Generation of a new anti-CD79b monoclonal antibody and CD79b CAR T cells for B cell malignancies

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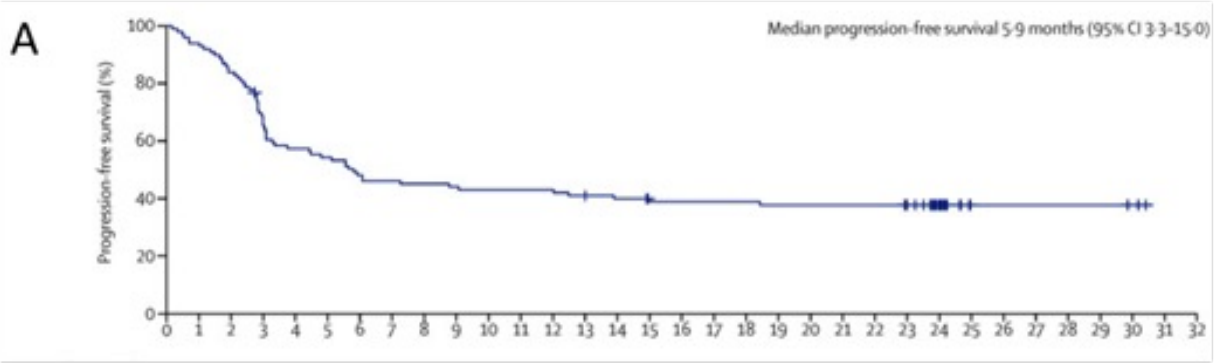
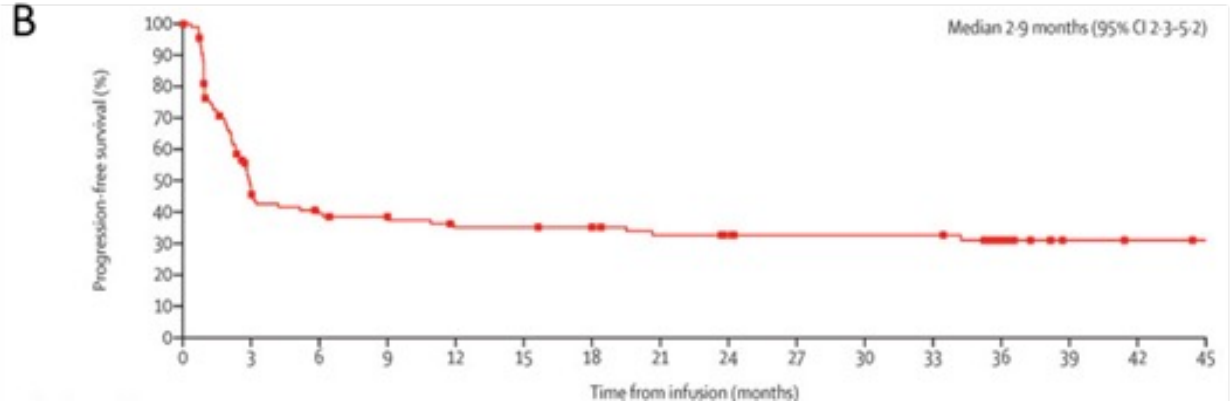
Disclosures: Perugia scFv CD79b sequence (under patenting)

CD19 CAR-T CELL THERAPY FOR r/r DLBCL

Long term PSF of 101 pts ho received anti-CD19 CAR-T cells axi-cel in phase 2 ZUMA-1 (A) and of 115 patients who received anti-CD19 CAR-T cells tisa-cel in phase 2 JULIET (B)



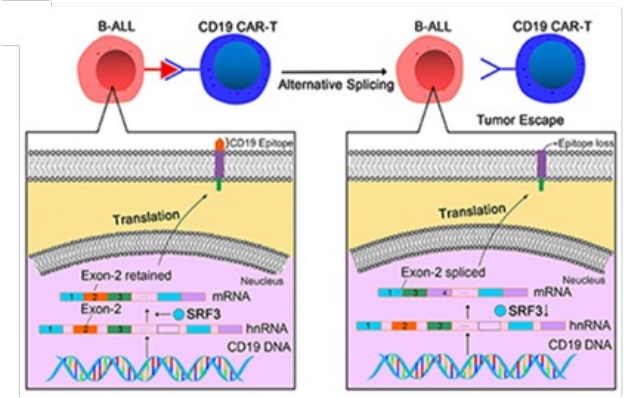
***Locke FL. et al., Lancet Oncol* 2019**



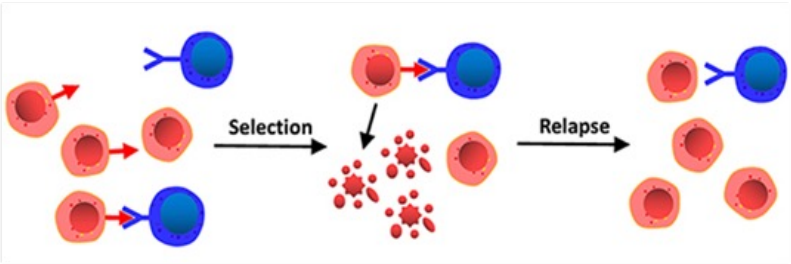
***Schuster SJ. et al., Lancet Oncol* 2021**

Antigen stability is fundamental for CAR T targeting

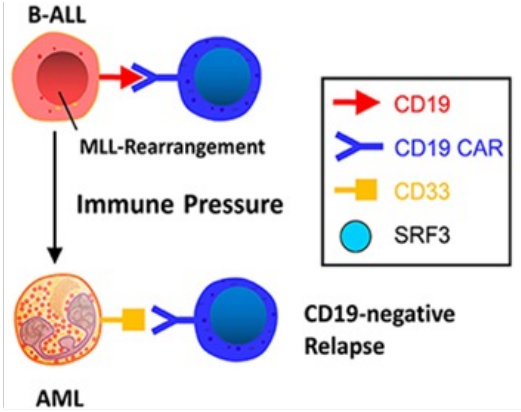
- The less stable the target is, the easier it is for cancer cells to escape from the killing of CAR-T cells (clinical response of CD22 CAR-T cells is inferior to that of CD19 despite almost equal expression pattern)
- **Antigen loss is the most common cause of relapse after anti-CD19 CAR-T cell in both B-ALL and DLBCL (30%)**



By mutation or alternative splicing



Selection of CD19- clones by immune pressure

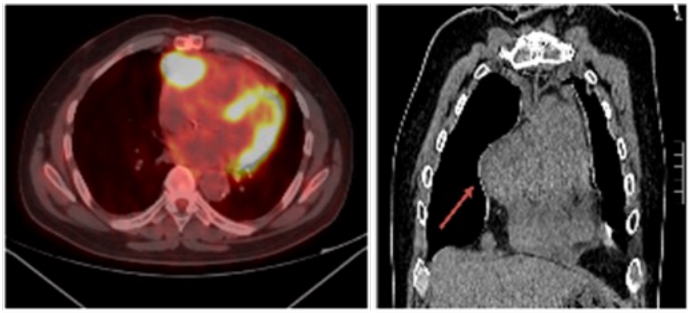


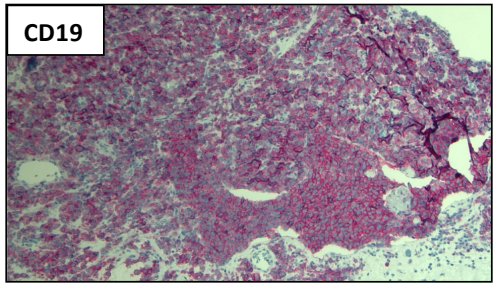
Lineage switch induced by immune pressure

1. Spiegel et al. Nat Med. 2021
2. Orlando et al. Nat Med. 2018

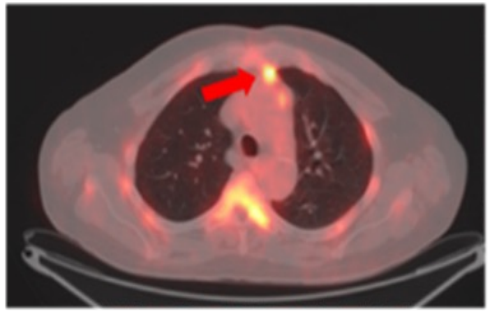
CD19-negative CNS relapse after CD19-directed CAR T cells in DLBCL

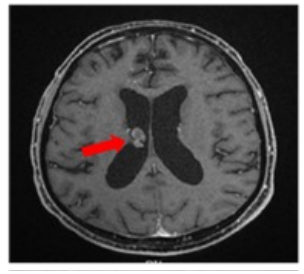
Primary Cardiac Lymphoma

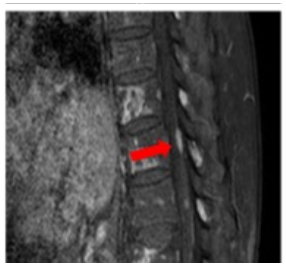


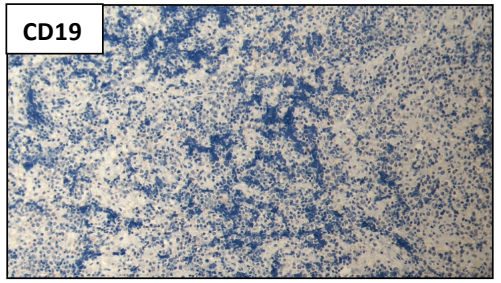


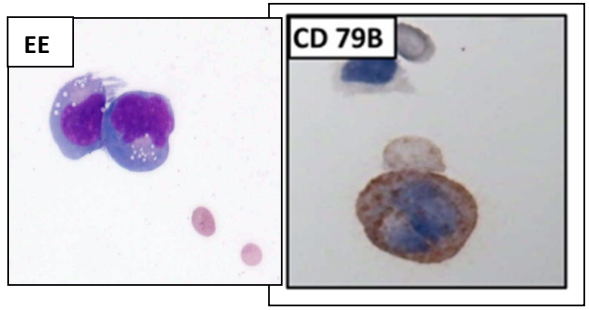
Relapse post CD19 CAR T treatment

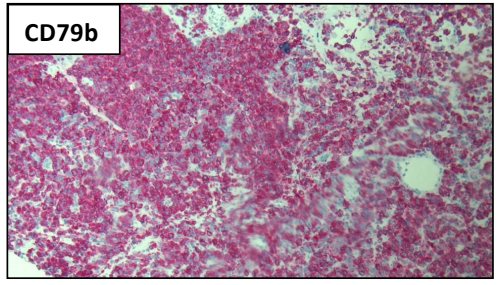






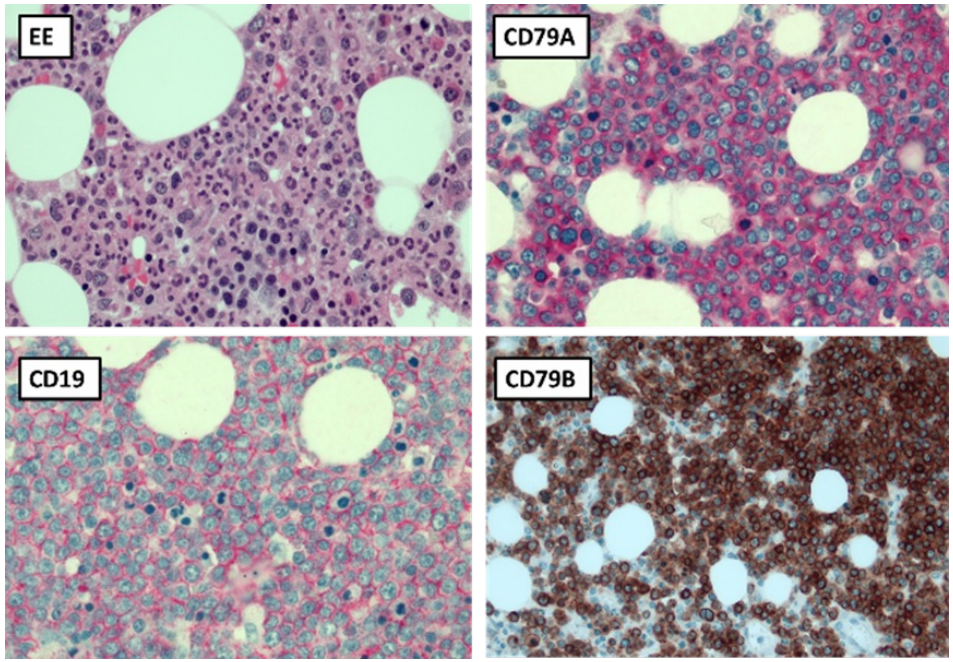


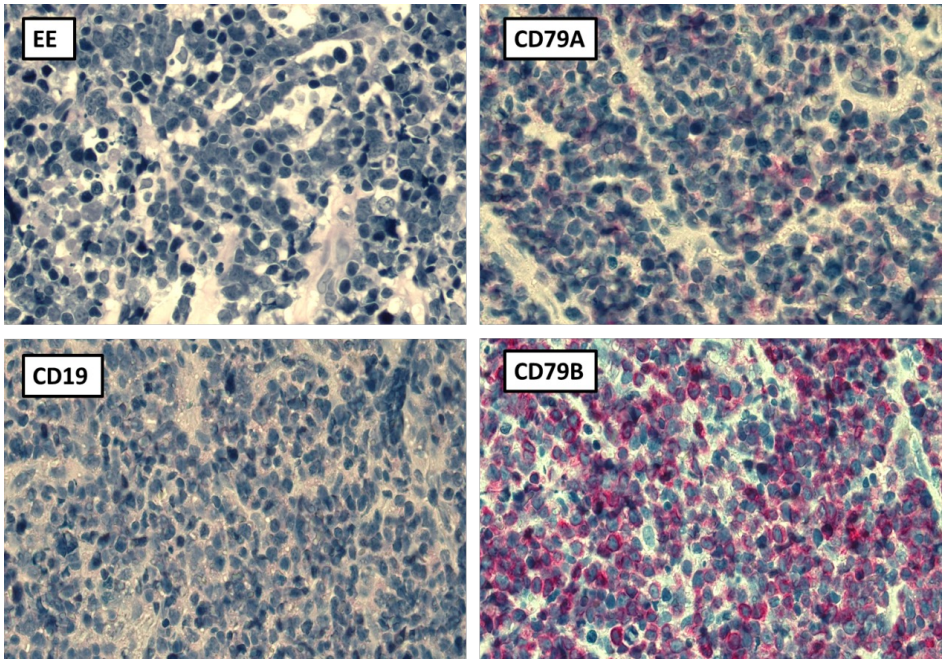




Perriello V et al. Am J Hematol 98:212-219, 2023

Wide loss of B-cell antigens after CD19-directed CAR-T cells in DLBCL

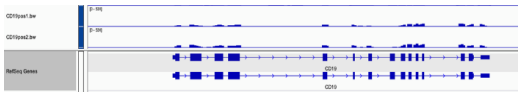




Nagler A, Falini B: How I treat DLBCL with CAR T cells. Br J Haematol, 201:396-410, 2023

