Expression Profiling in High Risk ALL: New Insights for Therapy and Novel Therapeutic Target Identification

NCI Meeting: Unique Characteristics of AYA Cancers
June 9-10, 2009

NCI CA114762 Strategic Partnerships to Evaluate Cancer Gene Signatures
Leukemia and Lymphoma Society Specialized Center of Research (7388-06)
The NCI TARGET and The Cancer Genome Atlas Projects

University of New Mexico Cancer Center
UNM High Performance Computing Center

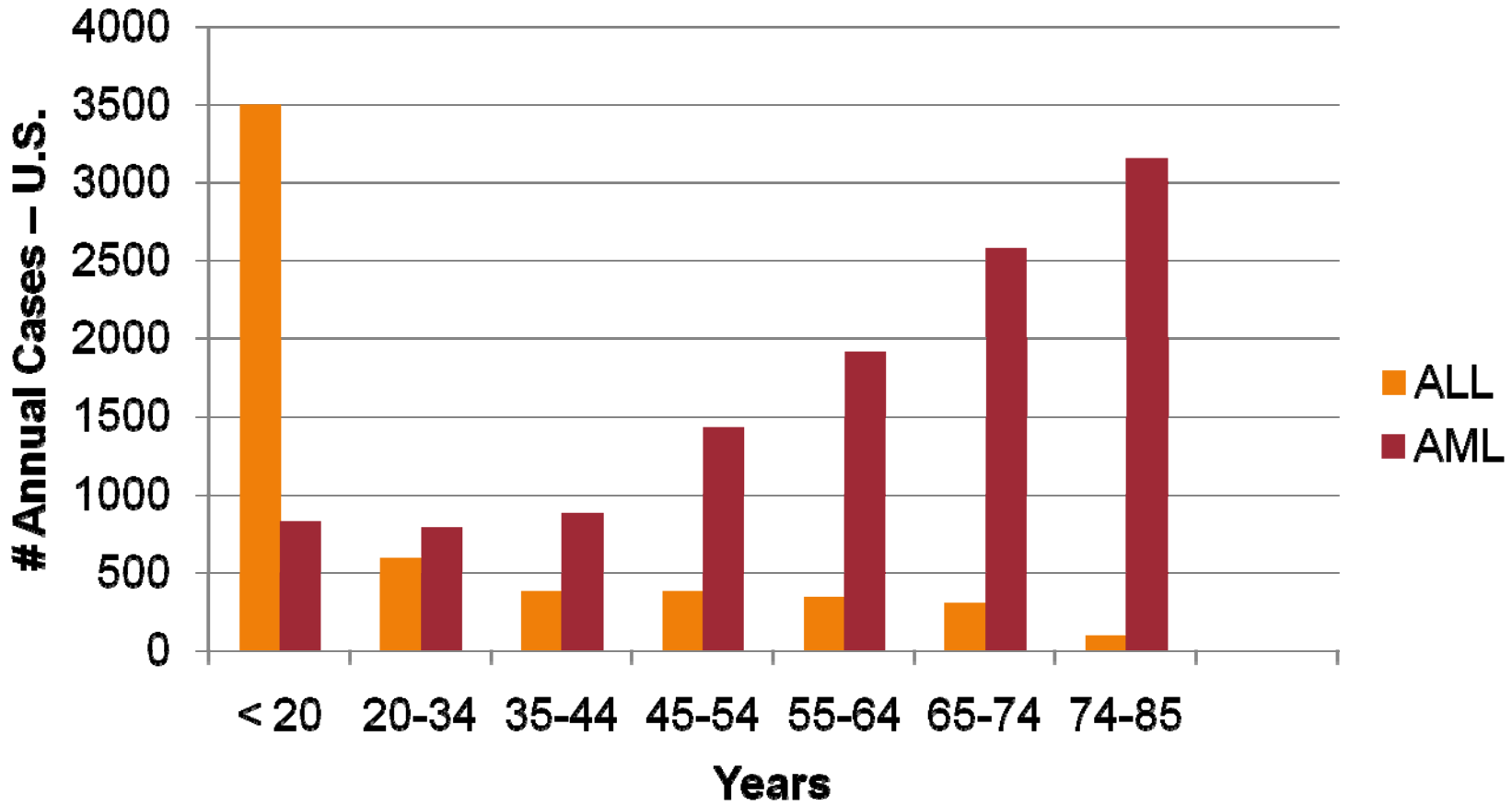
St. Jude Children's Research Hospital

NYU Cancer Center

National Cancer Institute

Children's Oncology Group /Southwest Oncology Group

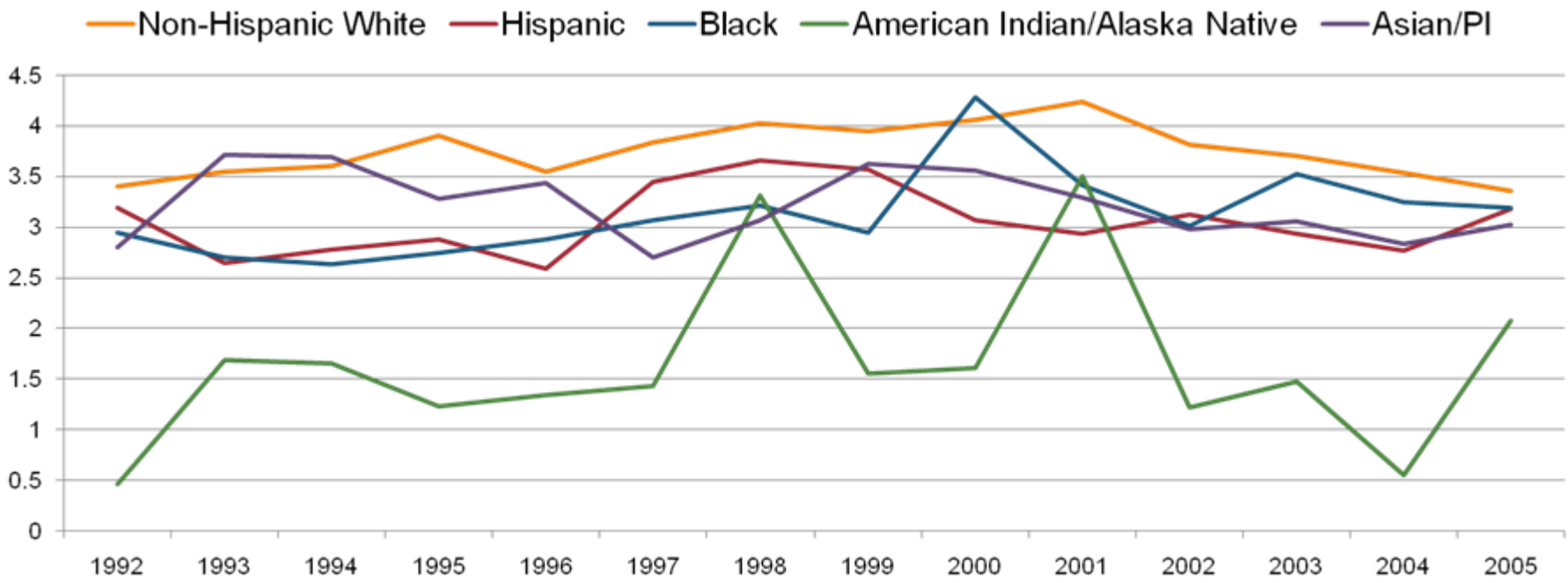
Annual U.S. Incidence of ALL and AML



http://seer.cancer.gov

Acute Myeloid Leukemia, 1992-2005

