

3rd Cuneo City ImmunoTherapy Conference (CCITC)

Immunotherapy in Hematological Malignancies **2023**

CUNEO
May 18-20, 2023

Spazio incontri Fondazione CRC

TOWARDS OFF-THE-SHELF ADOPTIVE
IMMUNOTHERAPY OF LEUKEMIA

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Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo, Italy
and Centro Interdipartimentale di Ricerca in Biologia Molecolare (CIRBM), Torino, Italy

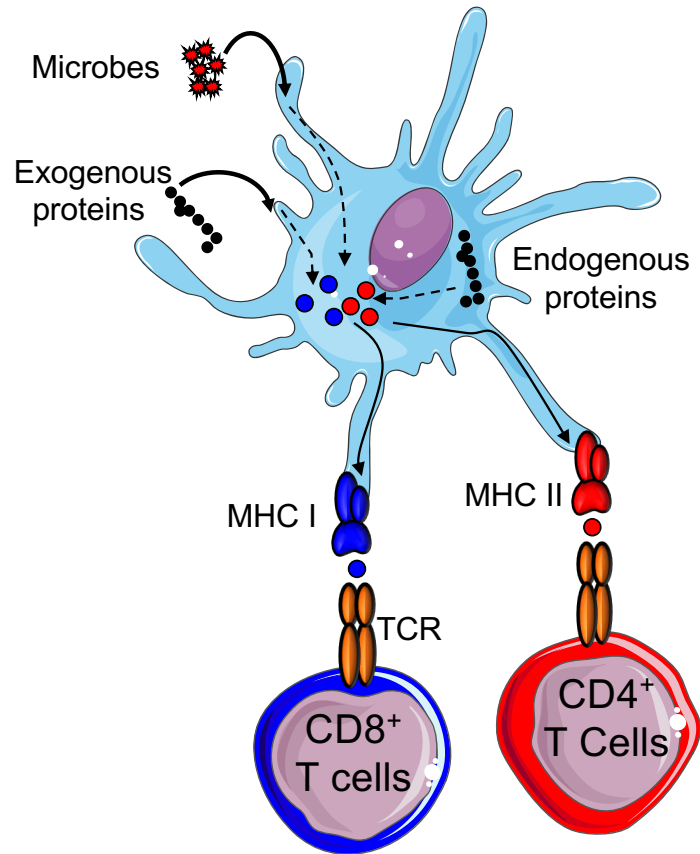
DICHIARAZIONE

Relatore: Giulia Casorati

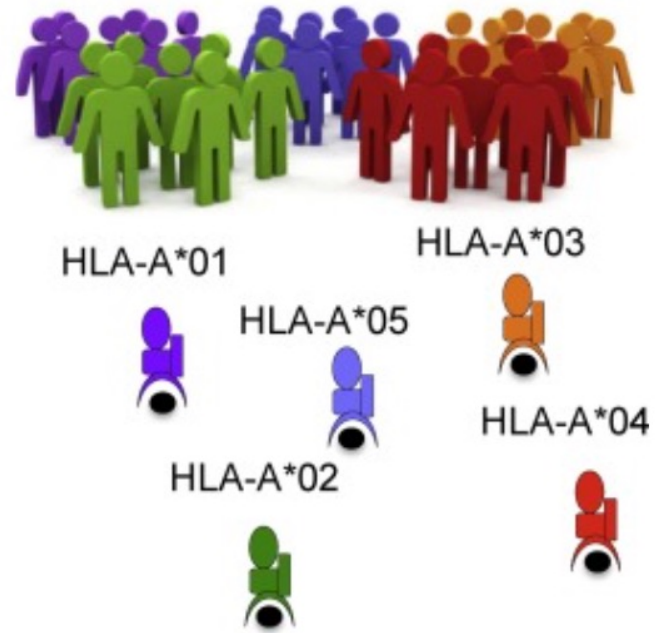
Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(AstraZeneca)**
- Partecipazione ad Advisory Board **(NIENTE DA DICHIARARE)**
- Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Altro

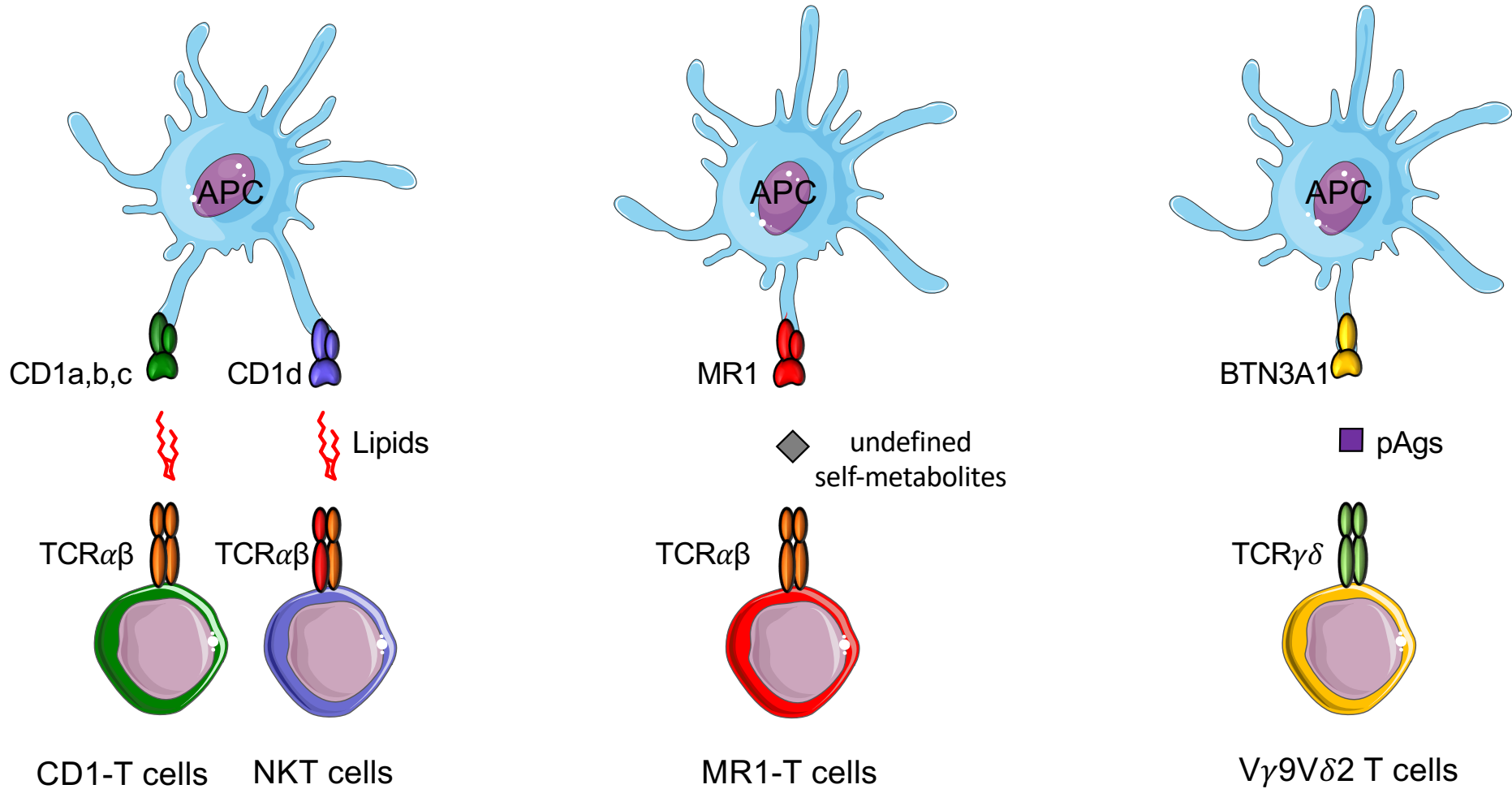
MHC-restricted T cells Specific for peptide antigens



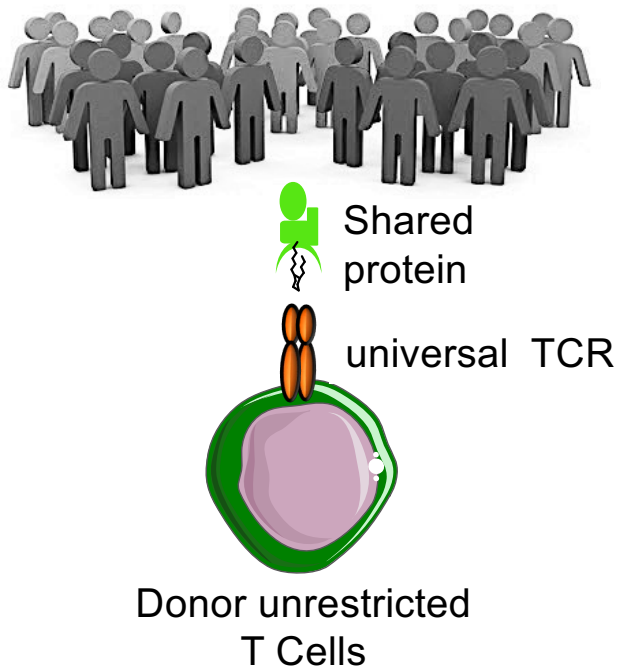
Polymorphic HLA genes



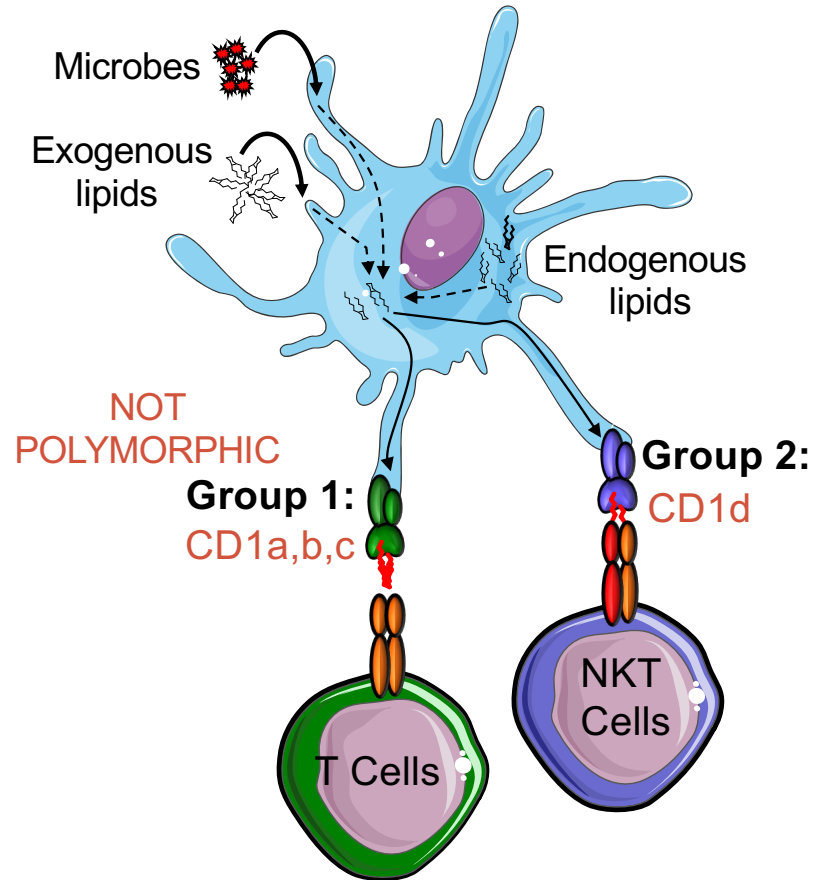
Exploiting T cells restricted for non-polymorphic antigen presenting molecules



Non-polymorphic antigen presenting molecules



CD1-restricted T cells Specific for lipid antigens



CD1 expression pattern

Group I CD1a, b, c

- Cortical immature tymocytes
 - mDCs (CD1a, b, c)
- Langherans cells (CD1a, c)
- Mono/Macrophages (CD1a, b, c variable)
- B cells (CD1c on a subset)

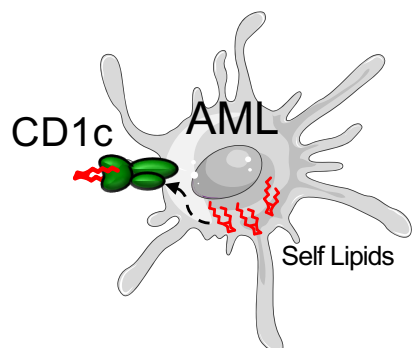
Group II CD1d

- Cortical tymocytes
 - mDCs
 -
- Mono/Macrophages
- B cells (most)
- Keratinocytes
- Hepatocytes
- Vascular smooth cells (gut, liver)
- Schwann cells

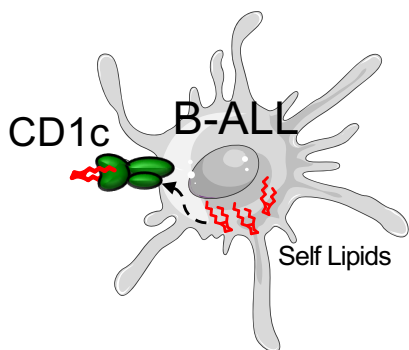
Group 1
CD1a,b,c

 LEUKEMIA

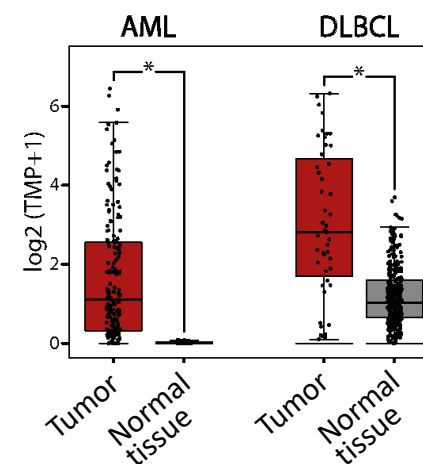
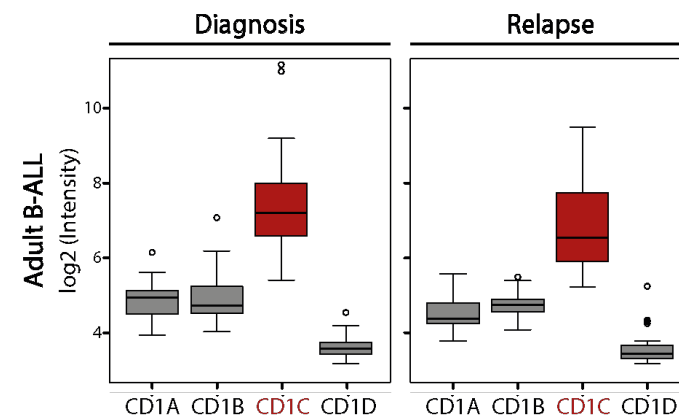
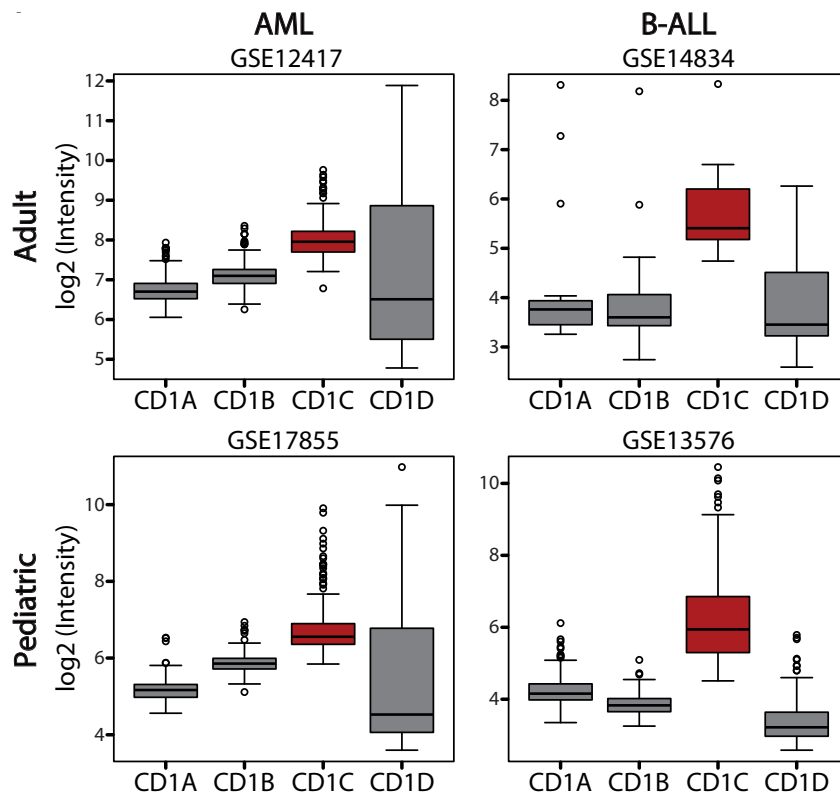
CD1c molecules are frequently expressed by primary acute leukemia blasts



