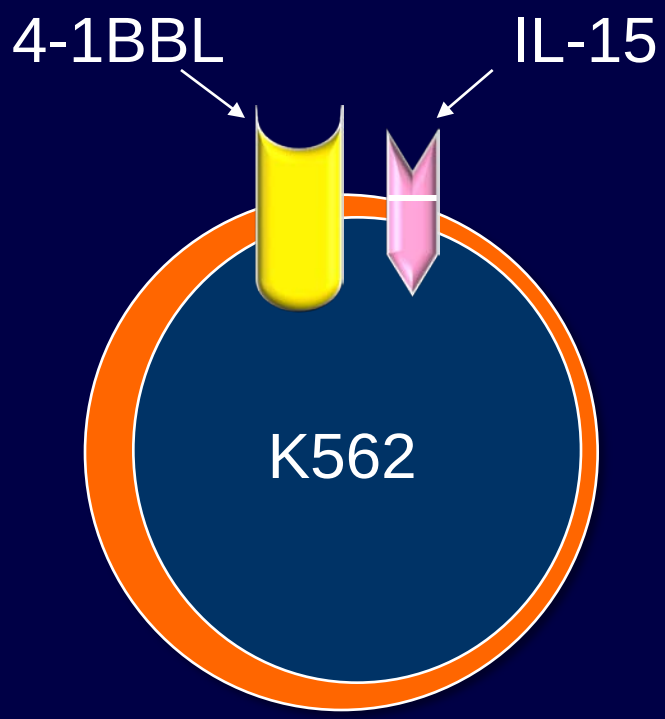
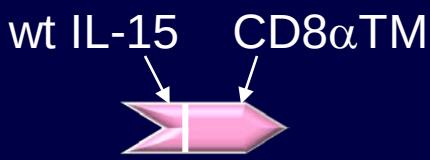
Dario Campana
Departments of Oncology and Pathology
St. Jude Children’s Research Hospital