Average Annual Age-Adjusted Incidence Rate per 100,000 (US 2000 standard)



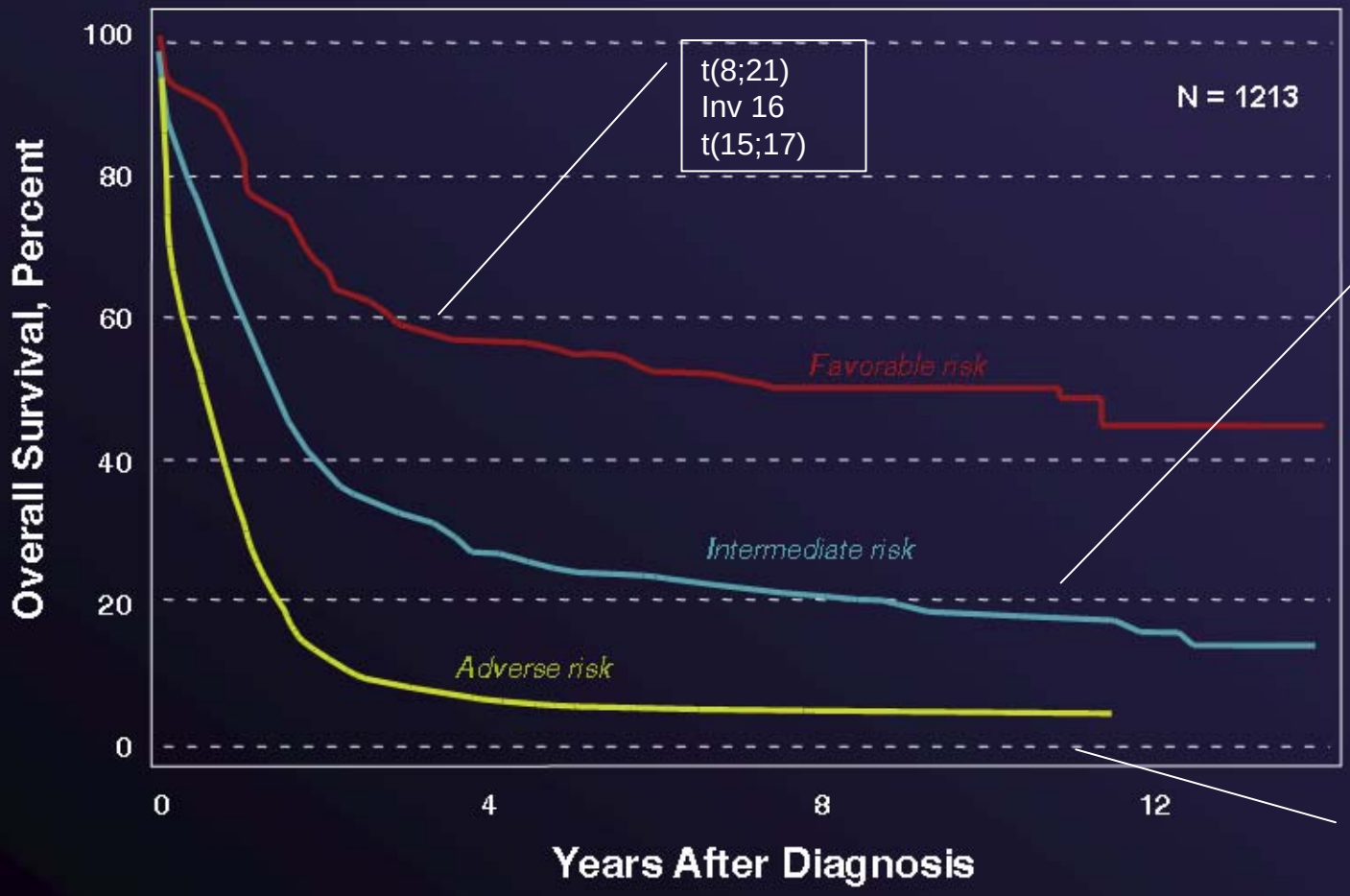
- AML incidence has been highest in non-Hispanic whites; African Americans and Hispanics have decreased CR rates and poorer OS
- Prognostic factors: age, de novo vs. 2º disease; genetic abnormalities
- Age is a continuous variable with poorer outcomes in each decade; disease biology/genetics become progressively more complex with age

http://seer.cancer.gov

AML: Karyotype and Prognosis

Results from Cancer and Leukemia Group B (CALGB 8641)

Overall Survival Patients Age 15 - 86 with Untreated AML



t(8;21)
Inv 16
t(15;17)

Normal Karyotypes

NPM1 - 53%
FLT3-ITD - 31%
FLT3-TK -11%
CEBPA - 14%
MLL- 8%
NRAS - 13%

Complex Cytogenetic Abnormalties

Byrd JC, et al. *Blood*. 2002;100:4325-4336.

