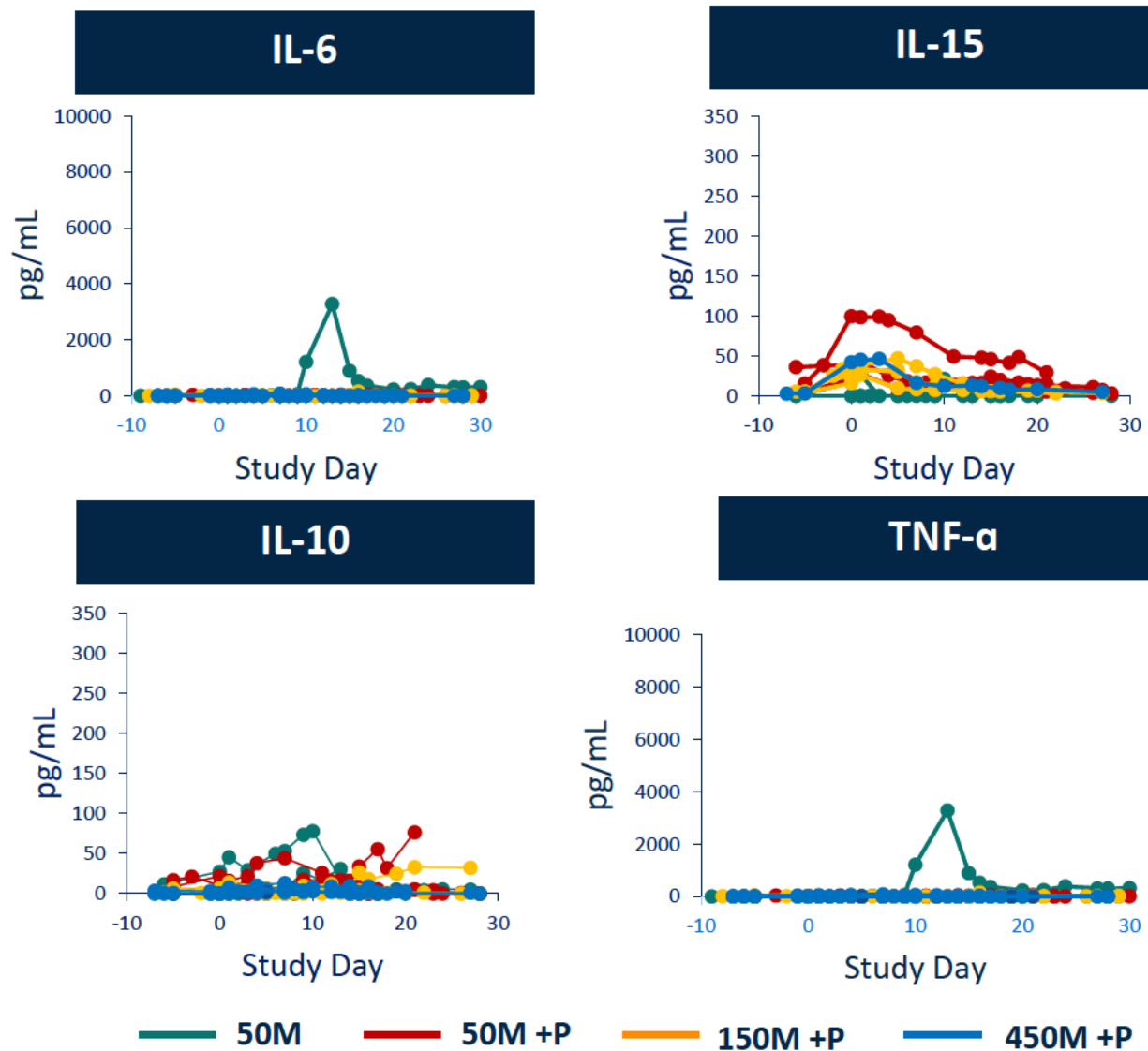
CAR T Product	CRS Grade 0-2 IL-6 level pg/ml	CRS Grade ≥3 IL-6 level pg/ml
AUTO3	7.49 (1.64, 3275)	NA
Yescarta	49.4 (3.5, 12109.7)	713.9 (152.5- 50705)

Low levels of cytokines are consistent with the observed low-grade CRS

Legend: 50M | 50M +P | 150M +P | 450M +P

* Data for all patients with 4 weeks efficacy follow-up

Preliminary Efficacy* Indication Of Dose Response

	50 x 10^6 No Pem (n=4)	50 x 10^6 D14 Pem (n=3)	150 x 10^6 D14 Pem (n=4)	450 x 10^6 D14 Pem (n=4)	450 x 10^6 D-1 Pem (n=3)
CR	1	1	2	2	2
PR	1	1	0	1	NA
NE	0	1	0	0	0
CRR	**25%**	**33%**	**50%**	**50%**	**66%**

- 450 million: ORR 5/7 (71%) and CR 4/7 (57%)
- All doses: ORR 11/18 (61%) and CRR 8/18 (44%)

Pre-CAR T-cells
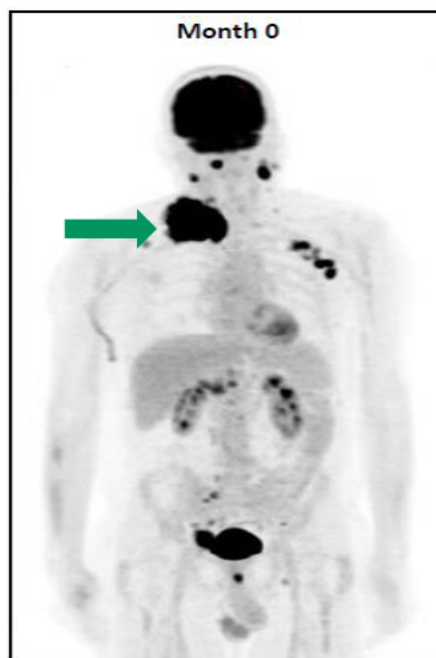
Month 0

Post-CAR T-cells
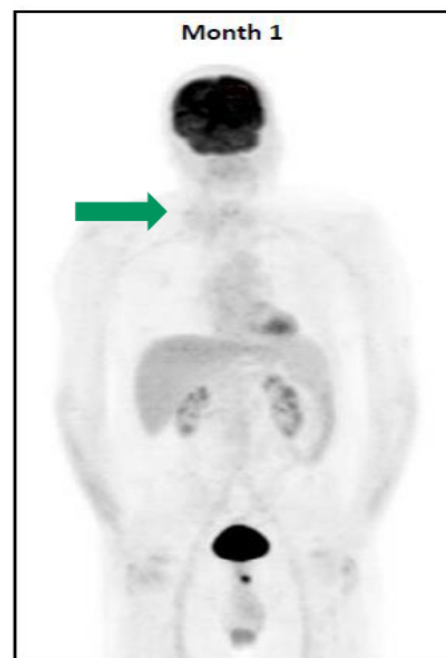
Month 1

Dose: 50 x 10^6
DLBCL: ABC, Primary refractory & refractory to RCHOP/RICE/RESHAP
No CRS or NT
CR duration 18 months+

* Per Lugano criteria; CR: complete response; PR: partial response; NE: not evaluable; PD: progressive disease
Data for patients with 4 weeks efficacy follow-up

21 January 2020 data cut-off

Duration of Complete Responses at All Dose Levels

Median Follow-up:
6 months (1 – 18 month)
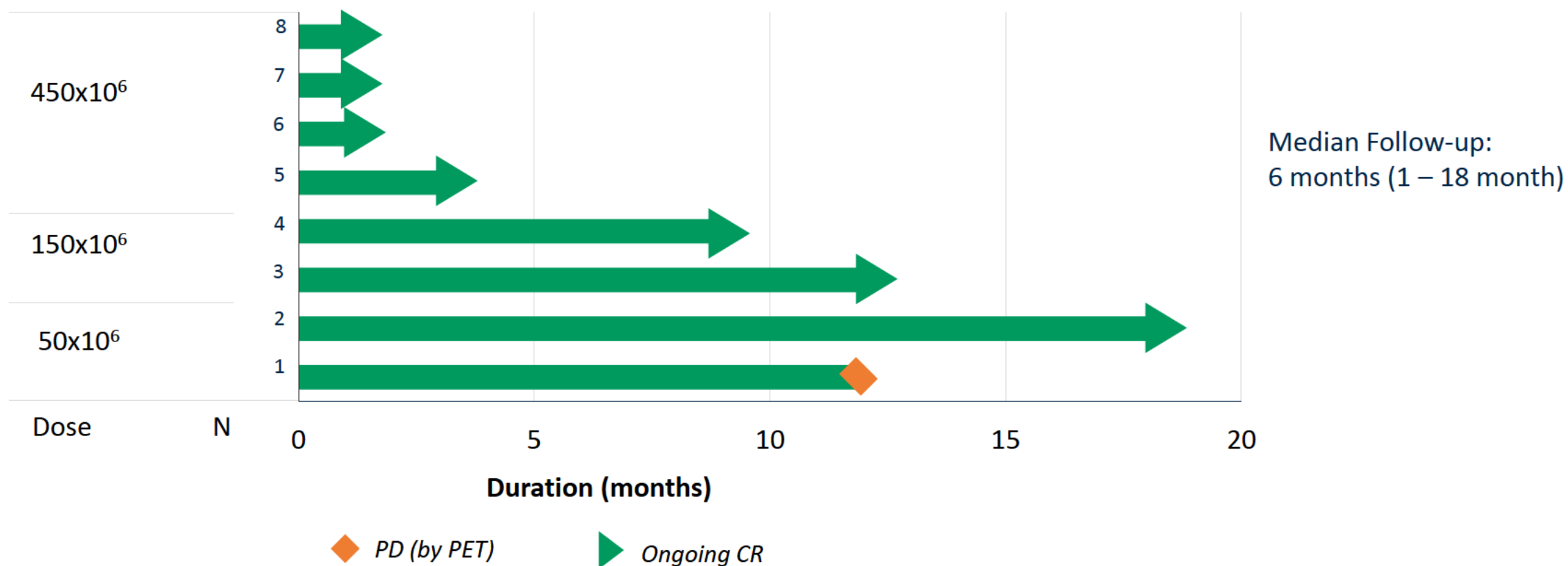
18 patients treated, 7 out of 8 (87%) CRs ongoing, 3 PRs not durable
7 of 7 (100%) CRs* are ongoing in AUTO3+ Pembro cohorts at a median f/u of 3 months (1-18m)

21 January 2020 data cut-off

AUTO3 and Approved CAR T Therapies In DLBCL

	[1]AUTO3 + Pembro ≥ 150 x10⁶ Dose	[2]YESCARTA	[3]KYMRIAH	[4]JCAR017
Best CR	55%*	54%	40%	53%
CRS ≥ grade 3	0%	11% #	23%	2% #
Neurotox any grade	0%	64%	21%	30%
Neurotox ≥ Grade 3	0%	28%	12%	10%

* All CRs ongoing at a median f/u of 2 months (1-12 month)

\# CRS rate achieved with intensive management

1. Data cut off 7 January 2020
2. Nellapu et al, 2017
3. Schuster et al., 2019
4. Abramson et al., 2019 (ASH)

Summary

Phase I Cohorts, ALEXANDER Study

- AUTO3 product was successfully manufactured for all patients

- Manageable safety profile, 0% ≥ Grade 3 CRS and 1/14 (7%) Grade 3 neurotoxicity with primary infusion
  - No neurotoxicity of any grade in patients treated with AUTO3+ Pembro

- Complete responses achieved without severe CRS, neurotoxicity or ICU care

- 7/8 CRs ongoing with a median follow up of 6 months (1-18 months)

- Pembrolizumab on D-1 x single dose is being evaluated further

AUTO3 Next Generation - Enhancing AUTO3

- Improve function in immunosuppressive environment
  - dSHP2 - Block PD1-PDL1 axis to restore T cell function
  - dnTBR2 - prevent activation of immunosuppressive SMAD signaling pathway
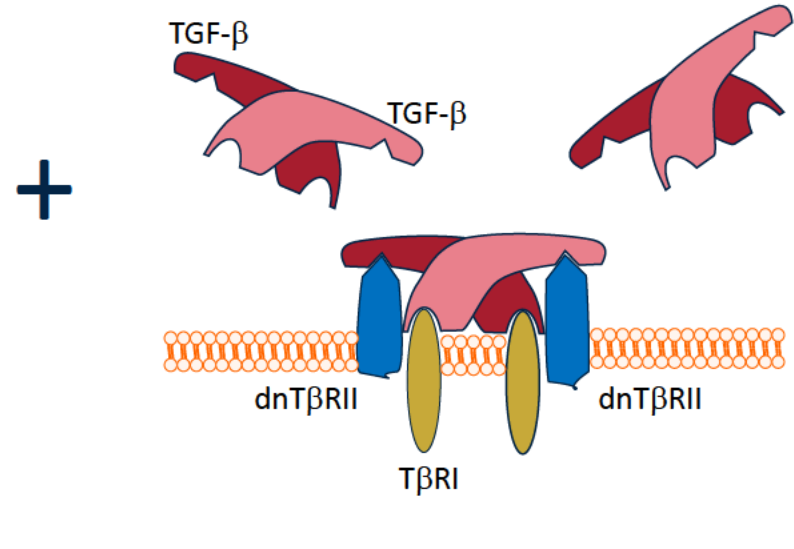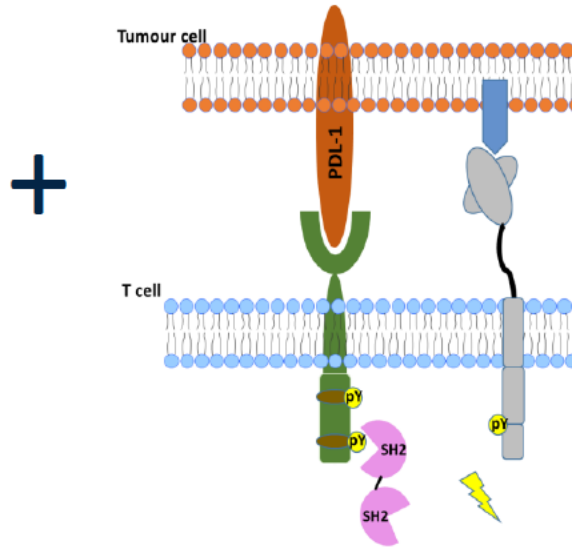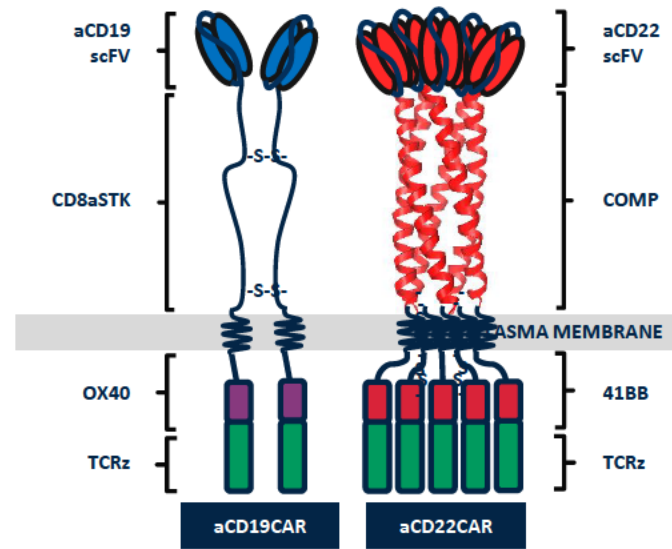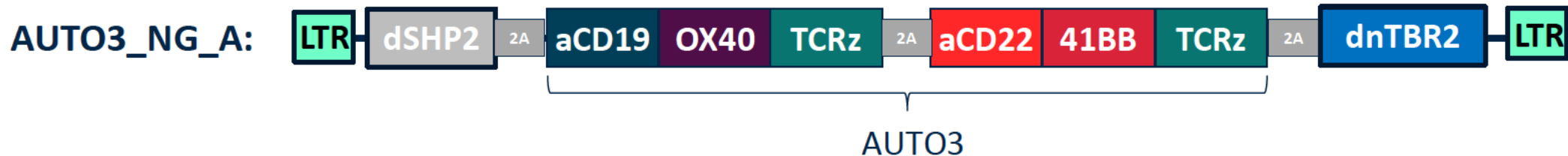
Methods for Blocking Immune Checkpoint Signaling
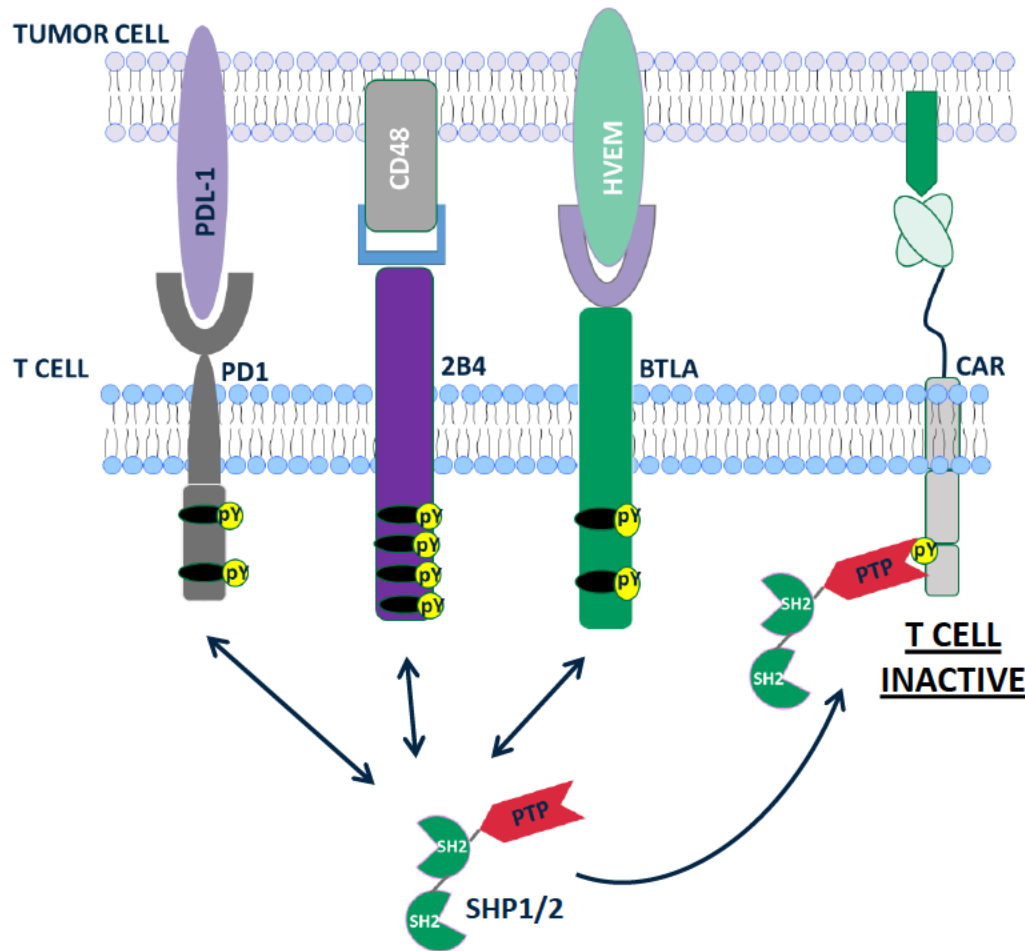
- Ligation of inhibitory receptors on the surface of tumor cells impair a T cell's ability to kill the tumor

