DART: groundwork and outlook

Immunotherapy in Hematological Malignancies 2018
1st Cuneo City Immunotherapy Conference (CCITC)

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Giorgio Inghirami
Department of Pathology and Laboratory Medicine
Weill Cornell Medicine, New York, NY

Center of Experimental Medicine and Research (CeRMS) University of Turin, Italy

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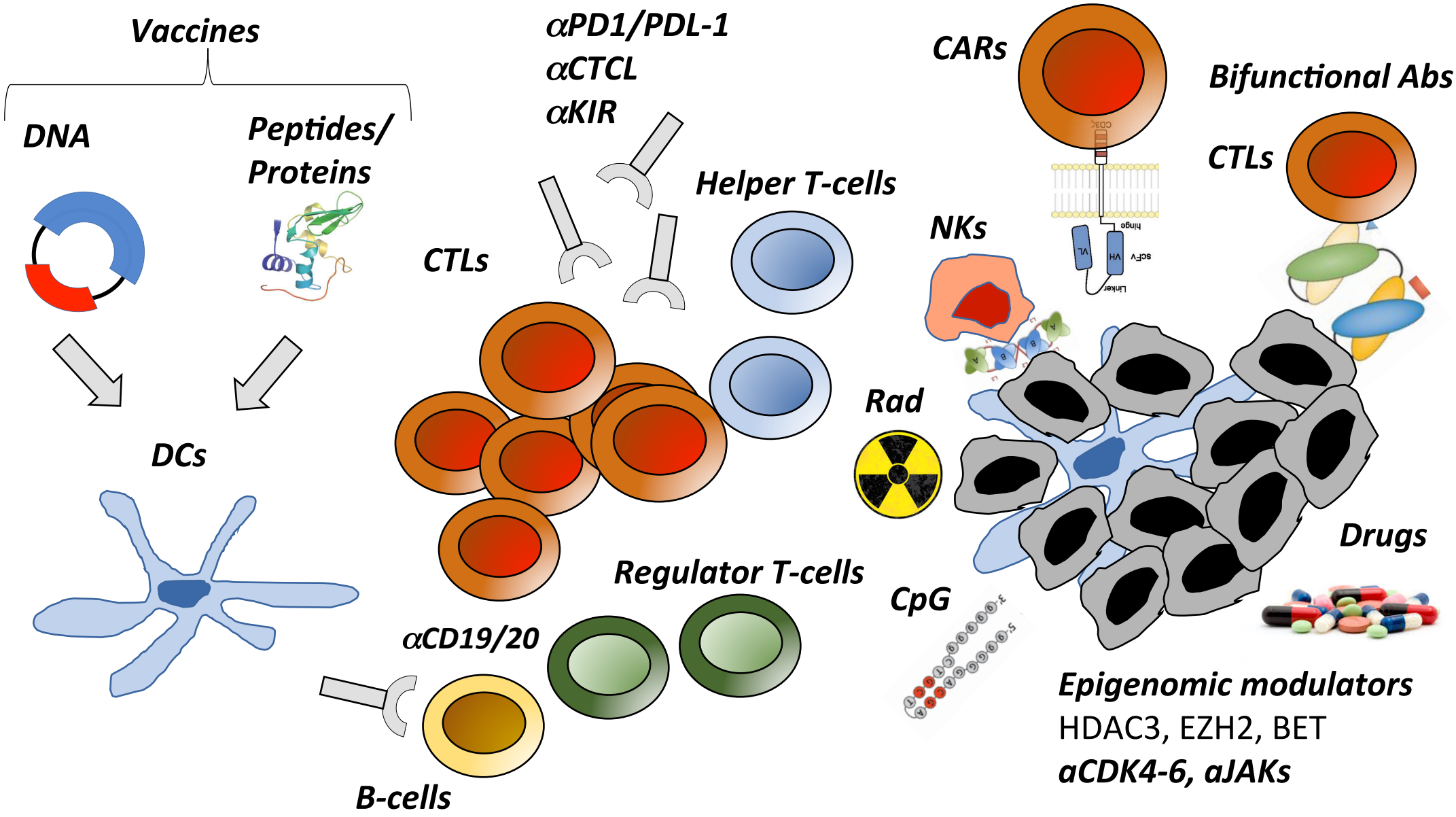
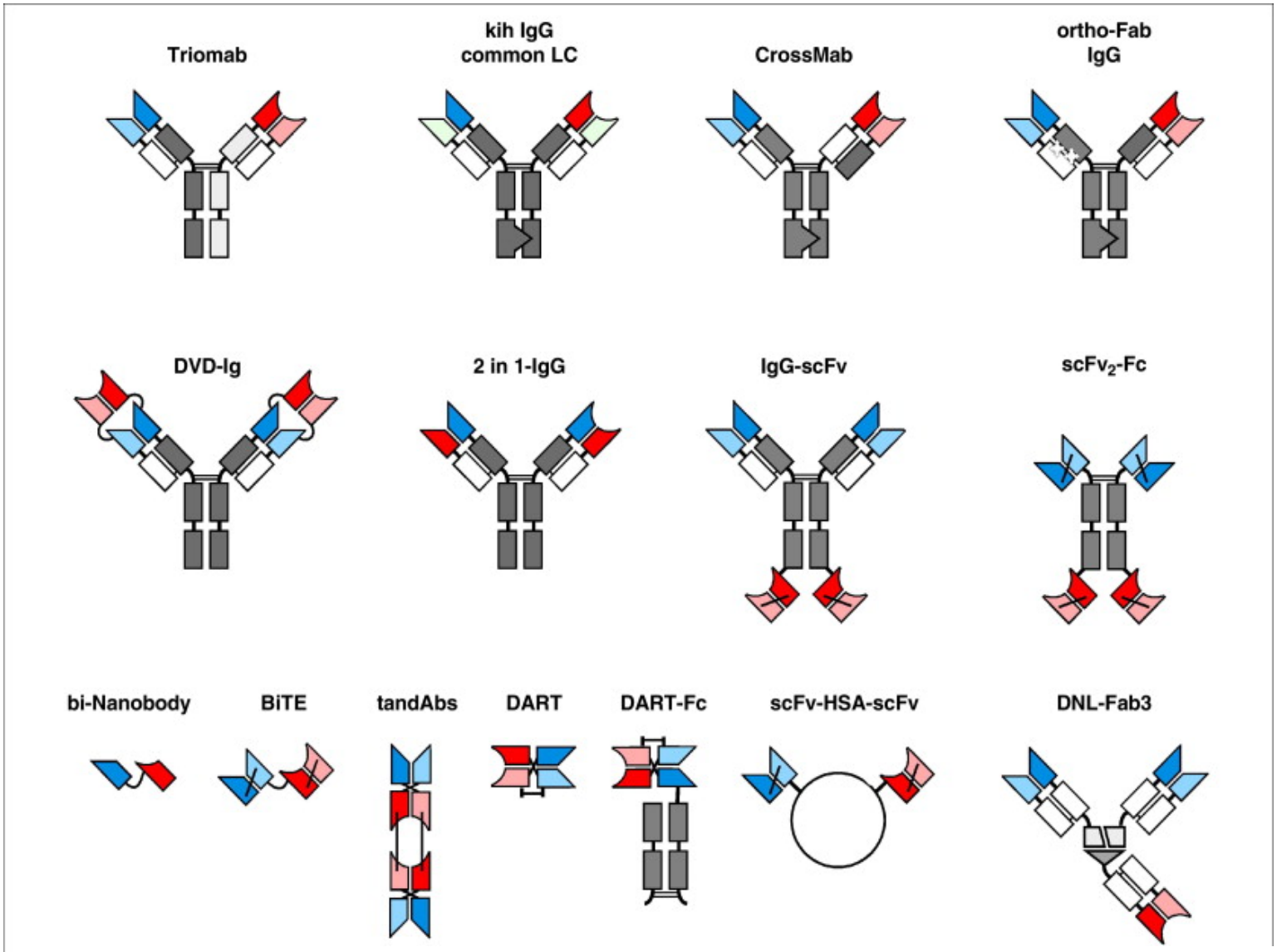
Con la ricerca, contro il cancro.
AIRC

someday is today
LEUKEMIA & LYMPHOMA SOCIETY OF CANADA

Immunotherapeutic strategies for the treatment of leukemia/lymphoma



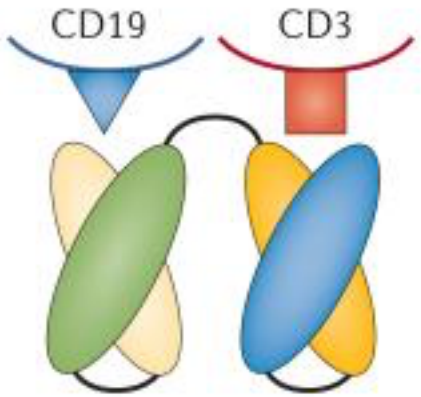
Bispecific antibodies (bsAbs) currently in clinical development or into the clinical arena



From Kontermann, RE and Brinkmann U. Drug Discovery Today, 2015

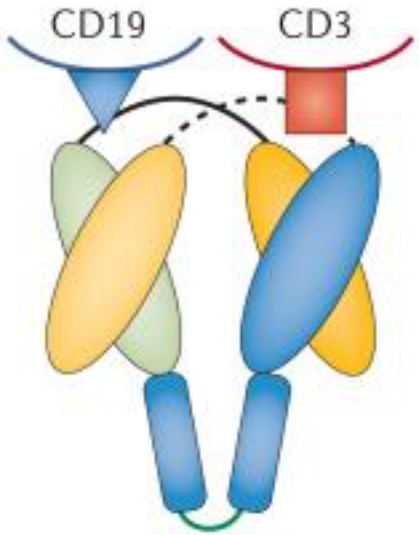
Dual Affinity retargeting Antibodies (DARTs)

Bispecific T-cell engager (BiTE®)

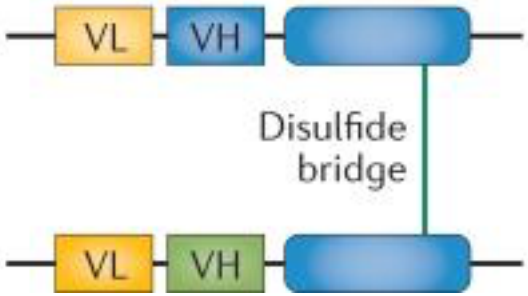


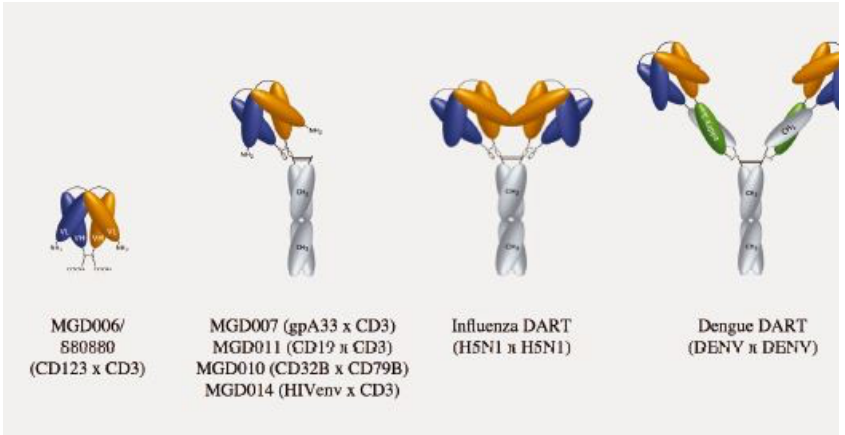
- Single polypeptide chain

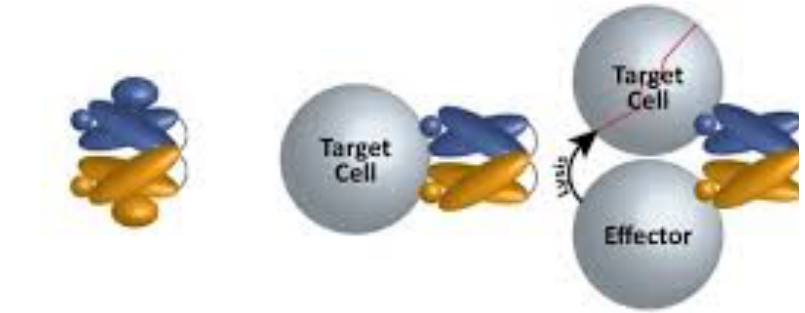
Dual affinity retargeting (DART)