AYA AML Outcomes

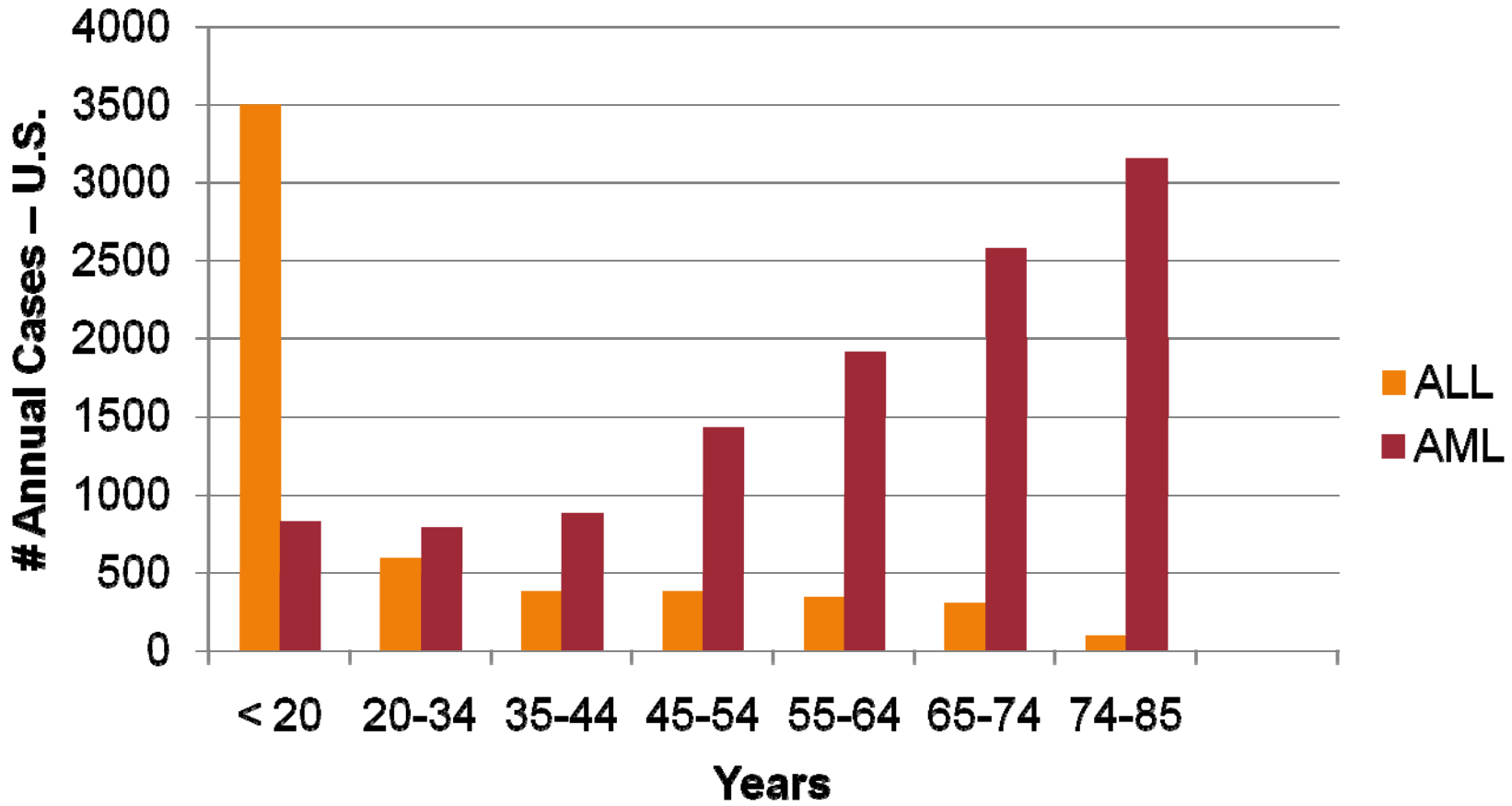
MRC AML 10 / AML 12 Trials: AYA AML Outcome Significantly Better
Advani , Hunger, Burnett; Sem. Oncol. 36(3):213-226, June 2009

		OS	CR Deaths	Good Risk	Standard Risk	Poor Risk
2-15 Yrs	708	59	9	77	56	34
16-24 Yrs	541	47	14	61	43	24
25-39 Yrs	1259	46	15	70	41	13
				P=.08	P=.0003	P=.0007

MD Anderson: All Pts (1965-2008); AYA Outcome Better than Older Adults
Pemmaraju et al., ASCO J. Clin. Oncol. 27:15S, June 2009; #7051

		CR	3-YR RFS
16-21 Yrs	Diploid Karyotype	81	46
22-45 Yrs	Diploid Karyotype	75	36
46-60 Yrs	Diploid Karyotype	68	28
> 60 Yrs	Diploid Karyotype	54	22

