RAC Review (Dec 4-5 2012)

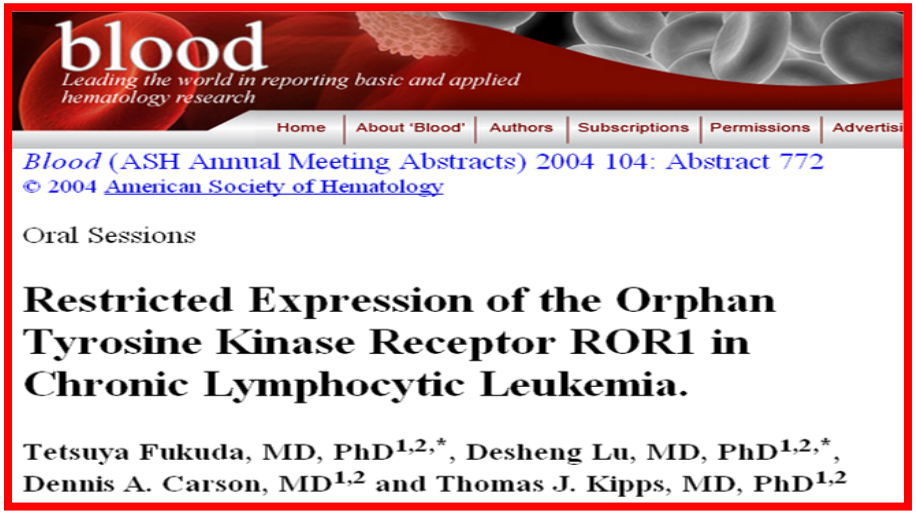
A study to infuse ROR1-specific Autologous T cells for patients with CLL

Presenters
PIs: Dr. William Wierda and Dr. Laurence JN Cooper, MDACC
Co-PI: Dr. Thomas J.Kipps, UC San Diego

Targeting ROR1

- Thomas Kipps MD,PhD
- William Wierda MD,PhD
- Laurence Cooper MD,PhD

ROR1 Is Expressed In CLL, But Not Normal Adult Tissues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Blood (ASH Annual Meeting Abstracts) 2004 104: Abstract 772
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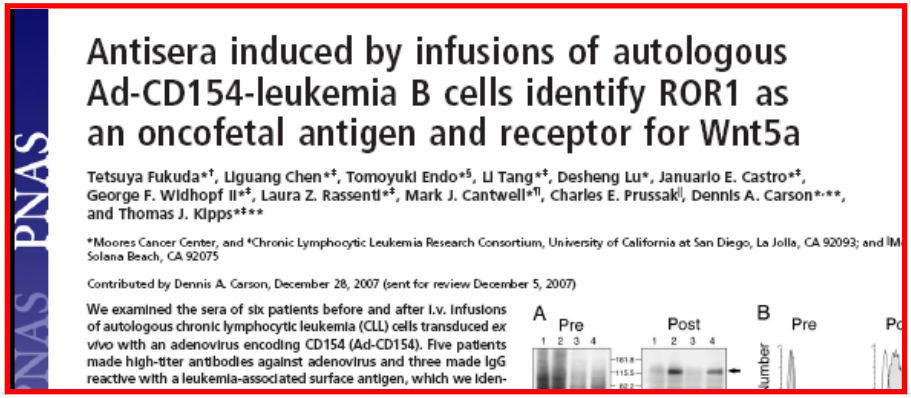
Oral Sessions

Restricted Expression of the Orphan Tyrosine Kinase Receptor ROR1 in Chronic Lymphocytic Leukemia.

Tetsuya Fukuda, MD, PhD[1,2,*], Desheng Lu, MD, PhD[1,2,*], Dennis A. Carson, MD[1,2] and Thomas J. Kipps, MD, PhD[1,2]

2004 ASH Annual Meeting. Blood, 2004,104 (11): 772


PNAS

Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a

Tetsuya Fukuda*†, Liguang Chen*‡, Tomoyuki Endo*§, Li Tang*‡, Desheng Lu*, Januario E. Castro*‡, George F. Widhopf II*‡, Laura Z. Rassenti*‡, Mark J. Cantwell*¶, Charles E. Prussak‖, Dennis A. Carson*,**, and Thomas J. Kipps*‡**

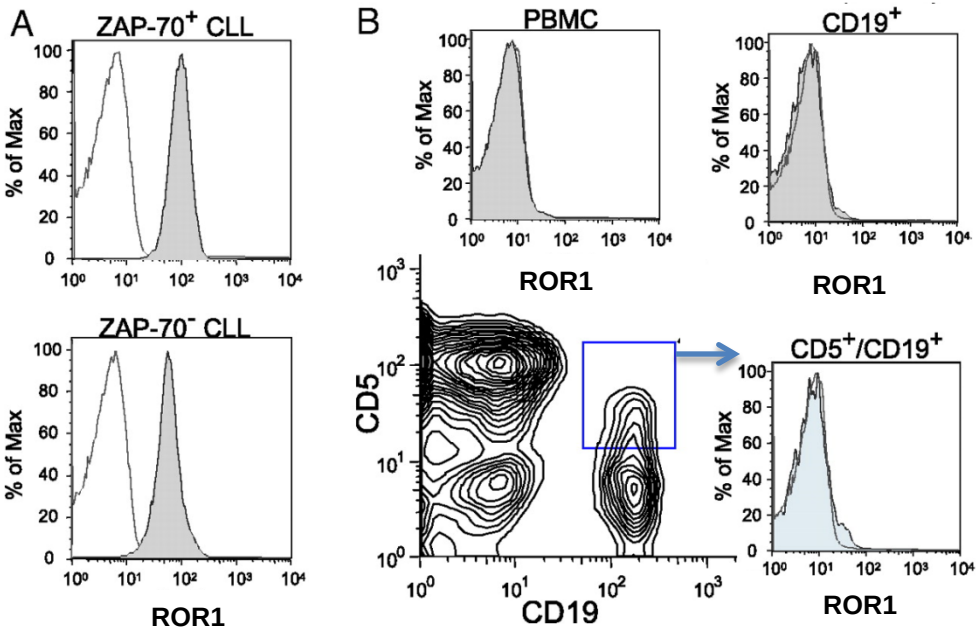
*Moores Cancer Center, and *Chronic Lymphocytic Leukemia Research Consortium, University of California at San Diego, La Jolla, CA 92093; and ‖M Solana Beach, CA 92075

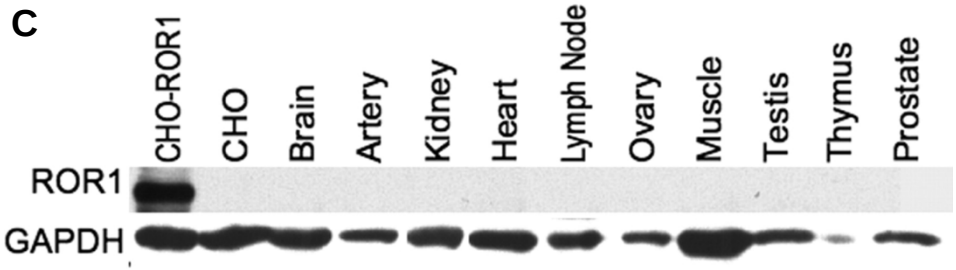
Contributed by Dennis A. Carson, December 28, 2007 (sent for review December 5, 2007)

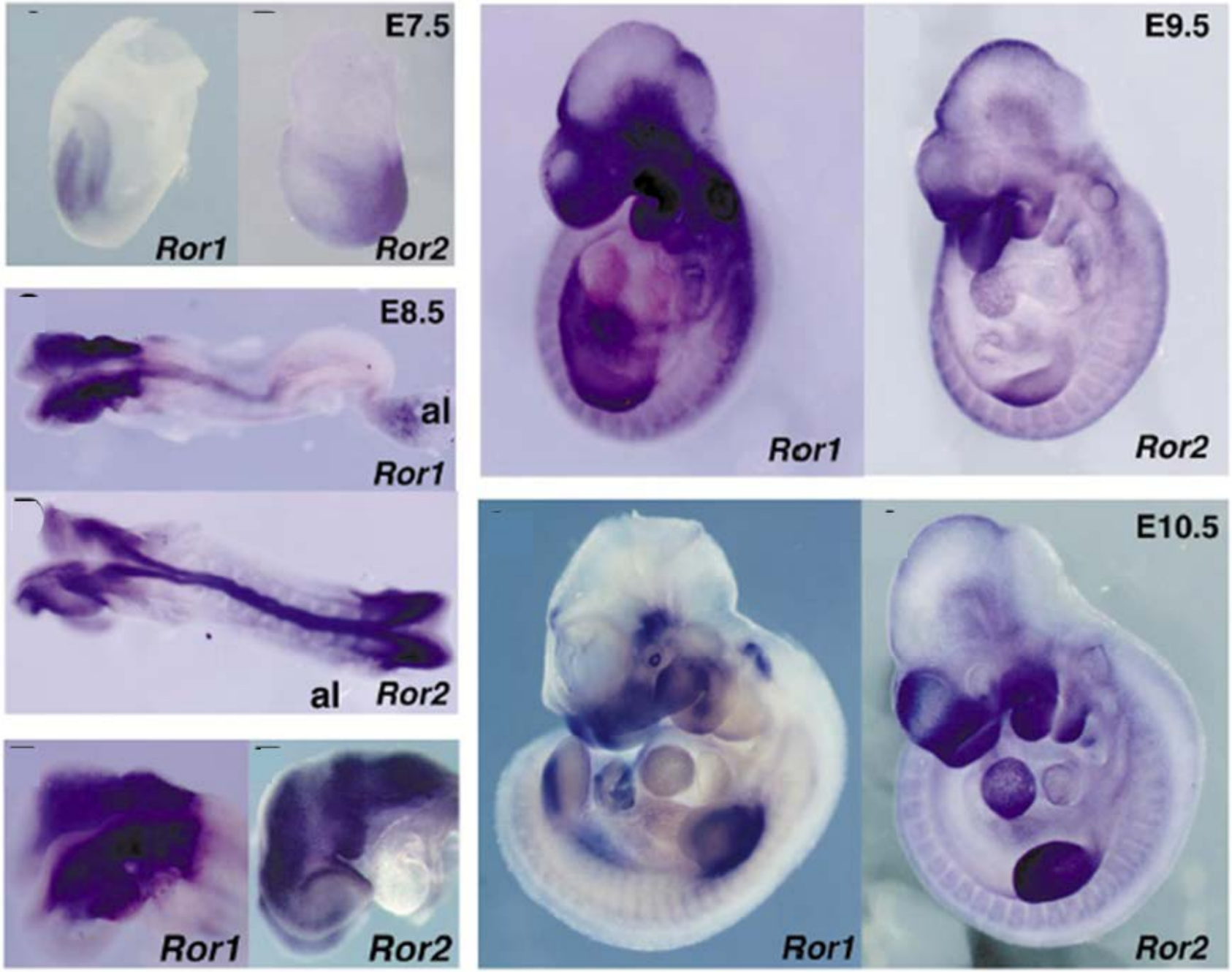
We examined the sera of six patients before and after i.v. infusions of autologous chronic lymphocytic leukemia (CLL) cells transduced *ex vivo* with an adenovirus encoding CD154 (Ad-CD154). Five patients made high-titer antibodies against adenovirus and three made IgG reactive with a leukemia-associated surface antigen, which we iden-