- Two polypeptide chains
- Interchain disulfide bridge







Ligand Targeting (i.e., cytokine blockade)

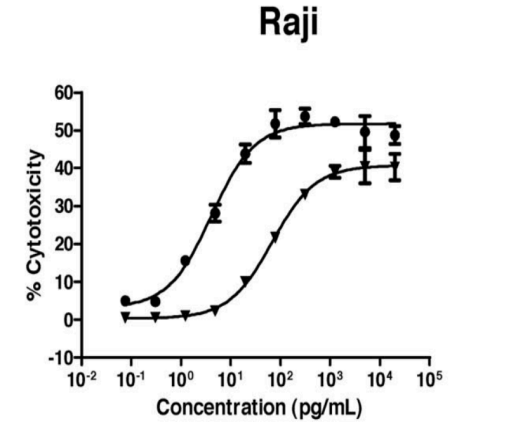
Signaling Modulation (i.e., suppression or blockade of an activating signal)

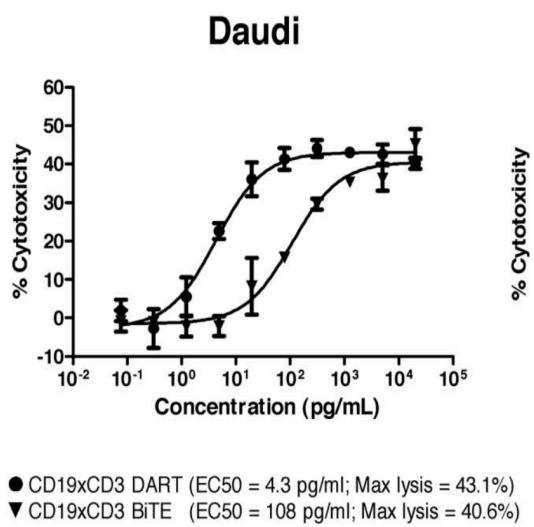
Redirected Effector Cell Killing

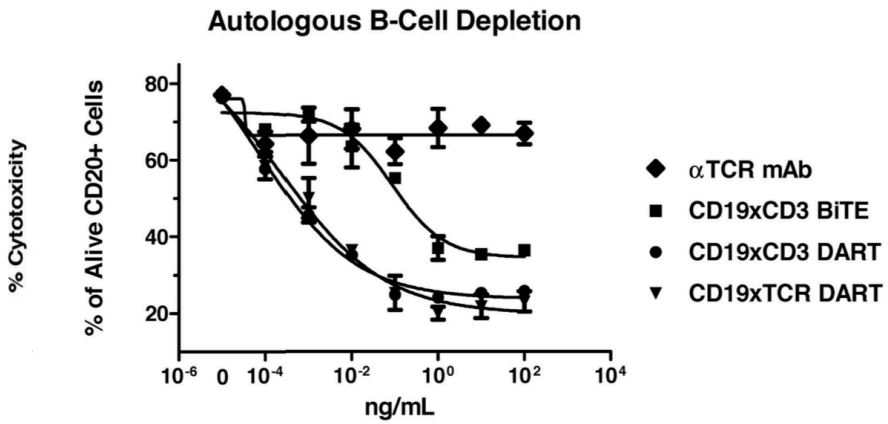
Application of dual affinity retargeting molecules to achieve optimal redirected T-cell killing of B-cell lymphoma

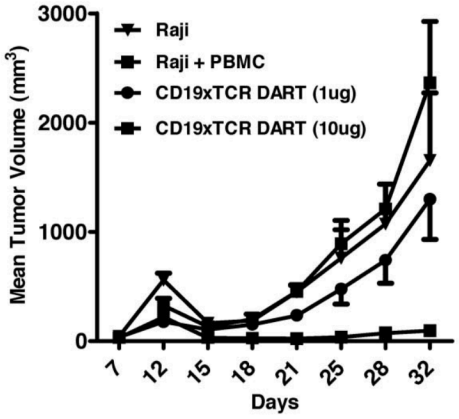
Paul A. Moore,[1] Wenjun Zhang,[1] G. Jonah Rainey,[1] Steve Burke,[1] Hua Li,[1] Ling Huang,[1] Sergey Gorlatov,[1] Maria Concetta Veri,[1] Sudeepta Aggarwal,[1] Yinhua Yang,[1] Kalpana Shah,[1] Linda Jin,[1] Sunan Zhang,[1] Leilei He,[1] Tengfei Zhang,[1] Valentina Ciccarone,[1] Scott Koenig,[1] Ezio Bonvini,[1] and Syd Johnson[1]

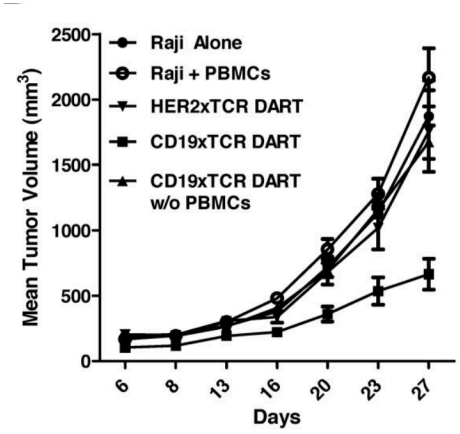
(*Blood*. 2011;117(17): 4542-4551)









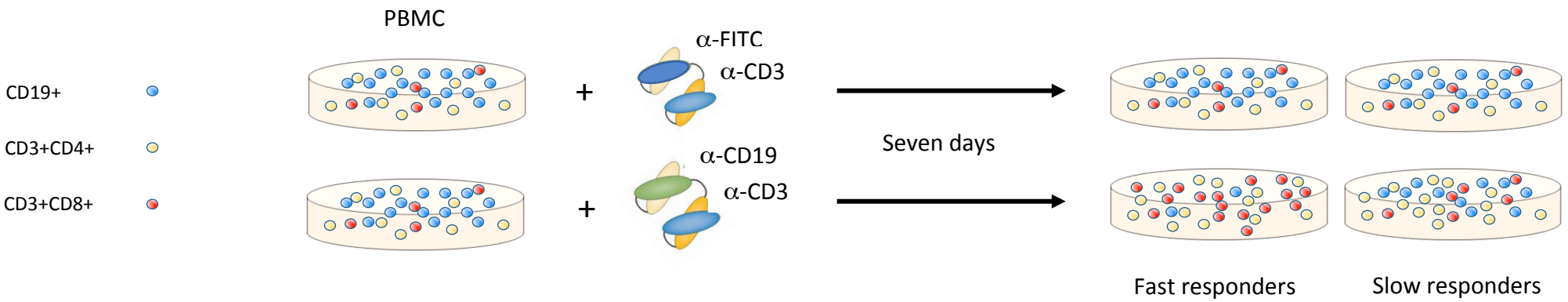


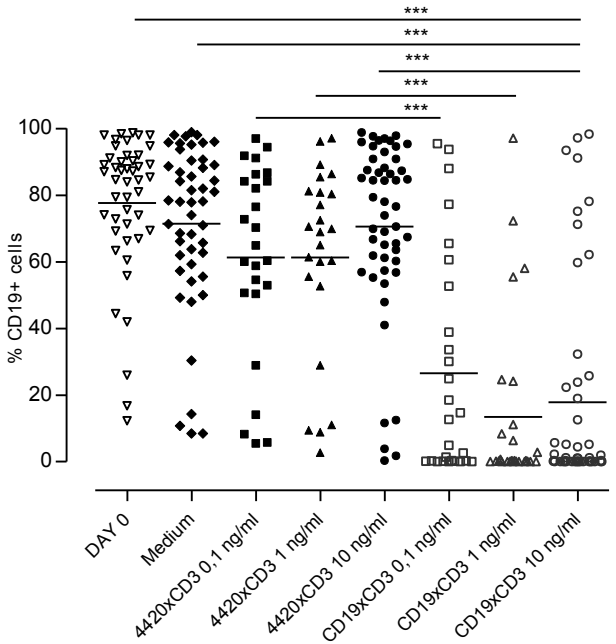
Patient cohort for DART-mediated preclinical studies

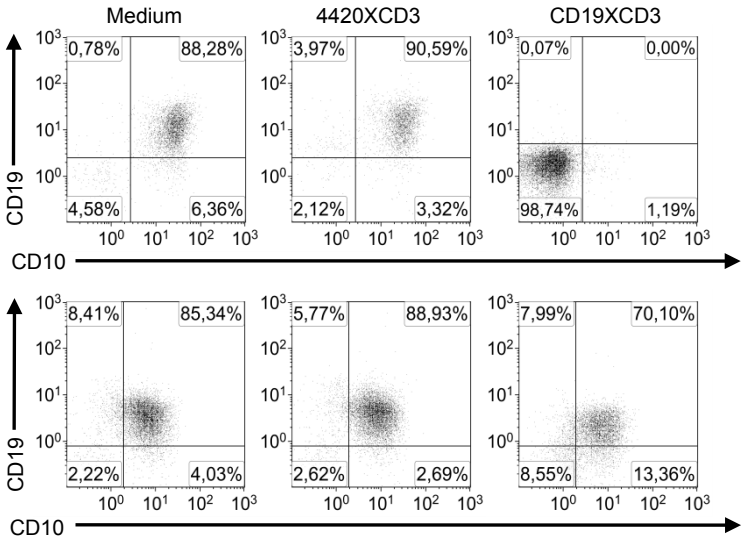
UPN	SOURCE	WHO 2008 CLASSIFICATION	DISEASE STATUS	%CD4 at day 0	%CD8 at day 0	%CD19 at day 0
PT# 1	SPL	B Splenic Lymphoma (not classified)	DGN	2.3	2	92
PT# 2	LN	B-CLL	DGN	N/A	N/A	N/A
PT# 3	LN	B-CLL	DGN	21	4.5	74
PT# 4	LN	FL	R/R (I REL)	N/A	N/A	N/A
PT# 5	LN	MCL	R/R (I REL)	N/A	N/A	N/A
PT# 6	LN	B-CLL	UNTREATED	9	3	87
PT# 7	LN	FL	DGN	50	21	26
PT# 8	PB	FL	DGN	21.21	4.84	60.55
PT# 9	PB	B-CLL	UNTREATED	2.99	1.53	91.95
PT#10	PB	SMZL	UNTREATED	3.66	1.95	55.8
PT#11	PB	B-CLL	R/R (REF)	1.45	0.37	96.39
PT#12	BM	B-CLL	R/R (REL)	3.5	6	90
PT#13	PB	B-CLL	R/R (REF)	2.67	1.98	87.94
PT#14	PB	B-CLL	R/R (II REL)	0.8	0.5	98
PT#15	LN	FL	DGN	3.52	0.89	86.26
PT#16	PB	B-CLL	R/R (I REL)	11.85	9.6	71.33
PT#17	PB	B-CLL	R/R (III REL)	2.88	2.01	89.14
PT#18	PB	MCL	DGN	3.26	7.33	85.38
PT#19	PB	FL	DGN	4.45	1.06	94.76
PT#20	BM	B-ALL	DGN	3.65	3.16	84.57
PT#21	PB	SMZL	R/R (REL)	56.55	16.88	12.25
PT#22	PB	B-CLL	R/R (REL)	0.43	0.22	98.72
PT#23	PB	B-CLL	UNTREATED	2.28	2.02	88.3
PT#43	PB	blastoid MCL	DGN	2.28	0.91	81.01
PT#44	BM	B-ALL	DGN	0.27	0.39	98.08
PT#45	PB	MZL	R/R (II REL)	11.1	4.58	66.24
PT#46	BM	B-CLL	DGN	5.61	3.29	69.28
PT#47	PB	B-CLL	DGN	5.32	3.17	87.19
PT#48	PB	B-CLL	DGN	19.72	2.1	66.9
PT#49	BM	B-CLL	DGN	4.27	2.07	79.35
PT#50	PB	MCL	DGN	1.28	1.16	96.74