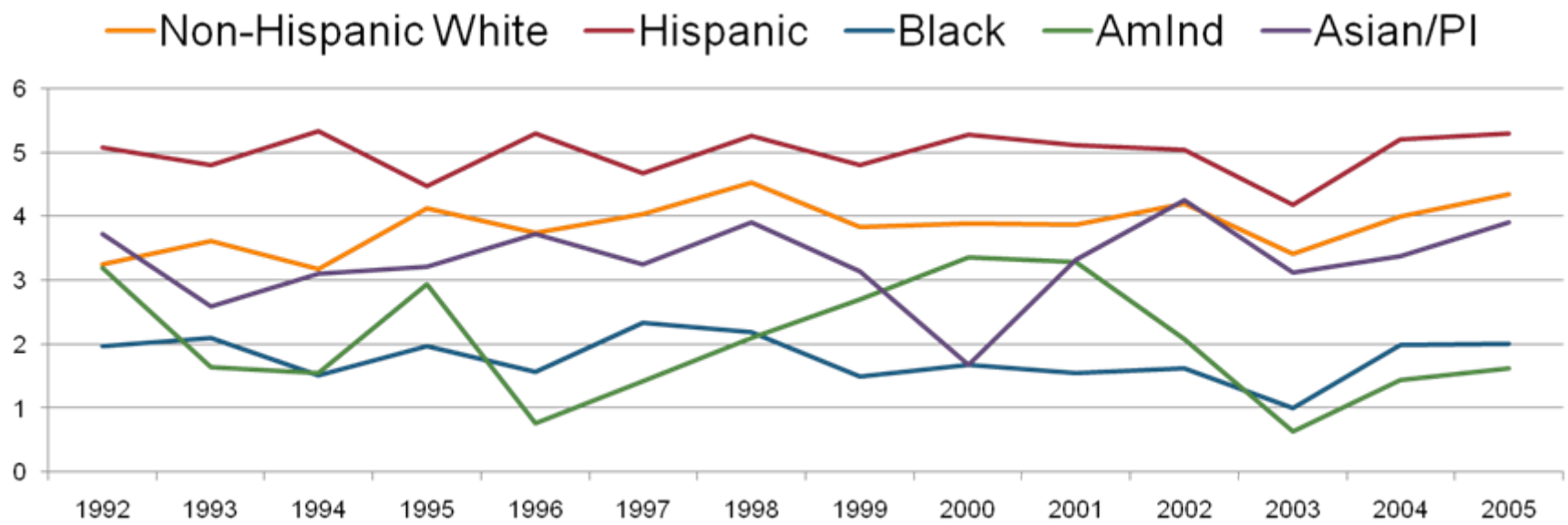
Annual U.S. Incidence of ALL and AML



http://seer.cancer.gov

Childhood Acute Lymphocytic Leukemia, 1992-2005

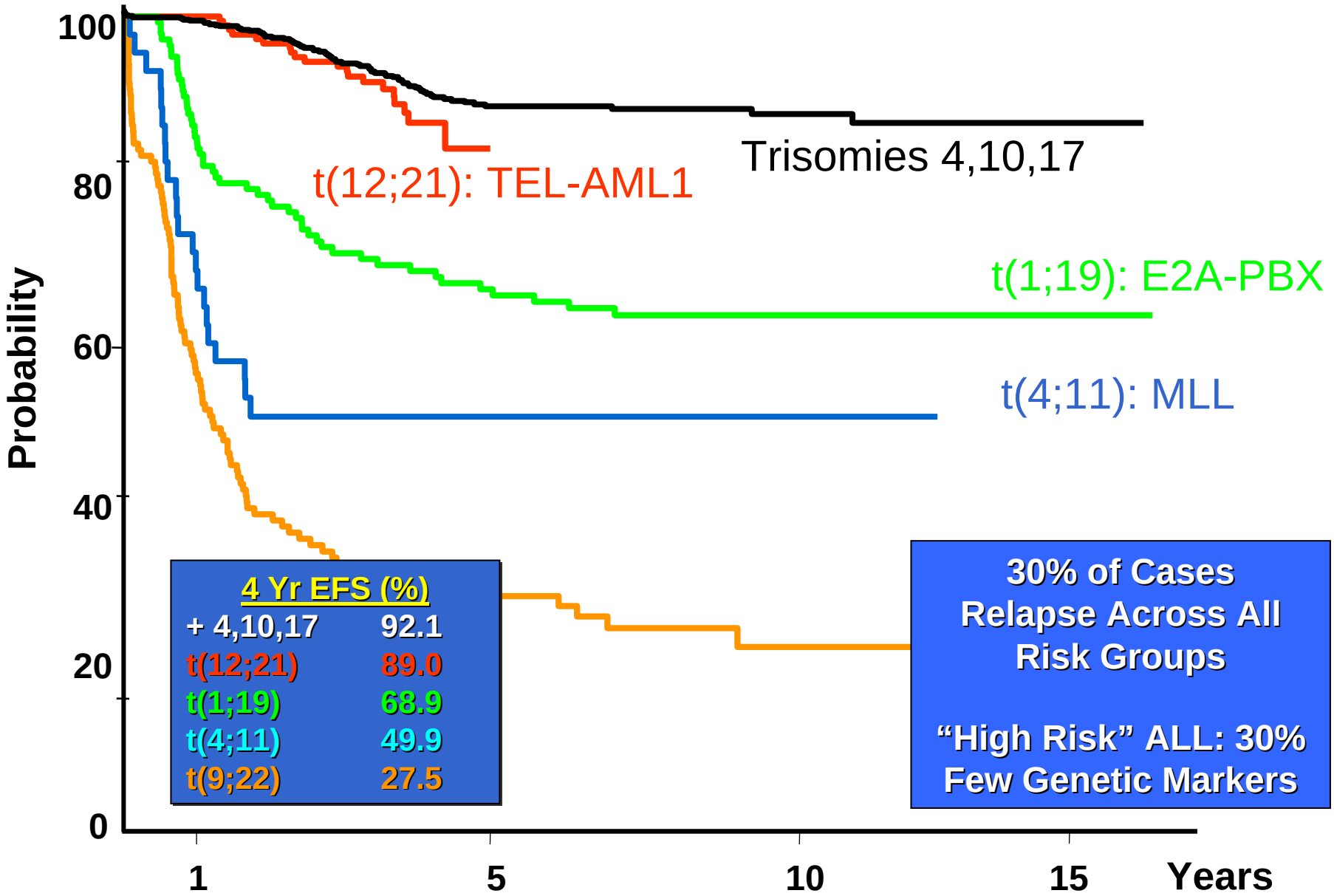
Average Annual Age-Adjusted Incidence Rate per 100,000 (US 2000 standard)



- Hispanic children and adolescents have the highest and African American children the lowest incidence rates.
- Hispanic, African American, American Indian, and Alaskan Native children have poorer outcomes and decreased overall survivals
- ALL survival begins to decrease dramatically after puberty

http://seer.cancer.gov

Outcome in Pediatric Acute Lymphoblastic Leukemia (ALL)