A Pre Post B Pre Pc

2008, PNAS, 105(8):3047-52

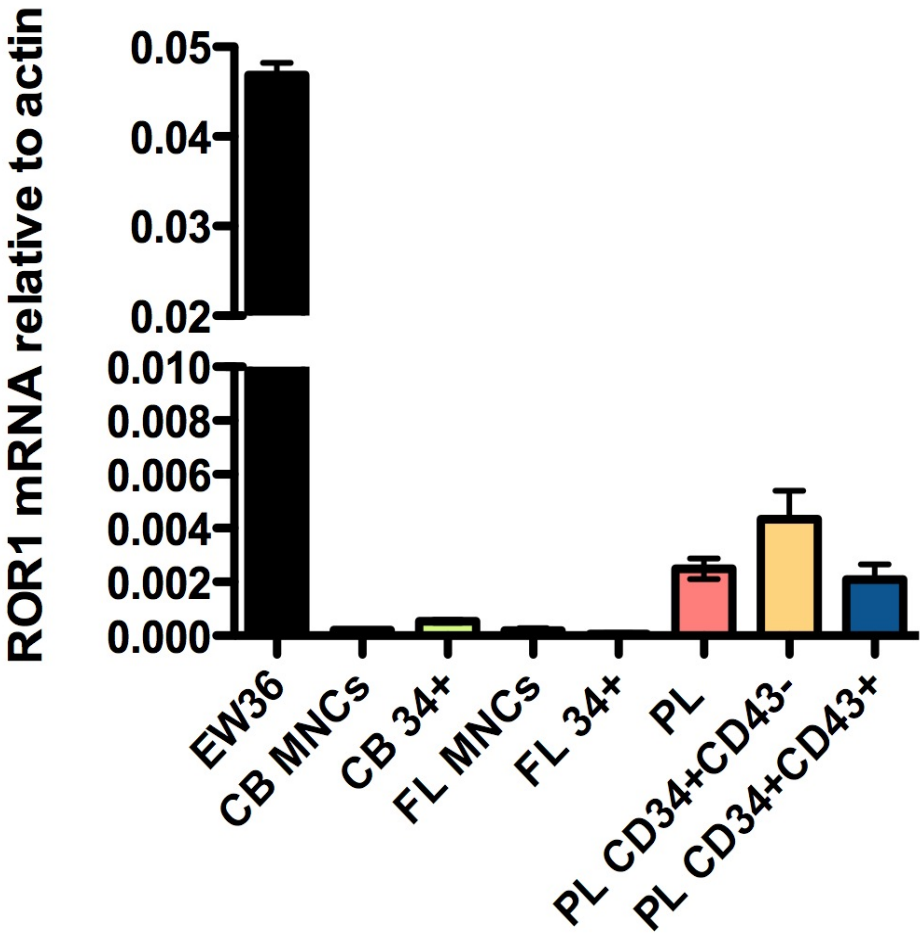




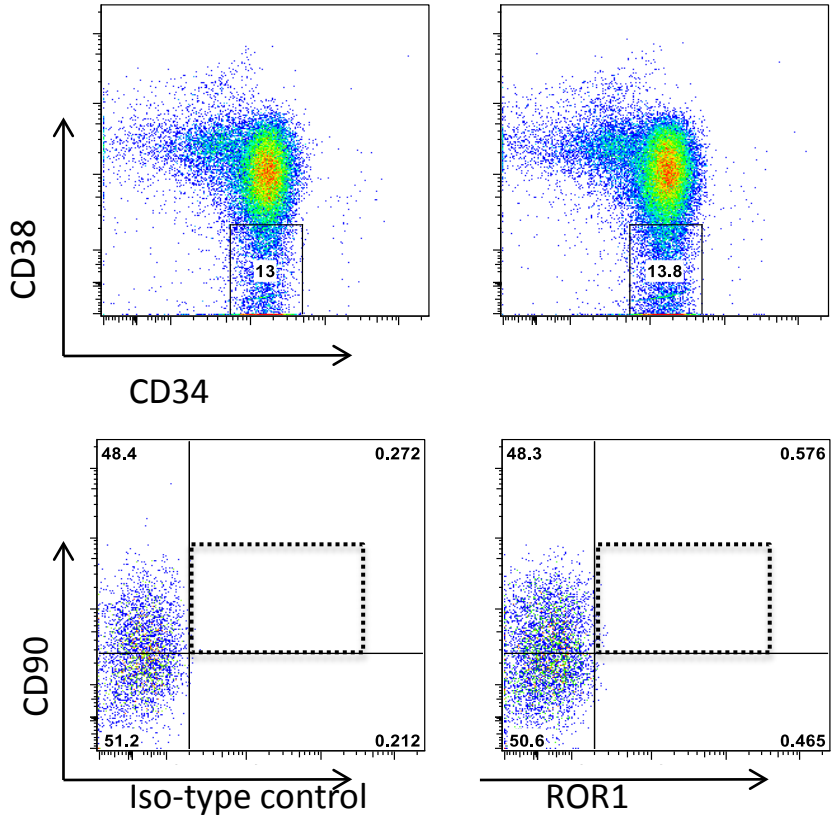


Matsuda, T. et al, Mech Dev 105-6, 2001

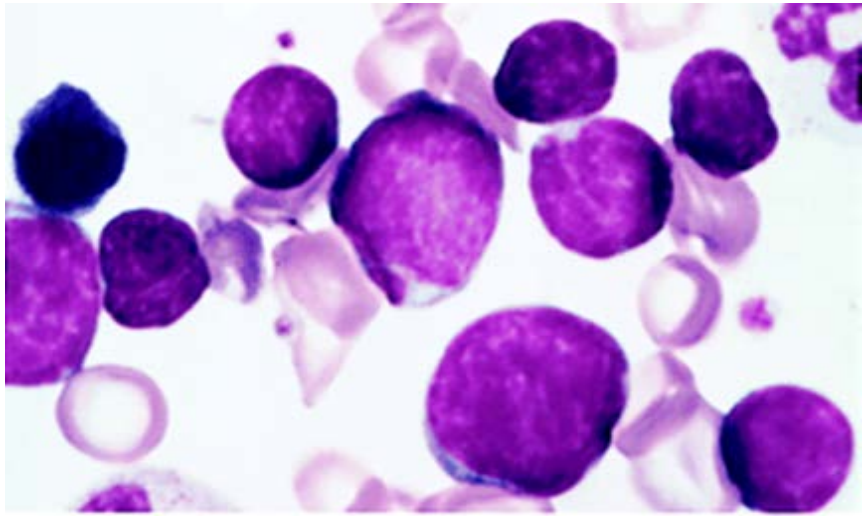
ROR1 Is Not Expressed In Cord Blood Or Fetal Liver Hematopoietic Stem And Progenitor Cells

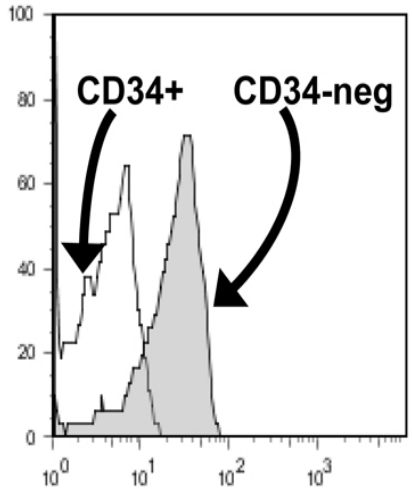


Cord Blood Mononuclear Cells



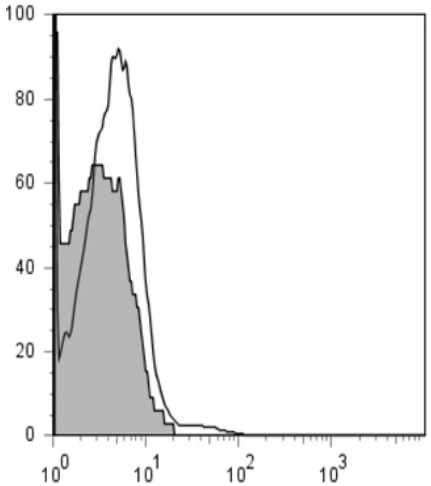
CD10^{+}/CD19^{+}/CD34^{-}/TdT^{-} Hematogones Express ROR1





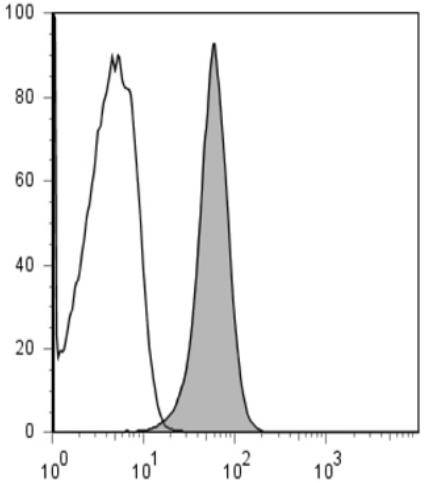
Blood B-lymphocytes

(CD5-neg, CD19+)



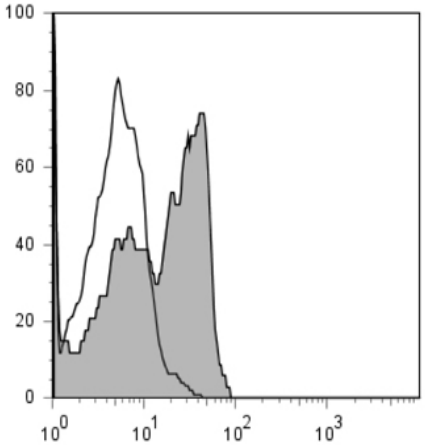
CLL

(CD5+, CD10-neg, CD19+)

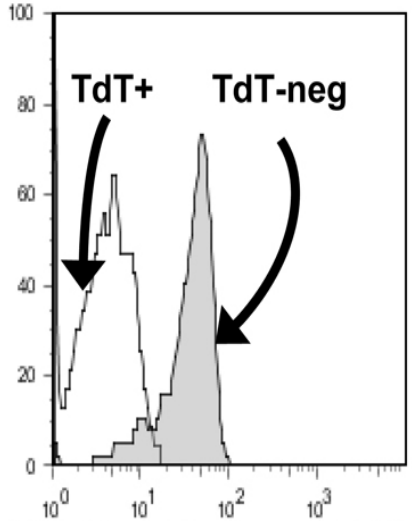


Hematogones

(CD45-dim, CD10+, CD19+)



FL4-H: 4A5-Alexa 647 (ROR1)

