INMUNOTERAPIA EN NEOPLASIAS HEMATOLÓGICAS:
implicaciones de la citometria de flujo

CIC Centro de Investigación del Cáncer

VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

800 AÑOS 1218 ~ 2018

IBSAL Instituto de Investigación Biomédica de Salamanca

CANCER RESEARCH UK

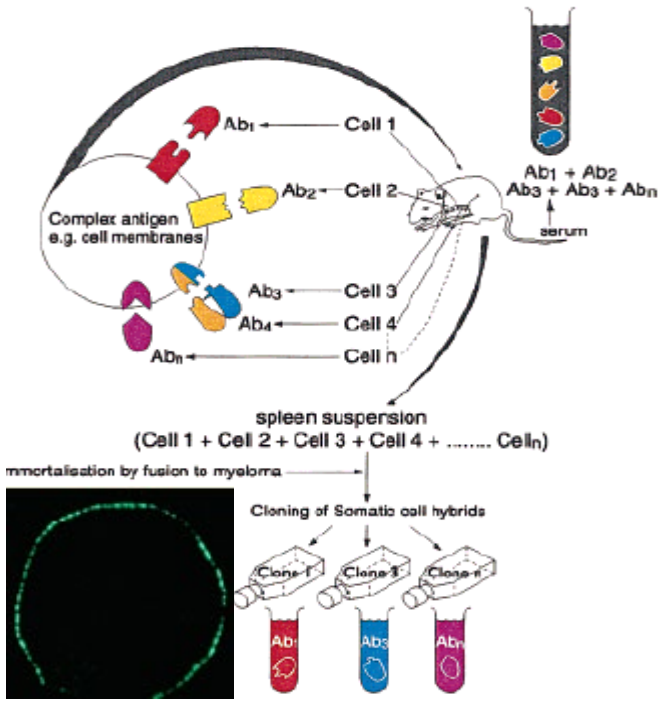
CANCER RESEARCH CENTER IBSAL, UNIVERSITY & UNIVERSITY HOSPITAL OF SALAMANCA

aecc Contra el Cáncer

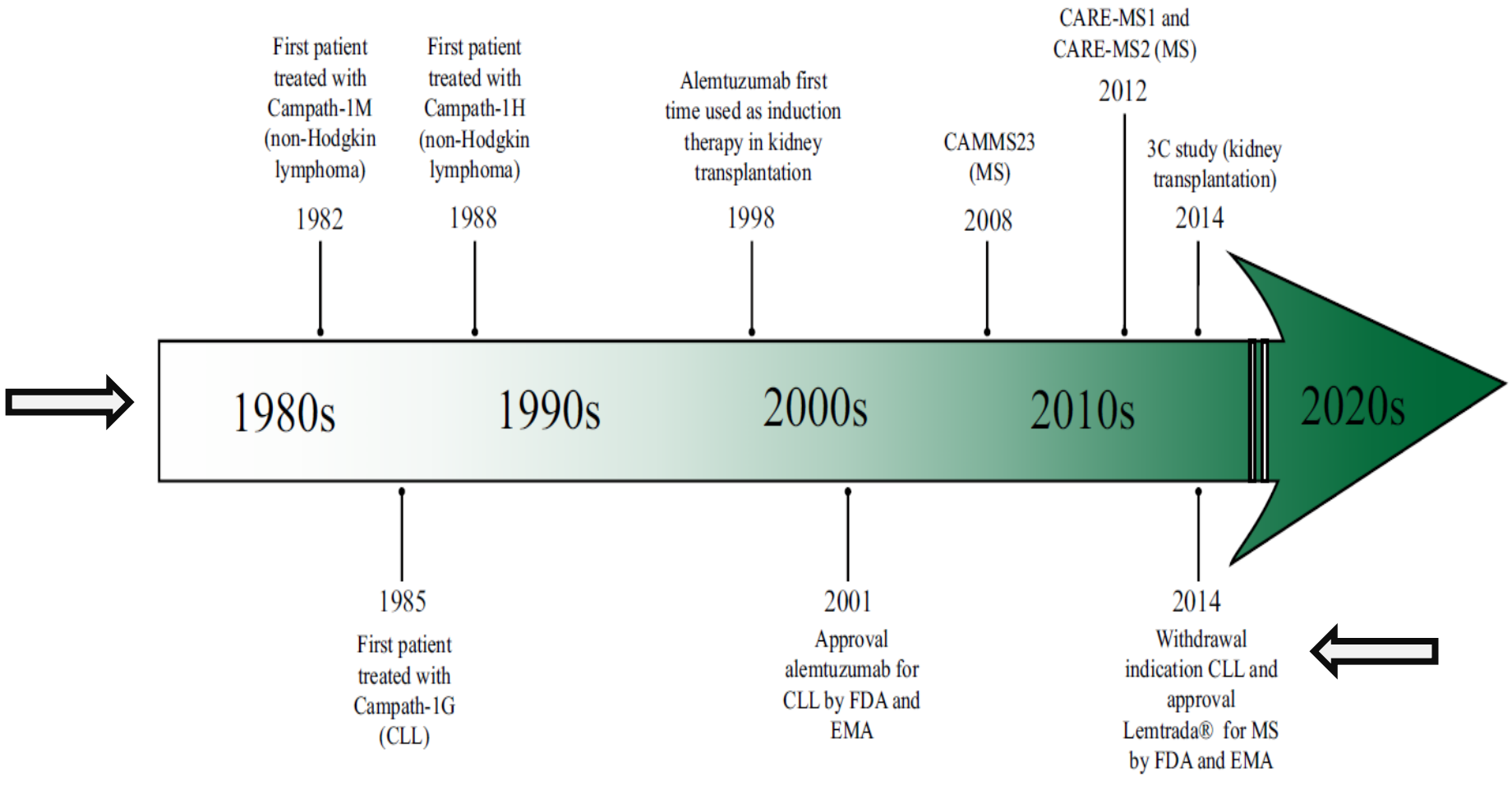
EuroFlow

6° Curso Práctico de Citometria de Flujo
Valencia, 28 de septiembre de 2023

FROM the MoAb TECHNOLOGY TO TARGETED ANTIBODY-BASED THERAPIES

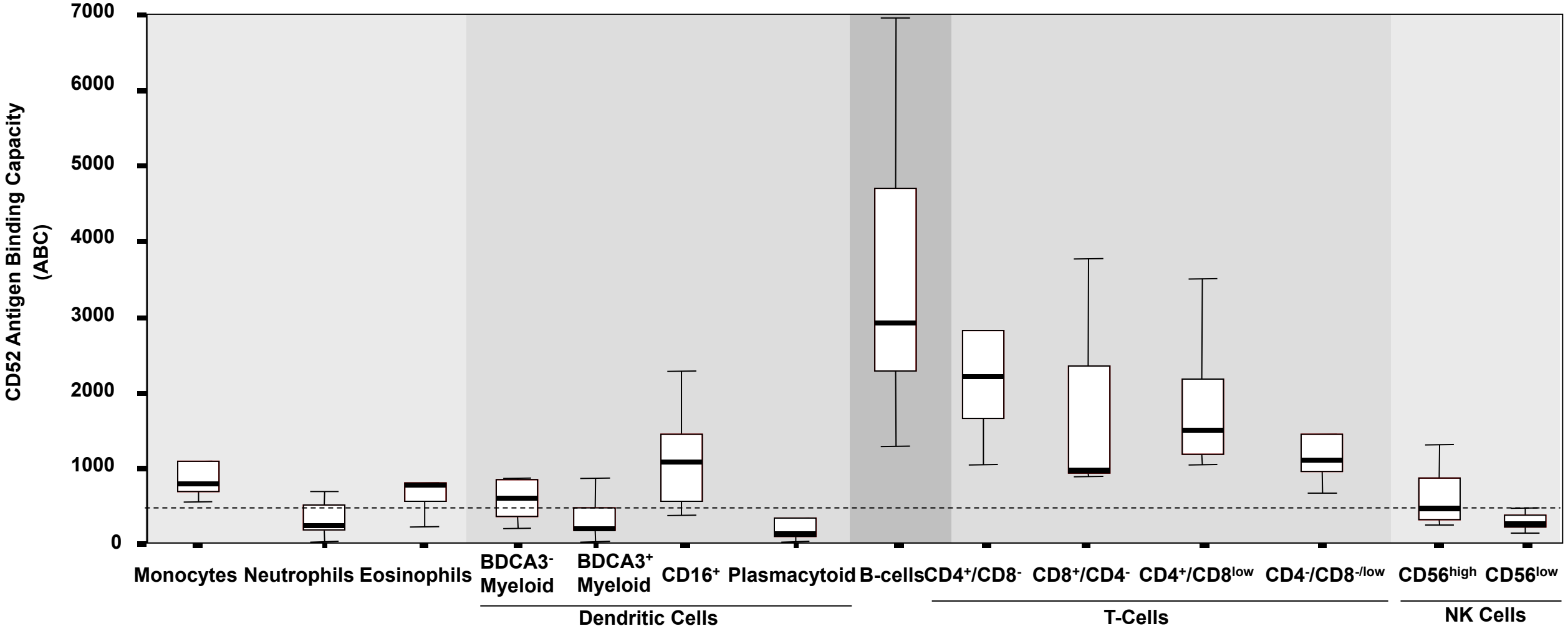


Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of pre-defined specificity. Nature 1975;256:495-497.



Van der Zwan et al, Clin Pharmacokinet, 2018, 57: 191-207

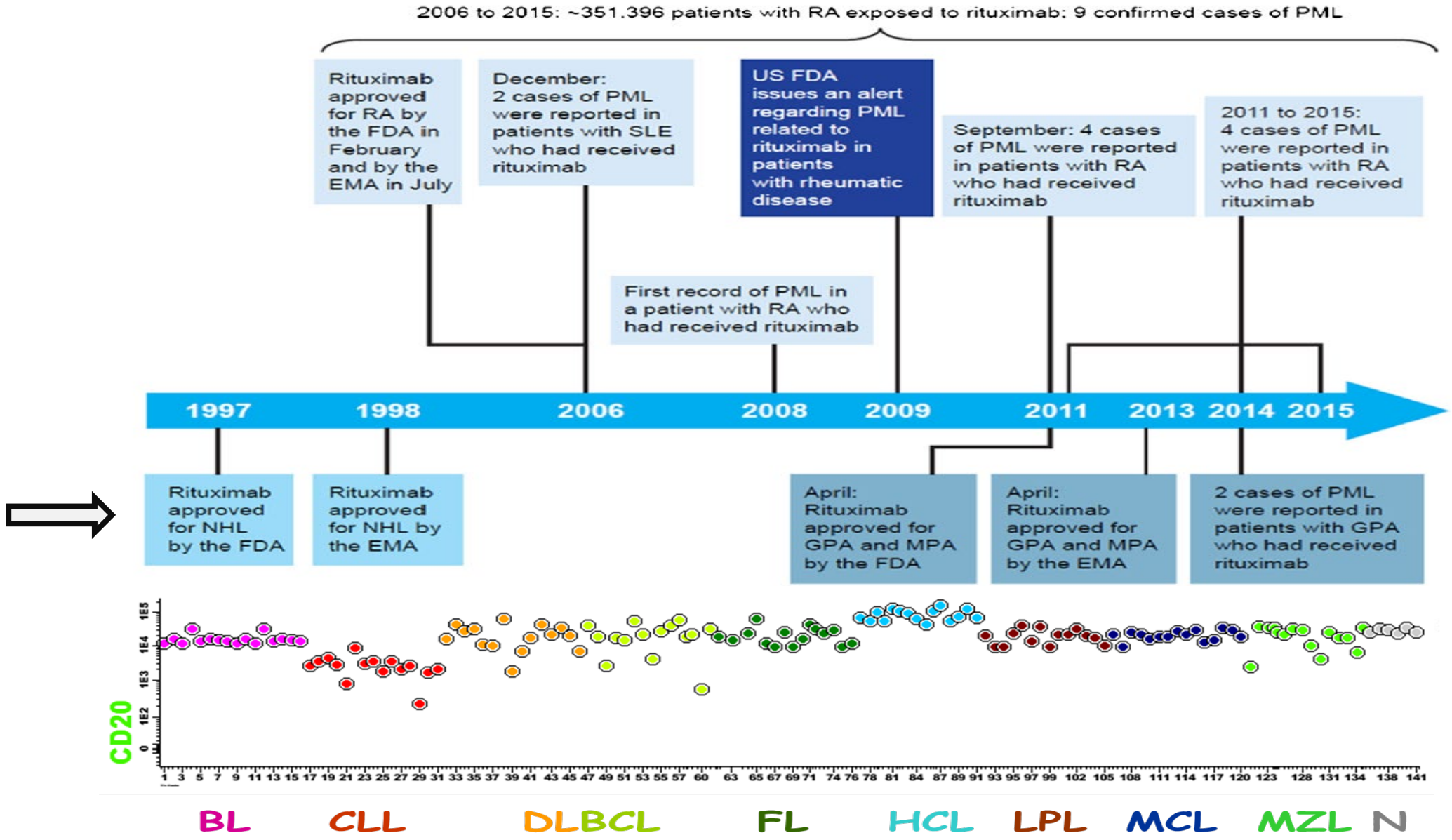
EXPRESSION OF CD52 (ALENTUZUMAB) BY NORMAL PB LEUKOCYTES



Evaluation of the pattern of expression of targeted cell surface proteins provides insight on tumor sensitivity to antibody targeted therapies as well as on its potential toxicity

Hernández-Campo et al, Cytometry B 2005

ANTI-CD20 (Rituximab) TARGETED ANTIBODY THERAPY



Berger et al, J NeuroVirol, 2018, 24: 323-331

Bottcher et al, Blood Adv, 2021, 6: 976-992

PB B-CELL PHENOTYPIC PROFILE

		Immature	Naive	Memory	Plasmablasts/PC
BCR-associated signaling molecules	CD5	++	-/+	-	-
	CD19	+	+	+	+[d]
	CD20	+	+	+	-
	CD21	+/++	++	++	-
	CD22	+	+	+	-
	CD45	++	++	++	+/++
	CD53	+	+	++	+[d]
	CD81	++	+	+	+
	sIgH	++	+	++	+[d]
T cell-associated molecules involved in B-T cell interactions	CD23	-/++	-/++	-	-
	CD25	-	-	+	-
	CD27	-	-	+	++
	CD40	++	++	++	+
	CD86	-	-	-	+
	CD95	-	-	-/+	+
	CD200	N.A.	+	-	N.A.
	HLA-DR	++	++	++	+/++
Other cell surface molecules	CCR6	+	+	+	-
	CD138	-	-	-	-/+[d]
	CD10	+	-	-	-
	CD24	+	+[d]	+	-
	CD38	+	-/+[d]	-/+[d]	+++
	CD43	-	-	-	+
	CD53	+	+	++	+[d]

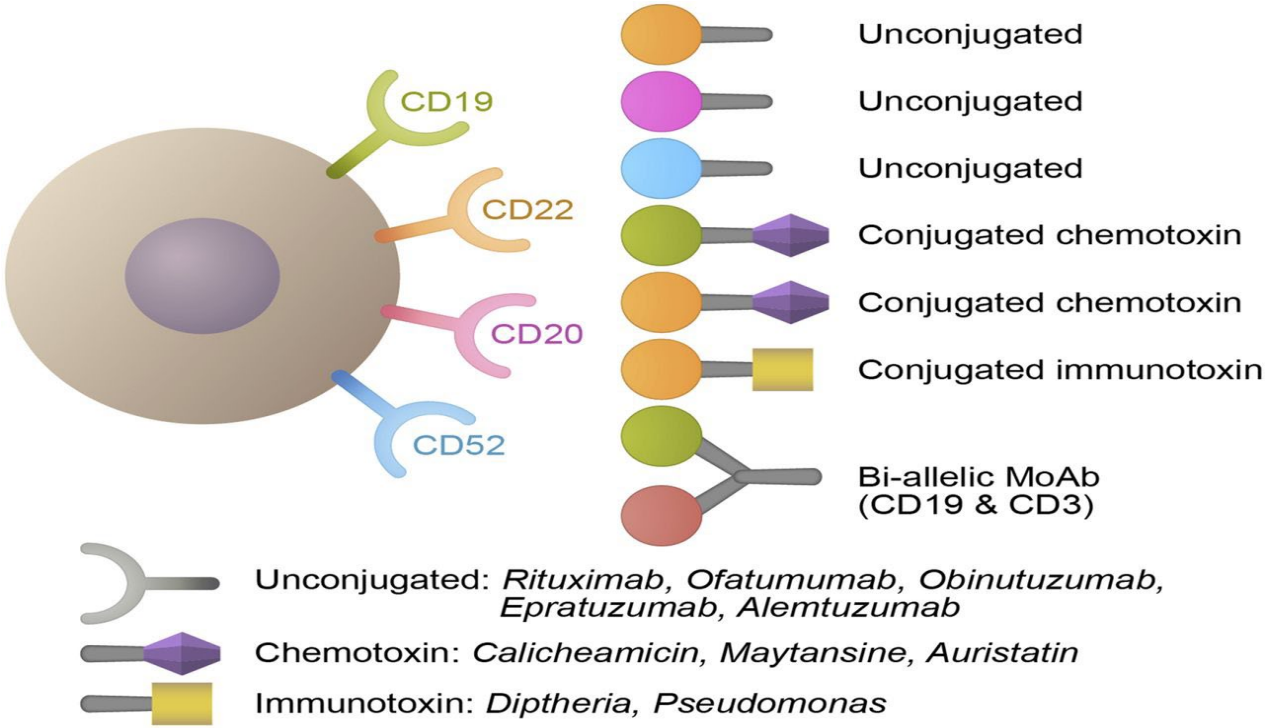
Caraux A Haematologica 2010

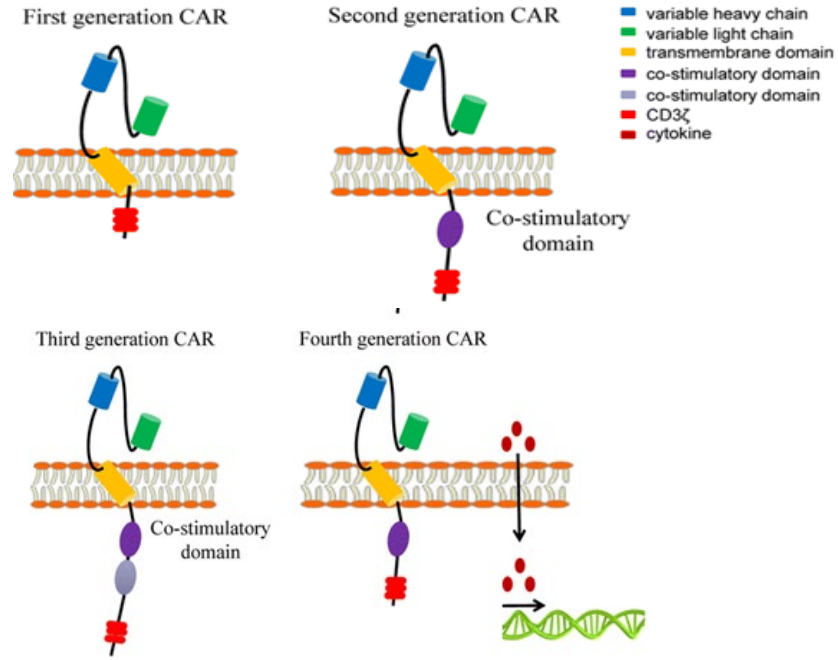
Perez-Andres M Clinical Cytomatry 2010

Targeted therapies in B-cell malignancies

Many B-cell targets: for example **CD19**, CD20, CD22, CD38, CD138, **CD269**…

Many different modalities: unconjugated Ab, toxin-conjugated Ab, bispecific Ab, BTK checkpoint blockers CAR T-cells…



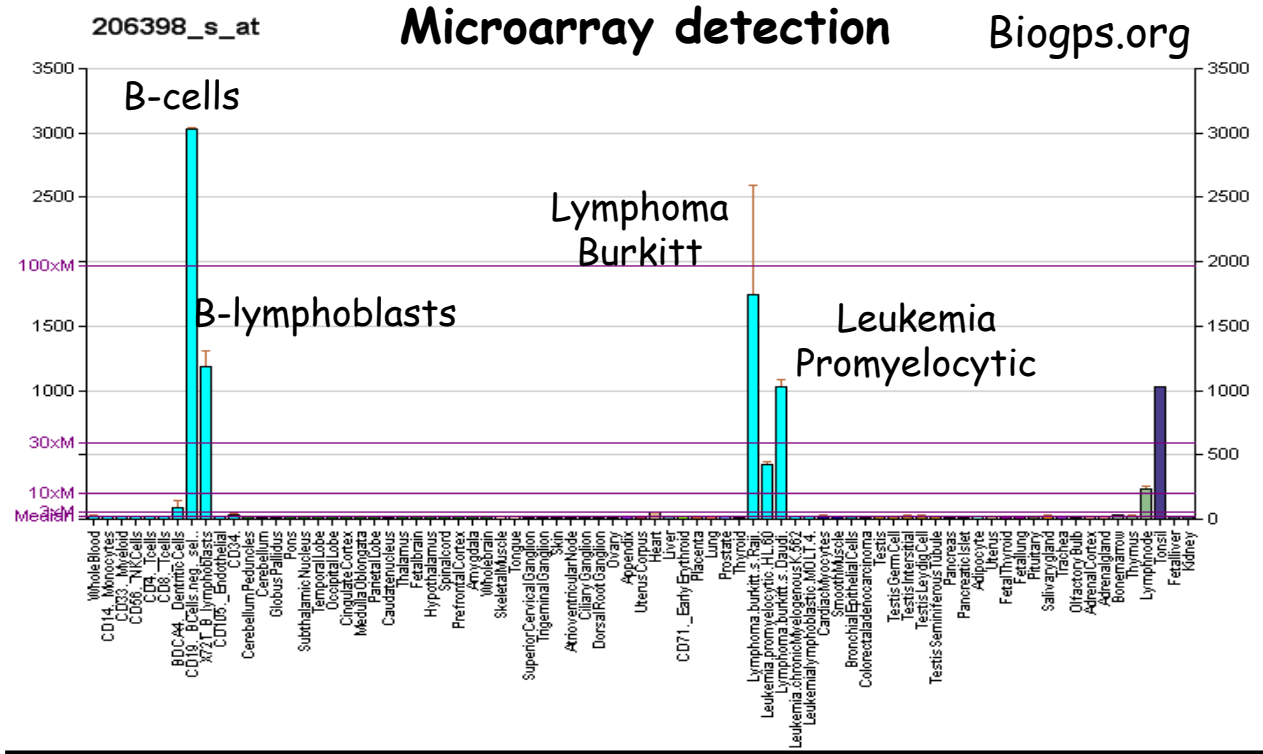


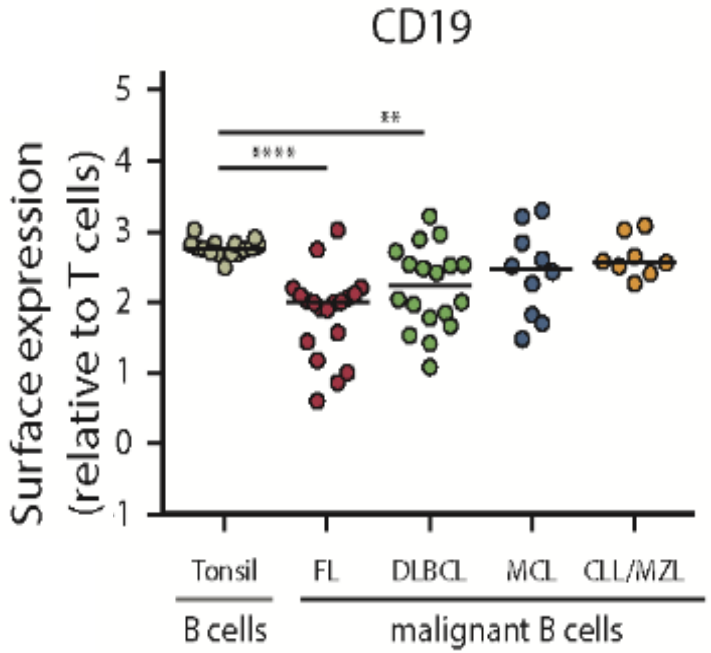
Jabbour et al. Blood 2015

Maus et al. Blood 2014; 123:2625; Singh AK & McGuirk JP, Lancet Oncol 2020, 21: e168-e178

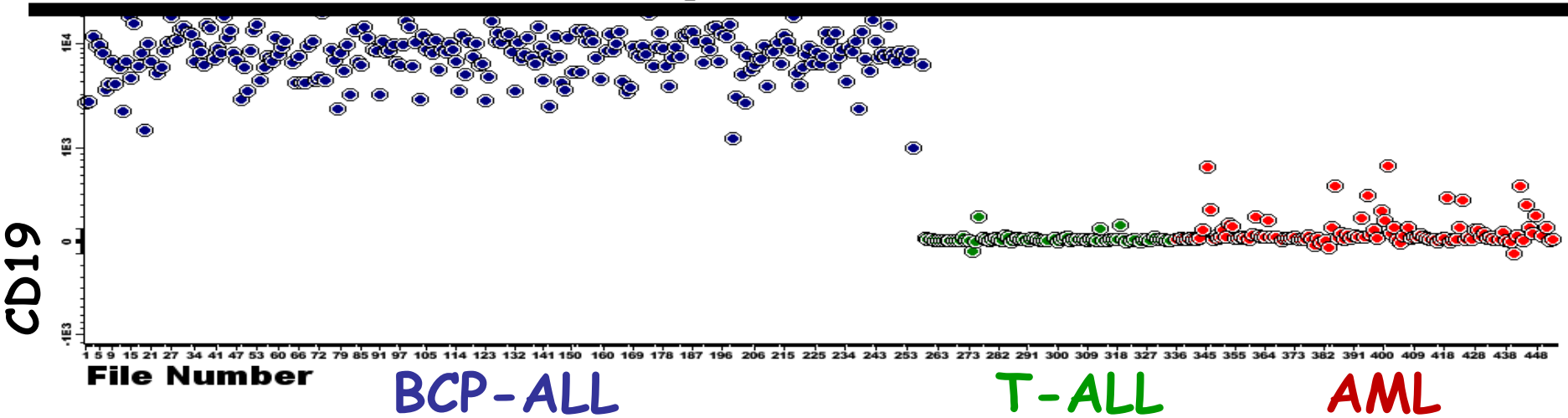
Differential CD19 Expression in normal and tumor B cells

