Nanoscale Liposomal CD22ΔE12-siRNA Formulation as a Potent RNAi Therapeutic Against B-cell Precursor Acute Lymphoblastic Leukemia

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DISCLOSURE STATEMENT

no conflicts, patents or financial interests

no corporate affiliations

Acknowledgments

Bioengineering *Hong Ma*

Biochemistry *Dorothea Myers, Zahide Ozer*

Proteomics *Martha Arellano*

Murine Leukemia Models

Anoush Shahidzadeh, Ingrid Cely, Cherish Flowers

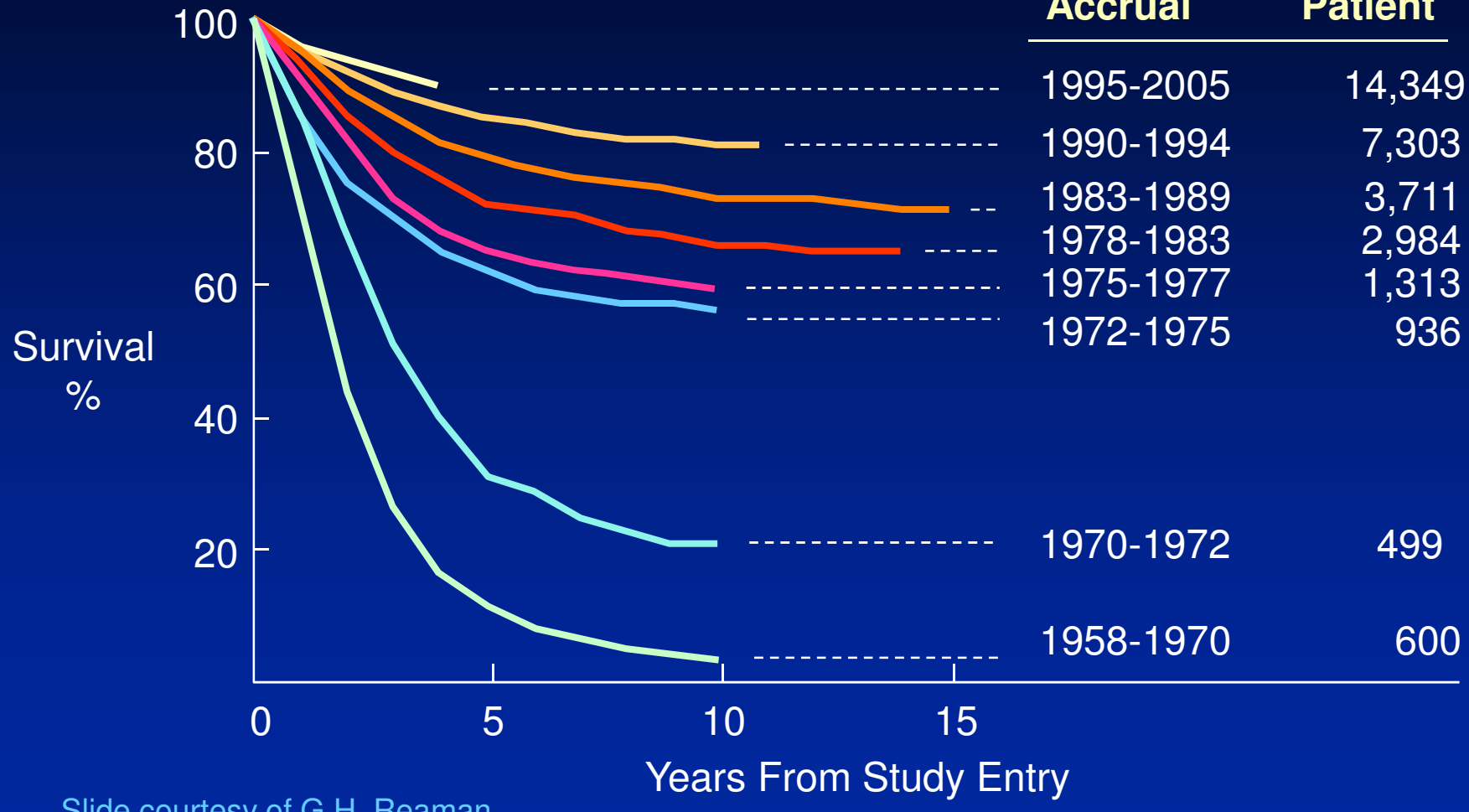
Bioinformatics *Sanjive Qazi*

Formulation *Jianjun Cheng, Seang Yiv*

Acute Lymphoblastic Leukemia (ALL)

- Most common form of childhood cancer
- 85-90% have B-lineage ALL
- Neoplastic expansion of B-cell precursors with a maturational arrest at discrete stages of B-lymphocyte ontogeny; therefore also referred to as B-precursor leukemia (BPL)

Survival of 31,695 Pediatric Patients with ALL Treated on CCG and COG Group Clinical Trials



Years of Accrual	No. Patient
1995-2005	14,349
1990-1994	7,303
1983-1989	3,711
1978-1983	2,984
1975-1977	1,313
1972-1975	936
1970-1972	499
1958-1970	600

Slide courtesy of G.H. Reaman

The Challenge

- With the progressive intensification of therapy, more than 80% of children with ALL achieve long term survival.
- To achieve this level of cure, children are exposed to very intensive therapies that have serious short and long term toxicities; and relapses occur across all risk groups; only 30% of children who relapse survive.
- Nearly 30% of children with ALL with “high risk” features have failed to respond to therapeutic intensification and require new therapeutic approaches for cure.
  - Can we uncover the underlying genetic abnormalities in this resistant form of disease and how can we identify new targets for therapy?

Slide courtesy of G.H. Reaman

CD22 Receptor

Inhibitory co-receptor of B-cells/B-cell precursors that acts as a negative regulator of multiple signal transduction pathways critical for B-cell homeostasis and survival

J. Immunol. 154:3096-3104 (1995); *Science* 269: 242-244(1995); *Immunity* 5: 551-62 (1996)

The inhibitory and apoptosis-promoting signaling function of CD22 dependent on recruitment of SHP-1 to the immunoreceptor tyrosine-based inhibitory motifs (ITIMs)

Cell 72: 767-78 (1993); *J Exp Med* 183: 547-60 (1996); *Immunity* 8: 497-508 (1998); *J. Biol. Chem.* 274: 2303-2307 (1999)

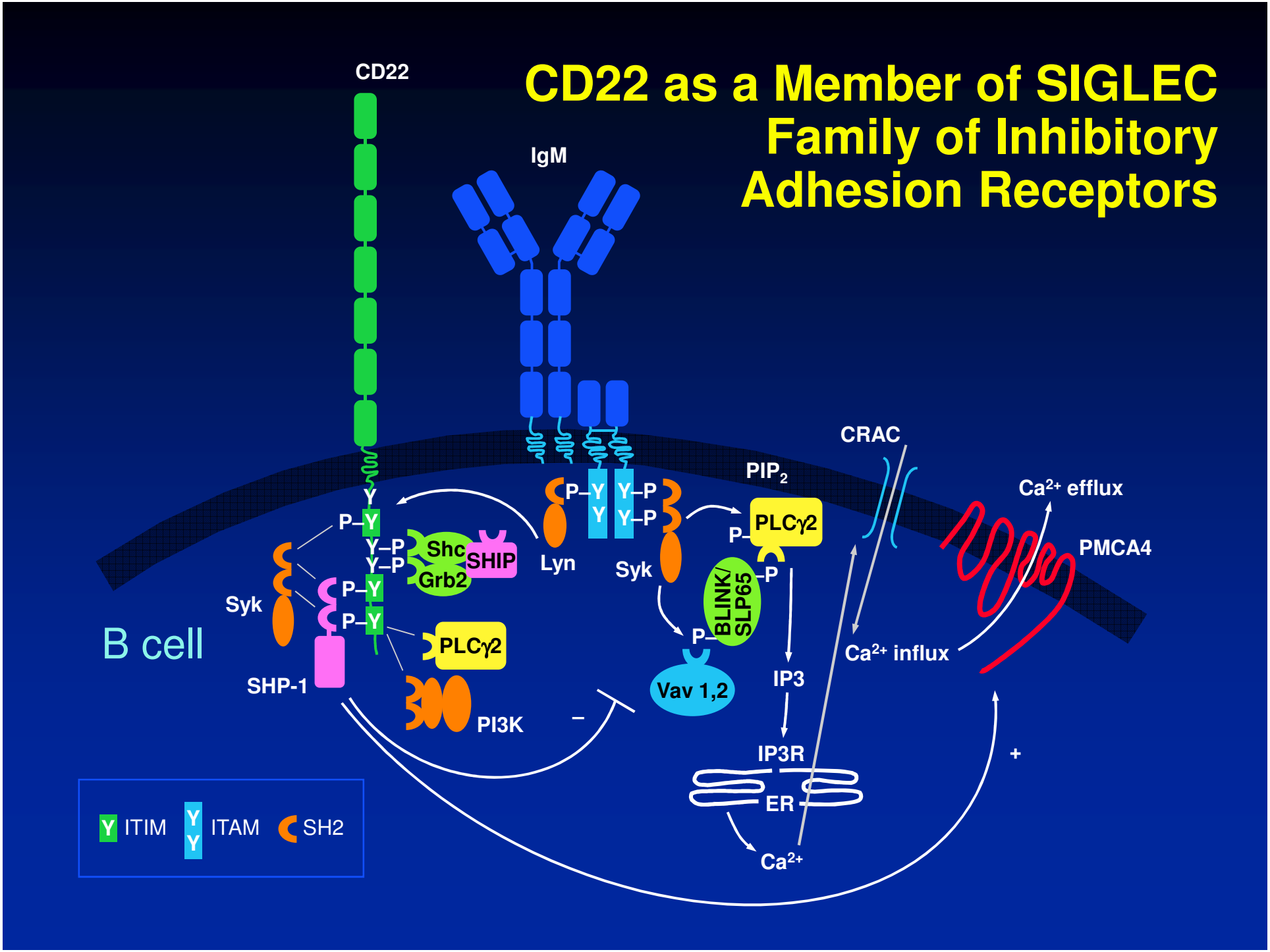
Disruption of the LYN-CD22-SHP1 signaling network can result in development of a B-cell lymphoproliferative state as well as systemic autoimmunity