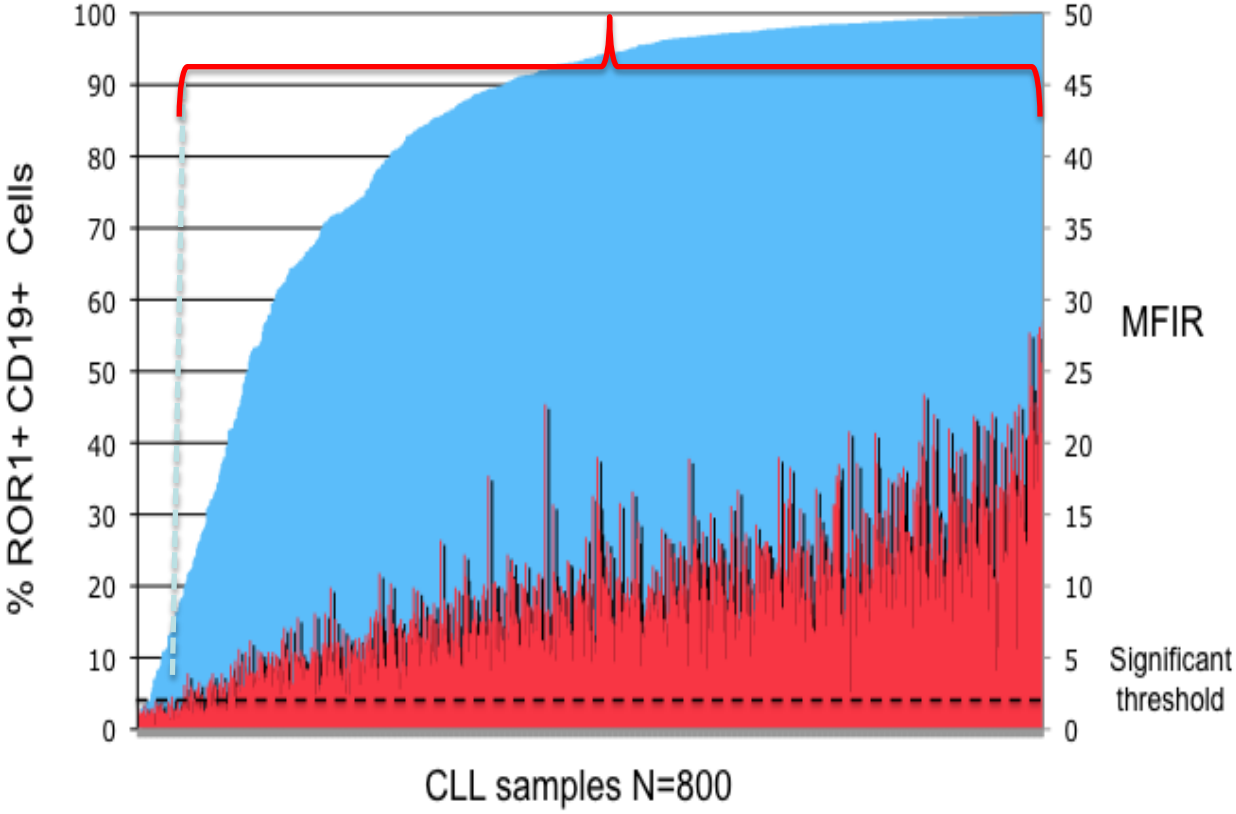


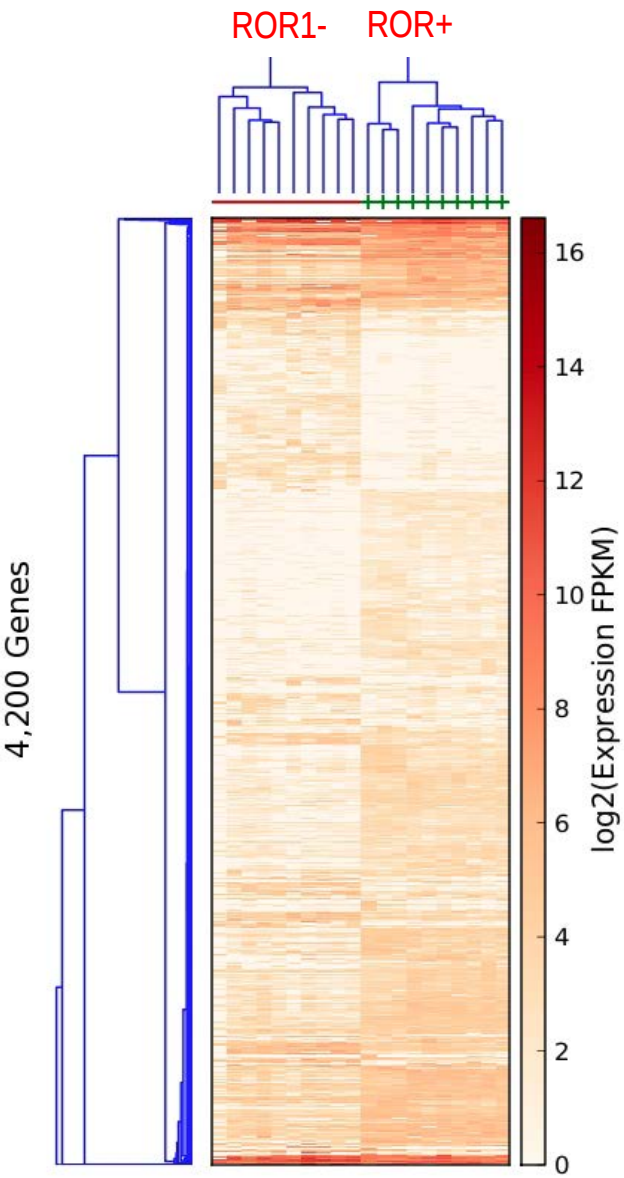
Broome, E. et al, Leuk Res. 35(10):1390–1394, 2011.

Expression Of ROR1 In CLL

The CLL cells of >94% of patients express ROR1 protein, which is not found on normal post-partum tissues

94% of Cases ROR1+ (N=800)

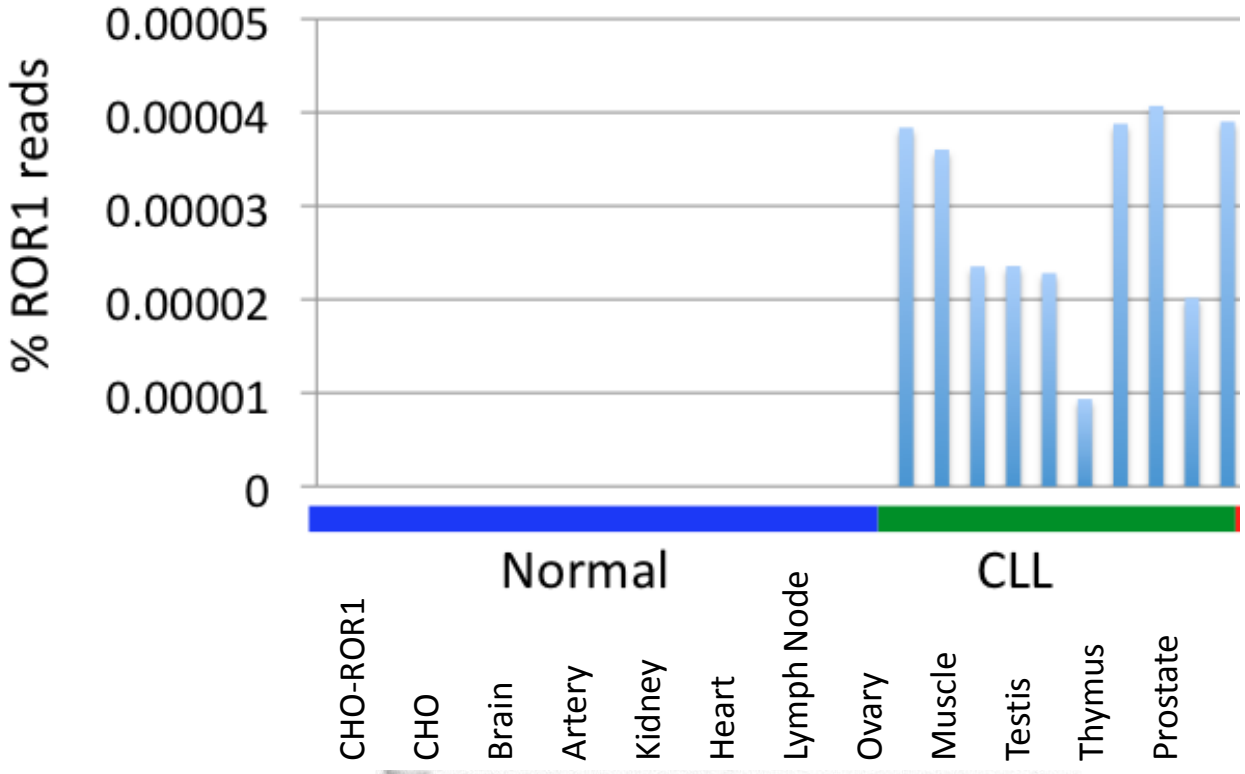


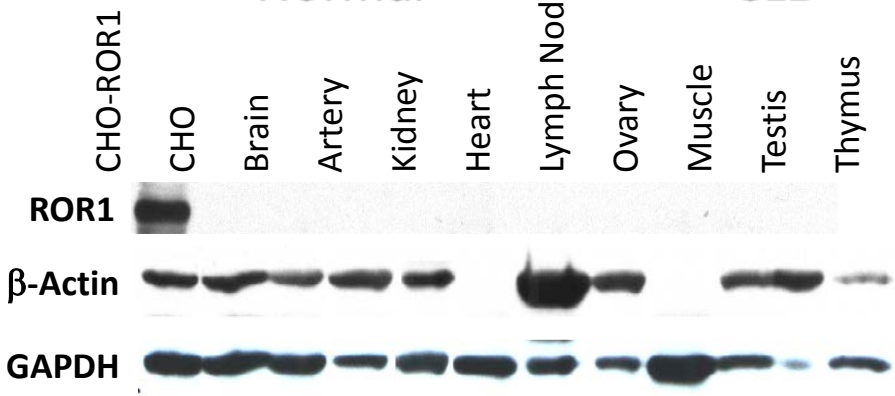


ROR1⁻ CLL has a distinct transcriptome signature

ROR1 Is A Cancer-Cell Specific Target

- Canadian Genome Center and CIRM Genomics Core performed paired-end RNA sequencing
- ROR1 mRNA was not observed in normal tissues



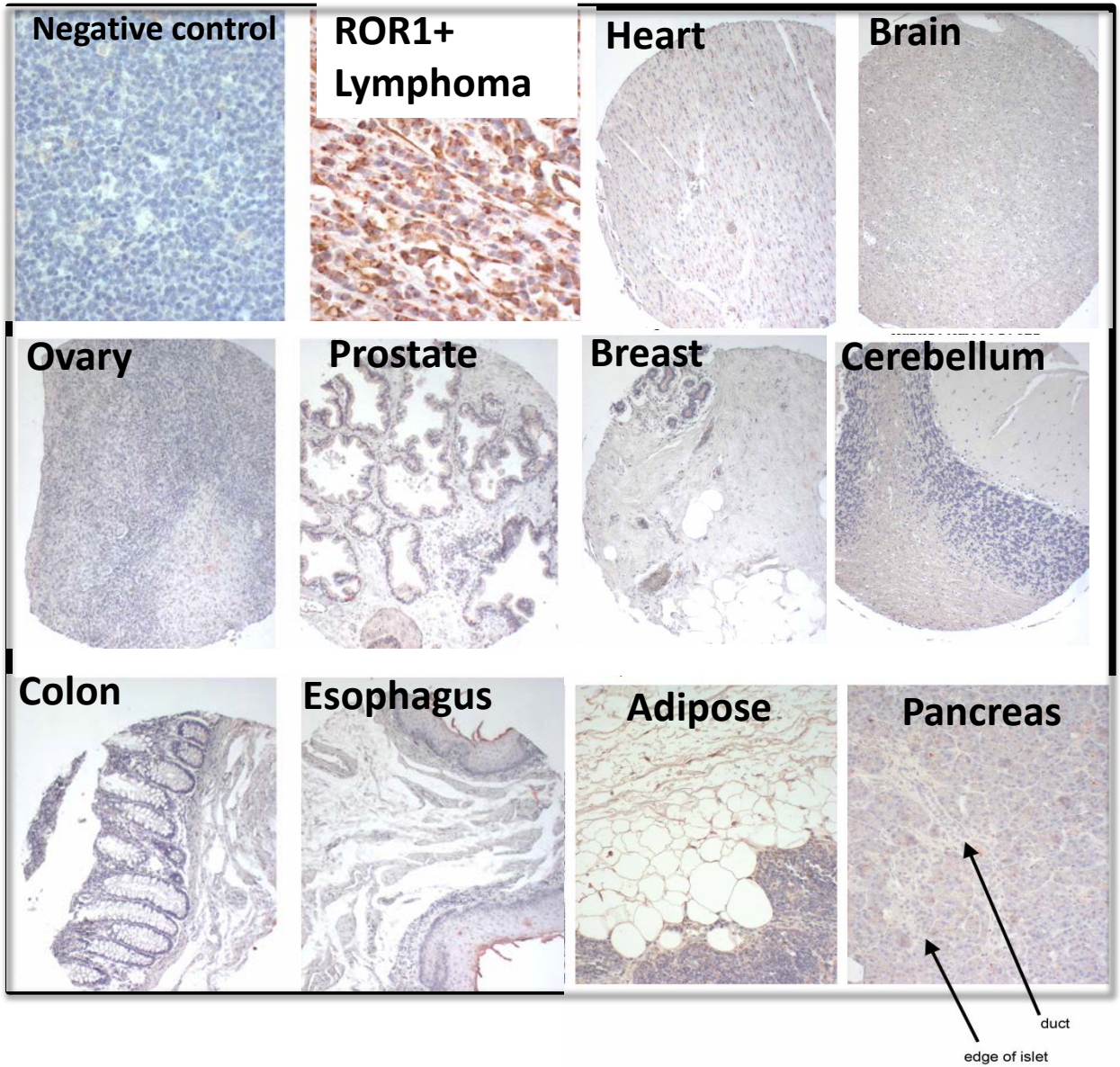


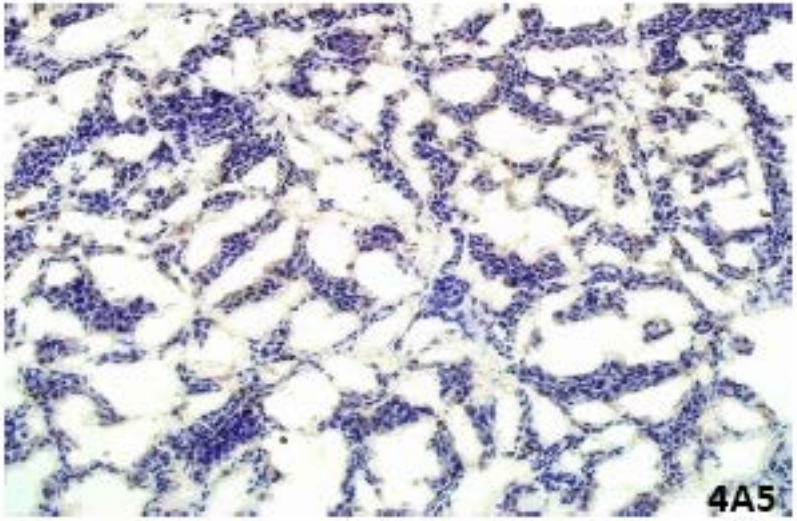
Normal tissues (RNAseq)

- adipose
- adrenal
- white blood cells
- skeletal muscle
- prostate
- lymph node
- lung
- brain
- breast
- colon
- kidney
- heart
- liver
- thyroid
- testes
- ovary