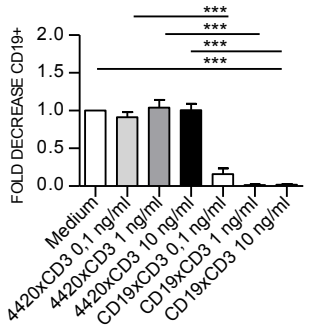
25 B-CLL/SLL,
7 B-ALL,
6 FCCL,
5 MgZL
5, MCL,
1 HCL,
1 Splenic NHL

CD19xCD3 molecule mediates autologous CD19⁺ B-cells depletion *in vitro*.

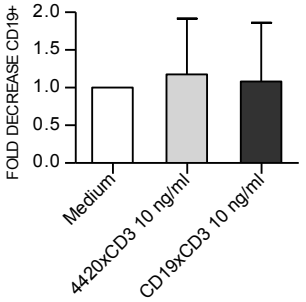








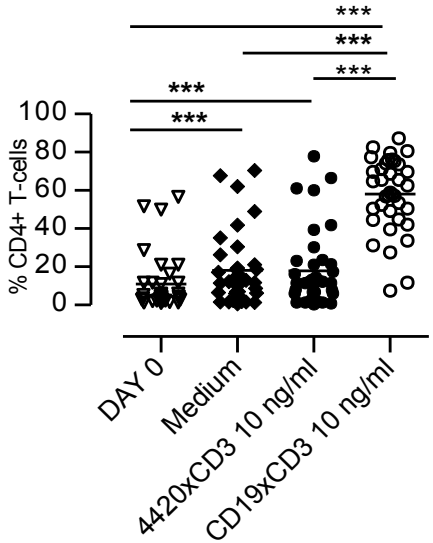
Fast responders

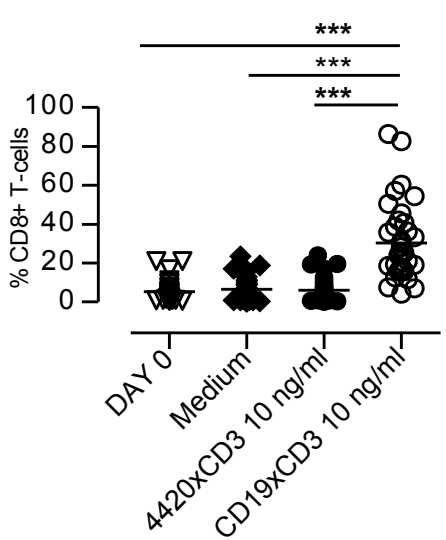


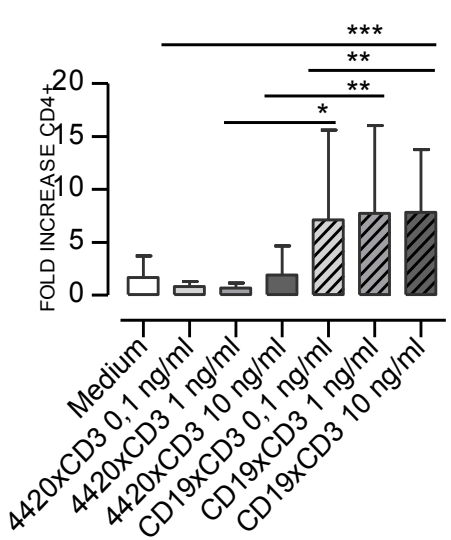
Slow responders

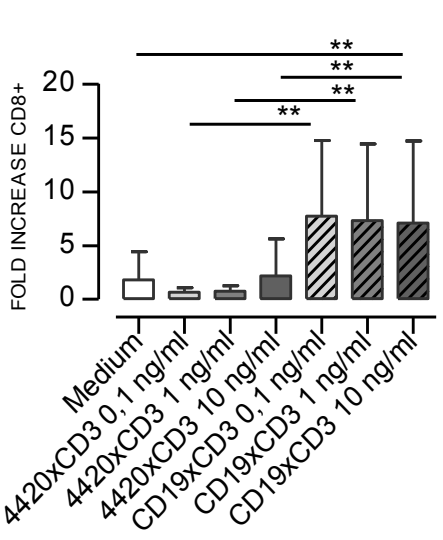
CD19xCD3 molecule mediates autologous expansion of T-cells *in vitro*.

Fast responders

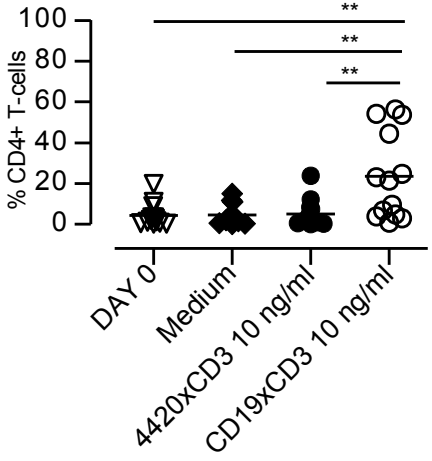


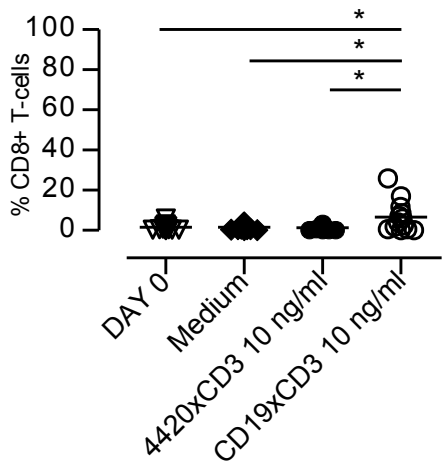


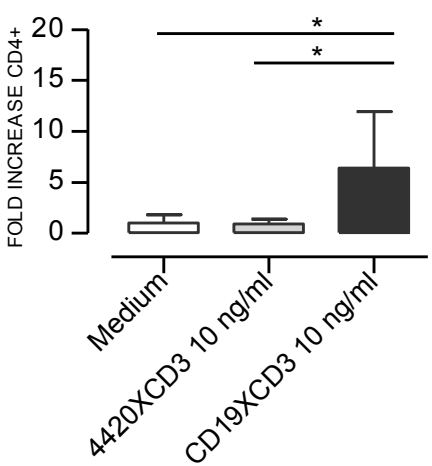


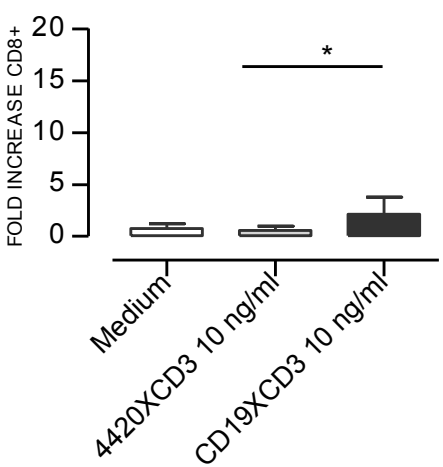


Slow responders





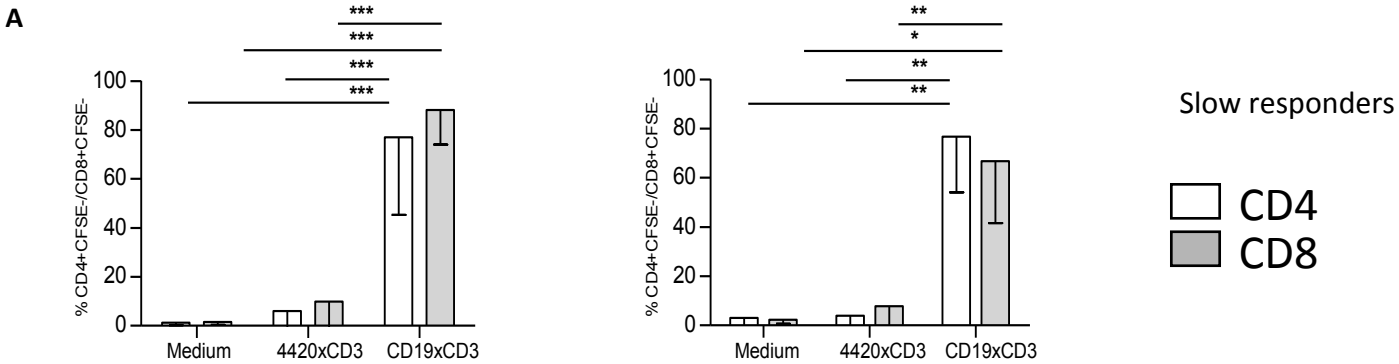




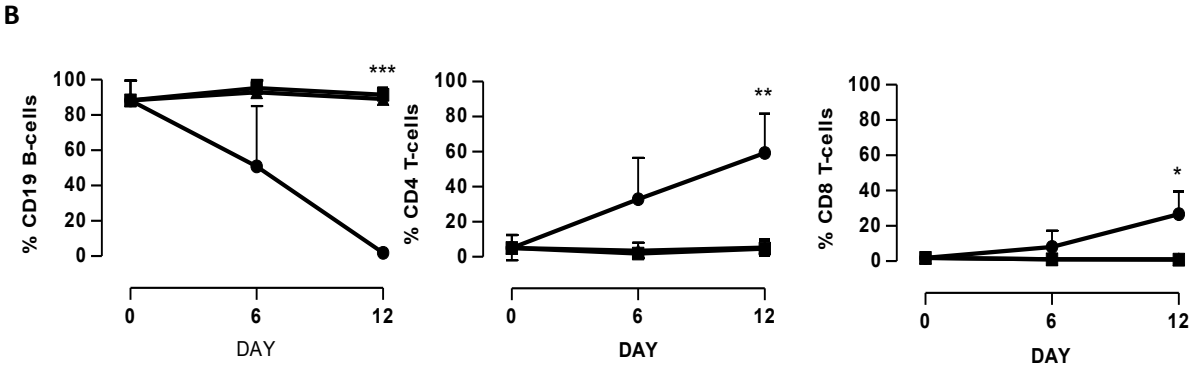
CD19xCD3 DART induces proliferation of CD4$^+$ and CD8$^+$ cells and leads to total eradication of neoplastic B-cells