- Many immune checkpoints act through a common T cell signaling pathway:
  - PD1 (PDL1 ligand)
  - 2B4(CD48 ligand)
  - BTLA (HVEM ligand)
  - TIGIT (CD155 ligand)
  - CTLA4 (CD80/CD86 ligands)

- Ligation of these inhibitory receptors leads to the recruitment and activation of SHP1/2 phosphatases

- Active SHP1/2 dephosphorylates the CD3z domain of the CAR or TCR and inactivates the T cell

Methods for Blocking Immune Checkpoint Signaling
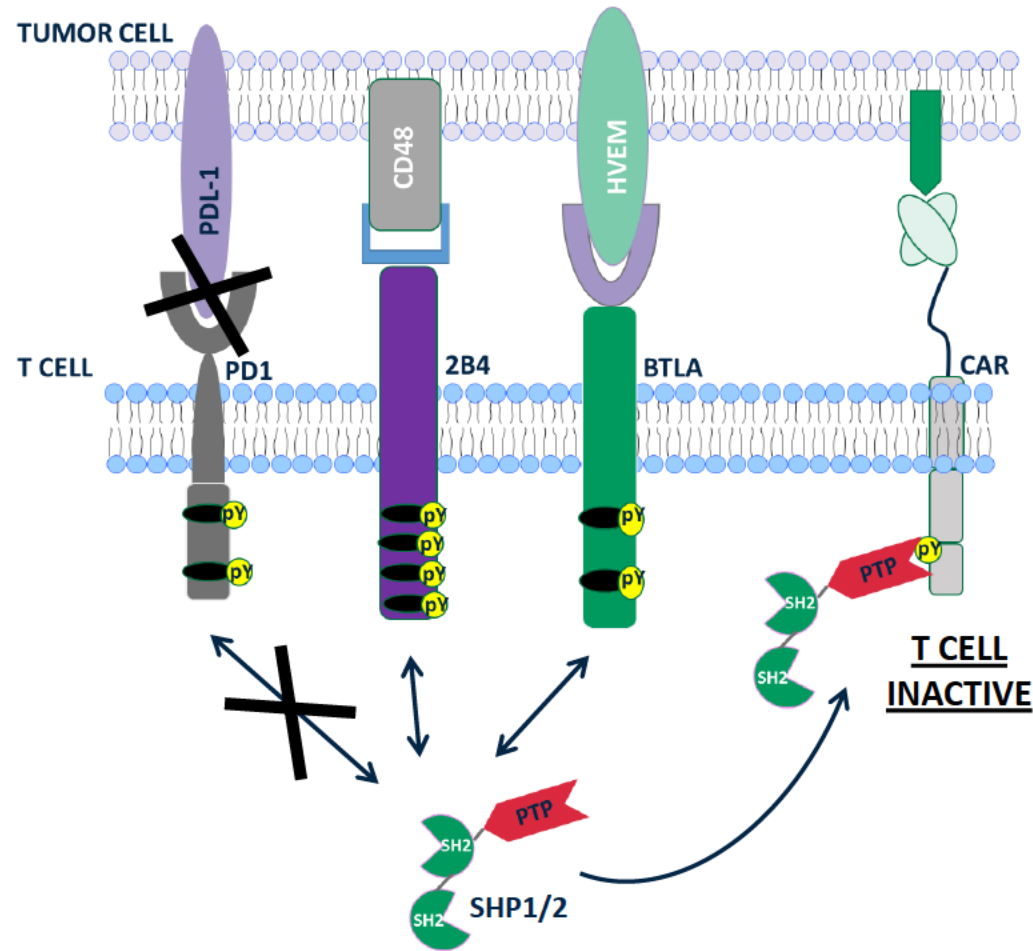
- Other approaches may only tackle one inhibitory receptor at a time using antibodies or gene editing

Methods for Blocking Immune Checkpoint Signaling
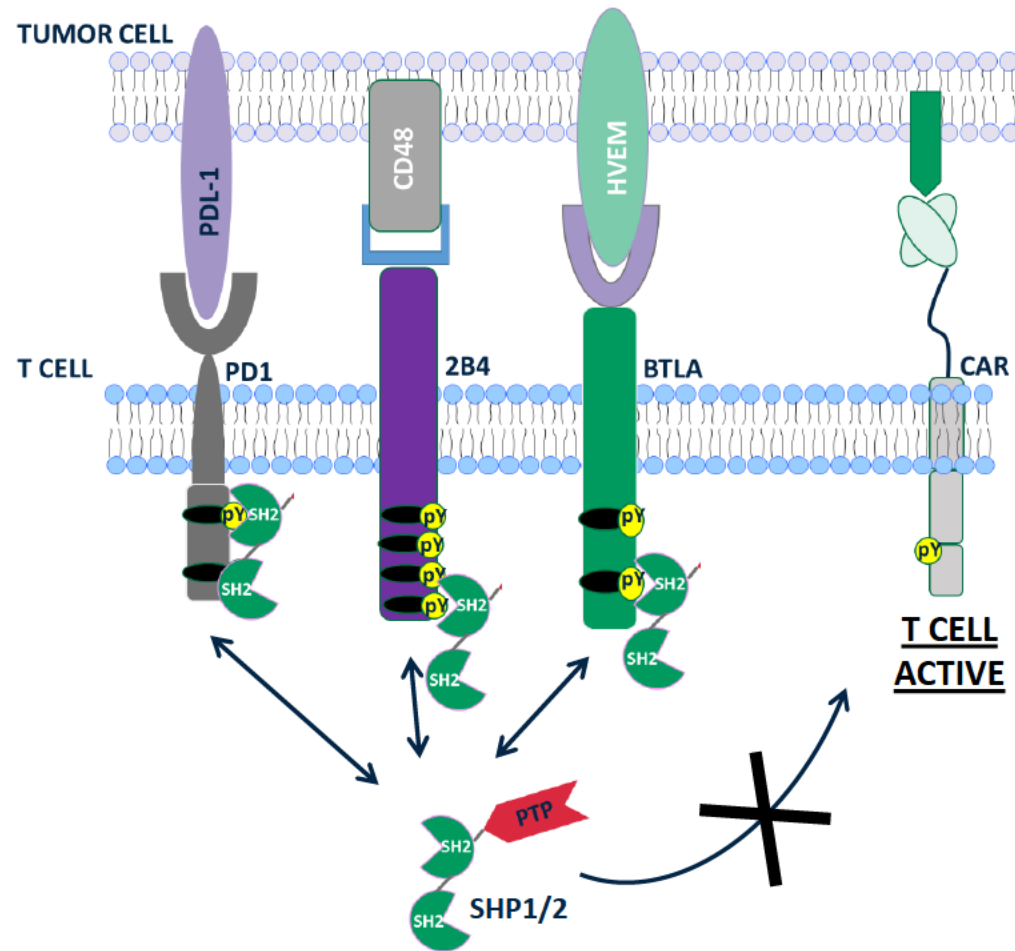
- Other approaches may only tackle one inhibitory receptor at a time using antibodies or gene editing

- We have designed a truncated SHP2 module that lacks the phosphatase domain and is unable to inactivate the T cell

- Unlike the antibody or gene editing approaches, this acts at the pinch point and is designed to simultaneously disarm multiple inhibitory receptors

Restoration of AUTO3 Activity by Using dSHP2

- AUTO3 with or without PD1 is inhibited by the PDL1+ target cells

- AUTO3_NG_A + PD1 persists to kill the PDL1+ targets effectively

Day 6
E:T 1:16

SupT1 NT
SupT1 CD19$^+$
SupT1 CD19$^+$ PDL1$^+$
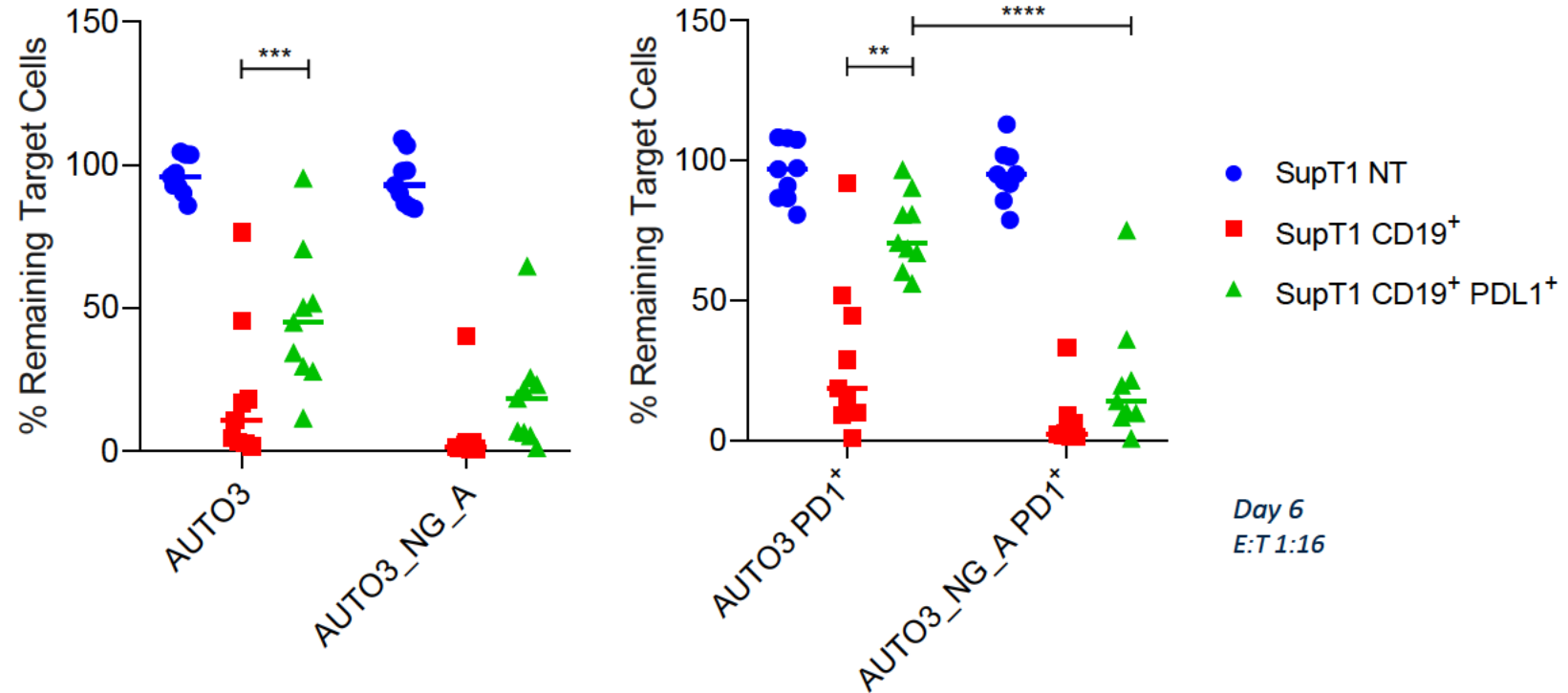
Our data demonstrates that the truncated SHP2 module also interacts with other inhibitory receptors including 2B4(CD48 ligand), BTLA (HVEM ligand), TIGIT (CD155 ligand) and CTLA4 (CD80/CD86 ligands)

Blocking TGFβ Signaling Using a Truncated TGFβ Receptor (dnTBR2)

TGF-β

TGF-β

TGF-β

TGF-β

Conventional Receptor

Truncated Receptor

Signaling
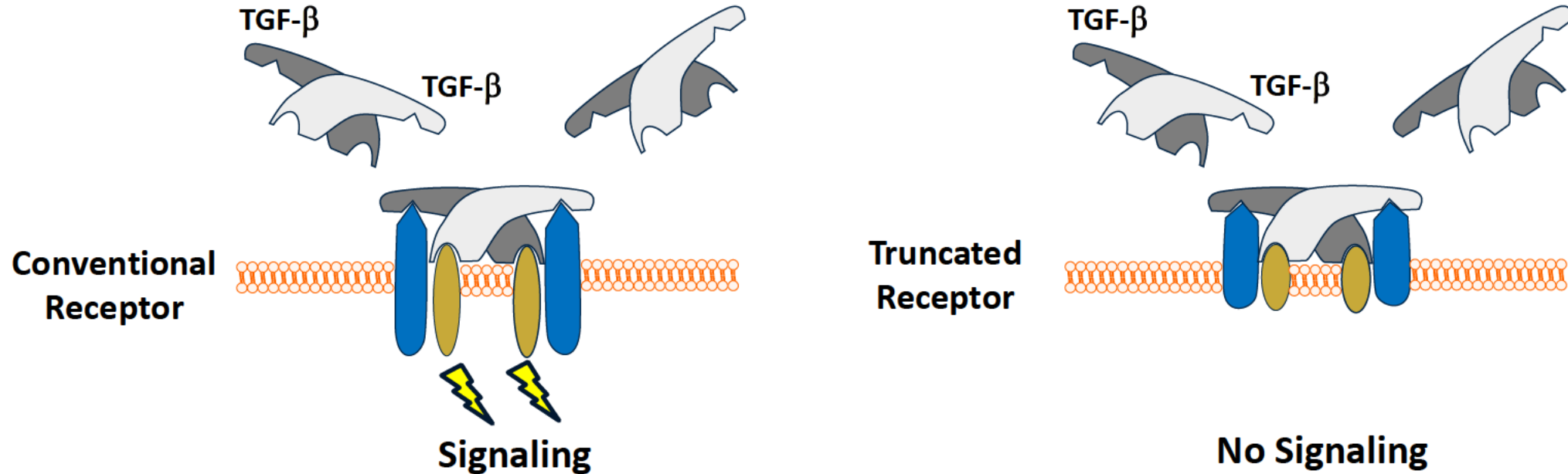
No Signaling

- TGF-β is a key negative regulator of immune cells with the potential to impair a T cell's ability to kill the tumor