51% adult AML
45% pediatric AML

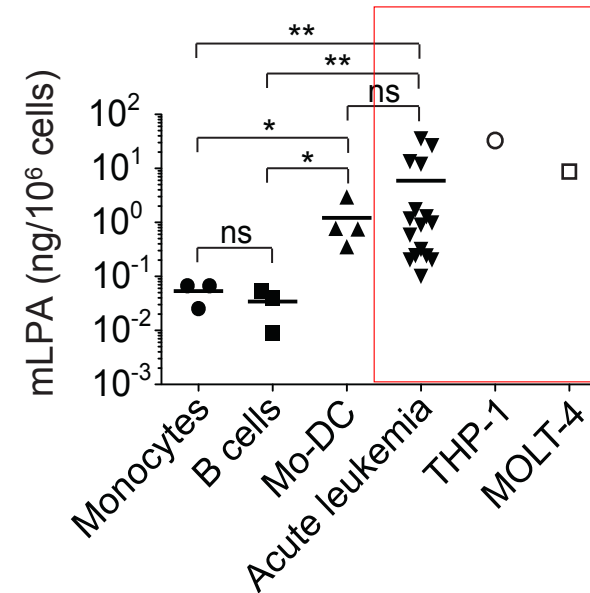
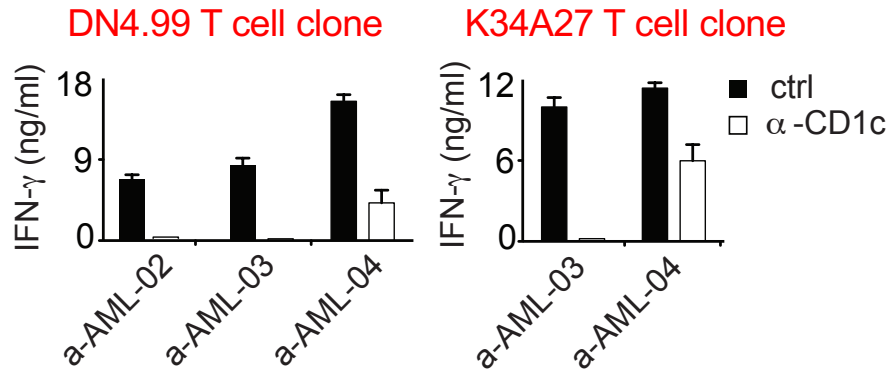
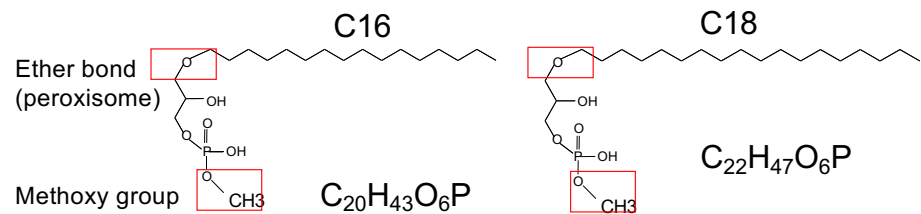
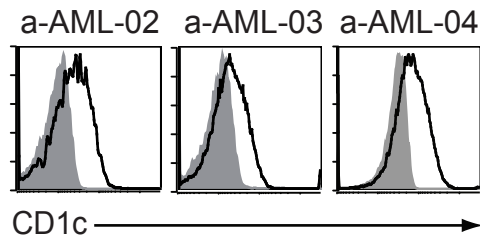


71% adult B-ALL
26% pediatric B-ALL

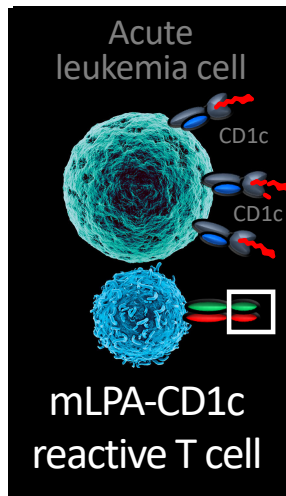


CD1c self-reactive T cell clones recognize acute leukemia expressing CD1c

methyl-lyso-phosphatidic acid Ag (mLPA)



Targeting CD1c+ malignancies with TCR-engineered T cells



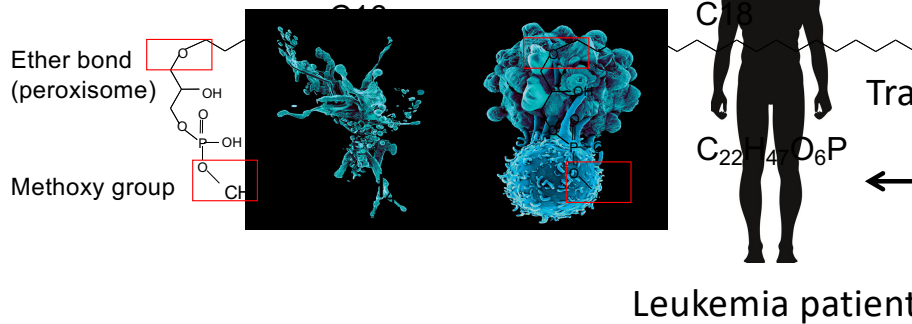
Lipid specific TCR gene

Leukemia-specific TCR vector

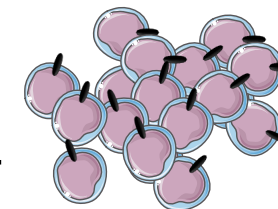


Transduction and expansion

methyl-lyso-phosphatidic acid Ag (mLPA)



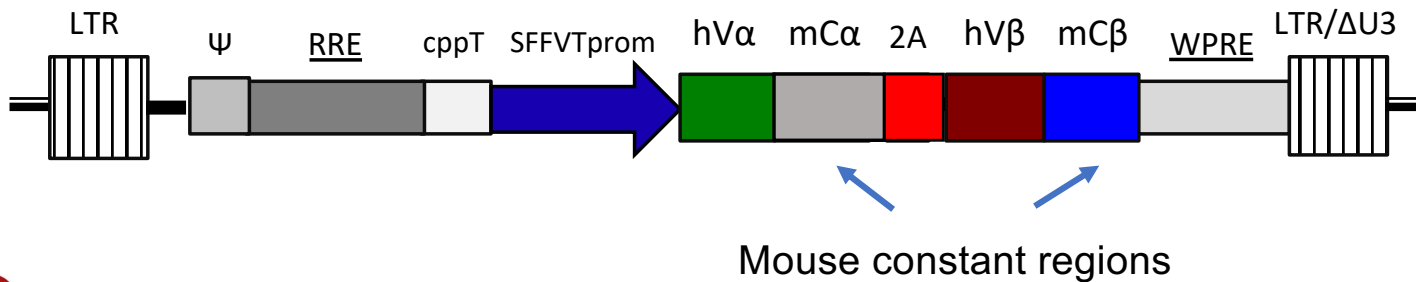
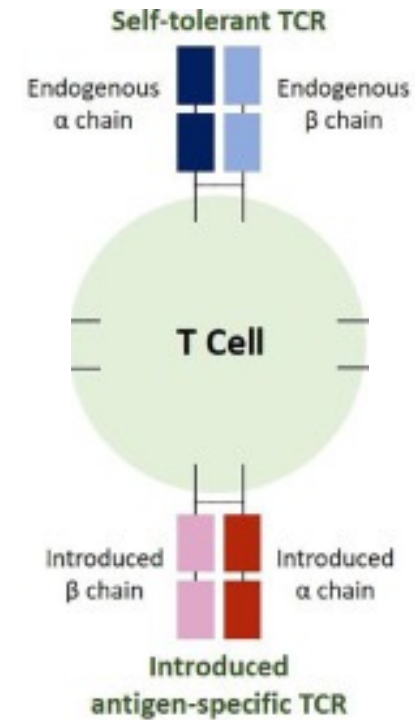
Transfer of leukemia-redirected T cells in patient



Transduced allogeneic/autologous T cells

Identification of the lead leukemia-specific TCR

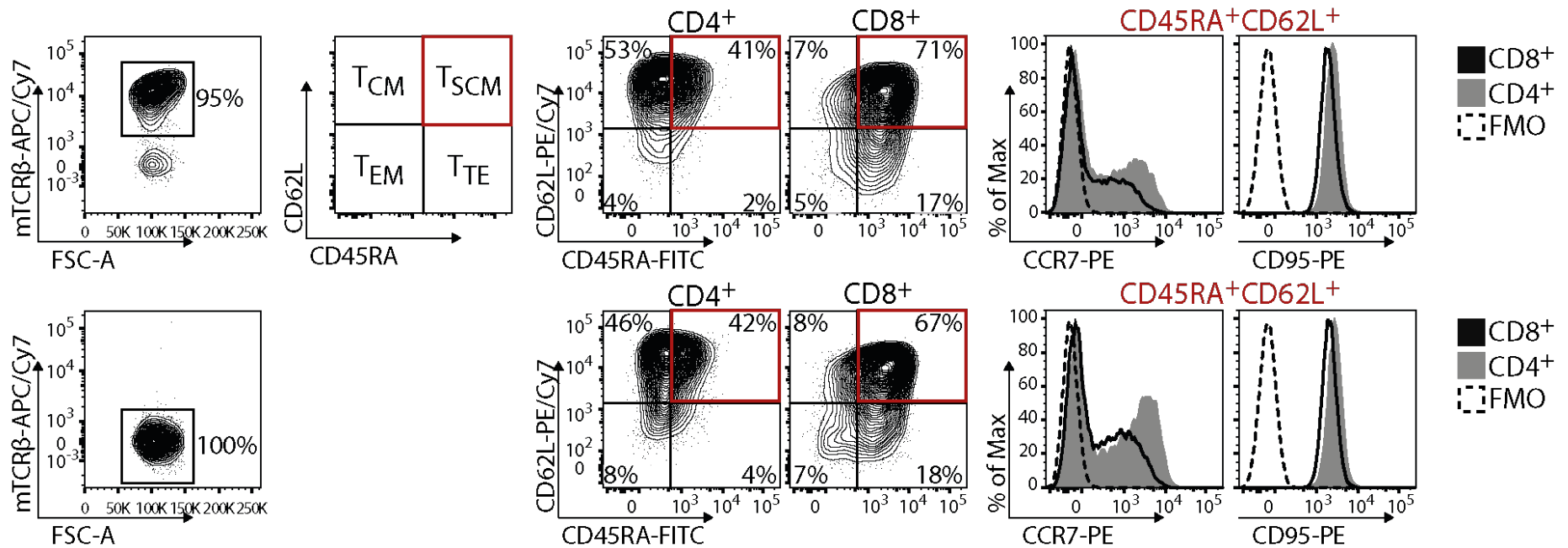
Clone Name	TCR V alfa	TCR V beta
DN4.99	TRAV38-2-J44 CAYRSP L NTGTASKLTFGTGTRLQVTL	TRBV28-J2.7 CASS PWV SYEQYFGPGTRL
PZP8A6	TRAV26-2-J44 CILRDVNTGTASKLTFGTGTRLQVTL	TRBV4-1-J2.3 CASS RLGL STDTQYFGPGTRLTVL
DN4.2	TRAV26-J53 CIL R LRGGSNYKLTFGKGLLLTVNP	TRBV4-1-J2.1 CASS PIMGLAATH NEQFFGPGTRLTVL
P8E3	TRAV38-1-J31 CAF A NNARLMFGDGTQLVVKP	TRBV28-J1.1 CAST DTG NTEAFFGQ GTRLTVV
DN7.6.16	TRAV13.1-J28 CAA P RGLGVTNSLSGRGPNSRSYQ	TRBV27-J2.1 TCASS LW WGT YNEQFFGPGTRLTVL