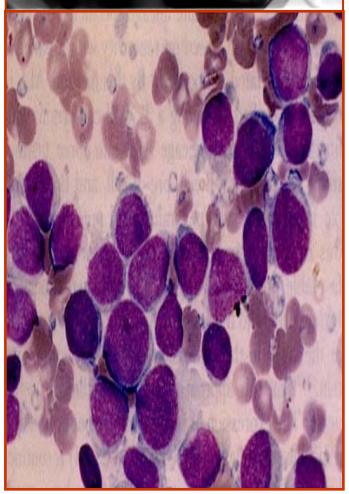
Association of ALL Prognostic Factors with Age in AYA ALL

	1-9 Yrs	10-14 Yrs	15-19 Yrs	20-39 Yrs	>40 Yrs	P-Value
* Trisomy / Hyperdiploidy	27	11	19	6	4	P=0.01
^ t(12;21)	27	12	7			P<0.01
* t(9;22)	1	3	4	12	19	P<0.01
*T-ALL	6	22	19	20	8	P=0.01
^ Prednisone Response	5	9	9			P=0.03
^ D 15 Marrow Response > 25 % Blasts	12	18	30			P=0.01

* MRC ALL Trials (1985 – 1992); ^ BFM Trials (1986-1999)

Barry and Silverman, Pediatric Malignant Hematology

Our Approach



- Focused on a Cohort of 207 Children with High Risk ALL Uniformly Treated on COG 9906 (Augmented BFM)

Sex:	70 Females; 137 Males
Race:	126 White; 51 Hispanic; 13 Black; 7 Asian; 3 American Indian/Alaskan Native; 7 Other
Age:	132 > 10 yrs; Mean: 13.5 Yrs
WBC:	108 > 50K
Genetics:	23: t(1;19), 21: MLL, Others Unknown
4 Year EFS:	61%
Predictors:	WBC ($p < 0.001$); Flow MRD ($p < 0.0001$)

- Performed comprehensive gene expression profiling of pre-treatment leukemic samples (Affymetrix U133 Plus2.0 (54,675 Probe Sets) and Affymetrix Human Exon 1.0 (1.5 Million Probe Sets))

NCI TARGET PROJECT

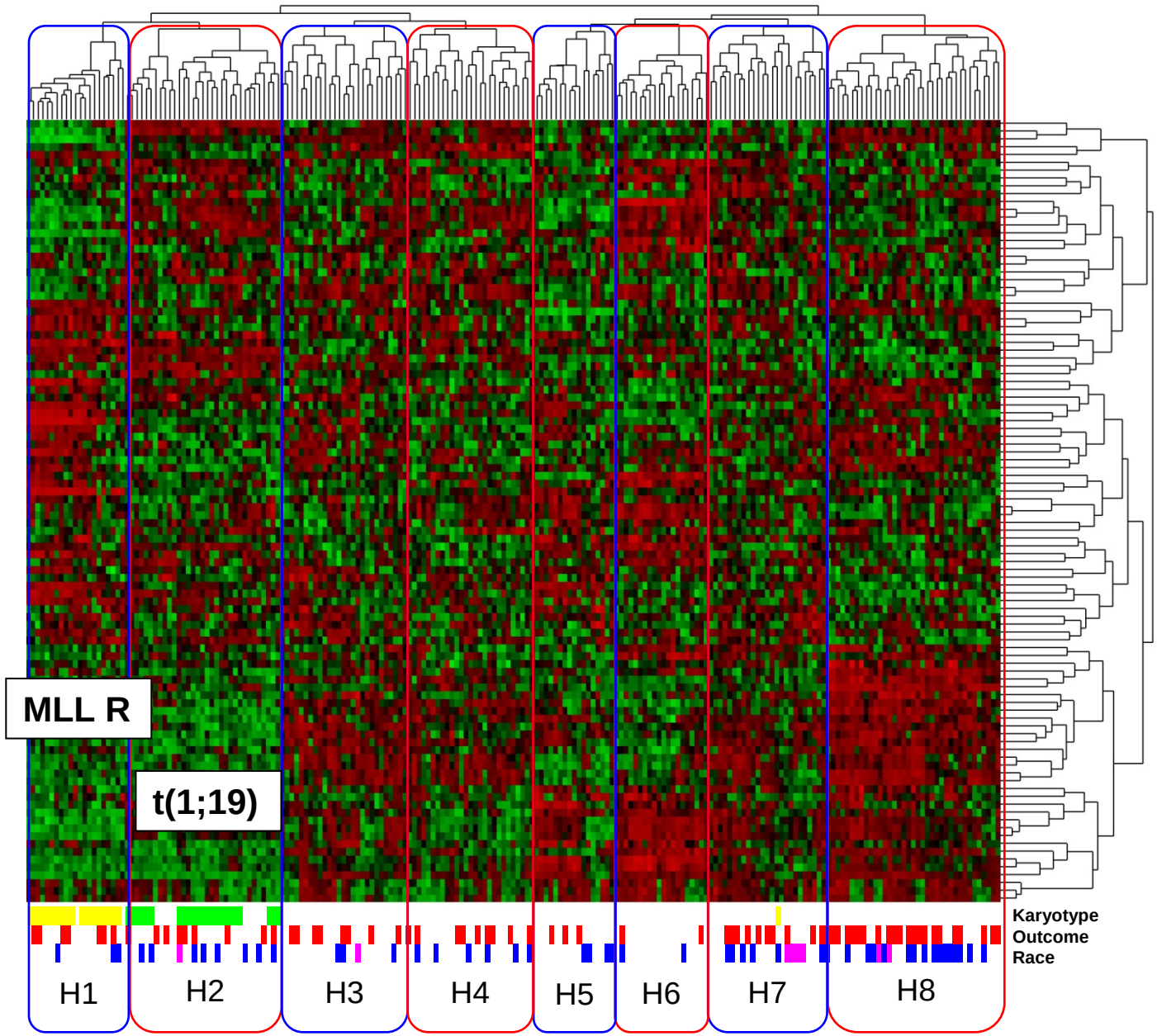
Therapeutically Applicable Research to Generate Effective Treatments