Adv Immunol. 88:1-50 (2005); *Nat Genet* 4:124-129 (1993); *Cell* 83: 301-311 (1995); *Science* 274: 798-801 (1996); *Nature* 384: 634-7 (1996)

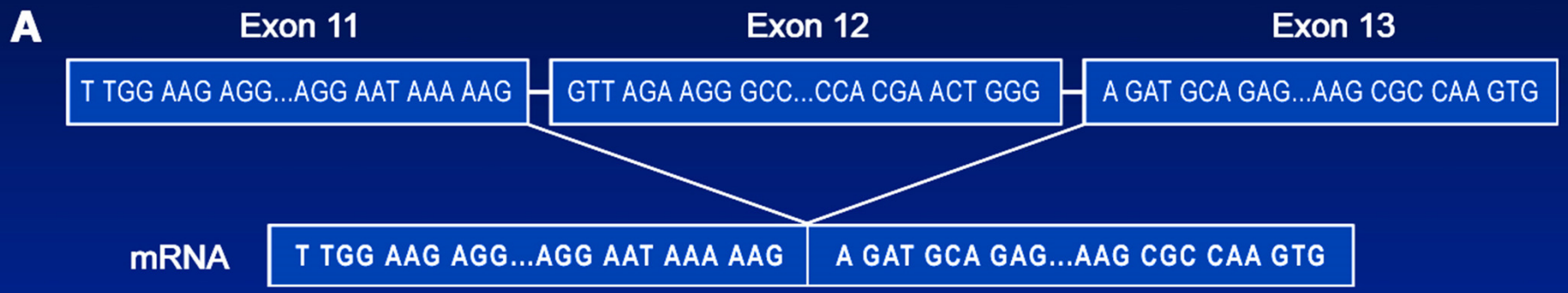
CD22ΔE12 caused by homozygous mutations associated with therapy refractory B-precursor leukemia in pediatric patients

Proc. Natl. Acad. Sci. USA, *107:16852-16857 (2010); BJH 156:89-98 (2012)*

CD22 as a Member of SIGLEC Family of Inhibitory Adhesion Receptors



CD22ΔE12 in B-Precursor Leukemia



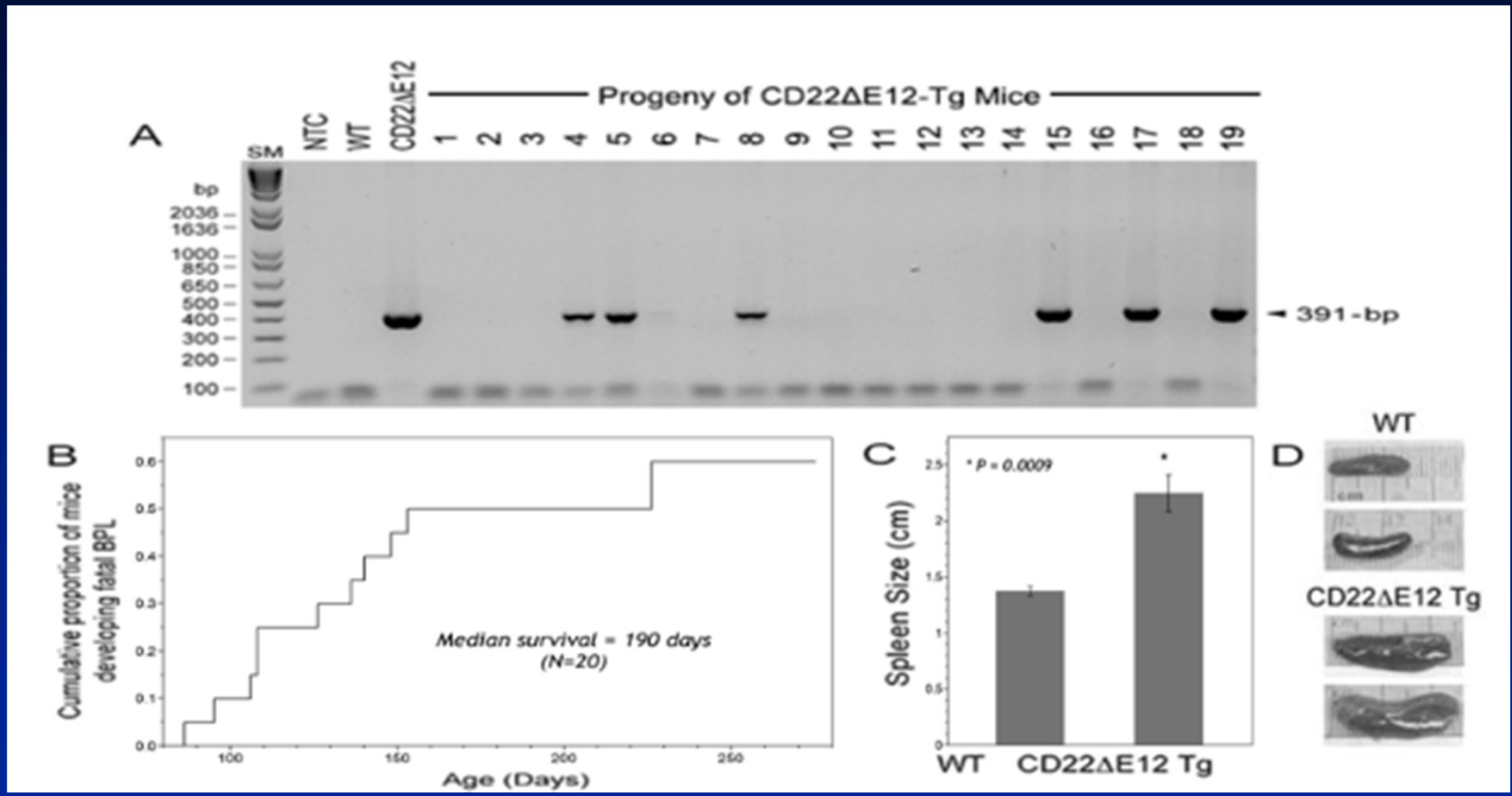
B

Ex 11
T TGG AAG AGG ACA CAG AGC CAG CAG GGG CTT CAG GAG AAT TCC AGC GGC CAG AGC TTC TTT GTG AGG AAT AAA AAG
W K R T Q S Q Q G L Q E N S S G Q S F F V R N K K