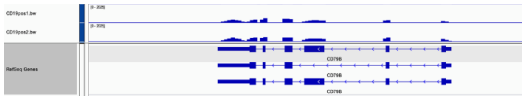
RNA sequencing at relapse post CD19-directed CAR T cells

RNA seq del CD19 <u>prima</u> della terapia con le cellule CAR-T anti-CD19

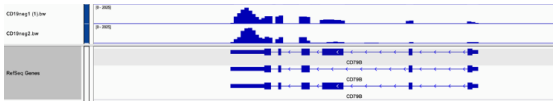


RNA seq del CD19 <u>dopo</u> la terapia con le cellule CAR-T anti-CD19

RNA seq del CD79B <u>prima</u> della terapia con le cellule CAR-T anti-CD19



RNA seq del CD79B <u>dopo</u> la terapia con le cellule CAR-T anti-CD19



Nagler A, Falini B: How I treat DLBCLwith CAR T cells. Br J Haematol, 201:396-410, 2023

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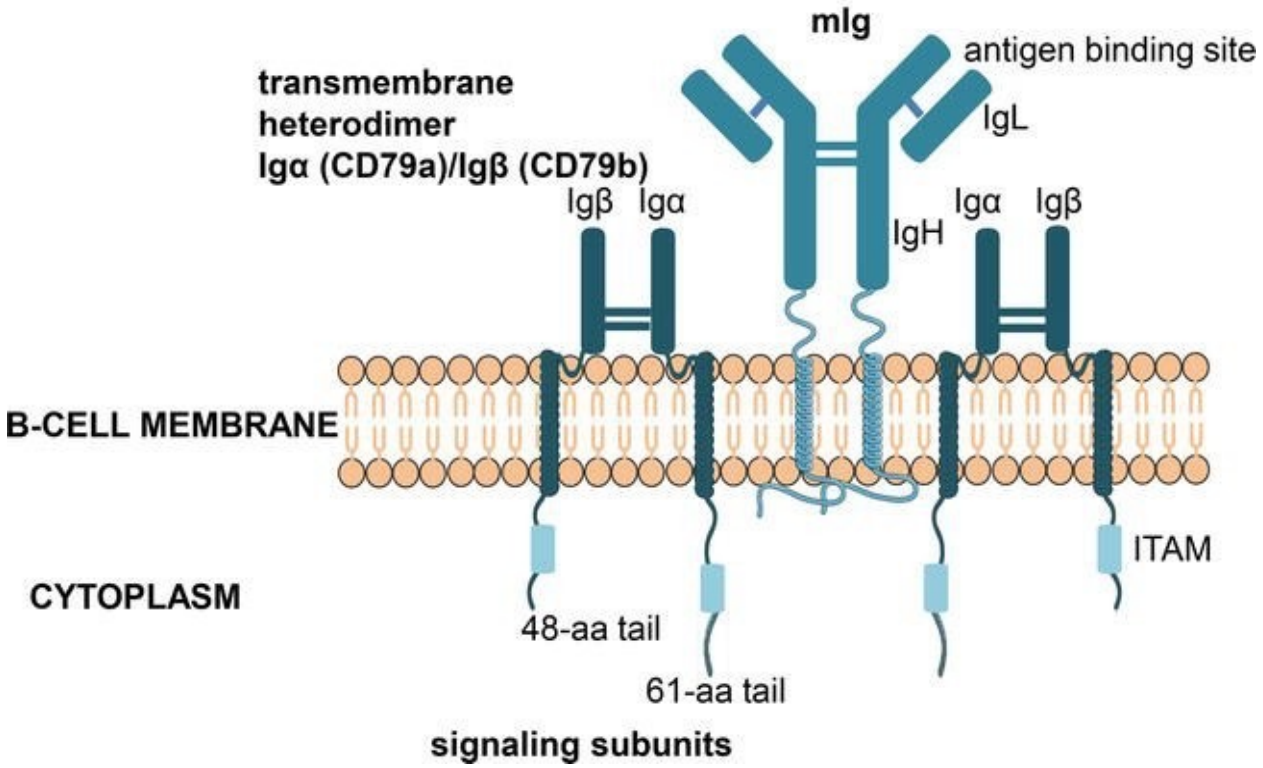
B-CELL ANTIGEN RECEPTOR COMPLEX



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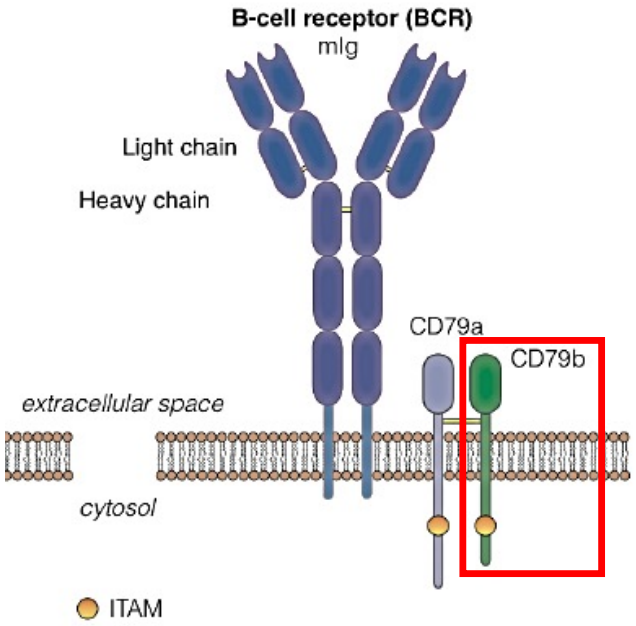
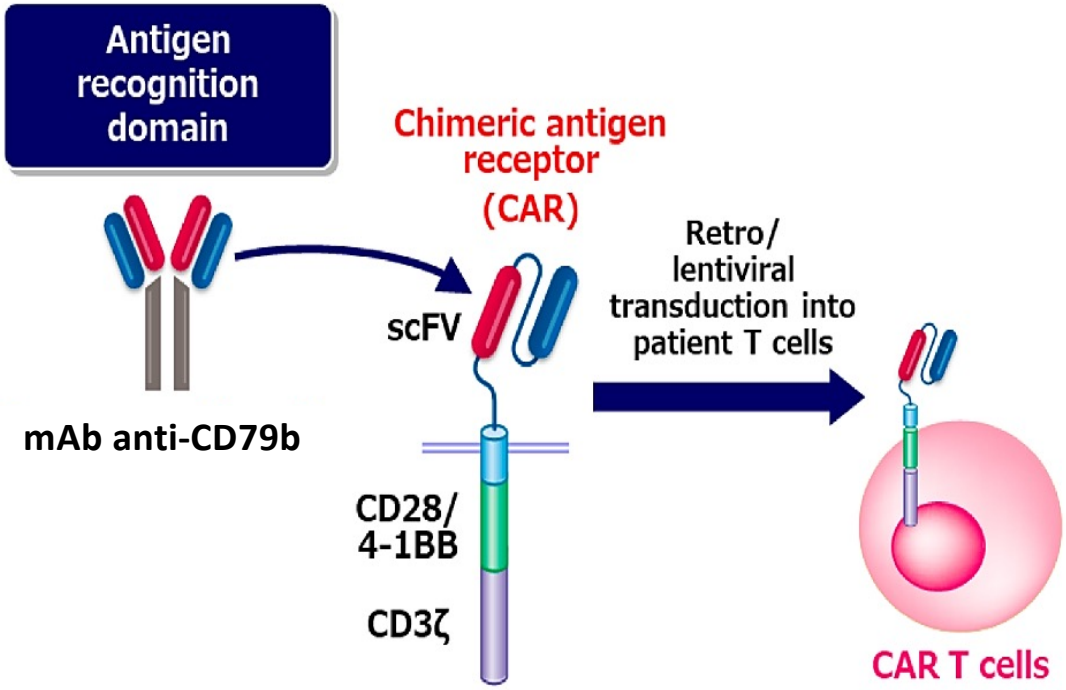
CD79b as an alternative B-cell marker for B-NHLs

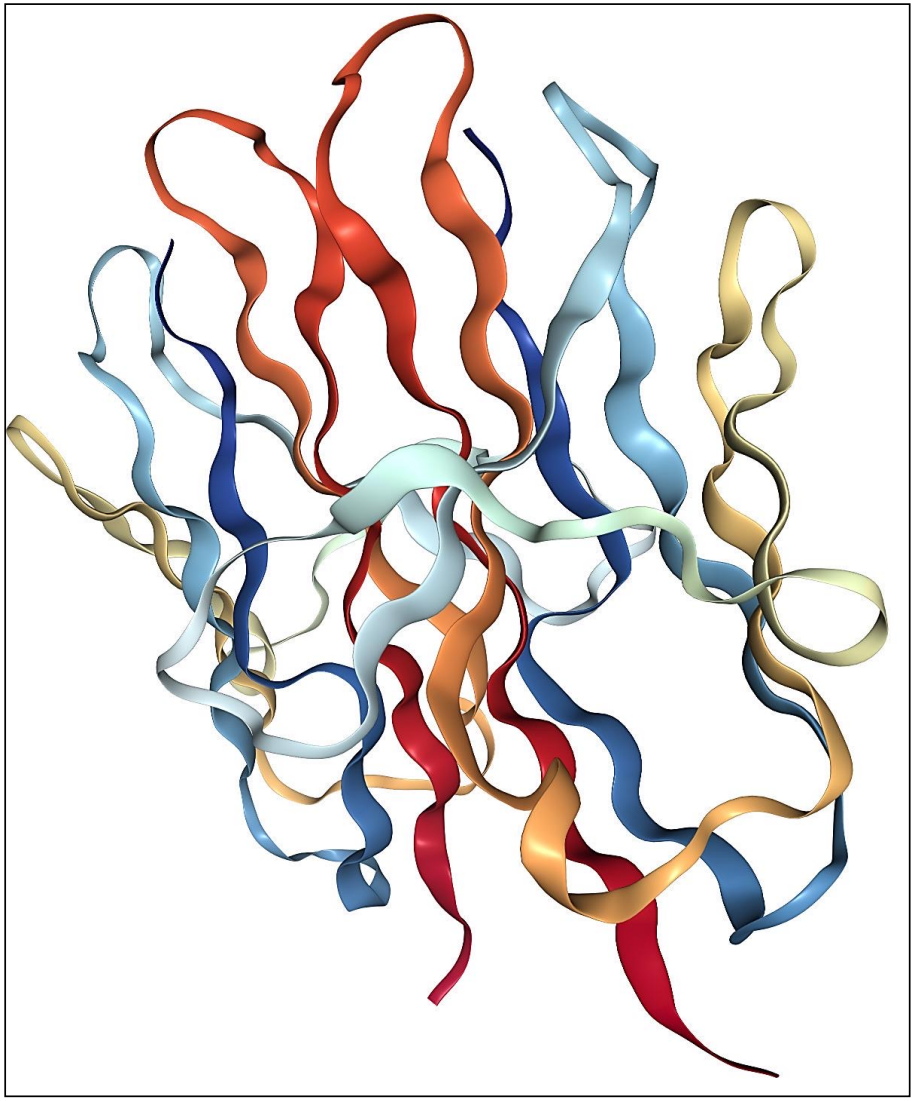
- Expressed in most B-NHLs, regardless of stage, subtype, cytogenetic and molecular characteristics
- Expressed only by mature B lymphocytes, thus reducing the risk of "on-target / off-tumor" toxicity
- As part of BCR, plays a role in supporting neoplastic proliferation and resistance to molecular drugs
- Stably expressed on the cell membrane of tumor cells, even at relapse after anti-CD19 CAR T cell therapy for DLBCL

GENERATION OF A NEW MONOCLONAL ANTIBODY ANTI-CD79b FOR DEVELOPING NEW CAR T CELLS





CD79b structure



Mice were immunized with a synthetic peptide corresponding to the entire extracellular portion of the CD79b molecule

> U5623EH200-1 (CD79b)

NdeI--ATG--His tag--CD79b--Stop codon--HindIII

Protein Length=144 MW=16701.7 Predicted pI=9.31 vector: pET30a

MGSSHHHHHHSSMARSEDRYRNPKGSACSRIWQSPRFIARKRGFTVKMHCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQ

NESLATLTIQGIRFEDNGIYFCQQKCNNTSEVYQGCGTELRVMGFSTLAQLKQRNTLKD..