A Method to Specifically Expand NK Cells



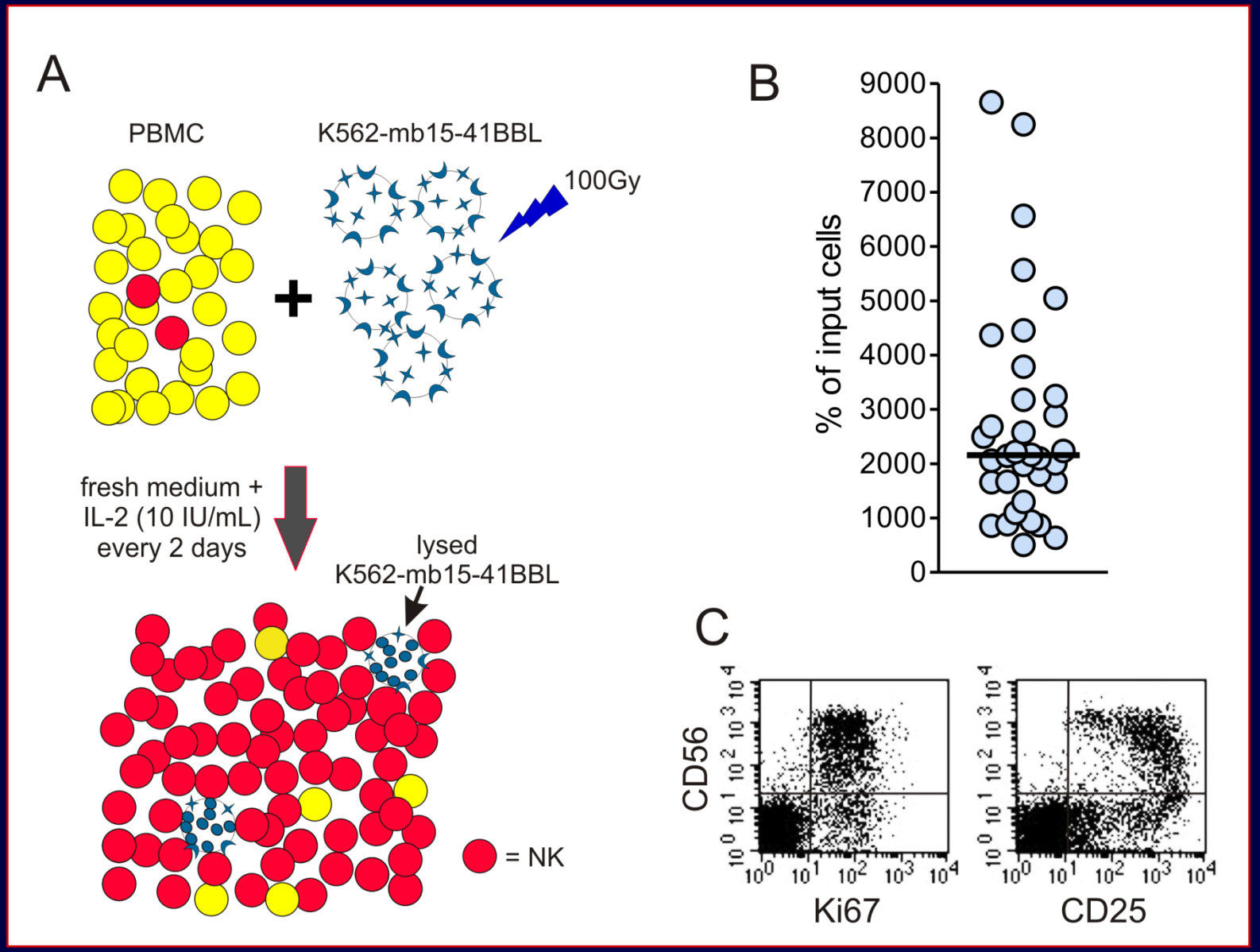


- NK cells express 4-1BB
- 4-1BB ligation results in stimulatory signals

- IL-15 stimulates NK cells
- It is more powerful in its membrane-bound form

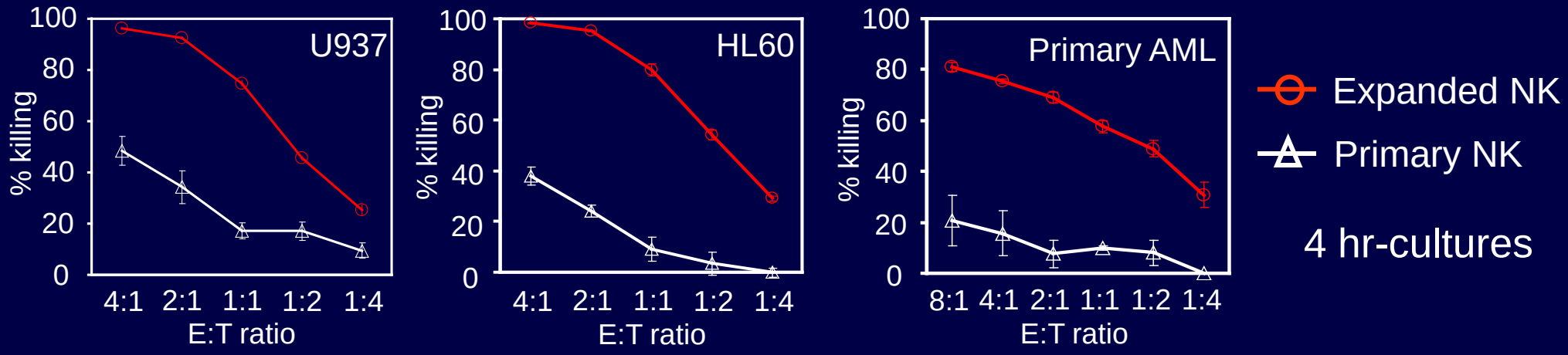
"K562-mb15-41BBL"