Comprehensive Molecular Analysis of High Risk ALL: 9906

- *Gene Expression Profiling (Cheryl Willman, UNM): Prediction of Outcome, Novel Cluster / Target Identification, Pathways Analysis*
- *Whole Genome SNP / LOH Analysis for Polymorphic Variation Predictive of Response and Toxicity on Leukemic Cells and Germline DNA (Mary Relling, St Jude)*
- *Whole Genome SNP / LOH Analysis for Copy Number Change to Identify Regions of Chromosomal Gain/Loss in Leukemic Cells (James Downing/Charles Mullighan, St. Jude)*
- *Comprehensive Sequencing of Selected Target Genes and Selected Cases for Whole Transcriptiome Sequencing (NCI CGAP)*

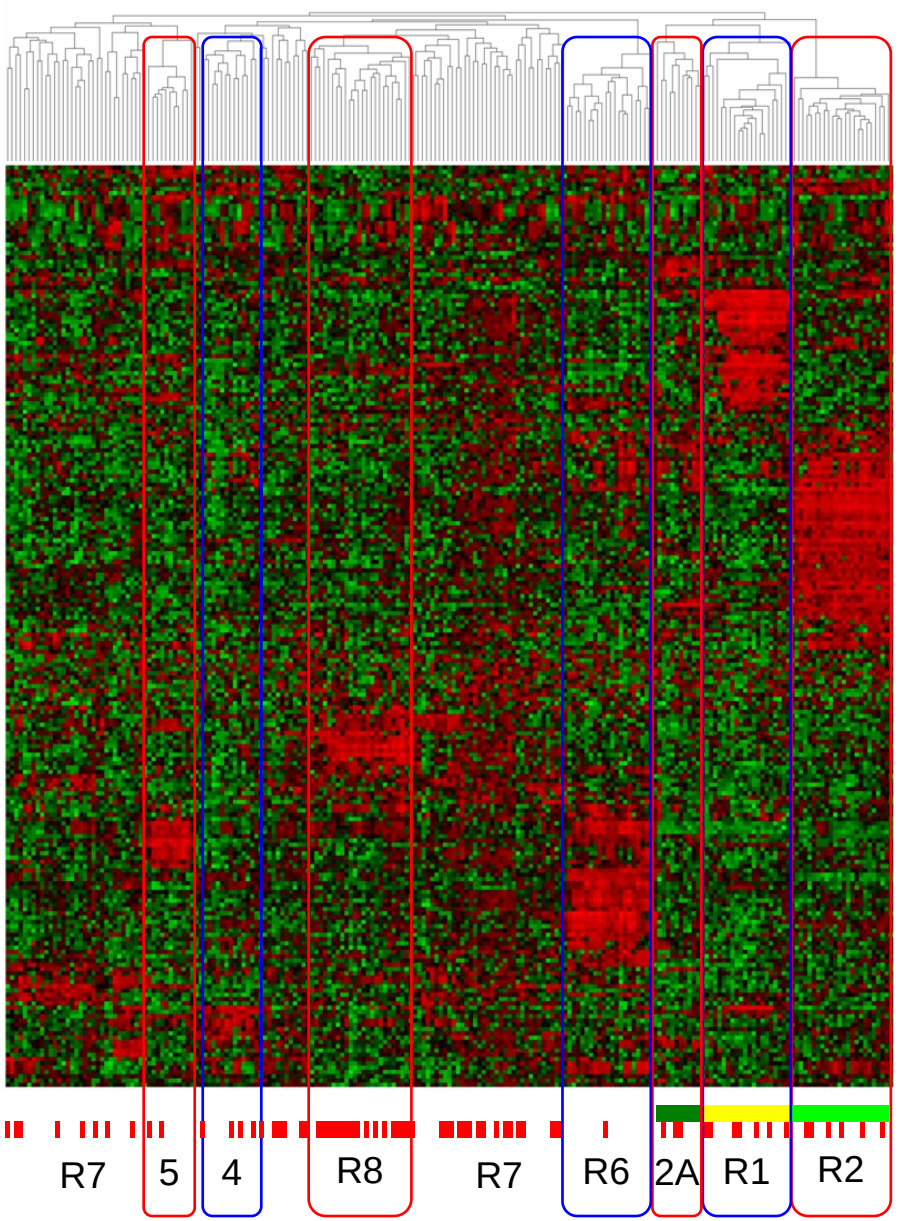
COG: **S Hunger, A Carroll, M Devidas, G Reaman**
NCI: **M Smith, K Dobbin, J Jacobson, D Gerhardt, J Zhang**