DNA sequence: 447bp

CATATGGGATCAAGTCATCACCACCACCACCACTCAAGTATGGCTAGATCAGAAGACCGTTACCGCAACCCGAAAGGCAGCGCGT

GCAGCCGTATCTGGCAGAGCCCGCGTTTCATTGCGCGTAAGCGTGGTTTTACCGTGAAAATGCACTGCTACATGAACAGCGCGAG

CGGCAACGTTAGCTGGCTGTGGAAGCAAGAGATGGACGAAAACCCGCAGCAACTGAAGCTGGAGAAAGGTCGTATGGAGGAAAGC

CAGAACGAAAGCCTGGCGACCCTGACCATCCAAGGTATTCGTTTCGAGGATAACGGCATCTACTTTTGCCAGCAAAAATGCAACA

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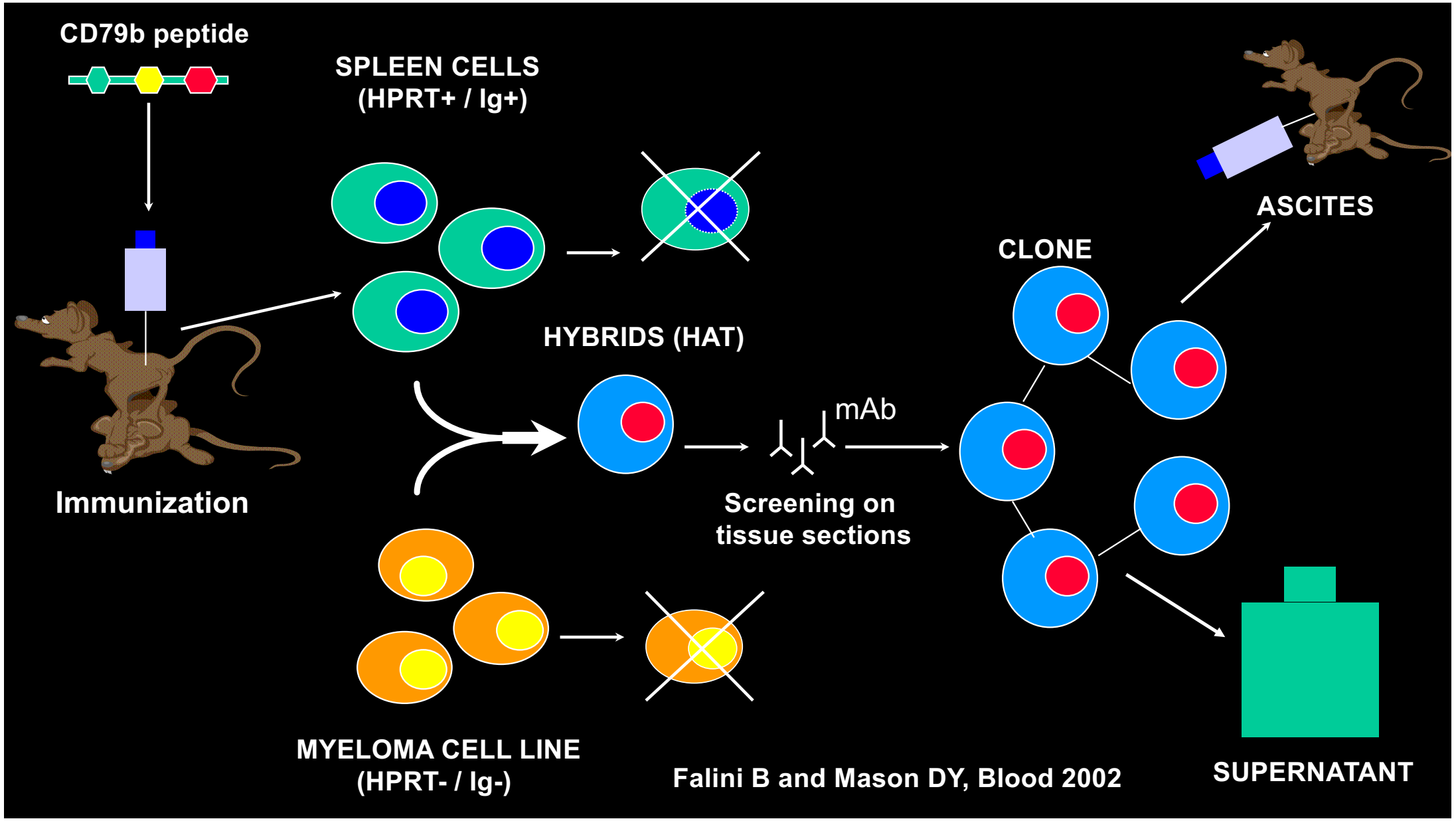
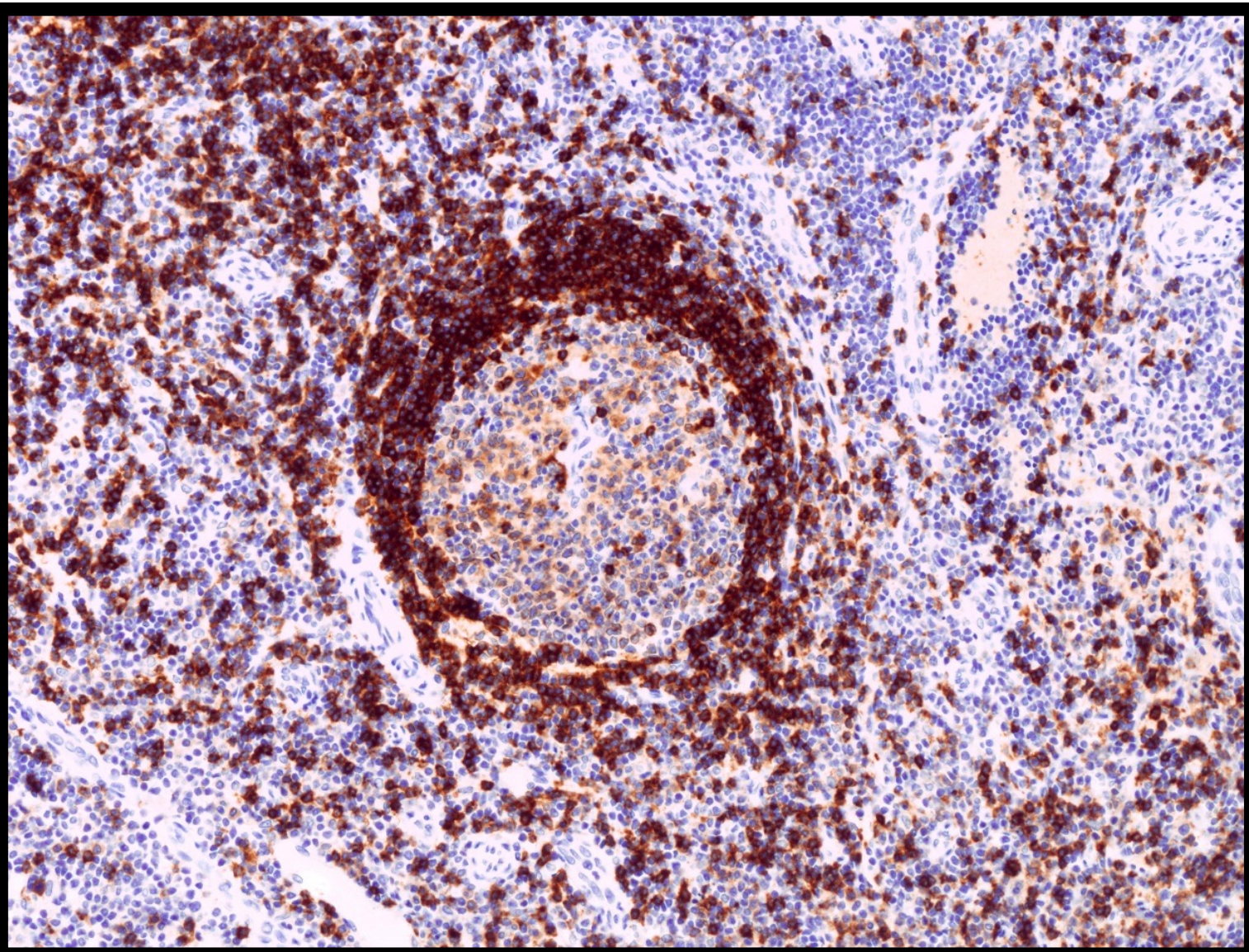
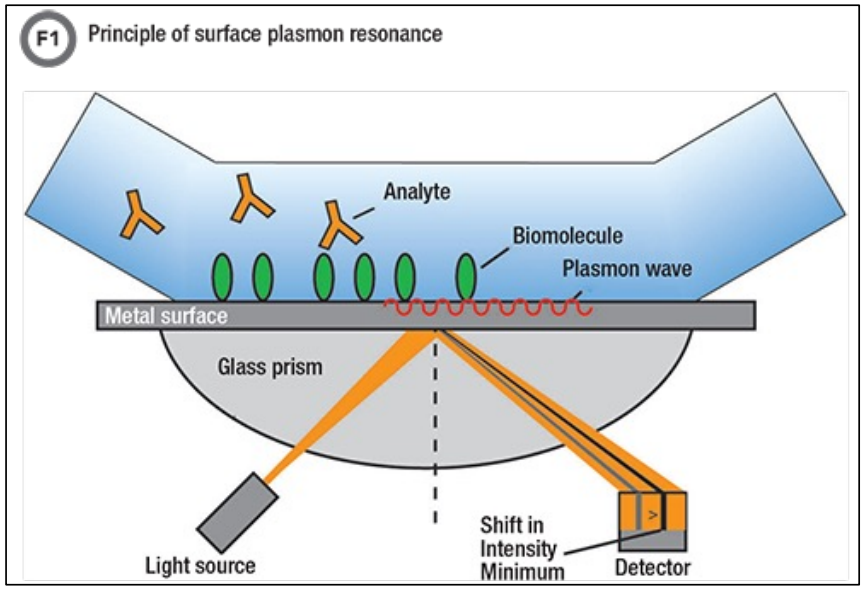
CCTGAAAGATTAATGAAAGCTT

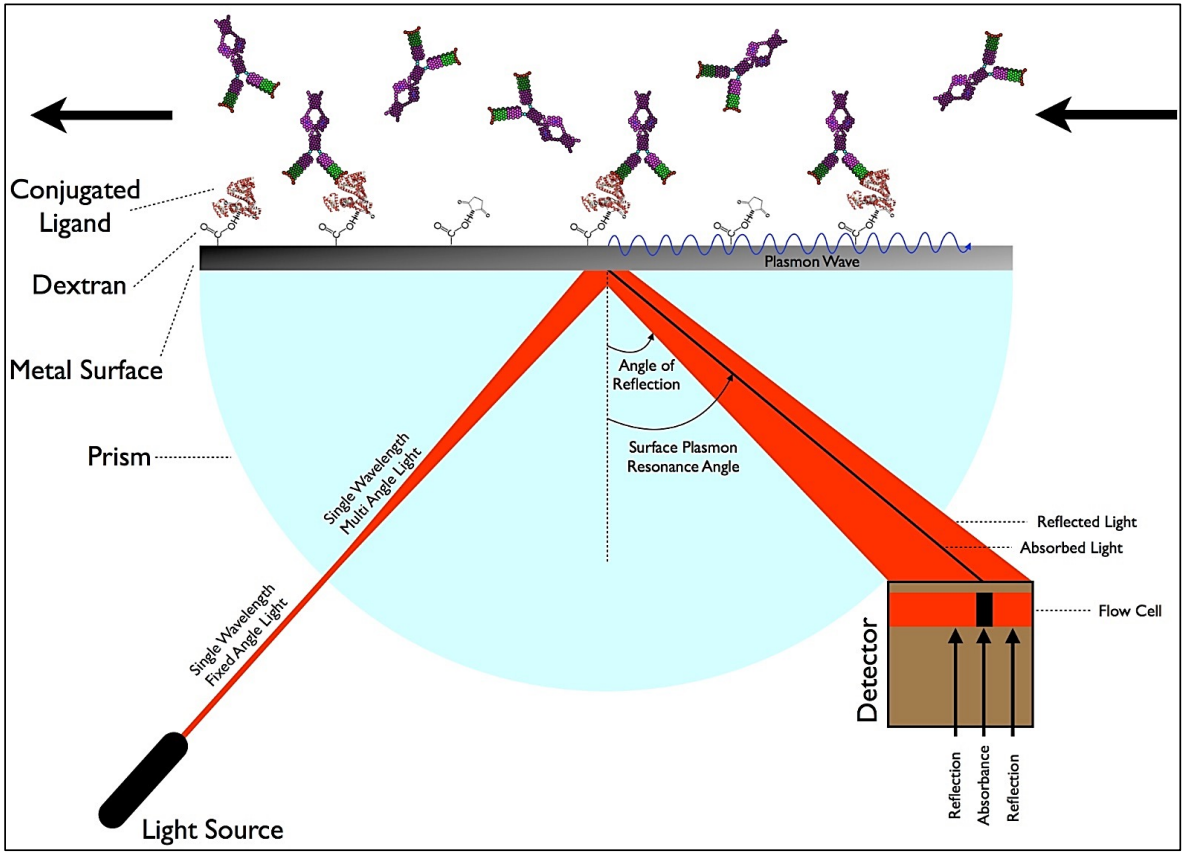

CD79b peptide
SPLEEN CELLS
(HPRT+ / Ig+)
ASCITES
CLONE
HYBRIDS (HAT)
mAb
Immunization
Screening on
tissue sections
MYELOMA CELL LINE
(HPRT- / Ig-)
Falini B and Mason DY, Blood 2002
SUPERNATANT



Screening on LN follicular hyperplasia: 2 anti-CD79b clones (PG 128, PG 151)

Surface plasmon resonance





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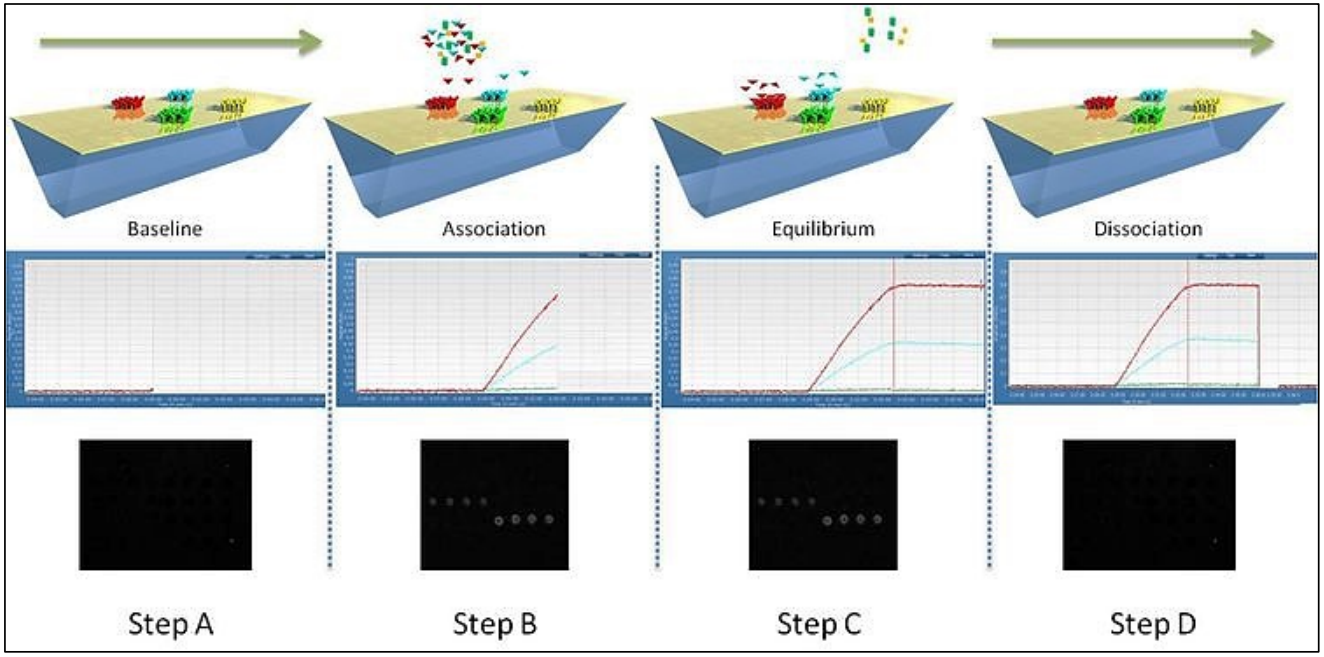
Surface Plasmon resonance