NK Activation and Expansion System

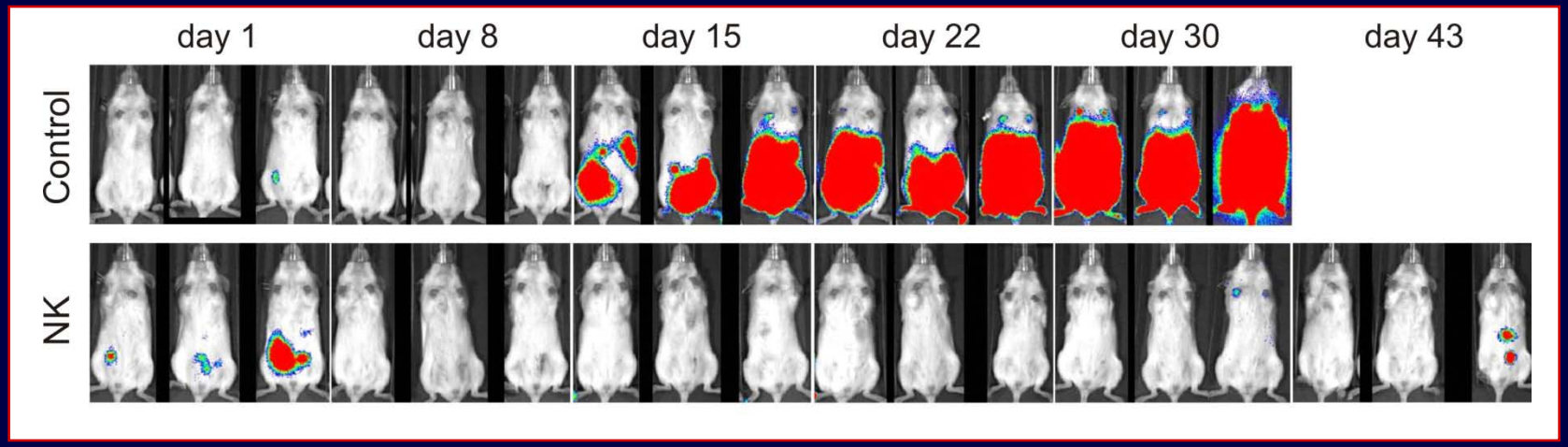


Expanded NK Cells are More Powerful Than Primary NK Cells Against AML

In vitro

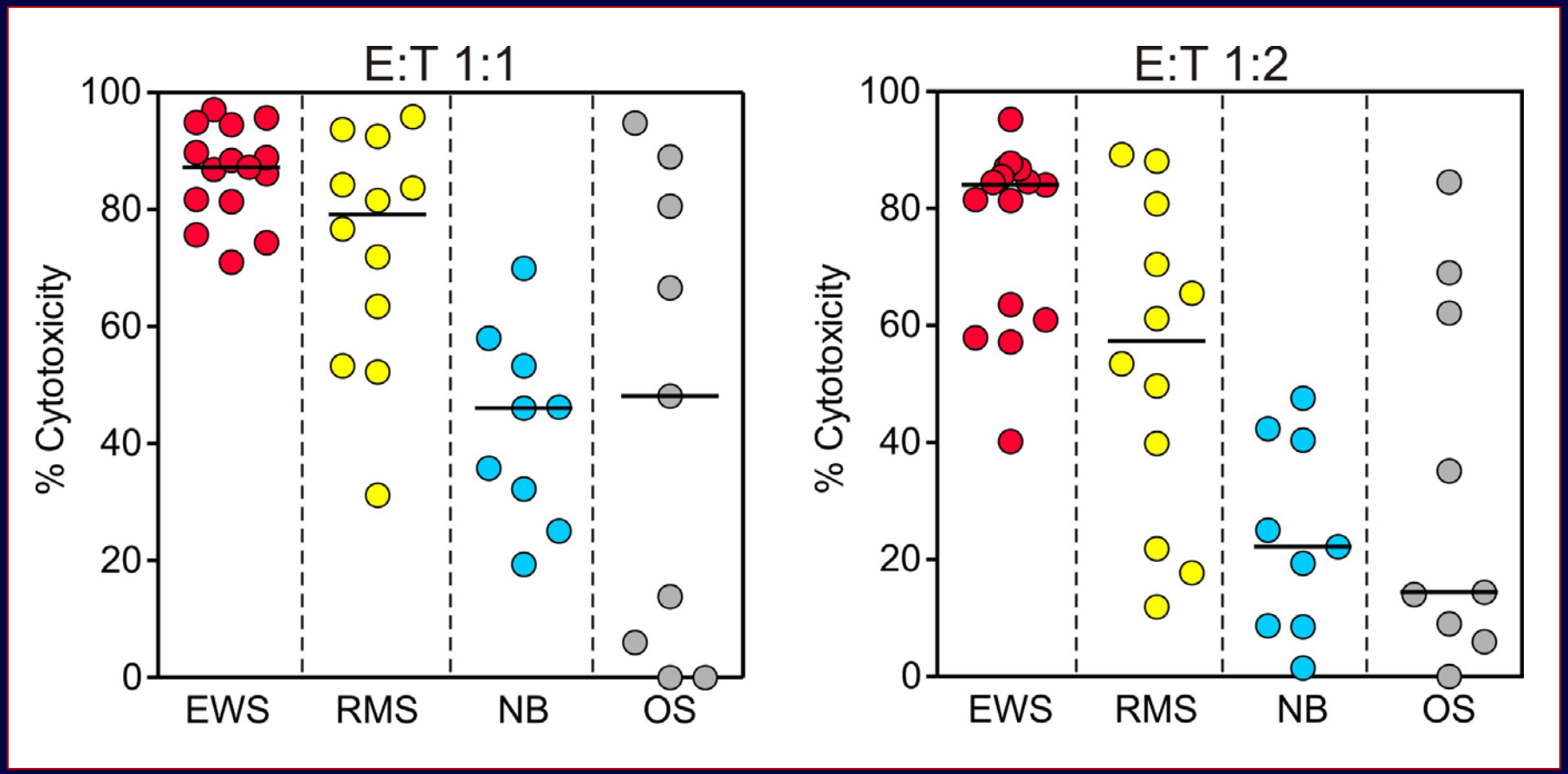


In vivo



Cancer Res 2009

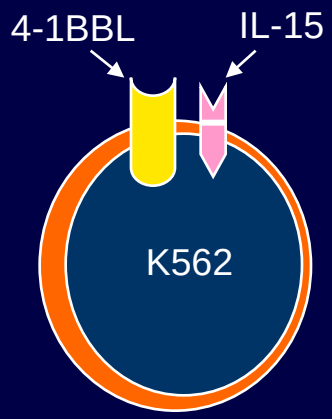
Relative Sensitivity of Childhood Solid Tumors to NK Cell Cytotoxicity



EWS: Ewing sarcoma; RMS: Rhabdomyosarcoma; NB: Neuroblastoma; OS: Osteosarcoma

Clin Cancer Res 2010

Production of Clinical Grade Reagents



K562-mb15-41BBL

Large-scale cultures
Median NK cell expansion after 7 days of culture:
91.5 fold (range, 33-141; n = 12)