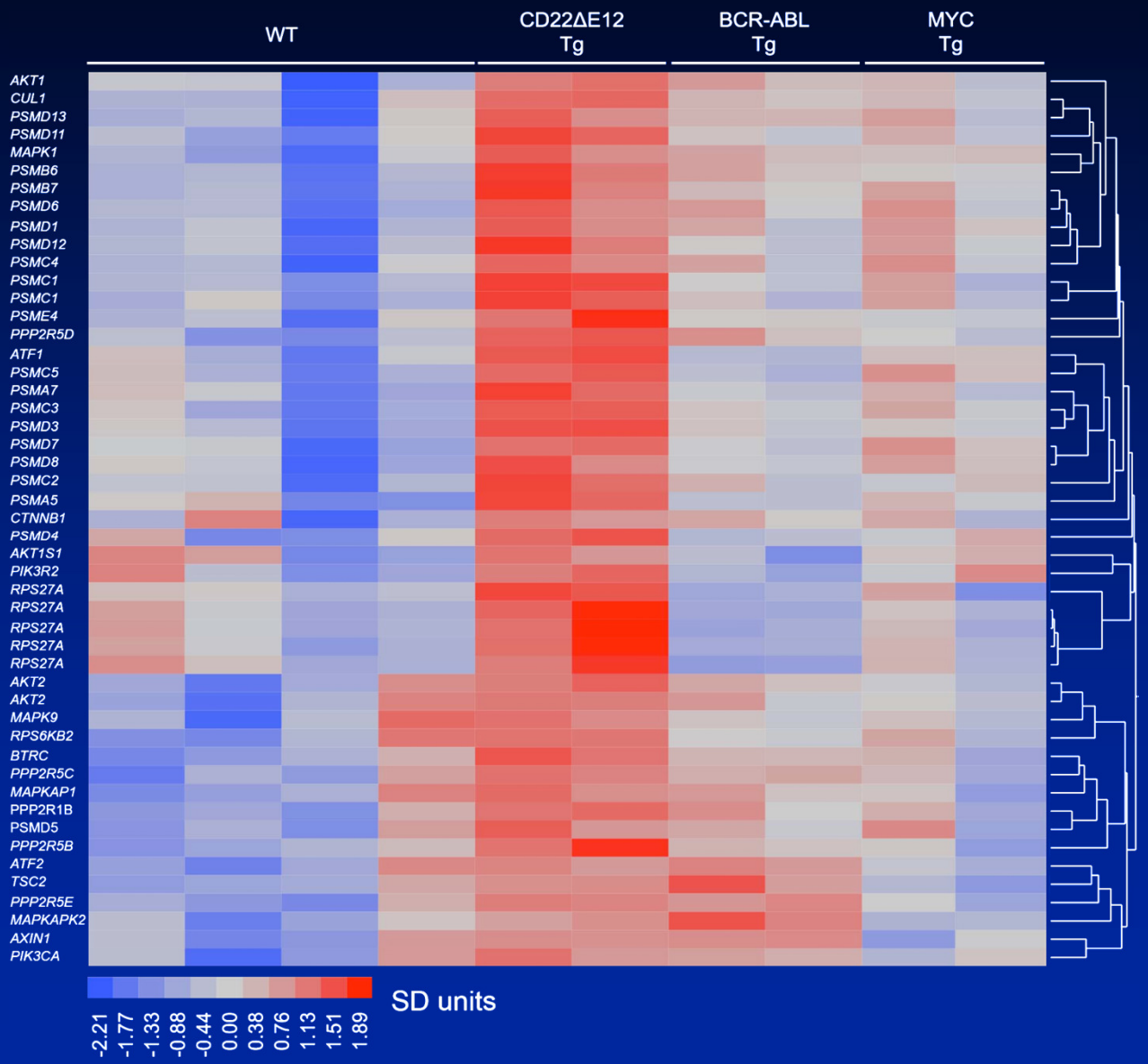
-Ex 12
AGA TGC AGA GTC CTC AGA GAT GCA GAG ACC TCC CCC GGA CTG CGA **TGA** CAC GGT CAC TTA TTC AGC ATT . . .
R C R V L R D A E T S P G L R STOP

+Ex 12
V R R A P L S E G P H S L G C Y N P M M E D G
GTT AGA AGG GCC CCC CTC TCT GAA GGC CCC CAC TCC CTG GGA TGC TAC AAT CCA ATG ATG GAA GAT GGC
I S Y T T L R F P E M N I P R T
ATT AGC TAC ACC ACC CTG CGC TTT CCC GAG ATG AAC ATA CCA CGA ACT GG . . .

CD22ΔE12 causes BPL in Transgenic Mice



B-Precursor Leukemia Developing in CD22ΔE12 Tg Mice is Characterized by a Unique Transcriptome

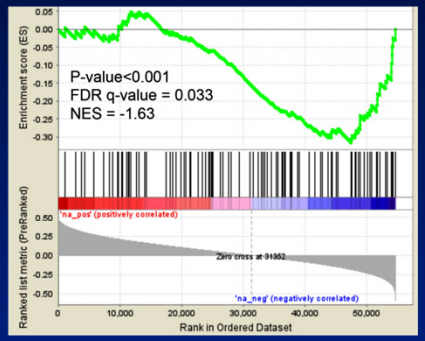


Striking Similarities Between Human vs. Mouse CD22ΔE12 Signature Transcriptomes

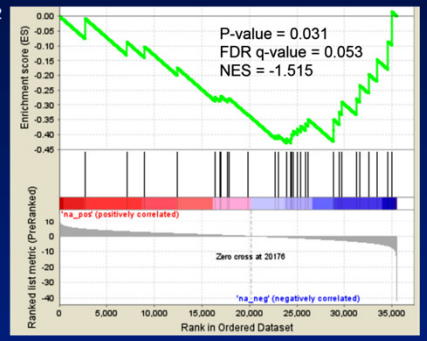
Human CD22ΔE12 Signature Transcriptome | **Mouse CD22ΔE12 Signature Transcriptome**

Gene set: MAPK pathway for nuclear events, Combined P-value = 0.0004

A1

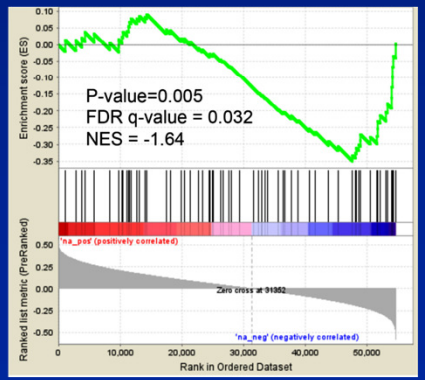


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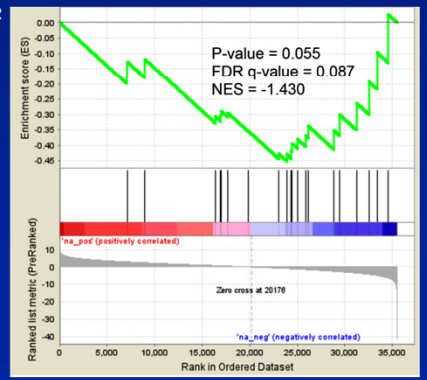


Gene Set: MAPK pathway, Combined P-value = 0.002

B1



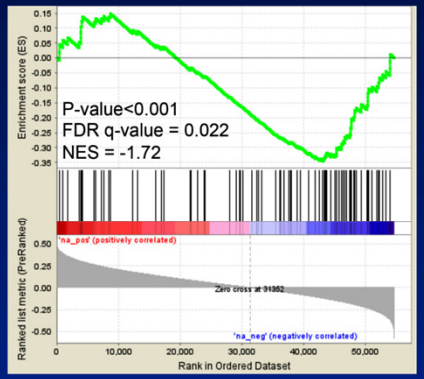
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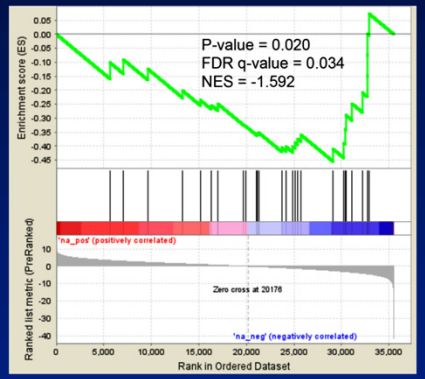
Human CD22ΔE12 Signature Transcriptome | **Mouse CD22ΔE12 Signature Transcriptome**