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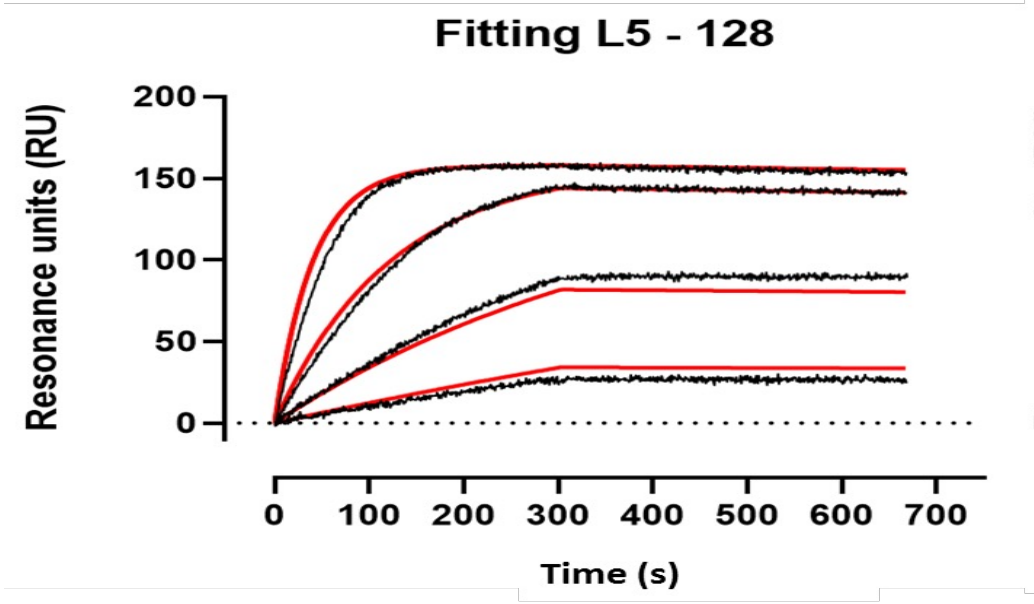
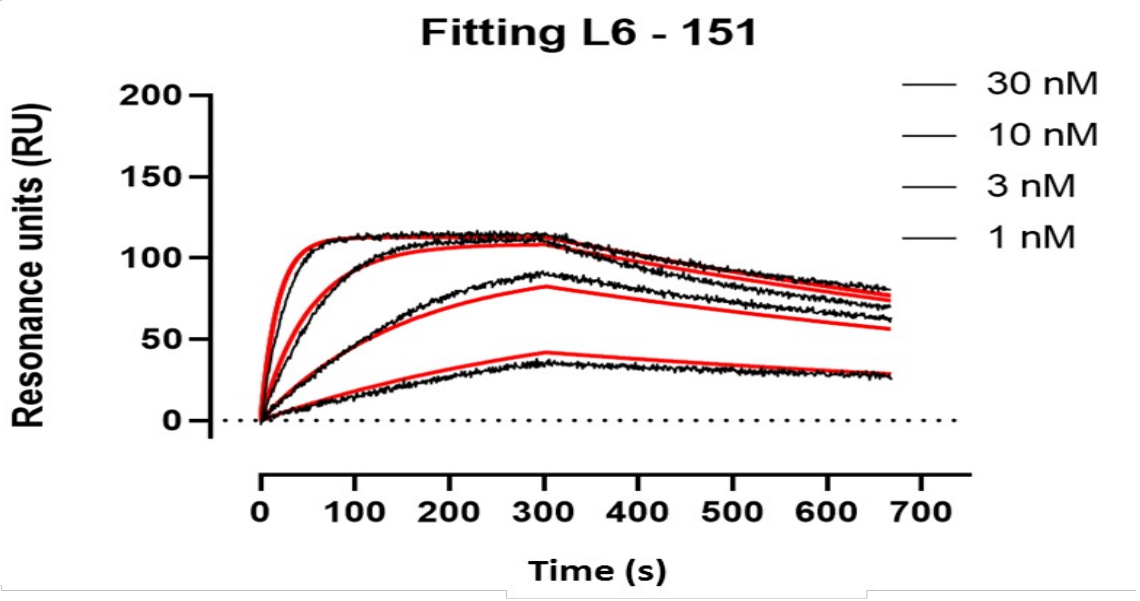
Surface plasmon resonance:CD79b specific binding and fitting



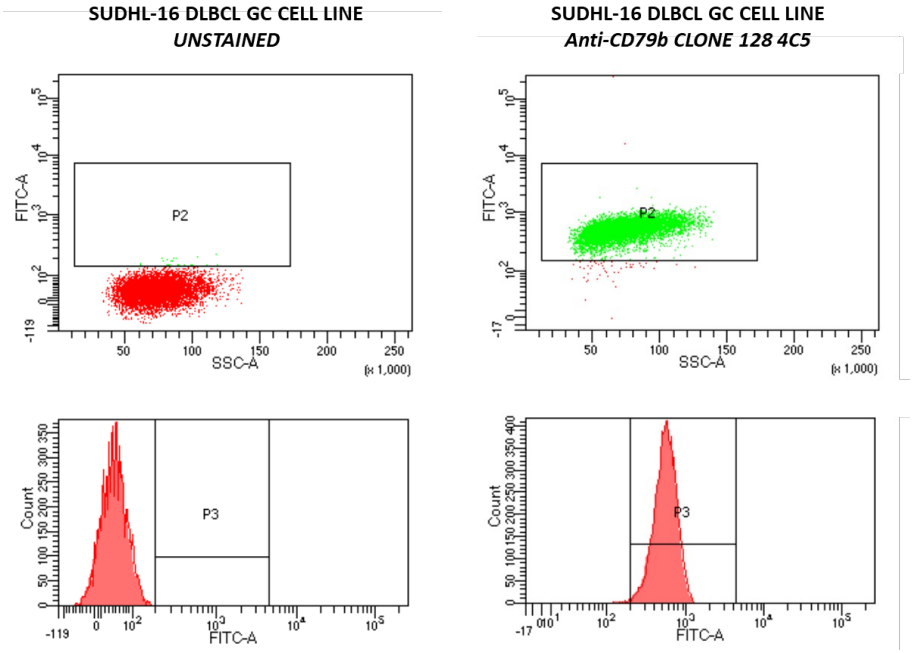
ka (1/Ms) 8,08E+05 **kd** (1/s) 4,96E-05
KD (M) 6,13E-11 **Rmax** (RU) 158,13



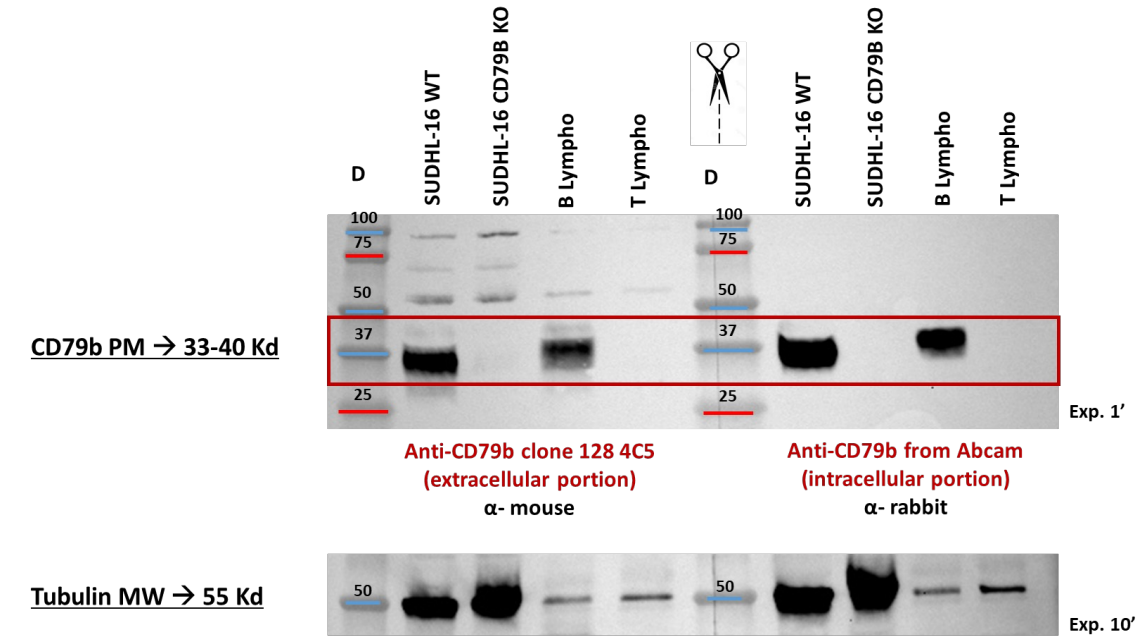
ka (1/Ms) 1,79E+06 **kd** (1/s) 1,05E-03
KD (M) 5,84E-10 **Rmax** (RU) 114,77