Fast responders

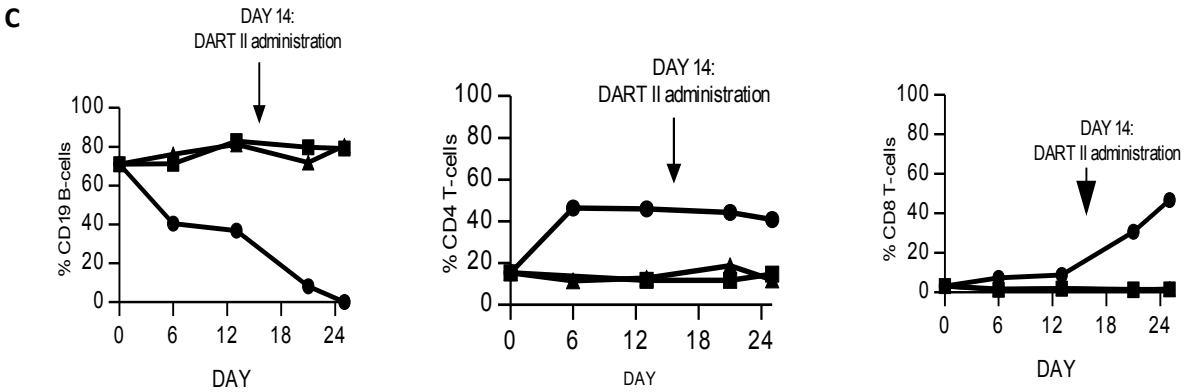
Slow responders



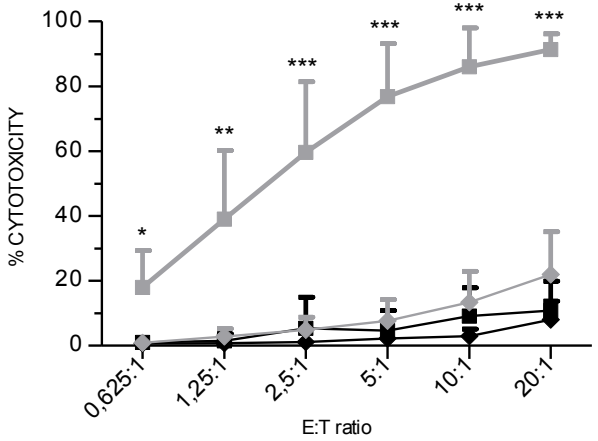
Slow responders



Very Slow responders

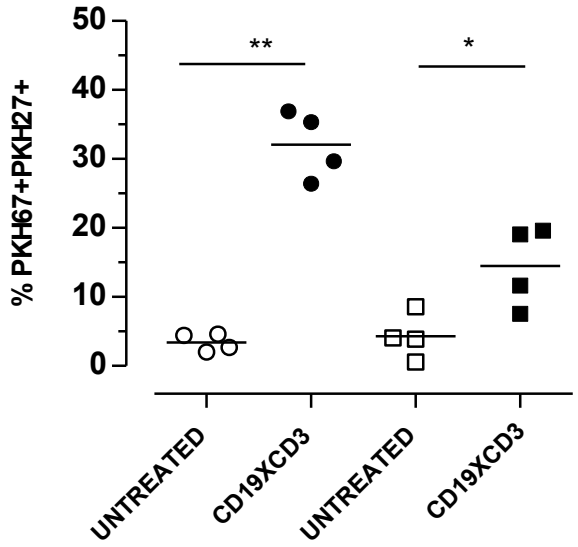


CD19xCD3 DART mediates expansion of cytotoxic T-cells selectively recognizing CD19+ target cells



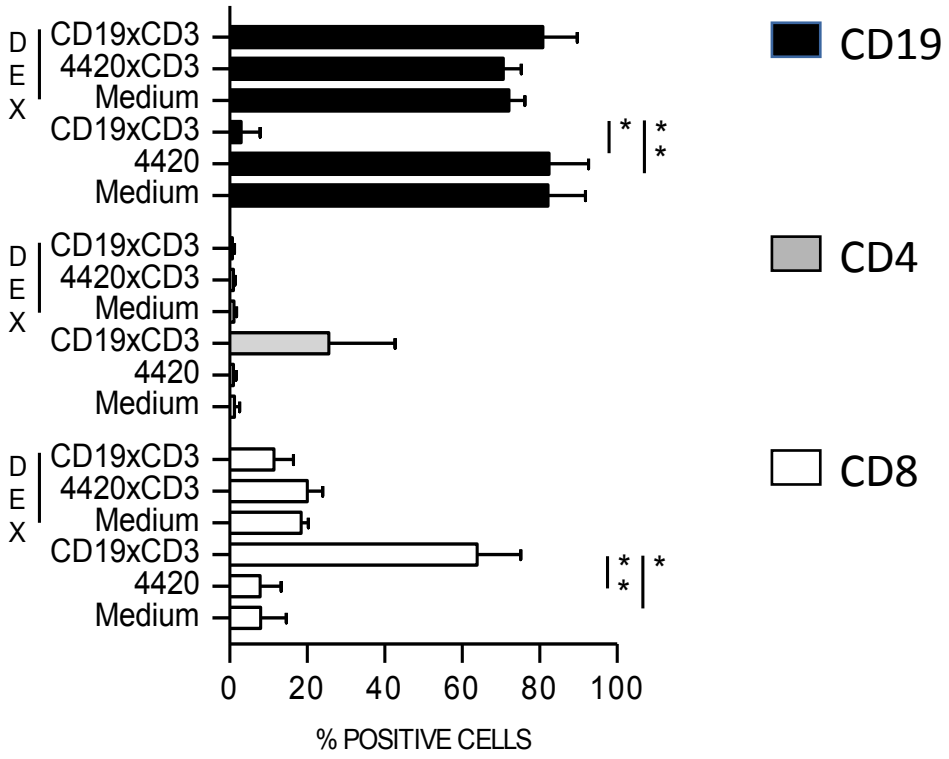
PBMC pre-treated with 4420xCD3
MEC 1 (■) THP1 (♦)

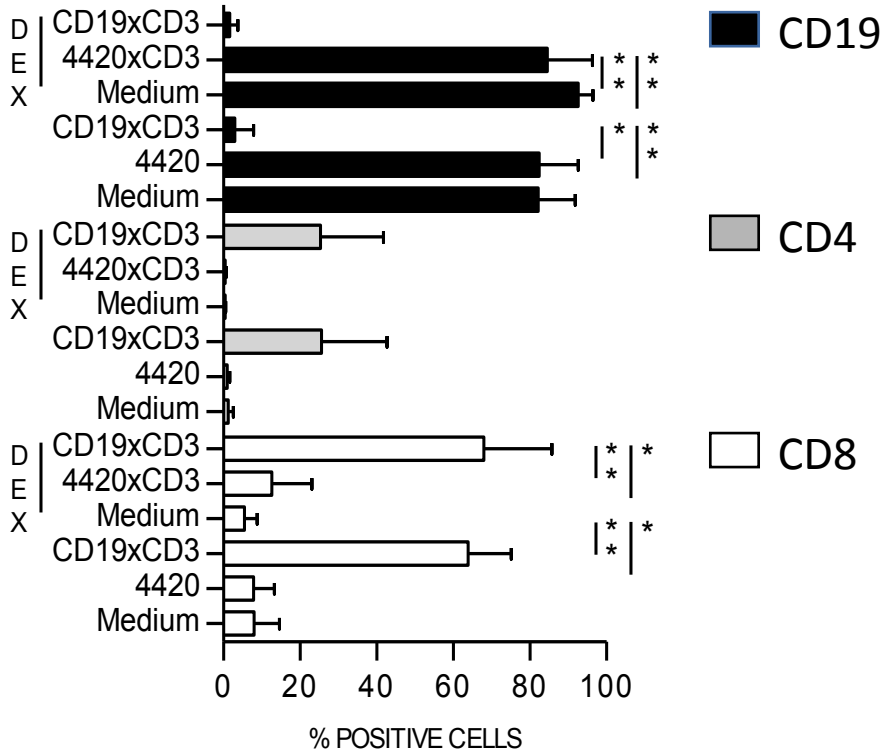
PBMC pre-treated with CD19xCD3
MEC 1 (■) THP1 (♦)



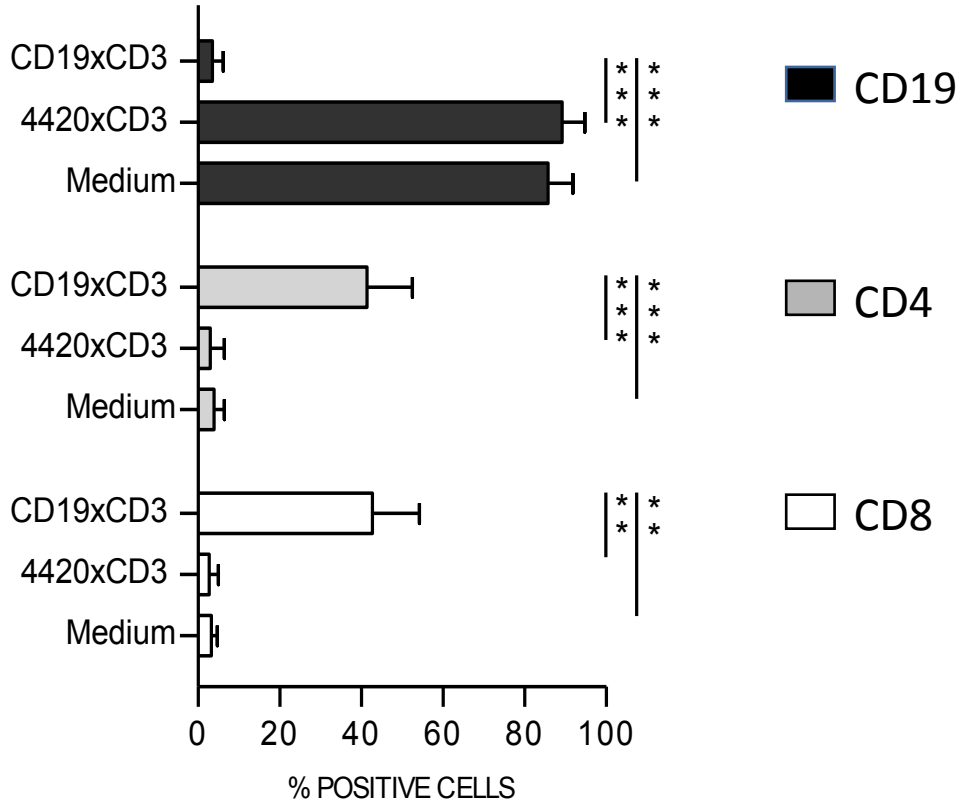
MEC 1 ( ) ○
THP1 (■)