- Our dnTBR2 module is designed to prevent our therapy from receiving the negative signaling

Restoration of AUTO3 Activity Using dnTBR2
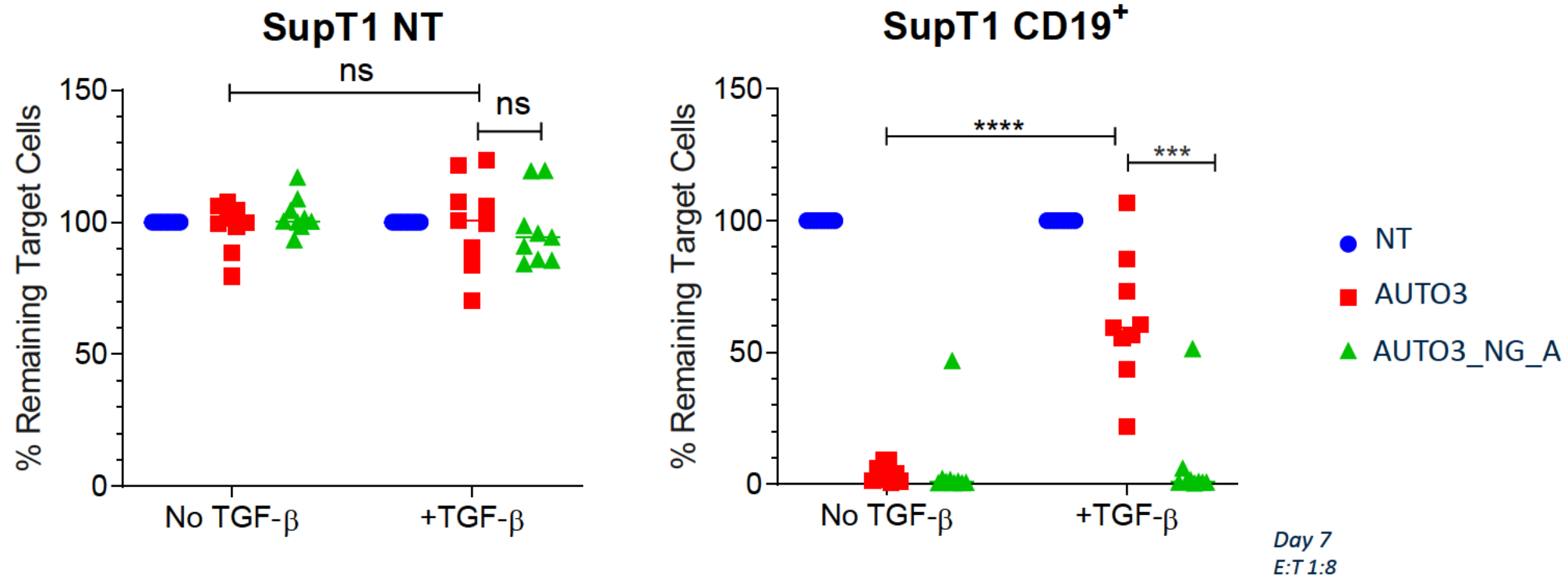
- AUTO3_NG_A is shielded from the immunosuppressive effects of soluble TGF-β

AUTO3 Next Generation: Additional Variants
Can we further improve the cytotoxic and proliferative capacity of AUTO3
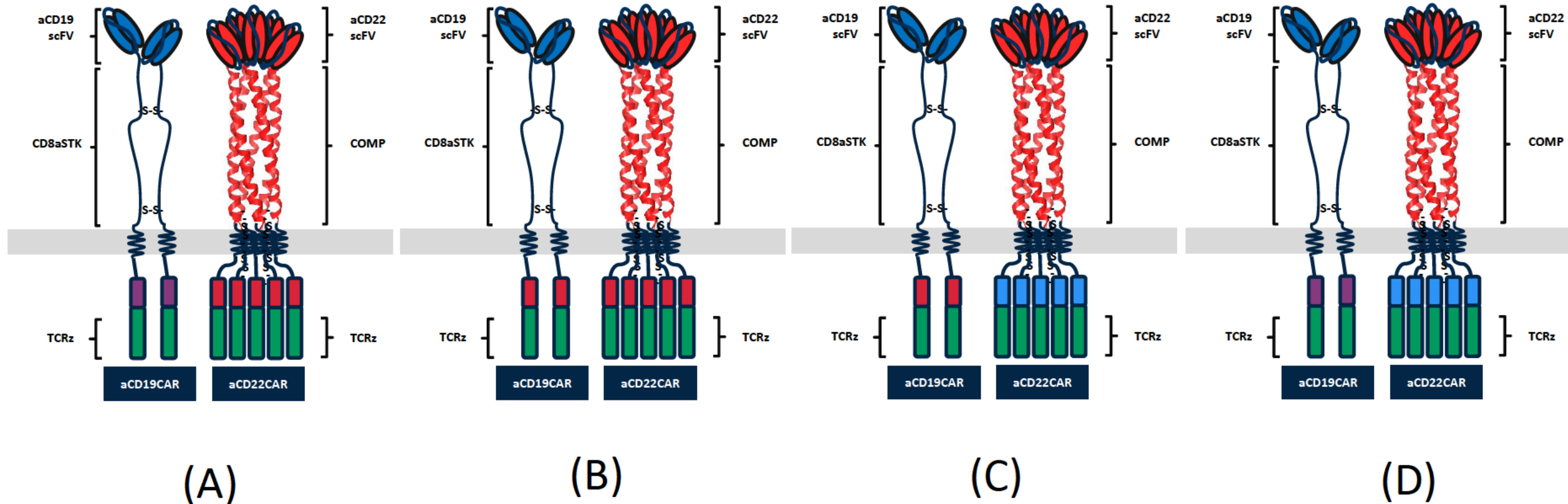
AUTO3NG Cytolytic Capacity

Differential ability to kill CD22+ targets

Day 3
E:T 1:1
n = 5, Stats– paired student t test
* P ≤ 0.05, ** P ≤ 0.01

Legend:
- SupT1 CD19$^+$ (blue circle)
- SupT1 CD22$^+$ (red square)
- SupT1 CD19$^+$CD22$^+$ (green triangle)

- Substitution of a CD28 on the anti-CD22 CAR enhances cytolytic capacity

AUTO3NG Proliferation

Enhanced Proliferation

AUTO3NG in vivo Efficacy

Tumor clearance with sub-optimal T cell dose
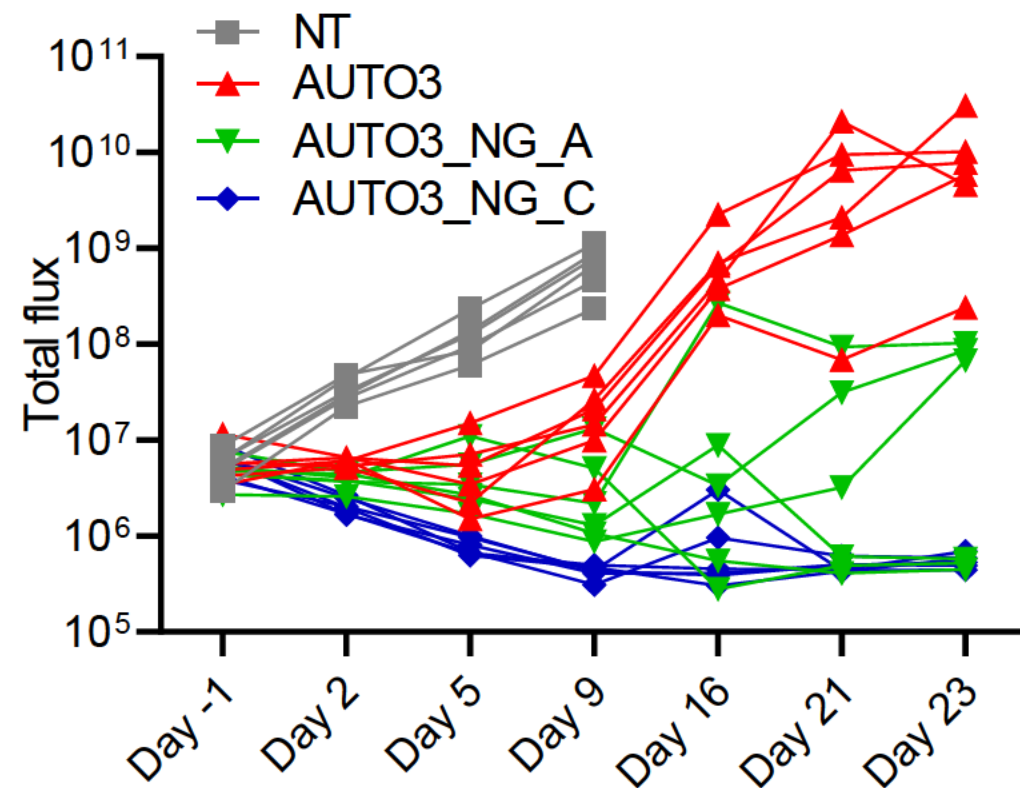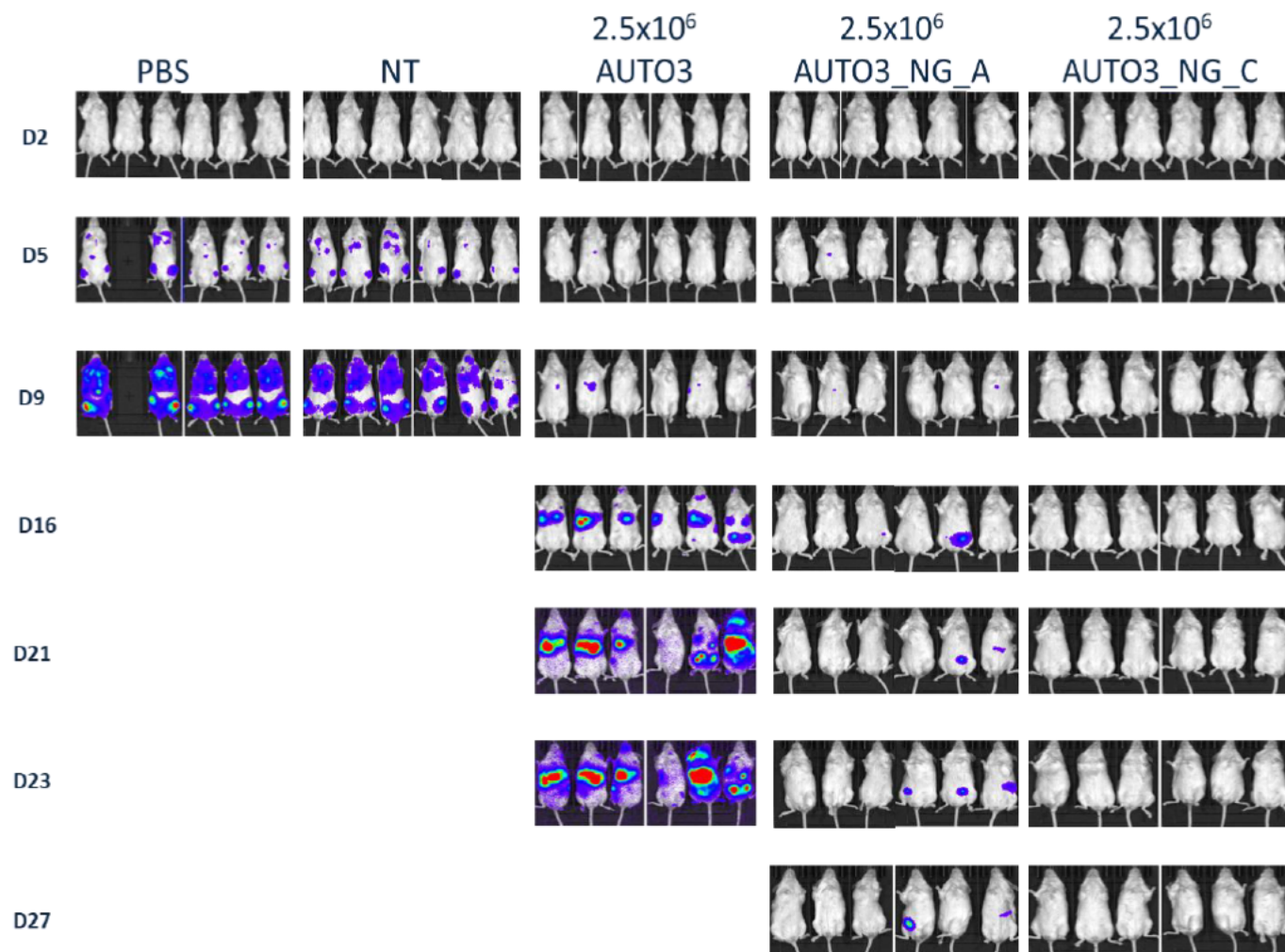
NB: PBS and NT cohorts terminated at day 9

AUTO3 Next Generation

Continuous improvement through module integration and CAR optimisation

dSH2 and dTGFβ may improve CAR efficacy

Efficacy

Safety

Potential to be safer due to targeted checkpoint blockade in only the infused CAR T cells

(Patient and Hospital)- Single dose similar to other products

Convenience

Cost of treatment

Designed to avoid cost of combinatorial therapy
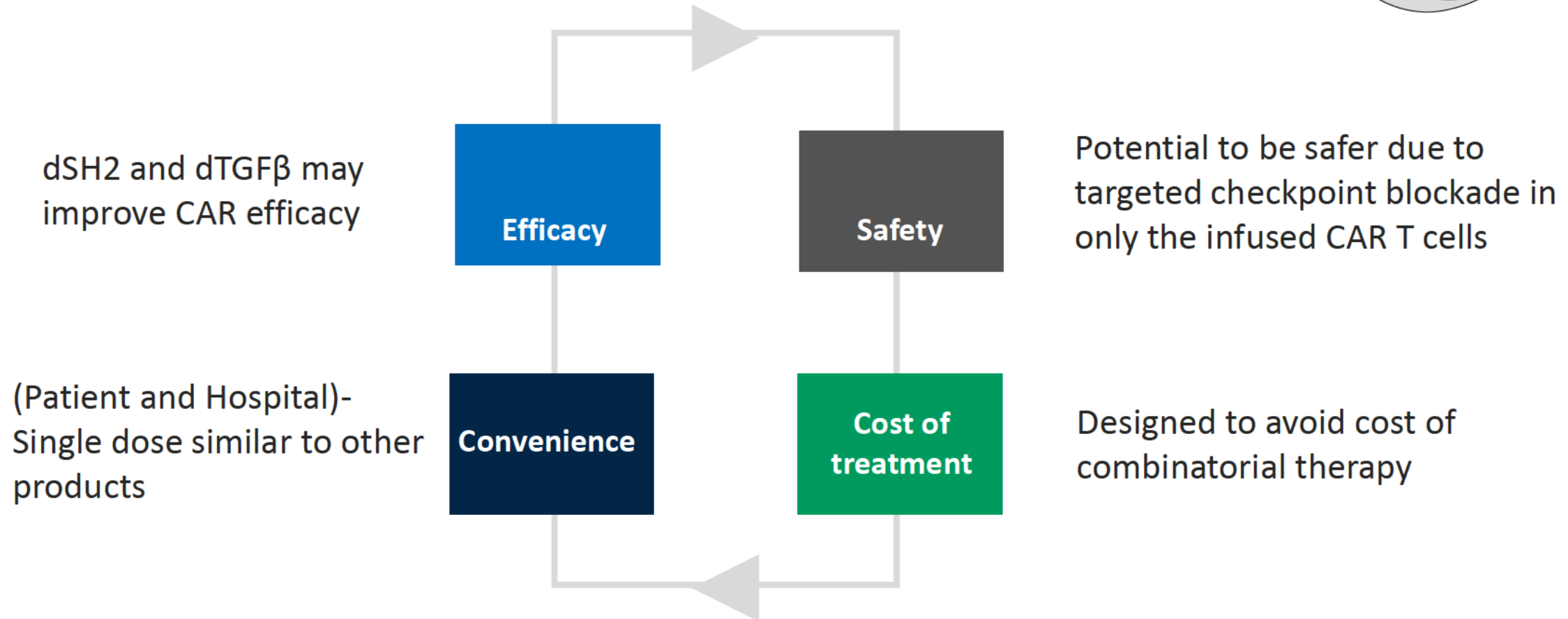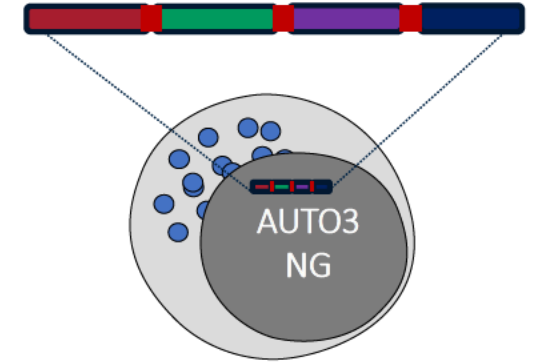
CAR T cell therapy for T Cell Lymphoma

T Cell Lymphoma

Disease is aggressive with very poor prognosis

DIAGNOSIS	5YR RELATIVE SURVIVAL (%)
B-CELL	
DIFFUSE LARGE B-CELL LYMPHOMA	54.8
FOLLICULAR LYMPHOMA	86.3
HODGKIN'S LYMPHOMA	84.9
T-CELL (NON-CUTANEOUS)	
PERIPHERAL COMMON, UNSPECIFIED	19.7
ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA	26.2
ANAPLASTIC LARGE CELL	
- ALK-	19.7
- ALK+	75.2
ENTEROPATHY ASSOCIATED LYMPHOMA	28.0

What is a Good CAR Target in PTCL?