Anti-CD79b mAb validation

mAb surface staining validation



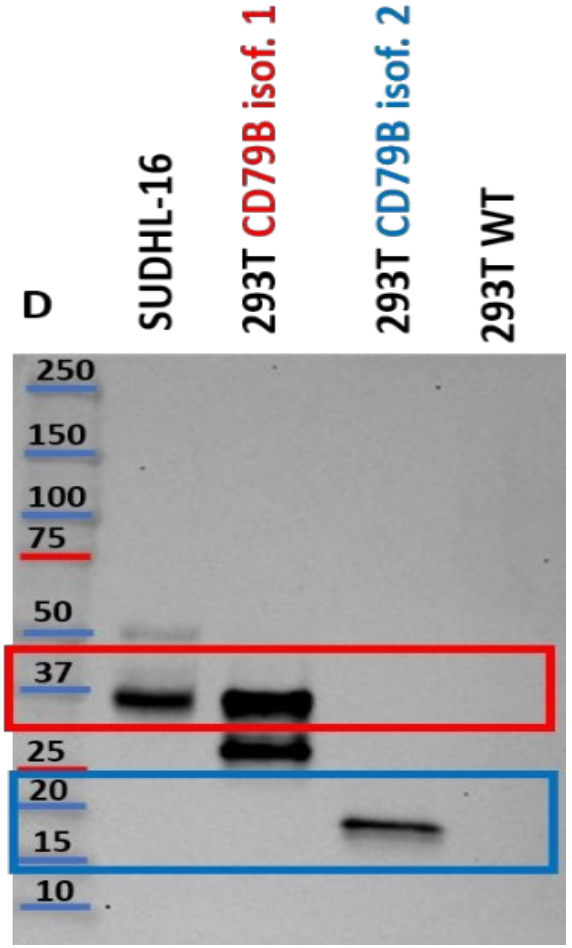
mAb target selectivity validation

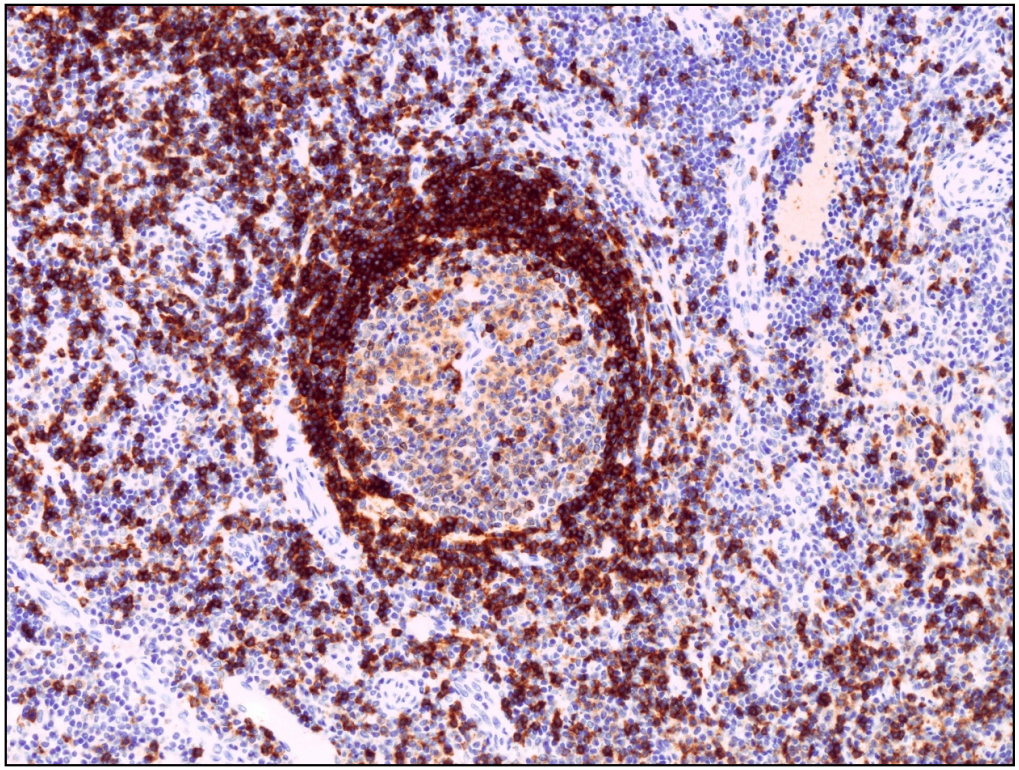


Clone 128 recognizes both the two isoforms of CD79b

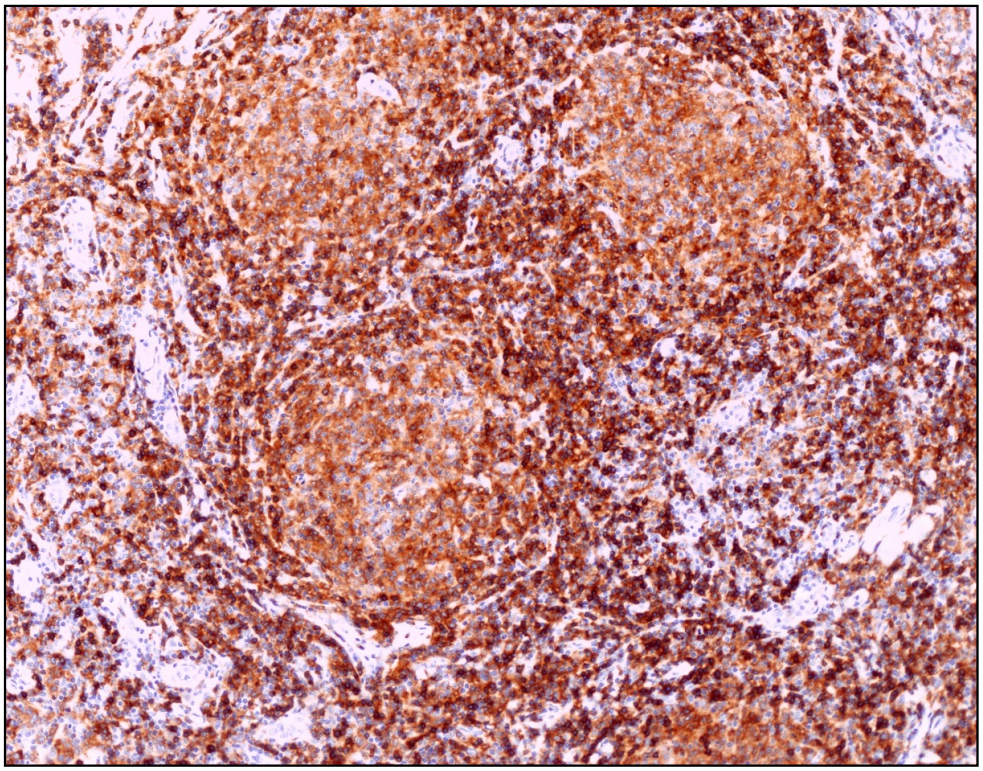
CD79b isof. 1= PM 33- 40 Kd

CD79b isof. 2= PM 15- 25 Kd



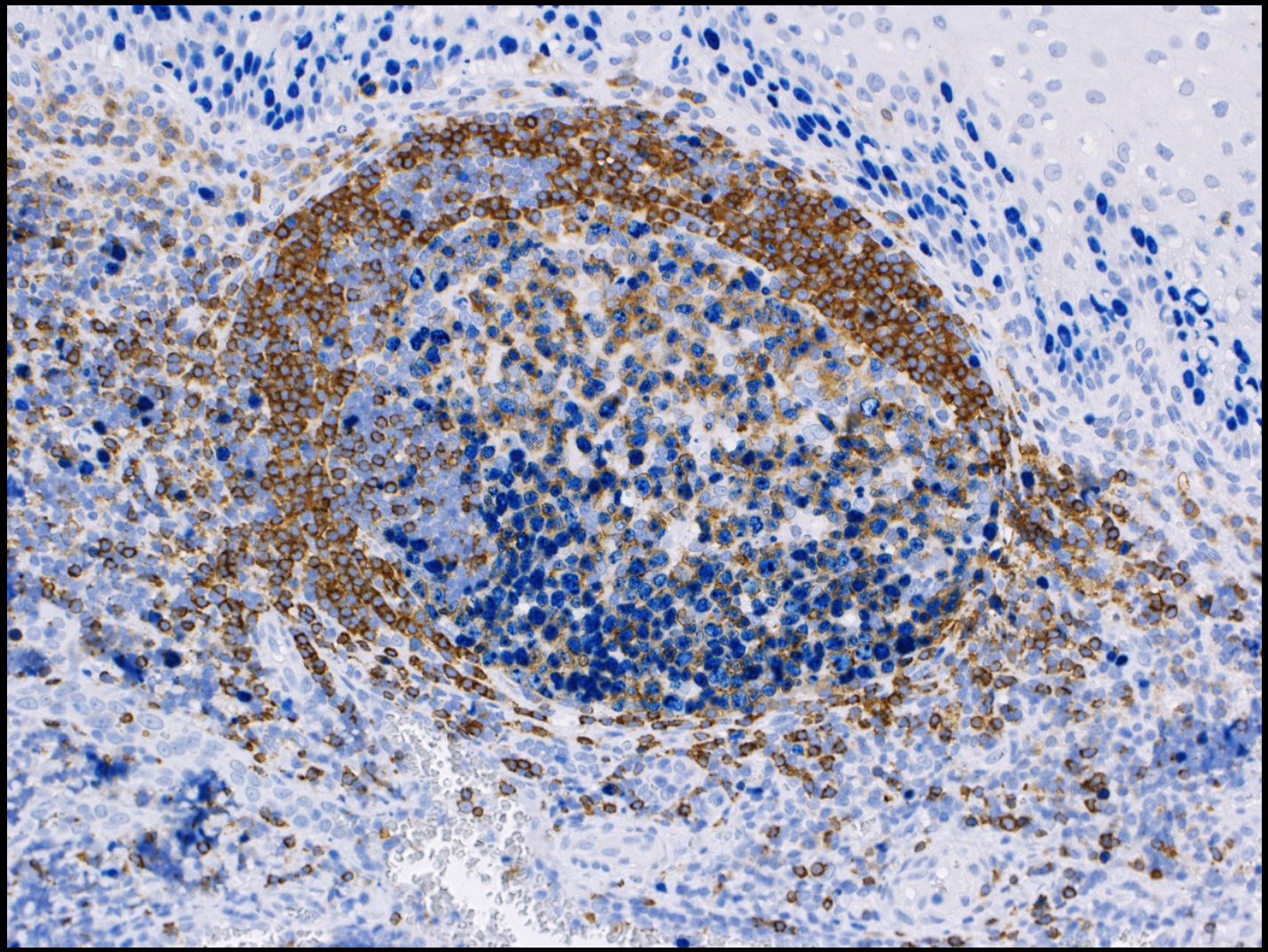


Follicular hyperplasia; CD79b is more strongly expressed in mantle cells as compared to GC cells

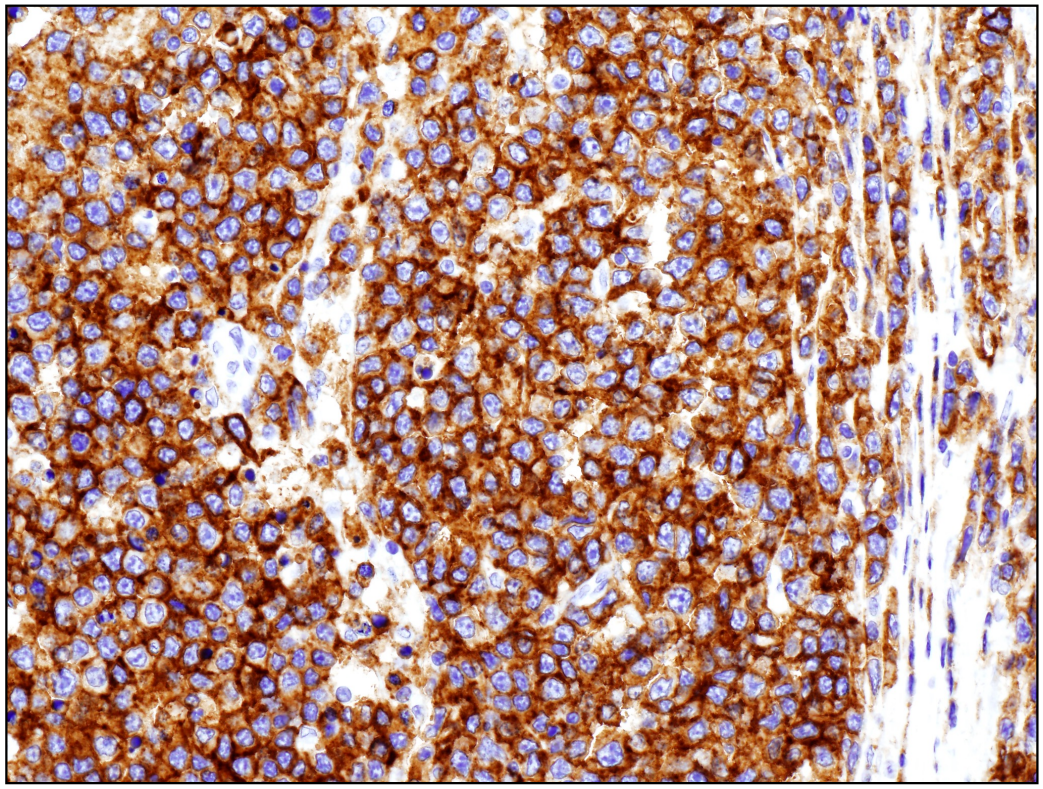


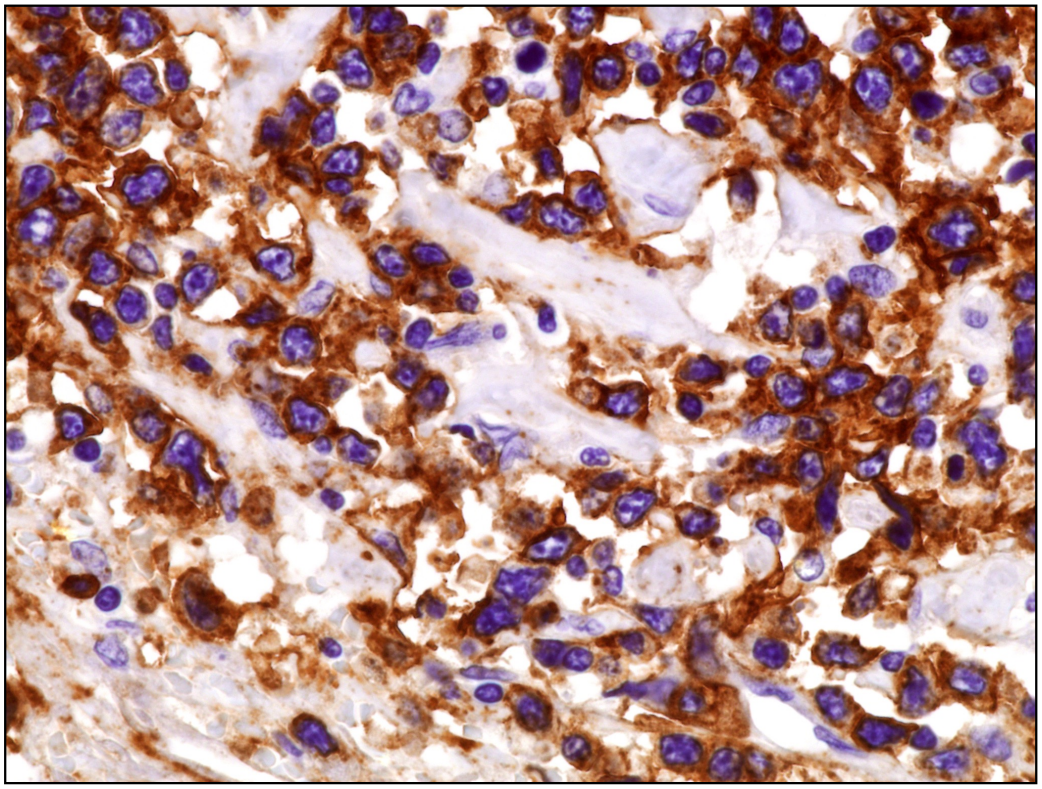
Follicular lymphoma: CD79b strongly expressed