Generated DN4.99 TCR T cells are enriched in Stem Cell Memory subset

b

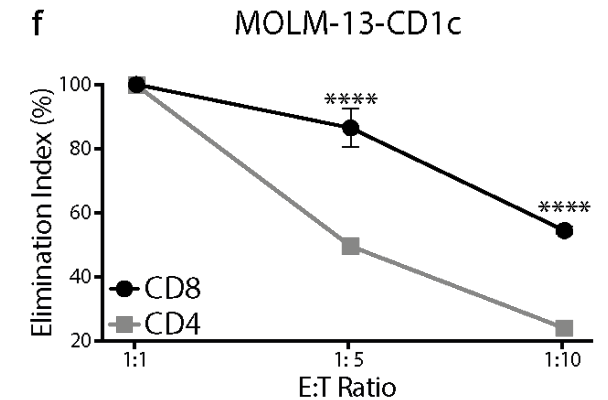
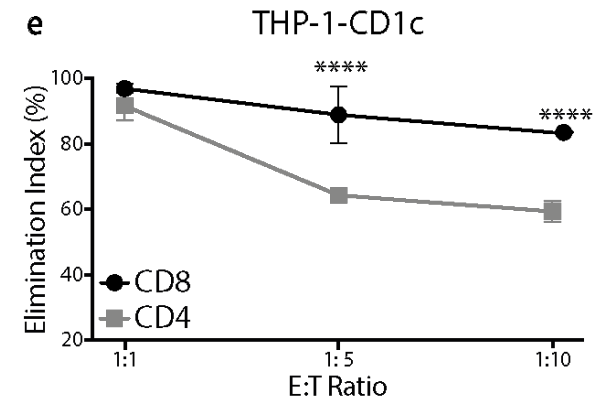
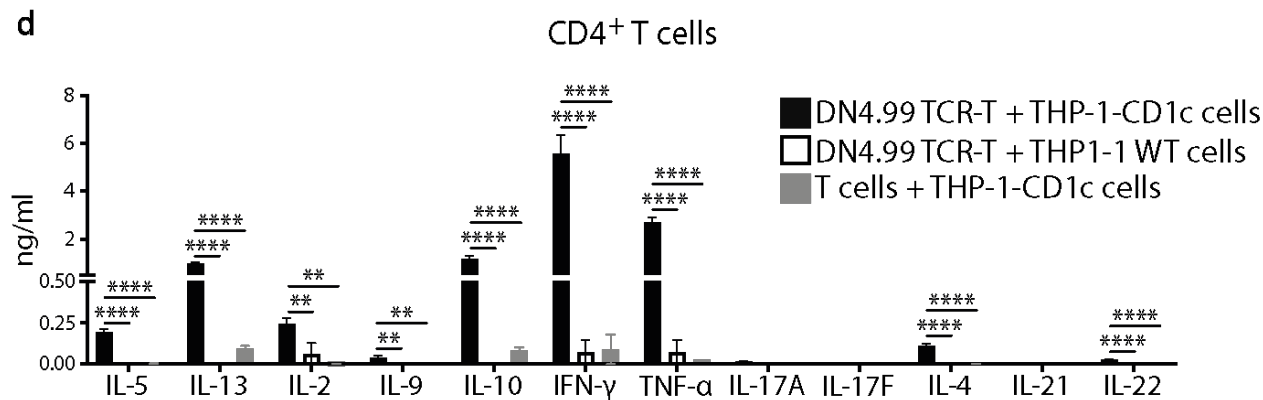
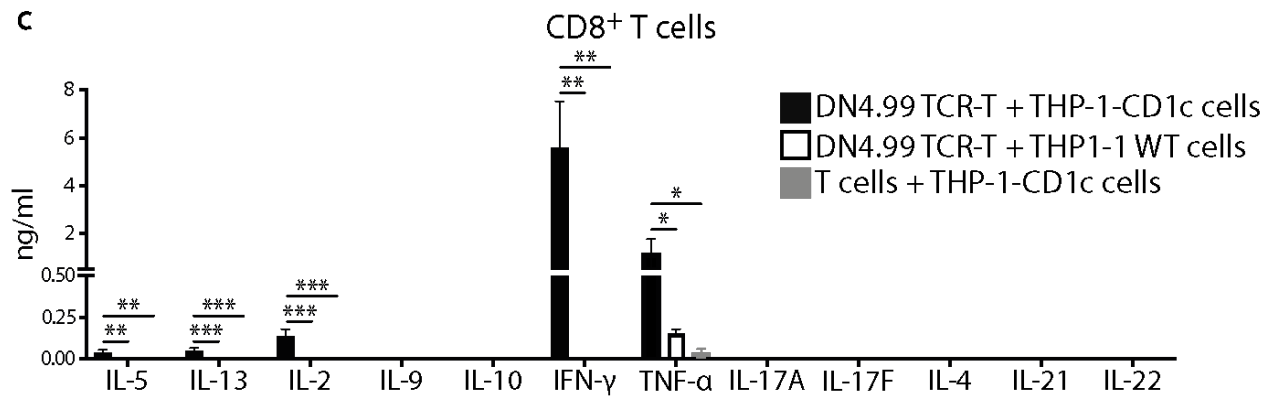
DN4.99 TCR-
T cells



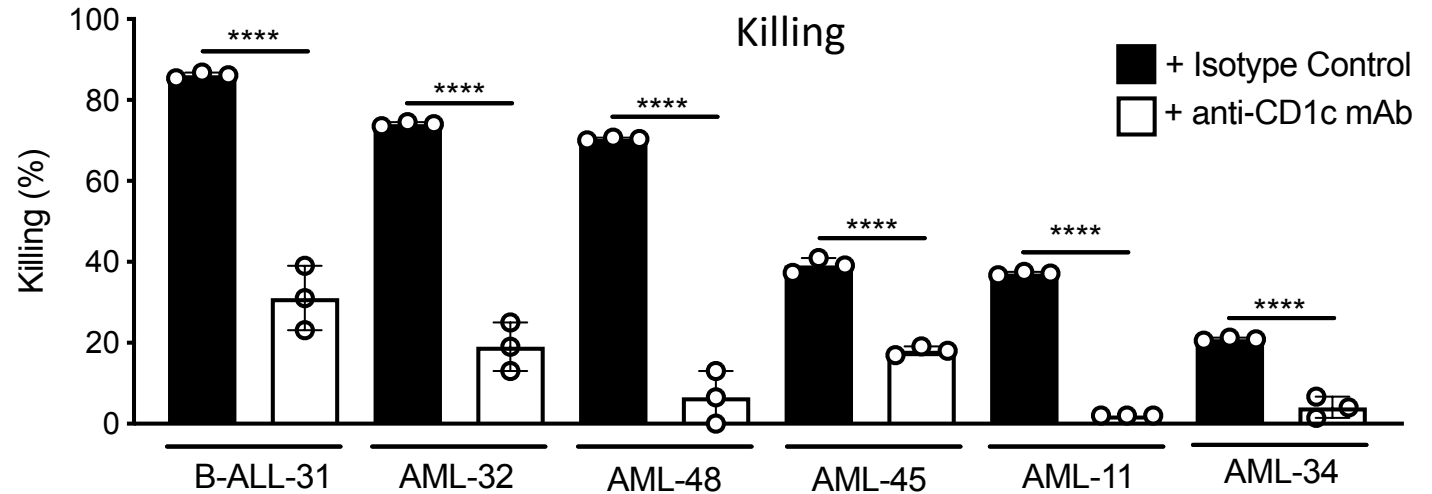
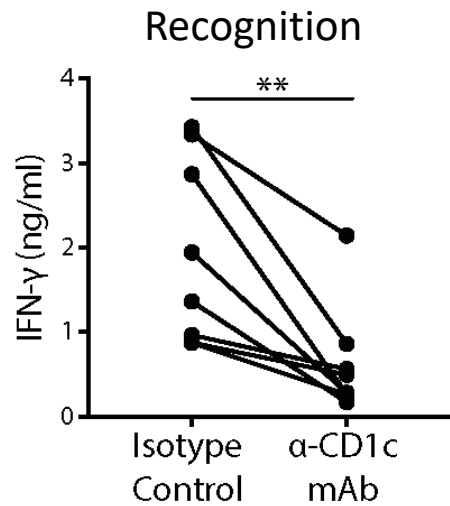
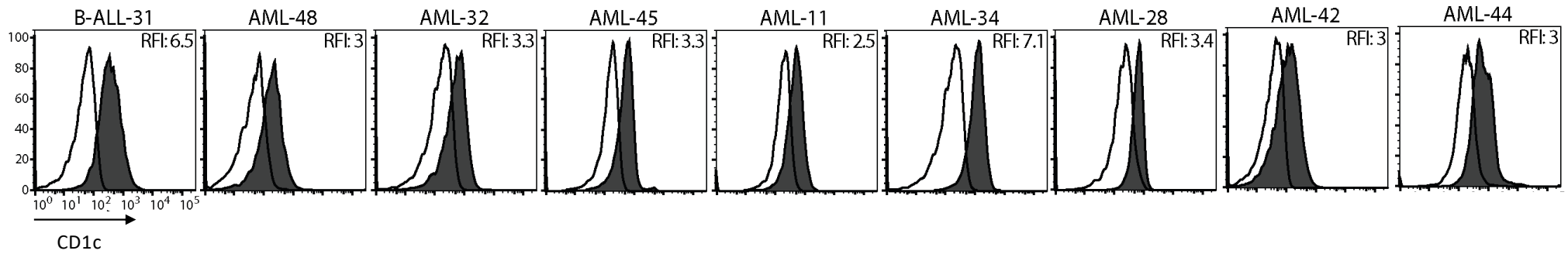
(anti-CD3/CD28 beads + IL-7/IL-15)

Consonni M. et al, Nat. Commun. 2021

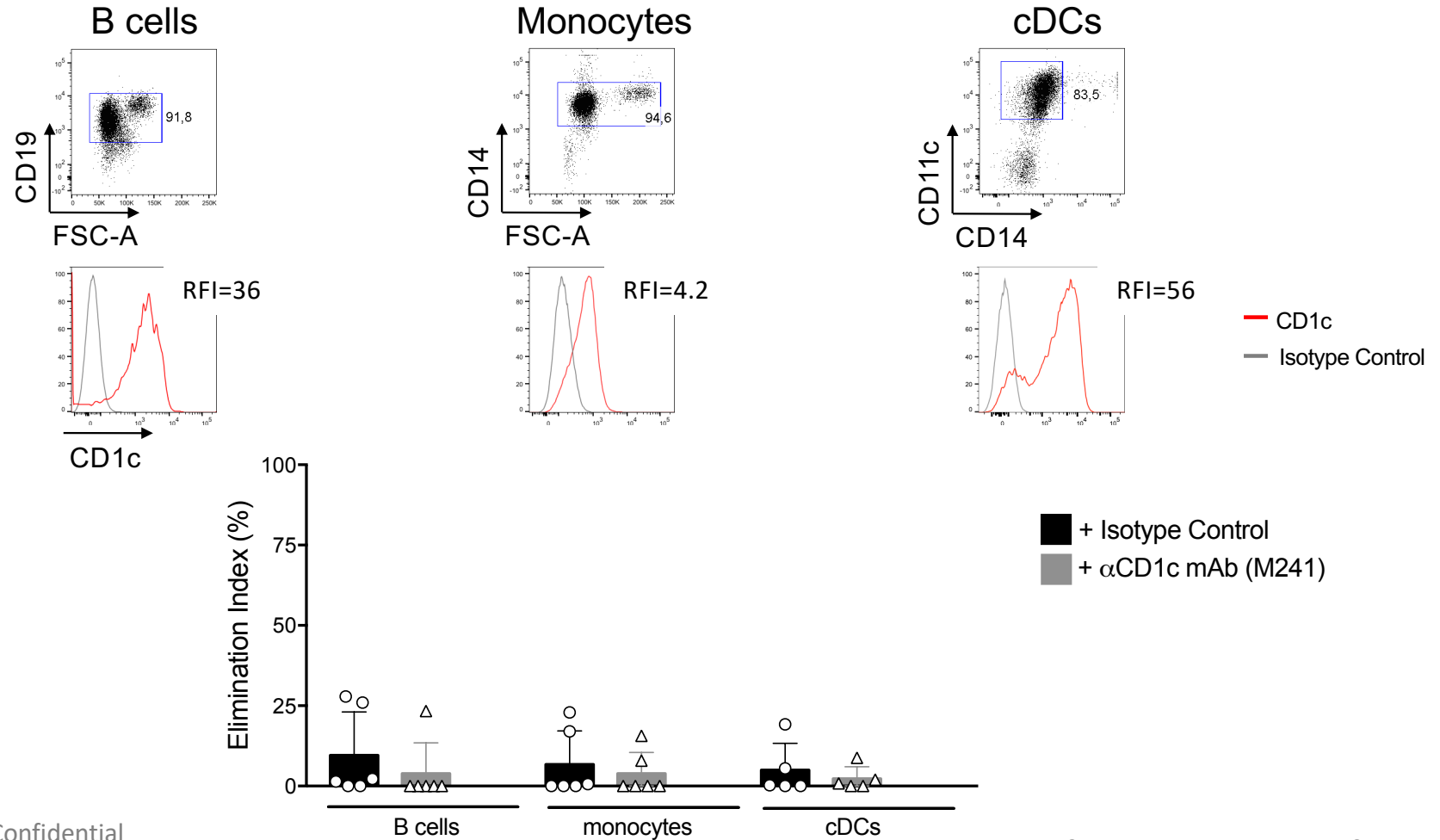
CD8+ T cells transduced with DN4.99-TCR produce mainly Th1 cytokines and kill leukemia targets better than CD4+ T cells



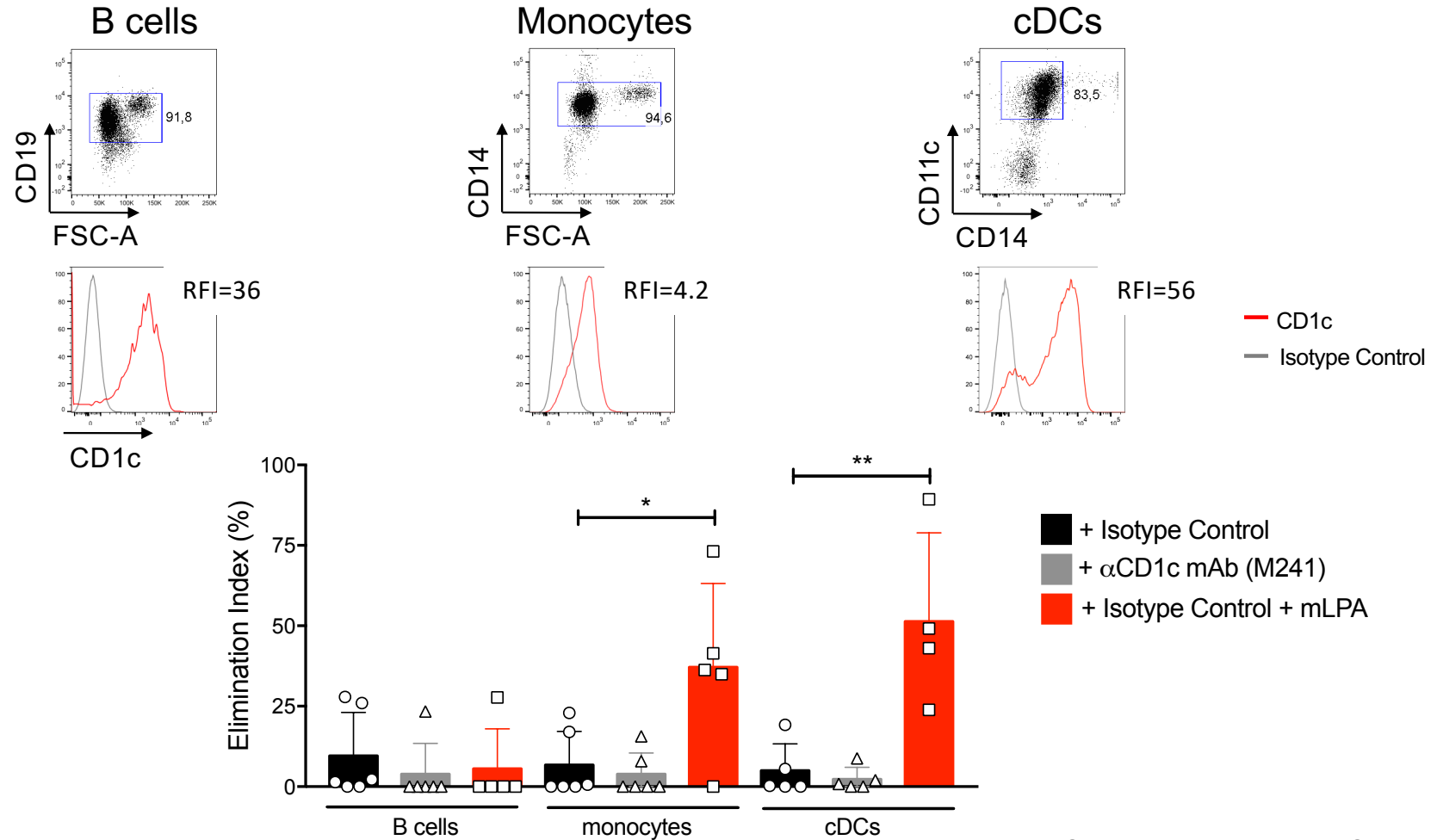
Primary T cells transduced with the DN4.99 TCR recognize and kill primary CD1c⁺ leukemia *in vitro*