		PTCL		AILD	
Antigen	No.	Positive (%)		No.	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
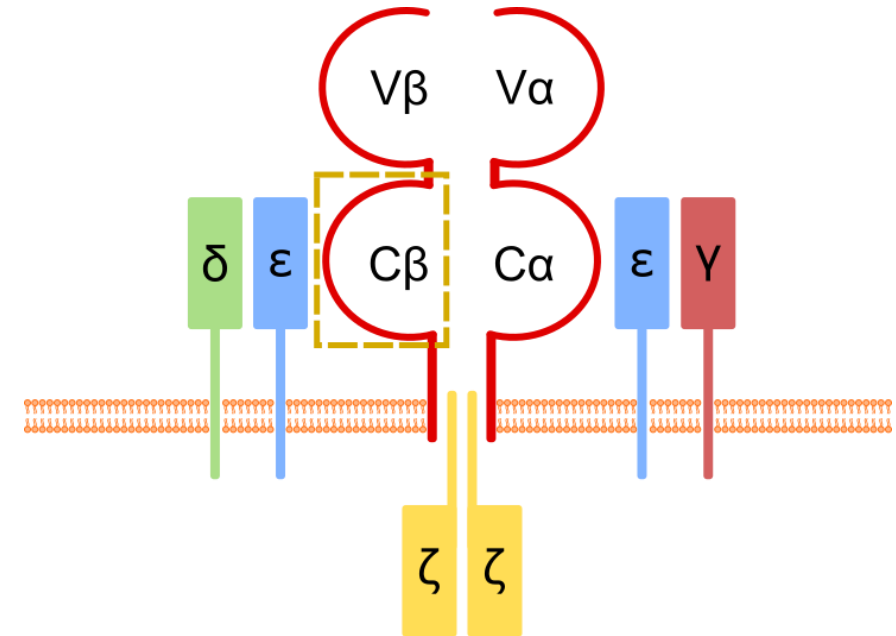
Went *et al. J Clin Oncol* (2006); **24**:2472-2479

The T cell Receptor Beta Constant Region is Duplicated
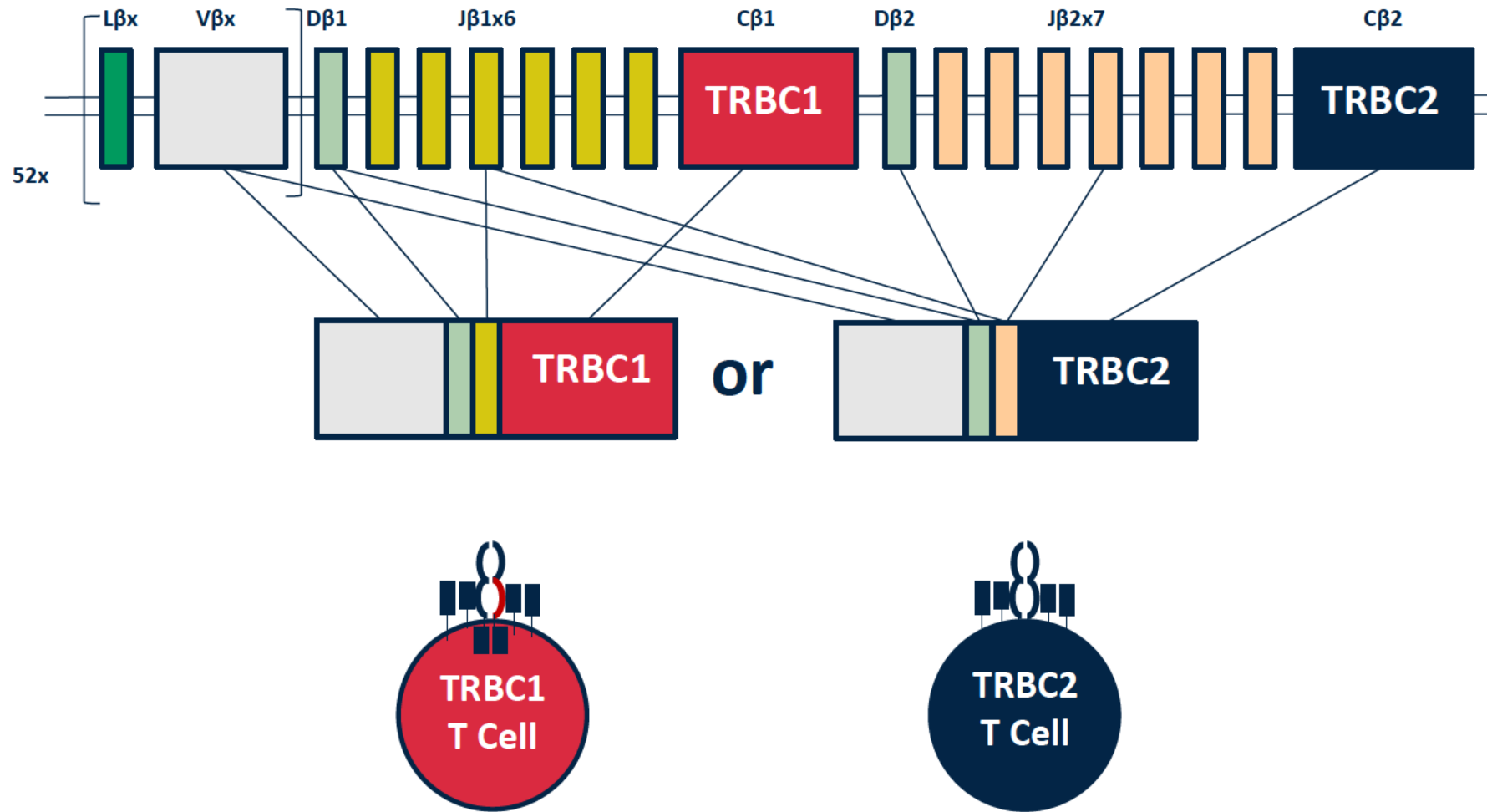
Targeting Strategy for T cell Lymphomas
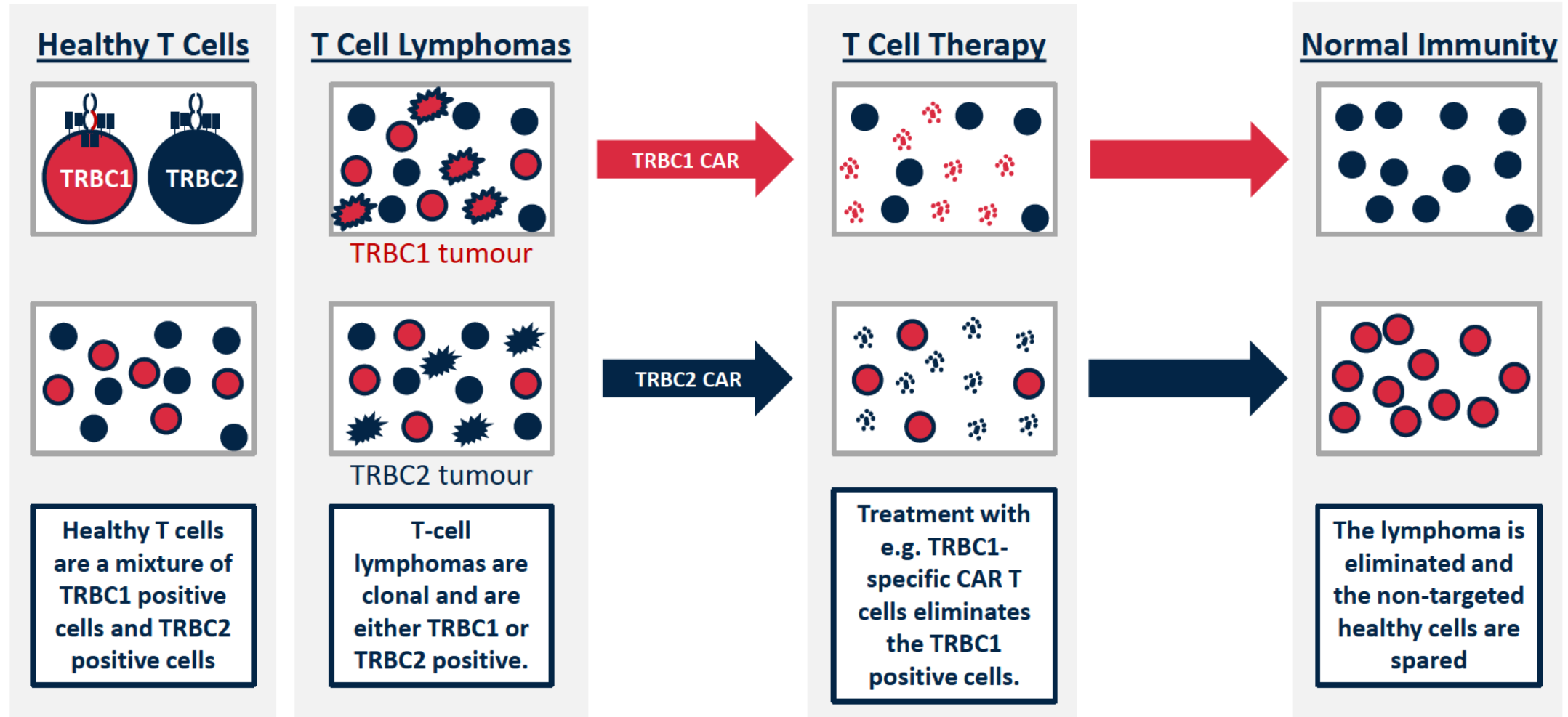
Challenges Targeting TCRβ

**Differences between TRBC1 and TRBC2 are small**

NK-KN 4/5   F-Y 36

```
TRBC1    1   EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGK
TRBC2    1   EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGK

TRBC1   51   EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQF
TRBC2   51   EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQF

TRBC1  101   YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSAT
TRBC2  101   YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSAT
```

V-E 135

TRBC1 Specific Antibody
Identification of a TRBC1 specific antibody
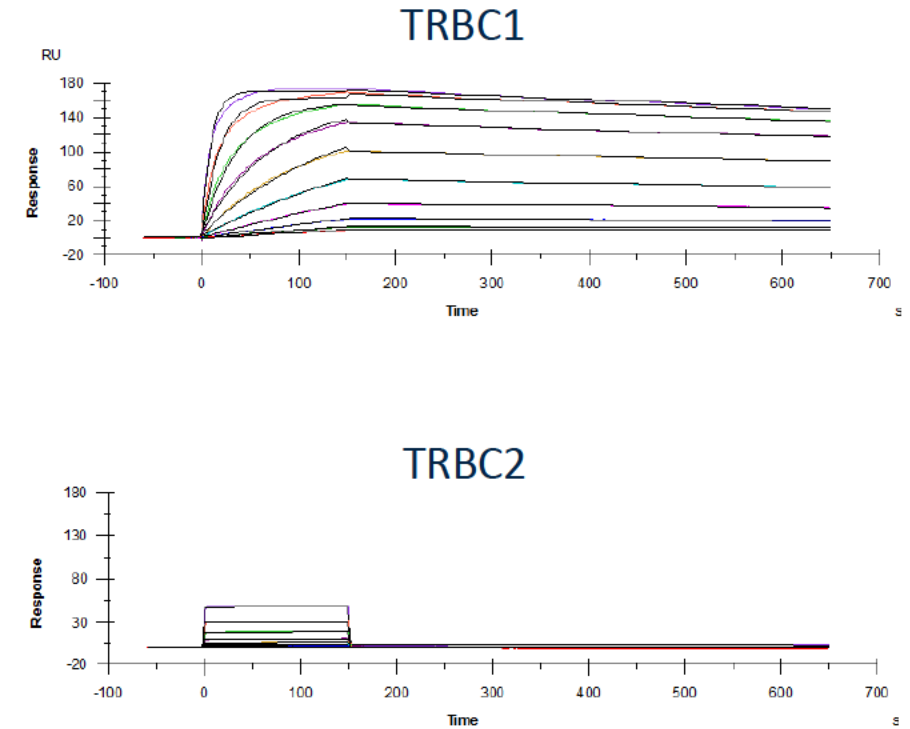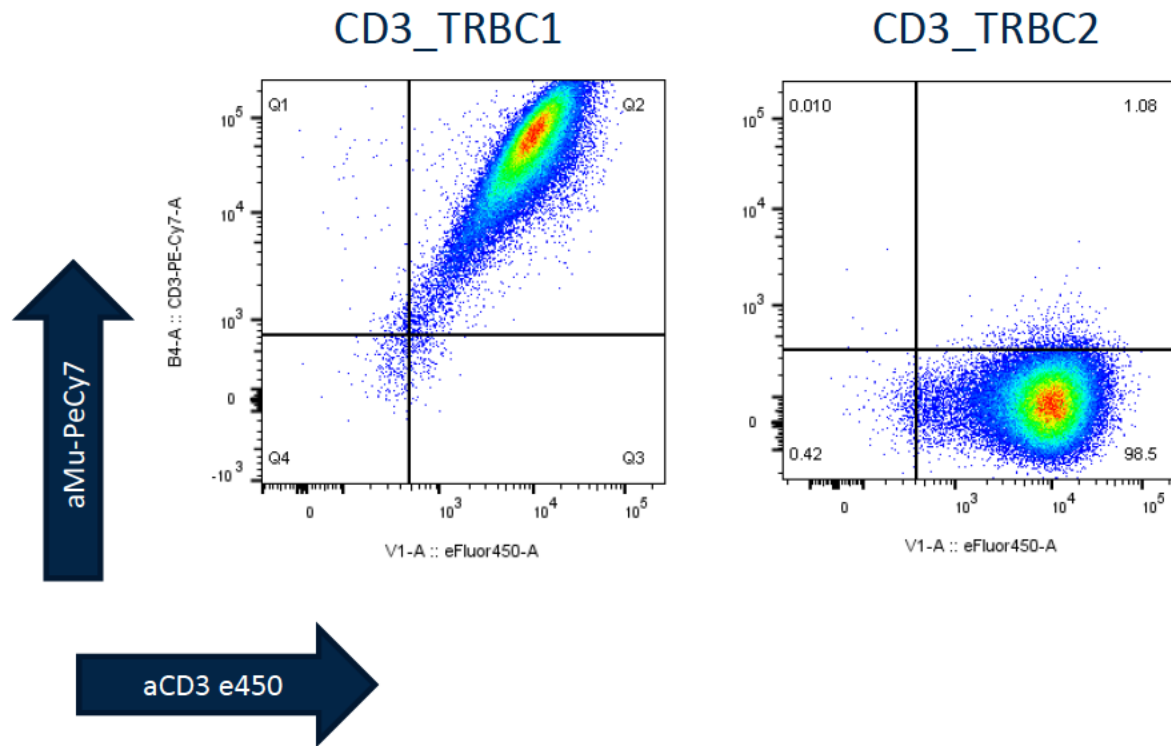
TRBC1 Specific Antibody
Identification of epitope required for recognition