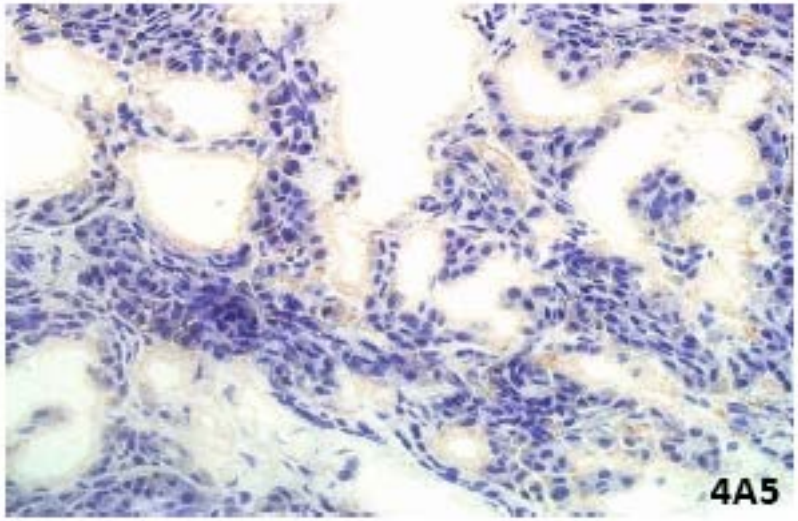
Specific Anti-ROR1 mAb Does Not Cross React With Normal Adult Tissues

Normal multi-tissue array (Biomax -FDA999)

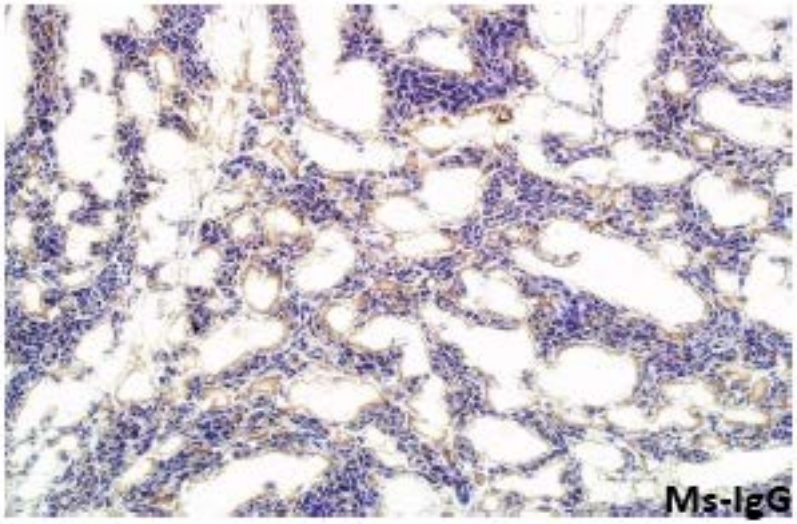




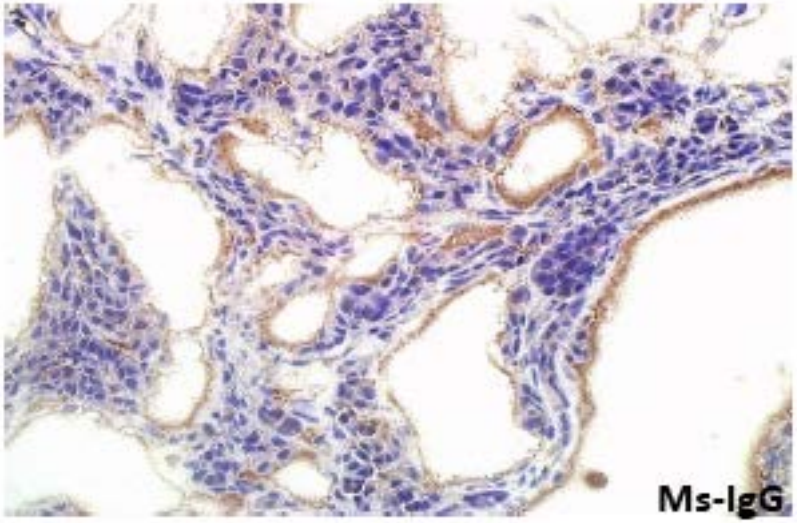
h-Pancreas(D3-0708004) mAb 4A5, 150µg/ml
Frozen tissue, x20, Score: 0



h-Pancreas(D3-0708004) mAb 4A5, 150µg/ml
Frozen tissue, x40, Score: 0



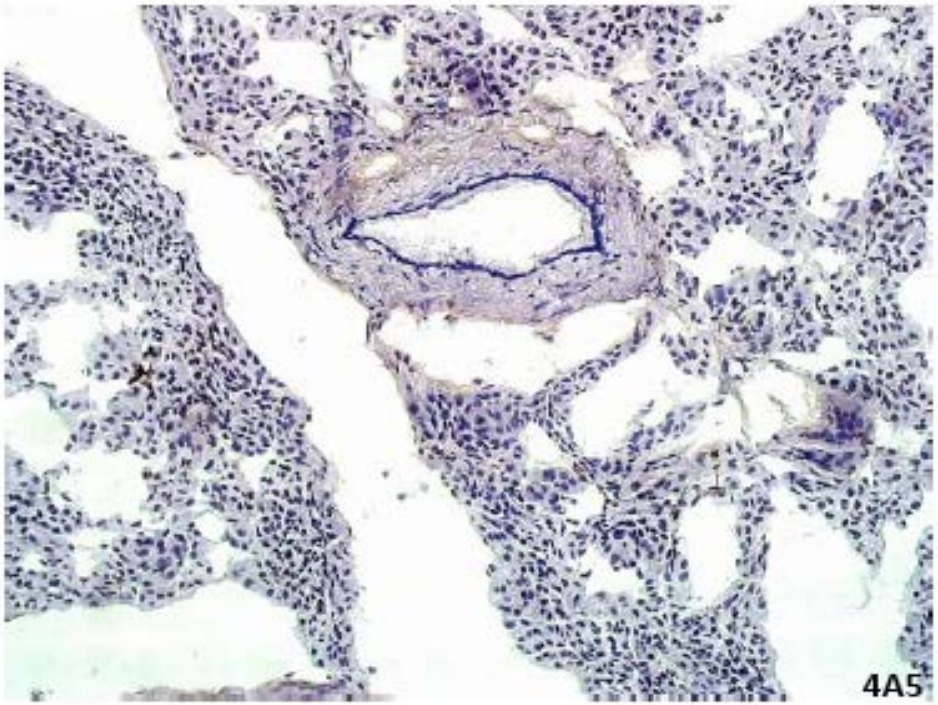
h-Pancreas(D3-0708004) Ms-IgG, 150µg/ml
Frozen tissue, x20, Score: 0



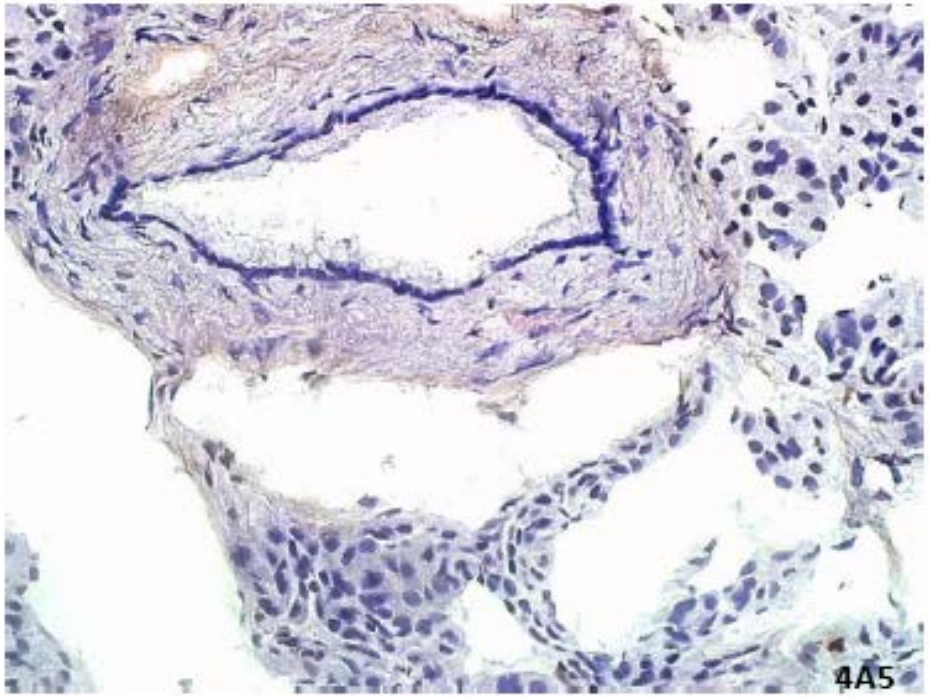
h-Pancreas(D3-0708004) Ms-IgG, 150µg/ml
Frozen tissue, x40, Score: 0

Alireza Ebrahimnejad, PhD, Comparative Biosciences Inc., Sunnyvale, CA 94085

4A5 Does Not React With Fresh-Frozen Pancreatic Tissue



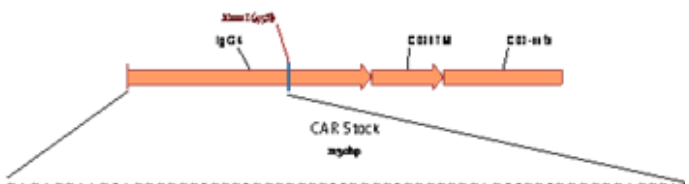
h-Pancreas(D3-0708004) mAb 4A5, 150µg/ml
Frozen tissue, x20, Score: 0



h-Pancreas(D3-0708004) mAb 4A5, 150µg/ml
Frozen tissue, x40, Score: 0

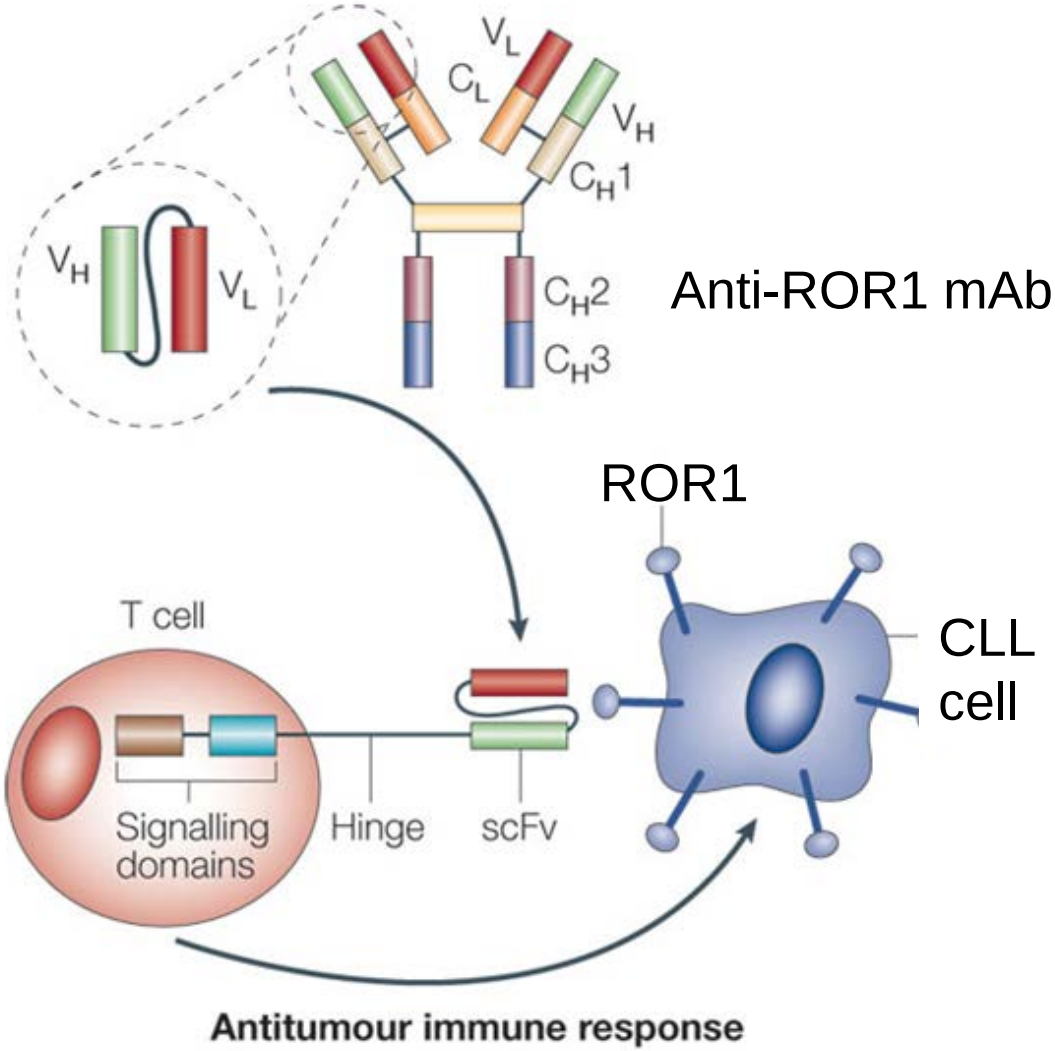
Alireza Ebrahimnejad, PhD, Comparative Biosciences Inc., Sunnyvale, CA 94085