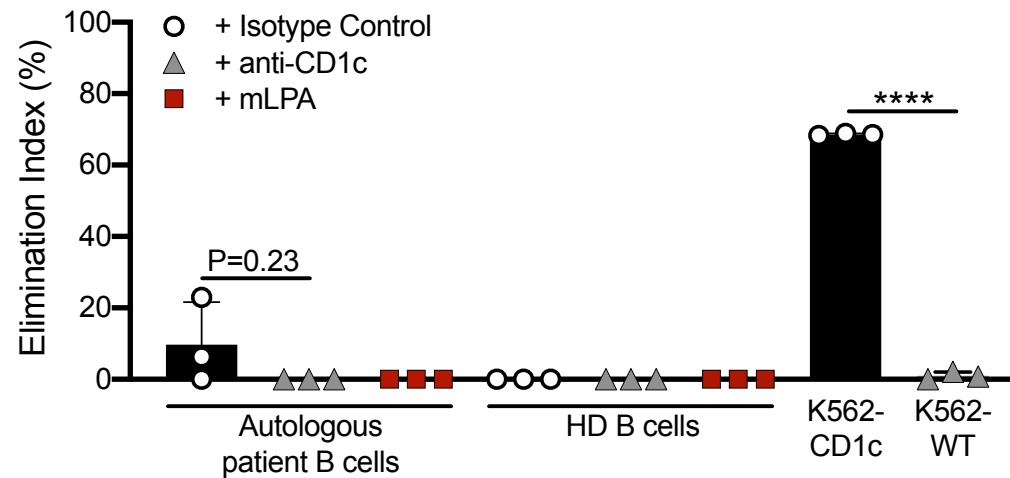
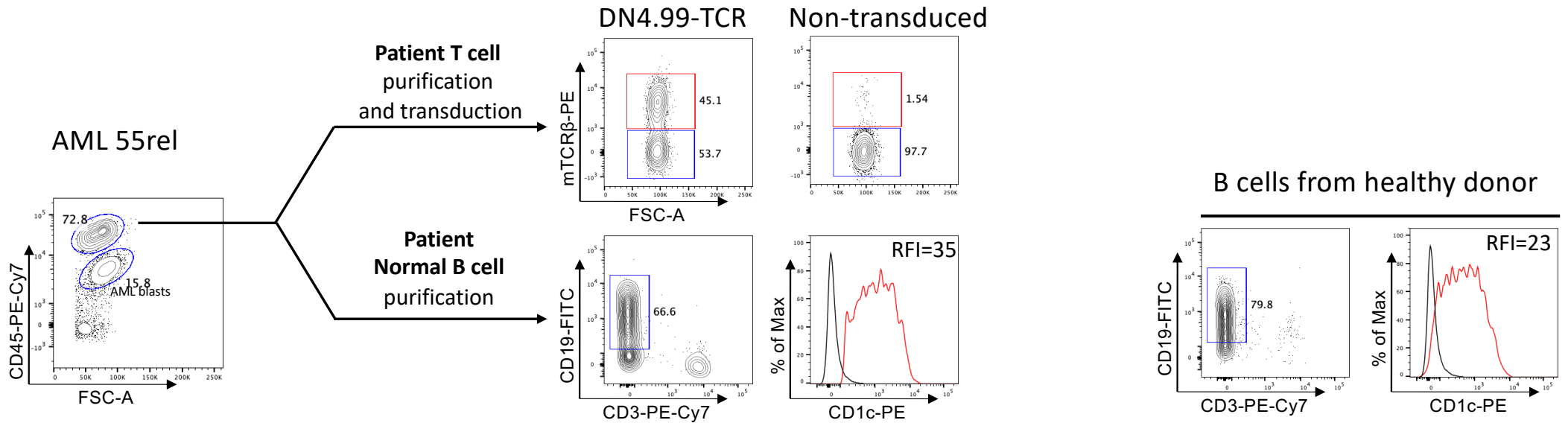
T cells transduced with the lead mLPA-specific TCR do not kill B cells, monocytes and DCs *in vitro*



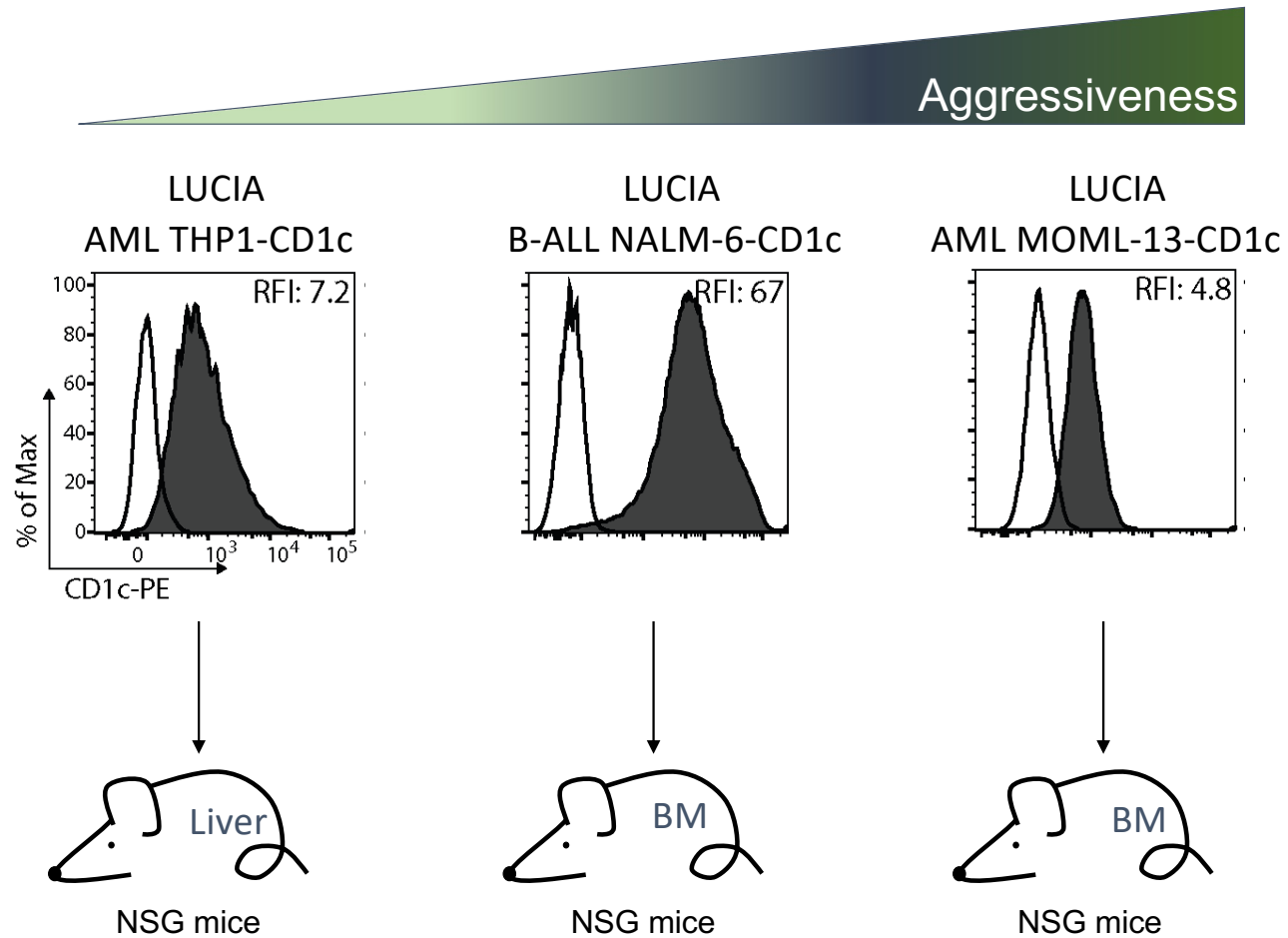
T cells transduced with the lead mLPA-specific TCR do not kill B cells, monocytes and DCs *in vitro*



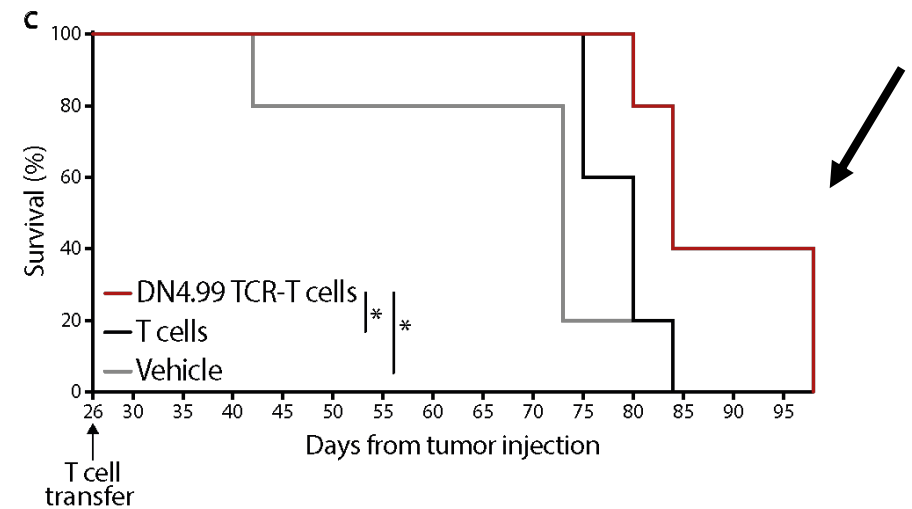
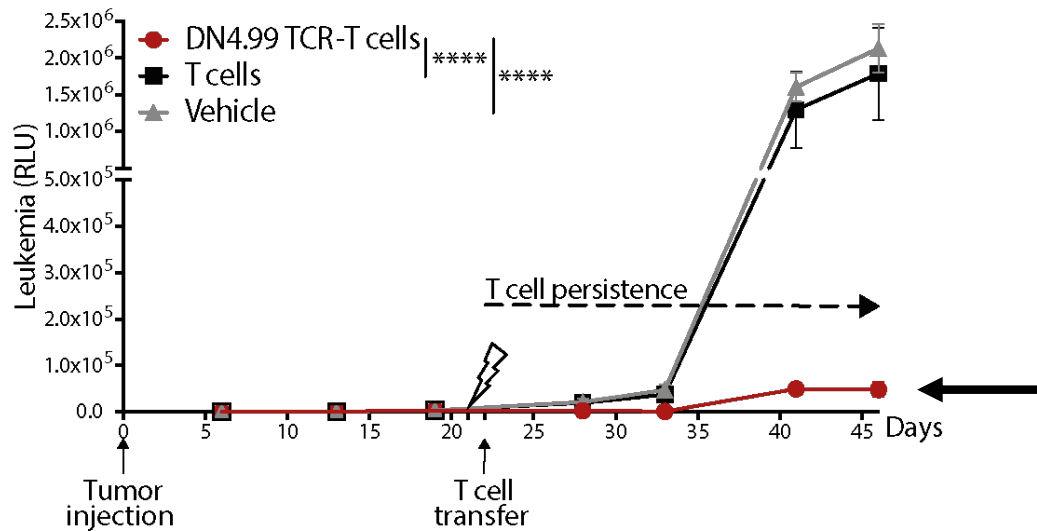
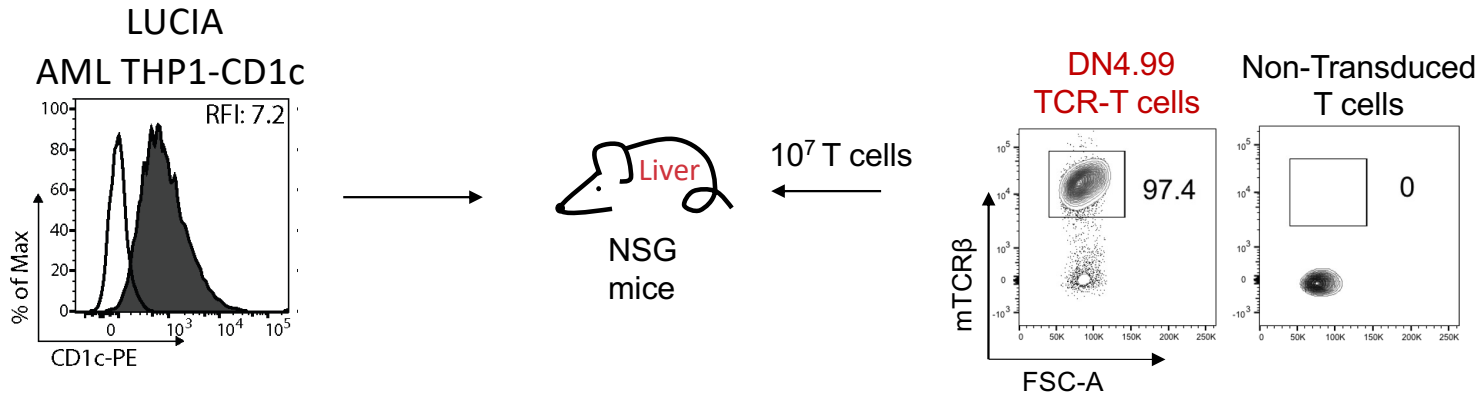
Normal B cells from an AML patient are not killed by DN4.99-TCR engineered autologous T cells