Hierarchical Clustering with 100 Probe Sets

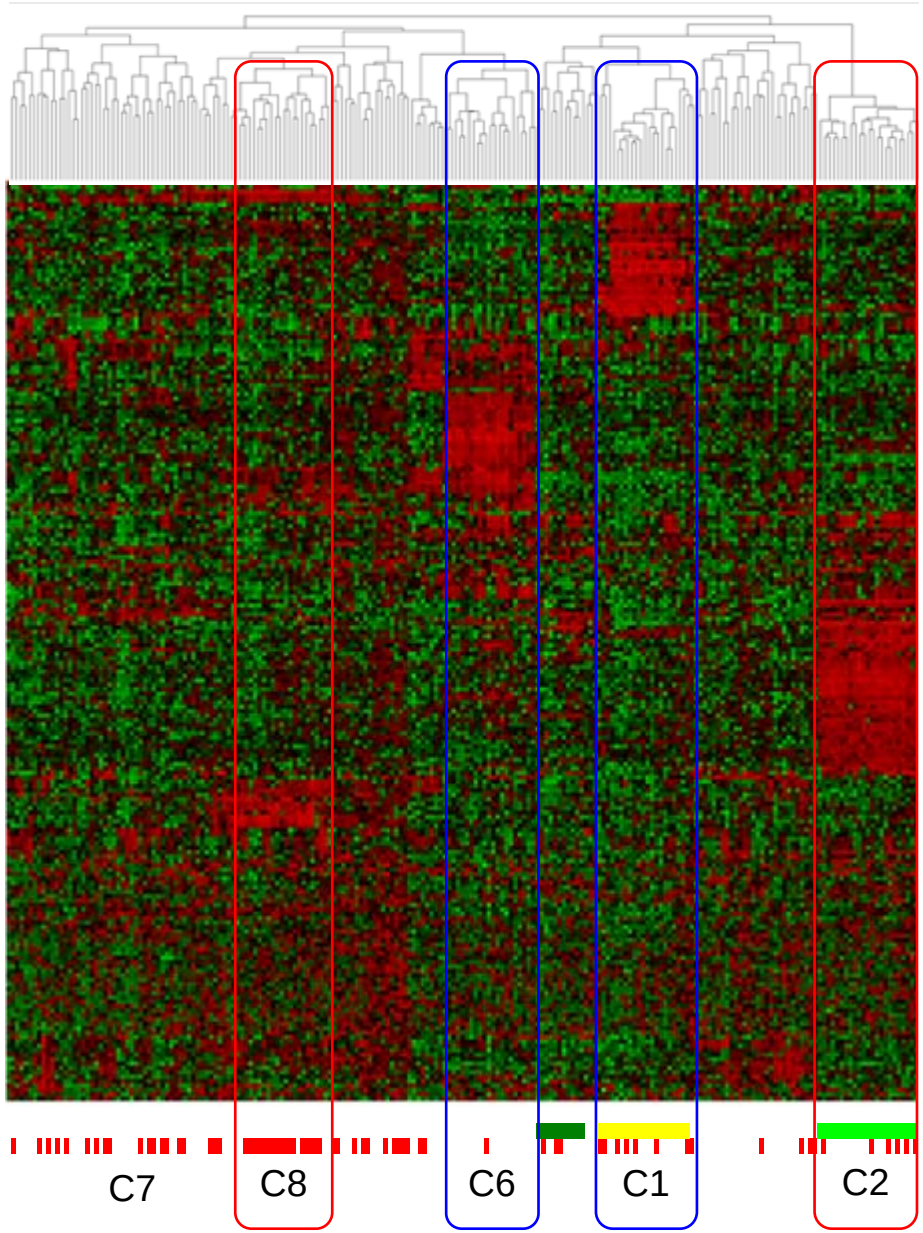


Harvey et al, Blood, in review

Clusters w/215 ROSE Genes



Clusters w/215 COPA Genes

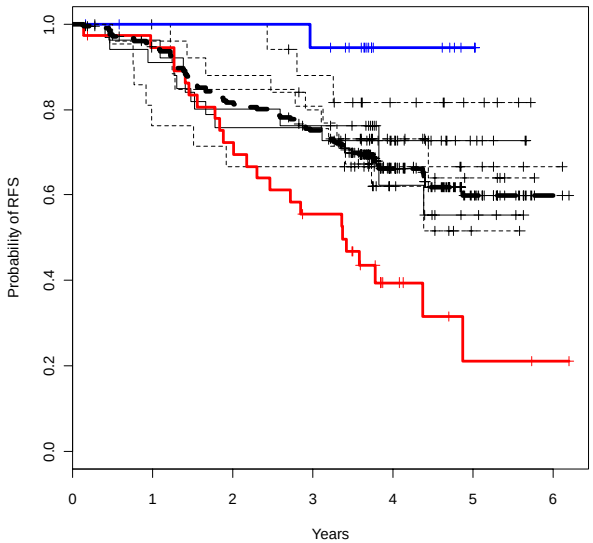


Harvey et al, Blood, in review

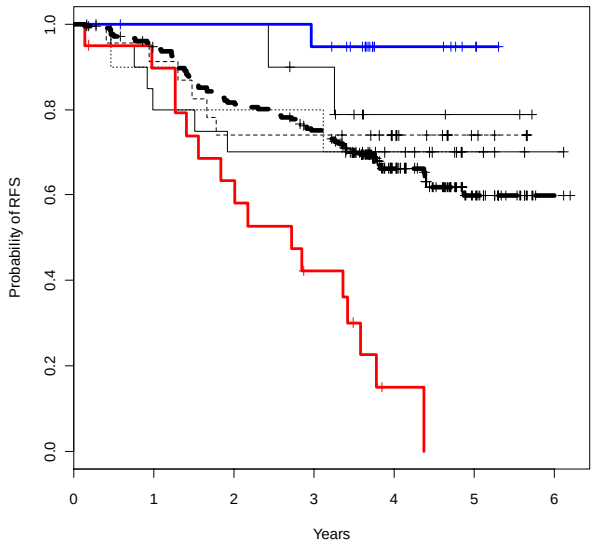
Association of Clinical and Outcome Features with ROSE Gene Expression Cluster Groups

	R1	R2	R2A	R5	R6	R8	Not Clustered	P-Value
# Cases / Cluster	21	23	12	10	21	24	96	
Median Age (Yrs)	4.7	13.1	15.2	14.5	14.5	14.1	11.8	
Sex (Male)	11 (52%)	11 (48%)	6 (50%)	8 (80%)	17 (81%)	17 (71%)	67 (70%)	0.118
Race (Hispanic)	4 (19%)	6 (26%)	2 (17%)	0 (0%)	3 (15%)	15 (63%)	21 (22%)	<0.001
MLL	21 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<0.001
TCF3-PBX1	0 (0%)	23 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<0.001
D29 MRD	9 (53%)	0 (0%)	1 (9%)	8 (80%)	6 (29%)	19 (83%)	24 (27%)	<0.001
Median WBC	125.8	67.2	49.6	31.5	26.0	153.8	45.0	
RFS - 1Yr	0.762	0.913	0.909	1.000	1.000	0.915	0.979	<0.001
RFS - 2Yrs	0.667	0.739	0.818	1.000	1.000	0.697	0.841	
RFS - 3Yrs	0.667	0.739	0.818	0.900	0.947	0.479	0.777	
RFS - 4Yrs	0.667	0.739	0.727	0.788	0.947	0.210	0.675	
RFS - 5Yrs	0.667	0.739	0.727	0.788	0.947	0.000	0.567	