EuroFlow





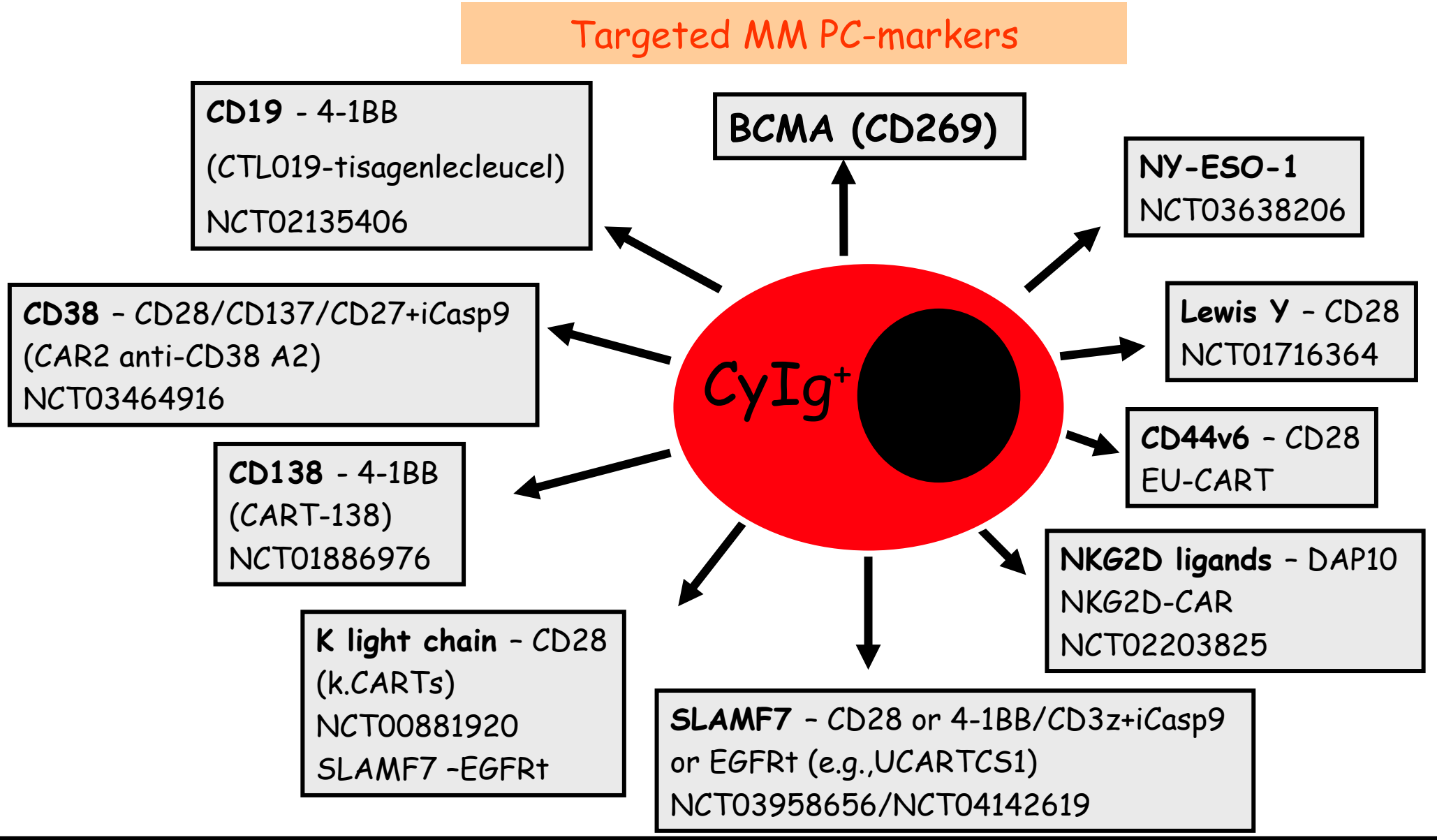
Köksal H et al. Blood Adv, 2019; 3: 1230–1243



Lhermitte et al. Leukemia, 2017; 3: 1230–1243

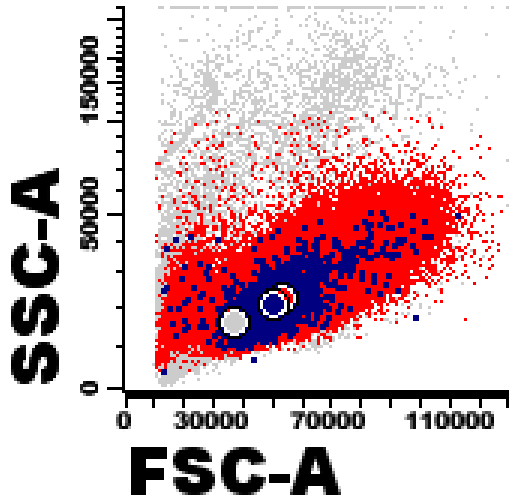
Lhermitte et al. Mod Pathol, 2020 (publish online)

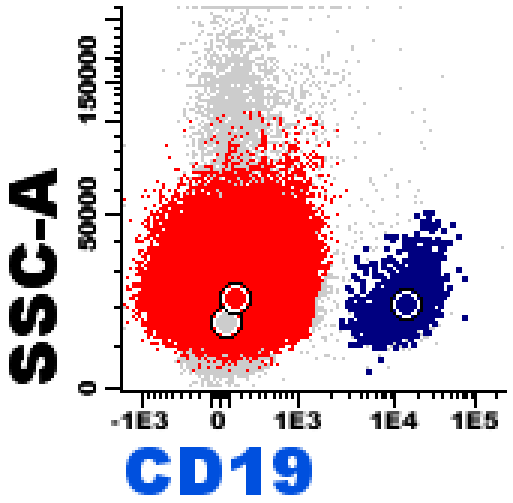
CAR T-cell targets in Multiple Myeloma plasma cells

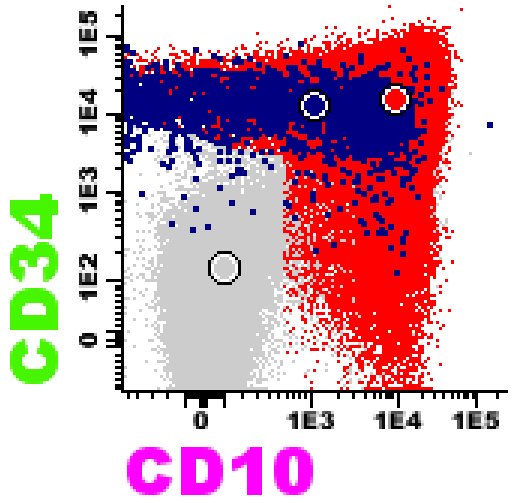


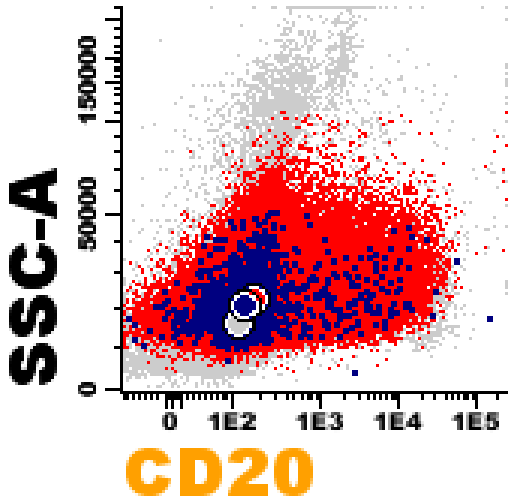
Teho & Chng, Blood Cancer J 2021, 11, 84: 1-18; Rawstron et al, Haematologica 2008, 93: 431-438; Caraux et al, Haematologica 2010, 95: 1016-1020

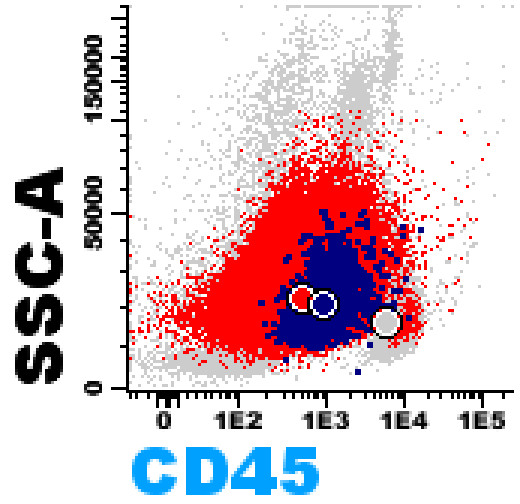
RESIDUAL CD19- BCP-ALL BLASTS AFTER BLINATUMUMAB OR CARTCD19 THERAPY

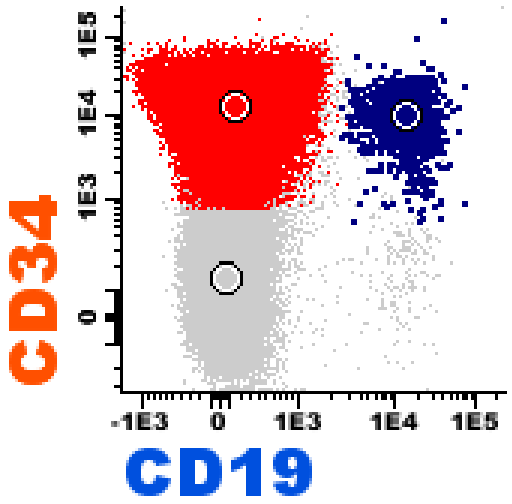


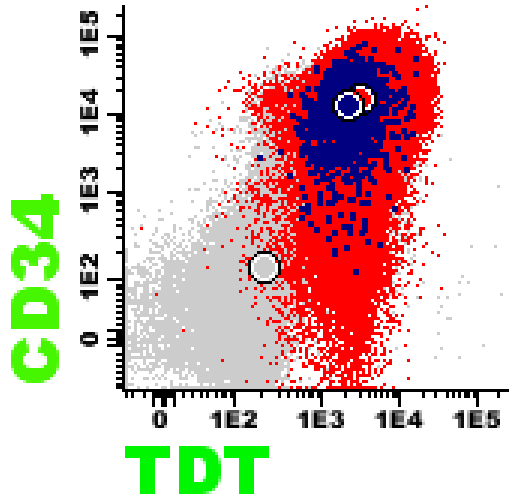


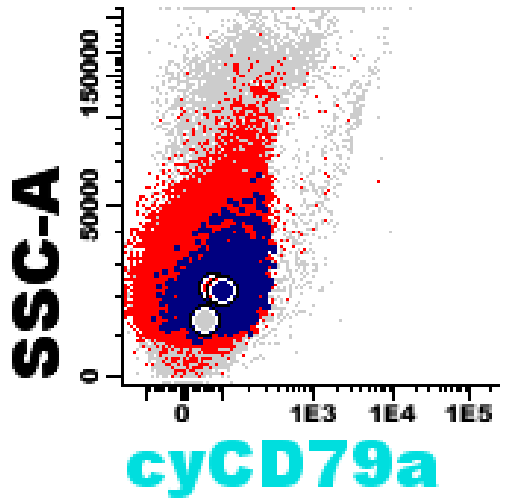












Orfao A et al. (unpublished observations)

FREQUENCY OF CD19-negative RELAPSES IN B CELL PRECURSOR (BCP) ALL

Trials	Treatments	Number of patients	Number of patients with CR	Number of patients with relapse	Number of patients with CD19− or CD22− relapse
Max S. Topp (2014) [27]	Blinatumomab	36	25	10	3
Arend von Stackelberg (2016) [28]	Blinatumomab	49+44	7+27	15	4
Elias Jabbour (2017) [30]	Blinatumomab	68	16	–	5
Ibrahim Aldoss (2017) [31]	Blinatumomab	65	27	20	5
E. Mejstríková (2017) [29]	Blinatumomab	70	27	19	4
Shannon L. Maude (2014) [32]	CAR-T (CD19)	30	27	7	3
Daniel W Lee (2015) [33]	CAR-T (CD19)	21	14	–	2
Cameron J. Turtle (2016) [34]	CAR-T (CD19)	30	29	11	2
Vinodh Pillai (2019) [35]	CAR-T (CD19)	166	155	67	39
Hanren Dai (2020) [36]	CAR-T (CD19/CD22)	6	6	3	1
Jing Pan (2020) [37]	CAR-T (CD19)	68	66	12	7
Terry J. Fry (2018) [38]	CAR-T (CD22)	21	12	8	7 (diminished CD22)

CD19- relapses: 68/166 (41%)

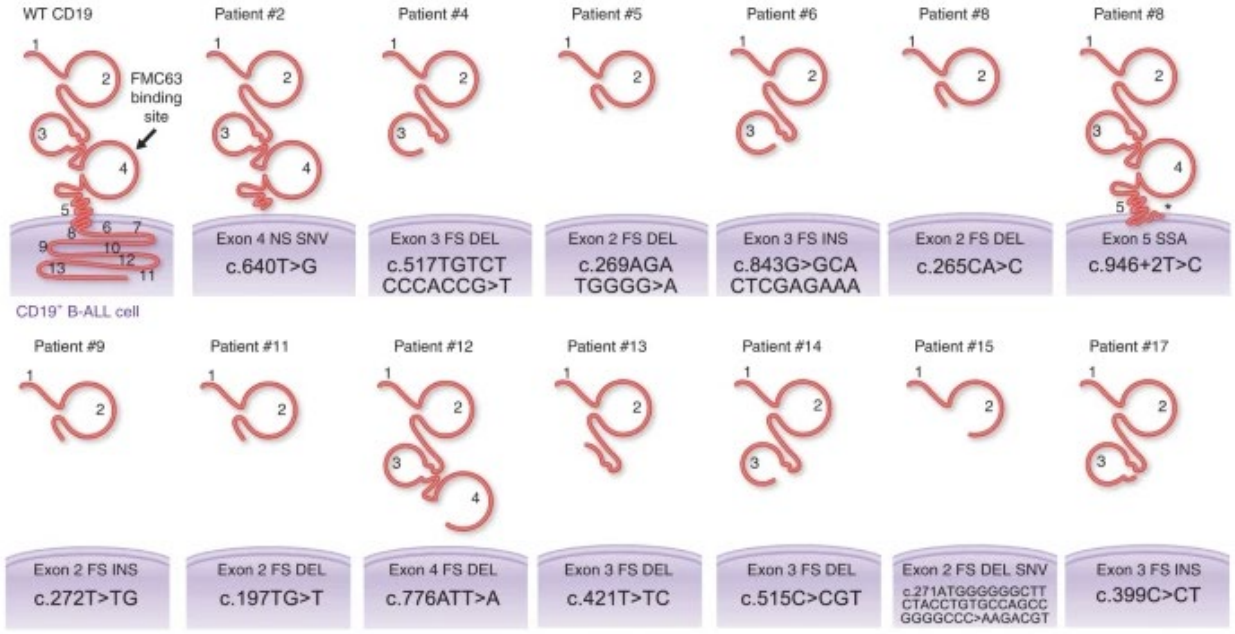
CD22^{lo} relapses: 7/11 (64%)

Liu et al, Int J Haematol 2021; 113: 337-343

CD19-negative relapses in BCP-ALL after treatment

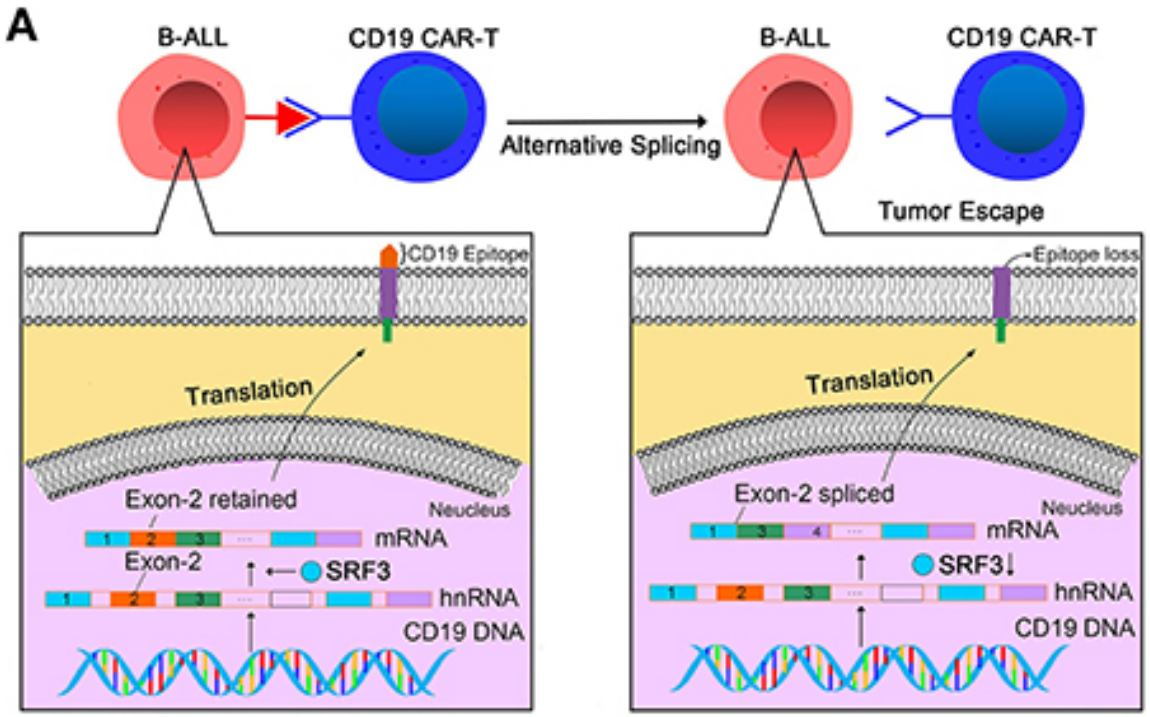
10–20% of B-ALL patients developed **CD19-negative relapse** after CD19 CAR T-cell treatment

Loss of exon 2 is the main cause of CD19-negative relapse, thereby lacking the CD19 epitope recognized by FMC63 of CAR-T cells



Orlando EJ et al. Nature Medicine 2018, 24; 1504:1506

SRF3: splicing factor whose function is to retain exon 2

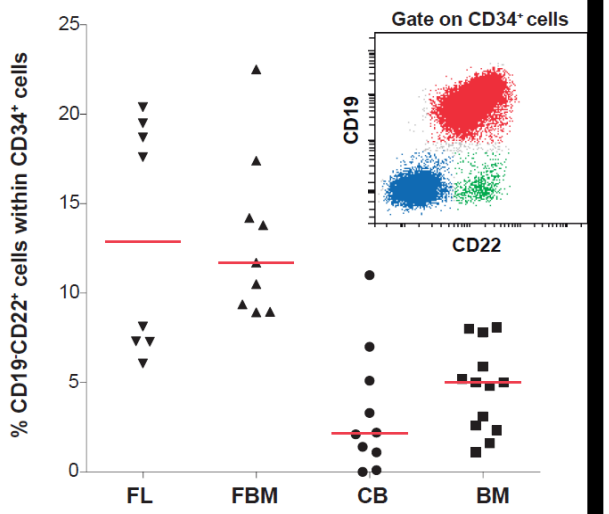


Xinjie X et al. Front. Immunol. 2019, 10:2664

CD19-negative LEUKEMIA BCP-ALL CELLS AT DIAGNOSIS AND DURING FOLLOW-UP AFTER THERAPY

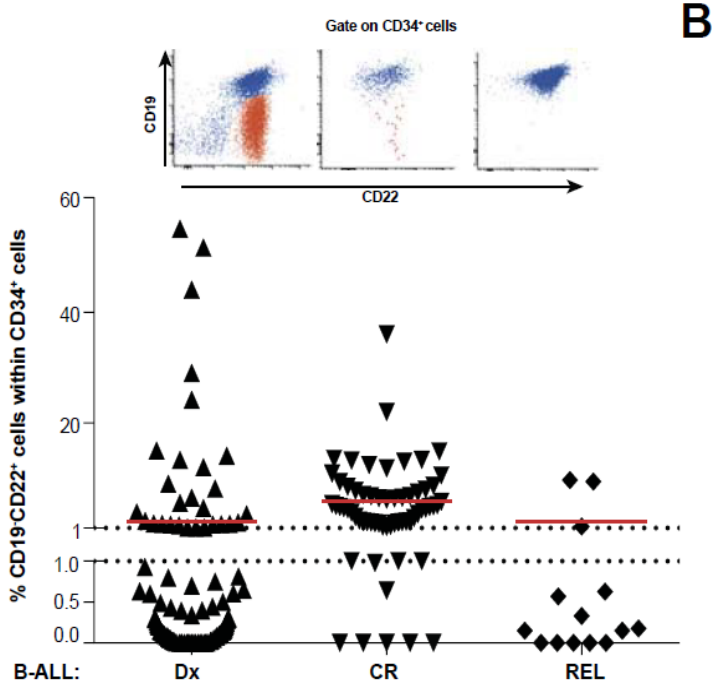
Normal B cell development/production

F



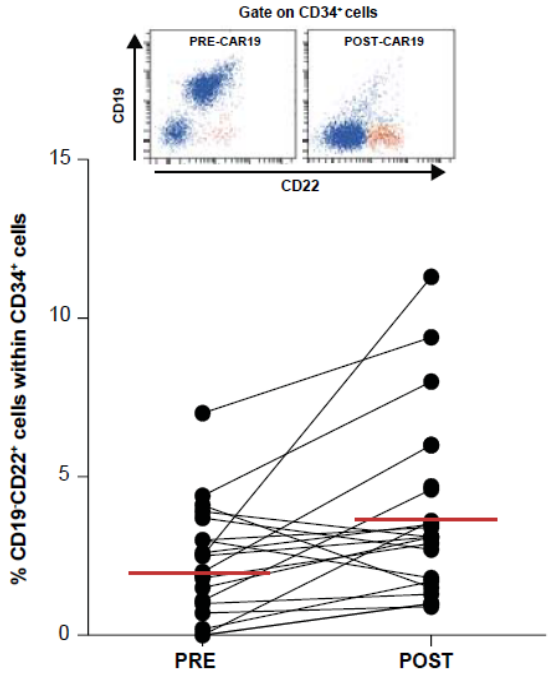
	FL	FBM	CB	BM
+ve / total (%):	8/8(**100%**)	9/9 (**100%**)	8/10 (**80%**)	13/13 (**100%**)
Mean ± SD:	(13.1± 6.4)	(13 ± 4.5)	(3.3 ± 3.4)	(4.6 ± 2.4)
Range:	(6-20)	(9-22)	(0-11)	(1.1-8)

CD19- CD22+ Leukemia BCP-ALL cells



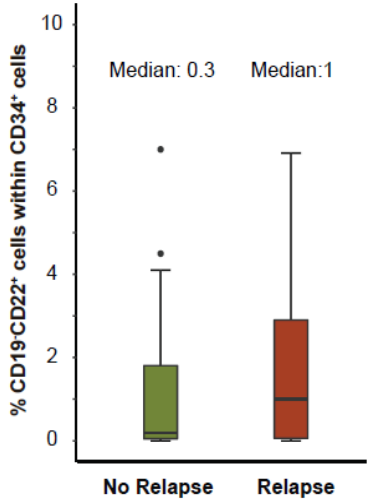
	Dx	CR	REL
+ve/ total (%):	114/159 (**72%**)	58/63 (**92%**)	10/15 (**66%**)
Mean ± SD:	(2.1±7.6)	(6.1±17.7)	(1.6±3.4)
Range:	(0-54)	(0-36)	(0-9.6)

B



B-ALL Patients treated with CAR19-T cells

C



Small subsets of CD19- BCP-ALL leukemia cells exist which might be responsable for tumor escape to CD19-targeted antibody and CART-cell therapies.

Bueno et al, Blood 2022

Pre-defined antibody panels for MRD monitoring in BCP-ALL

BIOMED1 5-antibody combinations (3-color-FITC/PE/PECy5):

TdT/CD10/CD19
CD10/CD20/CD19
CD34/CD38/CD19
CD34/CD22/CD19
CD19/CD34/CD45

Lucio et al, Leukemia, 2001; 15: 1185-92

COG 2-antibody combinations (4-color):

CD20-FITC
CD10-PE
CD45-PerCP
CD19-APC

CD9-FITC
CD34-PE
CD45- PerCP
CD19-APC

Borowitz et al, Blood, 2008; 111: 5477-85

DCOG BCP-ALL 2-antibody combinations (4-color):

CD34-FITC
CD19-PE
CD45-PerCP
CD22-APC

Tdt-FITC
CD19-PE
CD20-PerCP
CD10-APC

DCOG BCP-ALL 2-antibody combinations (6-color):

CD58-FITC
CD19-PE
CD45-PerCP
CD10-APC
CD22-PECy7
CD34-APCCy7

Tdt-FITC
CD19-PE
CD45-PerCP
CD10-APC
CD38-PECy7
CD20-APCCy7

Denys et al, Leukemia, 2013; 27: 635-41

COG 2-antibody combinations (6-color):

CD20-FITC
CD10-PE
CD38- PerCPCy5.5
CD58-APC
CD19-PECy7
CD45-APCH7

CD9-FITC
CD13+133-PE
CD34PerCPCy5.5
CD10-APC
CD19-PECy7
CD45-APCH7

Borowitz et al, Blood, 2015; 126: 964-71

SBTMO Consensus ALL MRD panels

Table 1 – Fluorochrome conjugated antibody panels for MRD detection in BCP-ALL by using 4-color multiparametric flow cytometry.