V>E, 135

F>Y, 36

NK>KN, 4–5

TRBC2

TRBC1

NT

αTRBC1

NK-KN 4/5   F-Y 36

```
TRBC1   1  EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGK
TRBC2   1  EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGK

TRBC1  51  EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQF
TRBC2  51  EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQF

TRBC1 101  YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSAT
TRBC2 101  YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSAT
```
V-E 135

Asn-Lys (C1 epitope)   Lys-Asn (C2 epitope)

TRBC1 Expression in Normal T Cells

Peripheral blood T-cells contain a mix of TRBC1 and TRBC2 cells
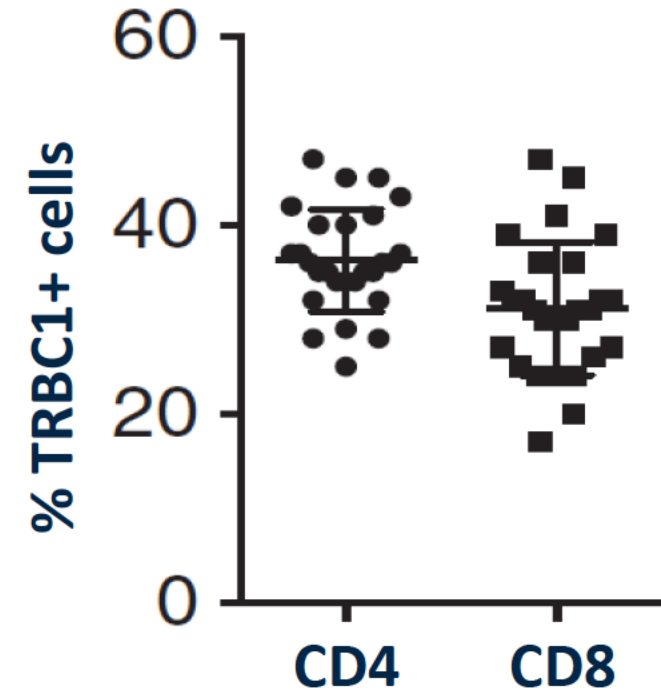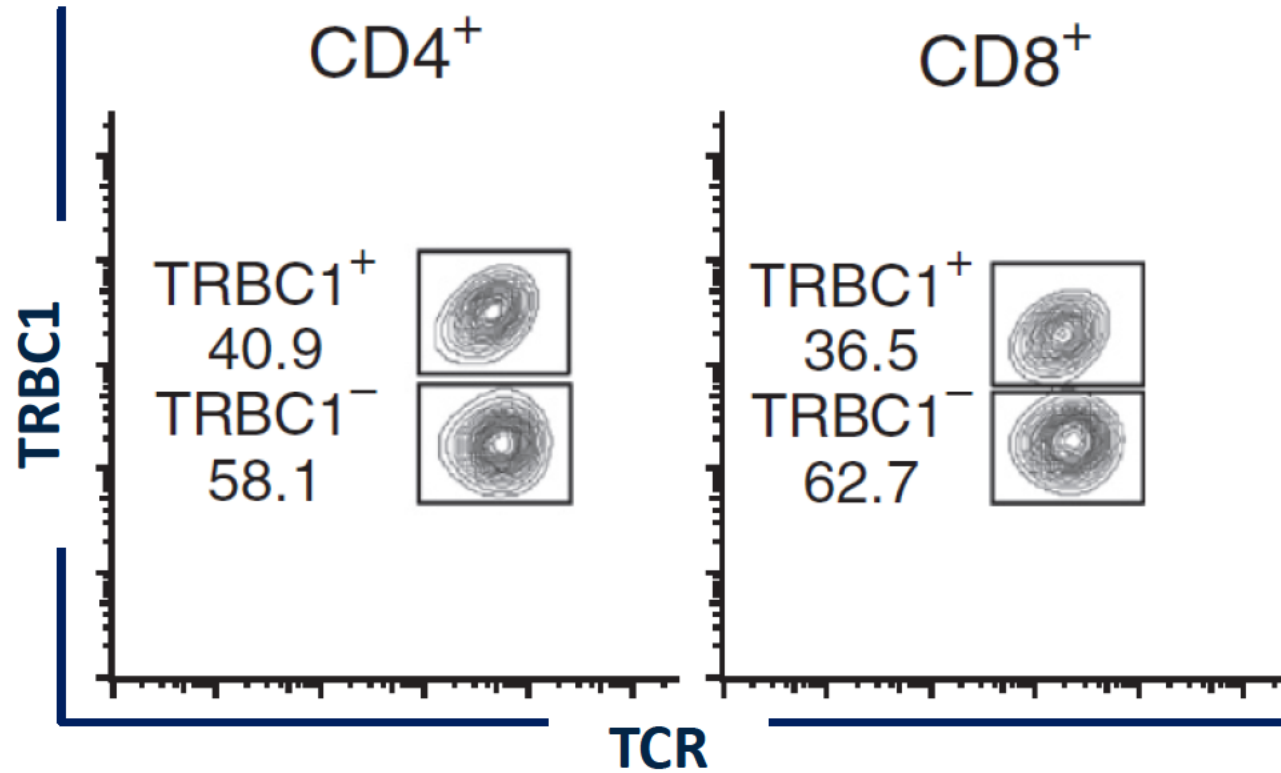
TRBC1 Expression in Primary T cell Cancers
Screening of patient samples with aTRBC1 antibody

CD3   TRBC1 mAb

TRBC1 –   PTCL-NOS Case 1

TRBC1 +   PTCL-NOS Case 2
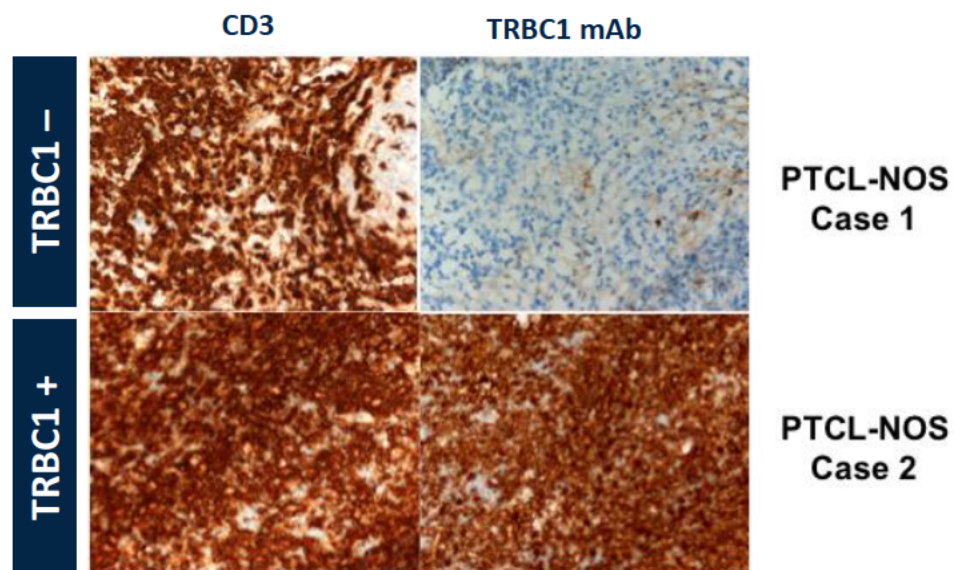
Diagnosis	TRBC1+ (%)	TRBC1-	Total
Anaplastic large cell lymphoma	5 (42)	7	12
Angio-immunoblastic T-cell lymphoma	2 (40)	3	5
Peripheral T-cell lymphoma, NOS	8 (44)	10	18
NK/T-cell Lymphoma	0 (0)	1	1
Sézary syndrome	1 (33)	2	3
T-acute lymphoblastic leukaemia/ lymphoma	2 (25)	6	8
Adult T-cell leukaemia/ 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

Impact of TRBC1 Depletion
TRBC1 depletion appears not to impact viral immunity
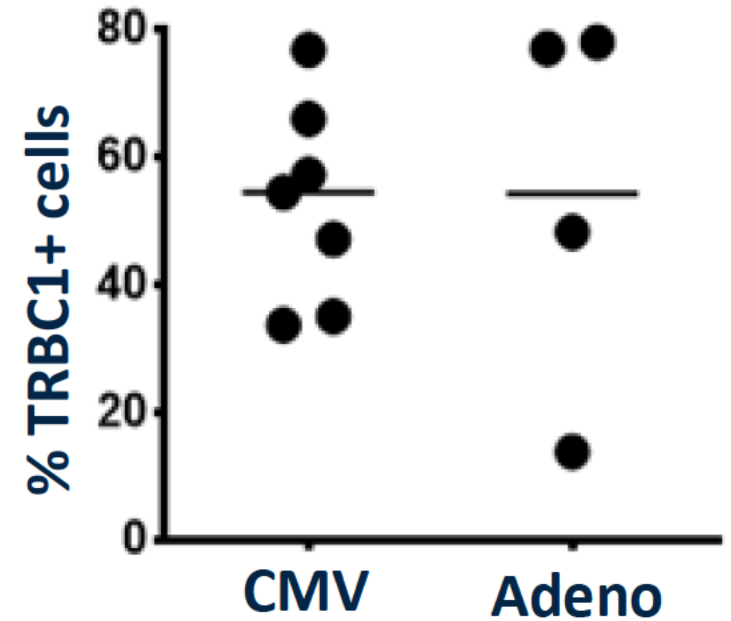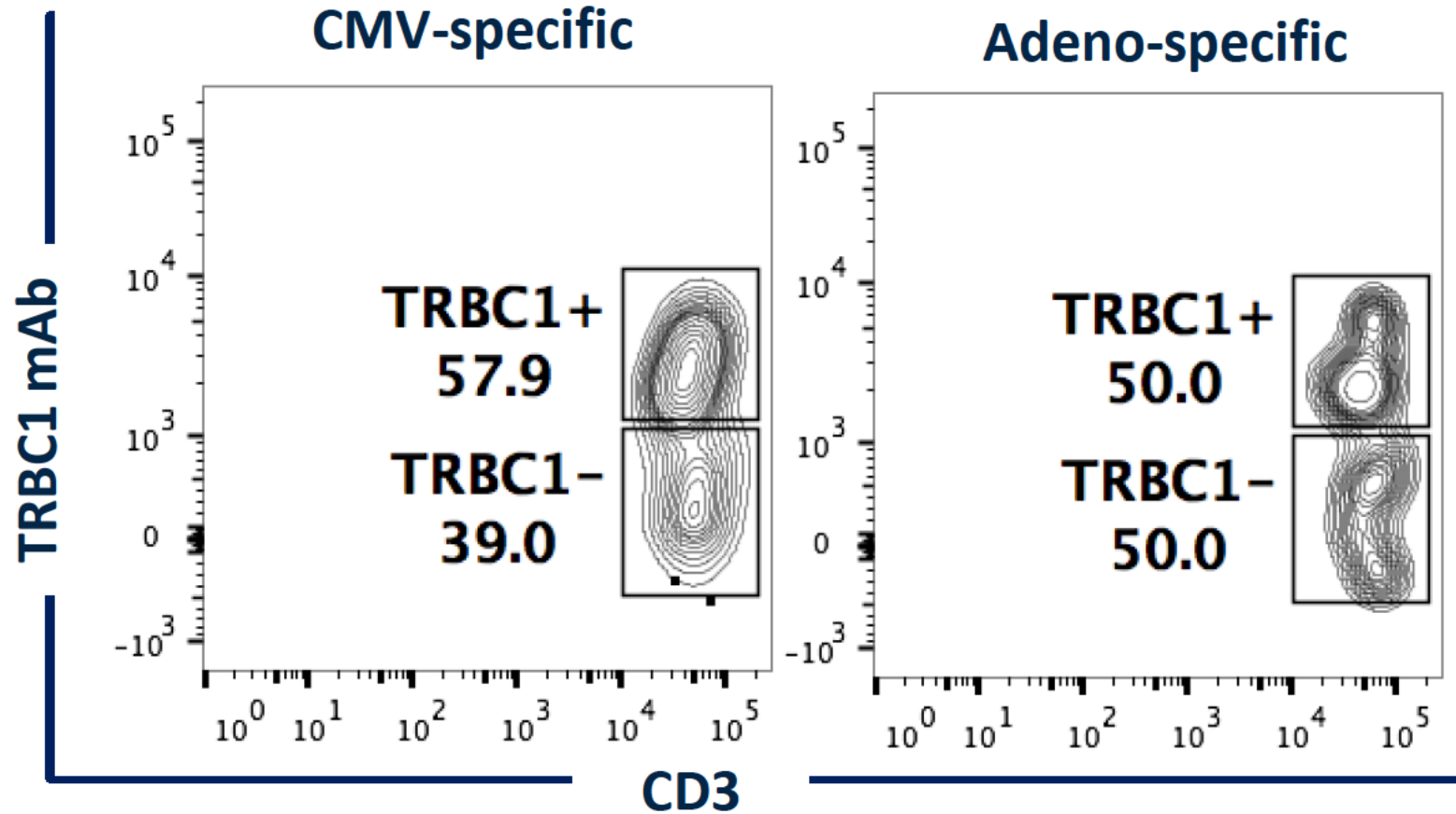
Generation of an Anti TRBC1 Chimeric Antigen Receptor