CD79 is expressed more strongly in MC than in GC cells

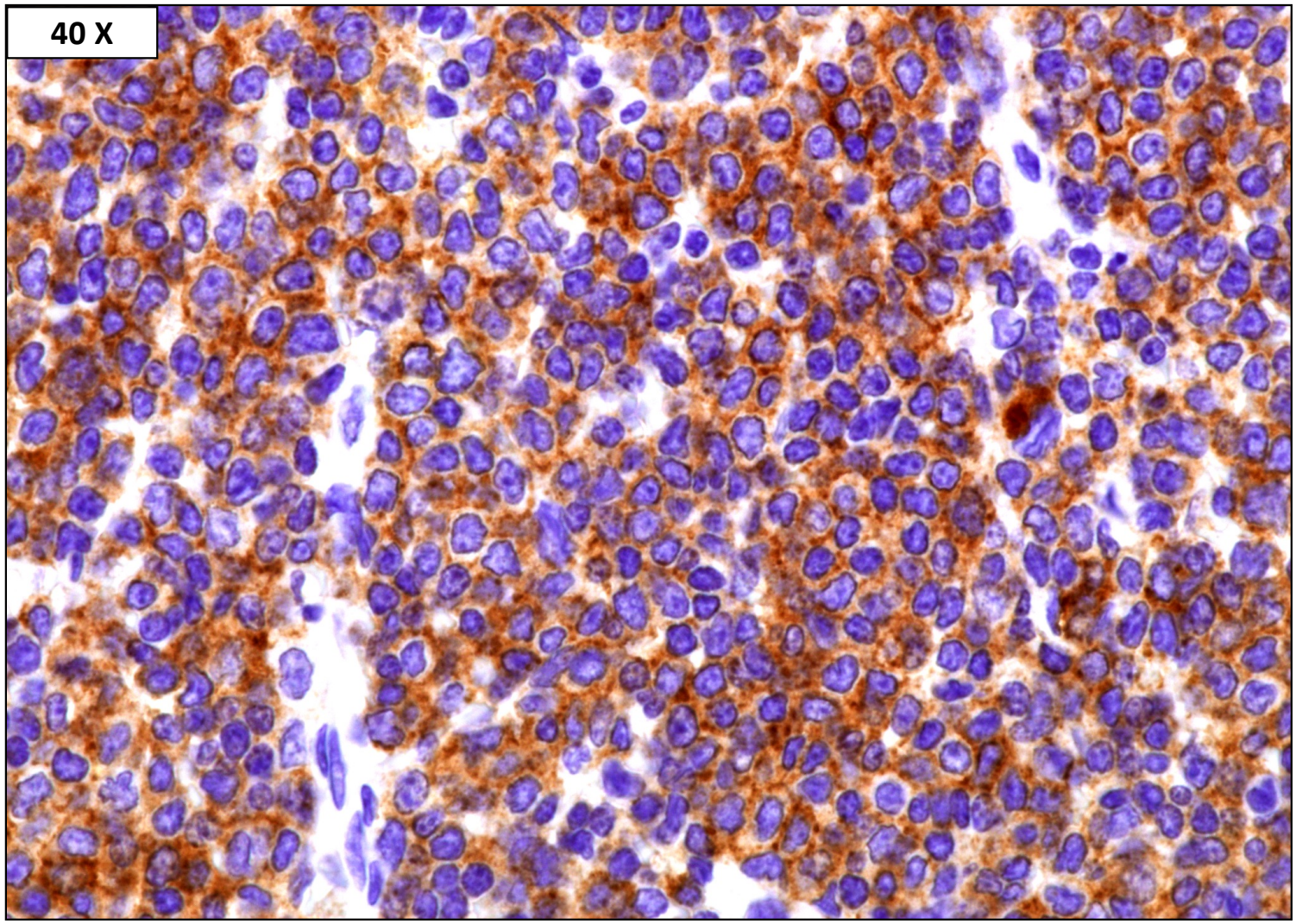


Double staining: CD79b (brown)/Ki-67 (blue)



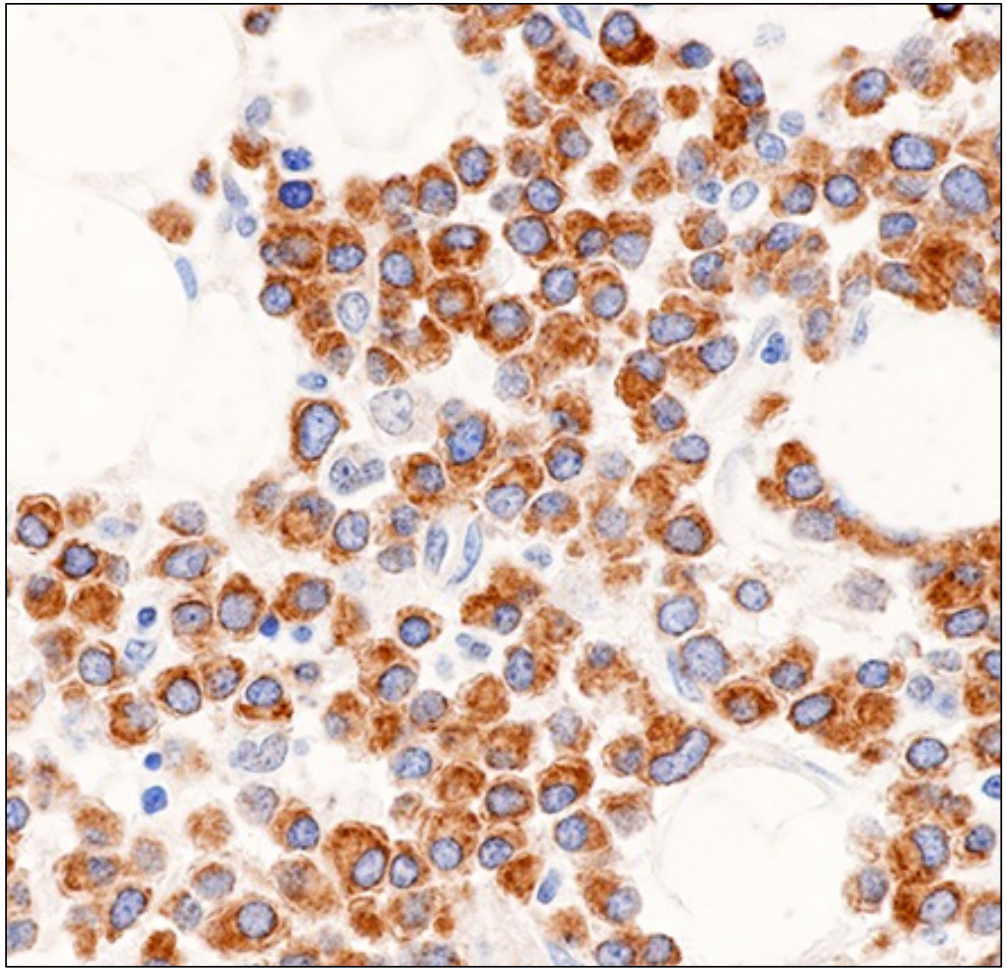


Diffuse large B-cell lymphomas (DLBCL) immunostained with mAb anti-CD79b (Perugia-clone PG-128)

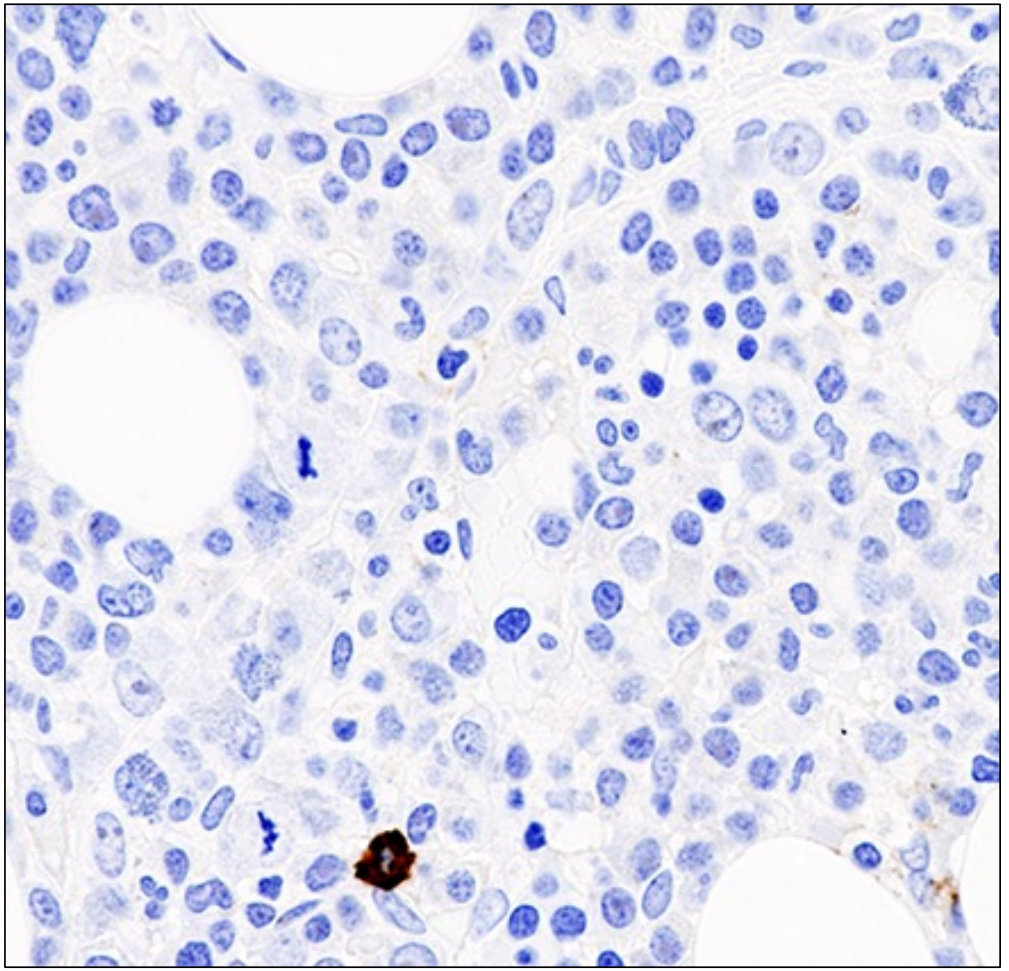


MCL blastoid variant: mAb anti-CD79b (Perugia clone)

Multiple myeloma: bone marrow biopsy

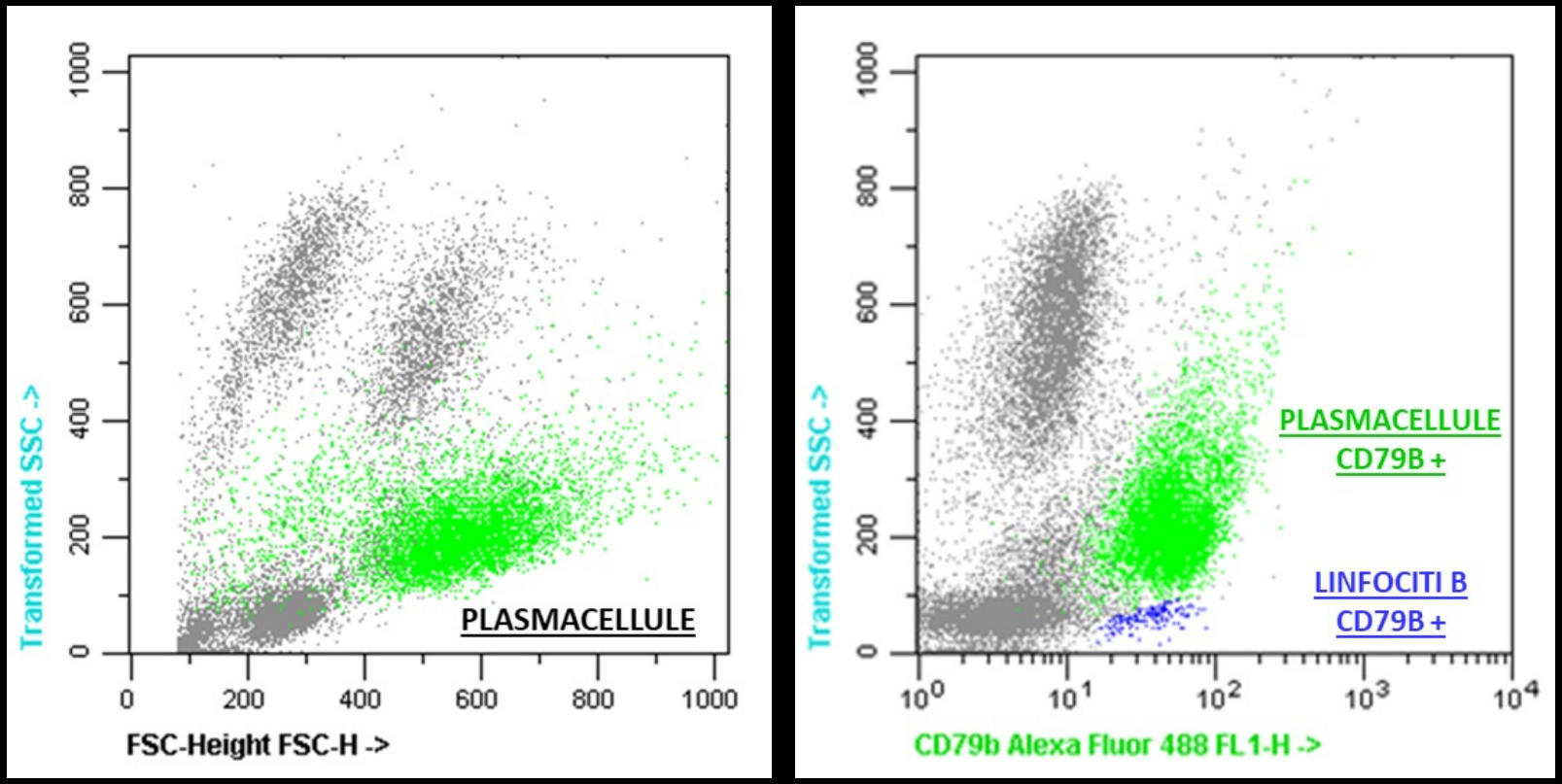


Expression of CD79b



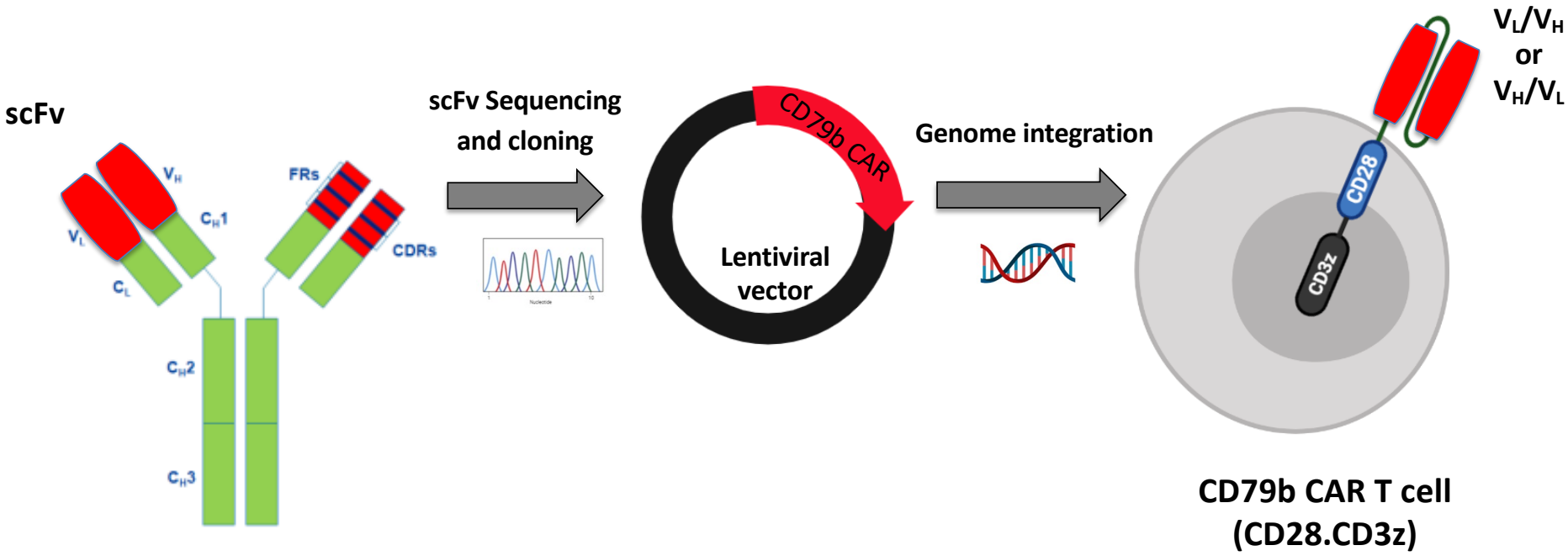
Expression of CD79a

Myeloma: flow cytometry of BM aspirate



Surface positivity with anti-CD79b mAb (Perugia clone PG-128)