Chimeric Anti-ROR1 T-cell Receptors

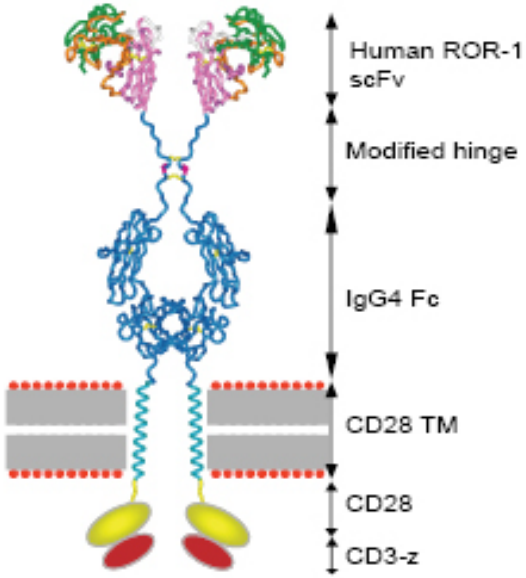


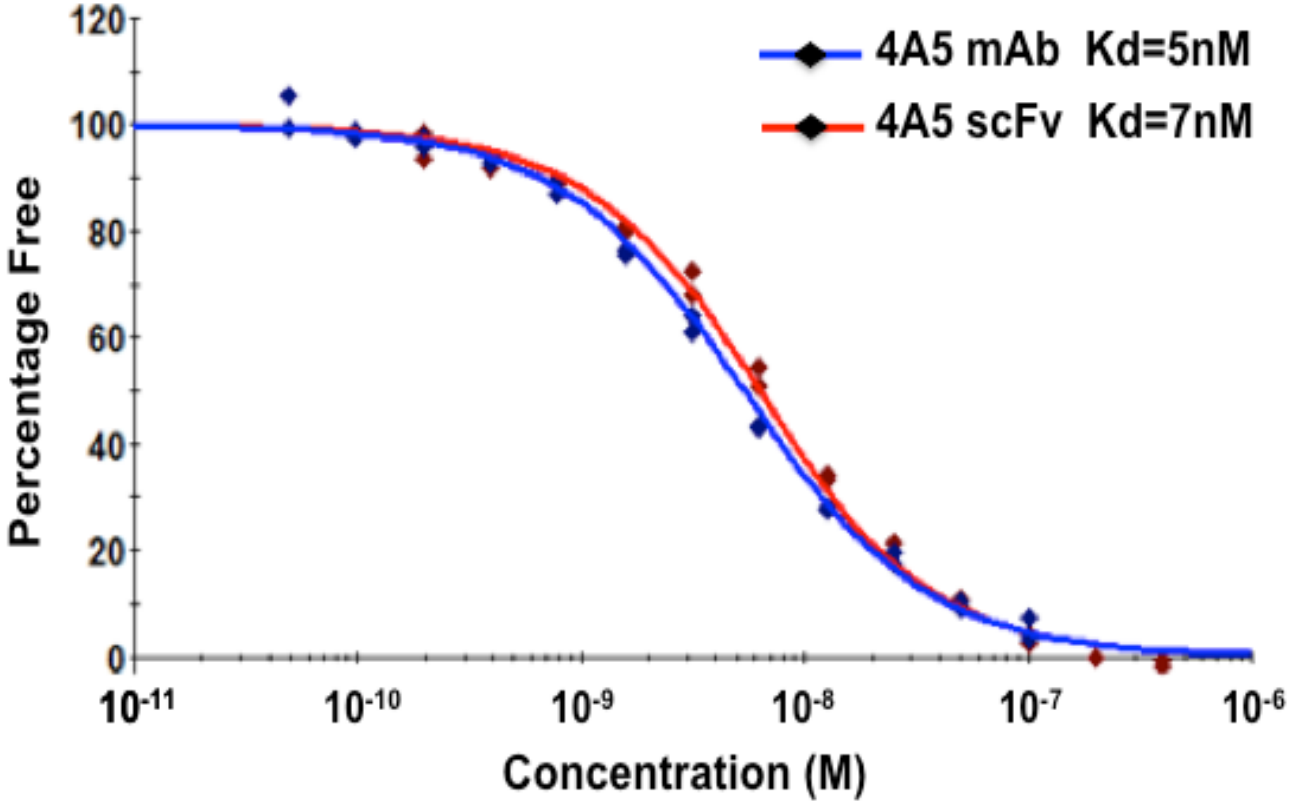
GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCAC
CGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTG
ACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAATAGCACCT
ACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAATACAA
GTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCCAA
GGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAAC
AATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTC; XmnI in yellow

A schematic for the cloning the ROR-1 scFv into the CAR is:



Affinity Of mAb & Single-chain Variable Region Fragment (scFv) For Recombinant ROR1





Comparison of Anti-ROR1 mAb and scFv

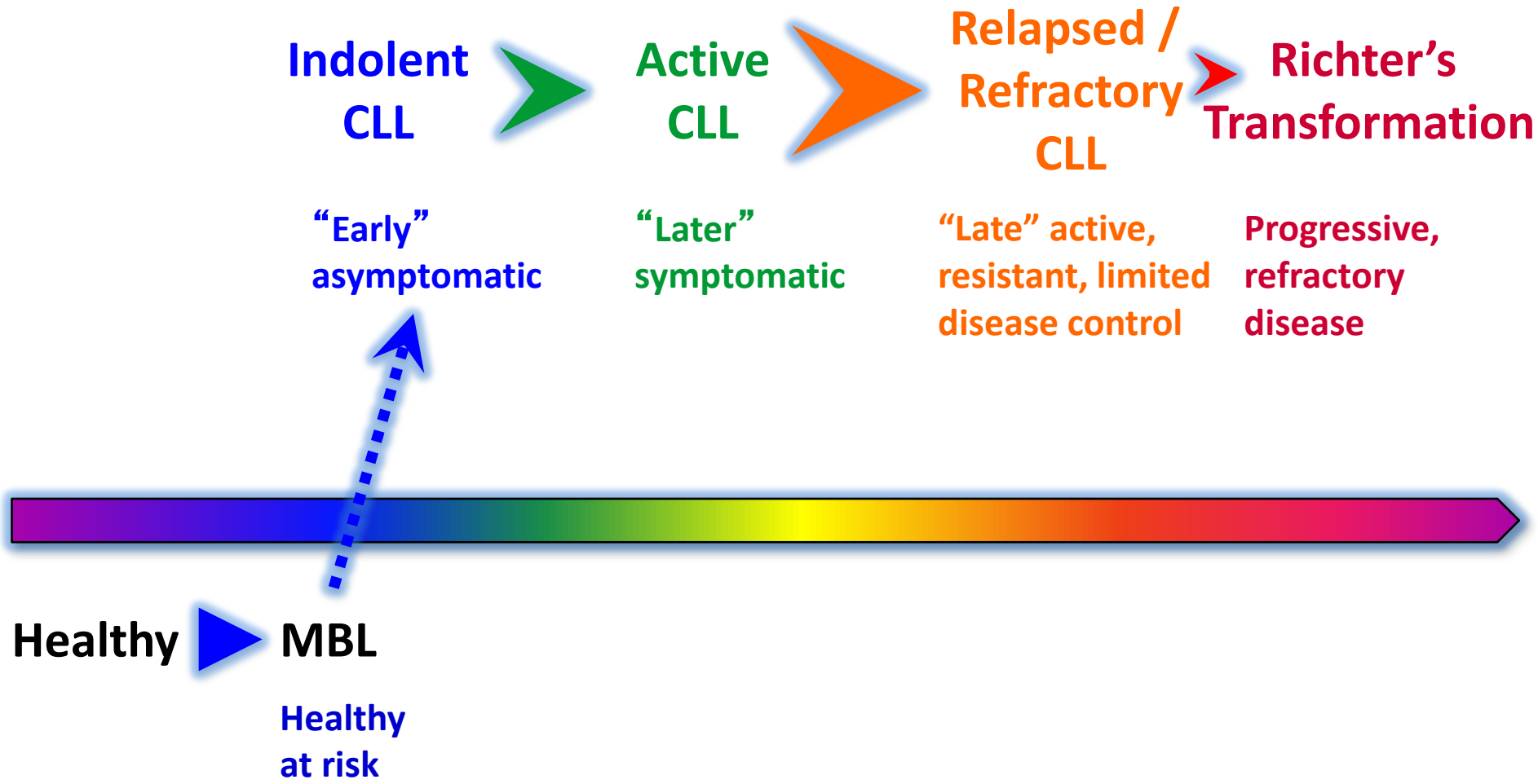
	Structure	Reactivity	Kd (10^{-9} M)	ROR1 Binding Region
4A5 mAb	Bivalent	Human	4.5	Ig Domain
4A5 scFv	Monovalent	Human	7.4	Ig Domain
2A2-IgG*	Bivalent	Human/mouse	0.8	Ig Domain
2A2-IgG*	Monovalent	Human/mouse	32.6	Ig Domain

** From Sivasubramanian et. al., mAbs 4:3, 1-13 May 2012*

Summary

- ROR1 is expressed in early embryonic development
- Expression of ROR1 attenuates during fetal development
- Normal post-partum tissues do not express ROR1 except for hematogones, unusual CD5 precursor B cells.
- ROR1 is expressed by virtually all cases of chronic lymphocytic leukemia
- We have generated highly specific, high-affinity mAb specific for human ROR1 from which we generated single-chain anti-ROR1 Fv of high affinity for ROR1
- Unlike anti-ROR1 mAbs or CAR developed by other investigators, our mAb do not cross-react with normal post-partum tissues

The Clinical Continuum of CLL – Finding Etiology



Outcomes in Relapsed / Refractory CLL

Responses to Regimens for Relapsed or Refractory CLL

Regimen	n	% Pts	% CR	% OR
CIT	244	49	23	67
mAb	101	20	11	42
Lenalidomide	32	6	6	34
Len + CD20 mAb	73	15	4	62
Other	51	10	0	22
Overall	501		14	54