Humanization of aTRBC1 binder and CAR construction
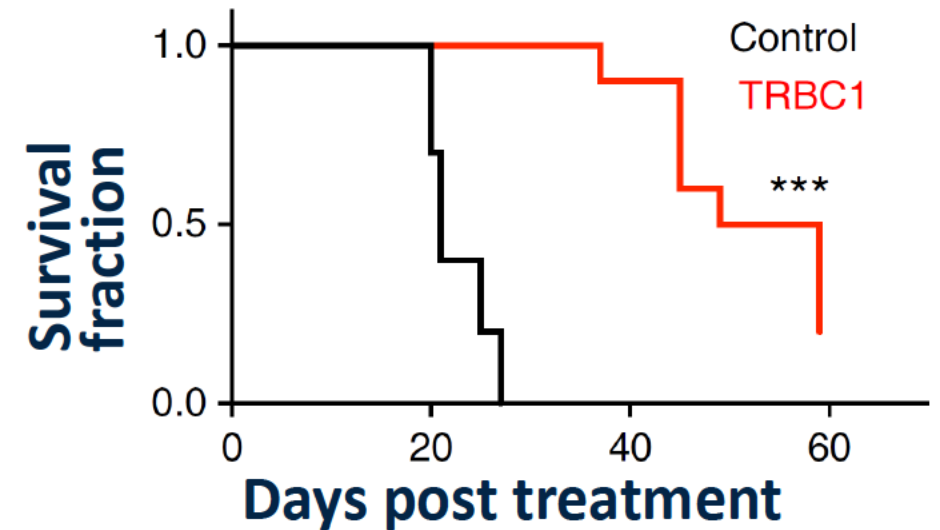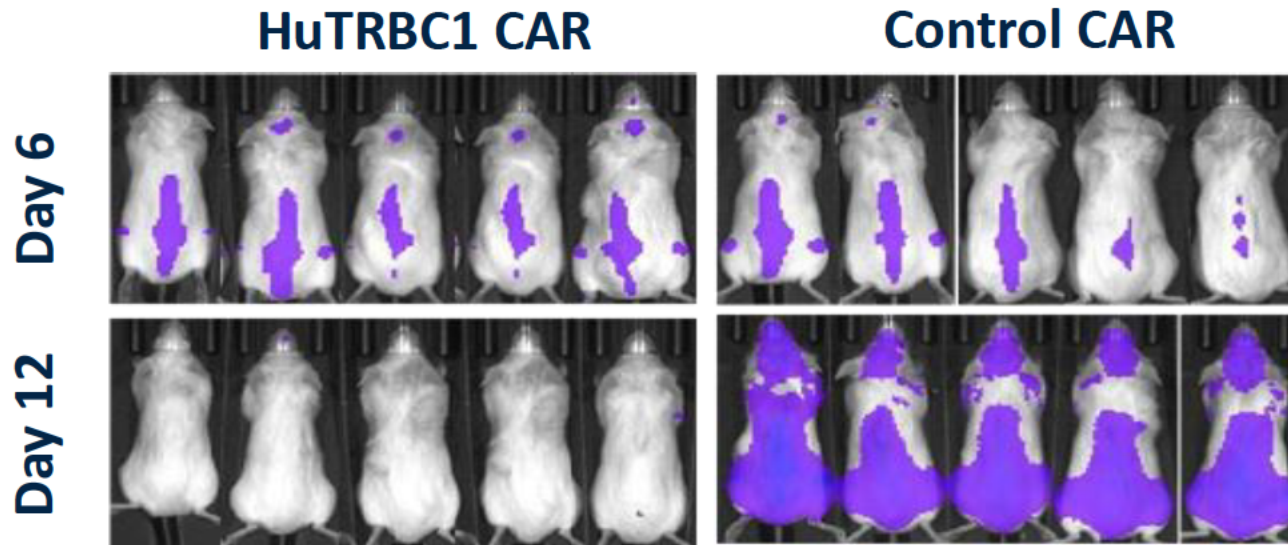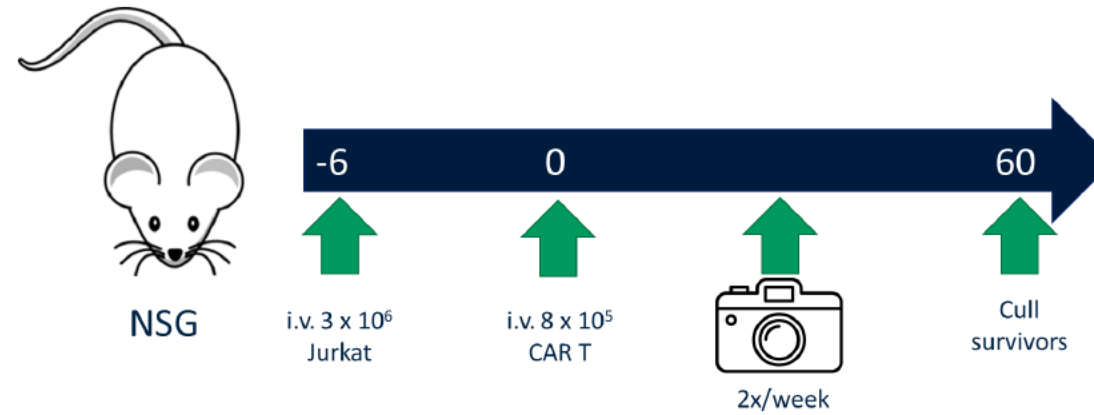
Humanization of aTRBC1 mAb
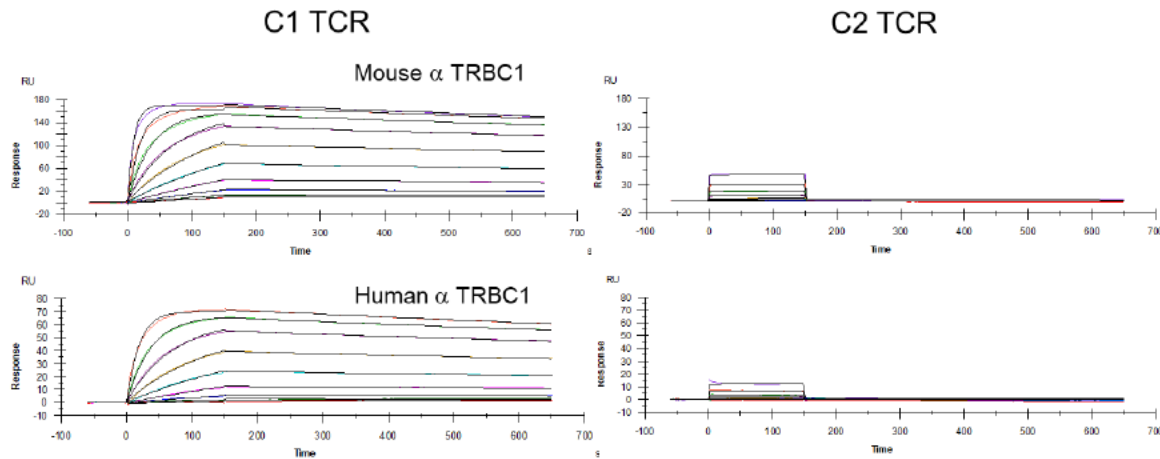
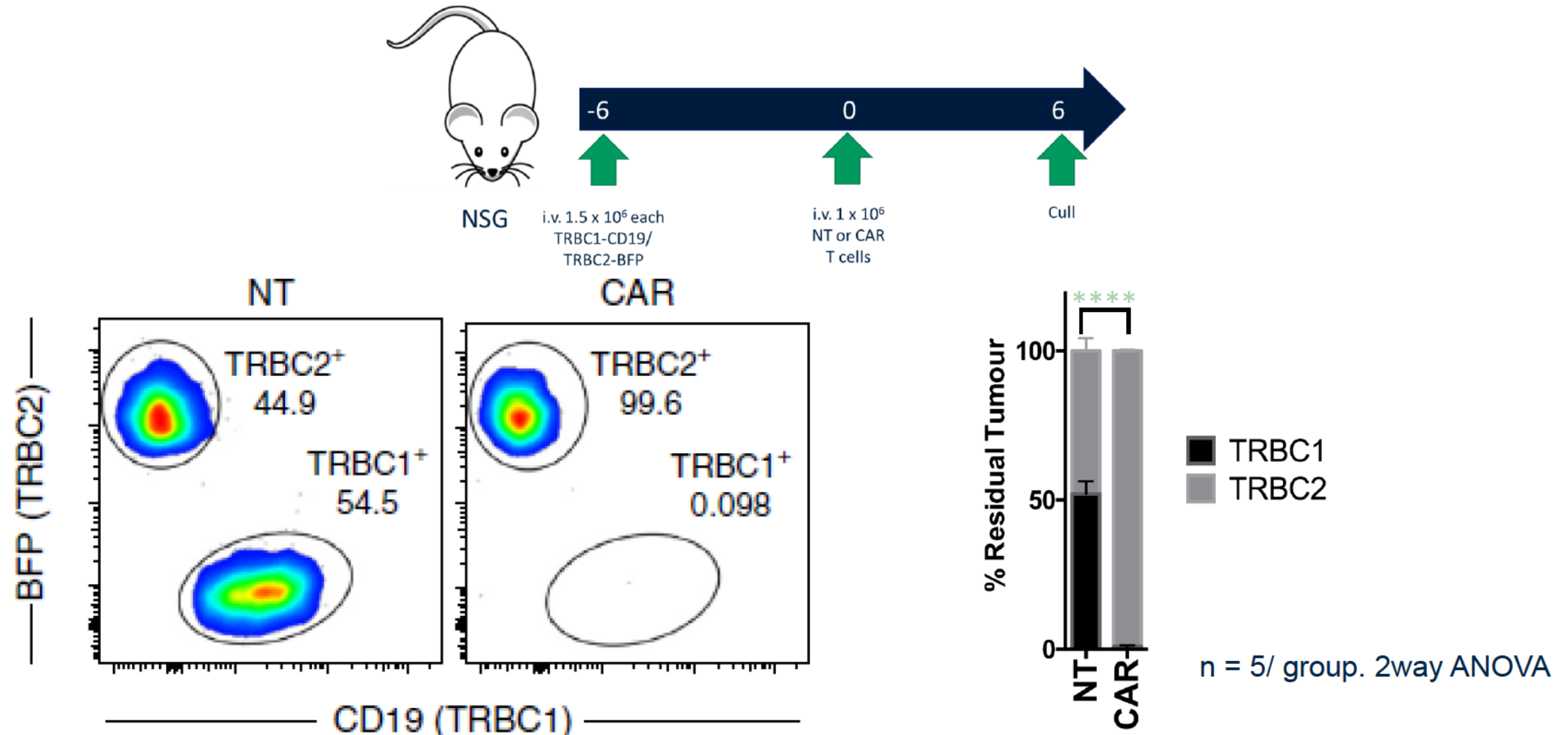| Binder	K_D
Mouse αTRBC1	2.41 ± 0.5
Human αTRBC1	2.96 ± 0.8

Construction of aTRBC1 Chimeric Antigen Receptor
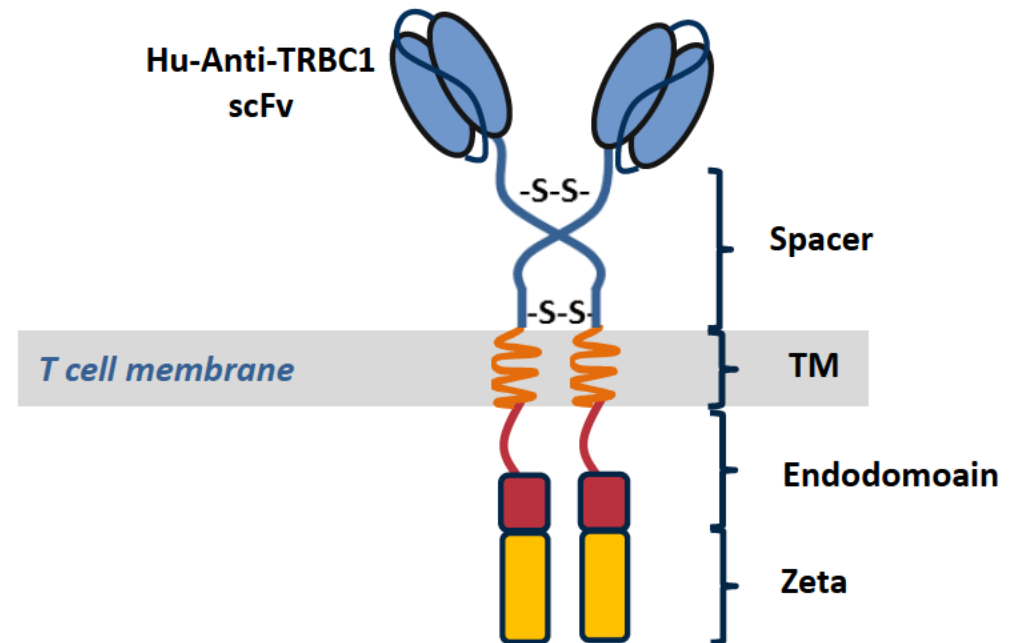
TRBC1 in-vivo CAR Activity

aTRBC1 CARs clear tumour in NSG model

TRBC1 in-vivo CAR Activity
aTRBC1 CARs selectively target TRBC1 cells in the presence of TRBC2 cells

AUTO4 Summary

- Targeting TCR beta constant chain isoforms may provide a generic approach to tackle the multiple subtypes of T cell-lymphoma

- Aim to preserve immunity, as viral immunity does not appear to be biased towards one TCR beta isoform

- Preclinical studies demonstrate utility of aTRBC1 CAR in vitro and in-vivo
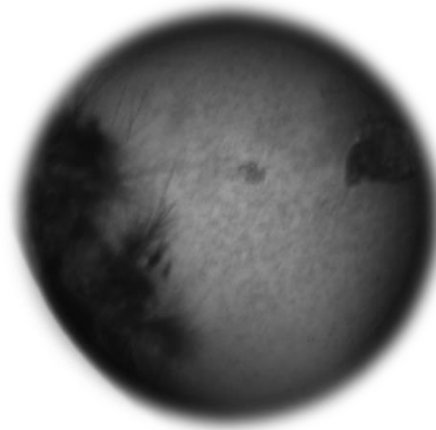
- AUTO4 clinical study in progress

Structural Studies on a Highly Specific TRBC1 Antibody
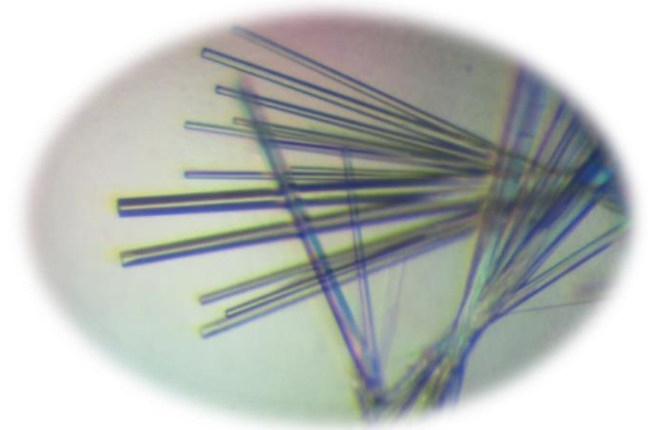
Generation of diffraction quality crystals
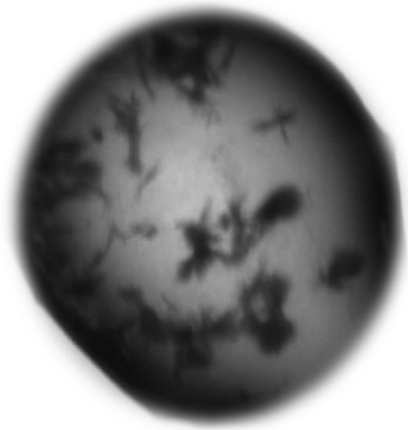
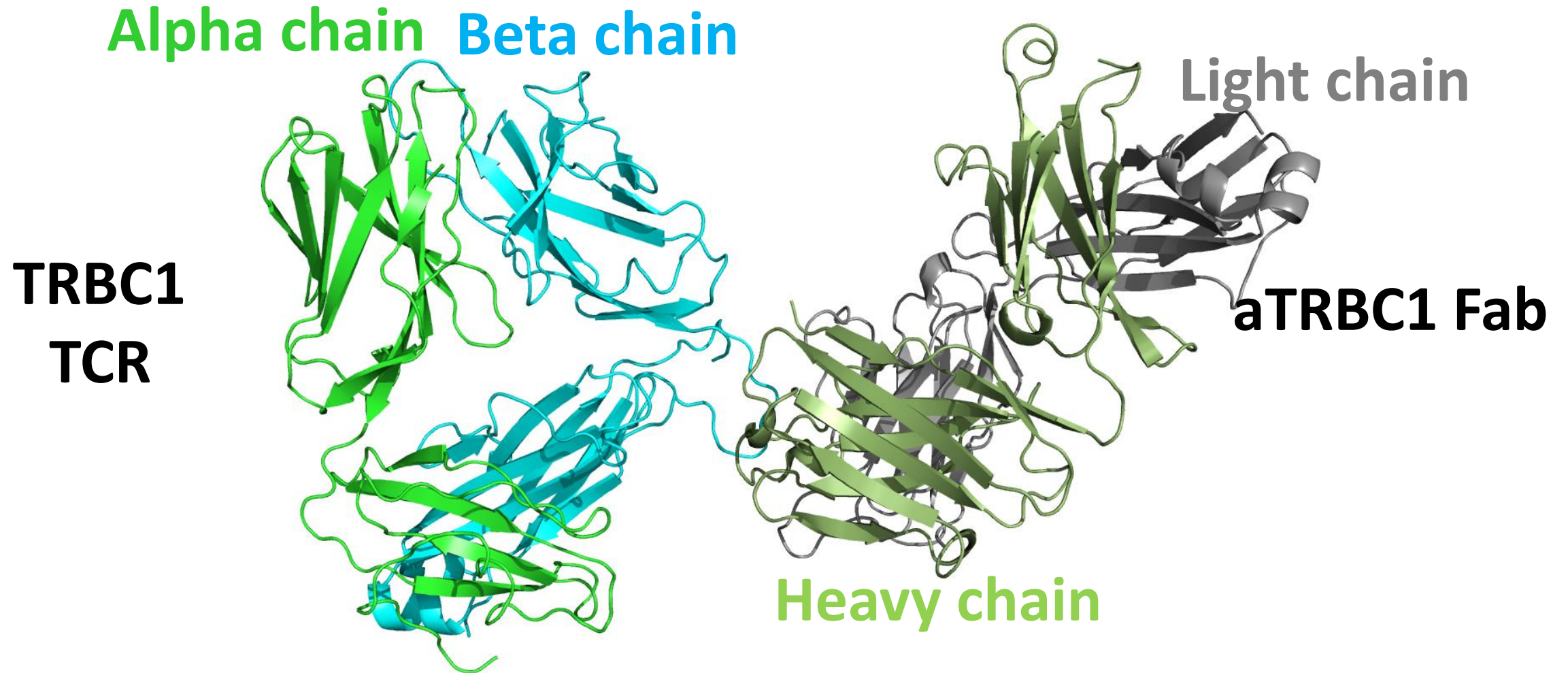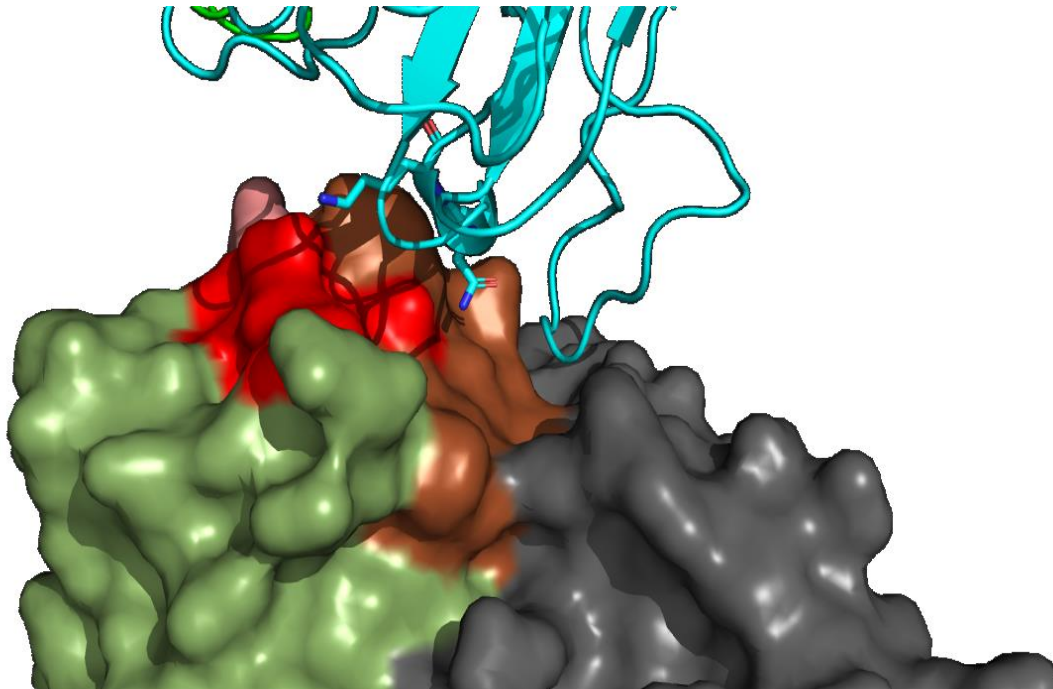MacroSol Screen
Initial hit