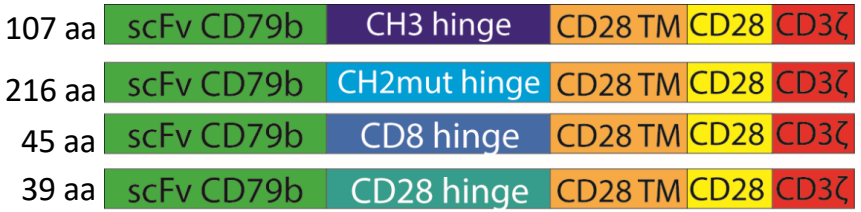
Generation of novel anti-CD79b CAR-T cells

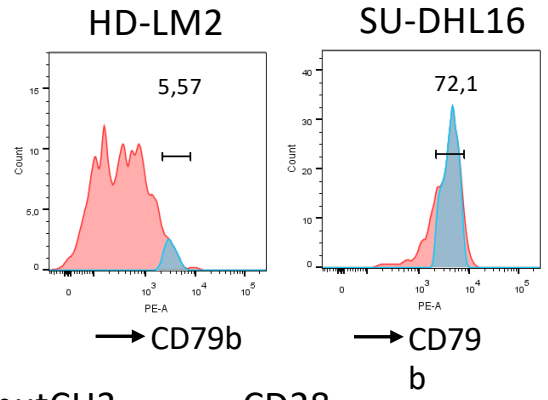


mAb anti-CD79b (Perugia clone PG-128)

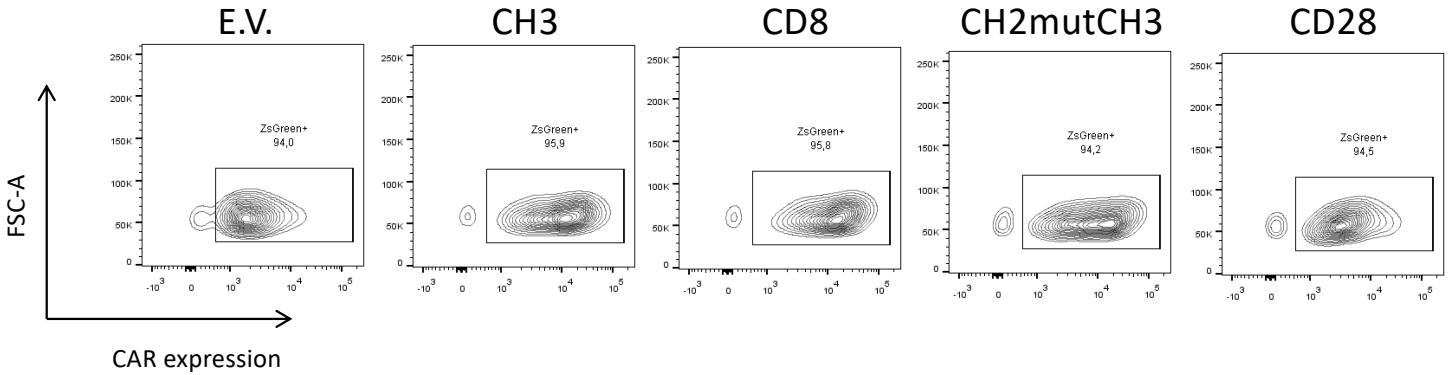
CD79b CAR T cell (CD28.CD3z)

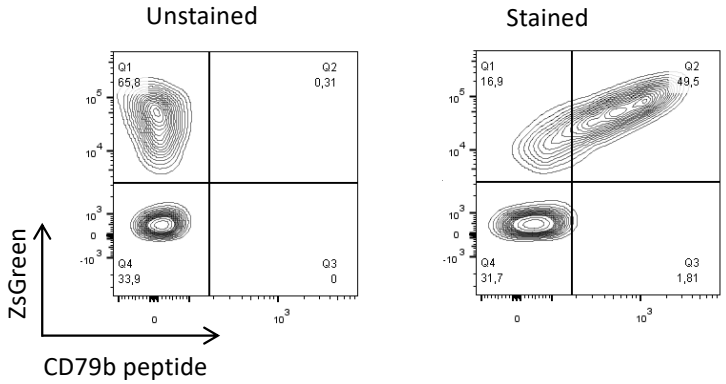
Extracellular CD79b CAR optimization





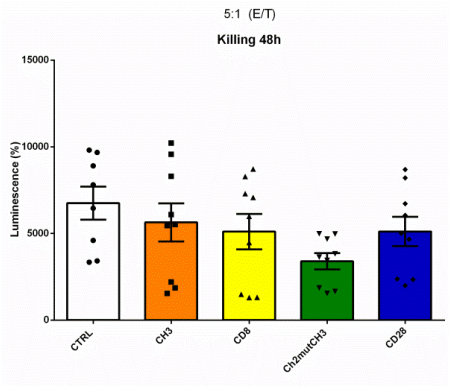
ZsGreen cell post-sorting

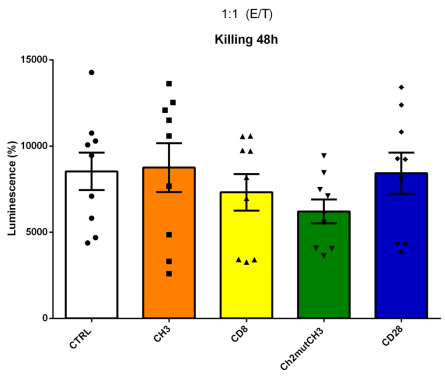


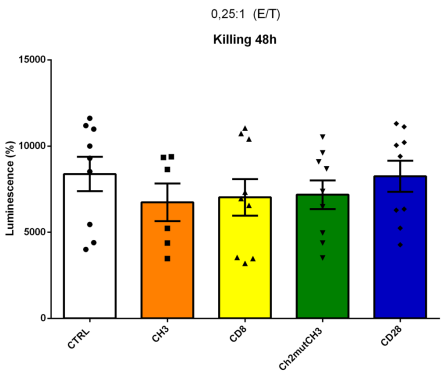


Luminescence assay (48 hours cytotoxicity)

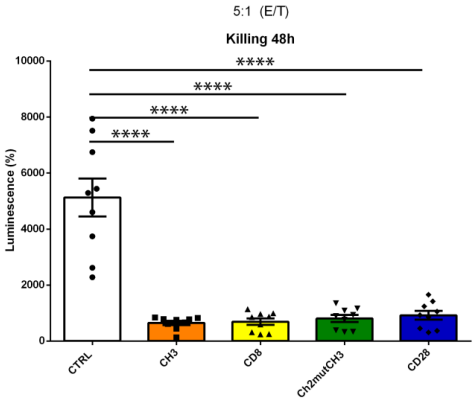
Versus HD-LM 2 (CD79b negative)

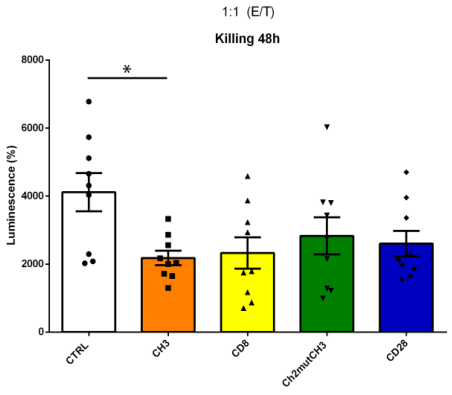


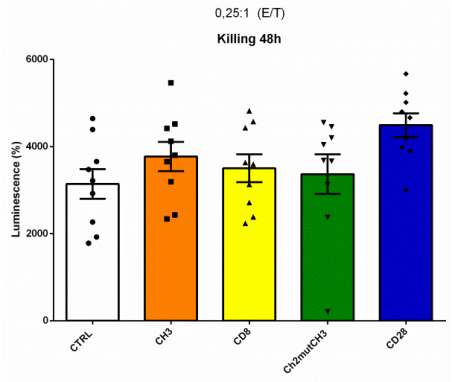




Versus SUDHL-16 (CD79b positive)

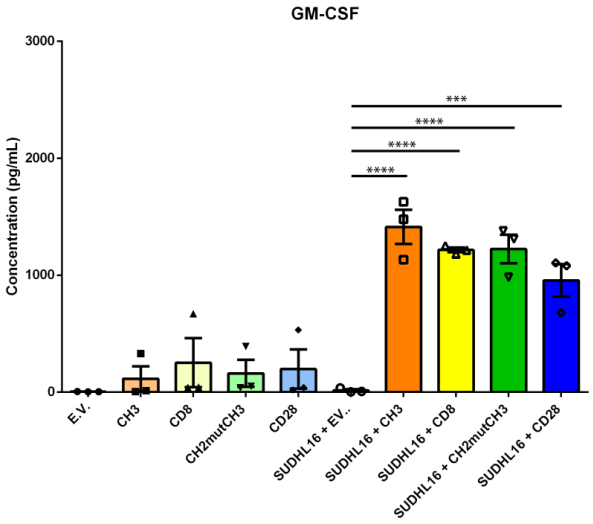


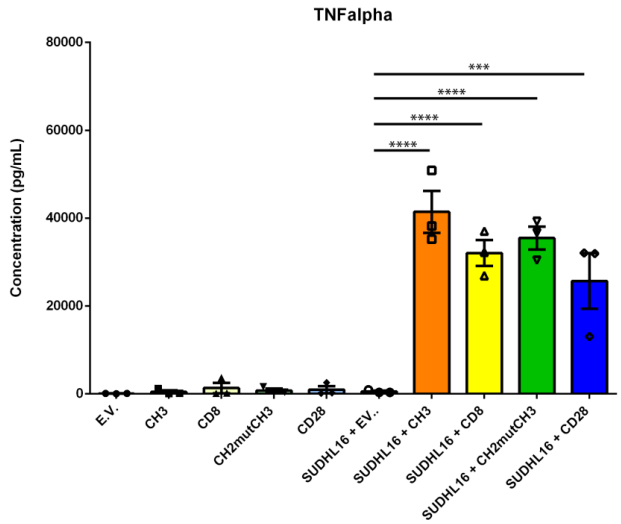


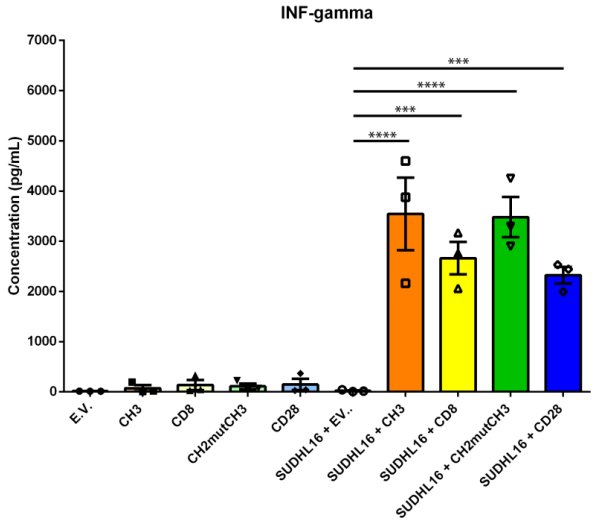


Values are expressed as mean ± SEM of 3 independents experiments

Cytokines detection after 24 hours co-culture (E:T ratio 1:3)