	FITC	PE	PerCP-Cy5.5	APC
Essential tubes				
1	CD20	CD10	CD34	CD19
2	CD45	CD66c/CD123	CD34	CD19
3	CD38	CD19	CD34	CD81
Recommended tube				
4	CD20/CD45	CD73/CD304	CD34	CD19
Optional tube[a]				
5	CD15/CD65	NG2 (7.1)	CD34	CD19

Abbreviations: FITC: fluorescein isothiocyanate; PE: phycoerythrin; PerCP-Cy5.5: peridinin chlorophyll protein/cyanin5; APC: allophycocyanin.
[a] According to antigen expression at diagnosis.

Table 2 – Fluorochrome conjugated antibody panels for MRD detection in T-ALL by using 4-color multiparametric flow cytometry.

	FITC	PE	PerCP-Cy5.5	APC
Essential tubes				
1	CD45	SmCD3	cyCD3	CD7
2	NuTdT	cyCD3	CD5	CD7
3	CD7	CD99	cyCD3	CD1
Recommended tubes[a]				
4	CD7	CD10+CD117	cyCD3	CD45RA
5	CD7	cyCD3	CD34	CD13+CD33
6	CD44	CD7	cyCD3	CD56

Abbreviations: FITC: fluorescein isothiocyanate; PE: phycoerythrin; PerCP-Cy5.5: peridinin chlorophyll protein/cyanin5; APC: allophycocyanin.

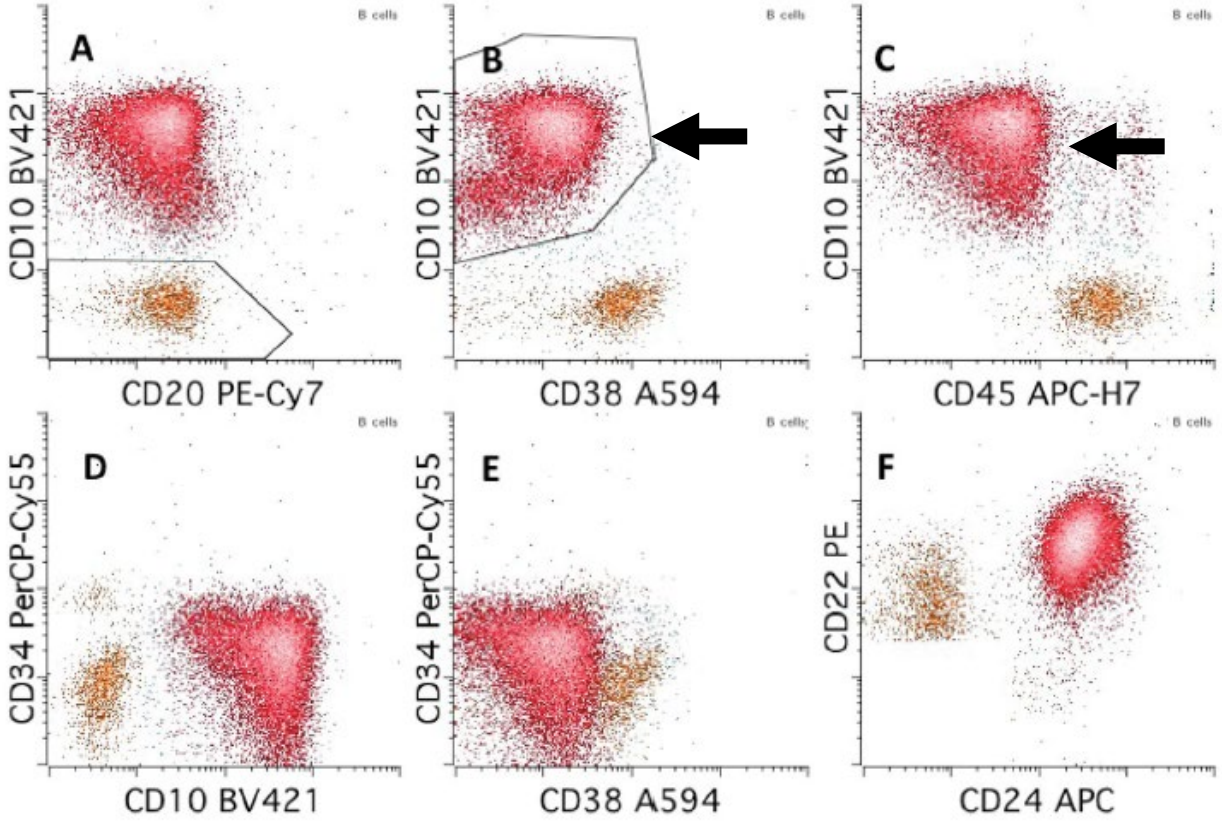
Ikoma-Colturato et al, Hematol Tranf Cell Ther, 2021; 43: 332-40

Progressive increased number of colors and backbone markers… but, with a few exceptions, no validation against other methods

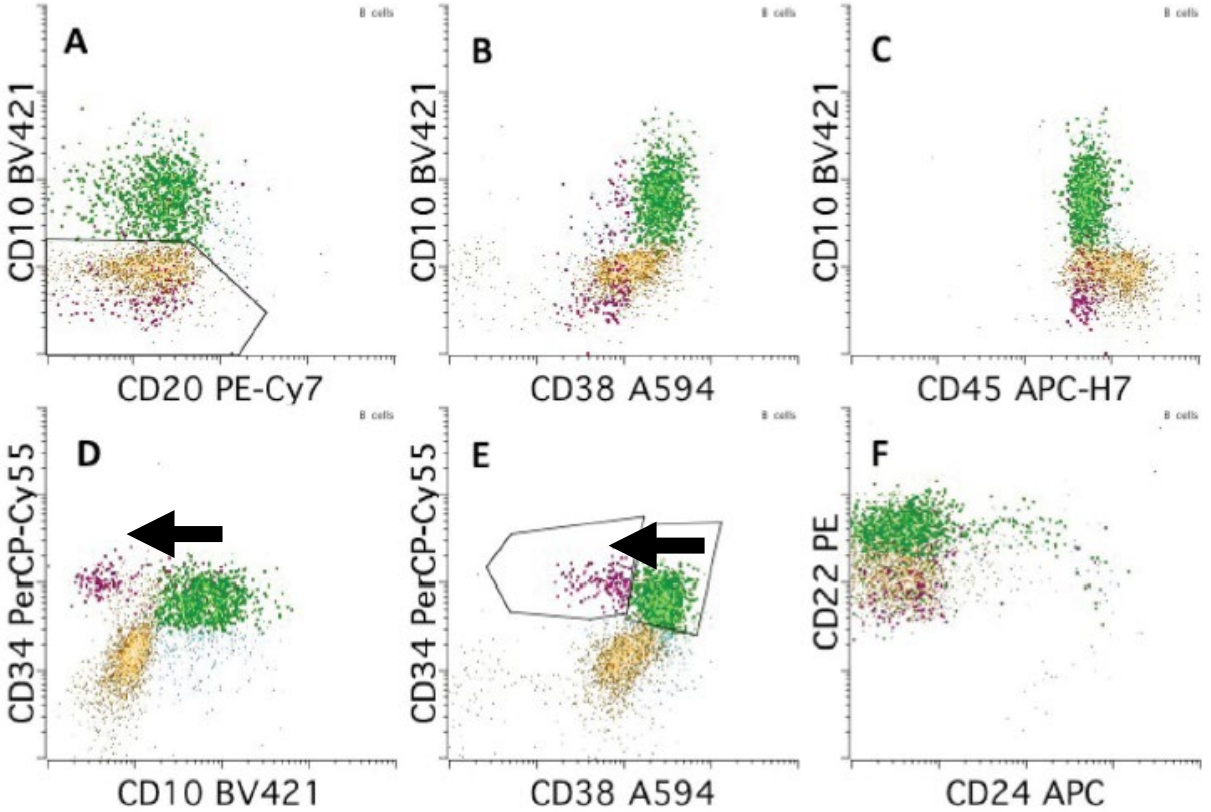
EuroFlow

IDENTIFICATION OF CD19-negative AND CD19+ NORMAL VS LEUKEMIC B CELL PRECURSORS

MRD+ Post-chemotherapy



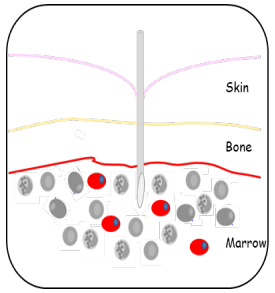
MRD+ Post-CD19 targeted therapy



Cheridan & Stetler-Stevenson, Curr Prot Cytometry 2018; 86: e44

MRD detection in adult ALL by Next Generation Flow (N=300)

BM sample



1st pull of BM aspirate

Sample preparation & staining

-Add lysing buffer to 0.3 to 3mL of sample containing >10 x 10^6

↓

-Incubate **>10 x 10^6 cells** in 100µL for 10 min (max volume 250 µL)

↓

-Wash with 2 mL PBS (1x)

↓

-Stain with antibody mixture

↓

-Add 2mL FACSLyse & incubate for 15 min

↓

-Wash with PBS (2x)

↓

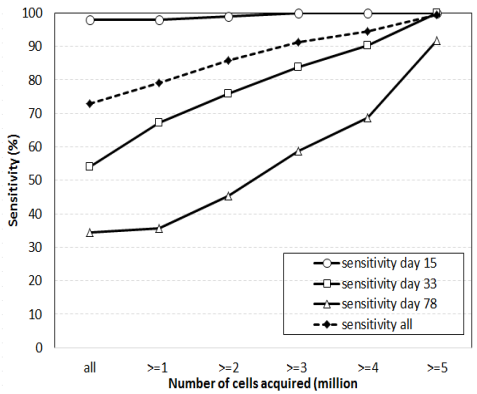
-Measure **>10x10^6 cells in 2 tubes** by FCM

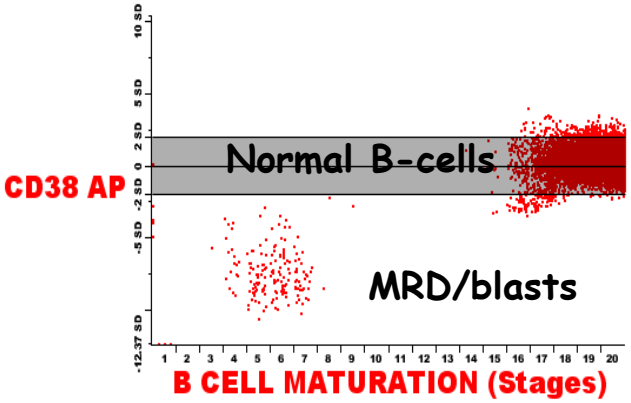
8-color EuroFlow antibody staining panels For BCP-ALL and T-ALL

PB	PO	FITC	PE	PerCPCy5.5	PE Cy7	APC	APCC750
CD20	CD45	CD81	CD66c/ CD123	CD34	CD19	CD10	CD38
CD20	CD45	CD81	CD73/ CD304	CD34	CD19	CD10	CD38

FCM Data acquisition and analysis

>10^7 BM cells measured
(2 tubes with >5 x 10^6 cells/tube)





Theunissen P, et al. Blood 2017, 129: 347-357

EuroFlow

EUROFLOW NGF MRD ALL PANEL
FOR DETECTION OF CD19-negative LEUKEMIA CELLS

Recommendation: make a reference image of the different cell populations in the normal bone marrow, including regenerating B-cell precursors, mature B cells, myeloid progenitors, basophils and erythroid precursors as a reference for normal expression patterns

Generate dot plot diagrams:
FSC-A/FSC-H
FSC-A/ SSC-A
CD45/SSC-A
CD10/CD20
CD34/CD20
CD38/CD45
plot every marker vs. CD10
clustering: e.g. APS or CA

Eliminate Doublets by FSC-A /FSC-H

In FSC-A/SSC-A gate on nucleated cells by roughly excluding debris (to be refined after identification of blasts)

Remove aggregates

Visually check for CD10 expression in CD10/SSC-A

Gate CD10+/++ cells with low / int SSC

Check for aberrant antigen expression / clustering of events in comparison to normal B-cell precursors and refine BCP-ALL population

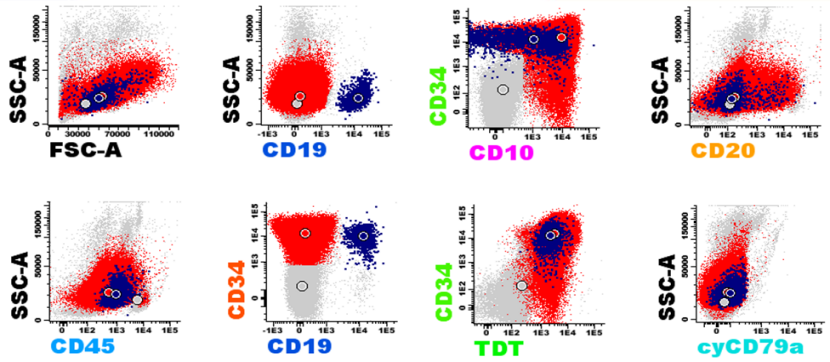
- Distribution of CD10/CD20 in combination with expected expression levels of CD34 and CD45
- Aberrant positivity of CD66c/CD123 or CD73/CD304
- Under-/overexpression of CD38 or CD81
- Increased SSC

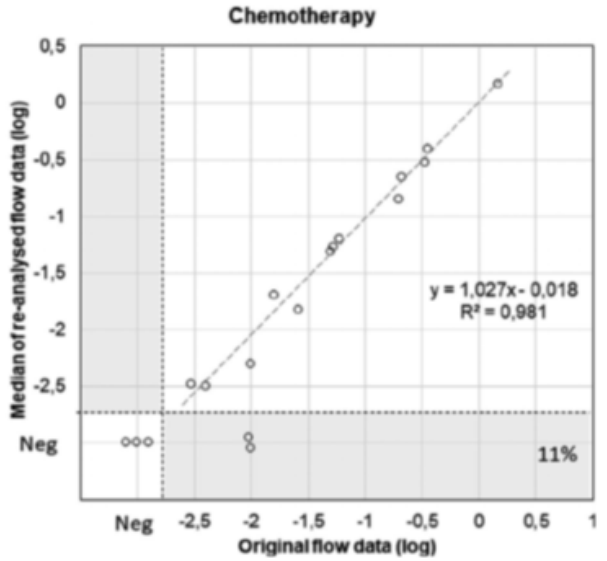
Gate CD10- cells with low SSC Check both CD34+ and CD34- populations

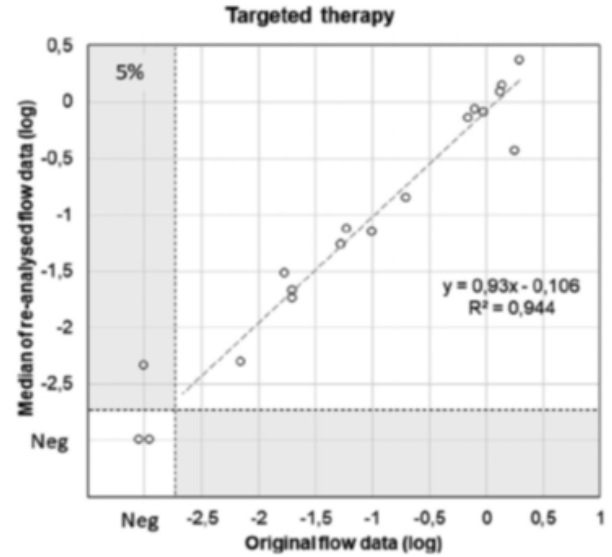
Check for clustering to discriminate from myeloid progenitors and refine BCP-ALL population

- Low SSC
- Aberrant positivity of CD66c/CD123 or CD73/CD304
- Under/-overexpression of CD38 or CD81

Back gate on CD45/SSC-A and FSC-A/SSC-A for uniform clustering of the BCP-ALL population



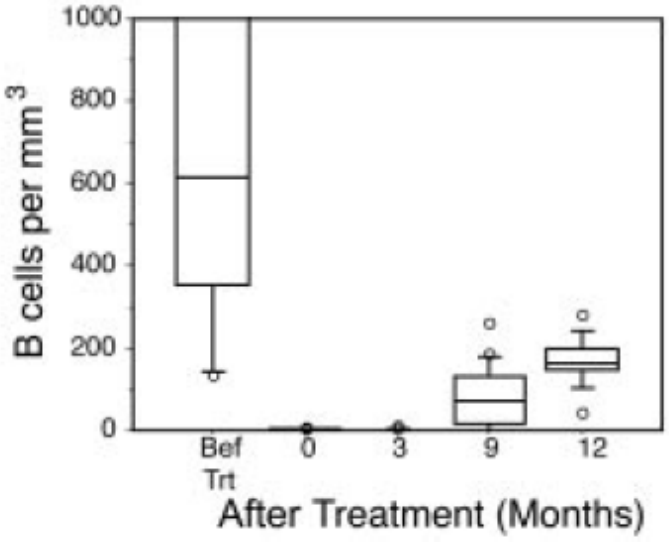


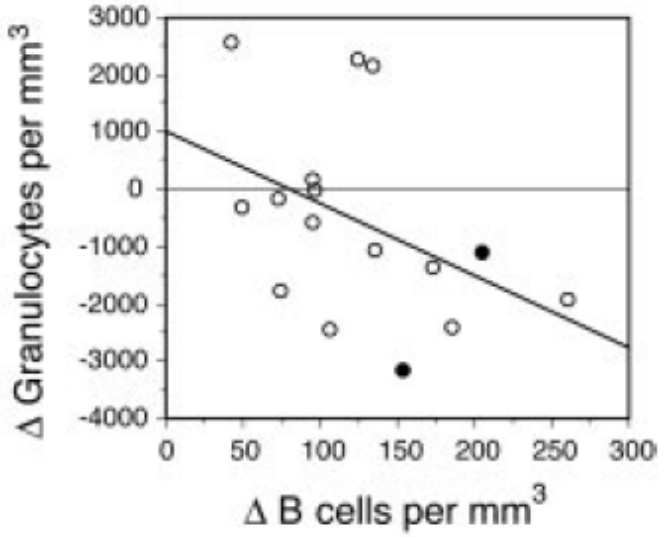


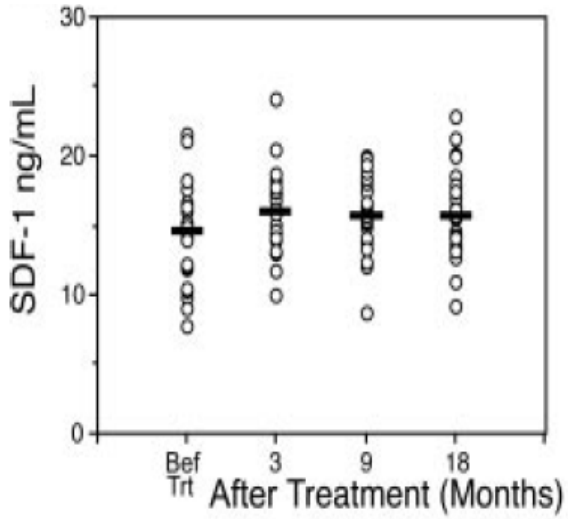
EuroFlow

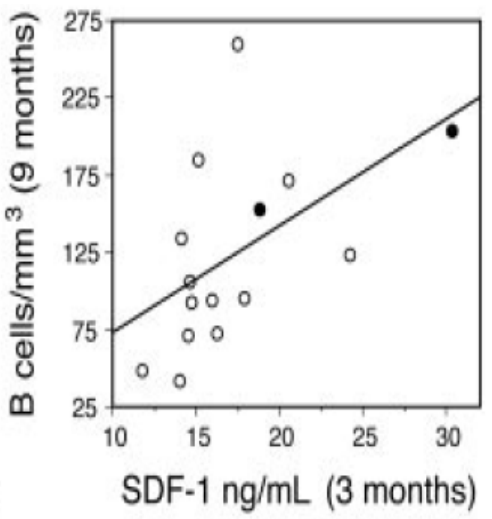
Verbeek et al, Br J Haematol 2021; Bueno et al, Blood 2022

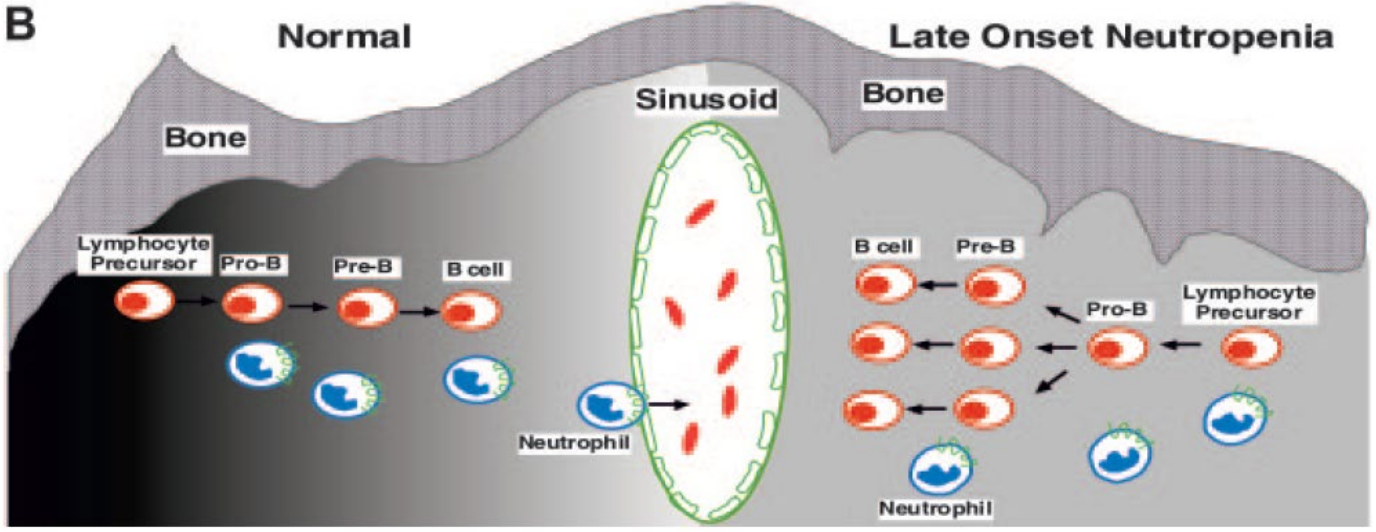
ANTI-CD20 (Rituximab) TARGETED ANTIBODY THERAPY







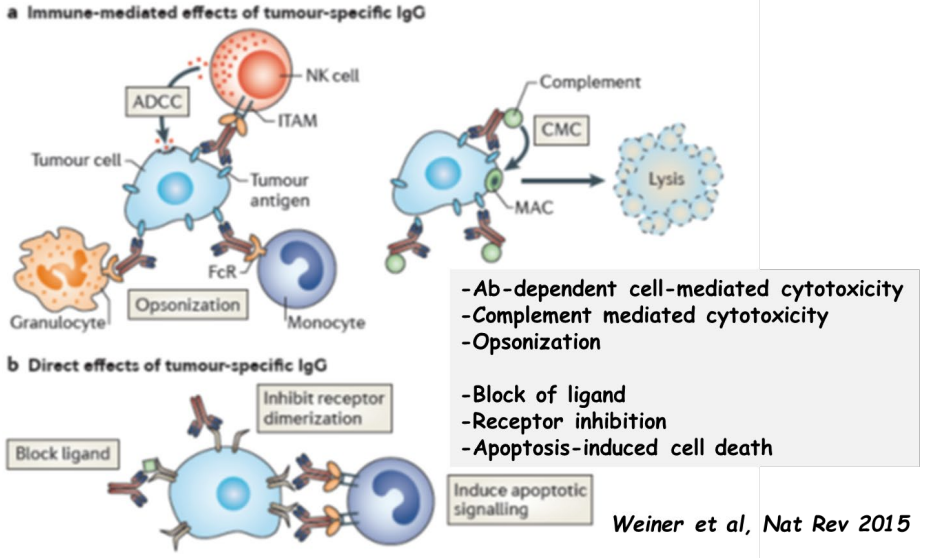




Recovery of B-cells retards neutrophil egression from BM to blood leading to delayed neutropenia after Rituximab

Dunleavy et al, Blood, 2005, 106: 795-802

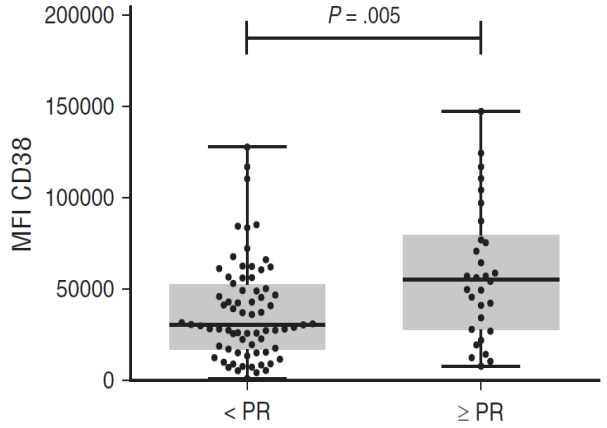
CD38-Targeted Antibody Therapy



-Ab-dependent cell-mediated cytotoxicity
-Complement mediated cytotoxicity
-Opsonization

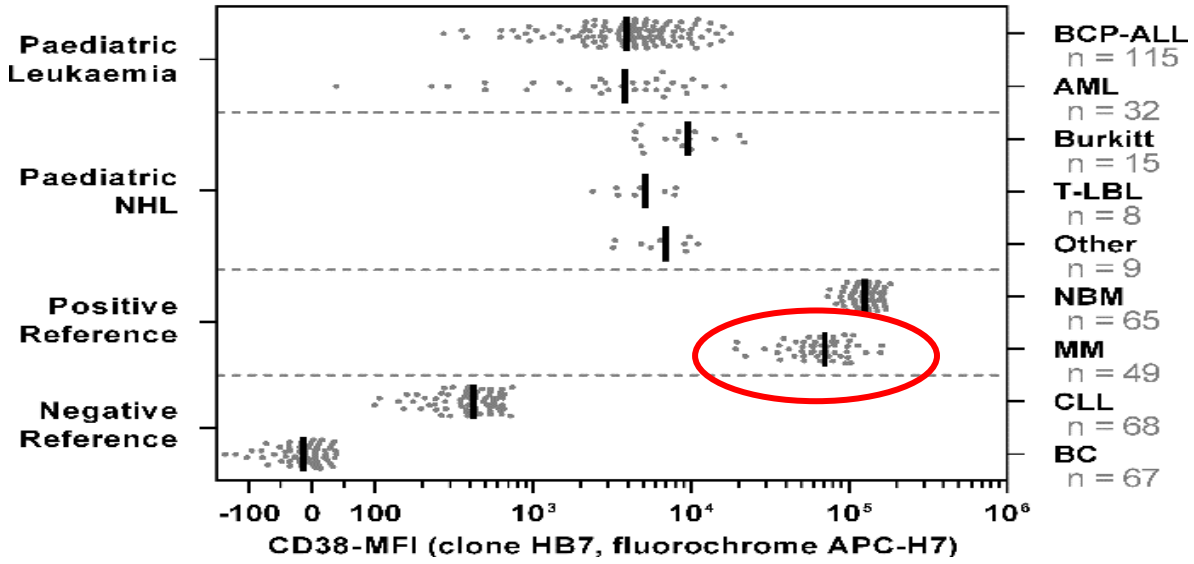
-Block of ligand
-Receptor inhibition
-Apoptosis-induced cell death