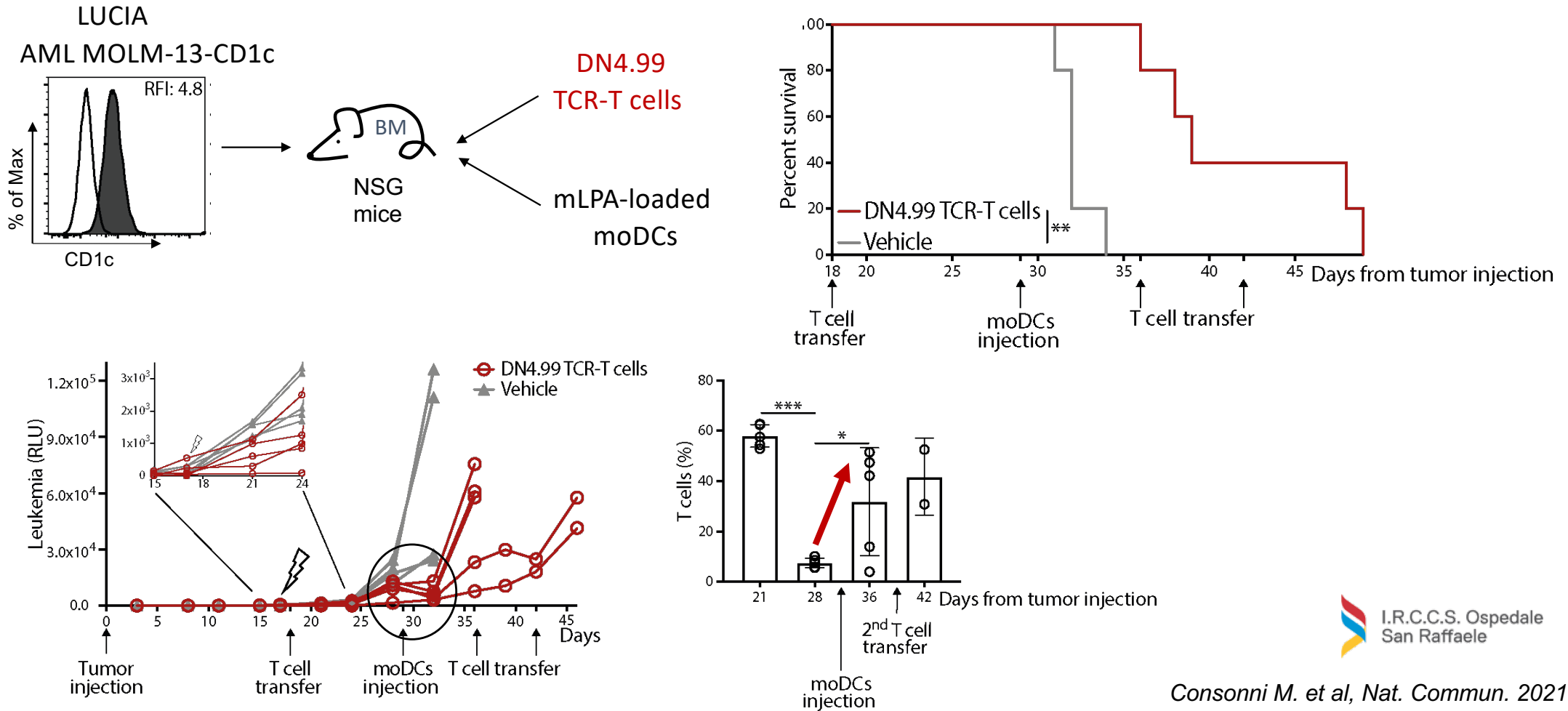
Leukemia mouse xenograft models



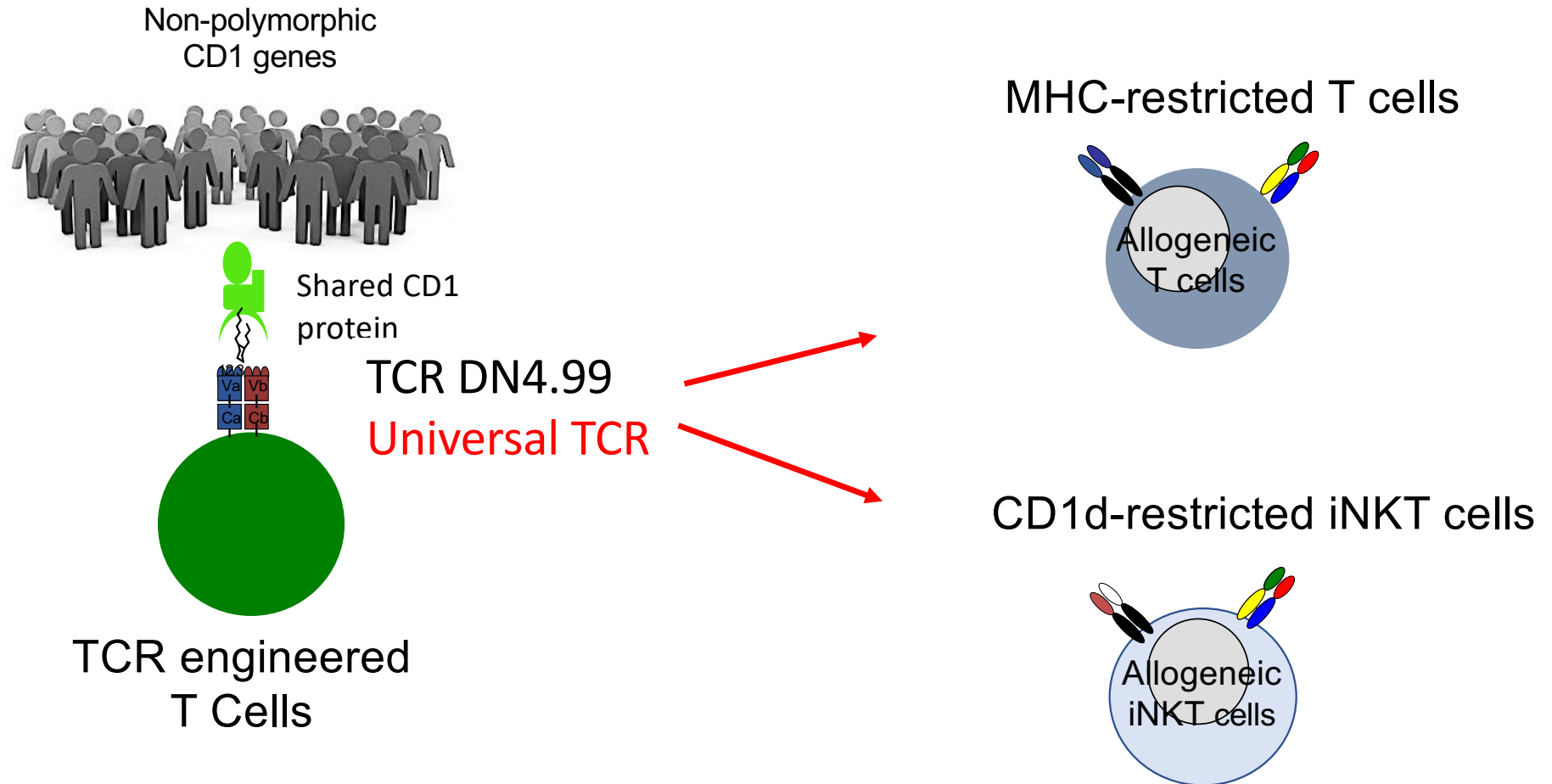
Adoptive immunotherapy with DN4.99 TCR-T cells delays THP-1 leukemia progression in mouse xenografts



The combination of multiple DN4.99 TCR-T cells injection and mLPA-loaded moDCs resulted in increased survival



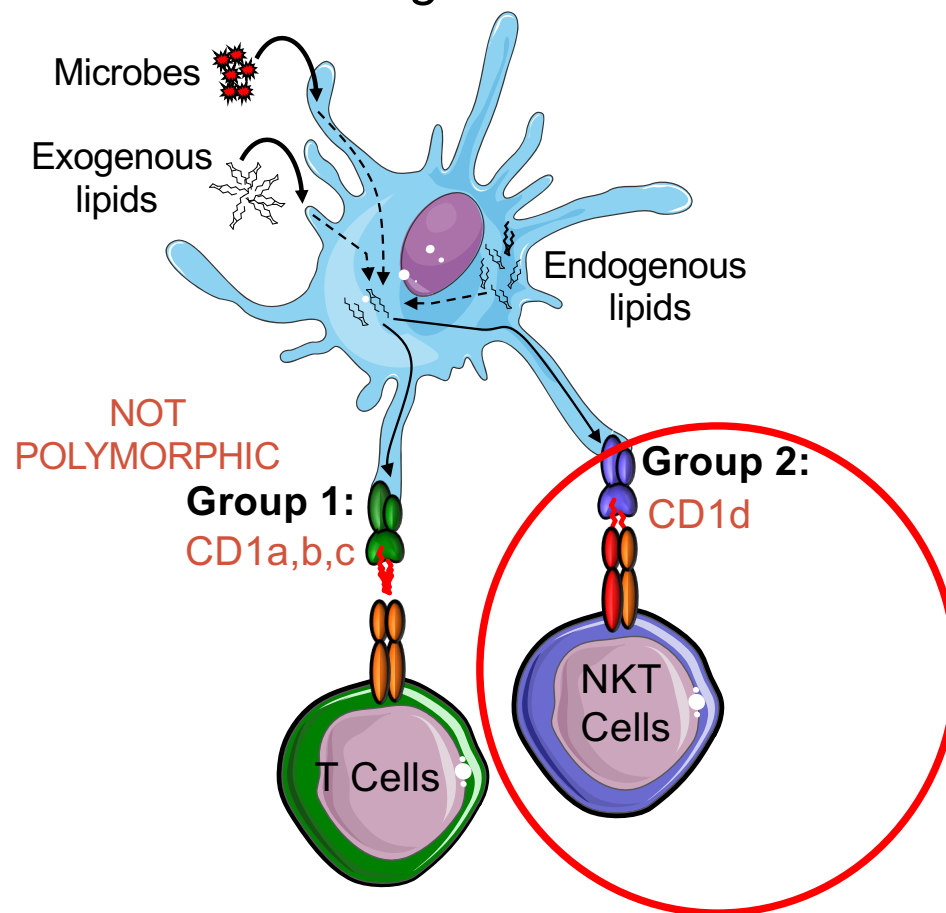
Selection of the optimal TCR recipient effector Towards an “off-the-shelf” platform



Appealing functional features of iNKT cells for ACT in the leukemia context

- Are restricted for the monomorphic CD1d molecule
- Home to the bone marrow (*Tian 2016*)
- Promote GVL while preventing GVHD (*Morris 2005; Pillai 2007; de Lalla 2011; Chaidos 2012; Rubio 2012*)
- Modulate the tumor microenvironment by eliminating suppressive macrophages (*Song 2009; Gorini 2017, Cortesi 2018, Delfanti 2022*)

CD1-restricted T cells Specific for lipid antigens

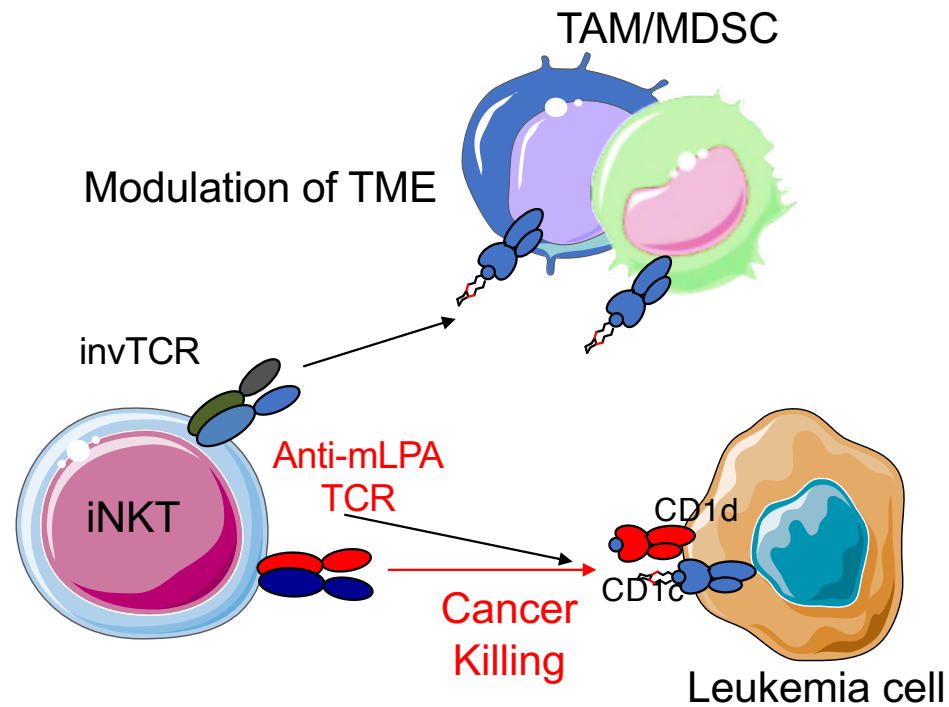


TUMOR IMMUNOLOGY

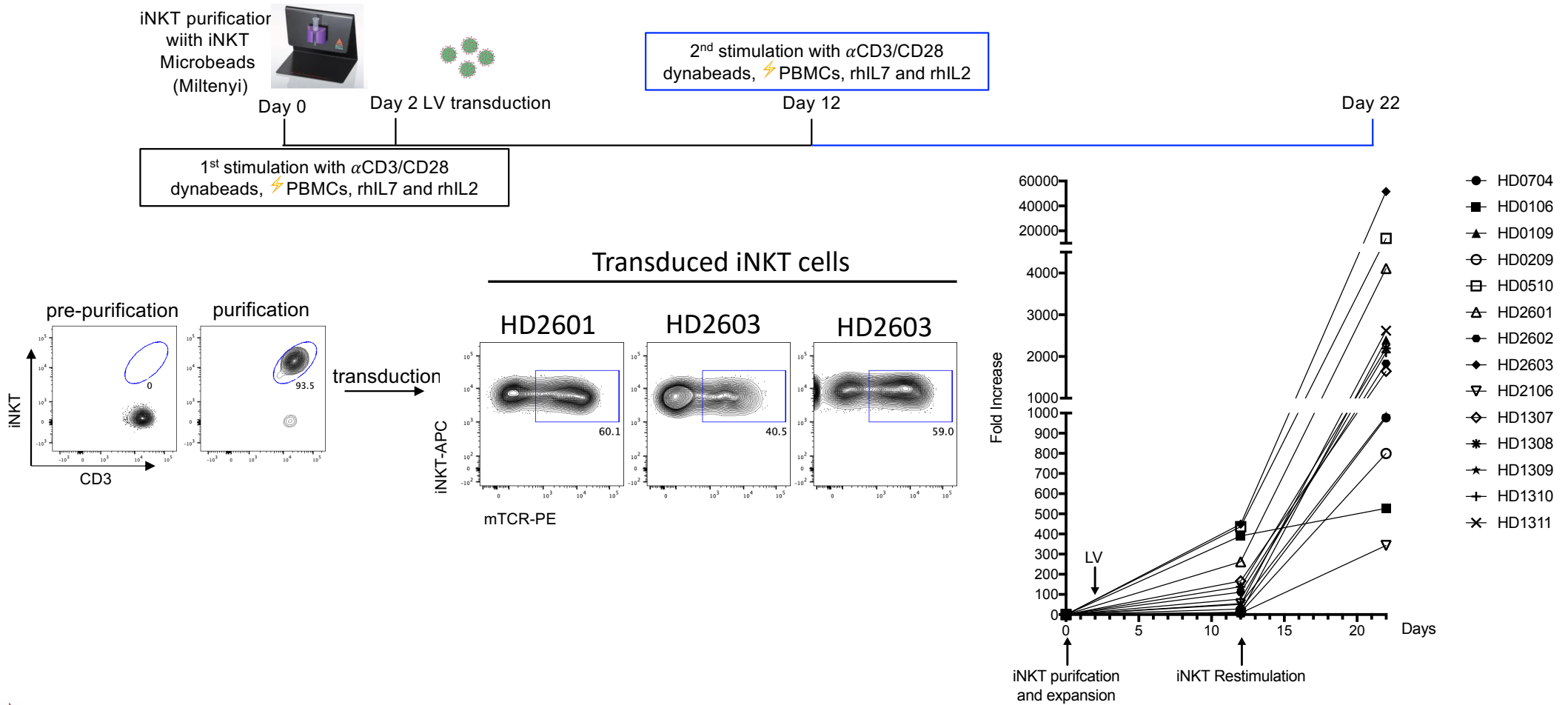
TCR-engineered iNKT cells induce robust antitumor response by dual targeting cancer and suppressive myeloid cells

**Gloria Delfanti¹, Filippo Cortesi^{1†}, Alessandra Perini¹, Gaia Antonini¹, Laura Azzimonti²,
Claudia de Lalla¹, Claudio Garavaglia¹, Mario L. Squadrito³, Maya Fedeli¹, Michela Consonni¹,
Silvia Sesana⁴, Francesca Re⁴, Haifa Shen^{5‡}, Paolo Dellabona^{1*}, Giulia Casorati^{1*}**