Optimisation →

Seeding →

Three-dimensional crystals

Crystal Structure of a TRBC1 Antibody in Complex with TCR


Alpha chain Beta chain Light chain

TRBC1 TCR

aTRBC1 Fab

Heavy chain

Crystal Structure of a TRBC1 Antibody in Complex with TCR

NK-KN 4/5

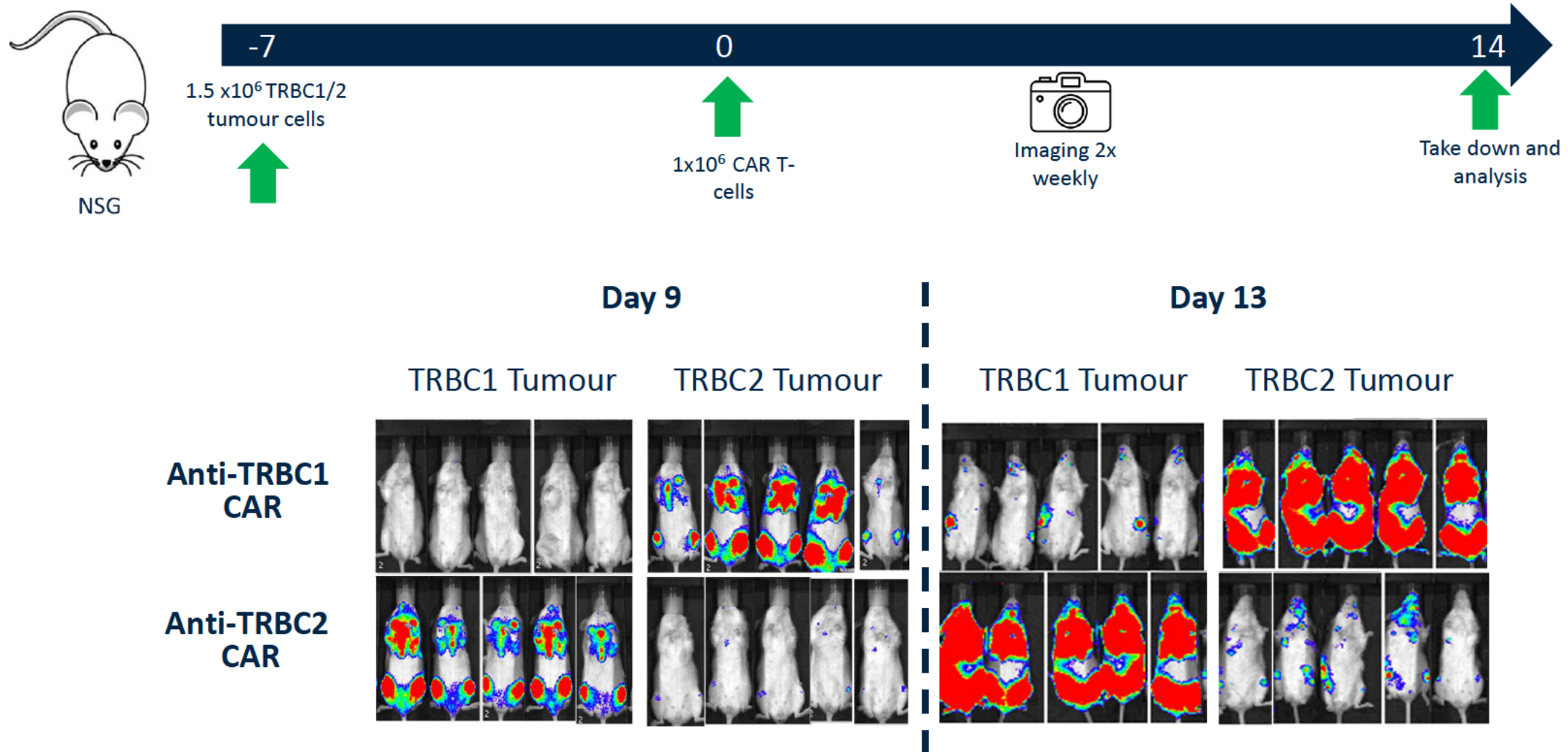|		
TRBC1	1	EDL NK VFPPEVAVFE
TRBC2	1	EDL KN VFPPEVAVFE

Crystal Structure of a TRBC1 Antibody in Complex with TCR
Engineering TRBC1 antibody specificity to create a TRBC2 specific antibody

■ WT- binder/ TRBC1   ■ Mut- binder/ TRBC1
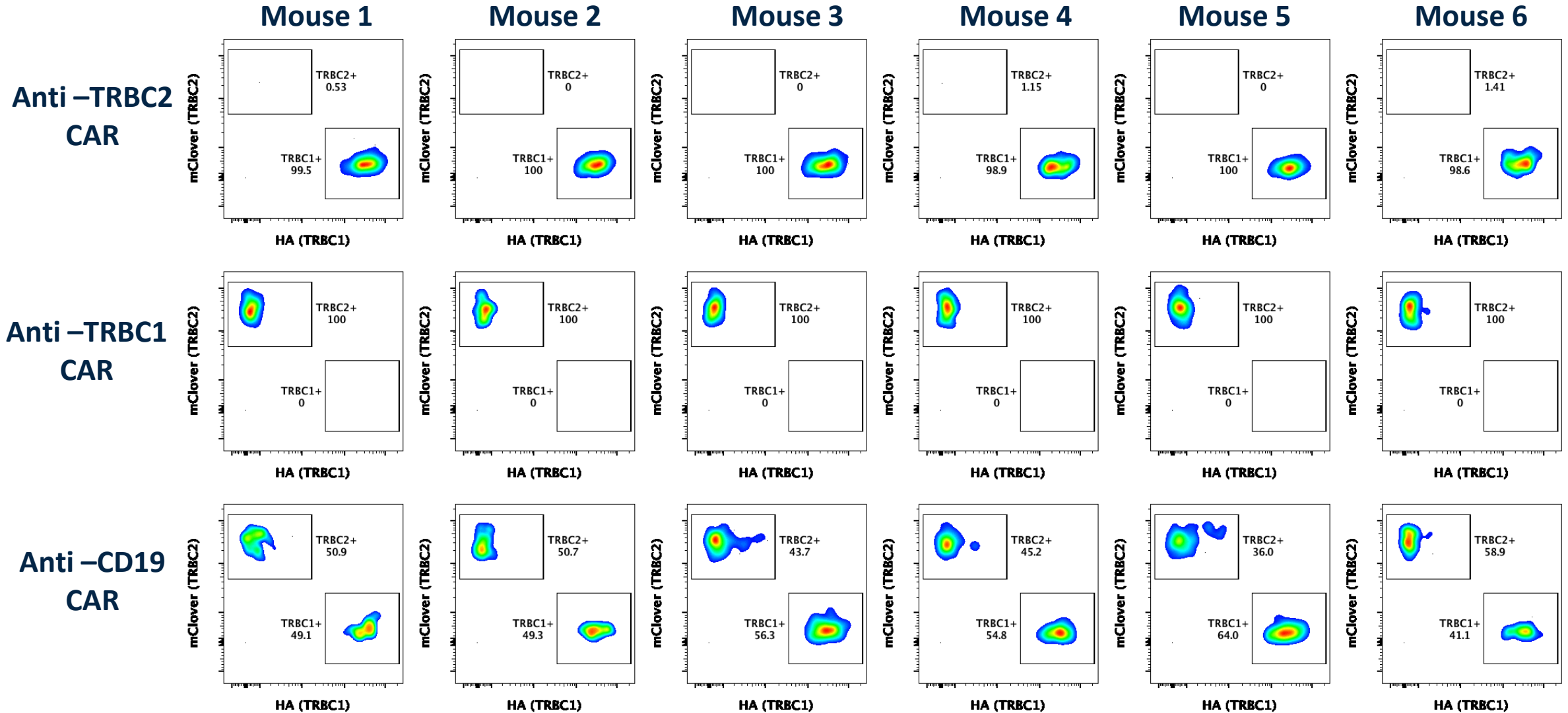▲ WT – binder/ TRBC2   ▲ Mut –binder/ TRBC2
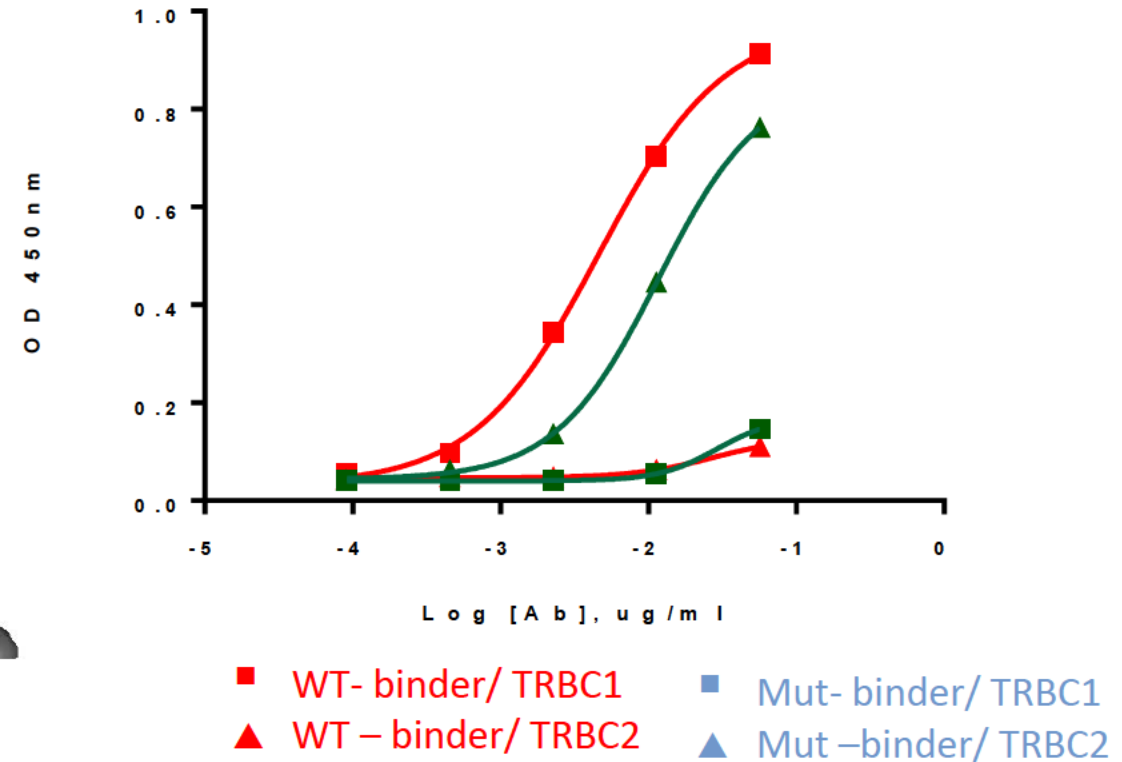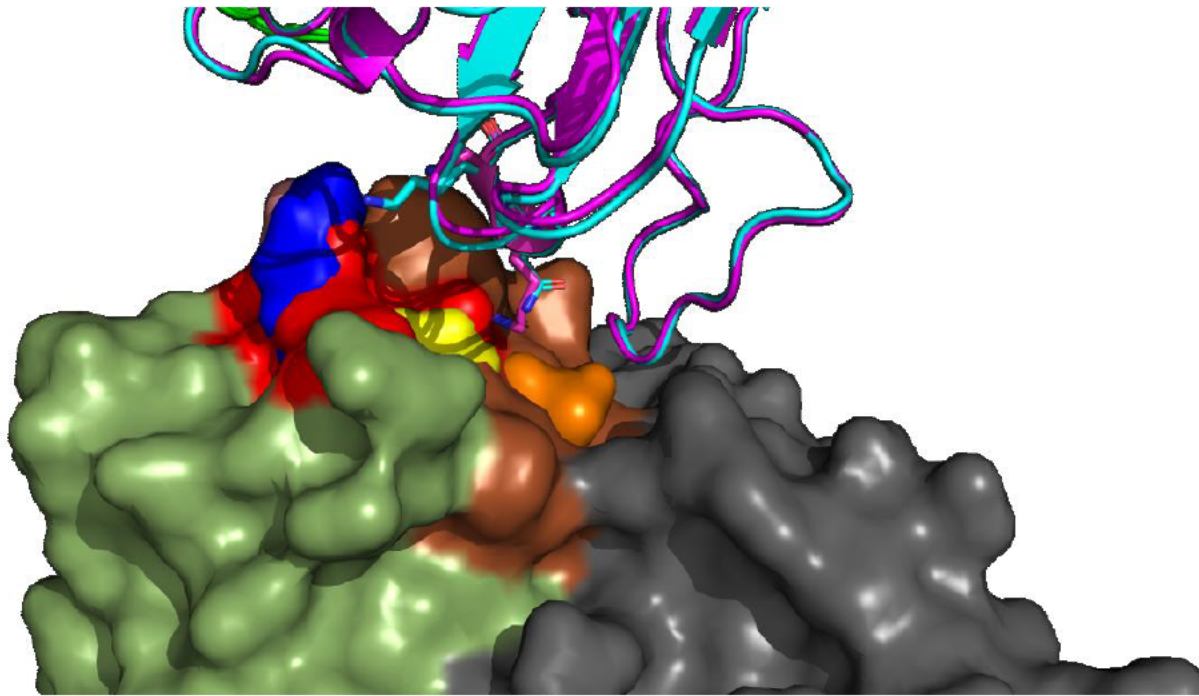
TRBC2 in-vivo CAR Activity