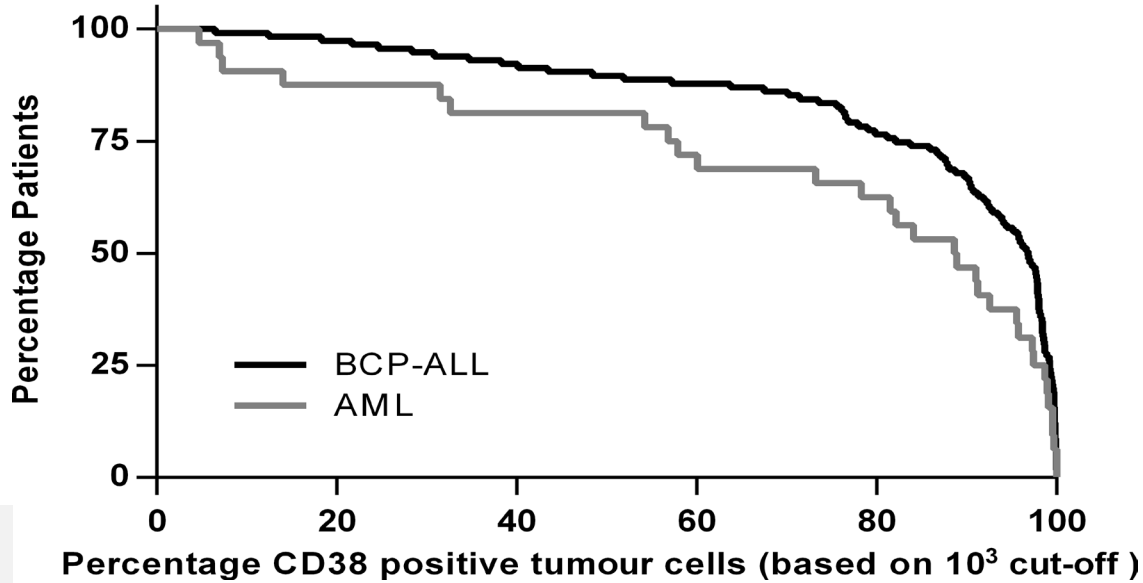
Weiner et al, Nat Rev 2015



'Response to the CD38-targeting antibody daratumumab is significantly associated with CD38 expression levels on the tumor cells'

Nijhof et al, Blood 2016;128:959-970



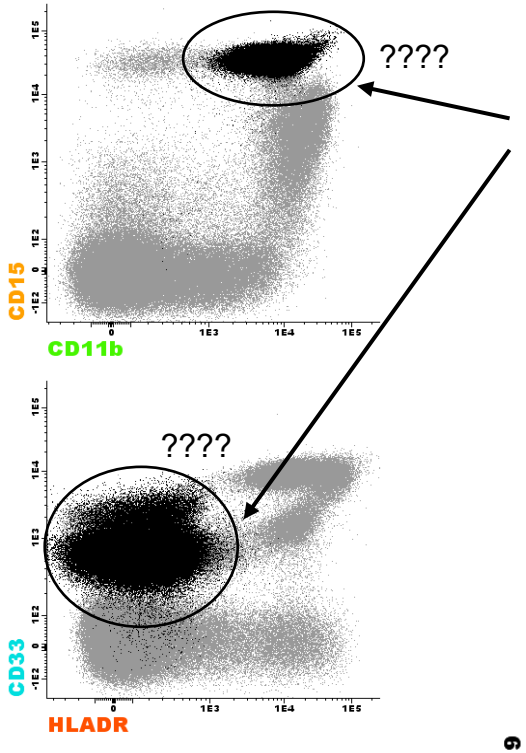


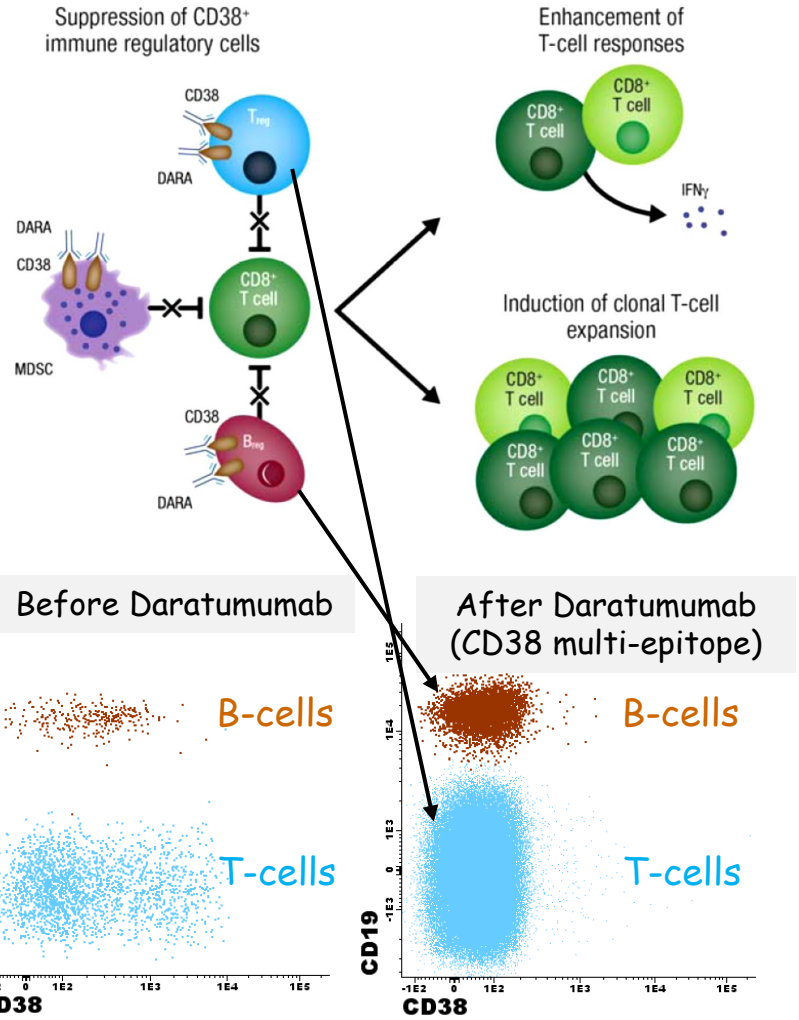
Bras et al, British Journal of Haematology 2016

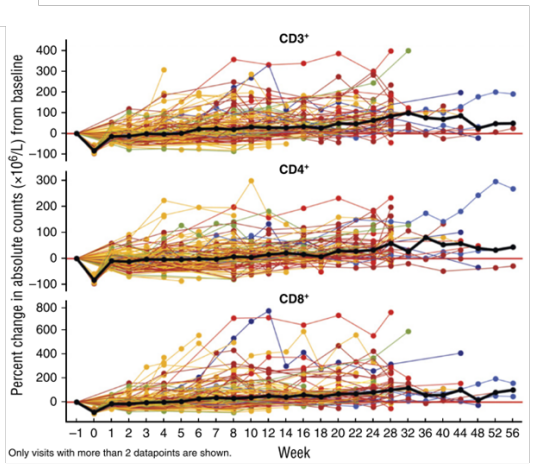
CD38+ regulatory T and B cells, and MDSCs are sensitive to Daratumumab treatment

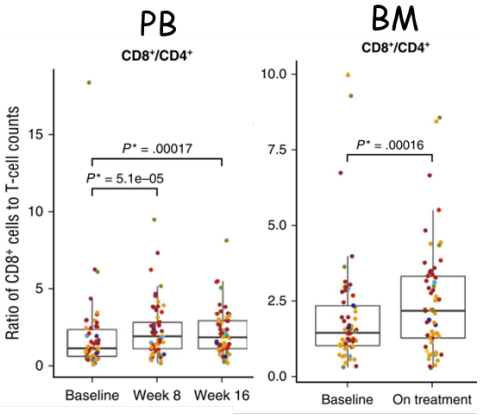
G-MDSCs phenotype in the literature[1]

$CD11b^+$ $CD14^-$ $CD15^+$ $CD33^+$ $HLADR^-$



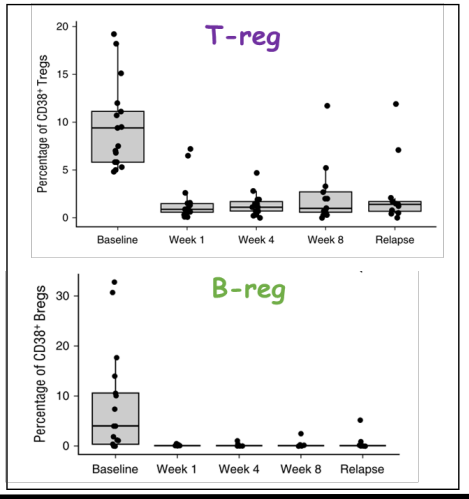






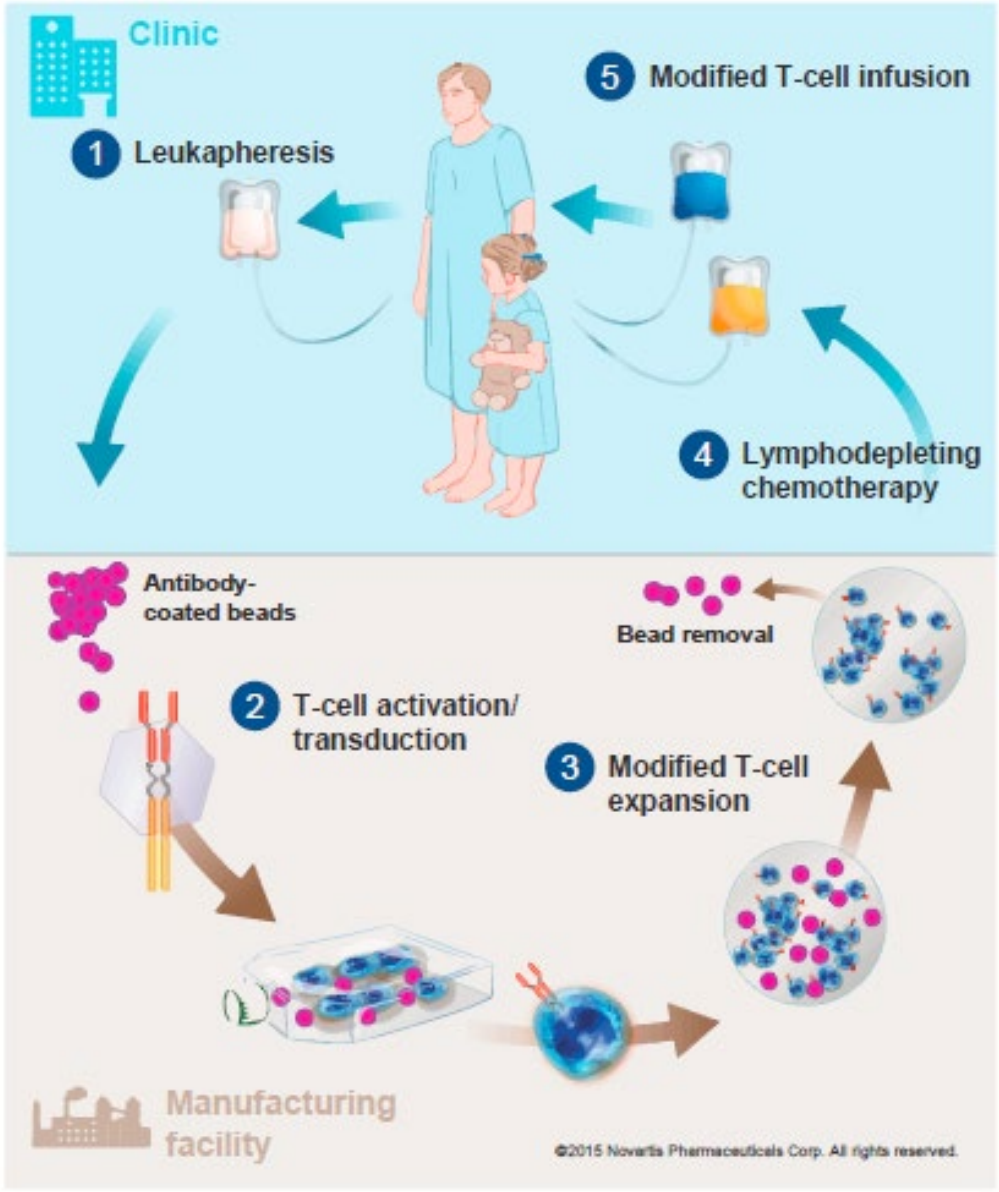
The black bold line shows the overall median percent change over time

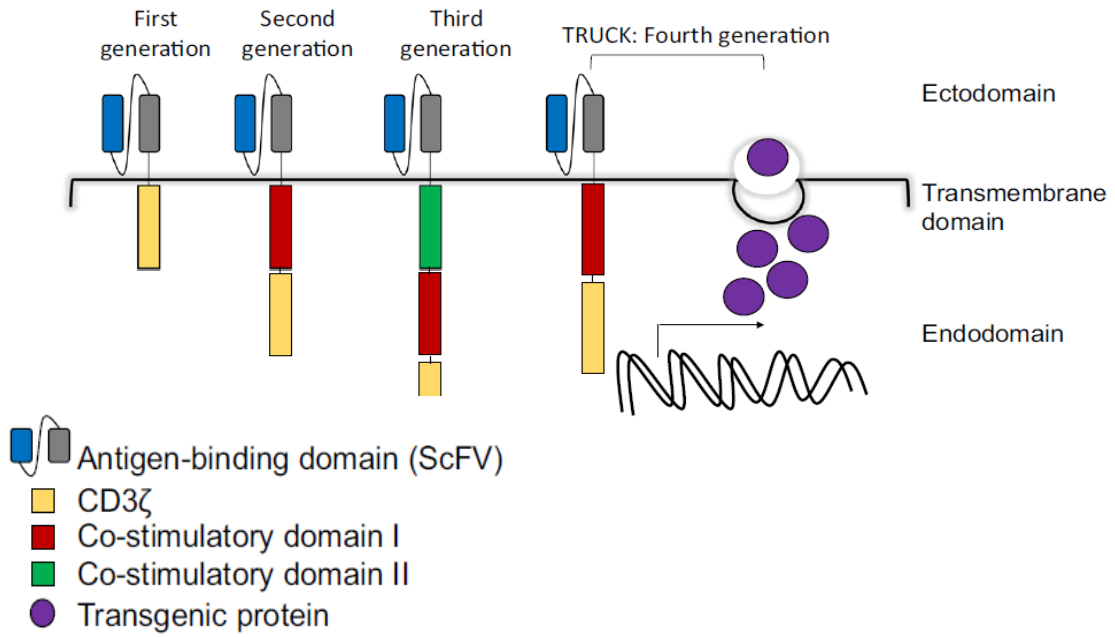
Daratumumab also eliminates the subset of **CD38 positive** T-reg cells and B-reg cells



Krejcik J, et al. Blood. 2016;128:384-94

CAR T-cell production: general scheme

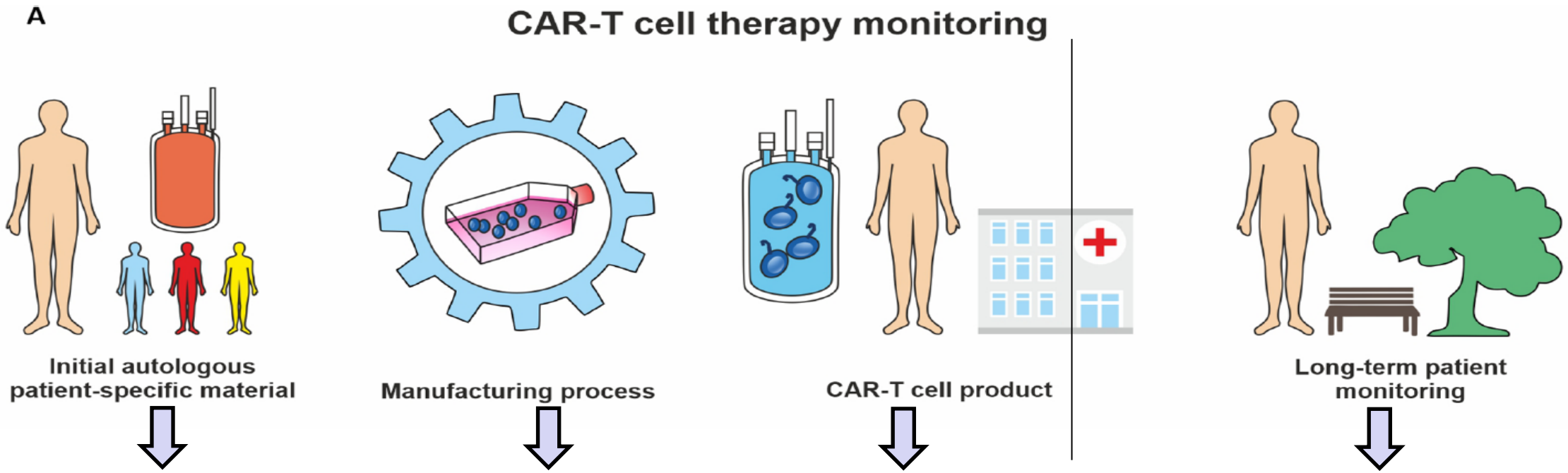




Tyagarajan et al, Meth Ther Meth Clin Develop 2020 16: 136-144

Teho & Chng, Blood Cancer J 2021, 11, 84: 1-18

When and how to monitor CAR targeted therapies?

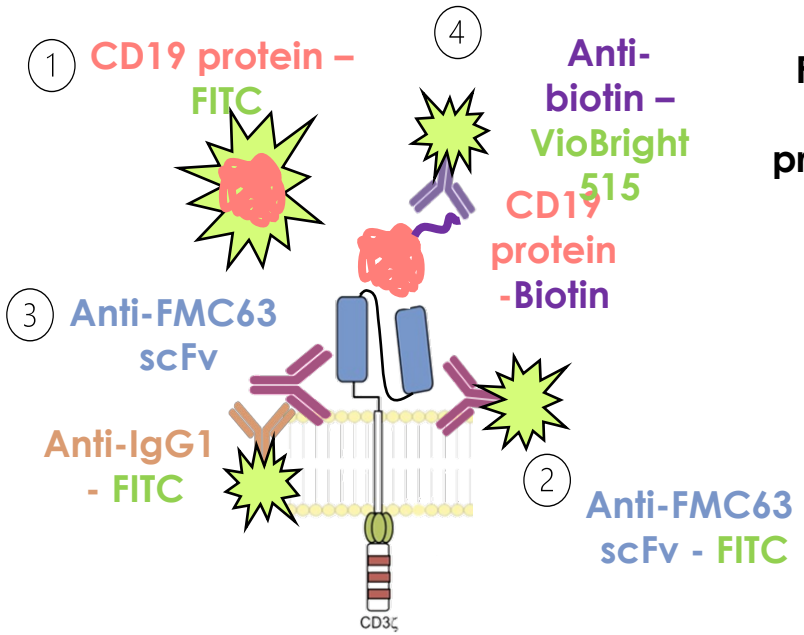


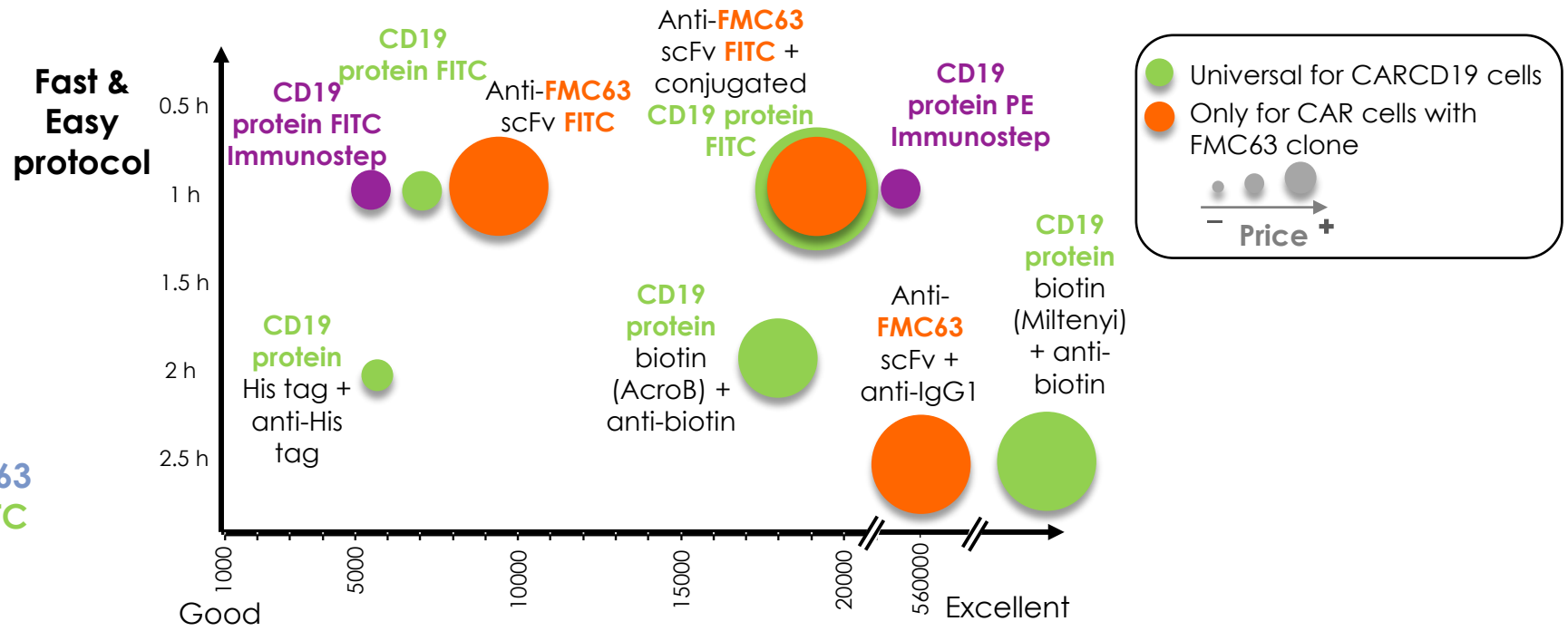
Pre-CART cell monitoring → Early + late post-CART monitoring

Need to monitor CART cell kinetics and clearance vs persistence for long-term disease control vs toxicity

Adapted from Blache et al, Front Immunol 2021; 12: 658314: 1-17

Monitoring CD19 CAR-T cells by flow cytometry: advantages and limitations of the different approaches





Advantages and limitations of CAR-T cell reagents

	Direct staining			Indirect staining		
Features	FITC CD19 protein	FITC anti-scFv FMC63	Conjugated CD19 protein + FMC63 (both in FITC or FMC63 FITC + CD19p PE)	CD19 protein His tag + anti-His tag	CD19 protein biotin + anti-biotin	anti-scFv FMC63 IgG1 + anti-IgG1
Target	**Universal** for all CD19 CAR-T	Specific for CAR-T with clone FMC63	Specific for CAR-T with clone FMC63	**Universal** for all CD19 CAR-T	**Universal** for all CD19 CAR-T	Specific for CAR-T with clone FMC63
CAR-T identification	Good	Very good	Very good but no signal improvement over FMC63 alone	**Poor**	Excellent	Excellent
Time consuming	Short	Short	Short	Acceptable	Acceptable	**Long**
Cost	Affordable	**Expensive**	**Expensive**	Affordable	Acceptable	**Expensive**
Allows binding capacity evaluation	**Yes**	No	**Yes**	**Yes**	**Yes**	No

Monitoring of CAR T-cell therapy in DLBCL:
Targeted cell populations

- Monitoring of infused (CAR) immune cells via cell surface and/or intracellular markers

- Monitor CAR T-cell composition

- Monitor CAR-therapy associated immune responses:

    - Innate immune cells
    - CD4+ T-cell subsets
    - Cytotoxic T and NK cell populations
    - Maturation-associated B-cell and plasma cell compartments

- Monitor persistence of circulating tumor cells (CTCs)

i.e. Monitoring of MRD levels and the residual immune cells (in blood) might contribute to understand the mechanisms involved in immune-escape and treatment failure.