PI3-K/mTOR pathway, Combined P-value = 0.0002

C1

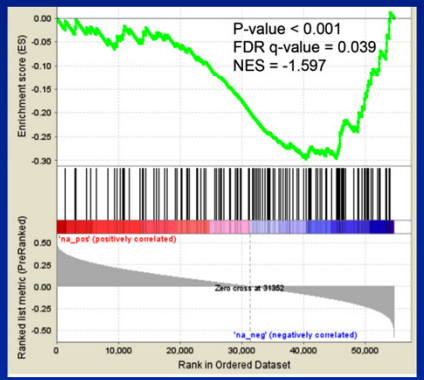


C2

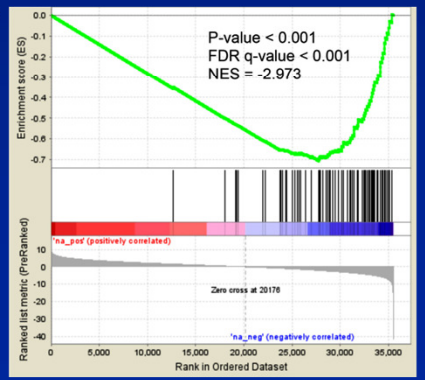


WNT pathway, Combined P-value < 0.0001

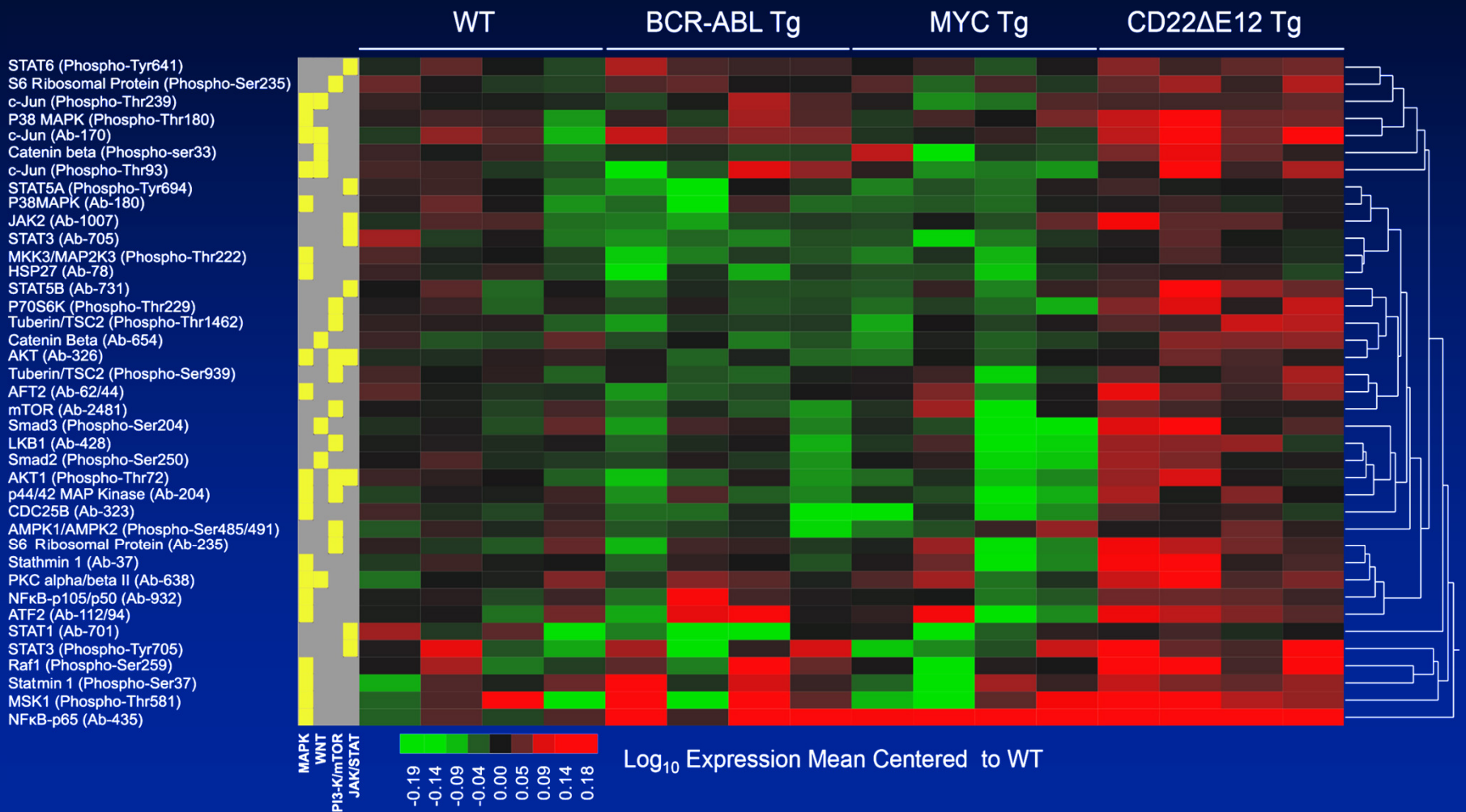
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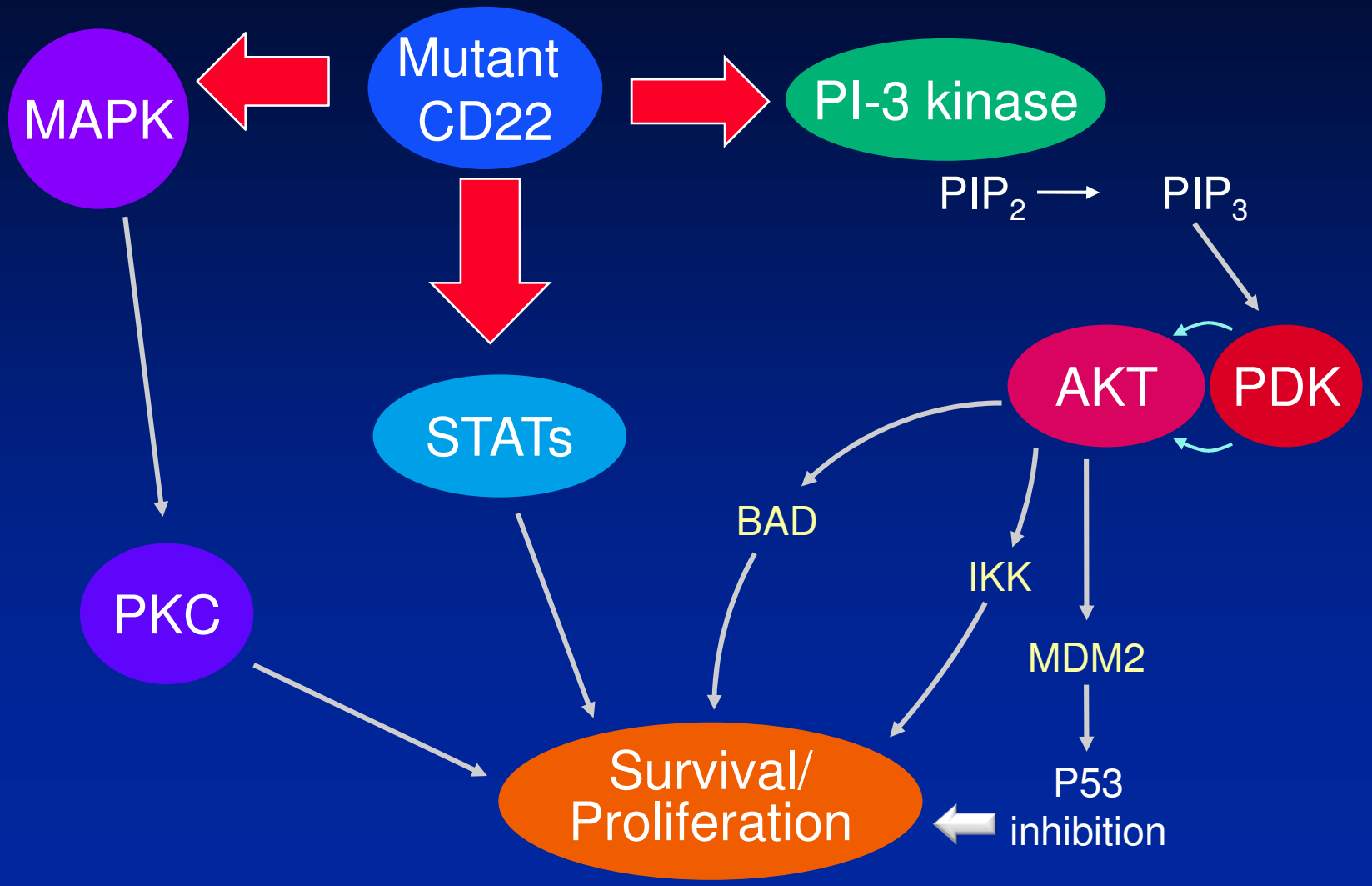
D2