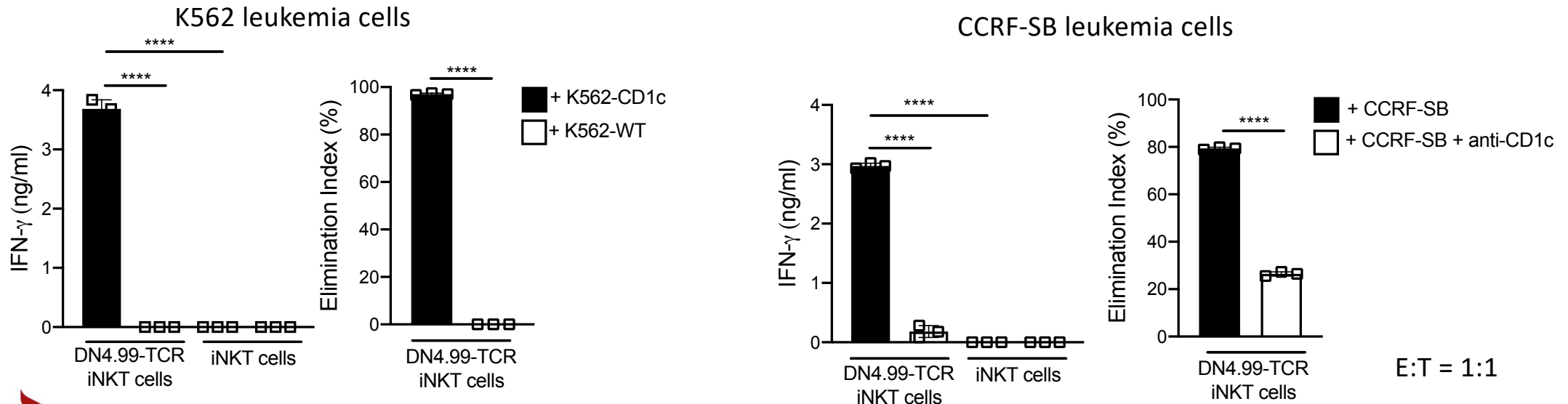
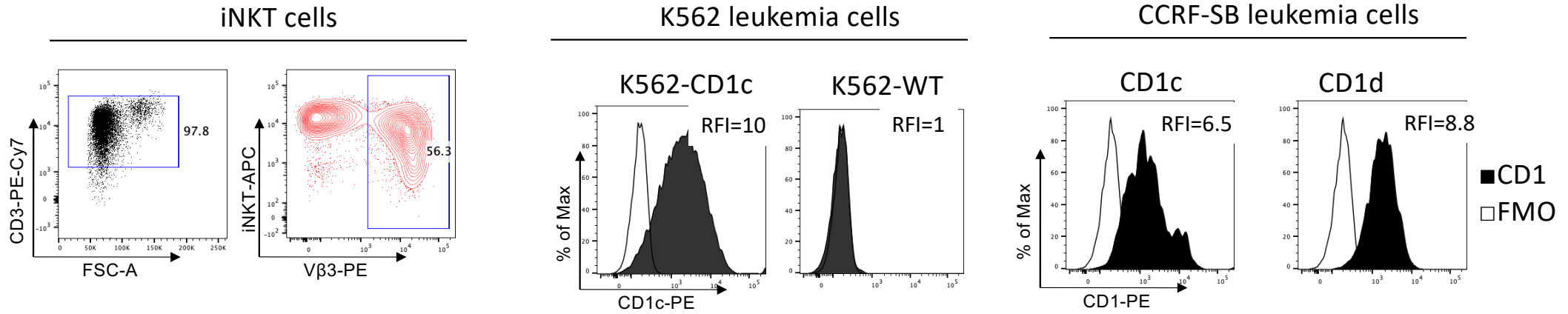
Redirecting iNKT cells against leukemia with mLPA-specific TCR



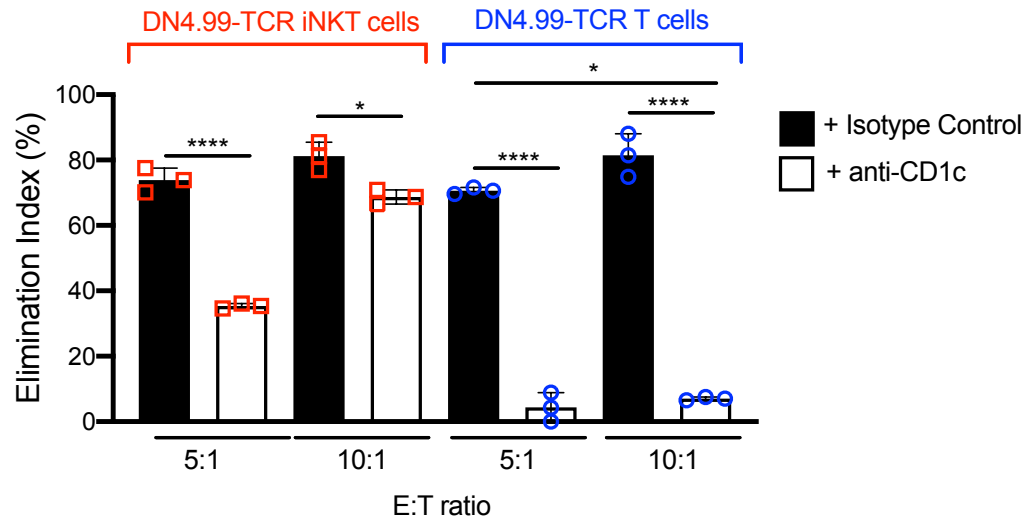
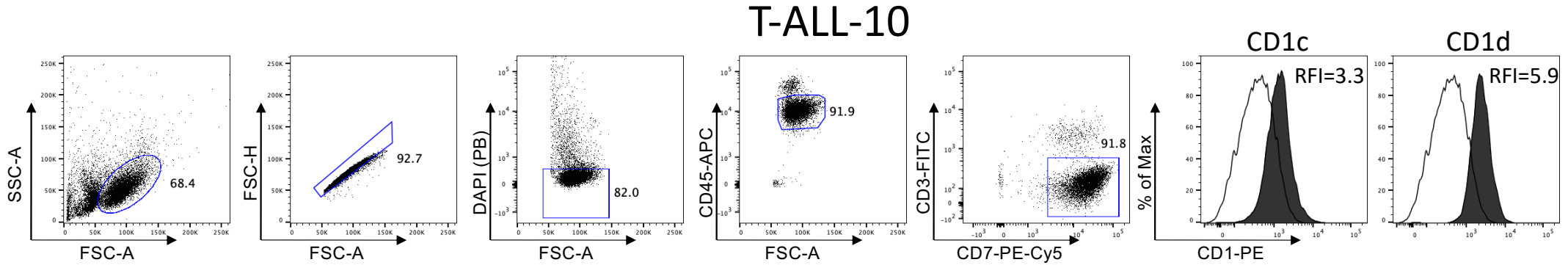
Efficient human iNKT cell expansion and transduction with exogenous TCR



Recognition and killing of K562 and CCRF-SB cells by DN4.99 TCR-iNKT cells



Combinatorial CD1c and CD1d recognition by DN4.99 TCR-iNKT on primary T-ALL



48h co-culture

Conclusions

- ✓ Human primary T and iNKT cells are efficiently redirected against diverse CD1c-expressing acute leukemias *in vitro* by engineering with a mLPA-specific TCR;
- ✓ The adoptive immunotherapy with mLPA-specific TCR-T cells significantly delay the progression of three models of leukemia xenograft in NSG mice, an effect that is boosted by mLPA-cellular immunization;
- ✓ iNKT cells engineered with mLPA specific TCR allow combinatorial targeting of CD1c and CD1d expressing targets
- ✓ Our results support the use of mLPA-specific CD1c-restricted TCRs as an attractive option for adoptive immunotherapy of leukemia across MHC barriers.

Acknowledgments



Experimental immunology unit

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Daniela Montagna

Experimental Hematology Unit

Chiara Bonini

Innovative Immunotherapies Unit

Monica Casucci
Barbara Camisa



worldwide
cancer research

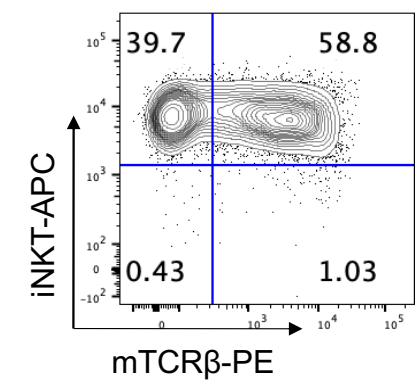
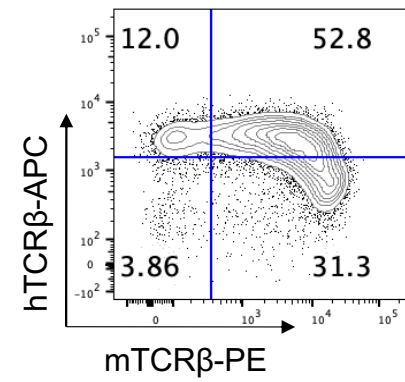
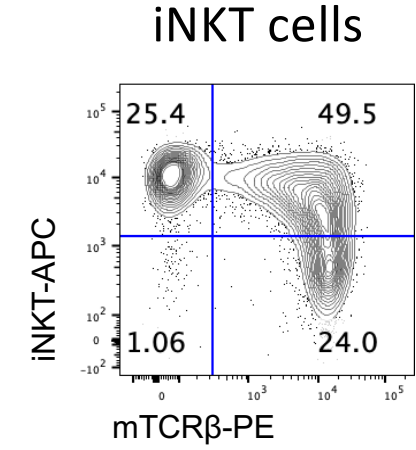
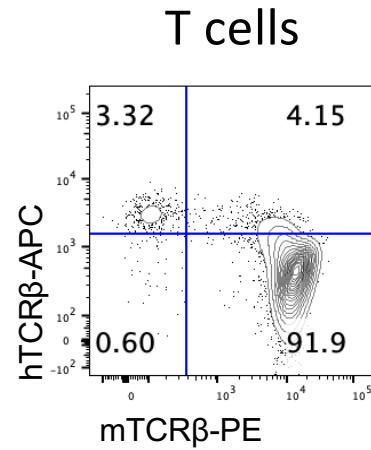
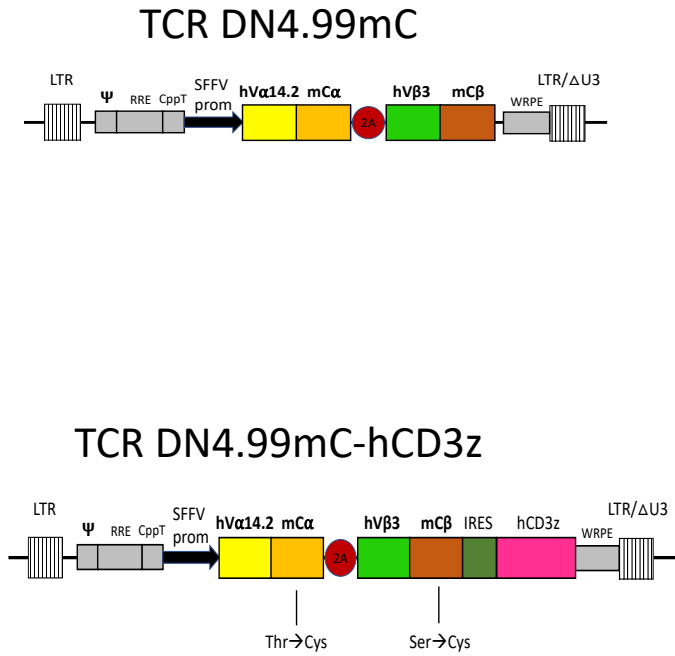
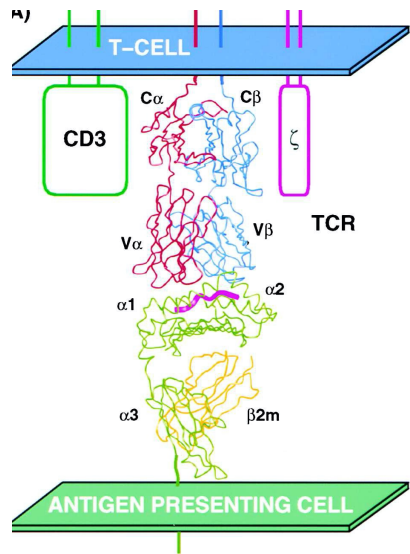


University of Milano-Bicocca,

Monza

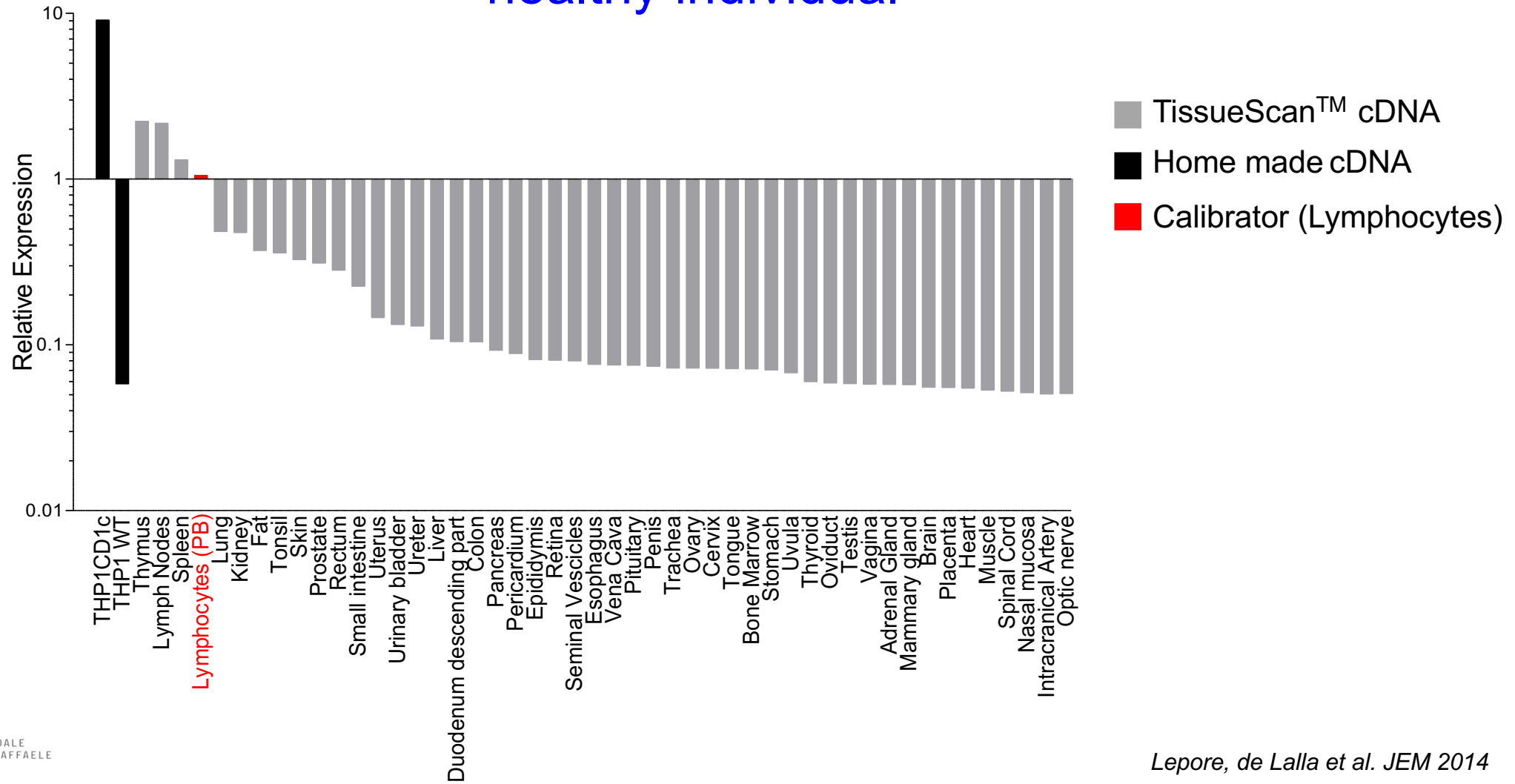
Marta Serafini
Alice Pievani
Sarah Tettamanti