Response to Salvage Treatment* by FISH

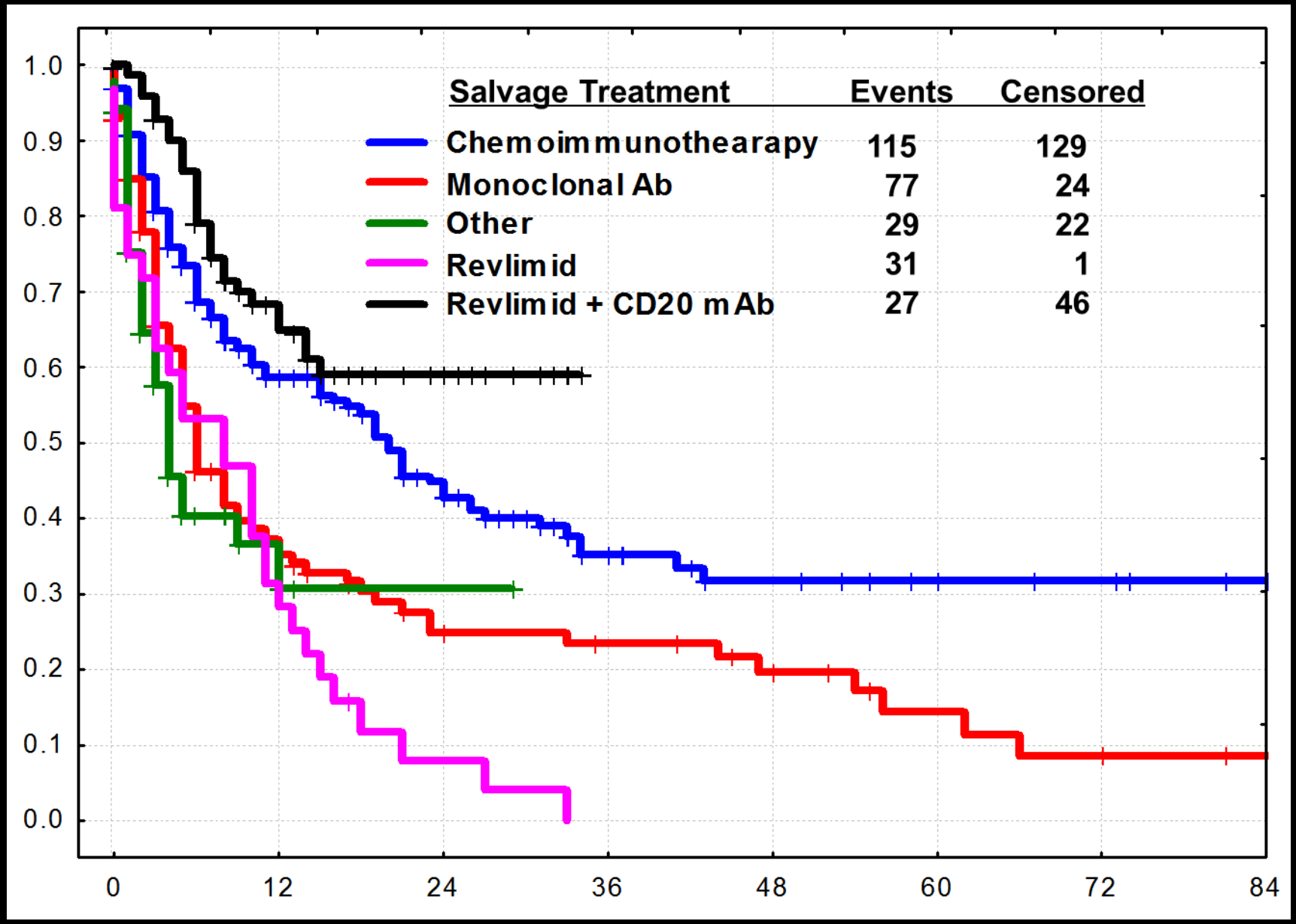
FISH	n	% Pts	% CR		% OR	
17p del	106	28	7	p=.013	40	p<.001
11q del	97	26	11		63	
+12	44	12	20		64	
None	61	16	26	p=.002	56	
13q del	66	18	12		68	p=.04
Overall	374		14		56	

*Includes: CIT; mAb; revlimid; revlimid+mAb; other

Multivariable Model for Time-to-Treatment Failure: Salvage Treatment (n=299)

Characteristic	Relative Risk	p-value
17p del *vs.* Others	1.58	.007
# Prior CLL Treatments	1.22	<.0001
IGHV Status (UM *vs.* Mutated)	1.94	.0009
HGB (gm/dl)	.86	.002
Chemoimmunotherapy *vs.* Others	.47	<.0001
Lenalidomide +CD20 mAb *vs.* Others	.32	<.0001

TTF by Treatment: Salvage (N=501)



Goals of the proposed trial

1. Avoid targeting of CD19 on normal B cells
   - One patient death due to opportunistic viral infection at NCI in patient that received CD19-specific CAR^+ T cells
     - *Kochenderfer et al., Blood. 2012 Mar 22;119(12):2709-20; Cooper et al., Blood. 2012.*

2. Determine the CAR design that improves therapeutic potential
   - It is not known whether signaling through CD28/CD3-zeta or CD137/CD3-zeta is superior and this can only be tested in human trials
     - Both CAR designs target normal $CD19^+$ B cells and have exhibited anti-tumor effects
     - Therapeutic potential is correlated with persistence

Precedent for competitive repopulation experiment co-infusing two populations of CAR$^+$ T cells

- Patients with non-Hodgkin' lymphoma (NHL) were simultaneously infused with 2 autologous T-cell products (CAR-CD19ζ and CAR-CD19-28 ζ)
- Objective was to determine the *in vivo* effects of co-stimulatory endodomain on fate of CAR-engineered T cells
- CD28 co-stimulation improves the *in vivo* expansion and persistence of CAR-modified T cells

Savoldo B, et al. J Clin Invest. 2011 May;121(5):1822-6.

Phase I ROR1-CAR-T for Rel/Ref CLL

- Rationale:
  - Surface ROR1 ubiquitously expressed on CLL B cells, not on normal B cells or normal tissues
  - CD19-CAR-T cells have clinical activity and were tolerated
  - Limited long-term disease control options for relapsed CLL
- Previously treated CLL/SLL:
  - Adequate kidney and liver fctn.; no active infection or autoimmunity; adequate counts; no pregnancy
- Primary endpoint:
  - 3+3 dose escalation to MTD
  - ROR1-CAR-T with 3 dose levels
  - Expansion to 12 pts
  - Multi-center study – MDACC & UCSD
- Secondary endpoints:
  - Tolerability, toxicities, response rate (CR/PR/OR), TTF, TTP, OS
  - Correlative laboratory studies

Phase I ROR1-CAR-T for Rel/Ref CLL

Primary Objective:

- Describe safety profile by identifying MTD and record *in vivo* persistence of autologous *ex vivo* expanded ROR1-specific T cells

Secondary Objectives:

- Assess feasibility of infusing autologous ROR1-specific T cells
- Obtain indication of clinical therapeutic activity by overall response (OR) rate [complete response (CR) & partial response (PR) rates]
- Study persistence of infused autologous ROR1-specific T cells
- Screen for development of host immune responses against the ROR1-specific CAR
- Additional correlative studies:
  - (i) trafficking of genetically modified T cells
  - (ii) development of oligoclonal sub-population(s) of infused T cells
  - (iii) emergence of genetically modified T cells with central memory, stem-cell, and/or naïve immunophenotypes
  - (iv) maintenance of ROR1R-redirected effector functions

Phase I ROR1-CAR-T for Rel/Ref CLL

Inclusion Criteria:

1. Patients with B cell CLL/SLL who have a 2008 NCI-WG/IWCLL indication for treatment
2. Patients who have been previously treated with a standard regimen or untreated with 17p del by FISH (high-risk)
3. At least 21 days from last cytotoxic chemotherapy
4. ECOG PS <2
5. Adequate hepatic function, defined as SGPT <3 x upper limit of normal (ULN); serum bilirubin and alkaline phosphatase <2 x ULN, or considered not clinically significant by the study doctor or designee. Lower than 2 x ULN is deemed as not clinically significant by the study doctor.
6. Able to provide written informed consent

Phase I ROR1-CAR-T for Rel/Ref CLL

Exclusion Criteria:

1. Positive βHCG in female of child-bearing potential (not post-menopausal for 12 months or no previous surgical sterilization or lactating females)
2. Patients with known allergy to bovine or murine products
3. Positive serology for HIV
4. Active, uncontrolled autoimmune phenomenon (AIHA, ITP) requiring steroid therapy
5. Presence of ≥G3 toxicity (CTC v4) from the previous Rx
6. Concomitant investigational agents
7. Prior allogeneic hematopoietic stem-cell transplantation if evidence of donor chimerism persists (patients with exclusively autologous hematopoiesis will be eligible)
8. Refusal to participate in the long-term follow-up protocol (2006-0676)

Phase I ROR1-CAR-T for Rel/Ref CLL

- **Treatment (MDACC & UCSD):**
  - **Collect autologous T cells for transduction and expansion (blood or pheresis)**
  - **Lymphodepletive condition with standard-dose FCR, FRB, or BR (D-5 to D-3)**
  - **Split-dose (D0=1/4 then D1=3/4) infusion of ROR1-CAR-T cells**
    - **$1x10^5$ transduced ROR1-CAR-T cells/kg (50% ROR1/CD28/CD3ζ & 50% ROR1/CD137/CD3ζ)**
    - **$1x10^6$ transduced ROR1-CAR-T cells/kg (50% ROR1/CD28/CD3ζ & 50% ROR1/CD137/CD3ζ)**
    - **$1x10^7$ transduced ROR1-CAR-T cells/kg (50% ROR1/CD28/CD3ζ & 50% ROR1/CD137/CD3ζ)**

Phase I ROR1-CAR-T for Rel/Ref CLL Lymphodepletive Chemoimmunotherapy

Day of Rx	Fludarabine, Cyclophosphamide, Rituximab (FCR) Regimen	Fludarabine, Bendamustine, Rituximab (FBR) Regimen	Bendamustin, Rituximab (BR) Regimen
D -5	• Fludarabine 25mg/m^2 • Cyclophosphamide 250mg/m^2 • Rituximab 375-500mg/m^2	• Fludarabine 20mg/m^2 • Bendamustine 30-50mg/m^2 • Rituximab 375-500mg/m^2	
D -4	• Fludarabine 25mg/m^2 • Cyclophosphamide 250mg/m^2	• Fludarabine 20mg/m^2 • Bendamustine 30-50mg/m^2	• Bendamustine 70-90mg/m^2 • Rituximab 375-500mg/m^2
D -3	• Fludarabine 25mg/m^2 • Cyclophosphamide 250mg/m^2	• Fludarabine 20mg/m^2 • Bendamustinem30-50mg/m^2	• Bendamustine 70-90mg/m^2
D -2			
D -1			
D 0	T cell infusion (25% of the genetically modified cells)		
D +1	T cell infusion [remainder (75%) of ROR1R-CAR-T cell dose]		

Phase I ROR1-CAR-T for Rel/Ref CLL

Evaluation	Within 30 (±7) days of T-cell Collection	Within 14 (±7) days of T-cell Infusion	Within 24 hours post 1st & 2nd part of split T-cell infusion (for Day 0 and Day 1)	Within 1-3 days post T-cell infusion	At 2 weeks (± 3 days)	At 3 weeks (± 3 days)	At 1 month (± 5 days)	At 3,6,12 months (± 14 days)
History and physical exam, including weight and vital signs (BP, HR, Temp)	X	X	X	X	X	X	X	X
Laboratory examinations: CBC, diff, PLT, ALC, T bili, SGPT or SGOT, Alk Phos, LDH, Alb, total protein, BUN, creatinine, NA^+, CL^-, K^+, CO_2, glucose	X		X	X	X	X	X	X
PT/PTT	X	X						
Serum immunoglobulin	X	X			X		X	X
Baseline: chest x-ray, 12-lead ECG	X							
Serum pregnancy test, if applicable	X	X						
Serology for CMV, HIV, HTLV, I/II, Hep B, Hep C, West Nile Virus, RPR, and T. cruzei	X							
Disease status to include CT and/or PET scan, bone marrow biopsy, molecular tests for MRD as clinically indicated		X						X
Serum for HAMA		X						
HLA typing		X						
Blood flow cytometry for T- & B-cell markers		X						
Adverse events assessment & grading	X	X	X	X	X	X	X	X
Research Tests		X	X	X	X	X	X	X

Safety

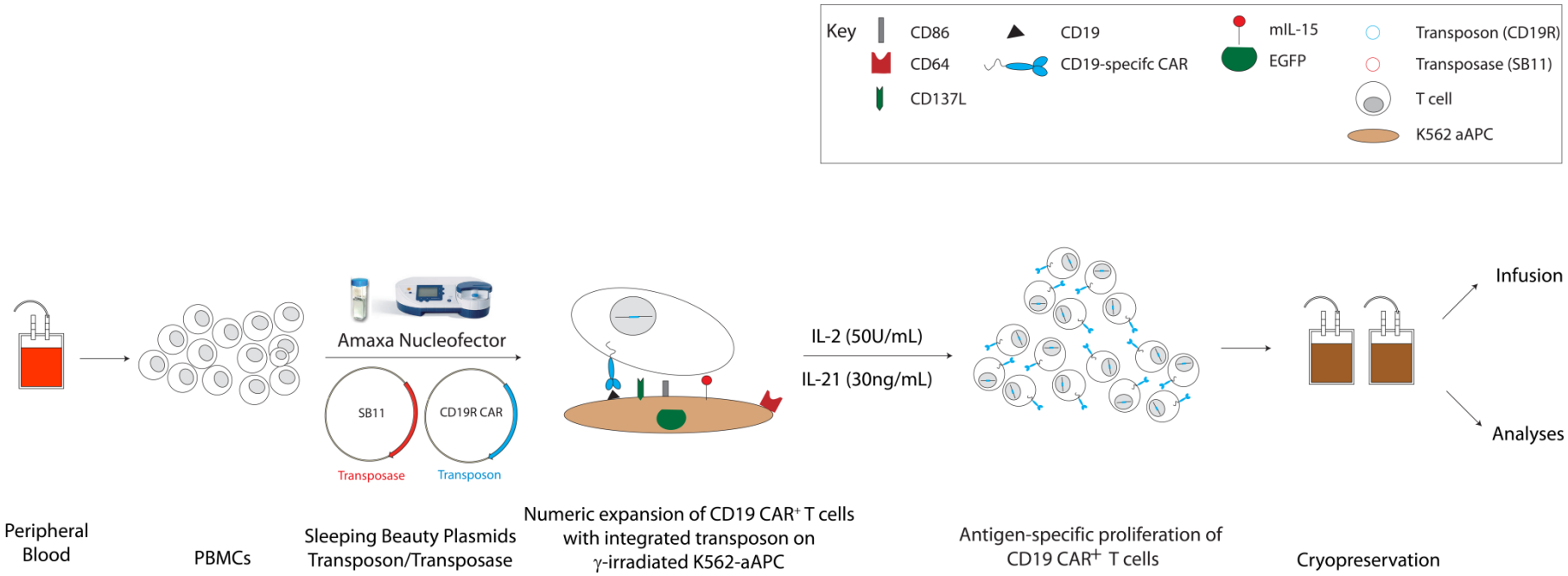
- Inter-patient dose escalation
- Splitting the infusion of T cells over two days
- "Pause" between patients
- Plan to provide corticosteroids and other immunosuppressive agents