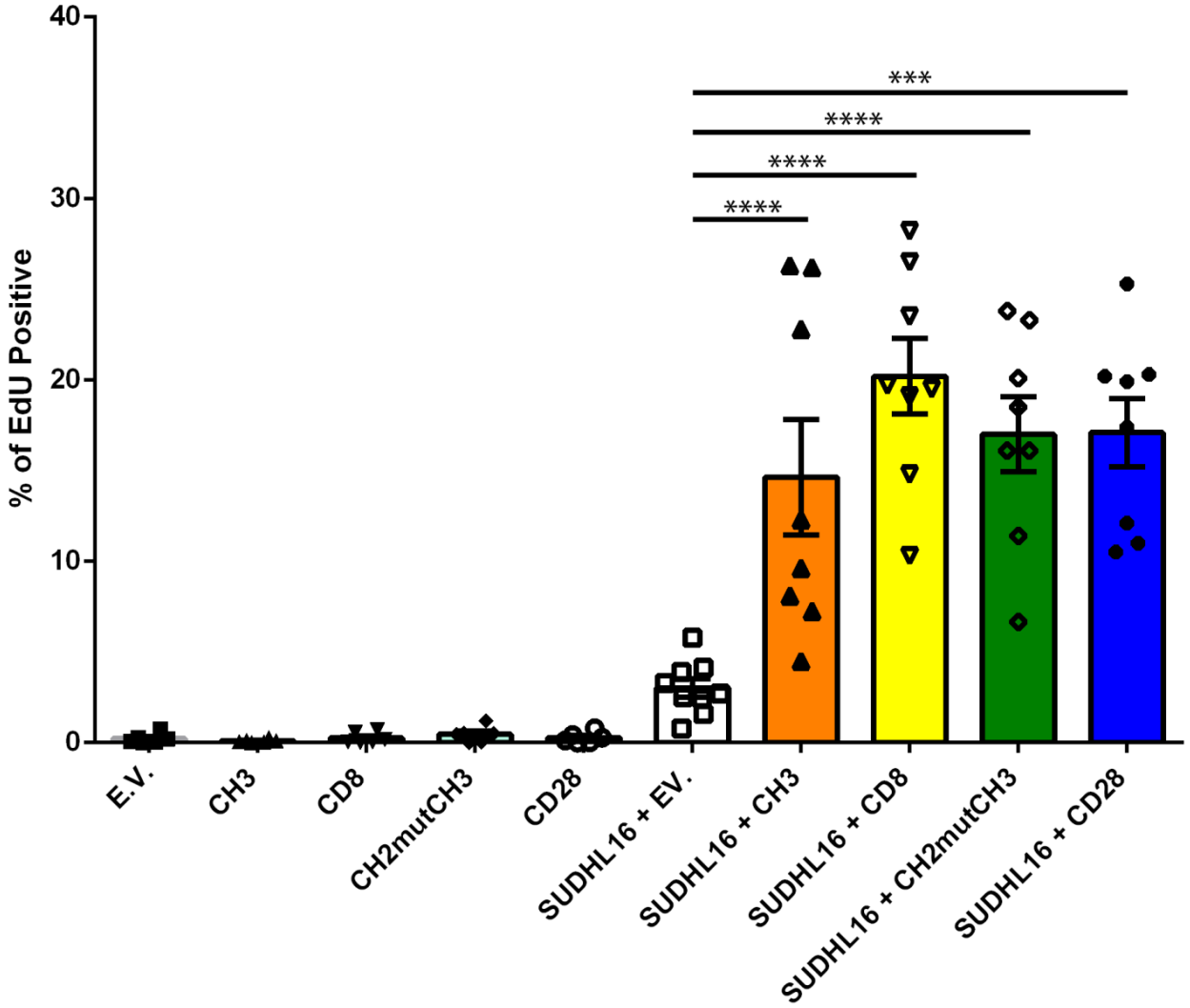






Values are expressed as mean ± SEM of 3 independents experiments

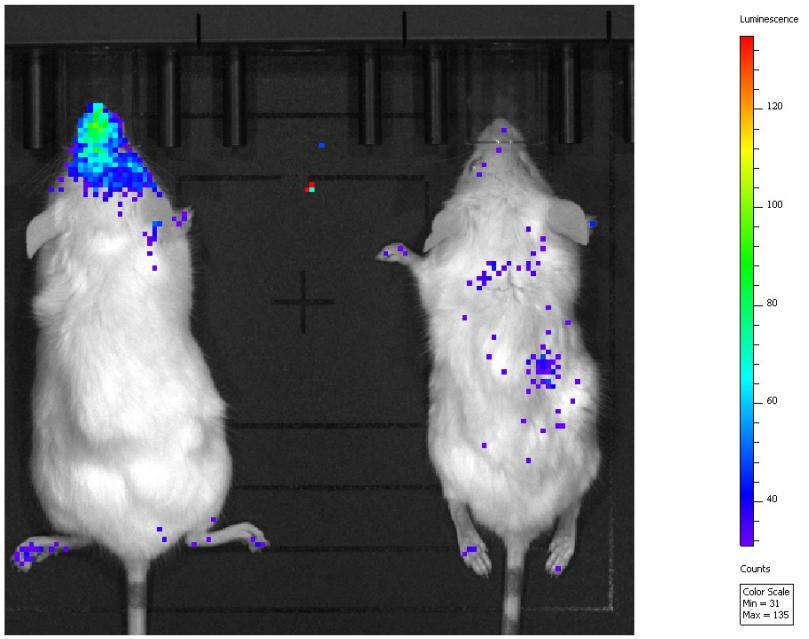
Proliferation of 72 hours co-culture of CAR and irradiated SUDHL-16 (E:T ratio 1:1)



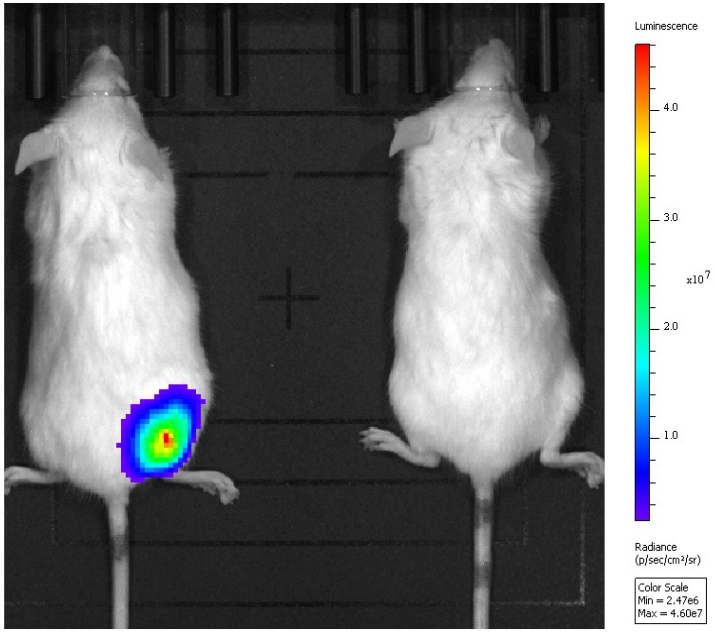
Values are exp

Selected constructs

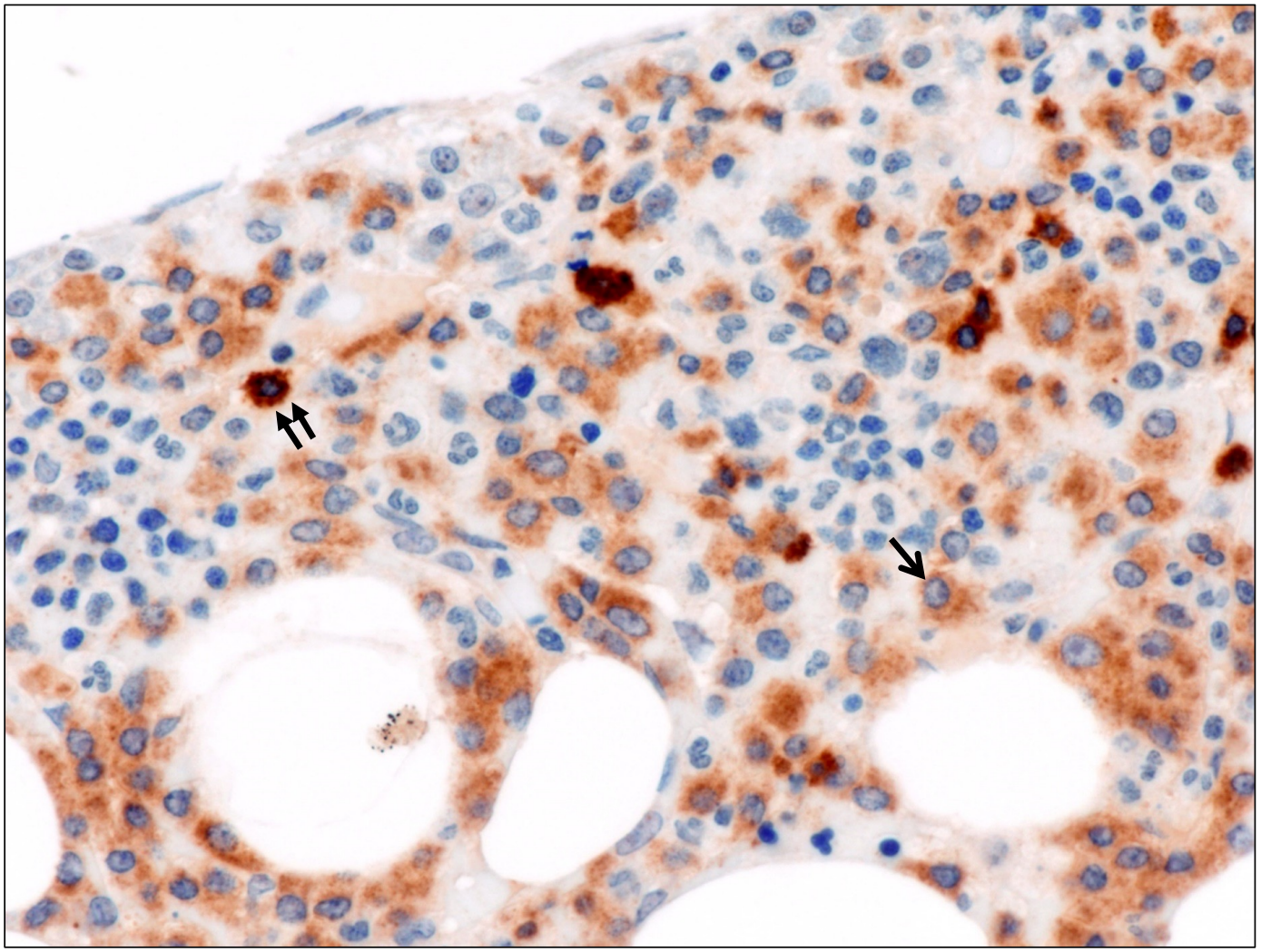
In vivo studies are ongoing



IV



SC



CD79b is expressed in MM at lower intensity than in B-cell lymphomas.
Can we increase the sensitivity of anti-CD79b CAR T cells in MM ?

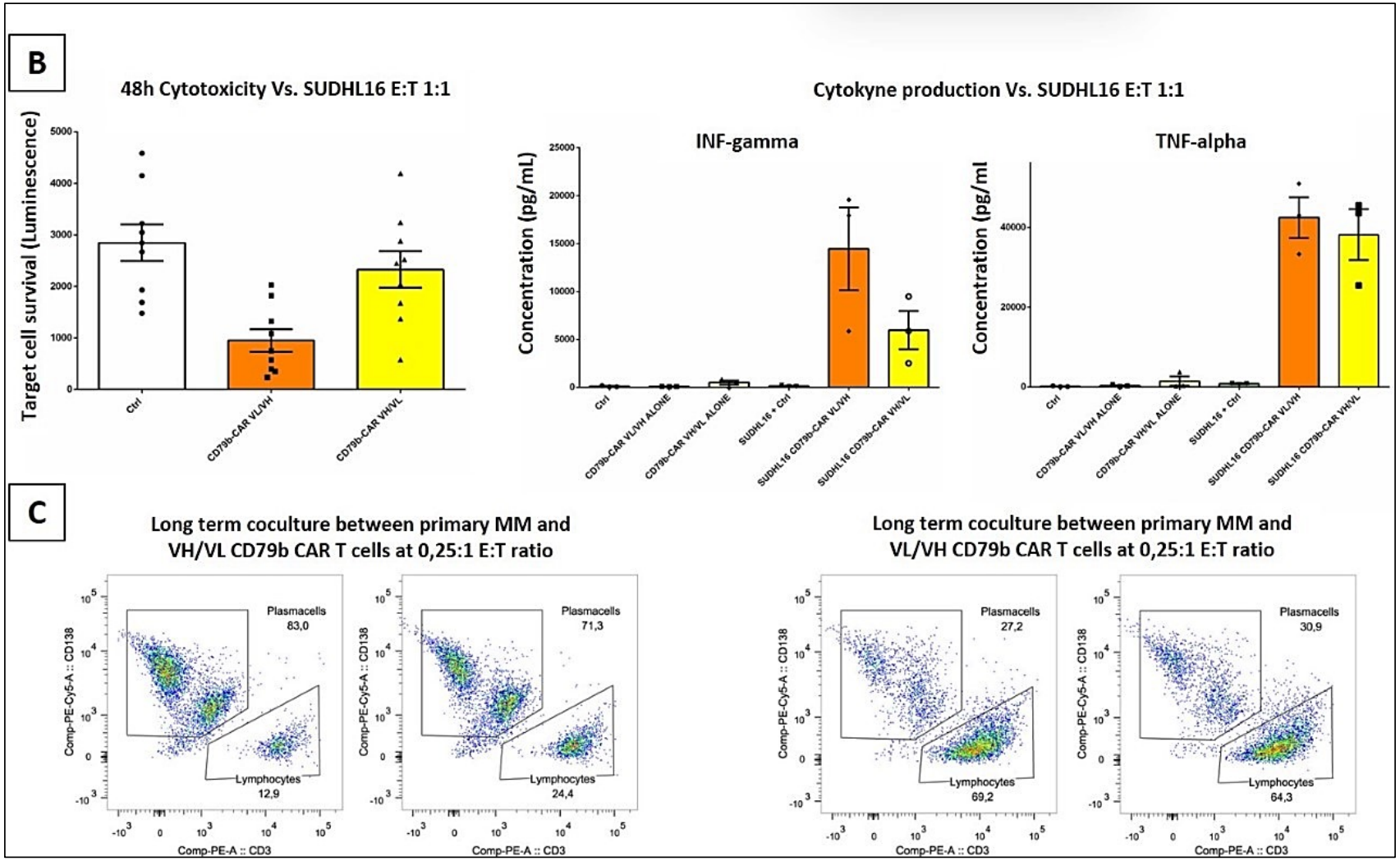
Artificial intelligence Alphafold2 program was used to predict 3D interaction between CD79b and VH/VL or VL/VH orientation of anti-CD79b mAb and the HADDOCK server for molecular docking analysis

Anti-CD79b
VH/VL scFv

CD79b Antigen
Anti-CD79b mAb

Anti-CD79b
VL/VH scFv

Anti-CD79b VL/VH SCFv orientation displayed lower binding energy tha VH/VL (HADDOCK score 54.2+/-14 vs 72.9+/-13.3), suggesting a higher association propensity to CD79b.



In Silico data were confirmed in vitro where VL/VH showed enhanced activity compared to VH/VL in terms of cytotoxicity against the SUDHL16 and primary myeloma cells.

Conclusions

We generated a novel murine mAb recognizing an epitope shared by the two isoforms of human CD79b

Perugia CAR-T cells carrying a murine scFv against CD79b are effective *in vitro* against B-NHL cell lines. Preclinical in vivo studies are ongoing

Artificial intelligence can be used to develop high affinity anti-CD79b CAR T cells, especially against multiple myeloma

The best CD79b CAR T construct will be used for a phase 1 clinical trial on patients with B-cell lymphomas and multiple myeloma relapsing after CD19- or BCMA-directed CAR T cells