Dexametasone pretreatment impair DART mediate T-cell expansion and CD19 eradication

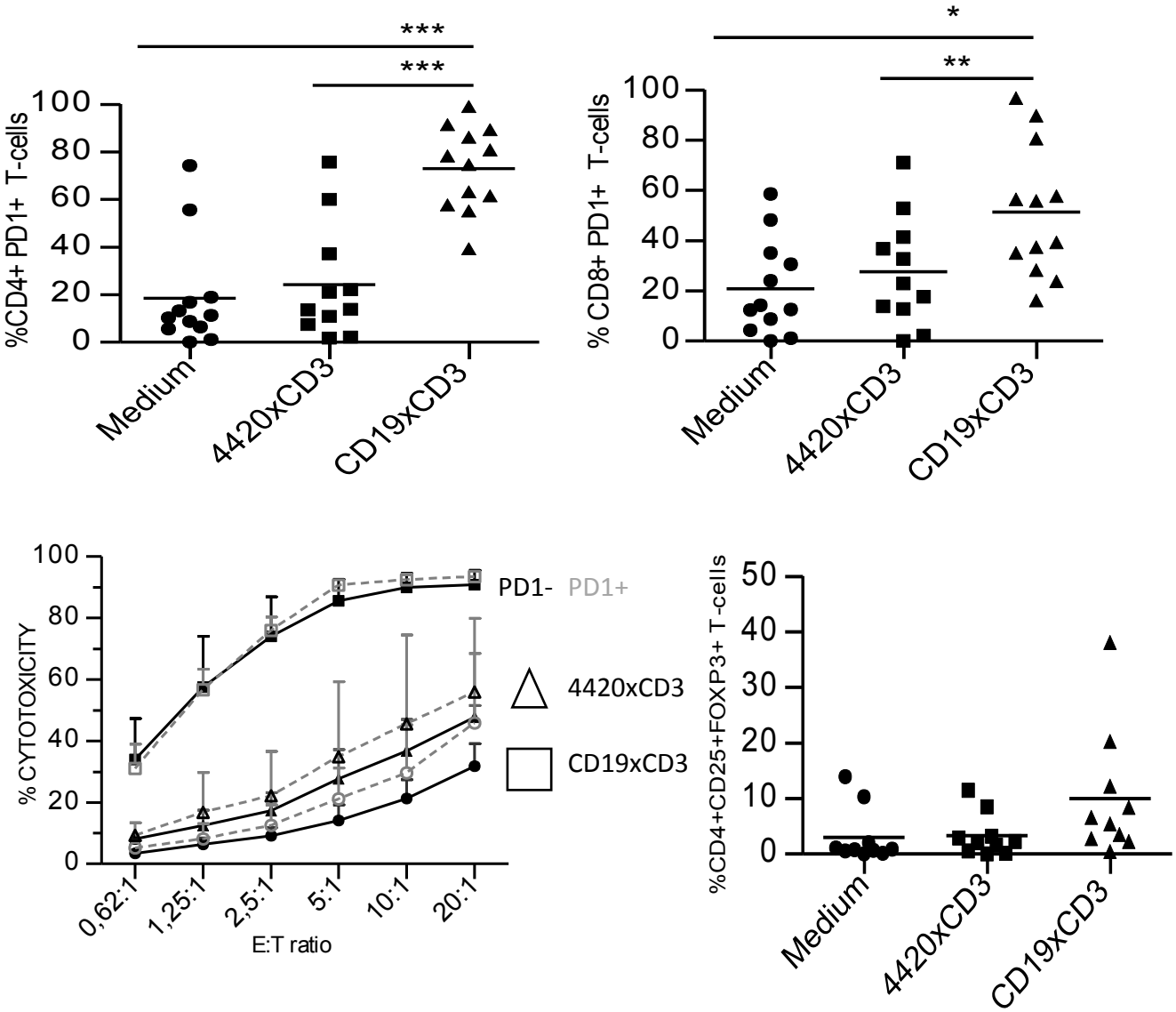




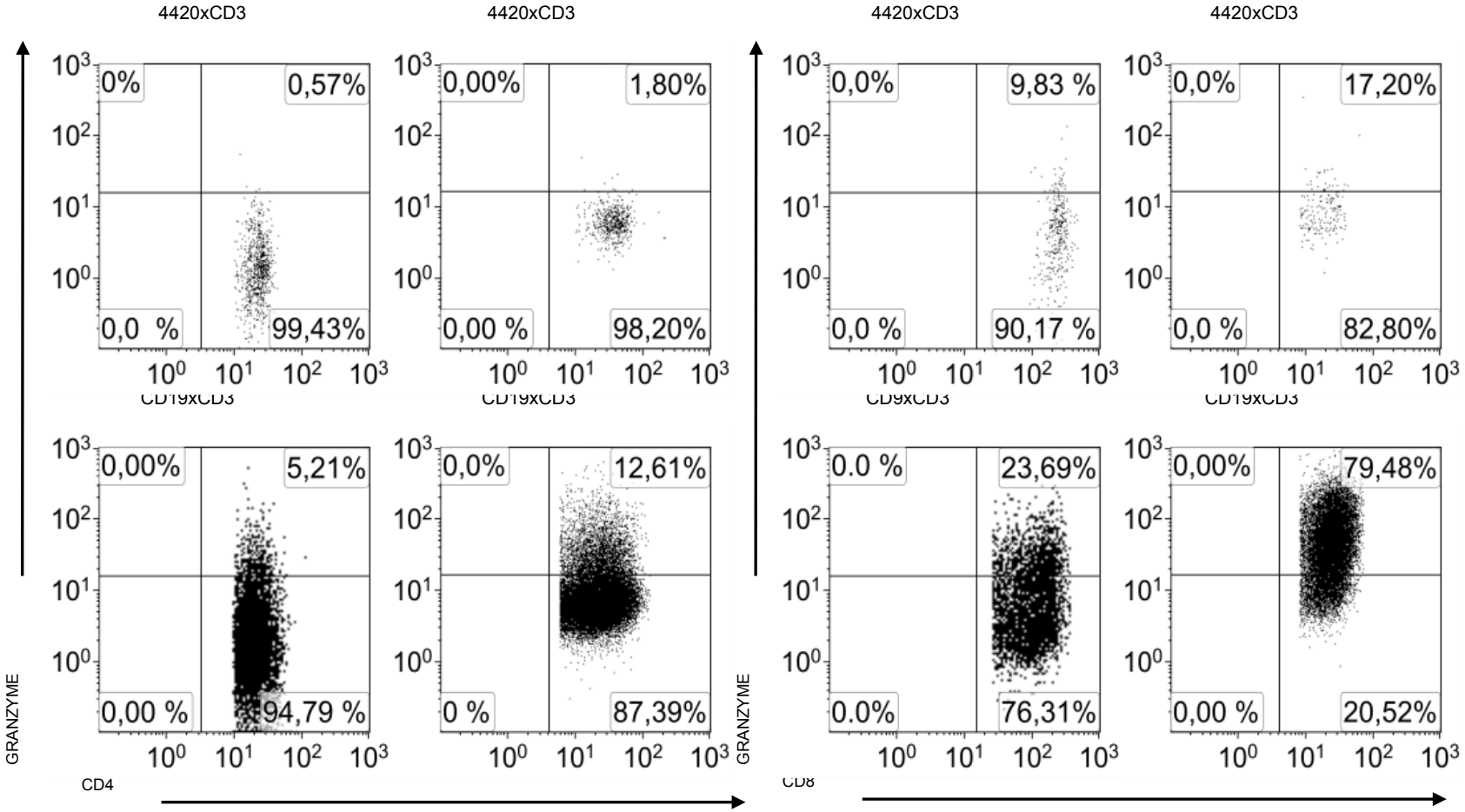
Pre-treatment with ibrutinib does not impair DART mediate T-cell expansion and CD19 eradication



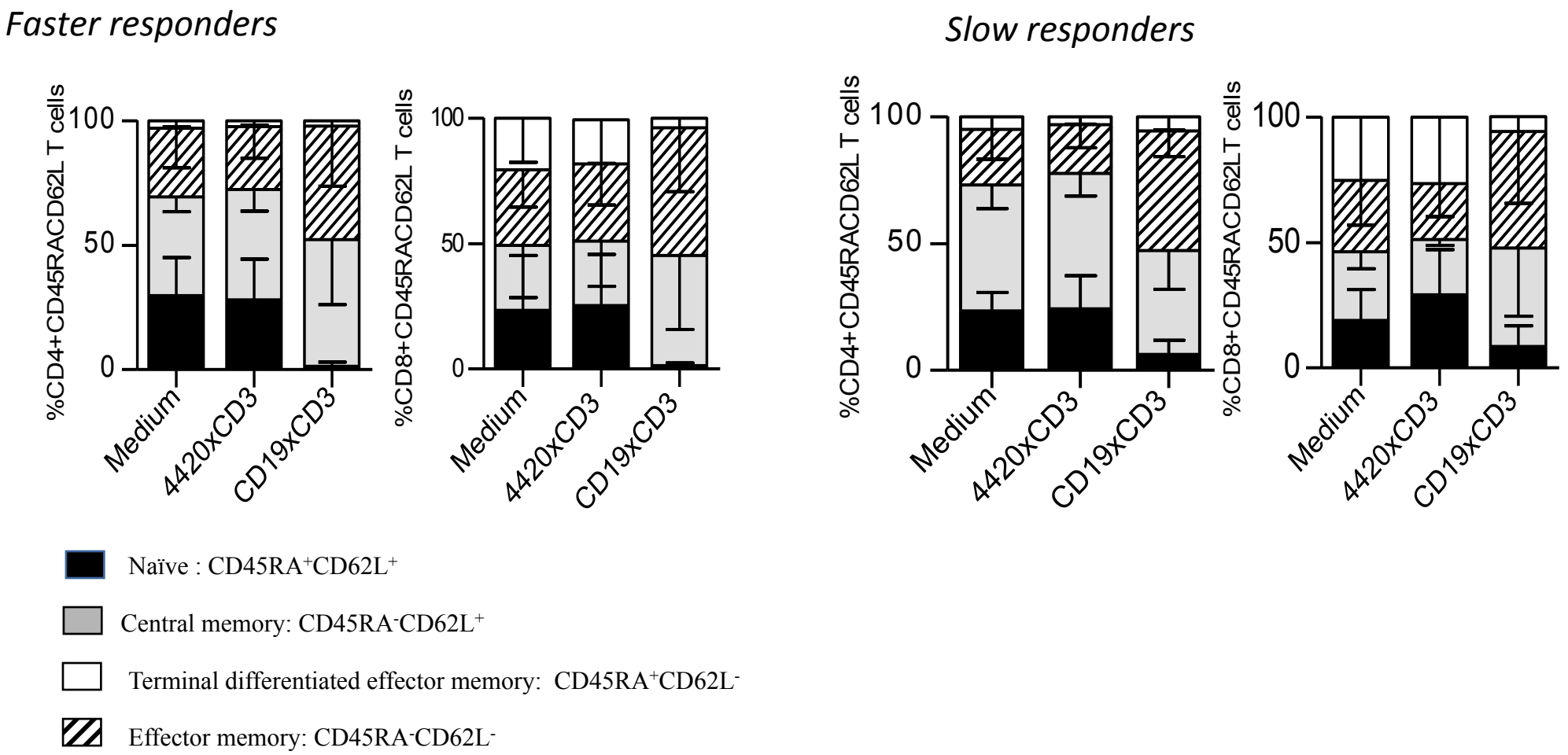
The effect of CD19xCD3 DART on autologous T-cell differentiation