CAR^{+} T-cell trials at MDACC

Trial	Agent	NIH-OBA	IND	Enrollment
ALL and lymphoma	Autologous CD19-specific T cells	0804-922	14193	4
ALL and lymphoma	Allogeneic CD19-specific T cells	0910-1003	14577	6
ALL and lymphoma	Allogeneic CD19-specific UCB-derived T cells	1001-1022	14739	Pending activation
CLL	Autologous CD19-specific T cells	1201-1142	15180	Pending activation
CLL	Autologous ROR1-specific T cells	1210-1192	Pending	Pending approval

2nd generation CD19-specific CAR signaling through CD28 and CD3-ζ

MDACC approach to generating clinical-grade CAR+ T cells



Sleeping Beauty (SB) Transposition : An alternative to retroviral based gene therapy

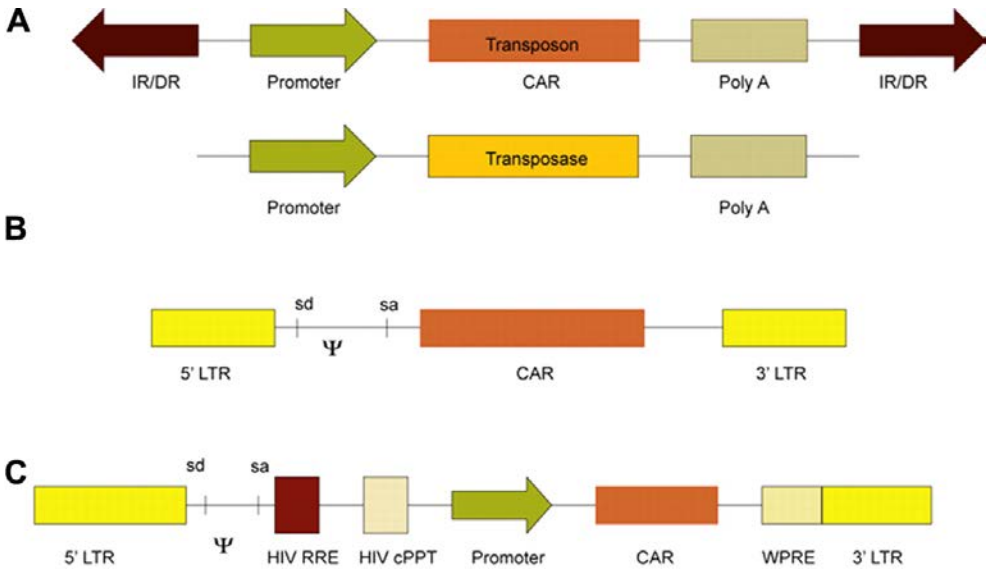


Fig 1. Vectors employed in CAR based gene therapy trials
Jena B, Dotti G, Cooper L et al., Blood. 2010

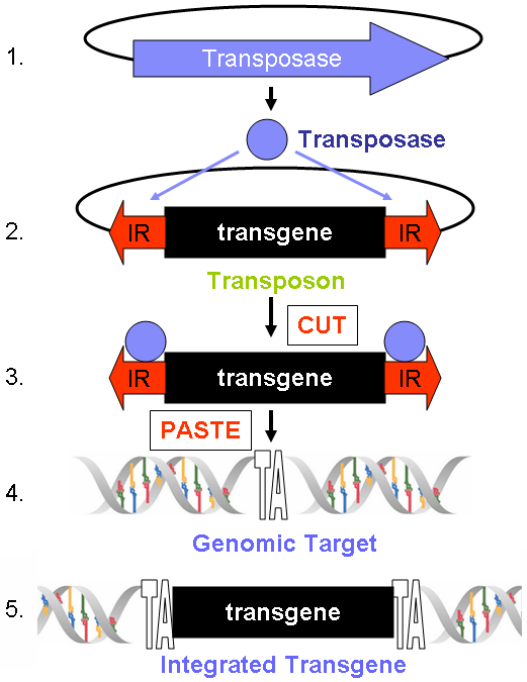
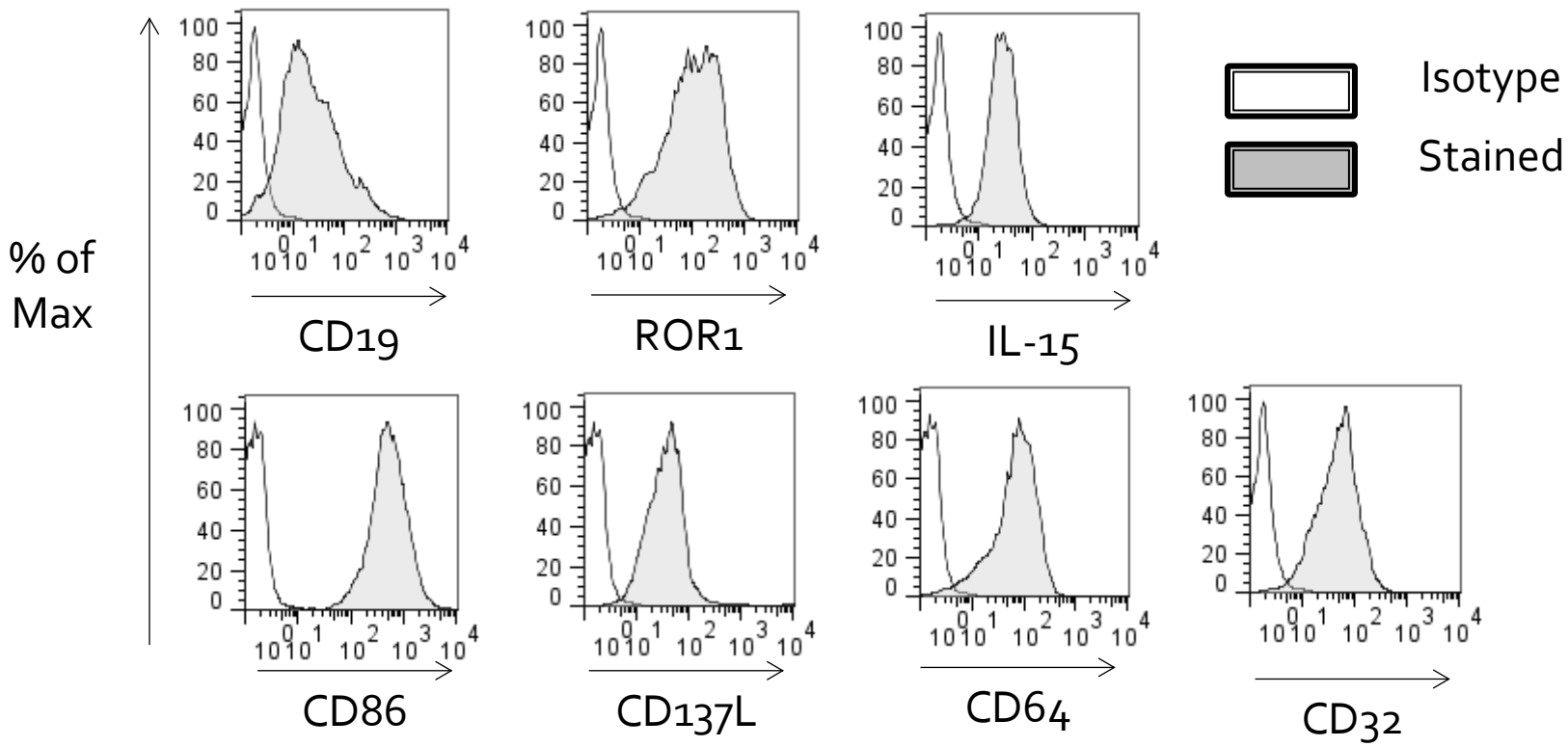
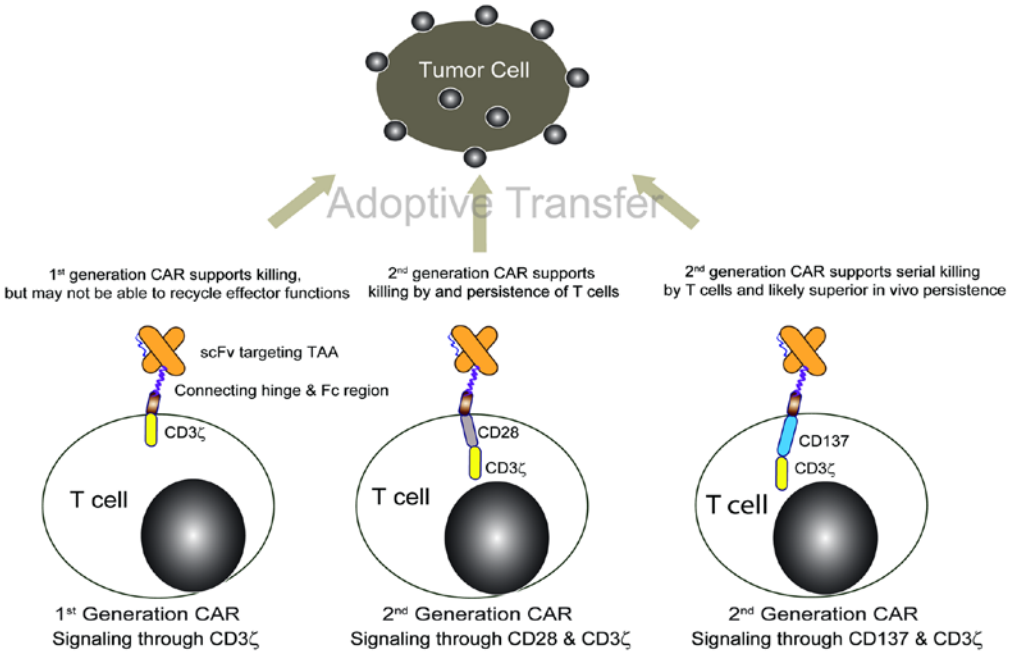


Fig 2. *Sleeping Beauty* transposition
Switzer K, Rabinovi ch B and Cooper et al., In Press

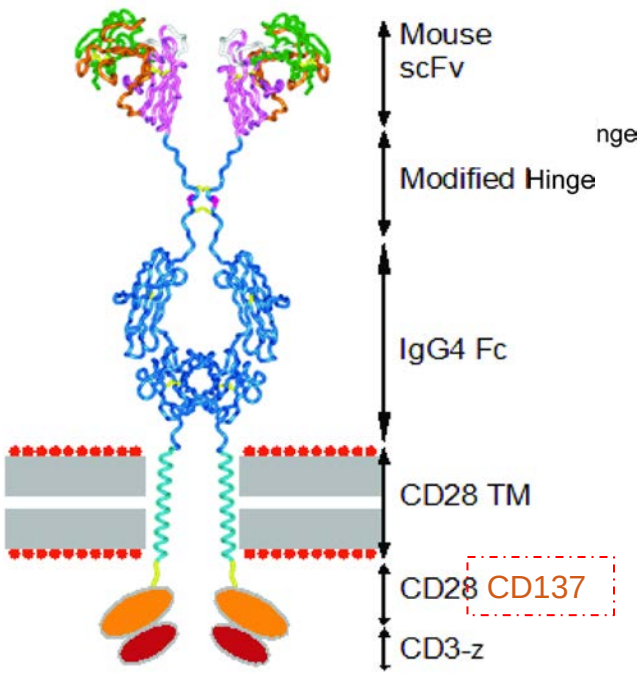
ROR1+ K562-aAPC (Clone 1) Expression of transgene