NKEXP

- Relapsed/refractory AML, T-ALL/LL, CML, JMML, Ewing sarcoma and rhabdomyosarcoma
- Leukapheresis of haploidentical donor; 7-day NK expansion with irradiated K562-mb15-41BBL cells + IL-2; T-cell depletion
- NK cell infusion: 1 x 10^6/kg, 1 x 10^7/kg and 5 x 10^7/kg; 3 patients in each group
- Cyclophosphamide 60 mg/kg; Fludarabine 25 mg/m^2/day x 5
  - IL-2: 1 million units/m^2 3x/wk x 2wks

NKEXP

- 3 patients treated (2 AML, 1 Ewing sarcoma); 1 patient ongoing (rhabdomyosarcoma)

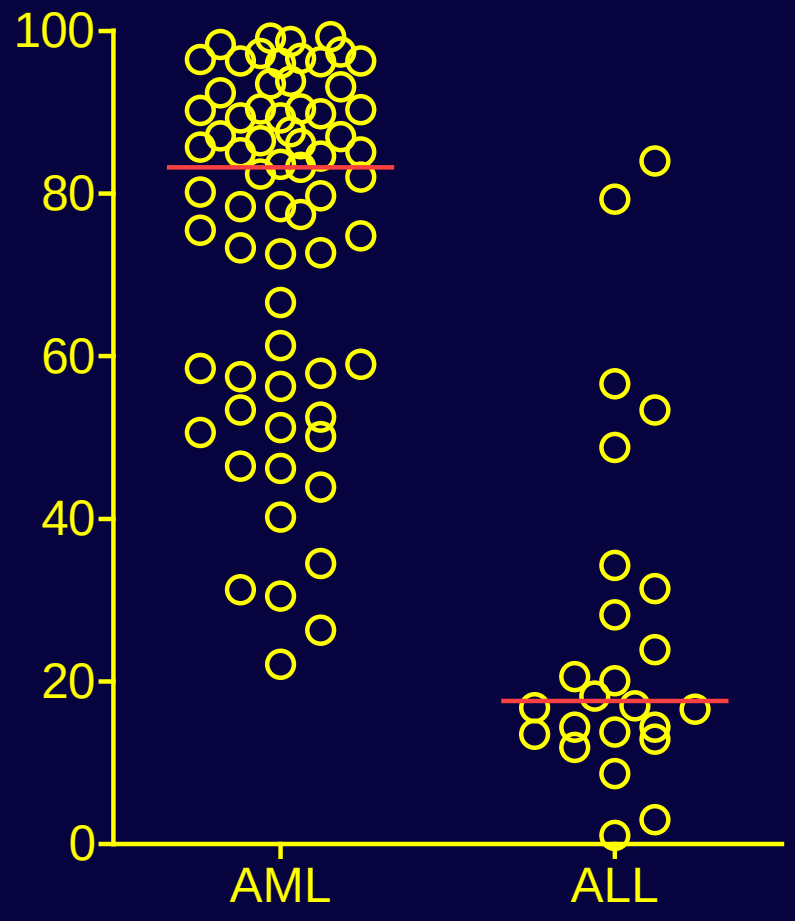
- NK cell expansion after T-cell depletion