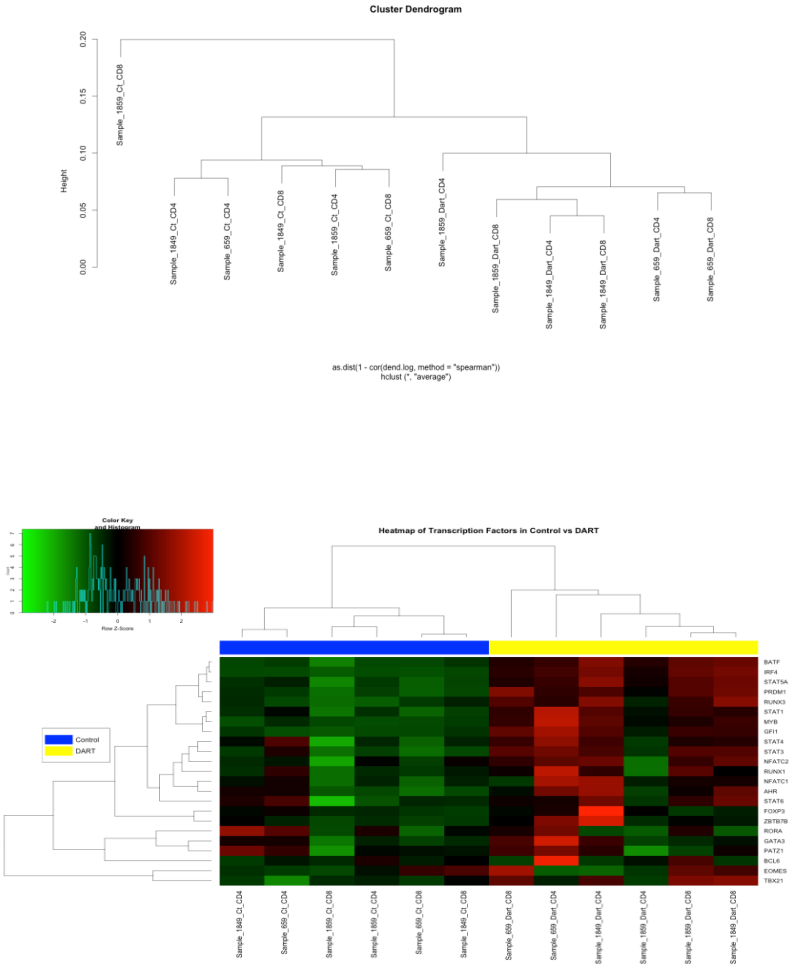
CD19xCD3 DART engagement leads to upregulation of cytoxic molecules in both autologous CD4 and CD8 cells