Co-infusion of two CARs

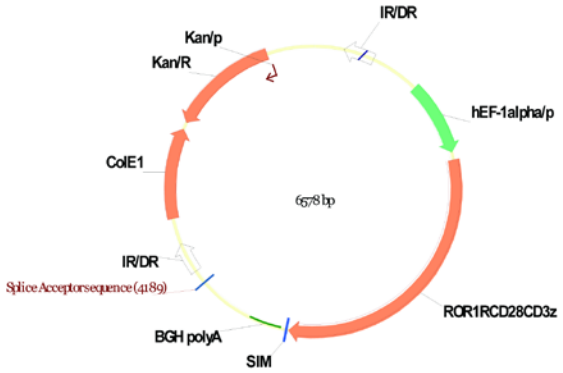


ROR1RCD28-CAR
ROR1RCD137-CAR

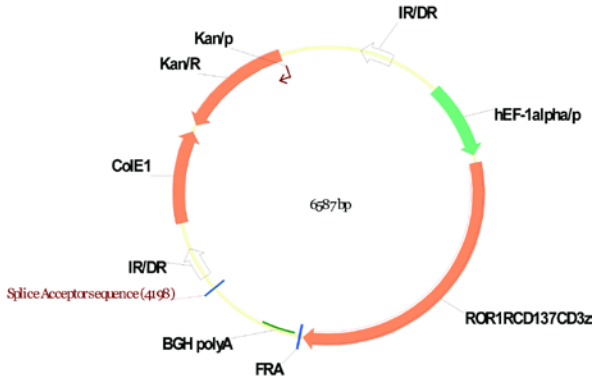


Sleeping Beauty (SB) plasmids

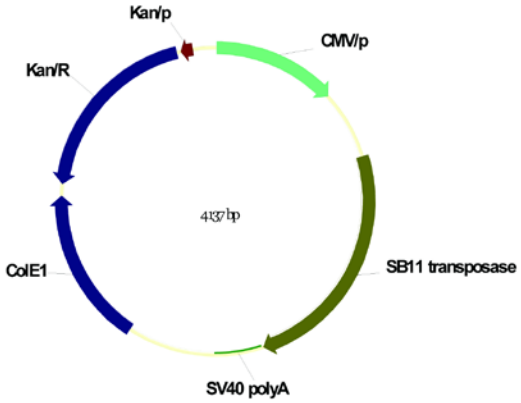
ROR1RCD28-CD3z



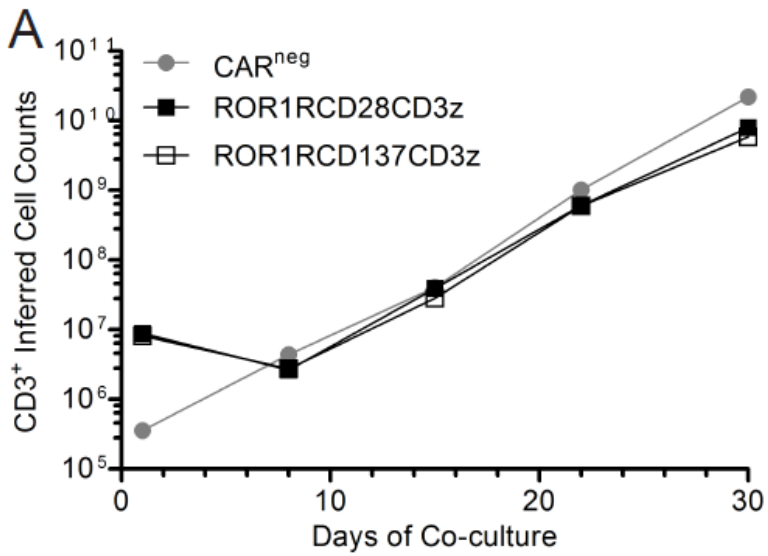
ROR1RCD137-CD3z

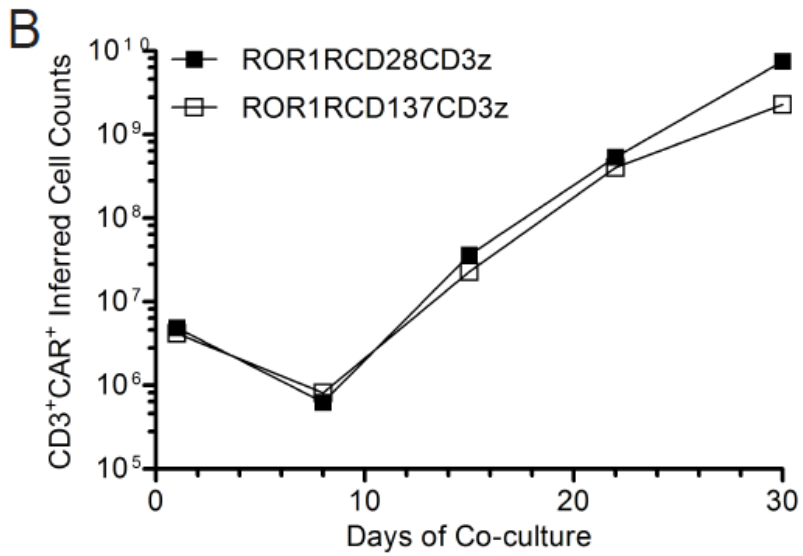


pKan-CMV-SB11 (Transposase)

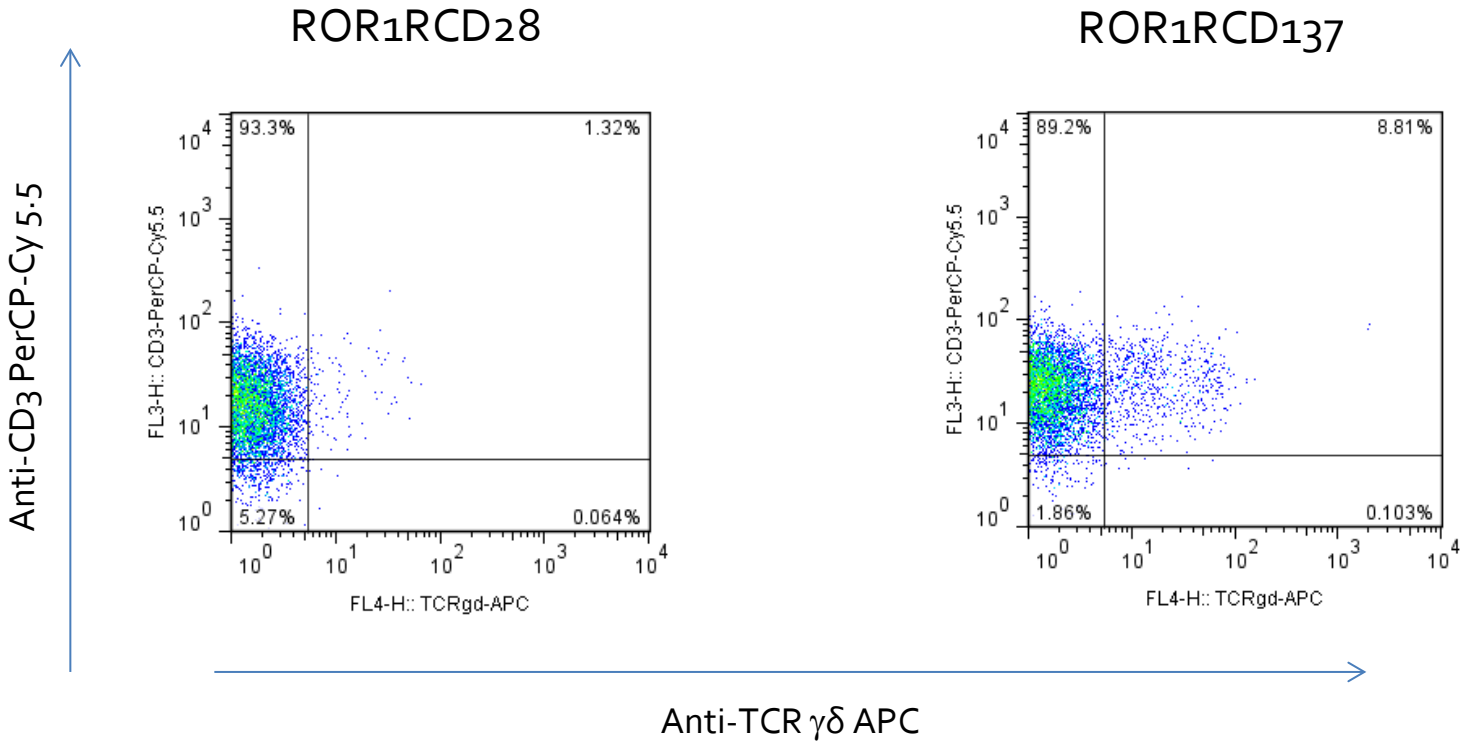


Propagation of ROR1RCD28 and ROR1RCD137 modified CAR$^+$ T cells on aAPC





Emergence of γδ-T cells in ROR1CD137 CAR⁺ T cells



Redirecetd lysis by ROR1-specific CAR^{+} T cells

Figure 1 Cytotoxic specificity of ROR1RCD28-T cells

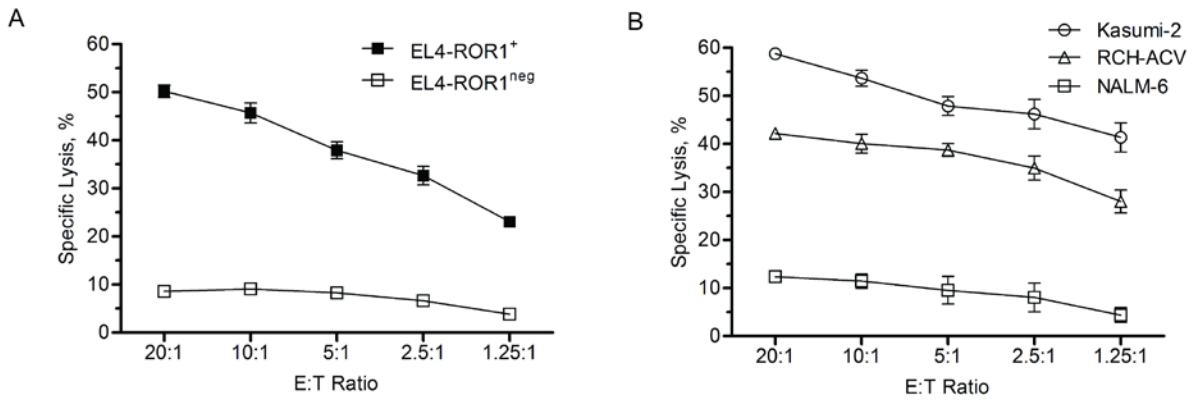
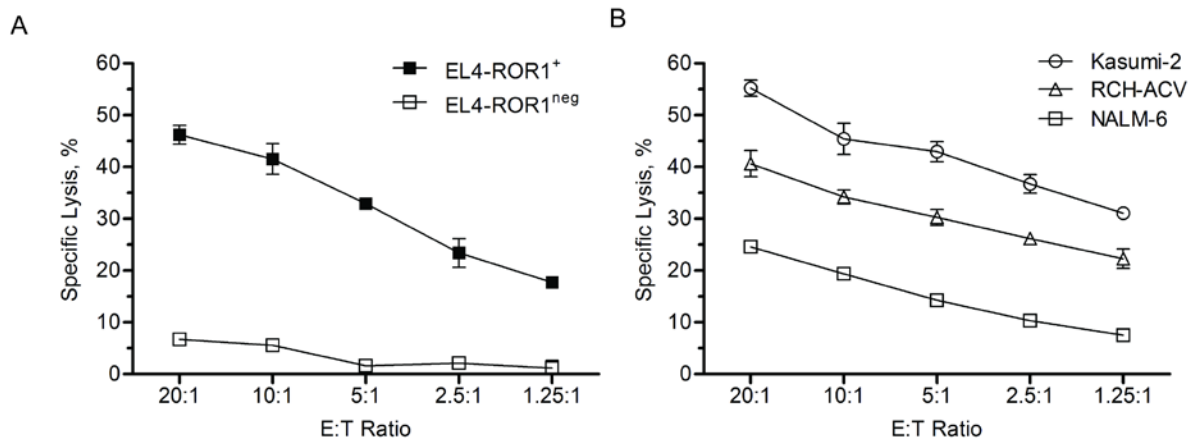


Figure 2 Cytotoxic specificity of ROR1RCD137-T cells



Summary

- Protocol addresses important questions:
  - Avoids inadvertent targeting of normal B cells
  - Tests CAR designs that imparts improved T-cell persistence and thus therapeutic potential
  - Provides investigational immunotherapy for patients with aggressive CLL
- Builds upon our current portfolio of CAR^+ T cells
  - Iterative changes to the electroporation and propagation of CAR^+ T cells

Acknowledgements

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