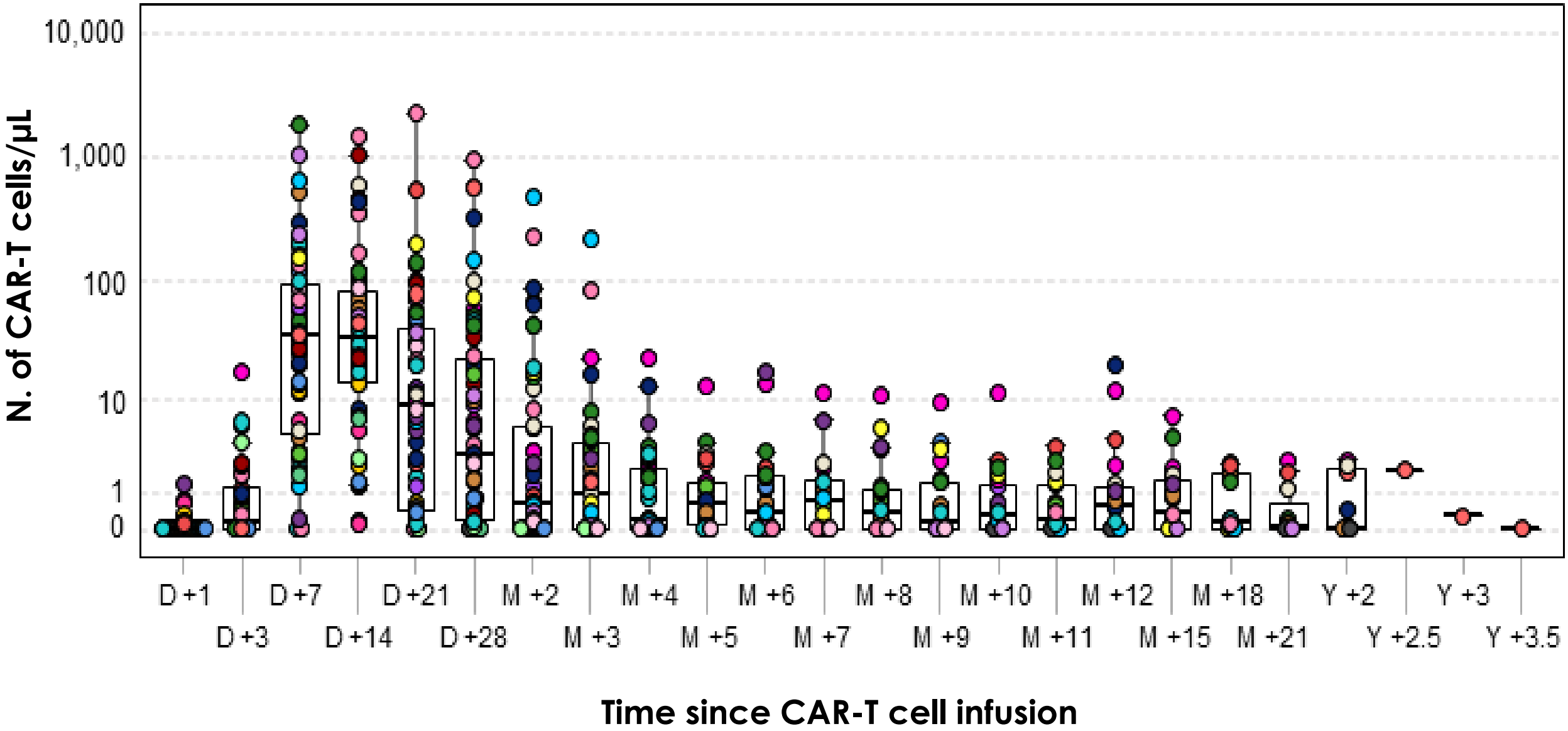
Monitoring of CAR T-cell therapy in DLBCL:
Targeted cell populations

- Monitoring of infused (CAR) immune cells via cell surface and/or intracellular markers

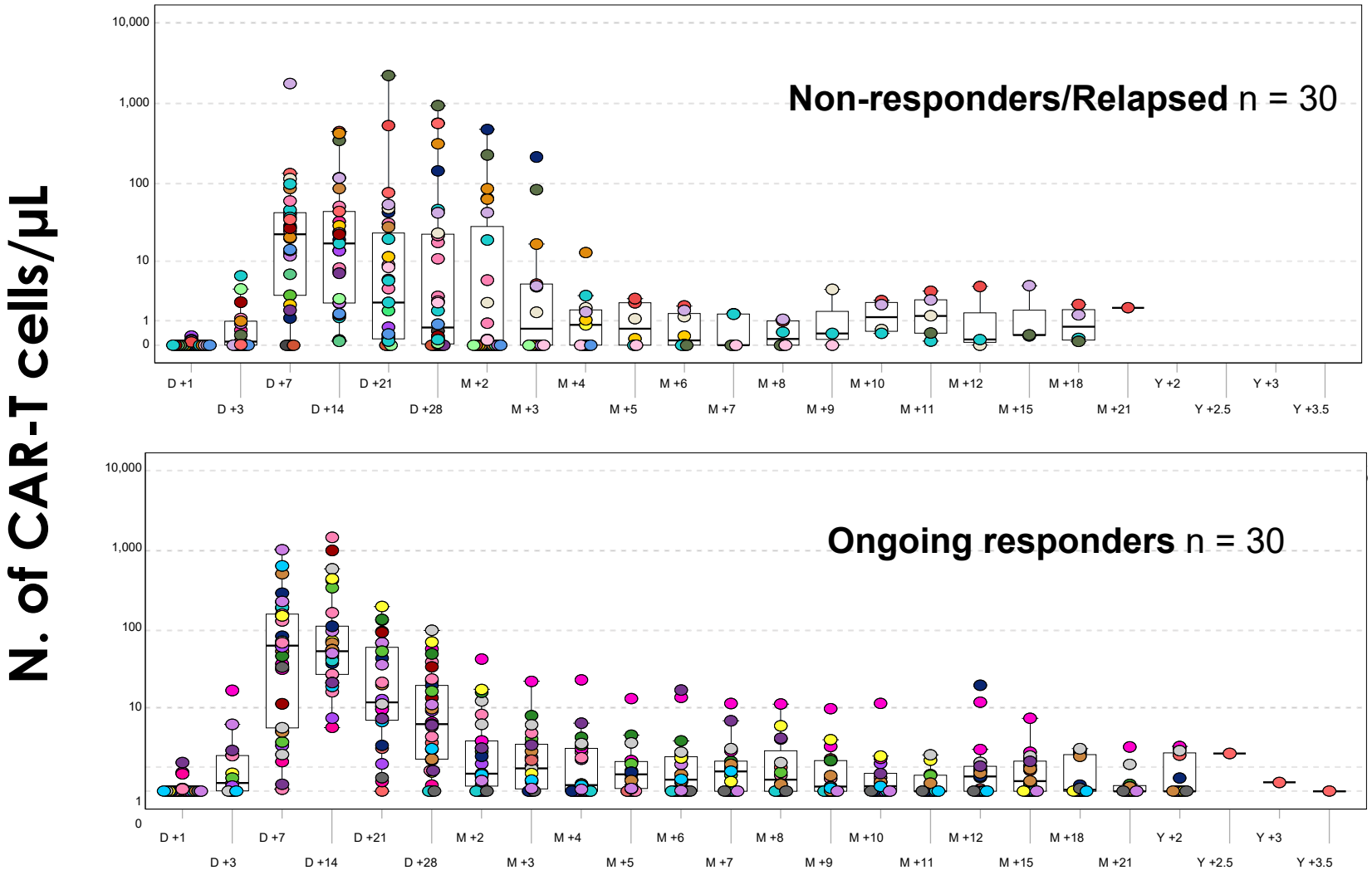
N. of CART cells expanded in vivo after infusion

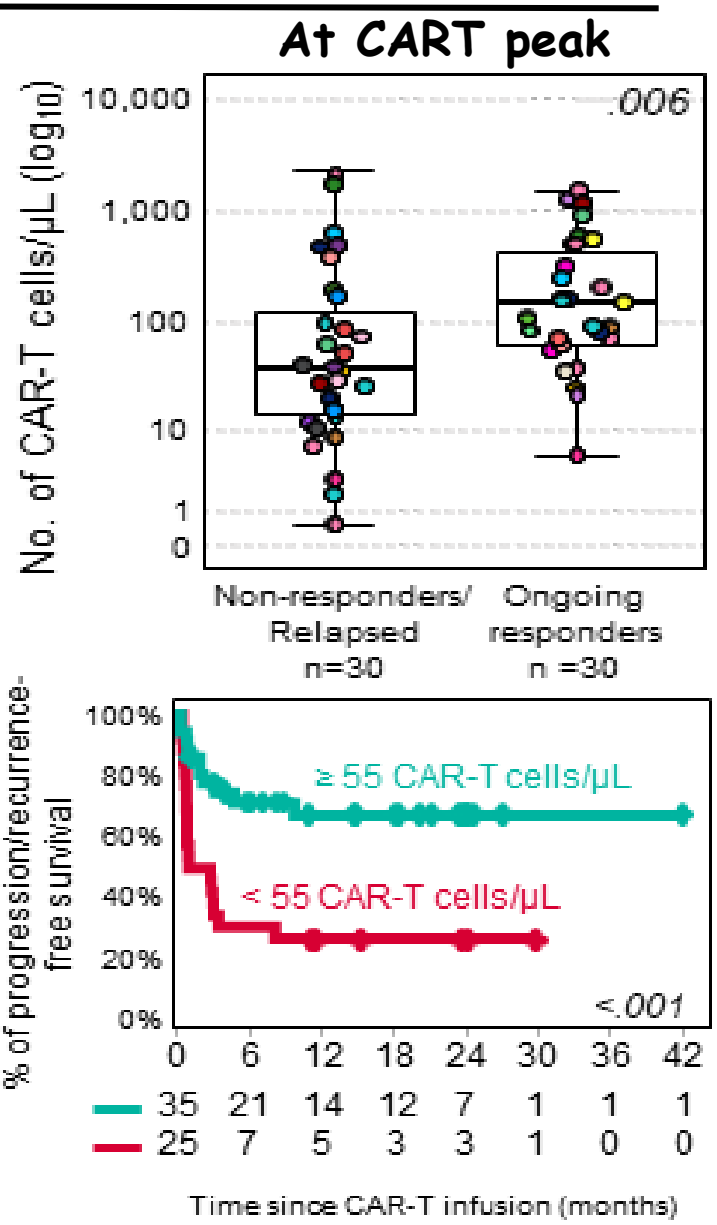
i.e. Monitoring the levels of a drug with highly variable composition and IC50 concentration in an individual patient basis

In vivo kinetics CAR-T cells in DLBCL (n=60)

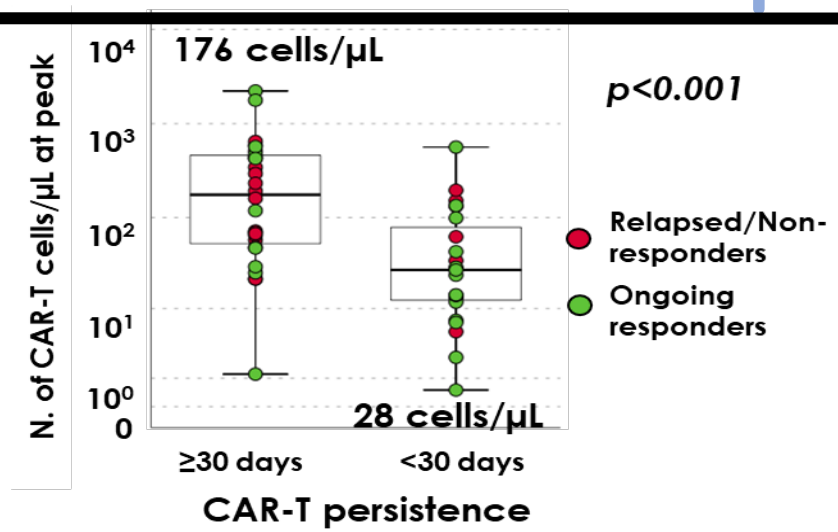


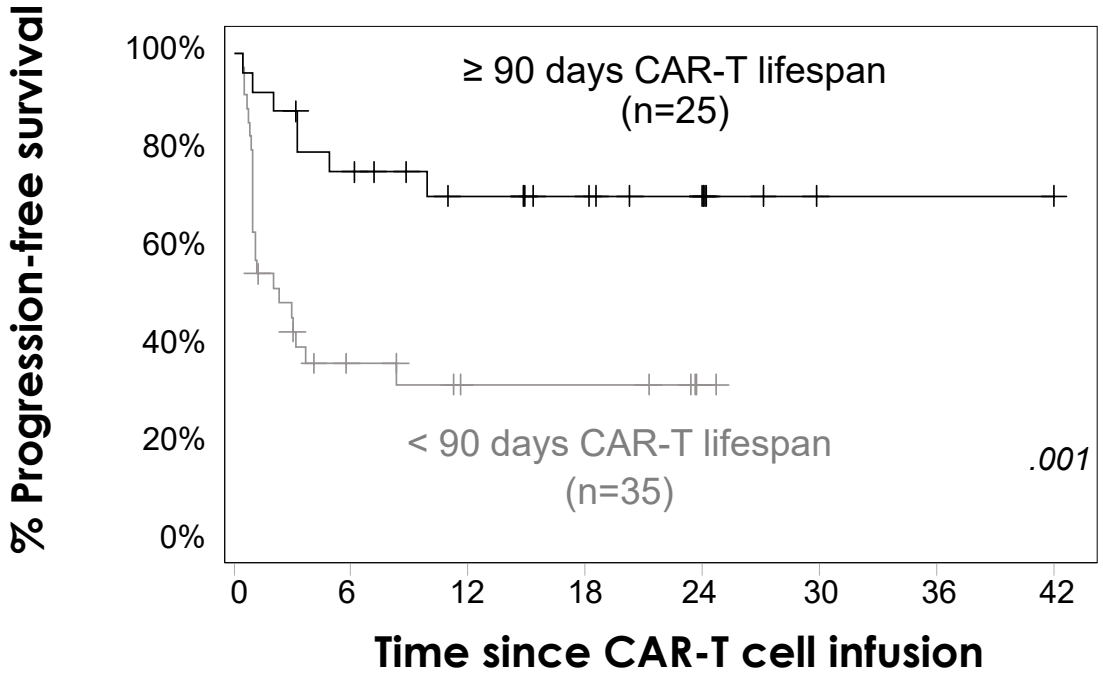
In vivo kinetics CAR-T cells in DLBCL: association with response to therapy

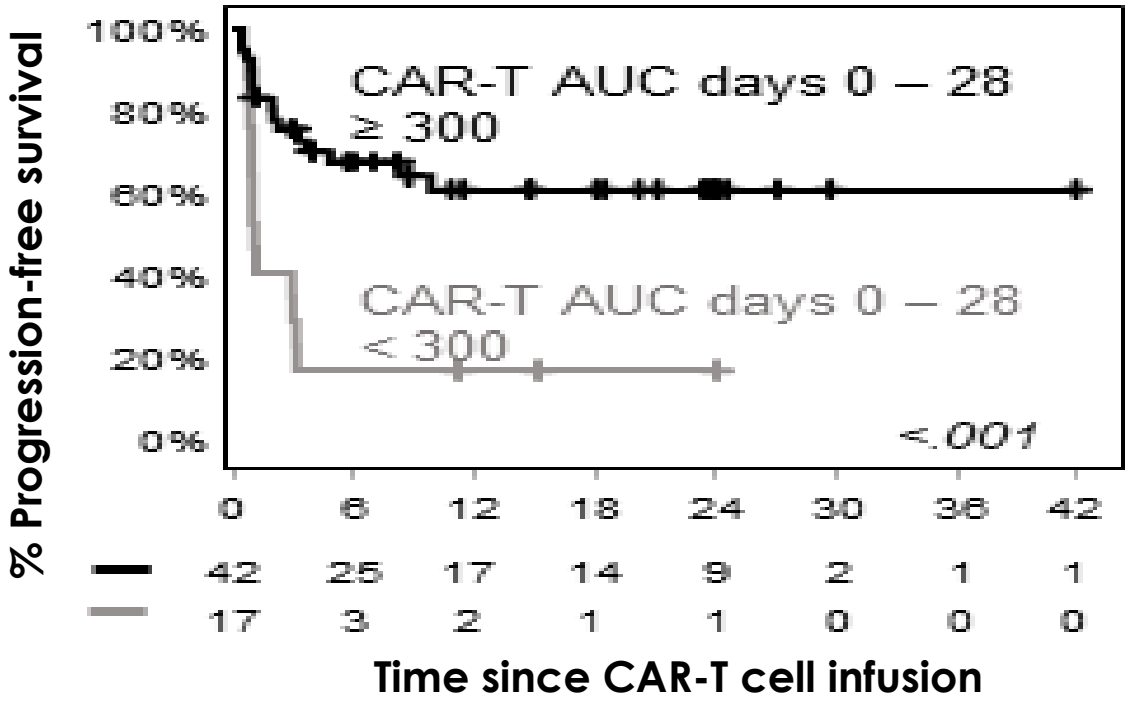




In vivo kinetics CAR-T cells in DLBCL: association with response to therapy







Monitoring of CAR T-cell therapy in DLBCL:
Targeted cell populations

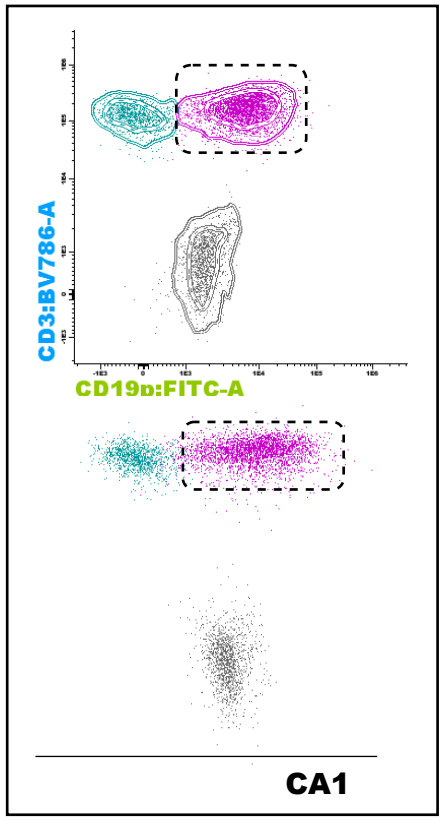
- Monitoring of infused (CAR) immune cells via cell surface and/or intracellular markers
- Monitor CAR cell composition.

DIFFERENCES IN CART CELL COMPOSITION?

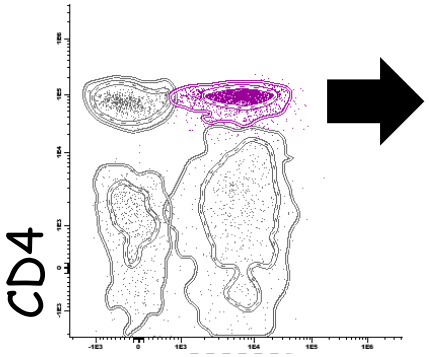
i.e. Monitoring the levels of the different components of a drug might contribute to explain differences for similar (total) drug concentrations

Circulating CAR-T cell composition

KTE-X19 (CAR-T-CD19)
75% of Tcells
5.1 cells/µl

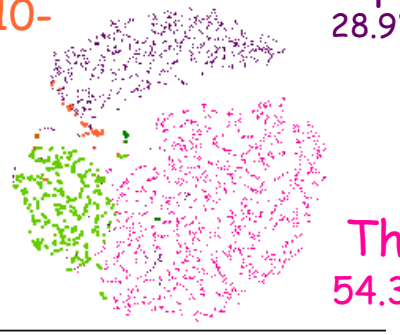


84% CD4+ CAR-T
(total CAR-T cells)
4.3 cells/µL



CD183+ CD194+ CD196+ CCR10-
1.4%

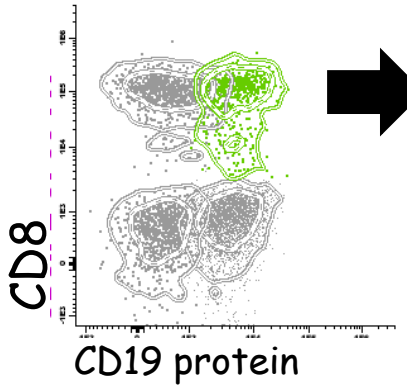
CD183+ CD194+ CD196- CCR10-
14.5%



Follicular helper
28.9%

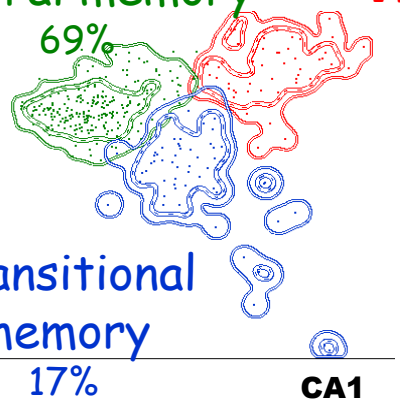
Th1
54.3%

Effector memory
14%



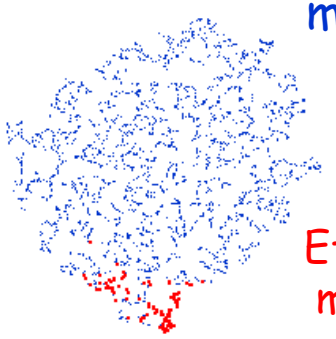
Central memory
69%

Transitional memory
17%

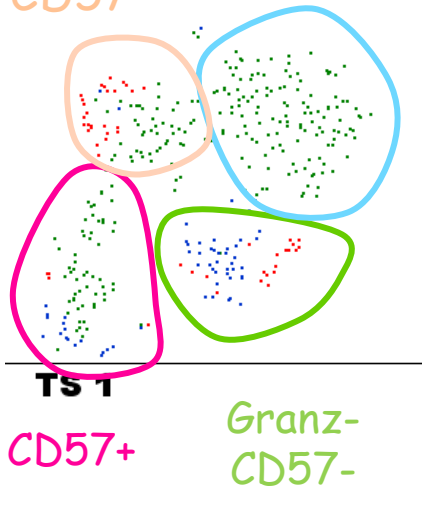


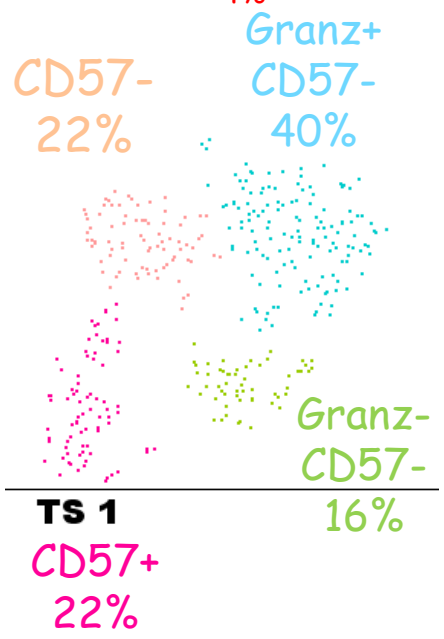
9.5% CD8+ CAR-T
(total CAR-T cells)
0.5 cells/µL