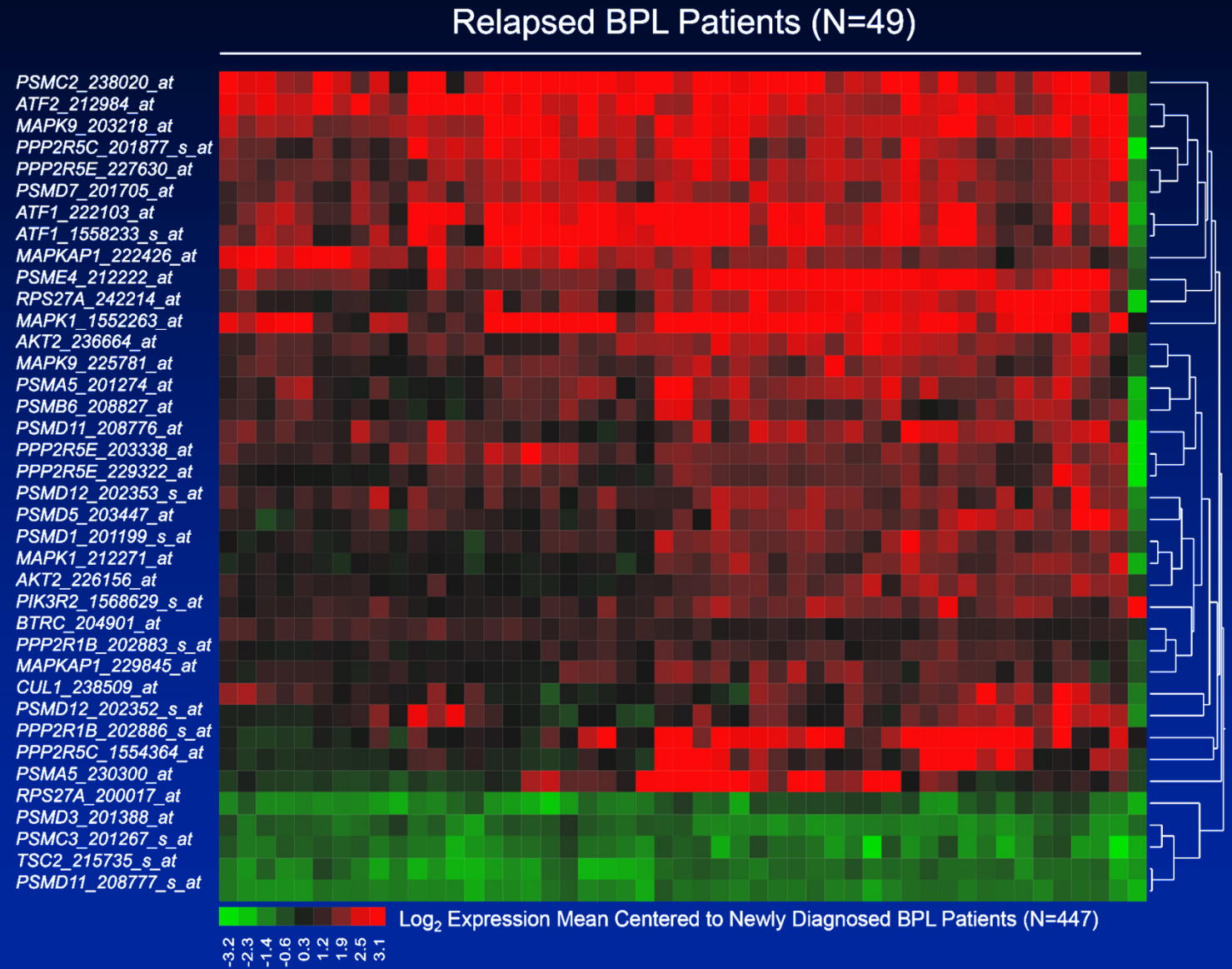
B-precursor Leukemia Developing in CD22ΔE12 Tg Mice is Characterized by a Unique Phosphoprotein Expression Profile



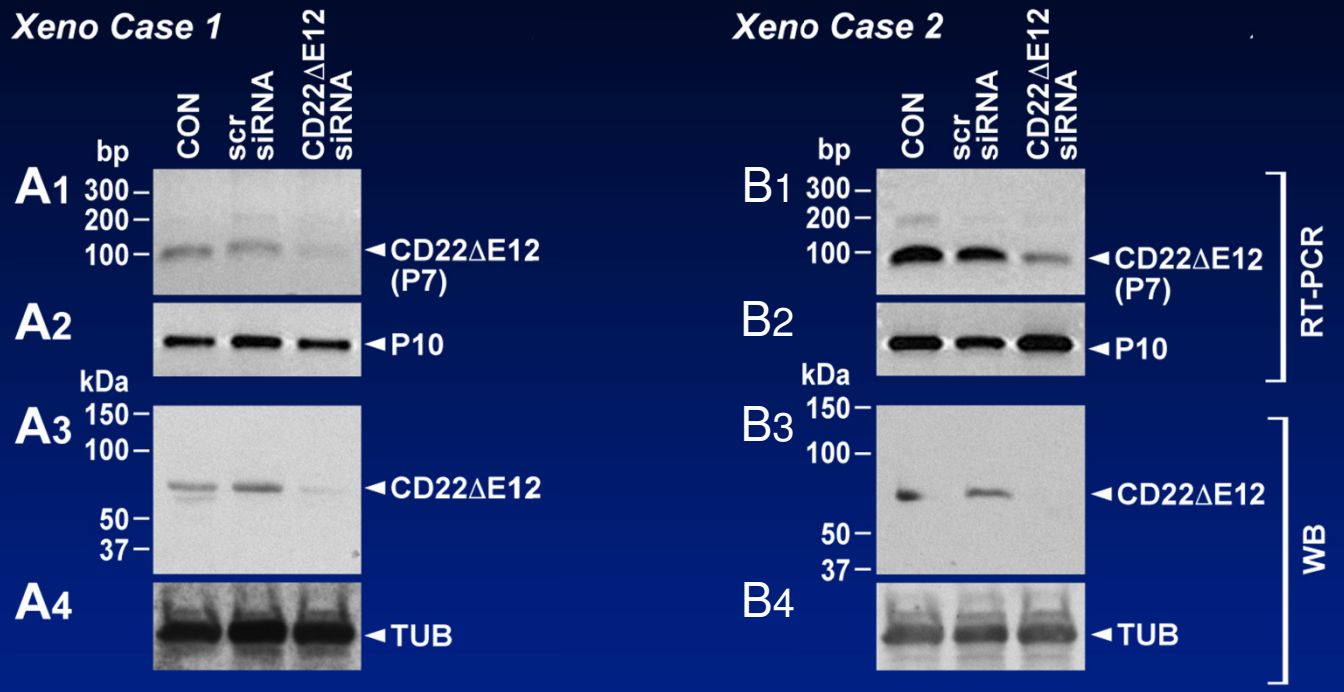
Mutant CD22 as a Master Regulator of Growth and Therapy Resistance of Leukemic Stem Cells

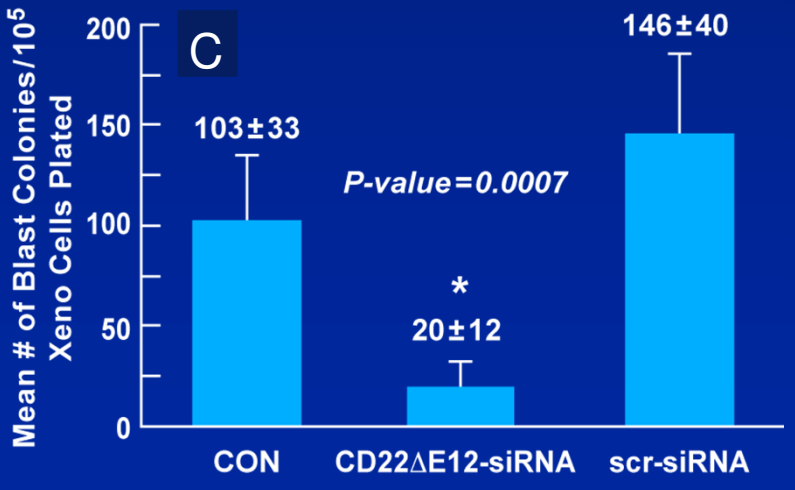


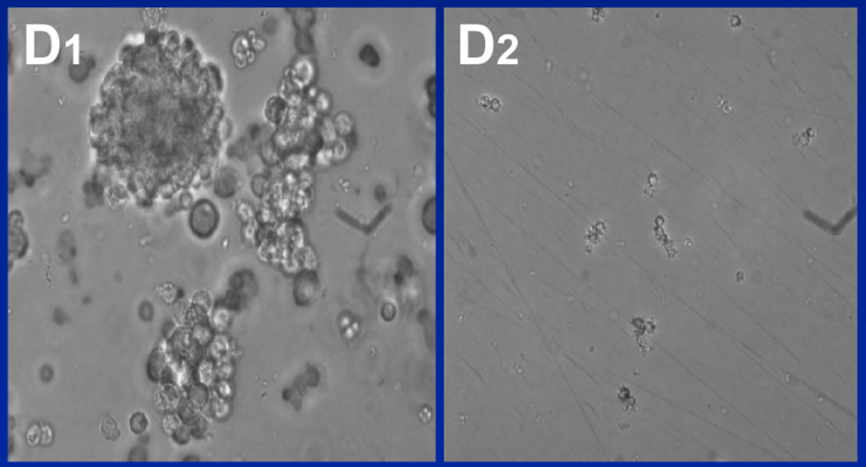
Expression of CD22□E12 Transcriptome in Relapse Clones from Pediatric BPL Patients