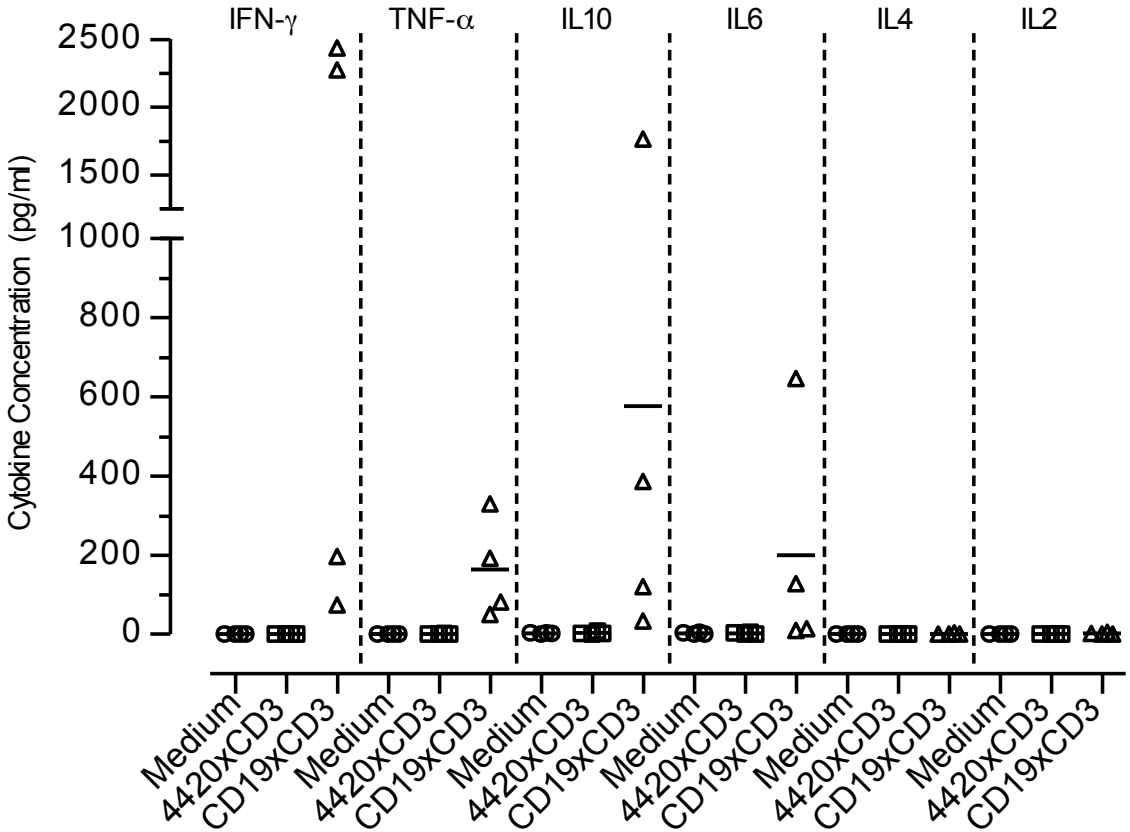


DART activated CD4⁺ and CD8⁺ displayed a central and effector memory phenotype

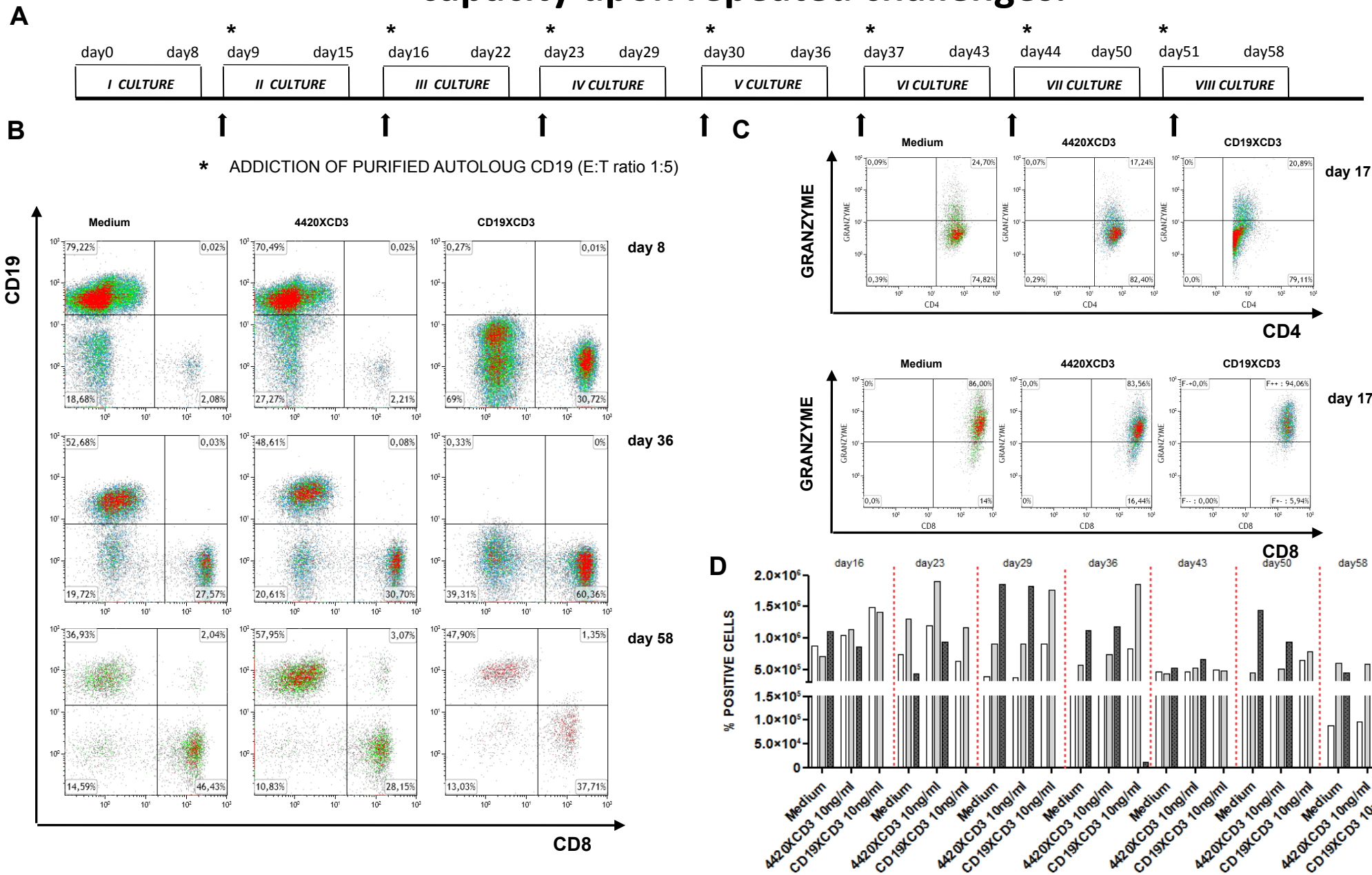


DART mediated signaling leads to a defined transcriptional profile

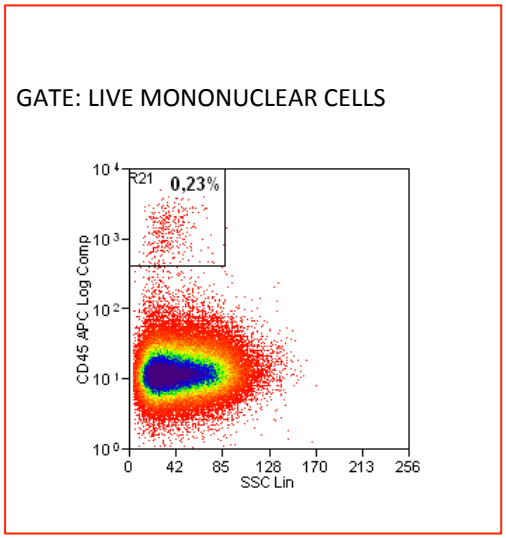


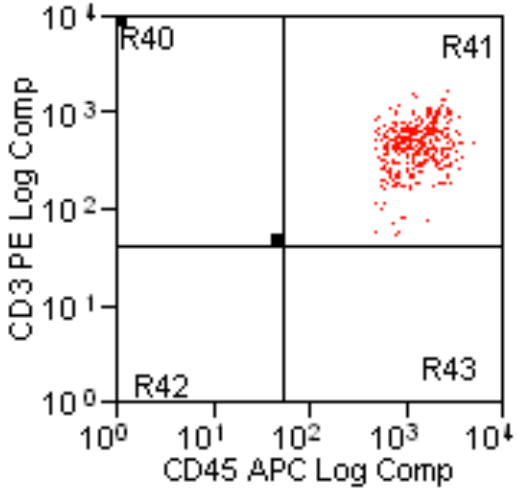


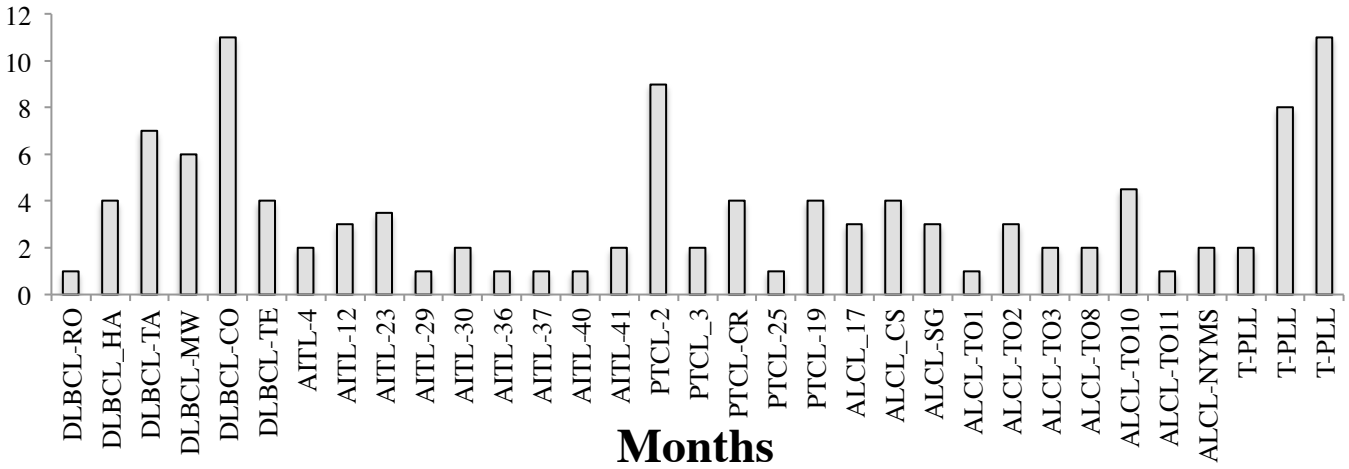
CD19xCD3 DART induces T-cell expansion and preserves cytotoxic capacity upon repeated challenges.



Graft versus host represents a fatal hurdle in the generation and maintenance of PDXT



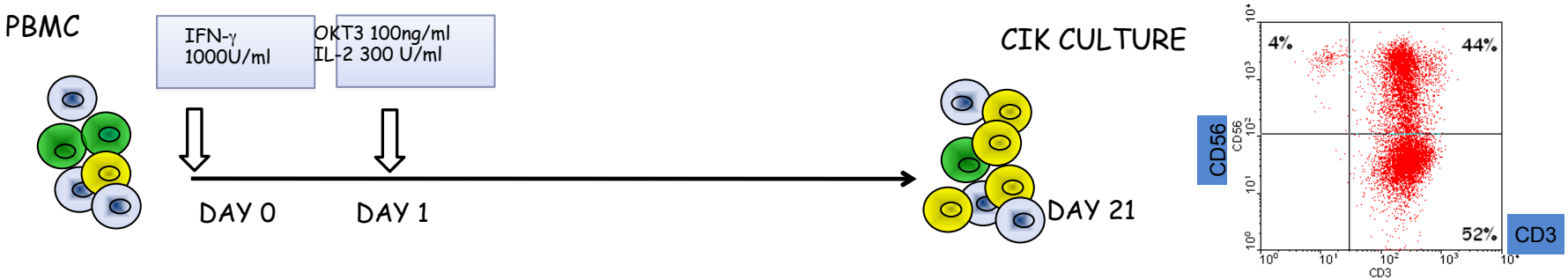




Expansion of Cytokine-induced killer (CIK) cells

- Cytokine induced killer (CIK) cells are heterogeneous subset of T lymphocytes with T-NK phenotype;

A. CIK cells can be expanded from peripheral blood mononuclear cells (PBMC) cultured with the timed addition of IFN-γ, Ab anti-CD3 and IL2;

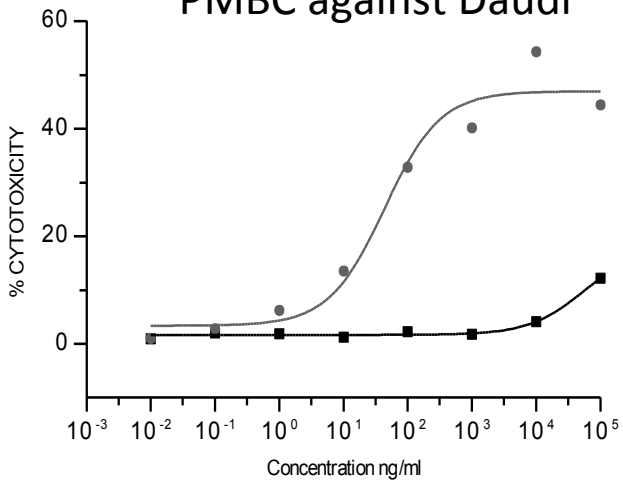


CIK cells are characterized by:

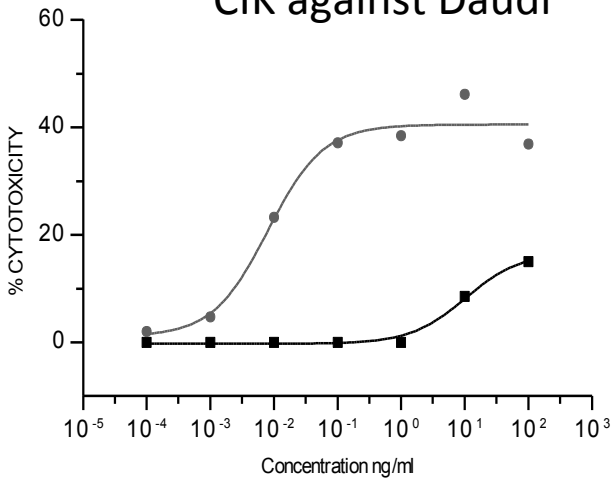
- high proliferative response
- MHC-unrestricted antitumor cytotoxicity in vitro and in vivo
- No GVHD effect in vivo

CD19xCD3 DART triggers cytotoxic activity of CIK cells against autologous and allogenic tumor target cells.

PMBC against Daudi



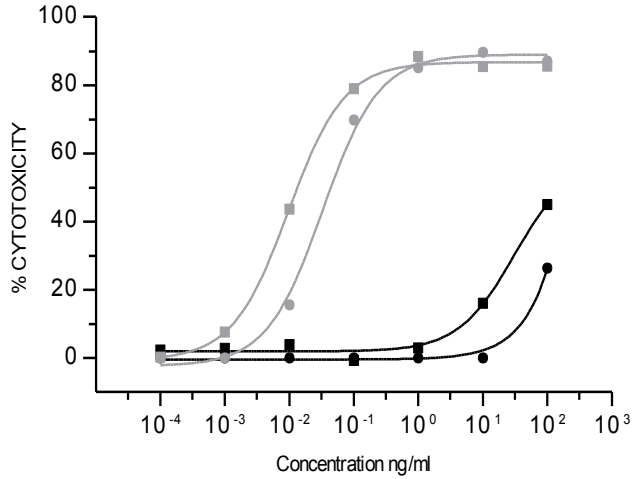
CIK against Daudi



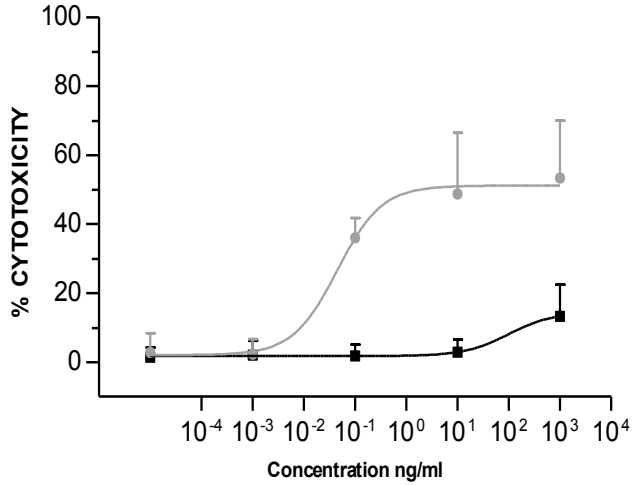
4420xCD3

CD19xCD3

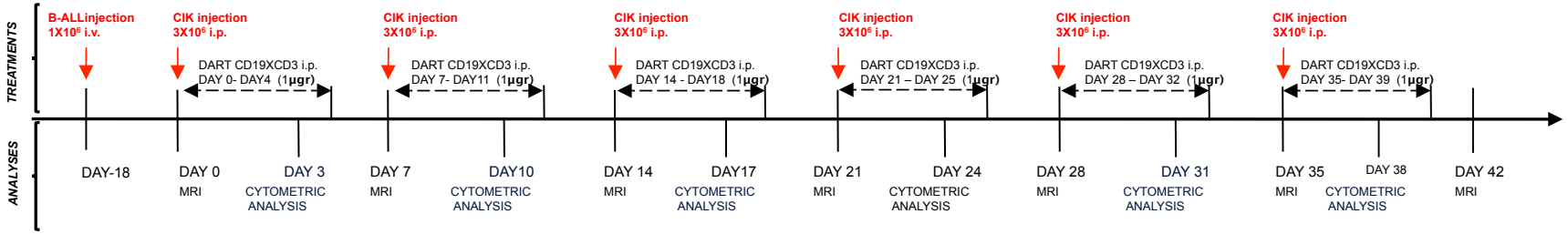
Autologous CIK against ALL (fast responder)



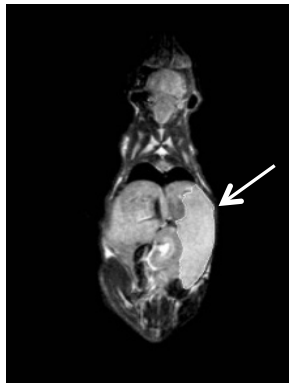
Autologous CIK against ALL (slow responder)



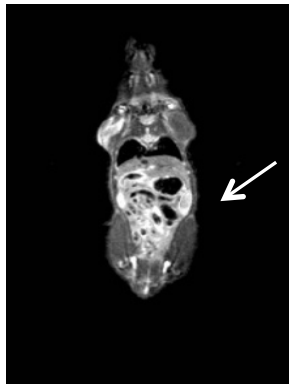
CD19xCD3 DART inhibits B-ALL patient derived tumor growth in NSG mice