  Patient 1: 8.7×10^9
  Patient 2: 5.0×10^9
  Patient 3: 3.3×10^9

- Persistence of donor NK cells

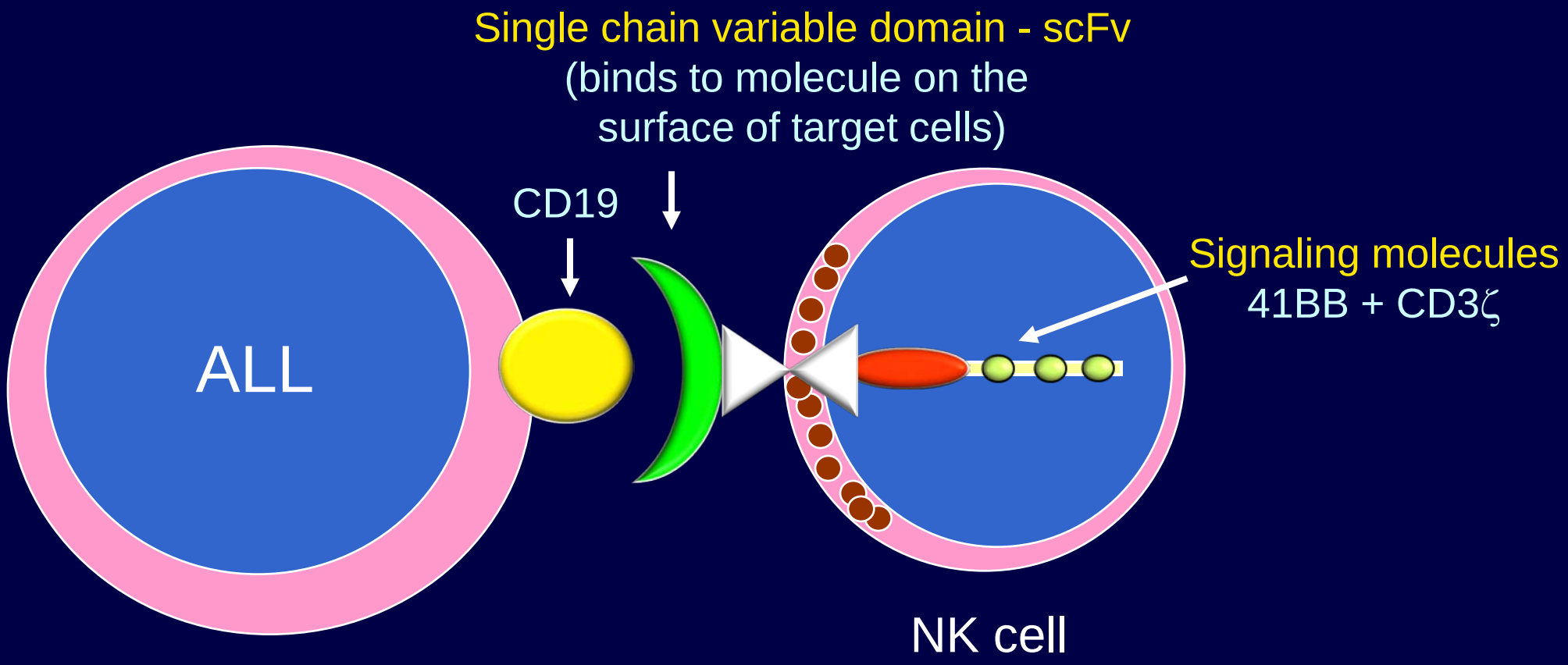
  Patient 1: day 15
  Patient 2: day 5
  Patient 3: day 12