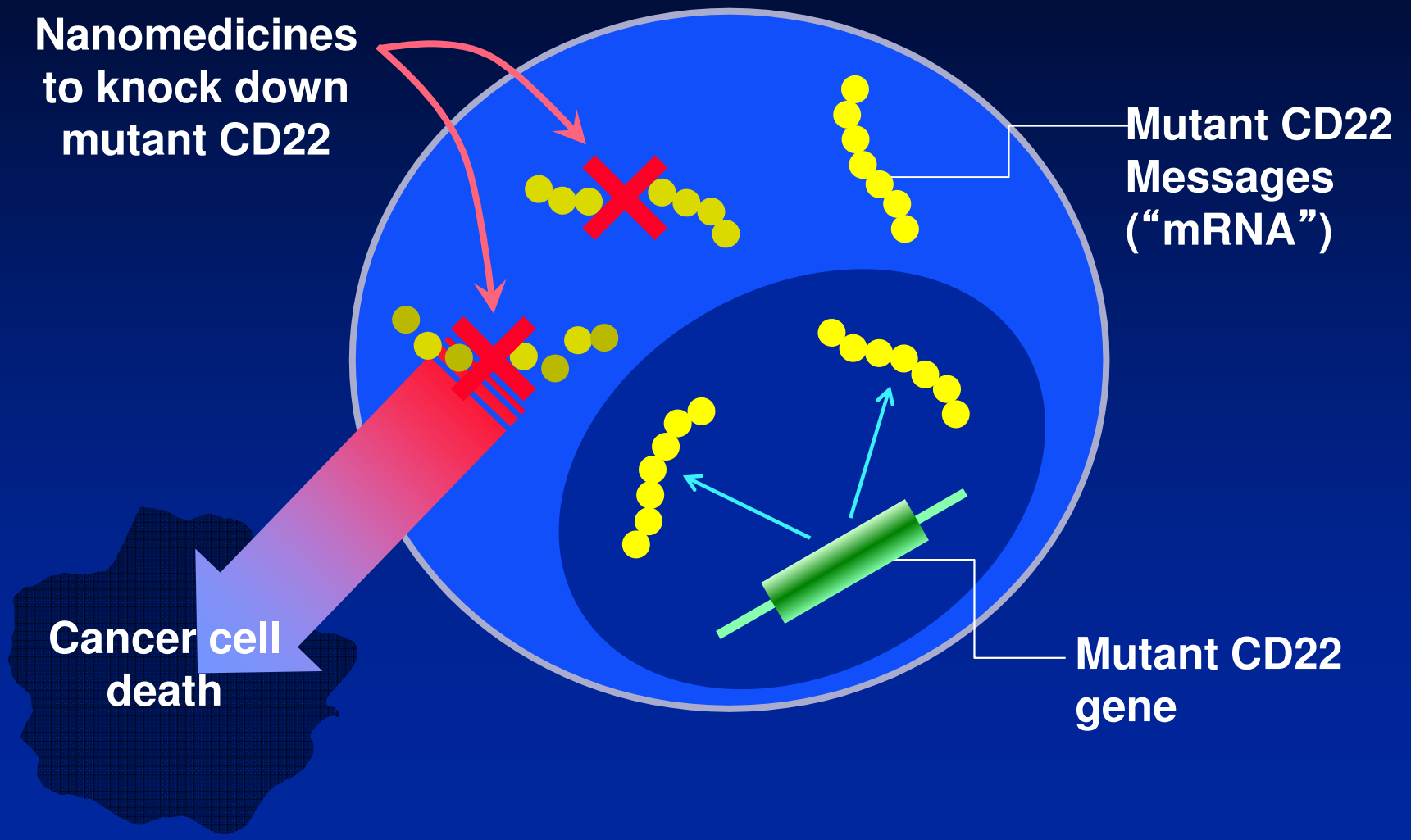
Effect of RNAi Knockdown of CD22ΔE12 Expression on Clonogenicity and Self-Renewal Rate of B-Precursor ALL Xenograft Cells







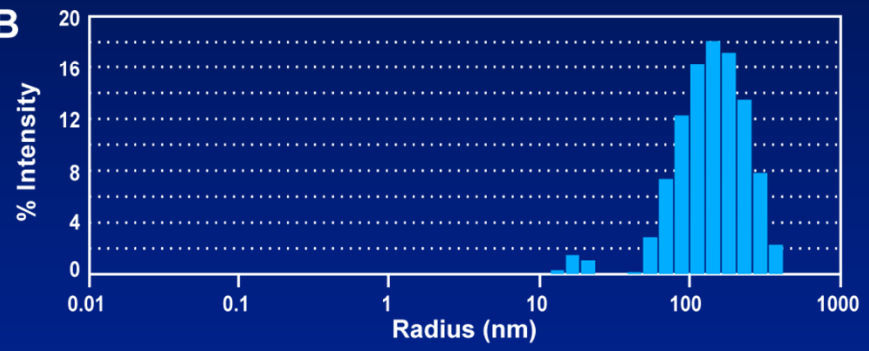
Destroying Leukemic Stem Cells by Using Rationally-Designed Nanomedicines to Knock Down Mutant CD22 Gene

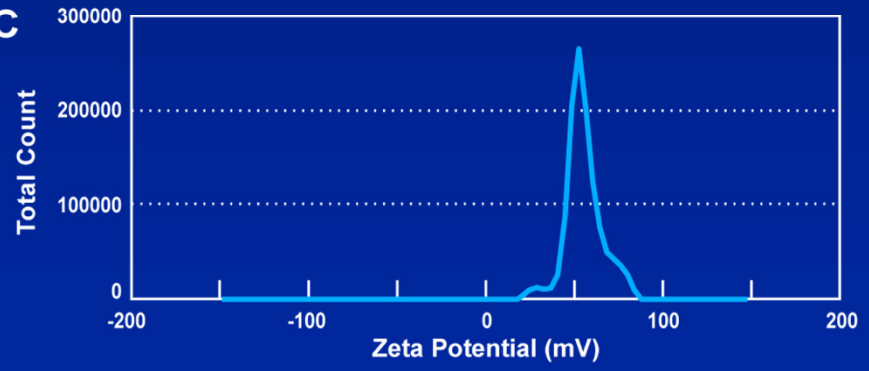


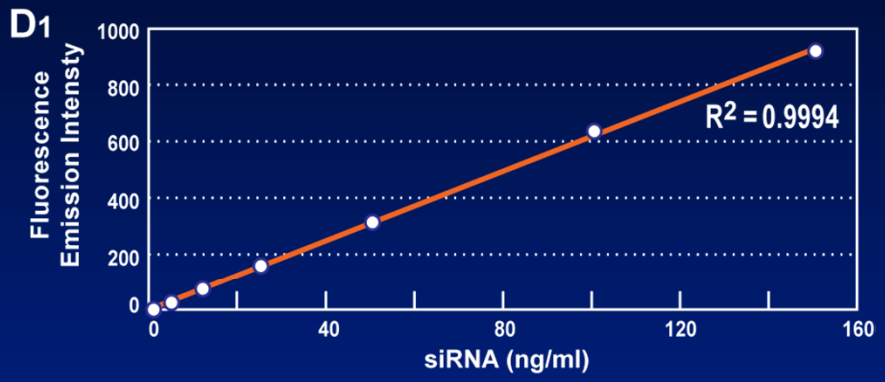
Liposomal Nanoformulation of CD22ΔE12-siRNA

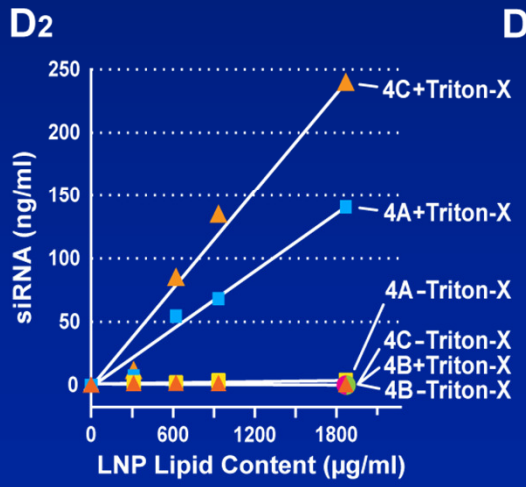
A

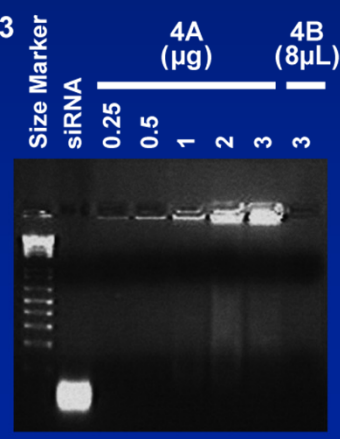
	DOTAP (mM)	DOPE (mM)	Radius (nm)	siRNA (μM)	Encapsulation of CD22 (%)	Zeta potential (mV)
4A	3.9	3.9	132.5 ± 4.7	33 ± 2	96.3 ± 0.7	54.8 ± 10.5
4B	3.9	3.9	70.1 ± 3.6	<1	N.A.	49.9 ± 16.5
4C	3.9	3.9	108.2 ± 3.4	60 ± 1	99.2 ± 0.5	26.7 ± 16.5