Transitional memory
96%



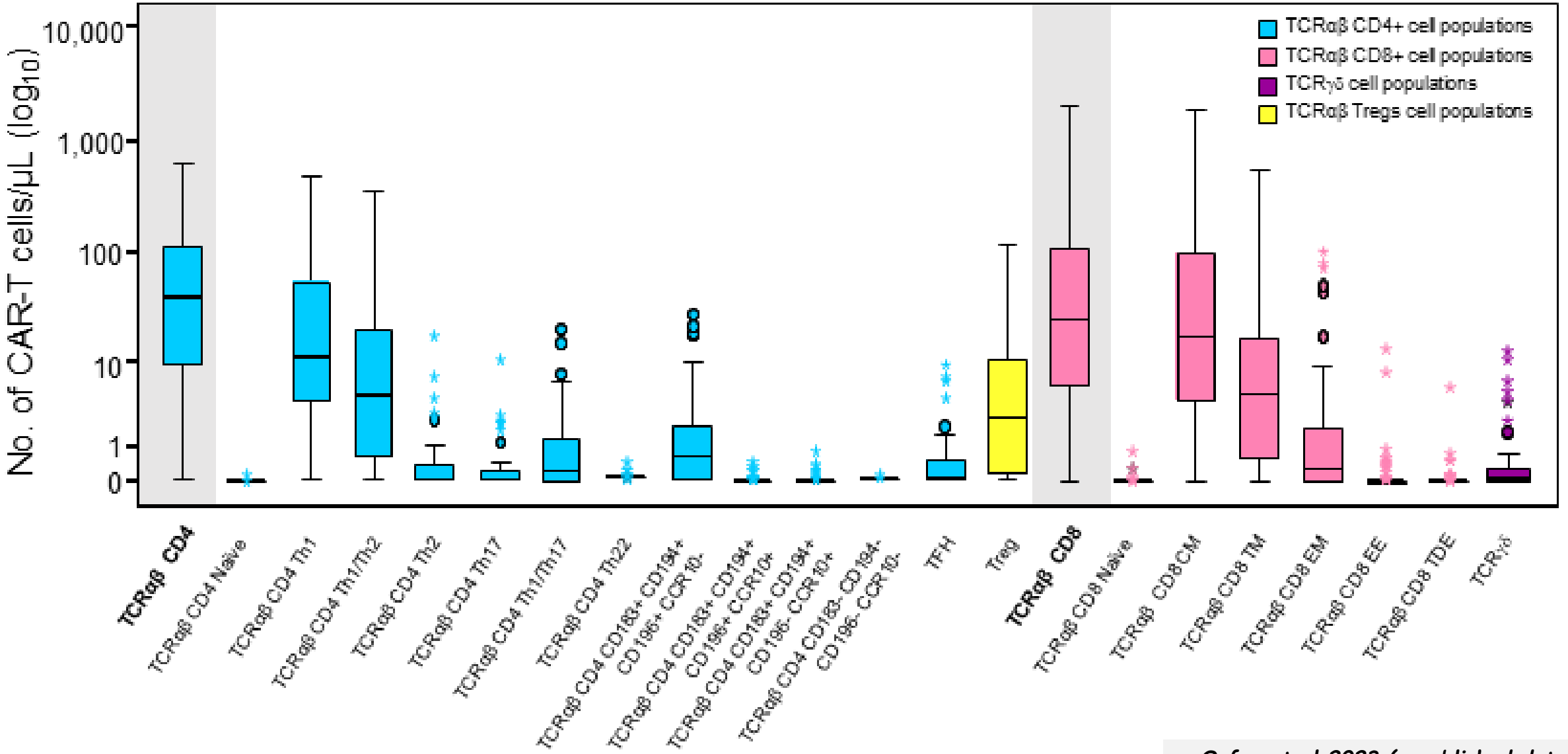
Effector memory
4%





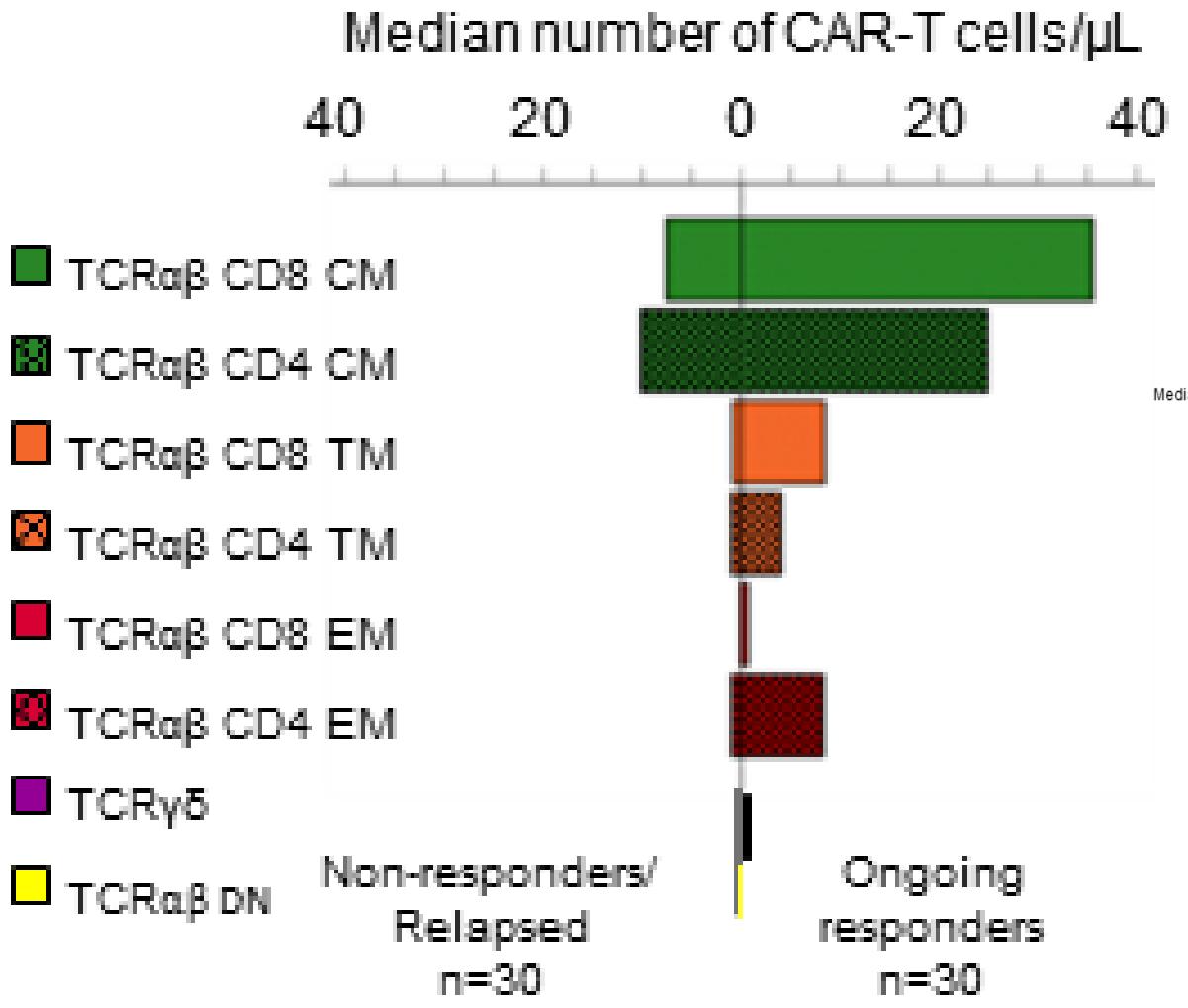
>145 populations of CAR-T cells

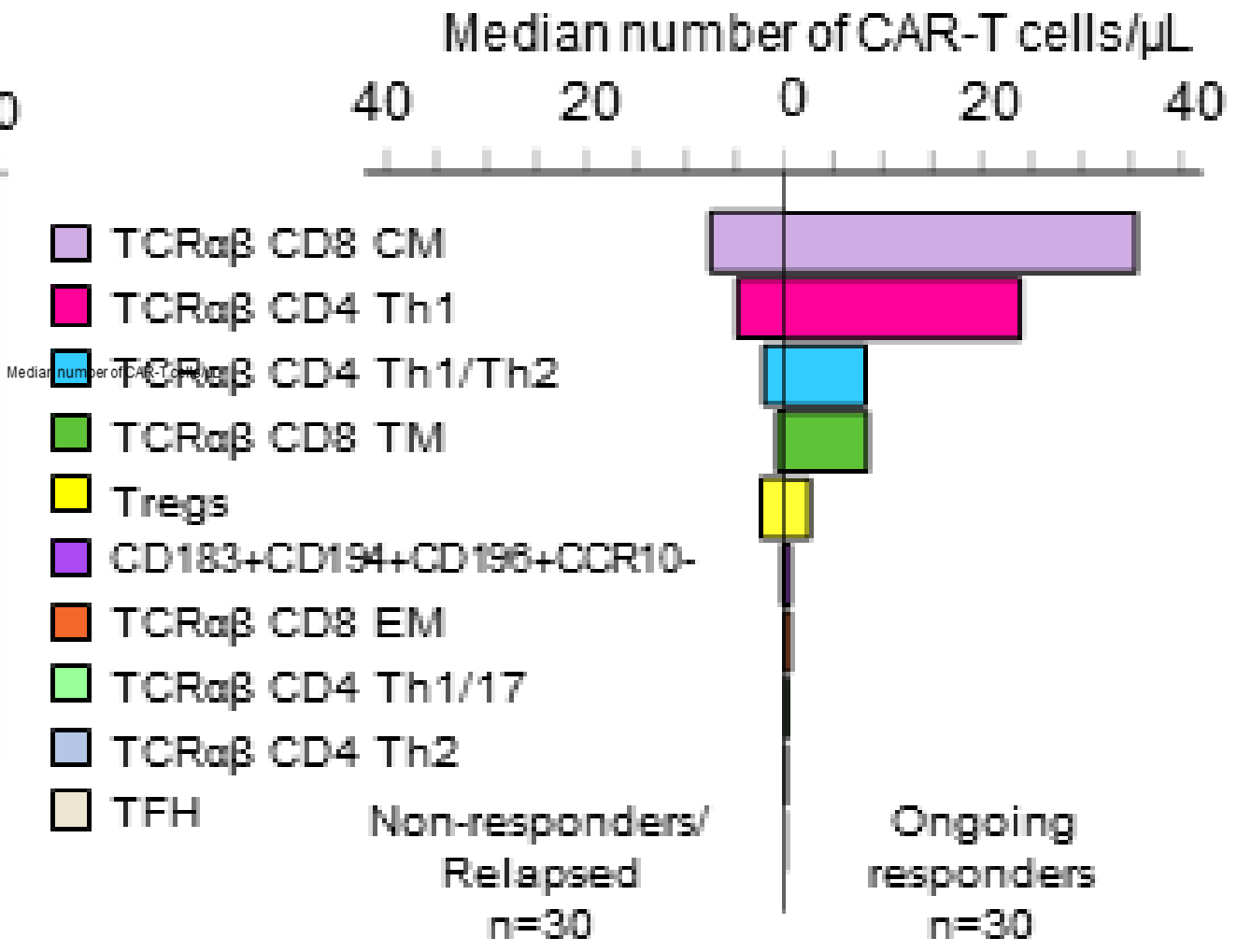
Post-infusion monitoring of circulating CAR-T cell populations in DLBCL (n=60)



Orfao et al 2023 (unpublished data)

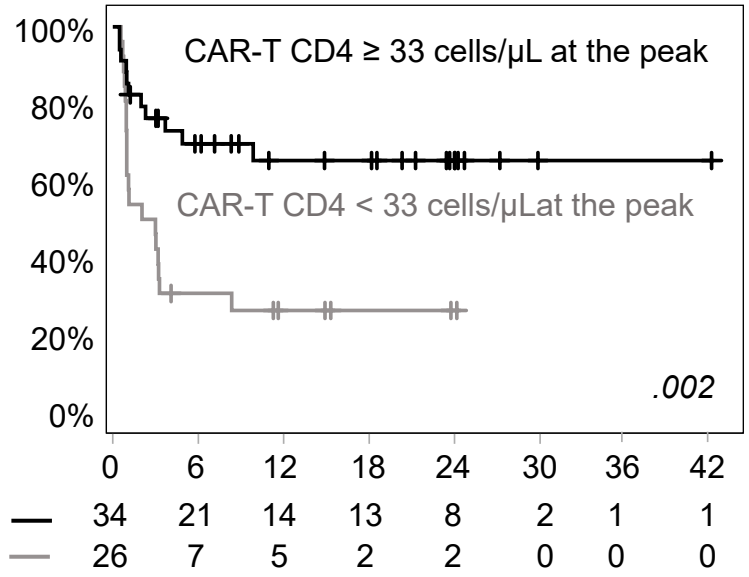
CAR-T cell **major populations at CAR-T peak in DLBCL:** ongoing responders vs. relapsed/non-responders

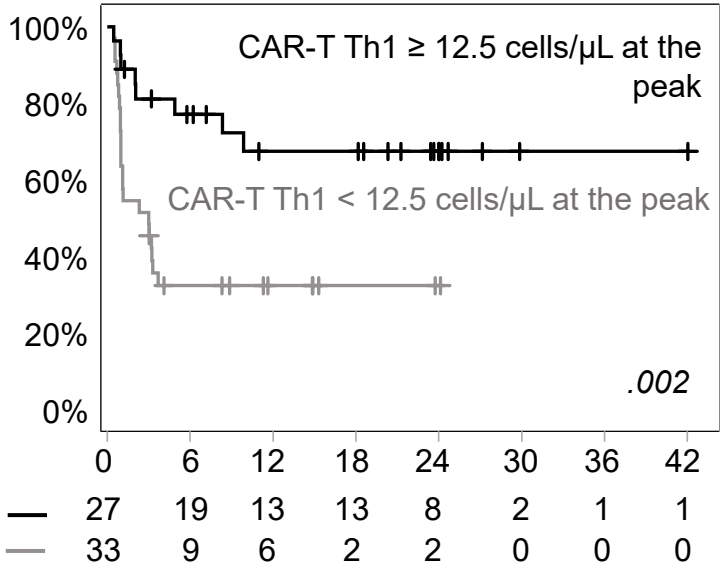


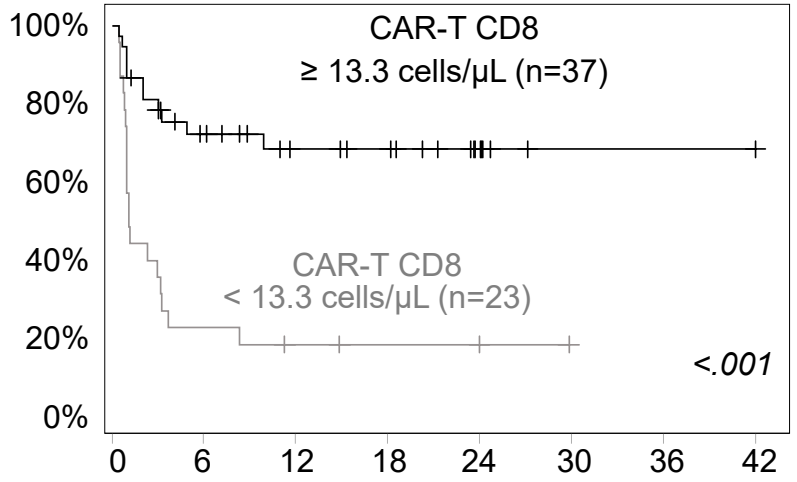


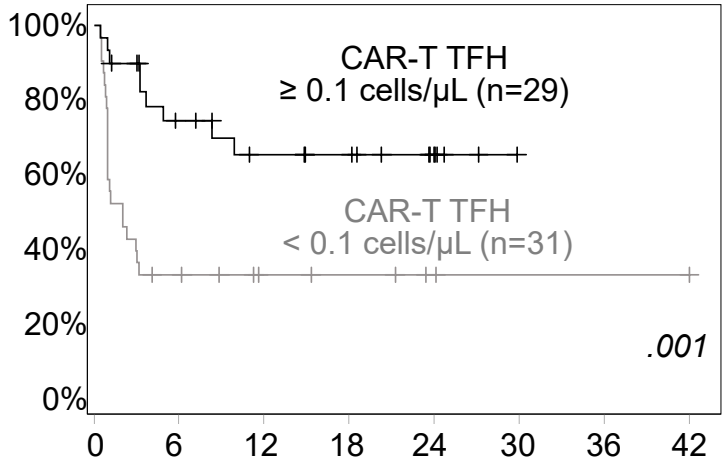
Orfao et al 2023 (unpublished data)

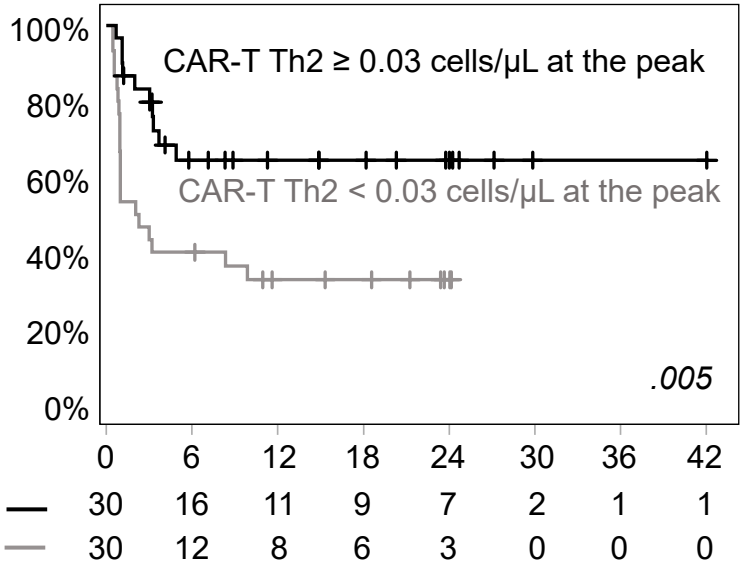
CAR-T TCRγδ cells at CAR-T peak have a favorable impact on progression-free survival of DLBCL patients

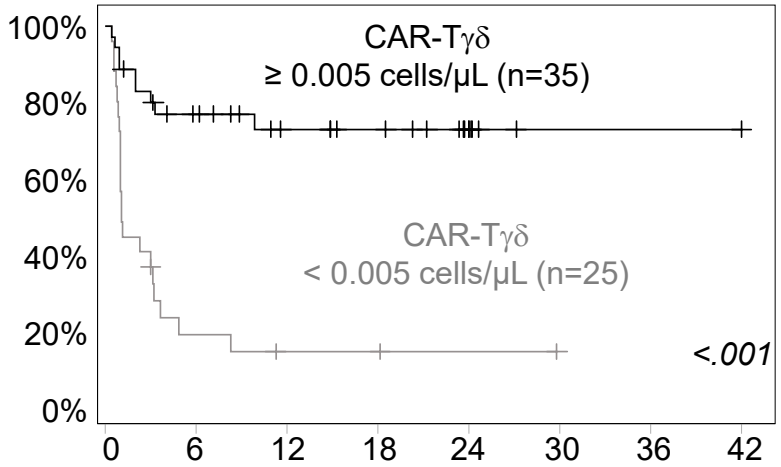








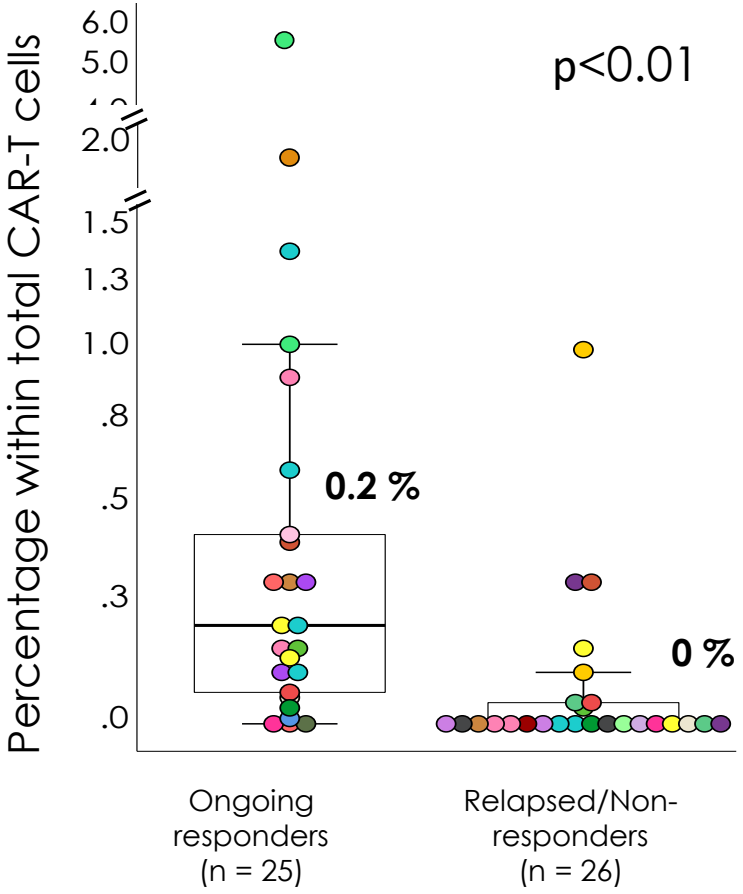


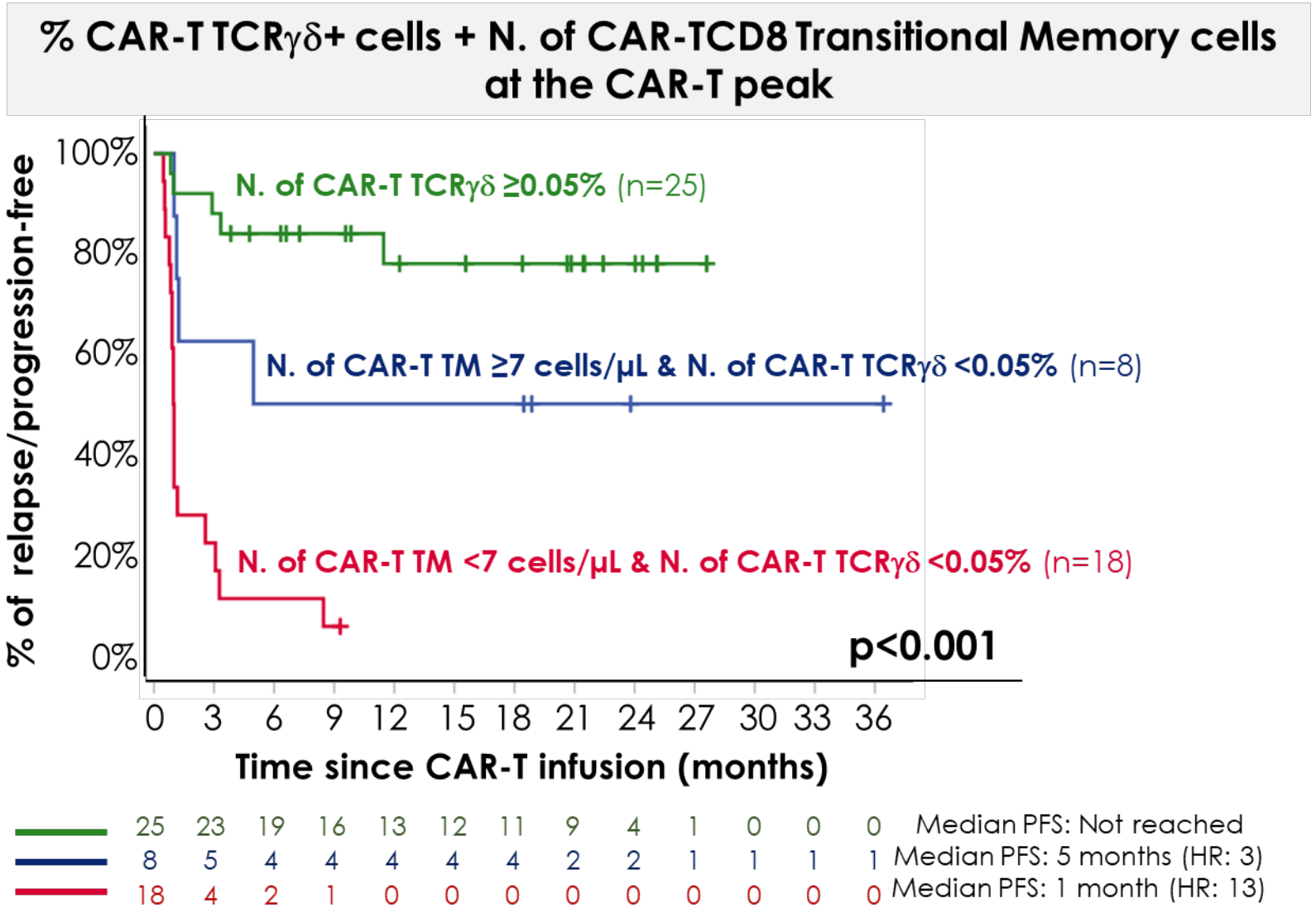


% of relapse/progression-free

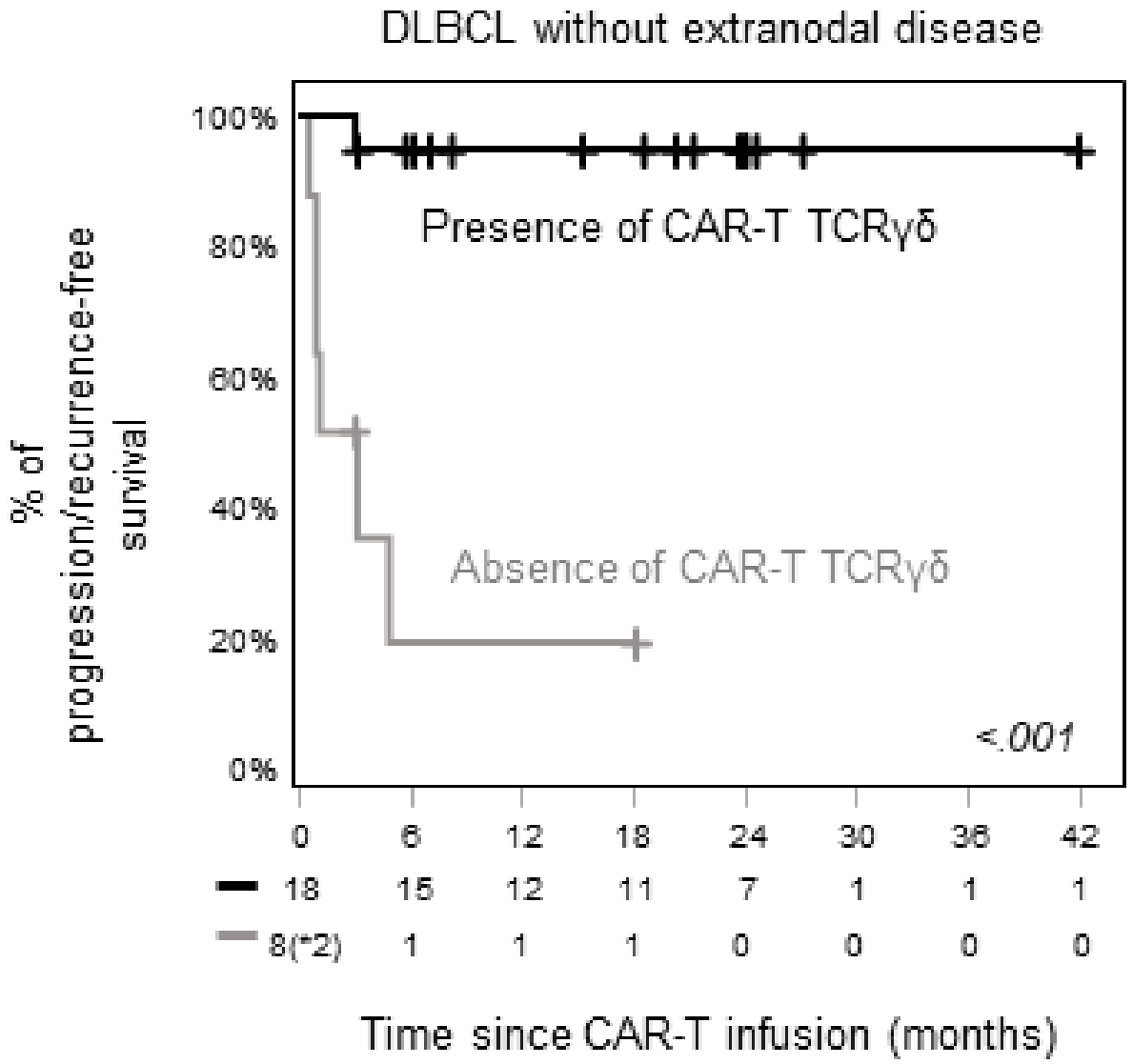
Time since CAR-T infusion (months)