Relative Sensitivity to NK Cell Cytotoxicity

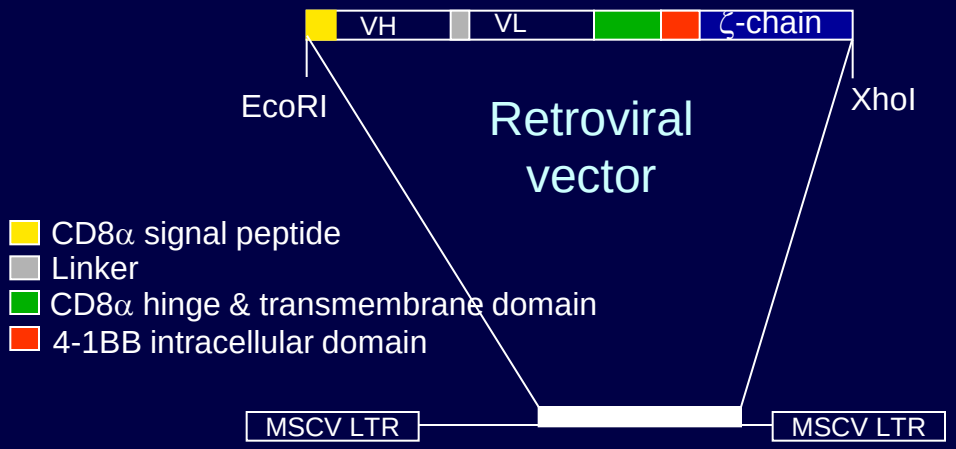


E:T 4:1
4 hrs

Redirecting the Specificity of NK Cells



Production of Clinical Grade Reagents



anti-CD19-BB-ζ

Large-scale transduction:
% NK cells with surface anti-CD19-BB-ζ: ~50%

NKCD19

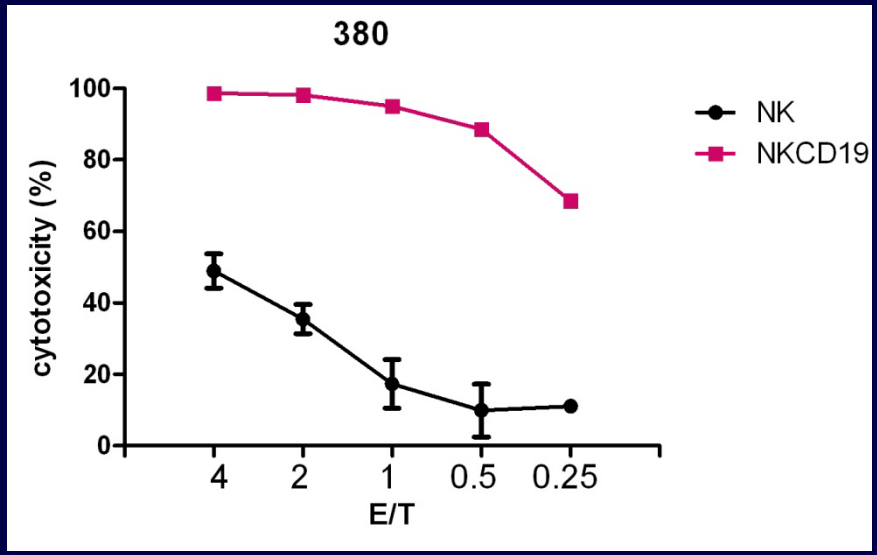
- Relapsed/refractory B-lineage ALL
- Leukapheresis from haploidentical donor; 7-day NK expansion with irradiated K562-mb15-41BBL cells + IL-2
- T-cell depletion and transduction with anti-CD19-BB-ζ
- NK cell infusion: 1 x 10^6/kg, 1 x 10^7/kg and 5 x 10^7/kg; 3 patients in each group
- Cyclophosphamide 60 mg/kg; Fludarabine 25 mg/m^2/day x 5
  - IL-2: 1 million units/m^2 3x/wk x 2wks

NKCD19

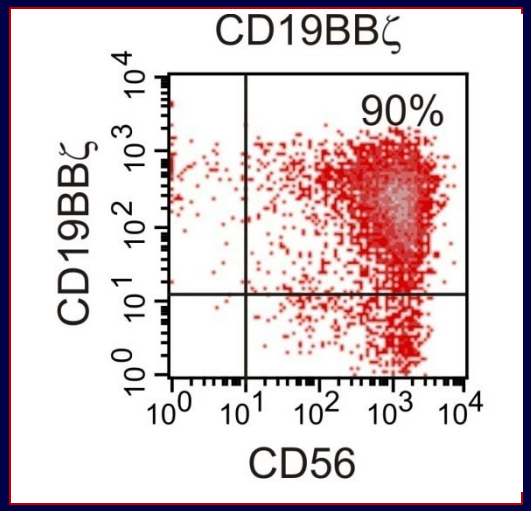
- 1 patient treated (B-lineage ALL)
- NK cell expansion after T-cell depletion

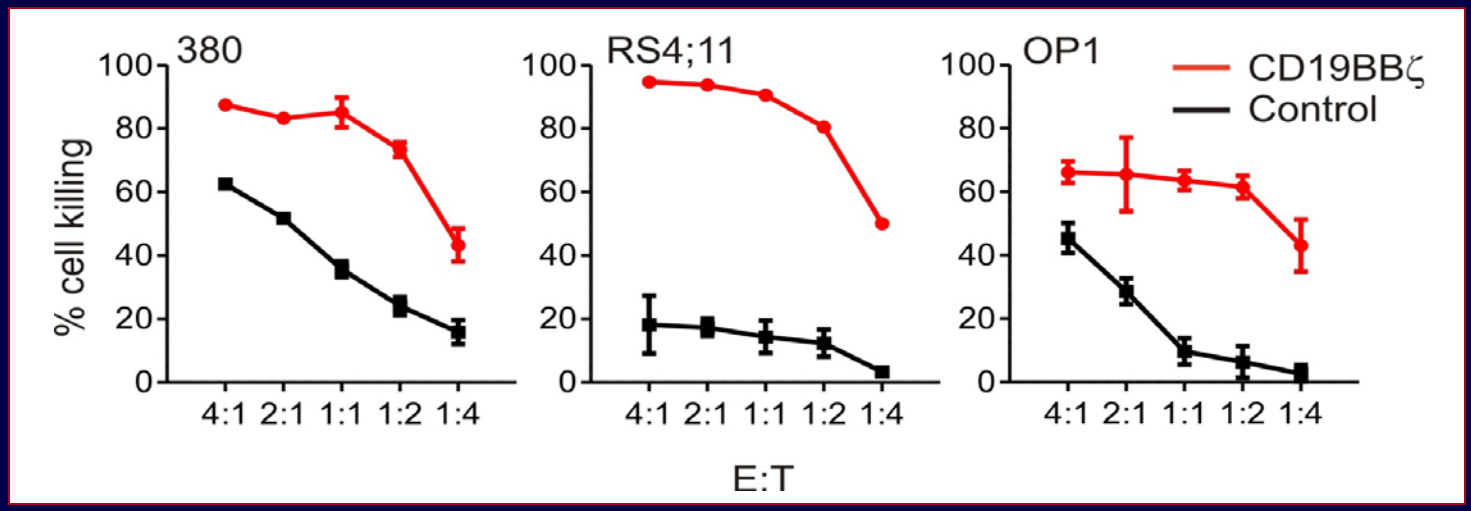
  Patient 1: 10.0×10^9
- NK cells expressing anti-CD19-BB-ζ receptor