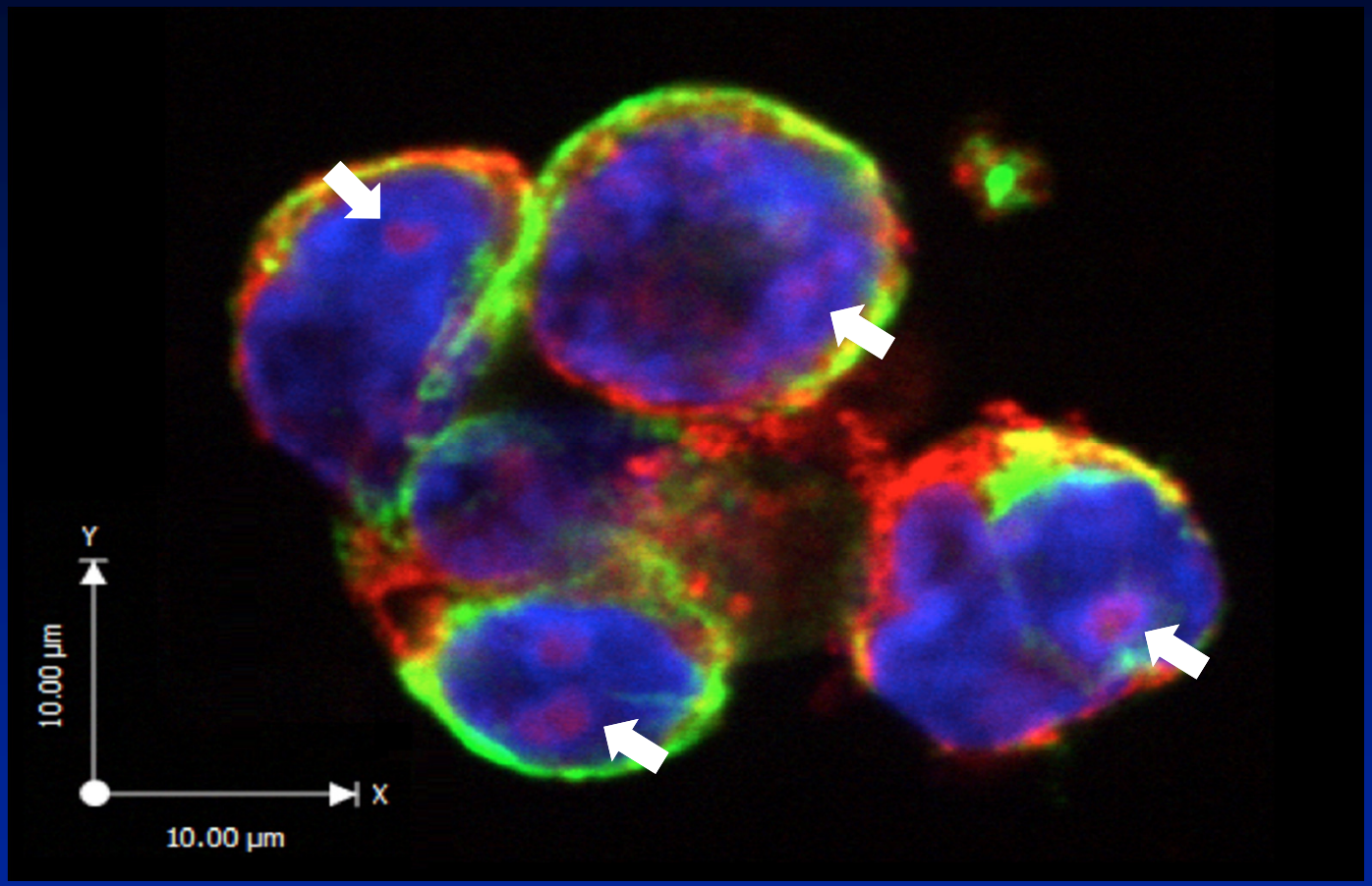




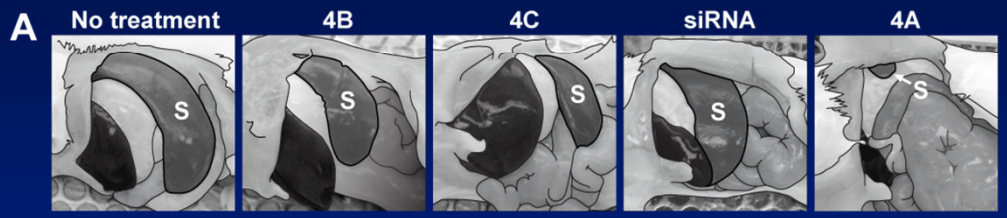


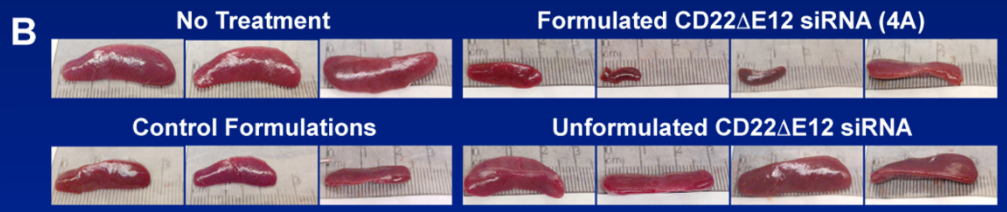


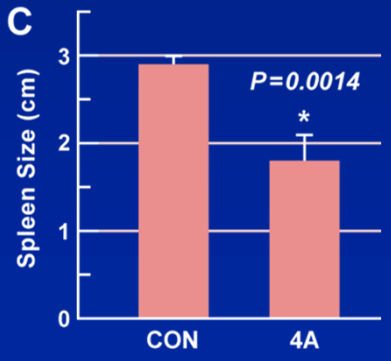
Nanomedicine Candidate Delivers RNA Therapeutics into BPL Cells for Knockdown of Mutant CD22



Potency of the nanoscale liposomal CD22ΔE12-siRNA formulation against LSC in BPL xenograft specimens