Addition of CD3z protein in DN4.99 TCR to favor co-expression of the exogenous and the endogenous TCRs



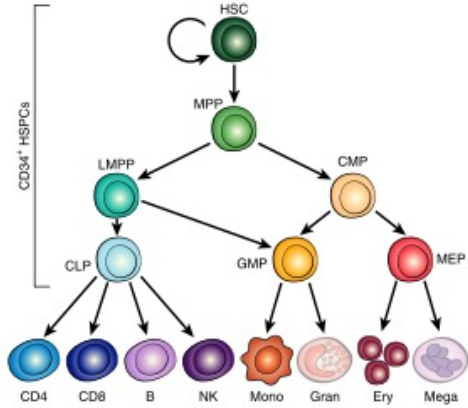
Consonni M. Unpublished confidential

Absence of CD1c expression in non-hematopoietic tissues of healthy individual

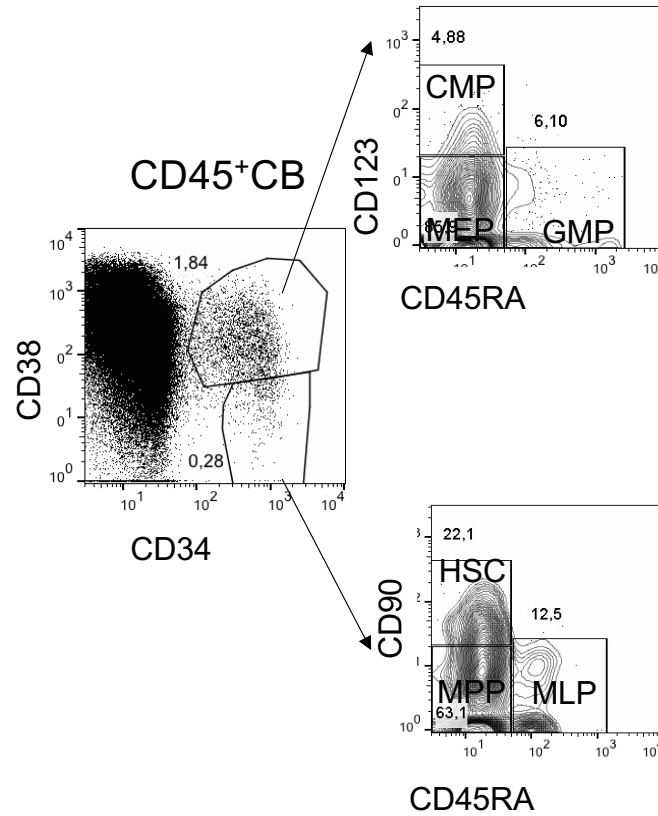


Absence of CD1c expression in normal hematopoietic precursors

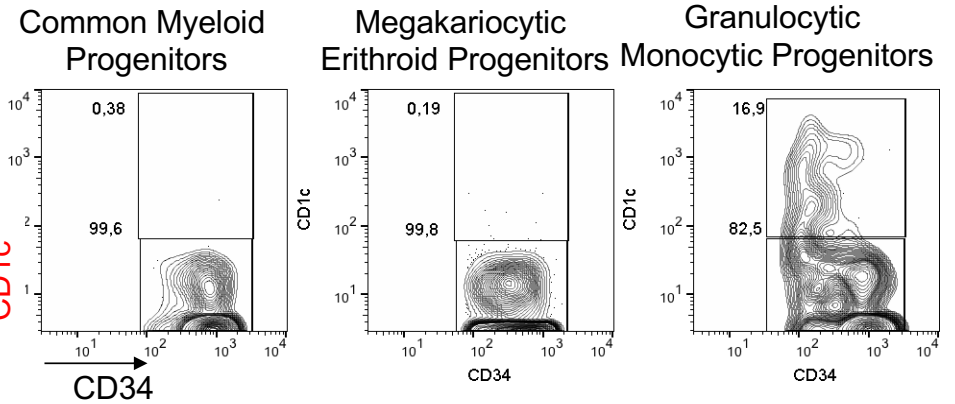
Cord Blood cells from healthy donors



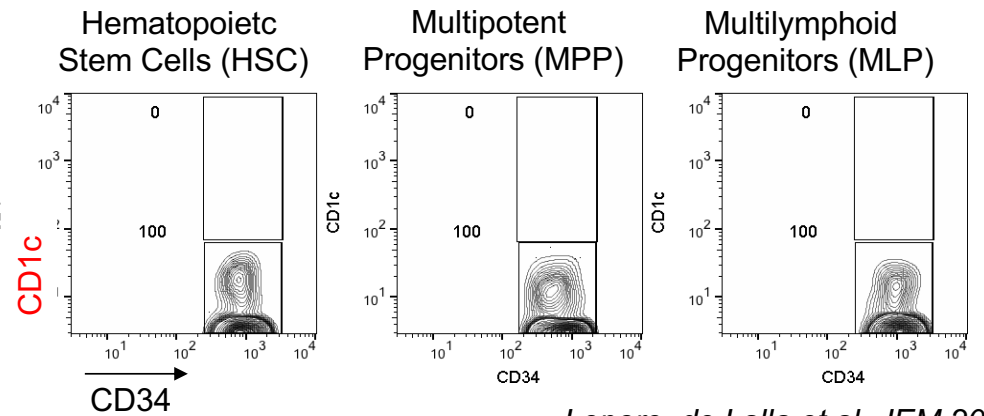
(adapted from Corces MR et al., *Nature Genetics* 2016)



CD38⁺CD34⁺(MORE MATURE PRECURSORS)



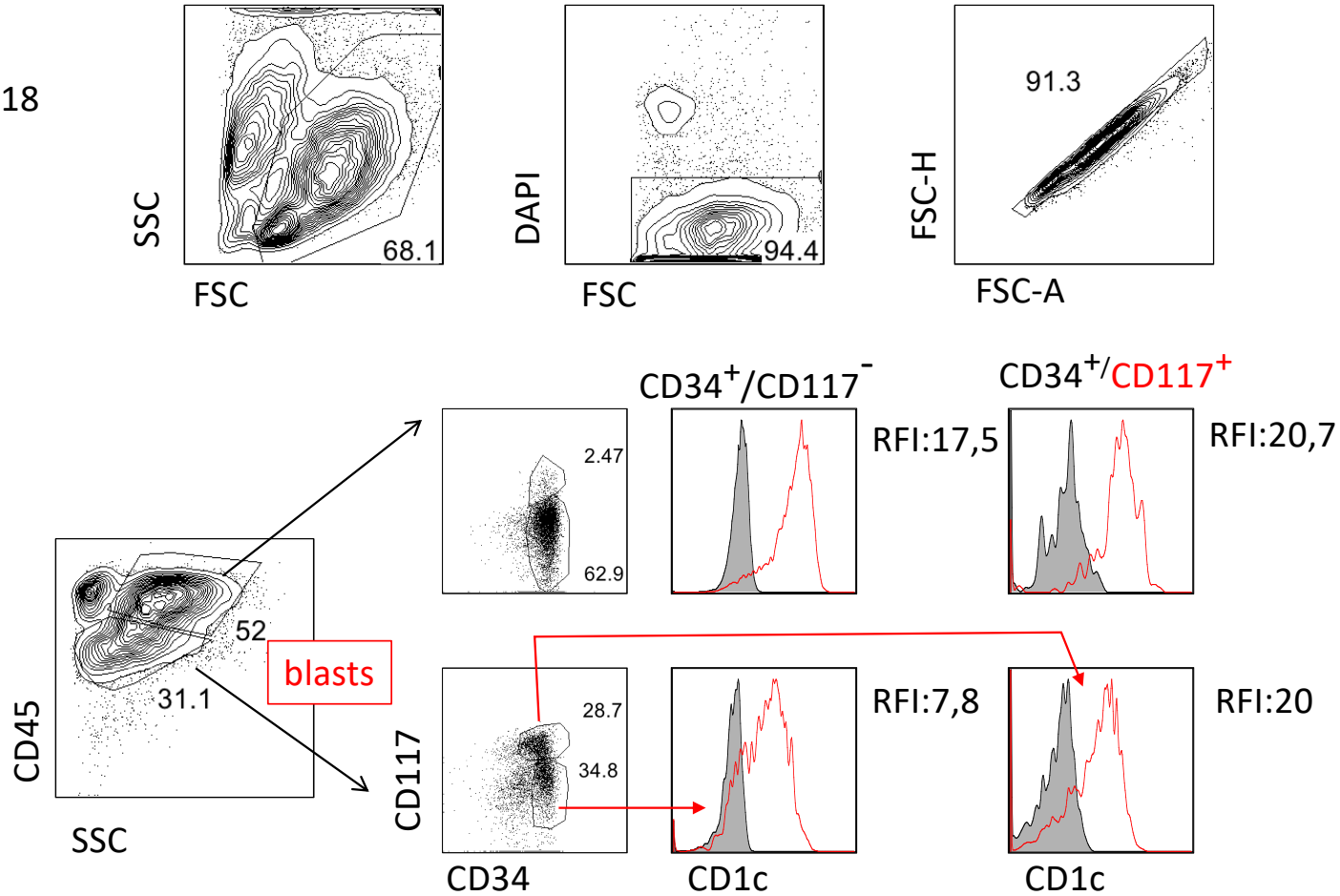
CD38⁻CD34⁺ (STEM CELLS)



Lepore, de Lalla et al. *JEM* 2014

CD1c is expressed on primary AML and ALL blasts displaying the LAIP (leukemia associated immuno phenotype) utilized to track relapses

Ex- Vivo a-AML-18



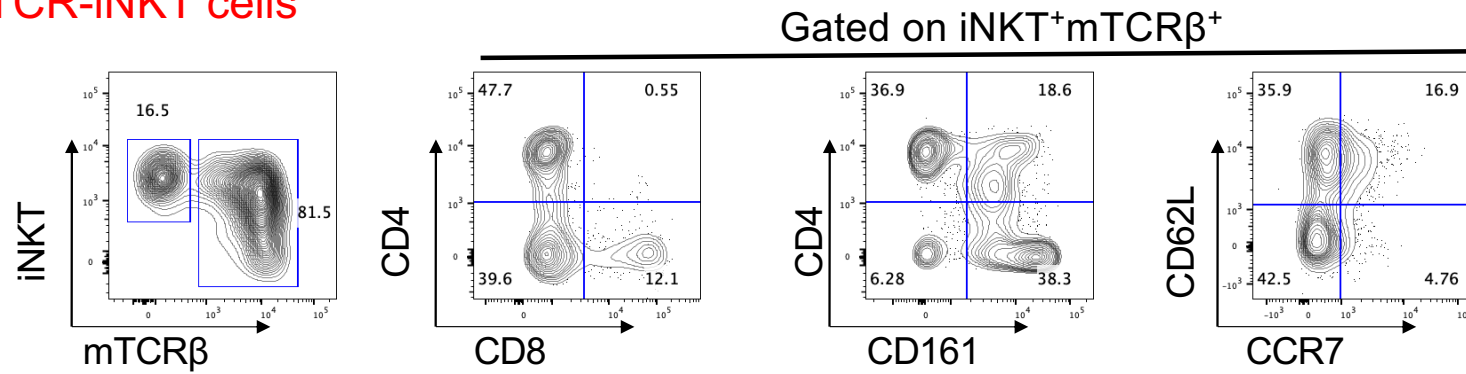
DIAGNOSIS: AML Fab M4 (presence of monoblasts>monocytes)
 LAIP= CD45^{low}, CD34⁺,CD33⁺ with asynchronous maturations CD117⁺ 38% CD117⁻ 62%