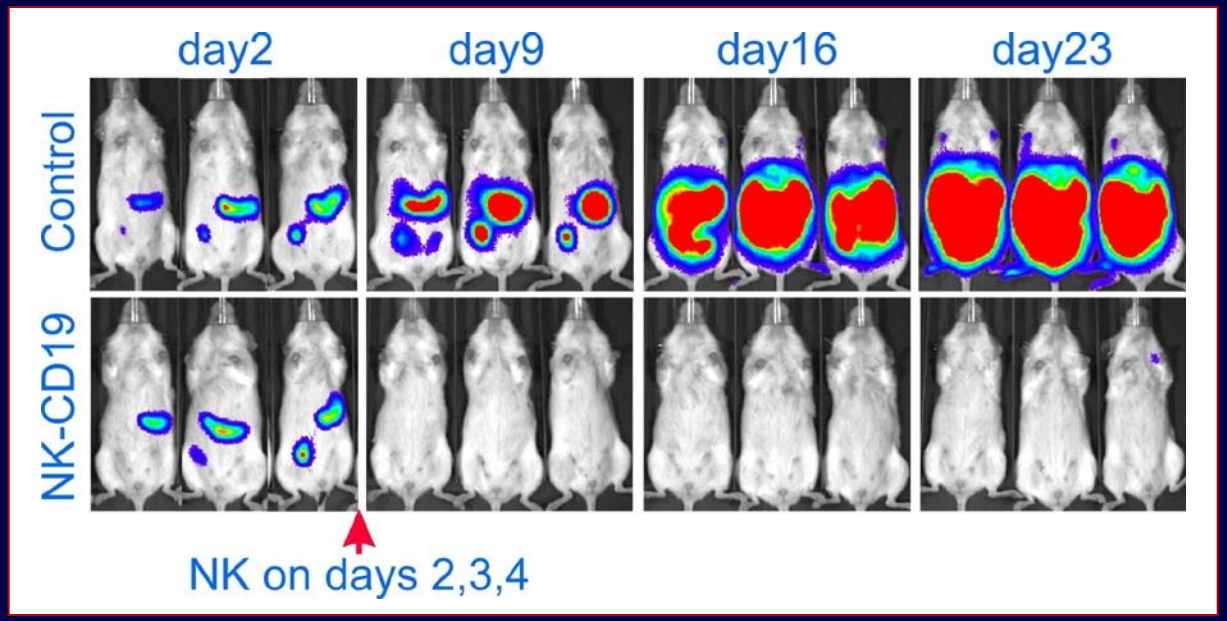
  53%



Expression of Anti-CD19-BB-ζ mRNA by Electroporation in NK Cells

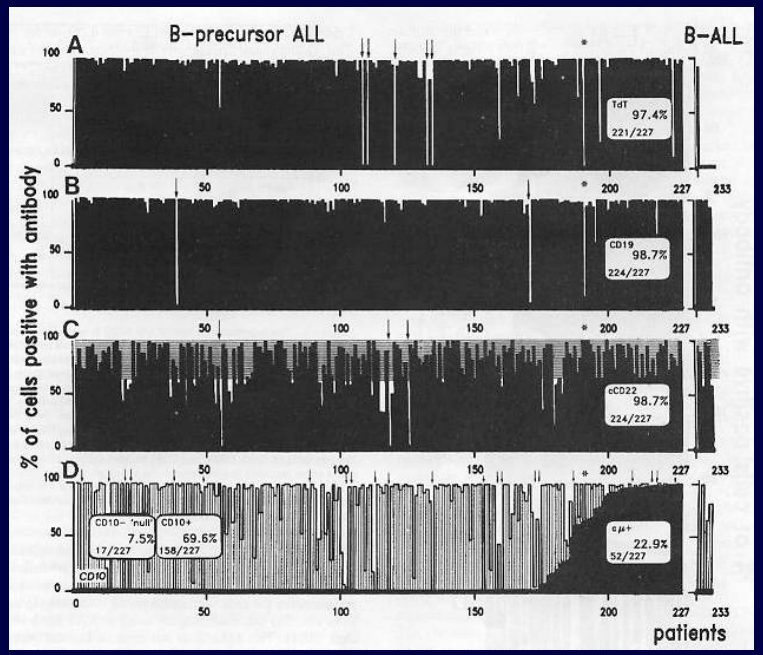






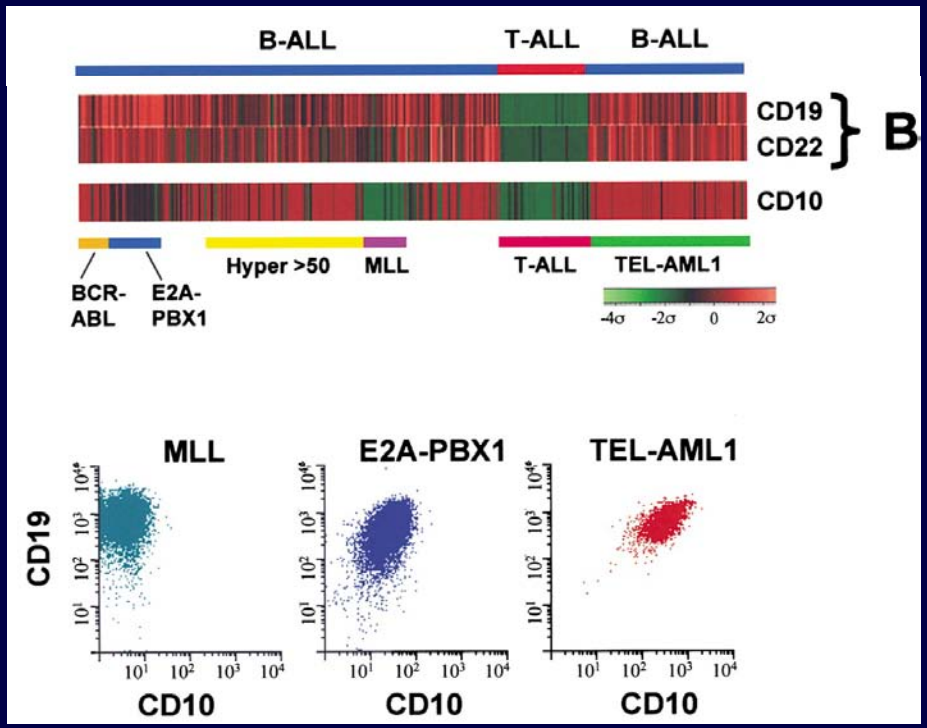
CD19 Expression in Acute Lymphoblastic Leukemia