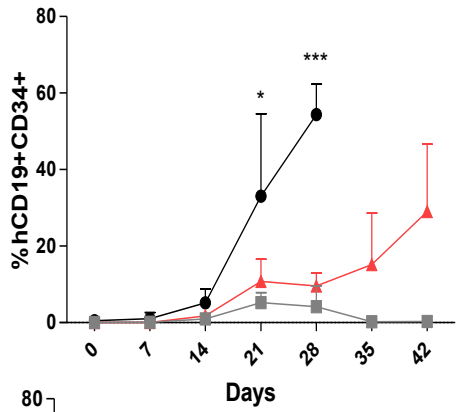


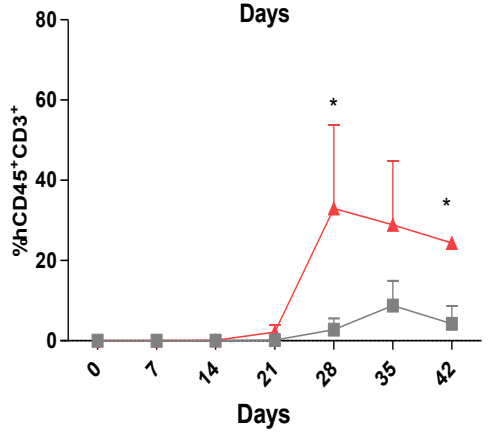
Control

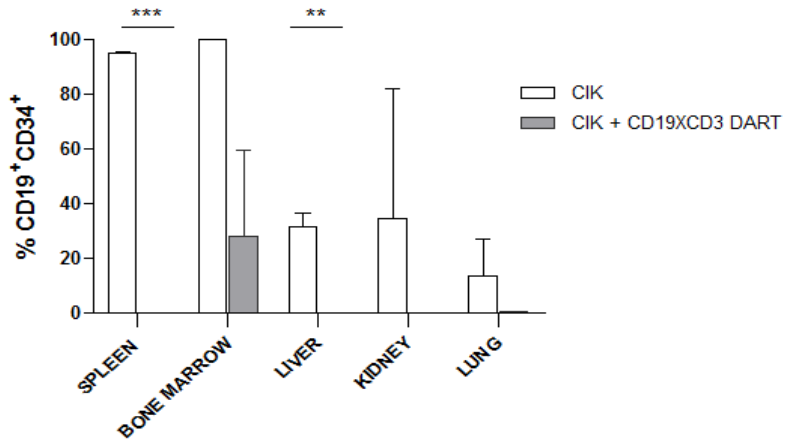


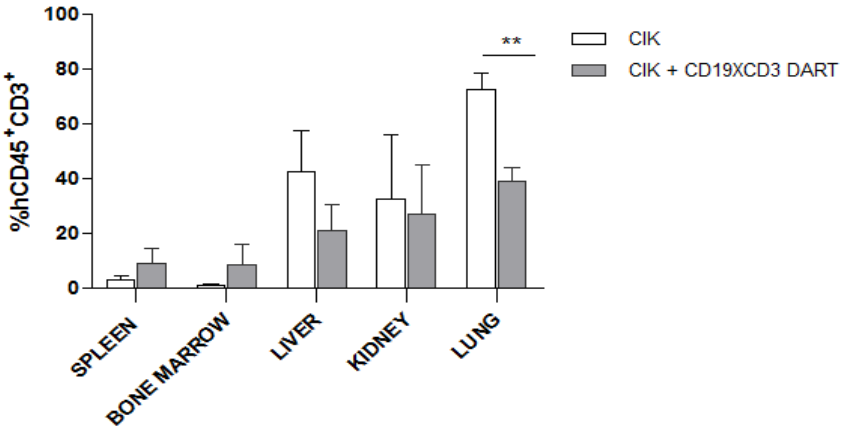
CD19xCD3 DART+ CIK



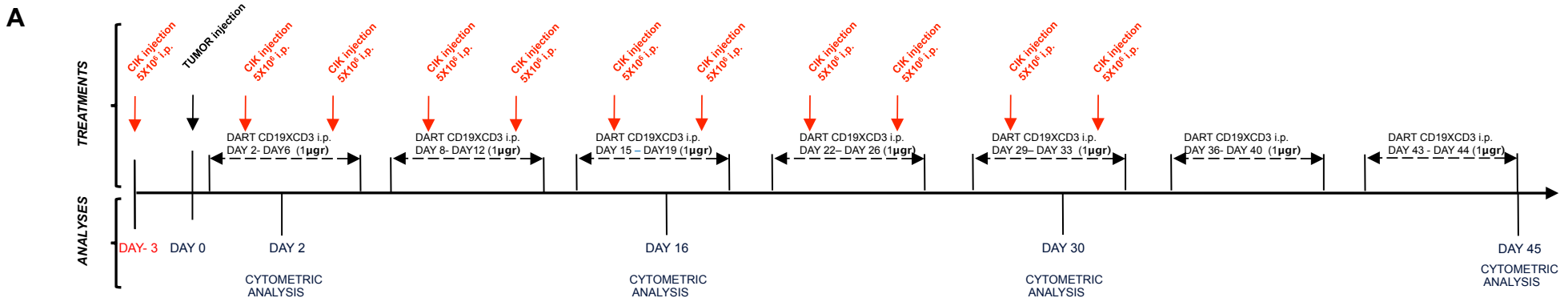


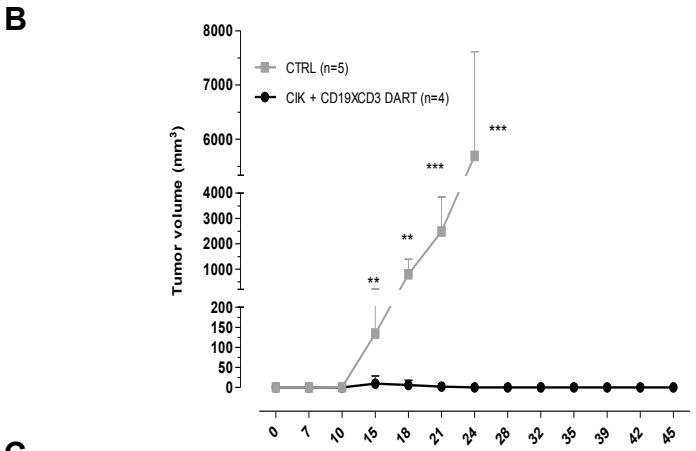


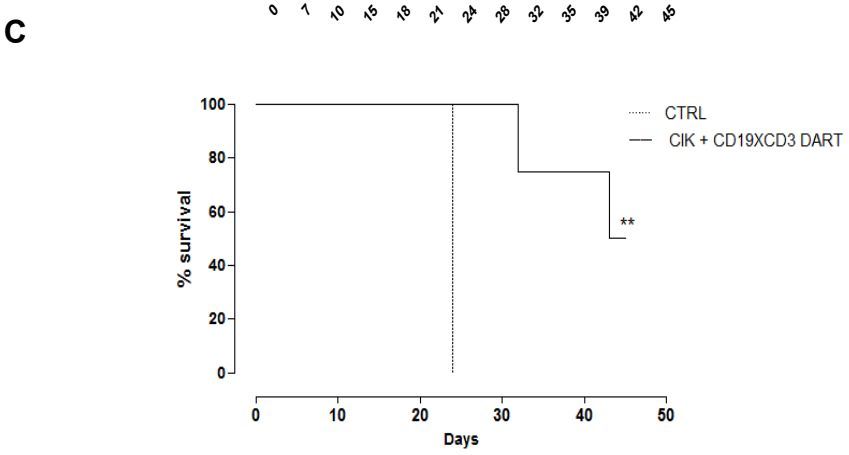


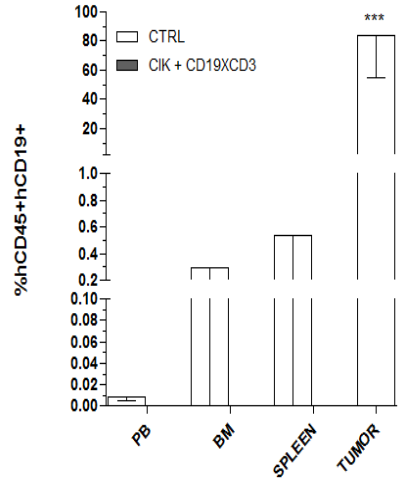


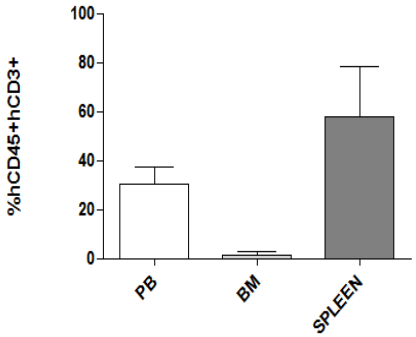
CD19xCD3 DART eradicates DLBCL patient derived tumor in NSG mice

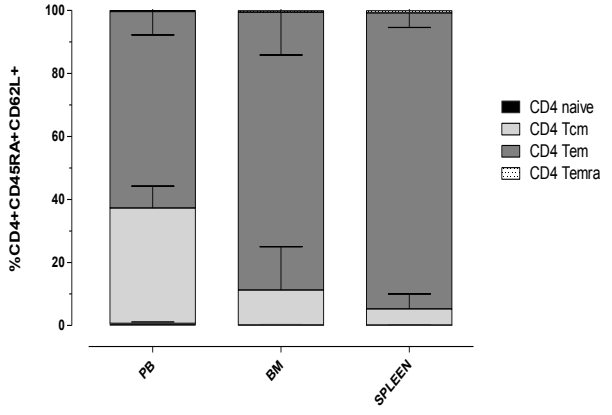


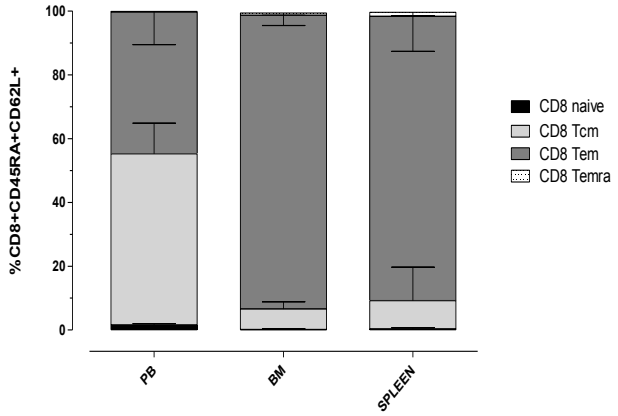












Conclusions

- CD19cCD3 DART elicits a strong activation of both CD4 and CD8 autologous cells leading to the eradication of CD19+ neoplastic B-cells,
- The CD19xCD3 DART identified two distinct subsets of patients which display distinct expansion of autologous T-cells: fast and slow responders. However delayed responses can be overcome by a prolonged or repeated DART exposure,
- Both CD4 and CD8 effector cytotoxic cells can be generated, but DART-mediated killing of $CD4^+$ cells into cytotoxic effectors required the presence of $CD8^+$ cells,
- Serial exposures to DART led to the exponential expansion of $CD4^+$ and $CD8^+$ cells and to the sequential ablation of neoplastic cells in absence of a PD-L1-mediated exhaustion,
- Patient-derived neoplastic B-ALL and DLBCL can be proficiently controlled/ eradicated in a PDTX model by DART-armed cytokine induced killer (CIK) cells


THANK YOU
Weill Cornell Medical College