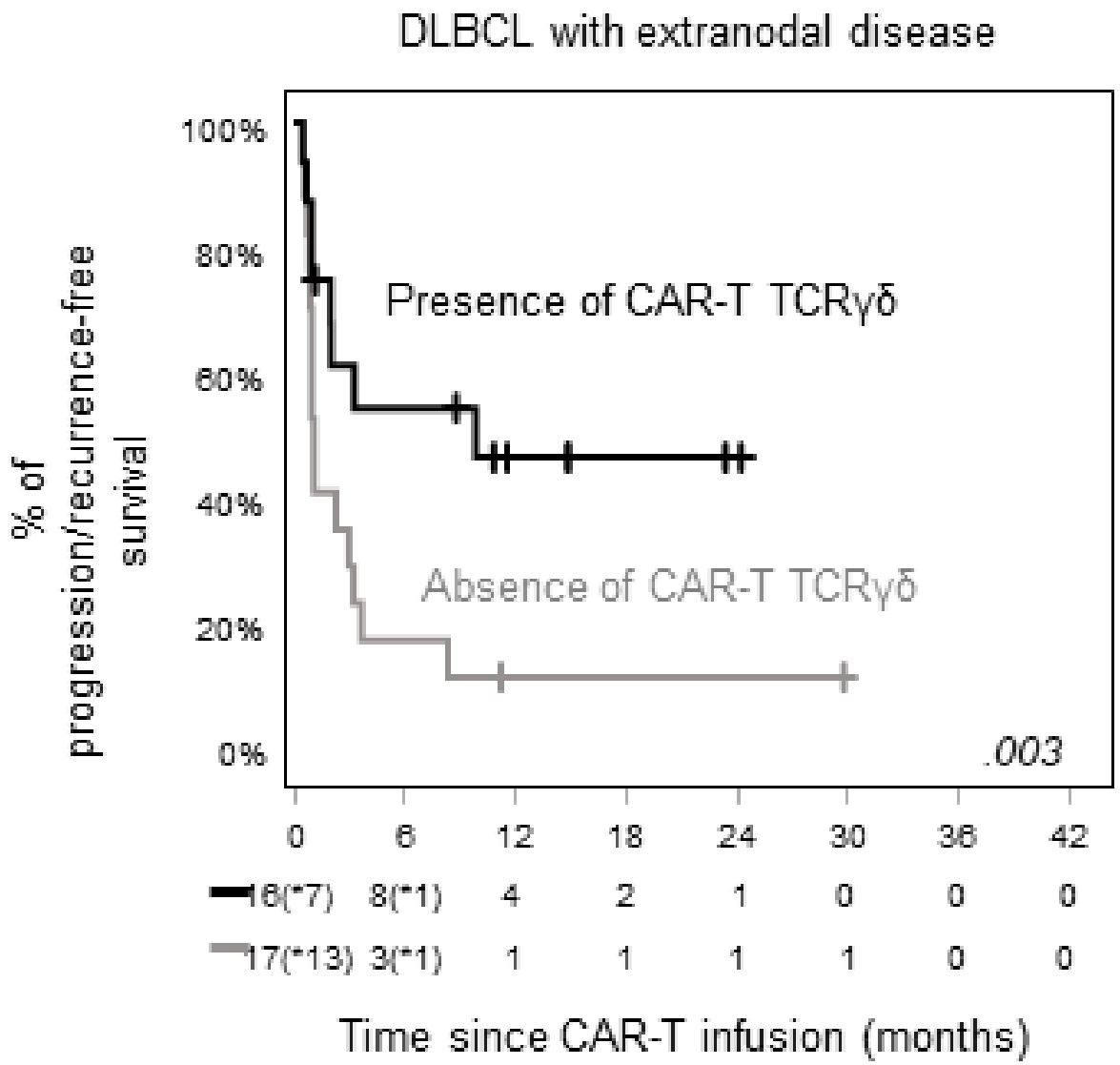
CAR-T TCRγδ cells at CAR-T peak have a positive impact on progression-free survival in DLBCL patients



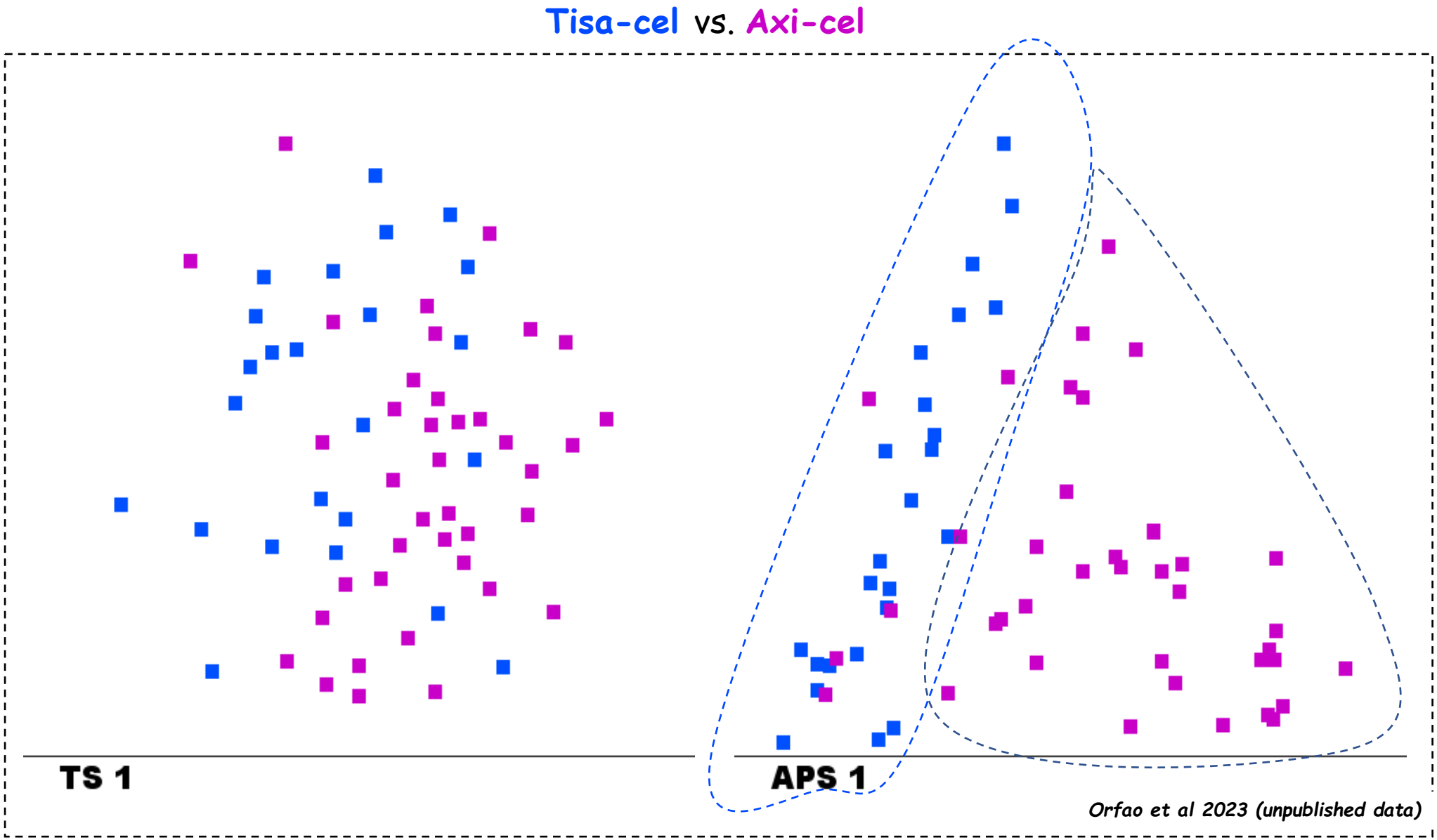


CAR-T TCRγδ cells at CAR-T peak impact on progression-free survival of DLBCL with and without extranodal disease at diagnosis

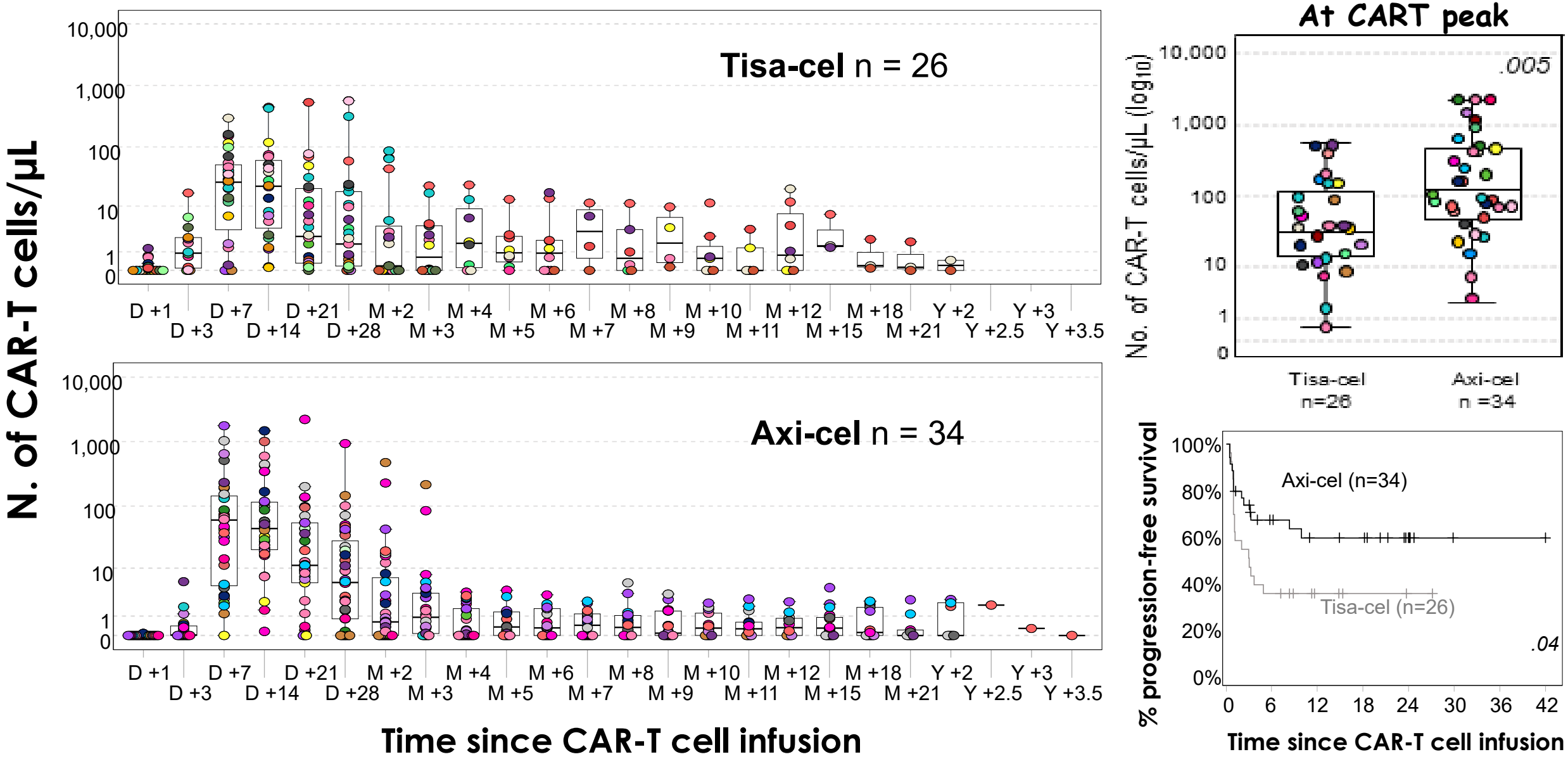




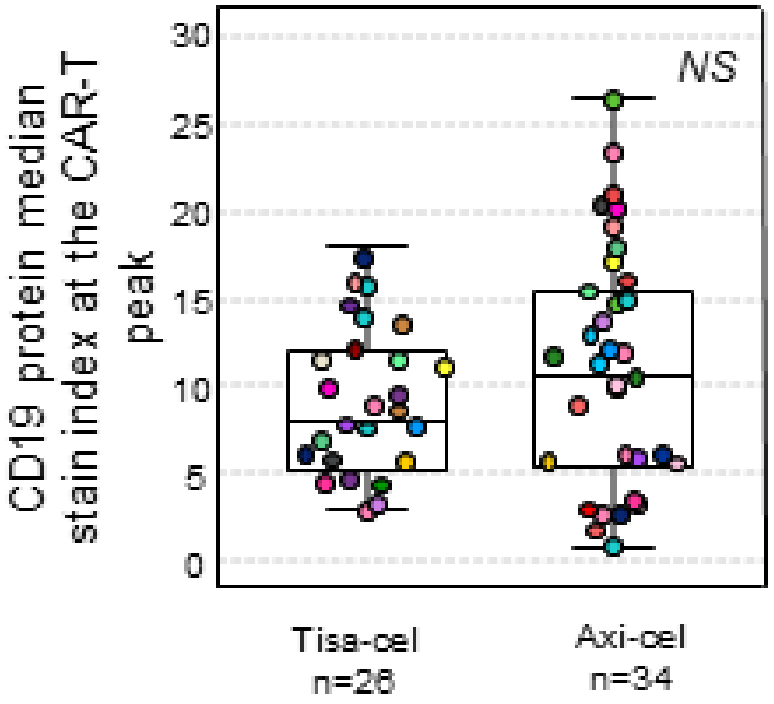
Distinct CART CD19 cell products might differ in their composition in blood after in vivo expansion

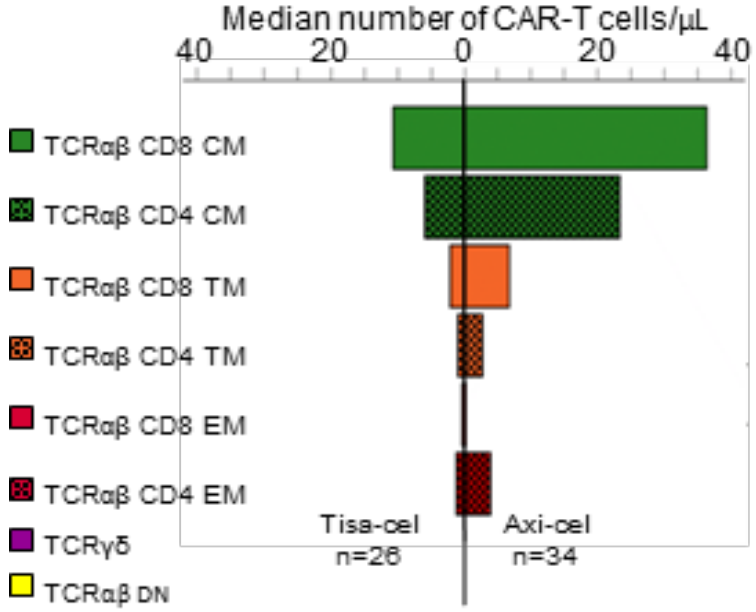


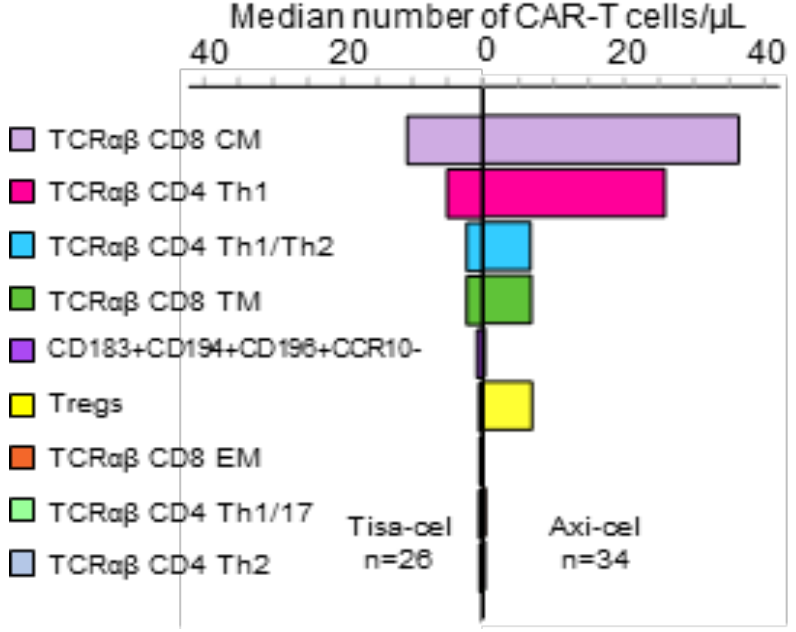
In vivo kinetics of CAR-T cells in DLBCL: association with CAR-T product



CAR-T cell **major populations at CAR-T peak in DLBCL:** association with CAR-T cell product







Orfao et al 2023 (unpublished data)

Monitoring of CAR T-cell therapy in B cell neoplasias:
Targeted cell populations

- **Monitoring of infused (CAR) immune cells via cell surface and/or intracellular markers**

- **Monitor CAR T-cell composition**

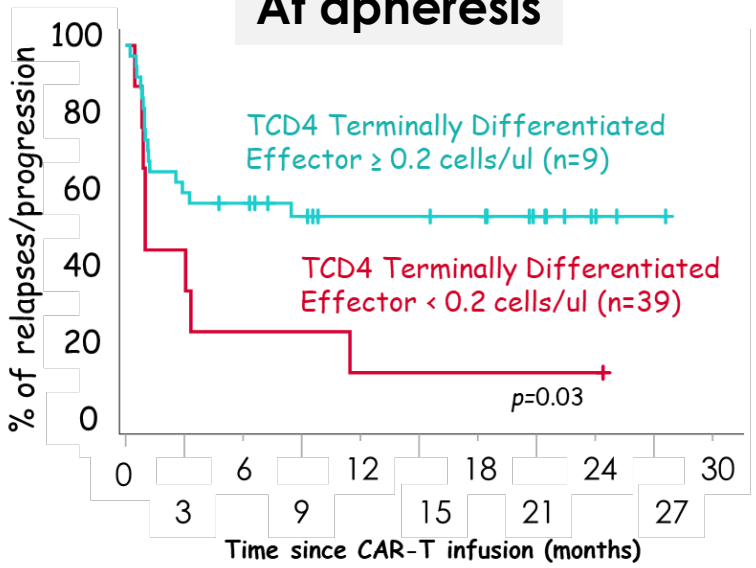
- **Monitor CAR-therapy associated immune responses:**

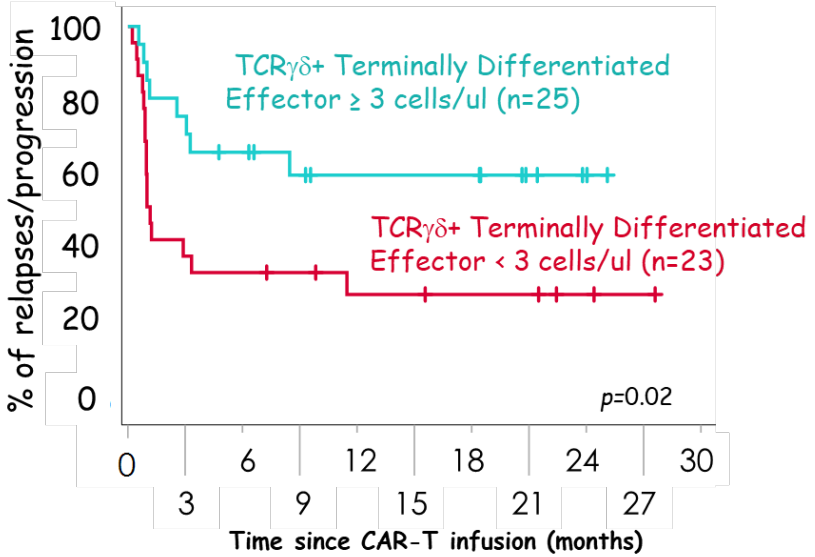
    - Innate immune cells
    - CD4+ T-cell subsets
    - Cytotoxic T and NK cell populations
    - Maturation-associated B-cell and plasma cell compartments

i.e. Assessing the immune status orior to therapy and monitoring the effect of CART therapy on residual immune cells (in blood) might contribute to understand the mechanisms involved in immune-escape and treatment failure.

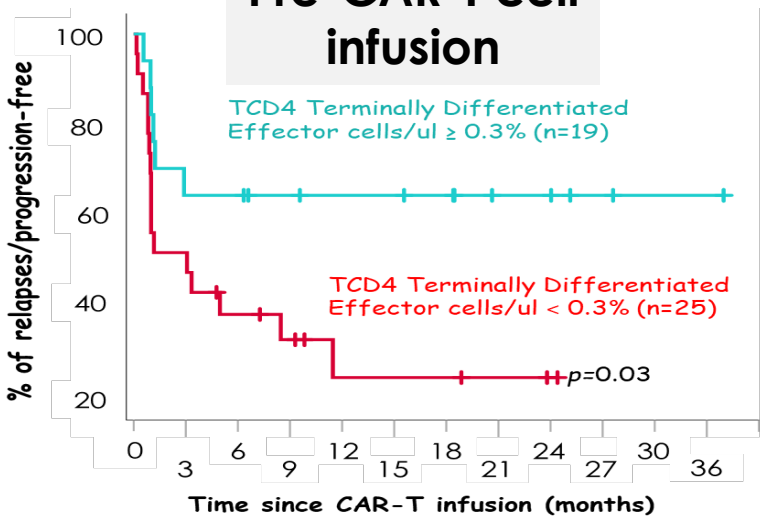
Blood T cell populations with independent impact on progression-free survival of DLBCL

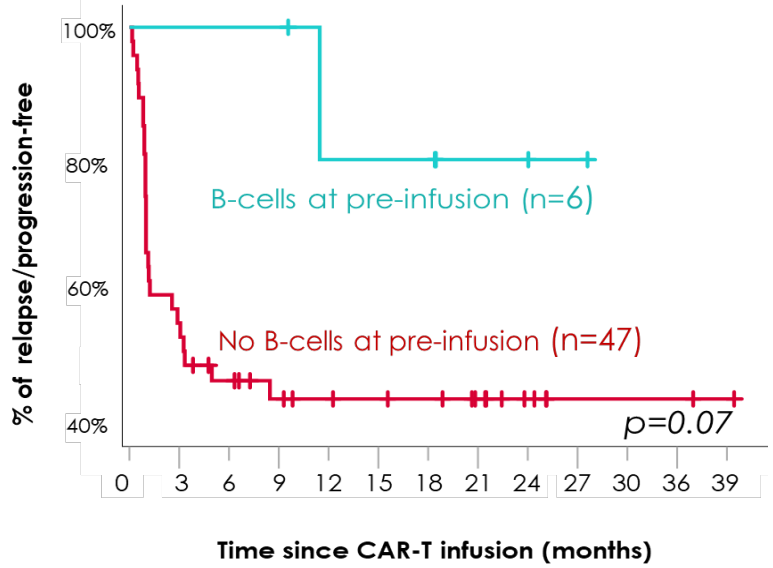
At apheresis



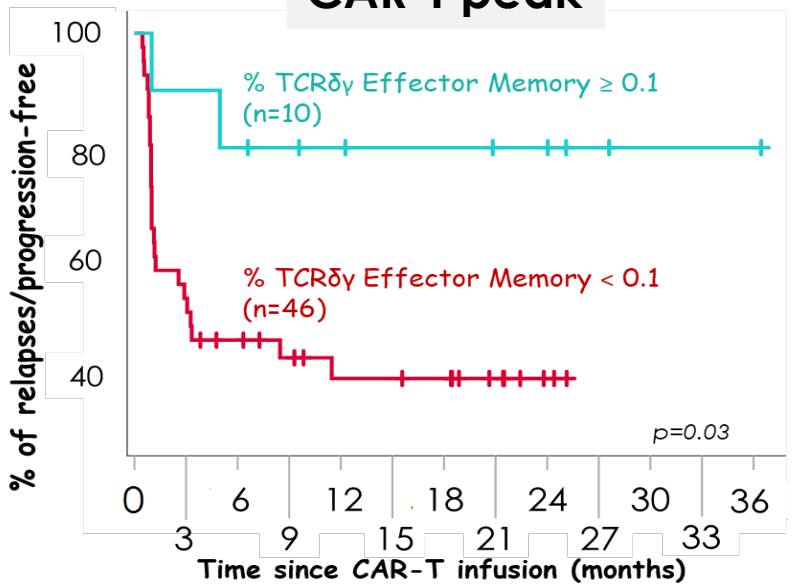


Pre-CAR-T cell infusion





CAR-T peak



The status of the patient immune system might play a critical role on disease outcome

Monitoring of CAR T-cell therapy in DLBCL:
Targeted cell populations

- Monitoring of infused (CAR) immune cells via cell surface and/or intracellular markers

- Monitor CAR T-cell composition

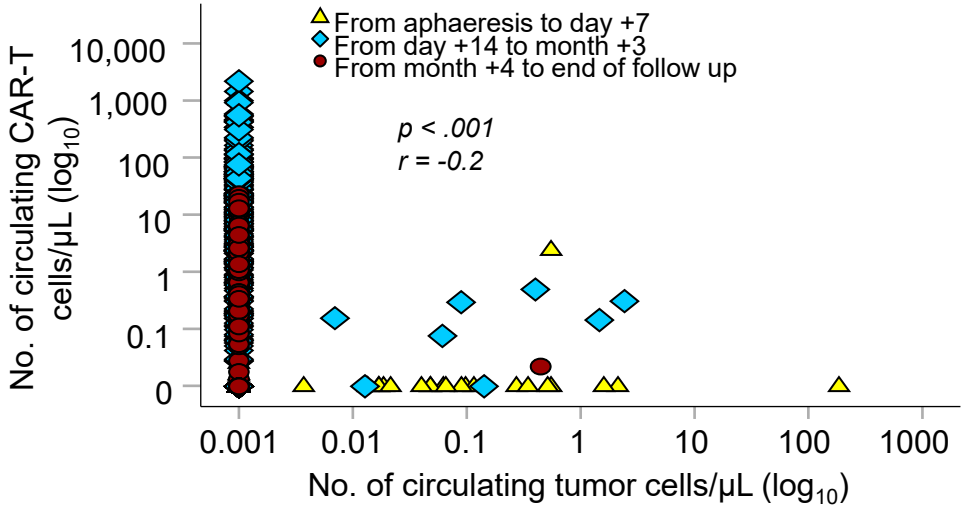
- Monitor CAR-therapy associated immune responses:

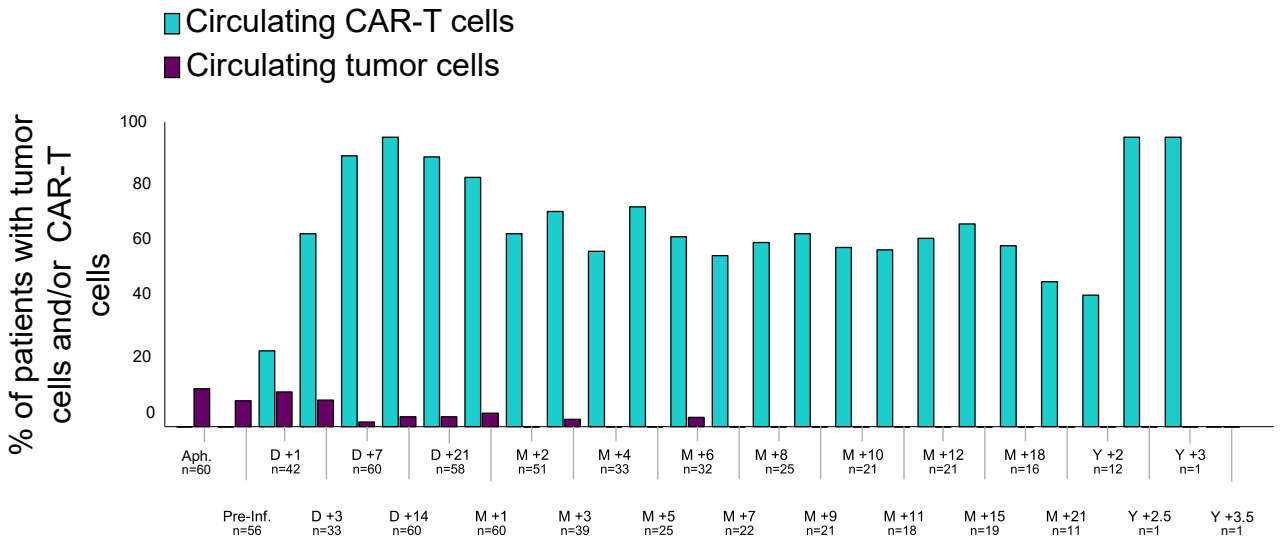
    - Innate immune cells
    - CD4+ T-cell subsets
    - Cytotoxic T and NK cell populations
    - Maturation-associated B-cell and plasma cell compartments

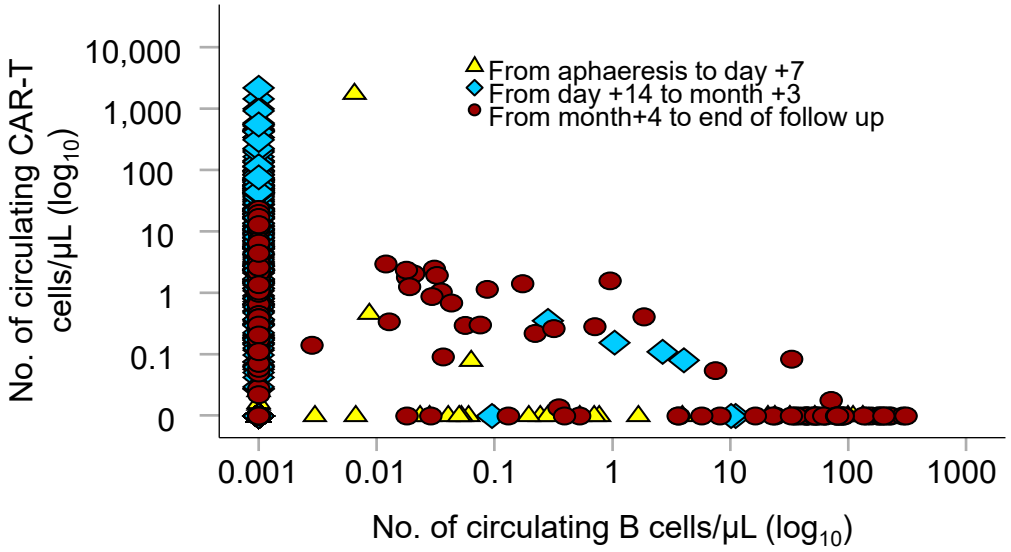
- Monitor persistence of circulating tumor cells (CTCs)

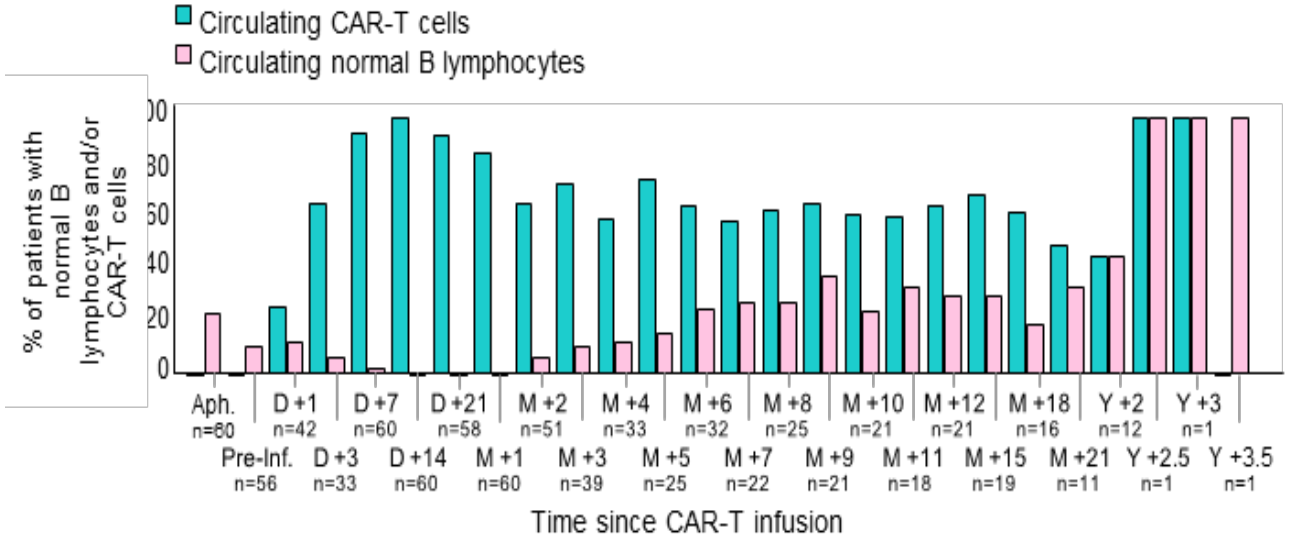
i.e. Monitoring of MRD levels and the residual immune cells (in blood) might contribute to understand the mechanisms involved in immune-escape and treatment failure.

Circulating CAR-T cells numbers in blood of DLBCL: correlation with tumor and normal B cell counts in blood



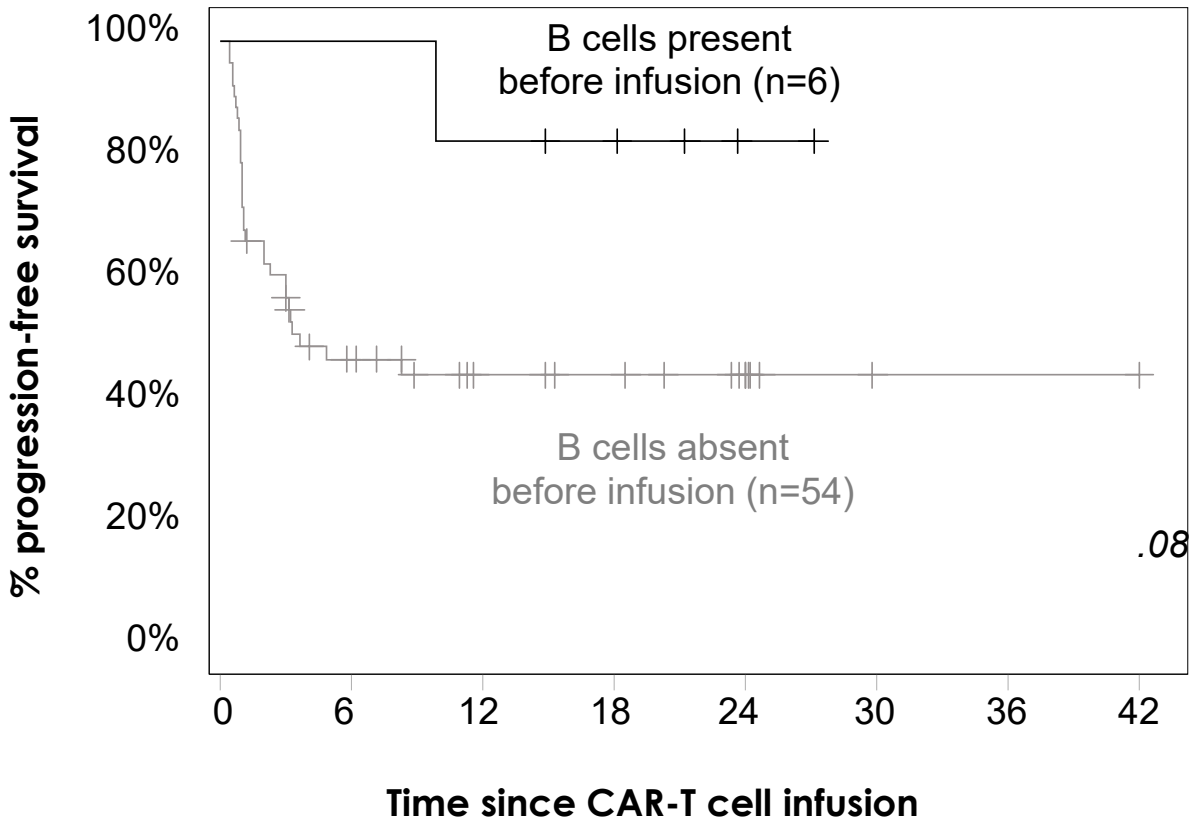




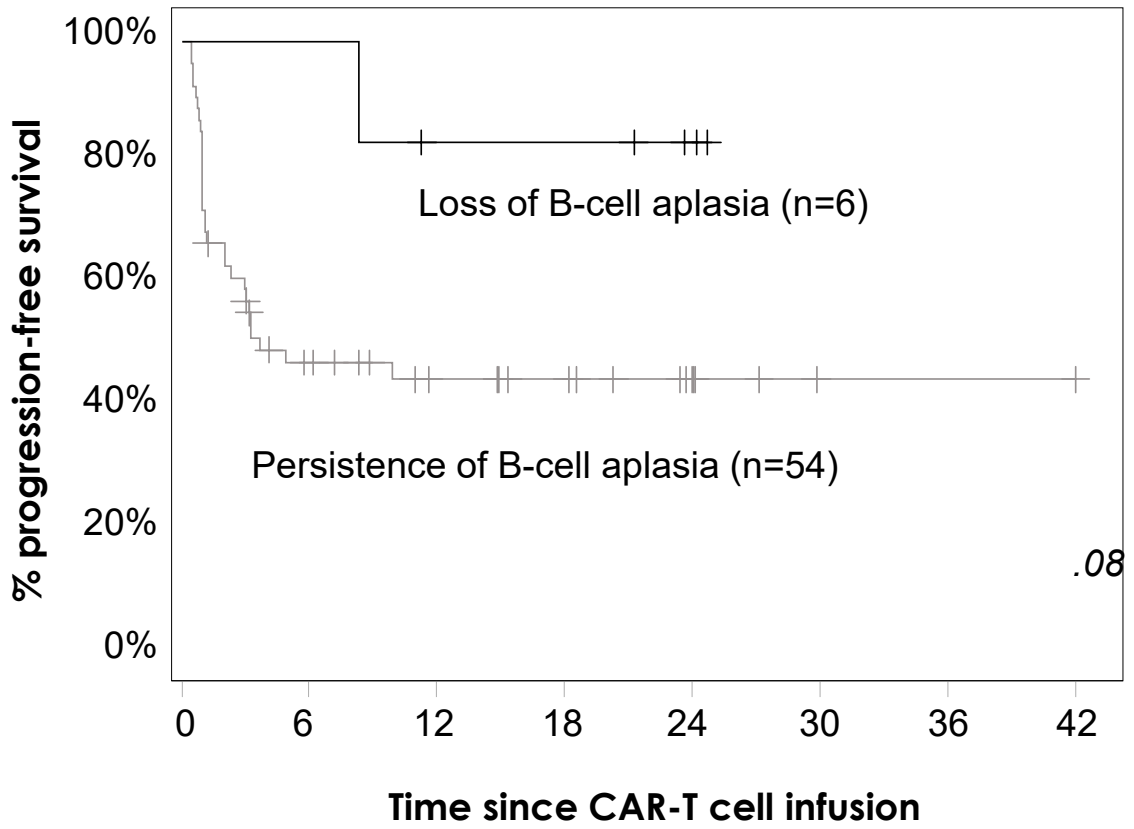


Blood B cell counts prior and after CART cell infusion: impact on progression-free survival of DLBCL

Pre-CAR-T cell infusion

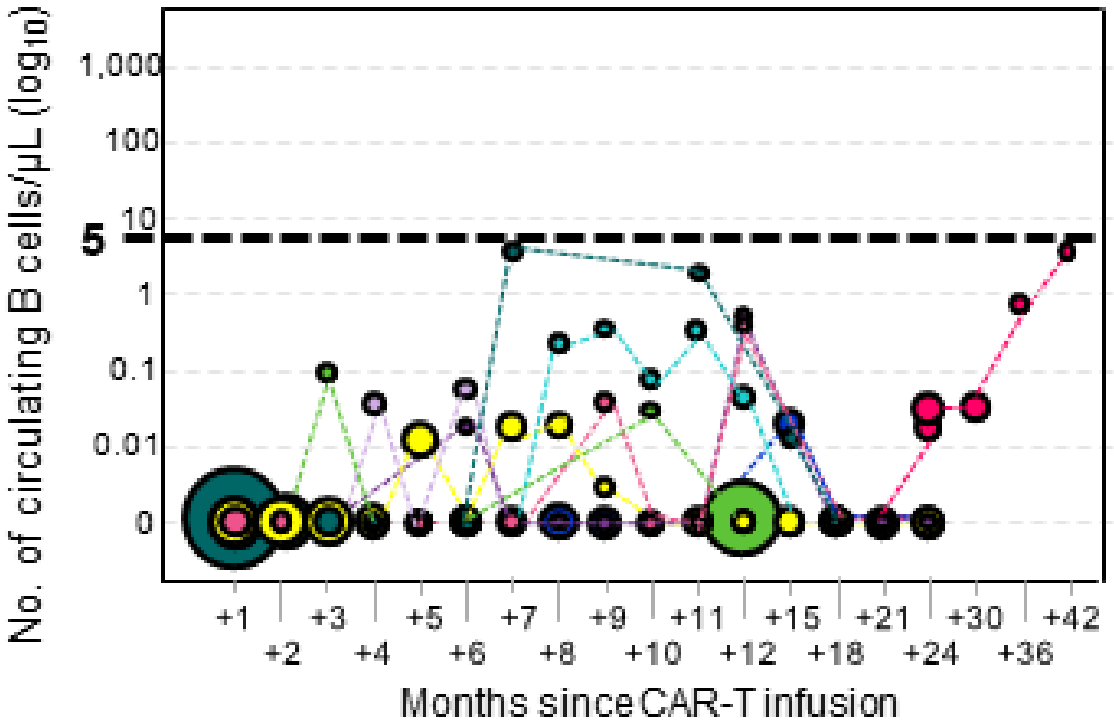


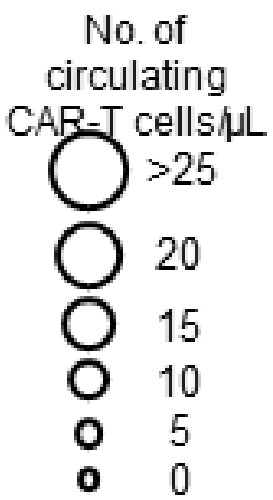
CAR-T peak



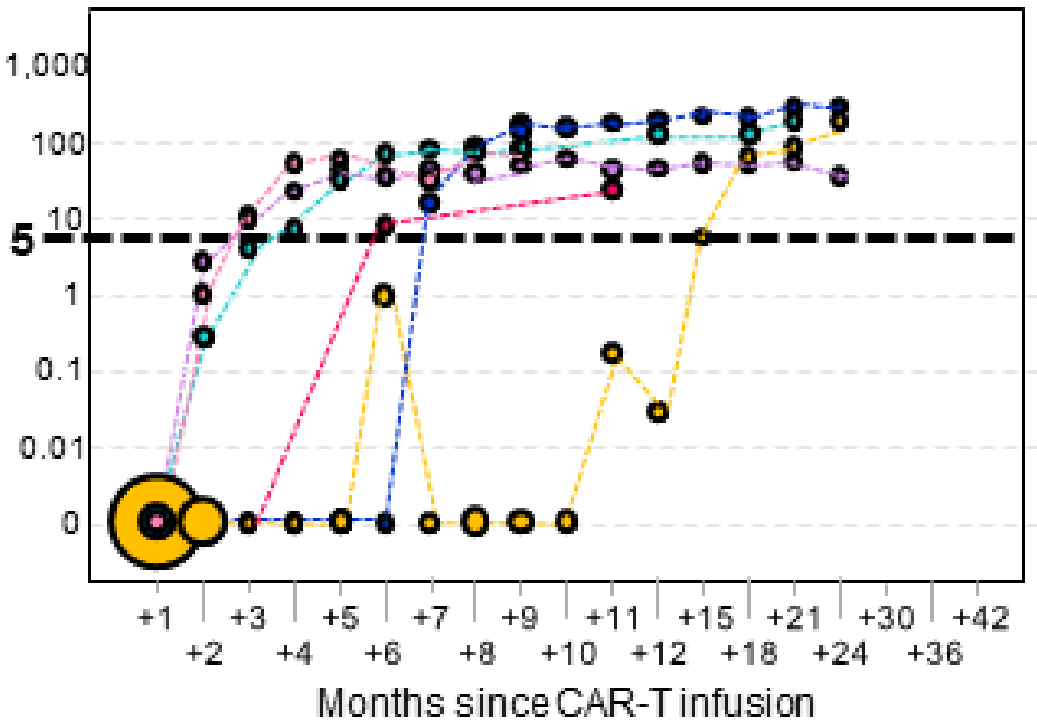
Blood B cell counts prior and after CART cell infusion: impact on progression-free survival of DLBCL

No/low B cell recovery





Higher B cell recovery



DLBCL: simultaneous MRD+CART CD19+T-cell+Immune Monitoring

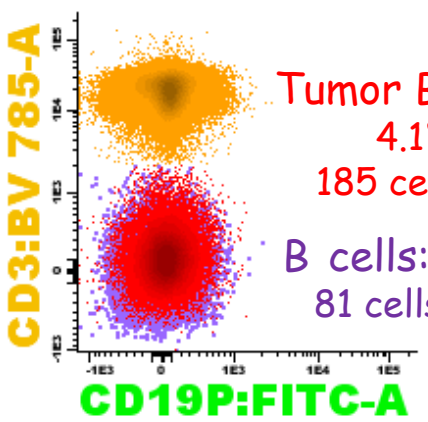
Apheresis

CAR-T infusion

CAR-T peak (day 14)

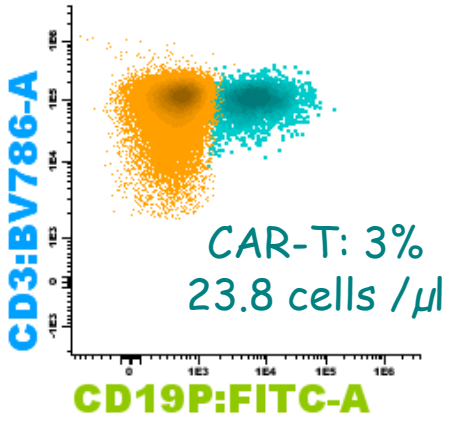
Relapse (Month 2)

T cells



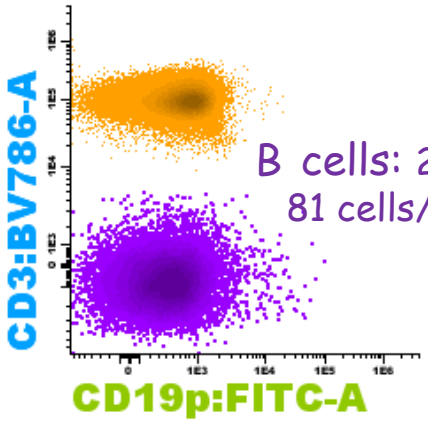
Tumor B cells:
4.1%
185 cells/µl

B cells: 1.8%
81 cells/µl



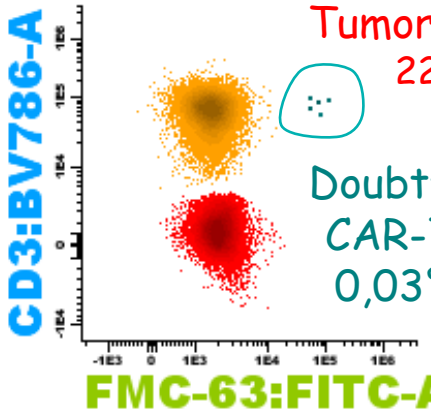
CAR-T: 3%
23.8 cells /µl

Peripheral blood



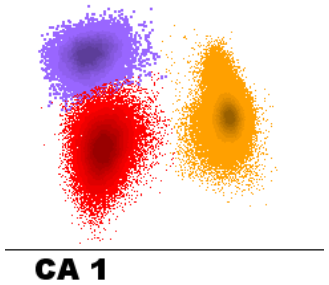
B cells: 2.1%
81 cells/µl

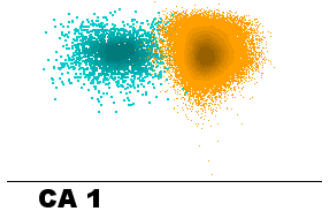
Breast biopsy

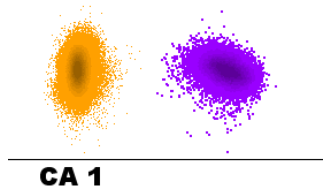


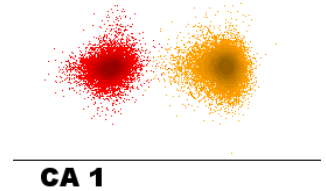
Tumor B cells:
22.3%

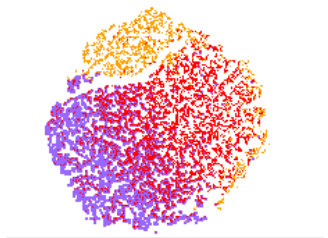
Doubtful CAR-T:
0,03%

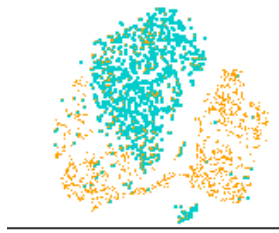


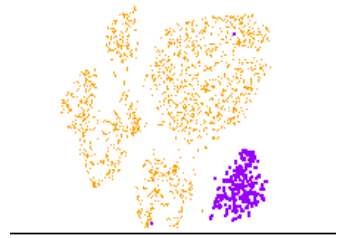


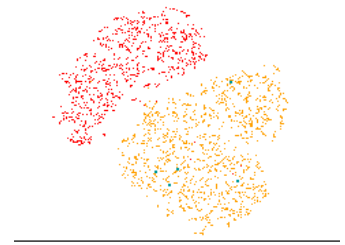












Orfao et al 2021 (unpublished data)

CONCLUDING REMARKS

EuroFlow

- Evaluation of the amount of protein expression on the tumor cell surface membrane, as well as other normal cells provides insight into the sensitivity to protein-targeted antibody-based and CART cell immunotherapy and its potential toxicity, respectively.

- An optimal target protein for antibody and CART cell therapy should be expressed at high levels in all individual leukemia cells, particularly on their precursor cells (i.e., leukemia stem cells)

- Monitoring of antibody targeted therapies aims at simultaneous monitoring of the beneficial effect of the drug (e.g. MRD), as well as drug-induced cytopenias of cell populations targeted by the antibody.

- In turn, in the settings of CART therapy, both MRD and drug-induced cytopenias are to be followed together with CART cell levels and their composition (prior to and after the infusion) and persistance after infusion.

- The specific normal and tumor cell populations to be monitored vary depending on the targeted protein, the underlying disease, and the specific drug used.

Acknowledgements & Collaborations in Immune Monitoring

VNiVERSiDAD D SALAMANCA

CANCER RESEARCH CENTER

Julia Almeida
Martin Perez-Andres
Elena Blanco
Juan Flores-Montero
Luzalba Sanoja-Flores
Daniela Damasceno
Juan Flores
Vitor Botafogo
Quentin Lecrevisse
Lourdes Martín
Sara Gutiérrez
María Herrero
Alejandro Hernandez
Sergio Matarraz
Maria Luz Sanchez
Paloma Barcena
Sheila Mateos
Alberto Orfao

IBSAL Instituto de Investigación Biomédica de Salamanca

Complejo Asistencial Universitario de Salamanca

Hematology Service

Ana Yeguas
Marta García Blázquez
Lucía López Corral
Ana África Martín
María Dolores Caballero
Alejandro Martín
Verónica González
Estefanía Pérez
Maria Belen Vidriales
Miriam López
Maria Victoria Mateos
Norma Gutiérrez
Marcos González

Junta de Castilla y León

Sacyl

LUMC Leiden University Medical Center

Department of Immunology

Cristina Teodosio
Magda Berkowska
Pieter van der Pol
Indu Khatri
Wouter van den Bossche
Kyra van der Pan
Annieck Diks
Gita Naber
Alita van der Sluijs
Sandra de Bruin
Sandra Vloemans
Rick Groenland
Bas de Mooij
Marieke Bitter
Bart Lubbers
Châtelaine Voets
Jacques JM van Dongen

peri scope

WP5 Task 5.6

USAL, Salamanca, ES
LUMC, Leiden, NL
RIVM, Bilthoven, NL
RUMC, Nijmegen, NL
UTU, Turku, FI
UOXF, Oxford, UK
US, Southampton, UK
MRC, Gambia, GM
ULB, Brussels, BE

CANCER RESEARCH UK

aecc Contra el Cáncer

INCAR

WP5

Concetta Quintarelli
Franco Locatelli
Sara Gorashian
Ignacio Criado
Silvia Zaninelli
Alessandro Rambaldi
Chiara Magnani
Chiara Buracchi
Giuseppe Gaipa
Andrea Biondi

erc European Research Council

EuroFlow

MUCHAS GRACIAS