224/227 (98.7%) samples of B-lineage ALL are CD19+



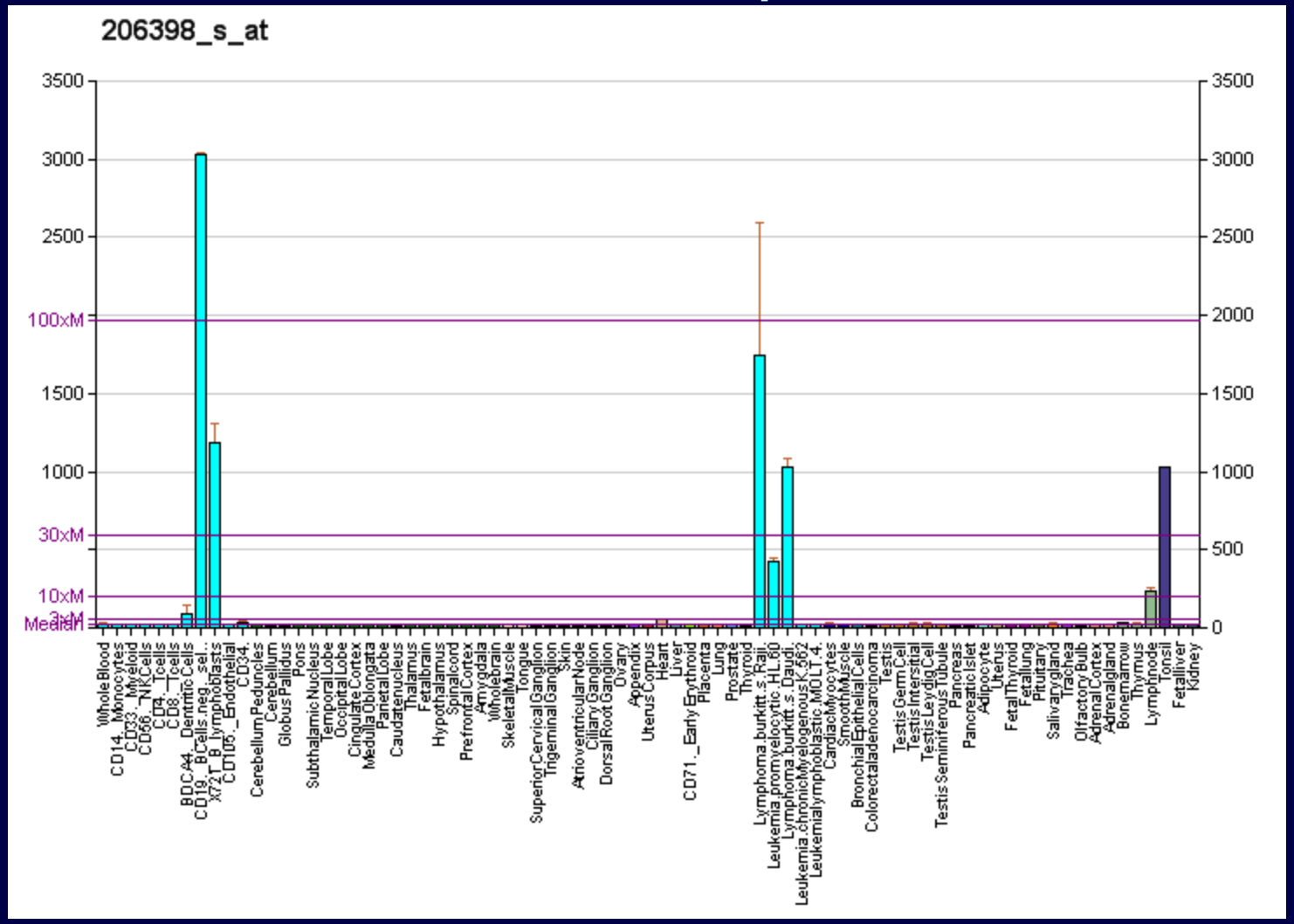
Janossy et al Leukemia 1989

All 327 samples of B-lineage ALL express CD19 mRNA



Yeoh et al Cancer Cell 2002

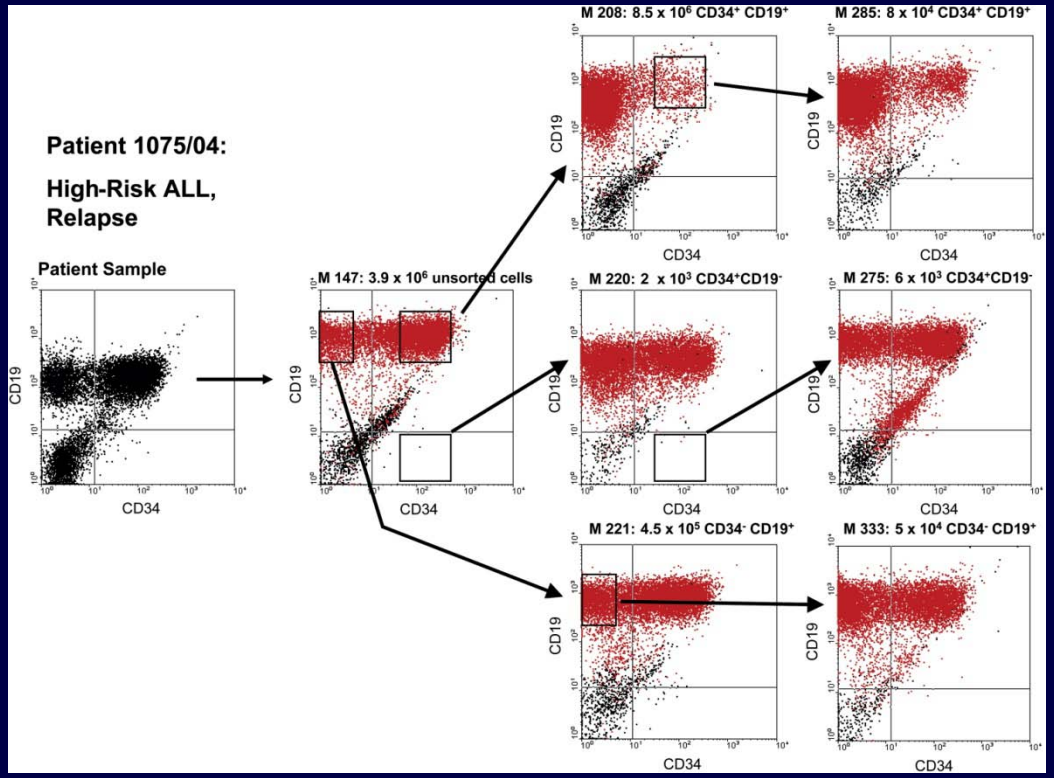
CD19 Gene Expression



http://commons.wikimedia.org/wiki/File:PBB_GE_CD19_206398_s_at_fs.png - Created by AndrewGNF from Su AI et al (2004). "A gene atlas of the mouse and human protein-encoding transcriptomes". *Proc. Natl. Acad. Sci. U.S.A.* **101** (16): 6062–7

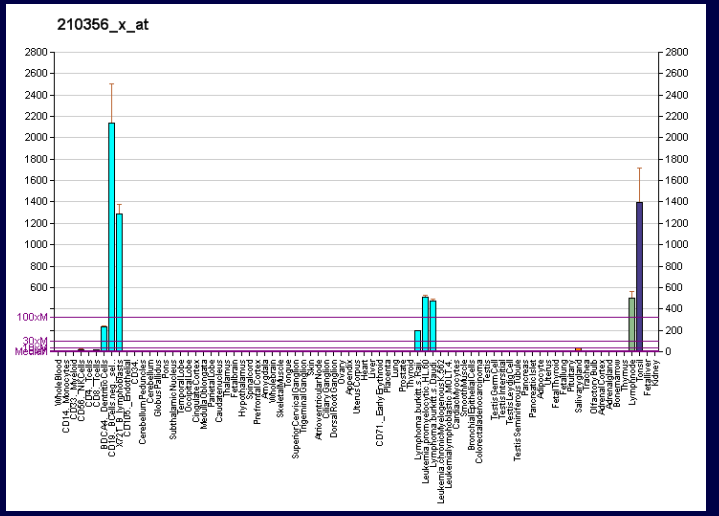
CD19 Expression in Acute Lymphoblastic Leukemia-Initiating Cells

Transplanted Subpopulation	Transplanted Mice	Engrafted Mice
Standard-risk ALL (n = 3)		
$CD34^{+}CD19^{-}$	2	0 (0%)
$CD34^{+}CD19^{+}$	5	4 (80.0%)
$CD34^{-}CD19^{+}$	18	11 (61.1%)
$CD19^{+}CD20^{-/low}$	3	1 (33.3%)
$CD19^{+}CD20^{high}$	2	1 (50.0%)
High-risk ALL (n = 7)		
$CD34^{+}CD19^{-}$	37	15 (40.5%)
$CD34^{+}CD19^{+}$	80	58 (72.5%)
$CD34^{-}CD19^{+}$	41	27 (65.9%)
$CD19^{+}CD20^{-/low}$	4	3 (75.0%)
$CD19^{+}CD20^{high}$	4	2 (50.0%)



Le Viseur et al Cancer Cell 2008

CD20 Expression in Acute Lymphoblastic Leukemia

- Childhood ALL: 169 (48%) of 353 children with B-lineage ALL had >20% CD20+ cells (Jeha et al. Blood 2006
  - No prognostic significance

- Adult ALL: 120 (47%) of 253 adults with B-lineage ALL had >20% CD20+ cells (Thomas et al Blood 2009)
  - Unfavorable prognosis