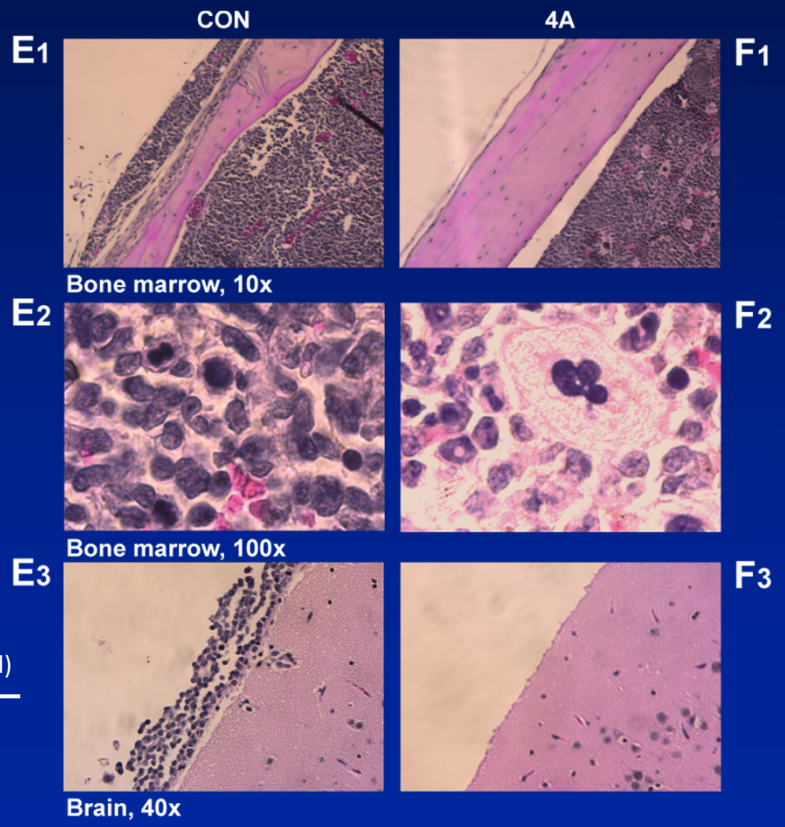




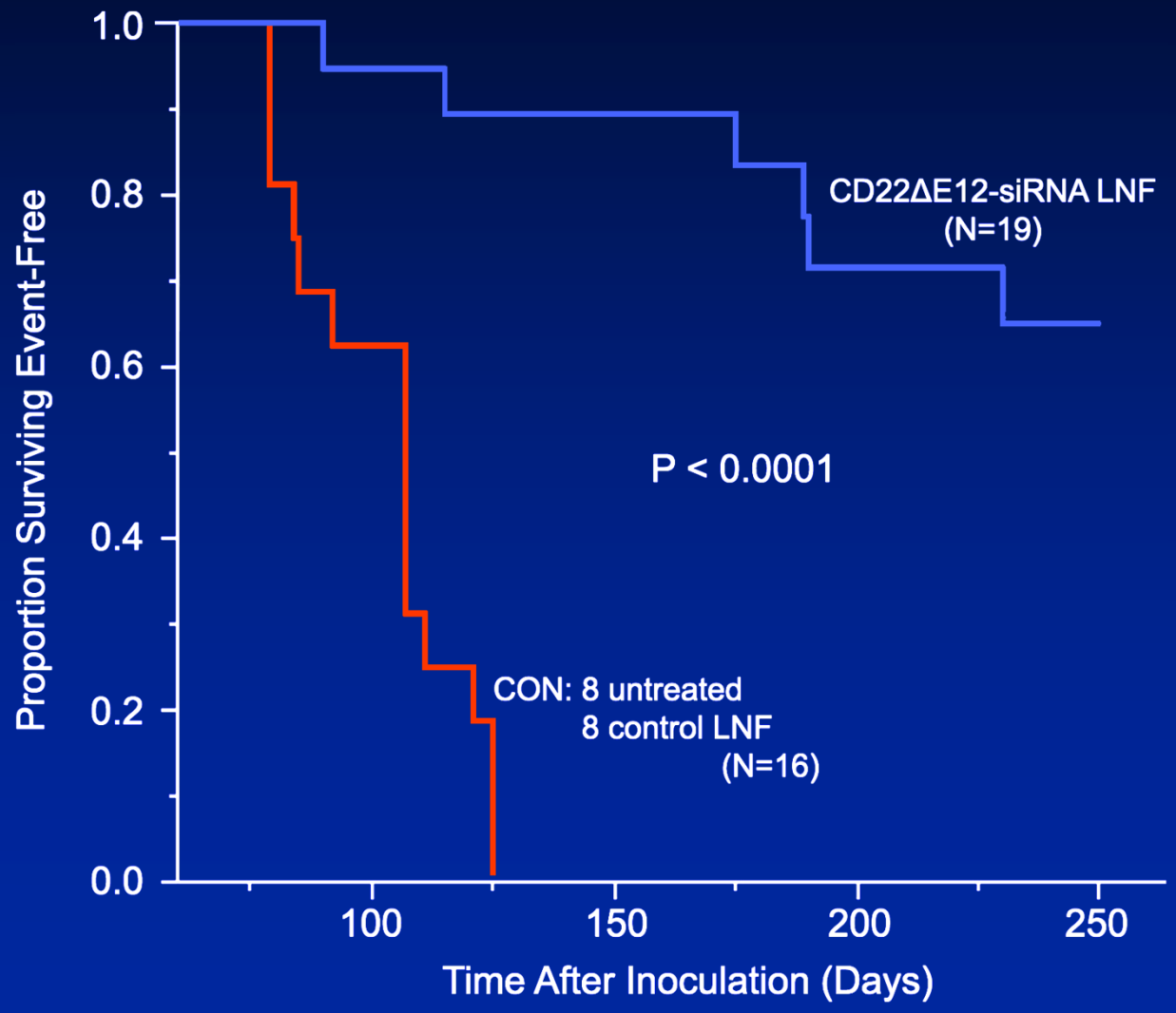


D

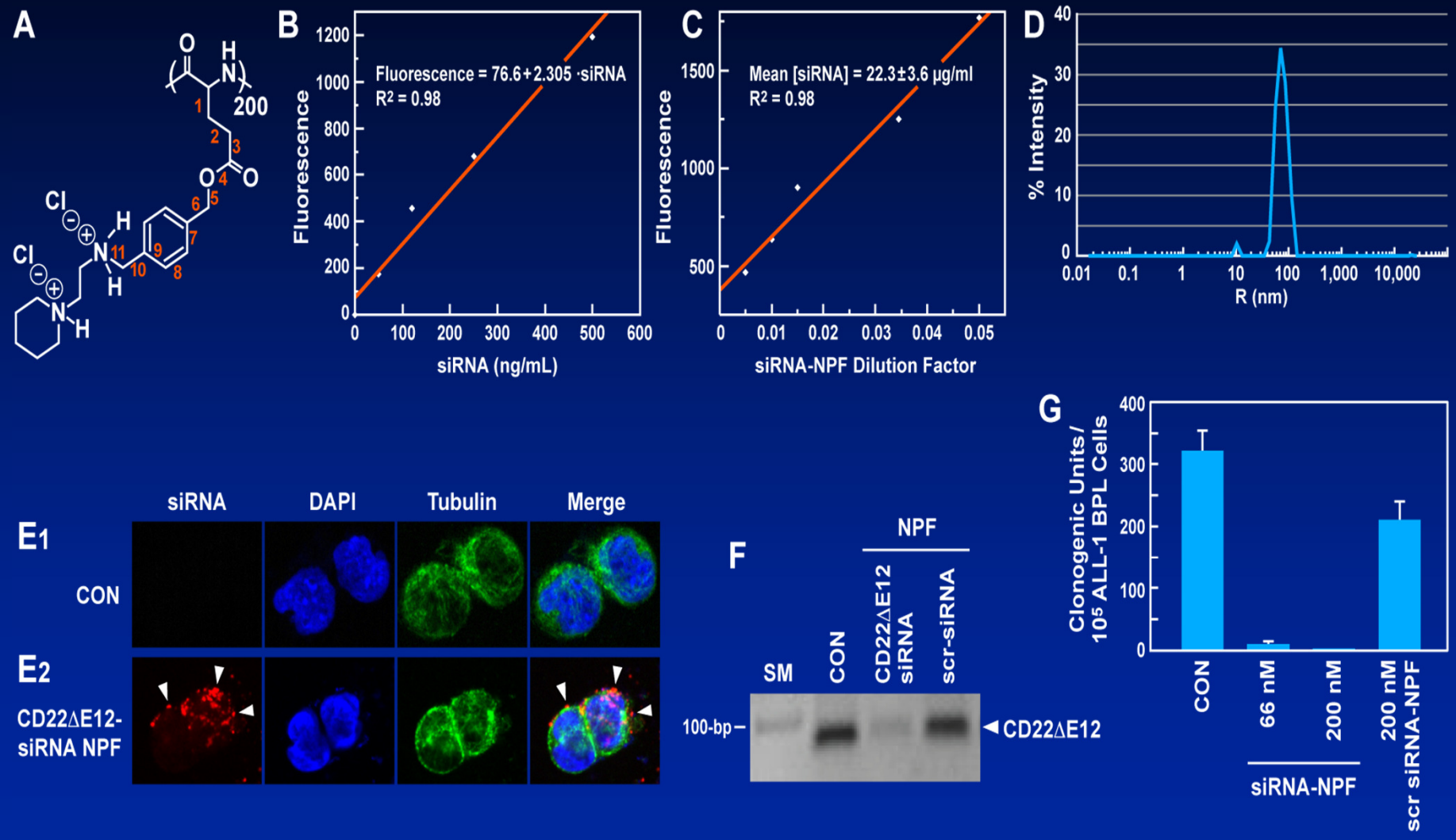
Leukemic Involvement	Treatment CON	Treatment 4A	P-Value (Fishers Exact, 2-tailed)
Bone Marrow	14/15	1/4	0.016
Meninges	14/15	1/4	0.016



In vivo anti-leukemic potency of CD22ΔE12-siRNA liposomal nanoformulation against relapse clones from pediatric B-precursor ALL patients



PVBLG-8-based Nanoformulations of CD22ΔE12-siRNA



Formulation platform: Yin et al., *Angewandte Chemie Internatl Ed,* 52:5757-5761, 2013

Antibody-like pRNA-3WJ Bivalent RNA Aptamers