aTRBC2 CARs clear tumour in NSG model

TRBC2 in-vivo CAR Activity
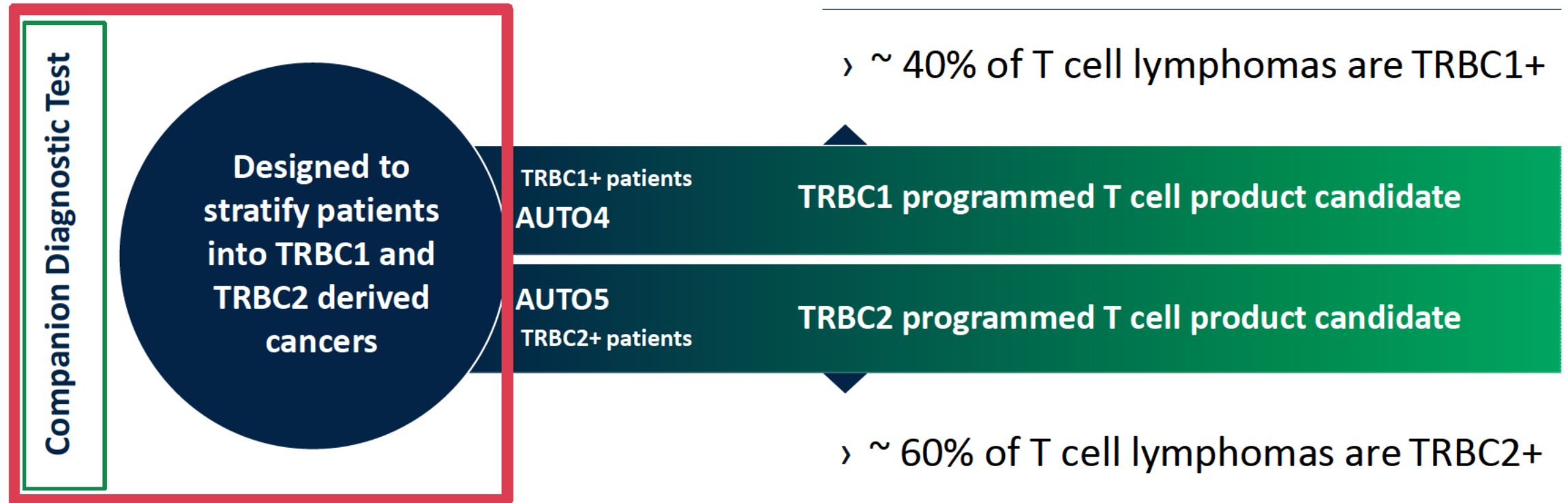## aTRBC2 CARs selectively target TRBC2 cells in the presence of TRBC1 cells

AUTO5 Summary

- Structural approaches have identified antibodies with specificity towards TRBC2

- AUTO 5 binding domains have been generated and tested in CAR format in vitro and in vivo

- Program is progressed towards IND

Addressing T cell lymphomas
Three key elements - AUTO4, AUTO5 and a companion diagnostic test

Companion Diagnostic Test

Designed to stratify patients into TRBC1 and TRBC2 derived cancers

TRBC1+ patients
AUTO4

AUTO5
TRBC2+ patients

› ~ 40% of T cell lymphomas are TRBC1+

TRBC1 programmed T cell product candidate

TRBC2 programmed T cell product candidate

› ~ 60% of T cell lymphomas are TRBC2+

AUTO4/5 Companion Diagnostic
Proof of concept data for Mi-Seq™ NGS based clonality Assay for TRBC1/2 stratification

J region usage correlates with C region usage
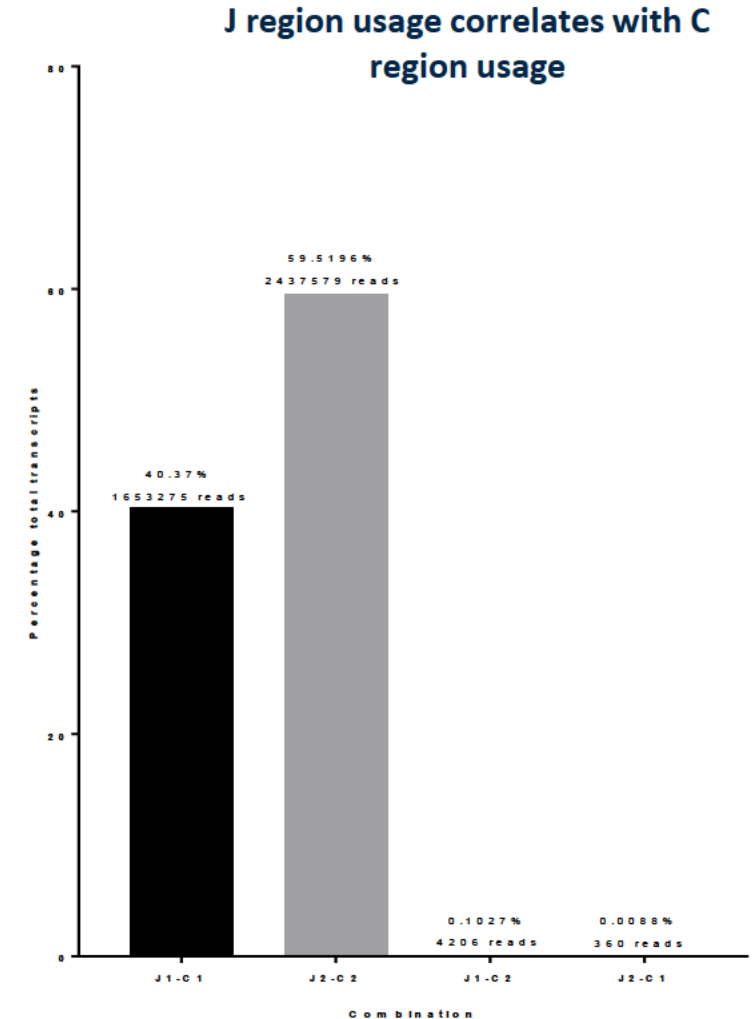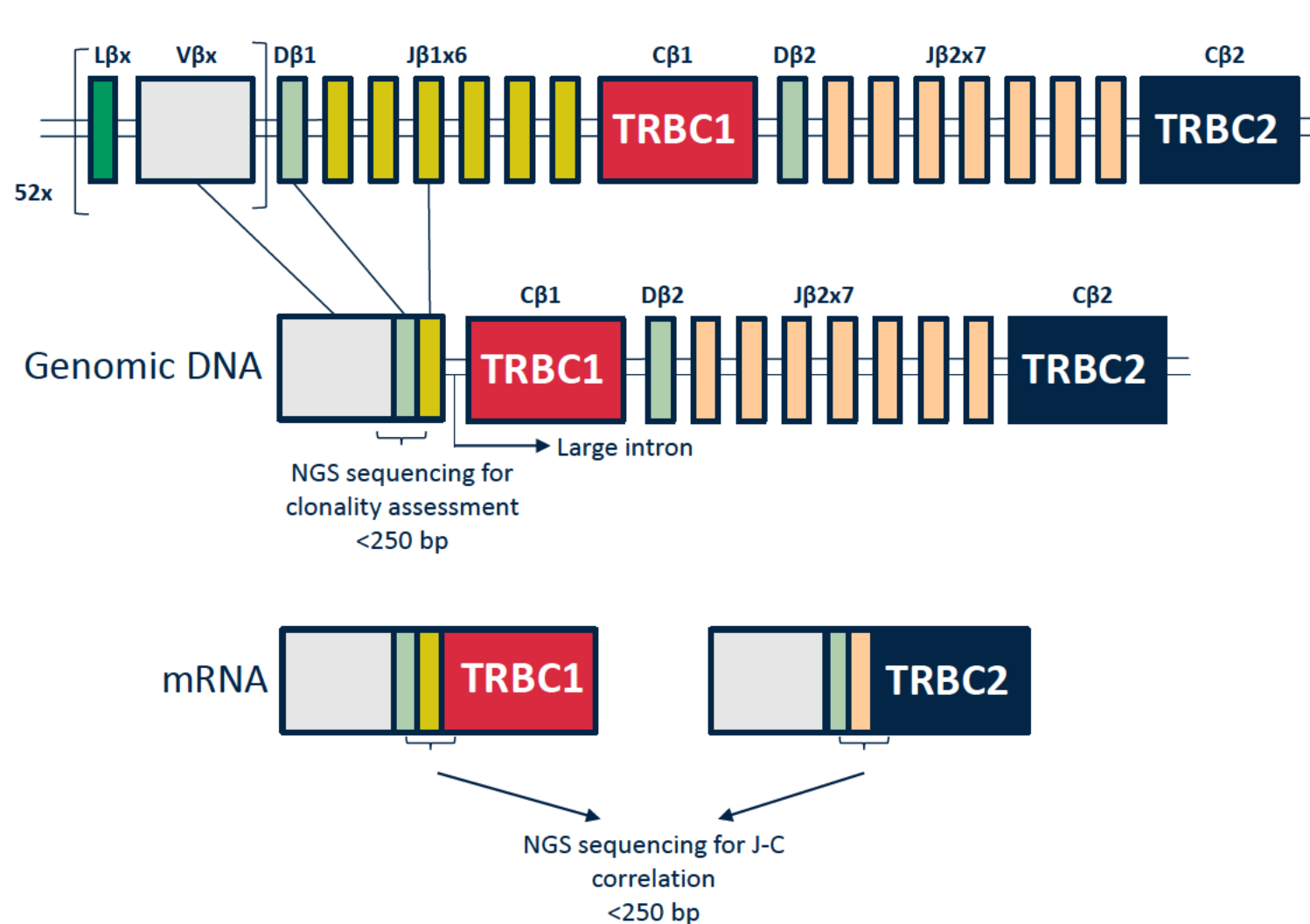
AUTO4/5 – Study Design and Status
Peripheral T-Cell Lymphoma

- Potential to be first in class therapies for T Cell lymphoma designed to avoid severe immunosuppression typically associated with current treatments
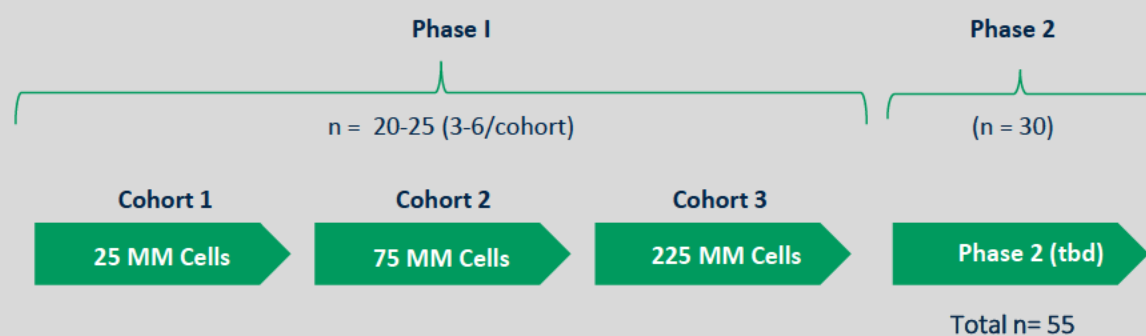
| Phase I			Phase 2	Status
n = 20-25 (3-6/cohort)			(n = 30)	Initiated AUTO4 Phase 1/2 trial in Q4 2018; first patient dosed
Cohort 1	Cohort 2	Cohort 3		AUTO4 Phase 1 data anticipated 2020
25 MM Cells	75 MM Cells	225 MM Cells	Phase 2 (tbd)	AUTO5 in preclinical development, will enter Ph 1/2 in late 2020
			Total n= 55	

- Expect to present initial Phase 1 data H2 2020

- AUTO5 Phase 1 to commence H2 2020

- Companion diagnostic development on-track

AUTO4-TL Phase I/II Study
Clinical outcome of patient 1

- 57 yr old with Angioimmunoblastic T cell lymphoma

- Past treatments include CHOP (CR) & IVE (refractory)

- AUTO4 Treatment
  - Treated with 25×10^6 anti-TRBC1 CAR T cells
  - No expansion of CAR T cells was noted, No CRS or neurotoxicity or T-cell aplasia was noted
  - Initial PET/CT at one month showed Complete Metabolic Response but subsequently had progression on day 71

Baseline PET/CT scan
Pre-AUTO-4 treatment
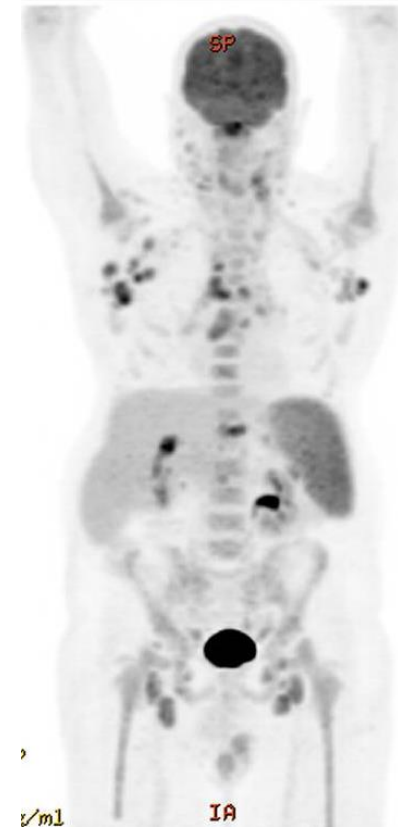
Month 1 PET/CT scan
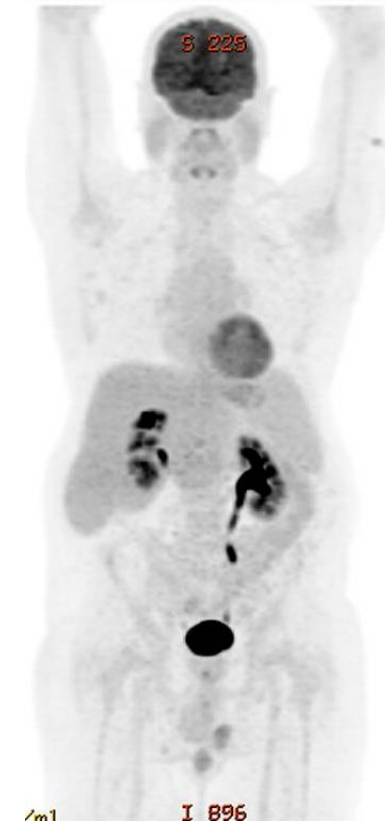
The UCL CAR T-cell Programme

STUDY	Target Disease	Status	Funder
COBALT	DLBCL	Complete	Bloodwise
CARPALL	**Paediatric ALL**	Complete	Children with Cancer
UCAR19	Paediatric ALL	Open	Cellectis/FP7
1RG-CART	Neuroblastoma	Complete	CRUK
CARD	CD19+ disease post allo	Complete	EU FP7 ATECT
ALLCAR19	**Adult ALL (PhI/II)**	Open	NIHR
AUTO3b	adult DLBCL	Open	Autolus
AUTO4	T-cell Lymphoma	Open	Autolus
CNS19	adult PCSNL	Pre-clinical	Wellcome Trust
GlioV3	GBM	Pre-clinical	Moulton Trust
ALLOKCAT19	B-cell cancers	Pre-clinical	MRC
X3-30	r/r HL	Pre-clinical	Moulton Trust

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AUTO1 study investigators

AUTO3 study investigators

AUTO4 study investigators

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