CdL

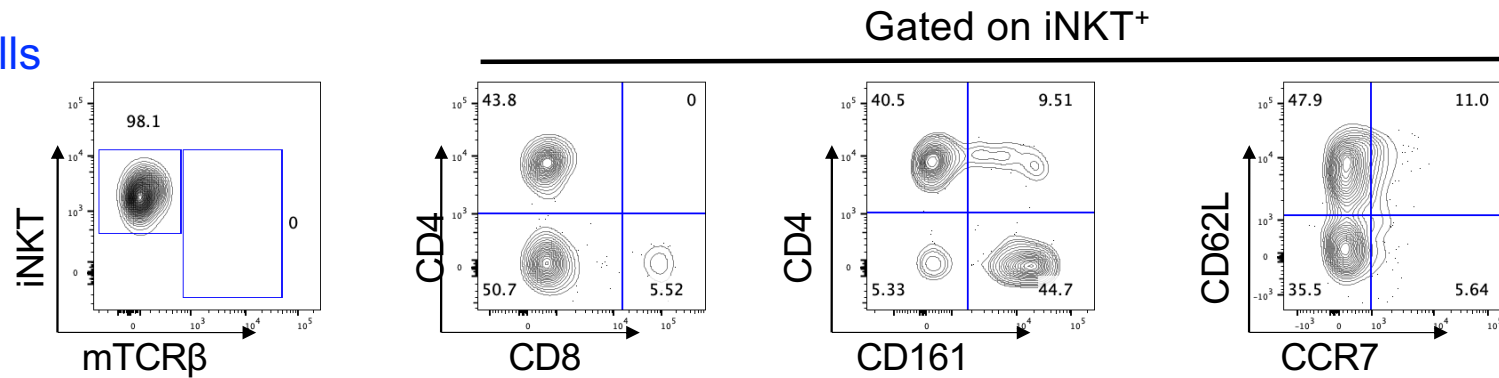
Unpublished
 confidential

Expanded DN4.99 TCR-iNKT express CD62L

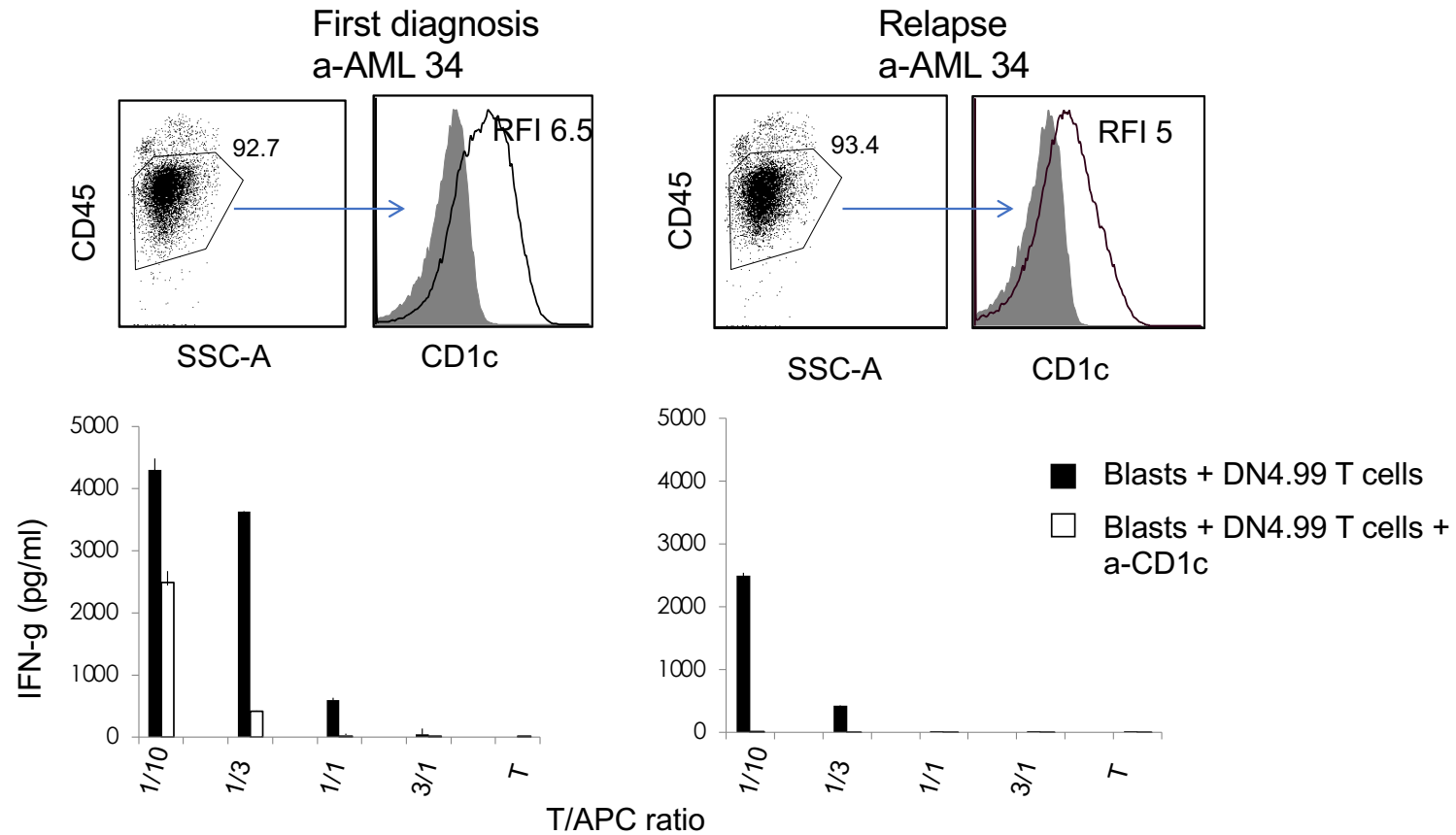
DN4.99 TCR-iNKT cells



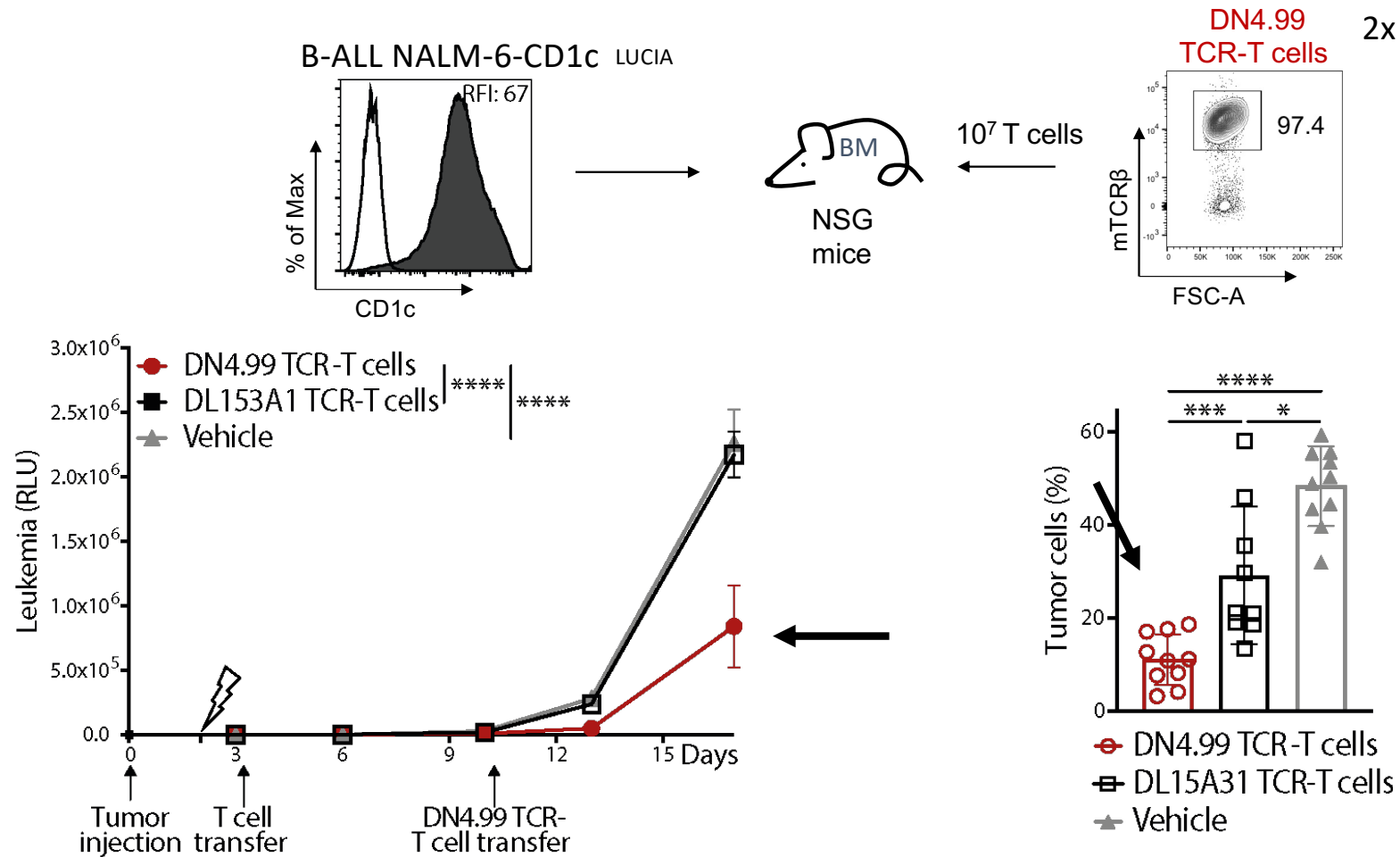
iNKT cells



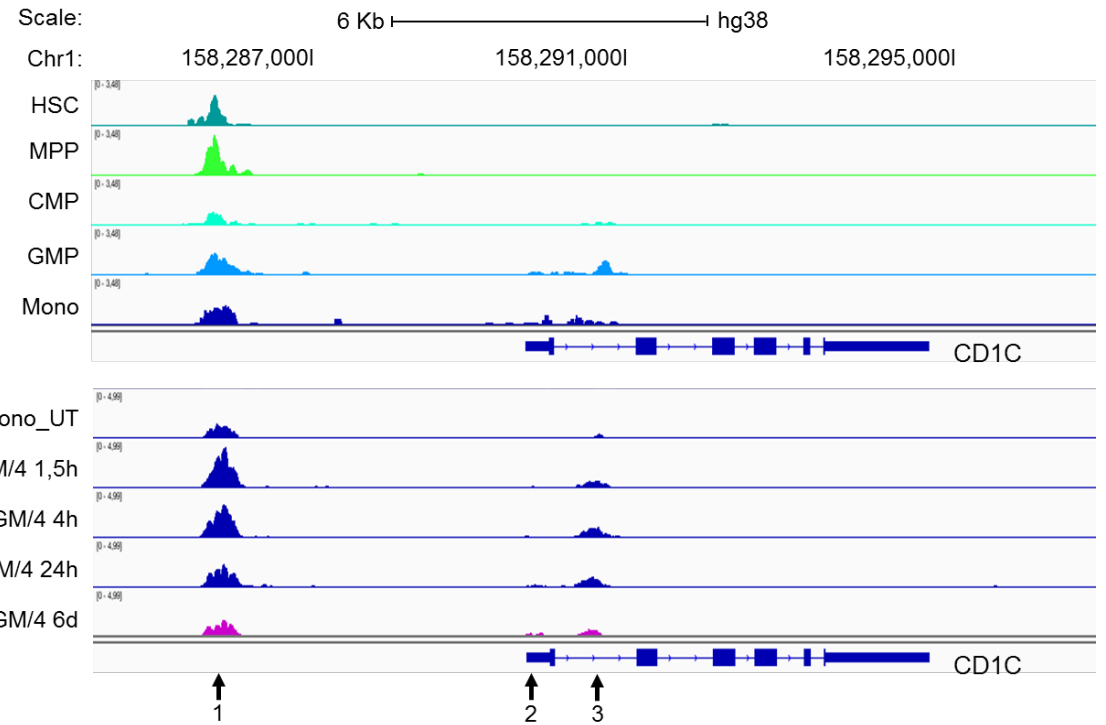
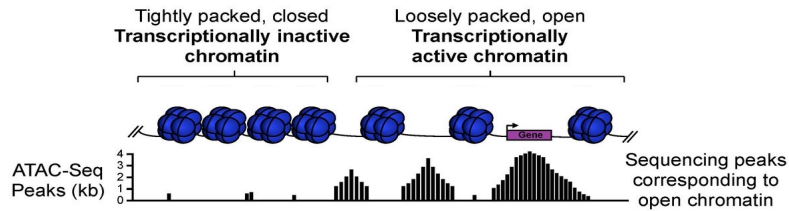
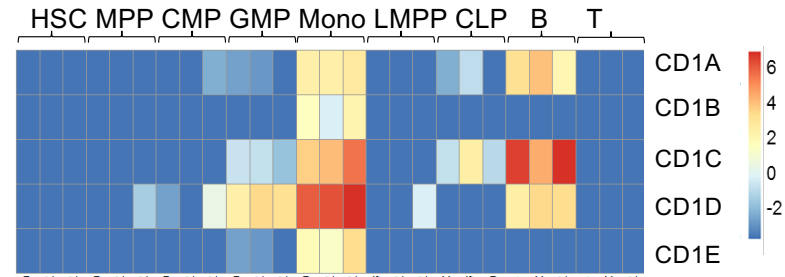
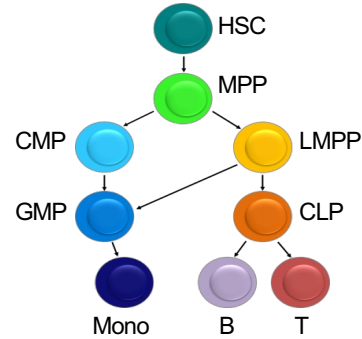
self-reactive CD1c restricted T cells recognize also relapsed leukemia blasts

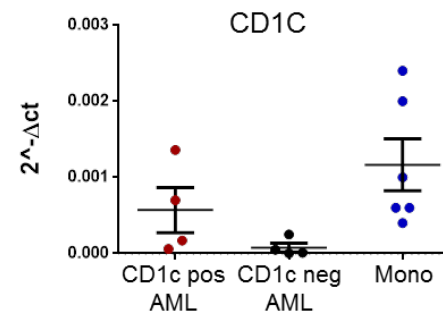
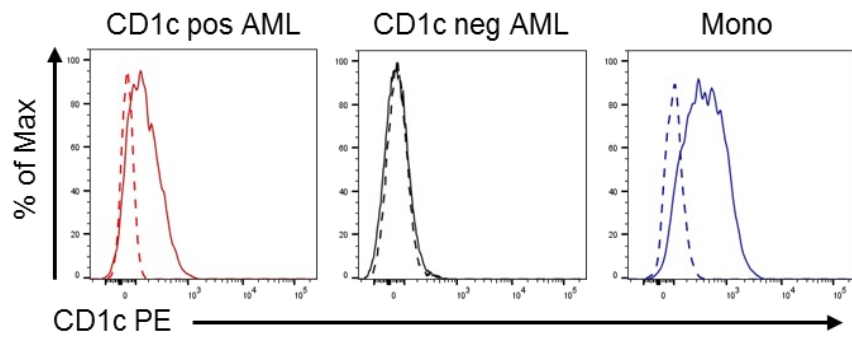


A second transfer of DN4.99 TCR-T cells significantly further delays **NALM-6** leukemia progression in mouse xenografts

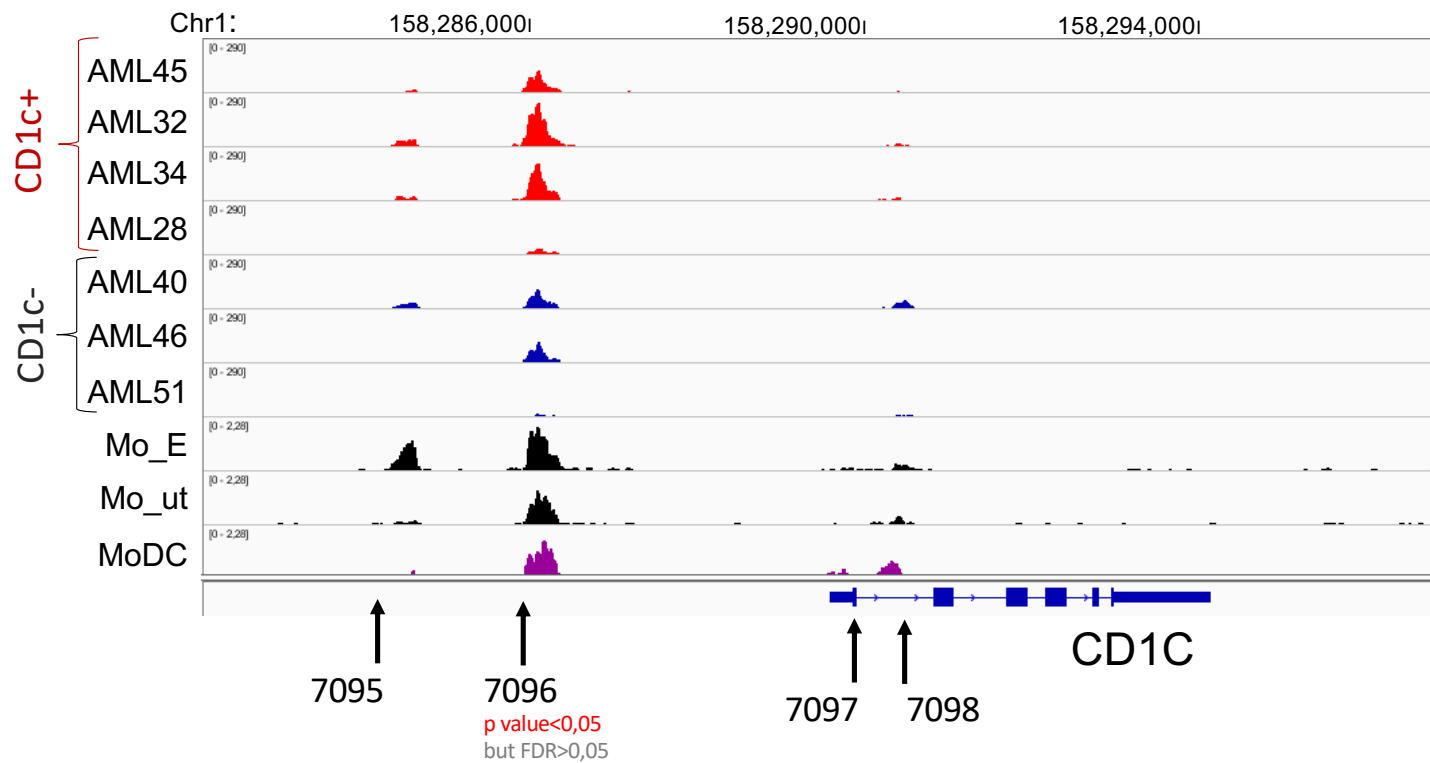


Step-wise epigenetic and transcriptional events determine CD1 gene expression profile along lymphoid and myeloid differentiation





Scale: 4 Kb | hg38



Adoptive cell therapy with engineered T cells is currently represented by two approaches