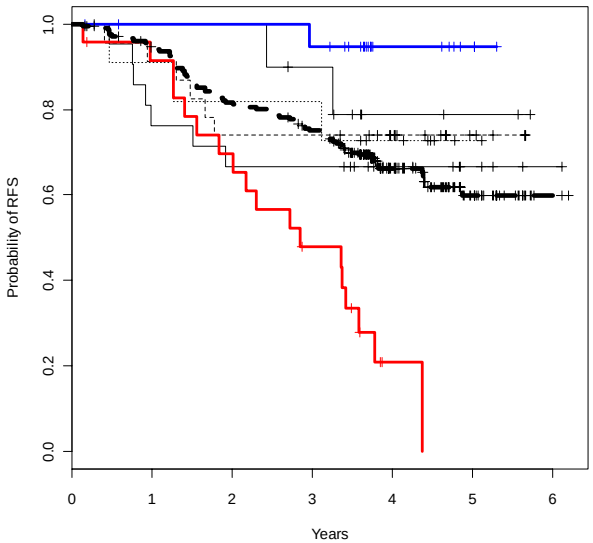
H-Clusters



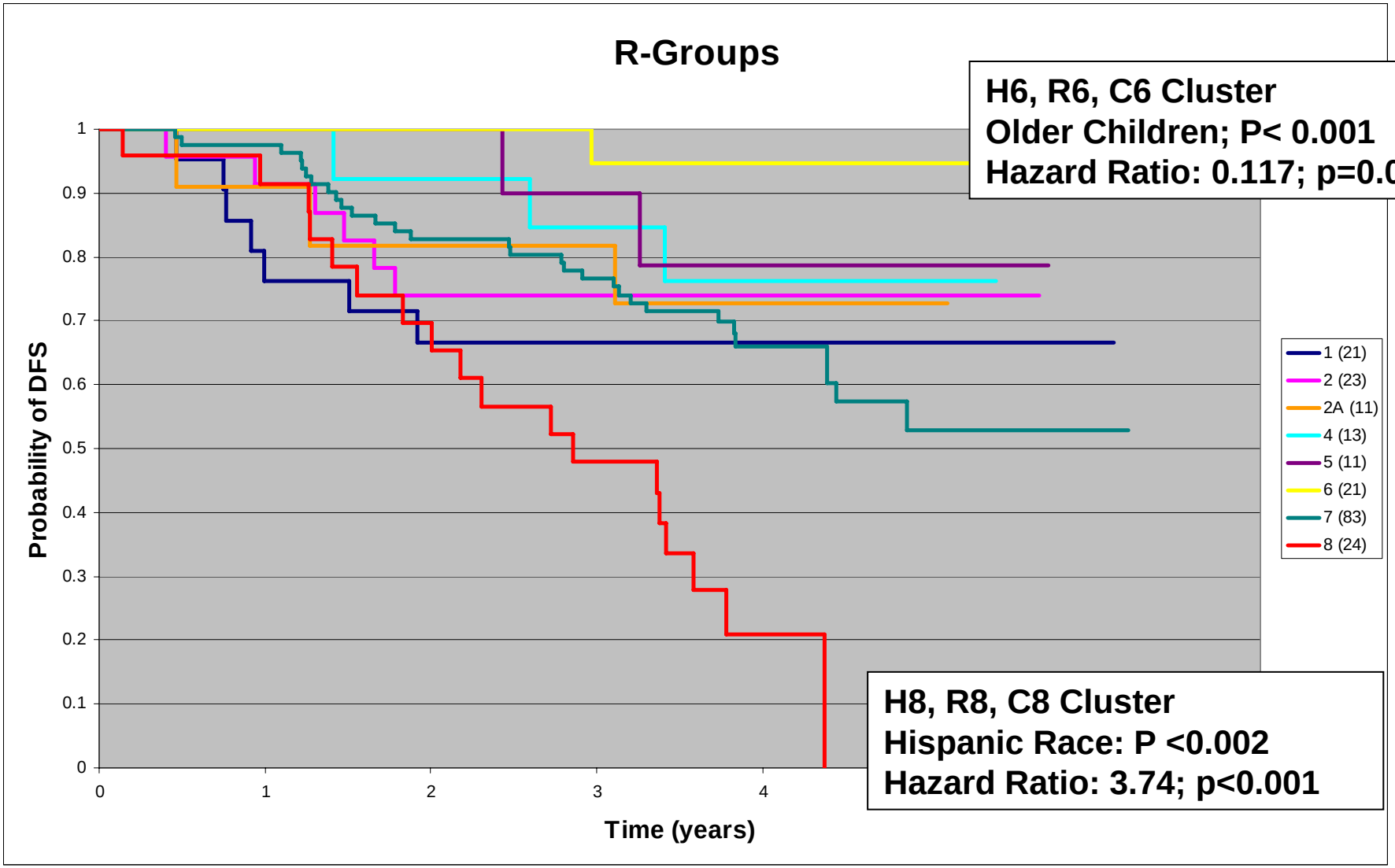
C-Clusters



R-Clusters

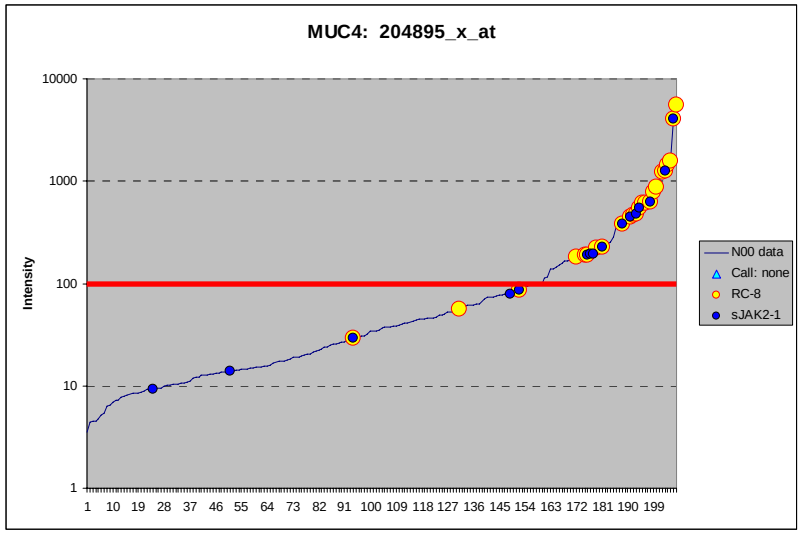


Disease Free Survival for ROSE Cluster Groups



Harvey et al, Blood, in review

MUC4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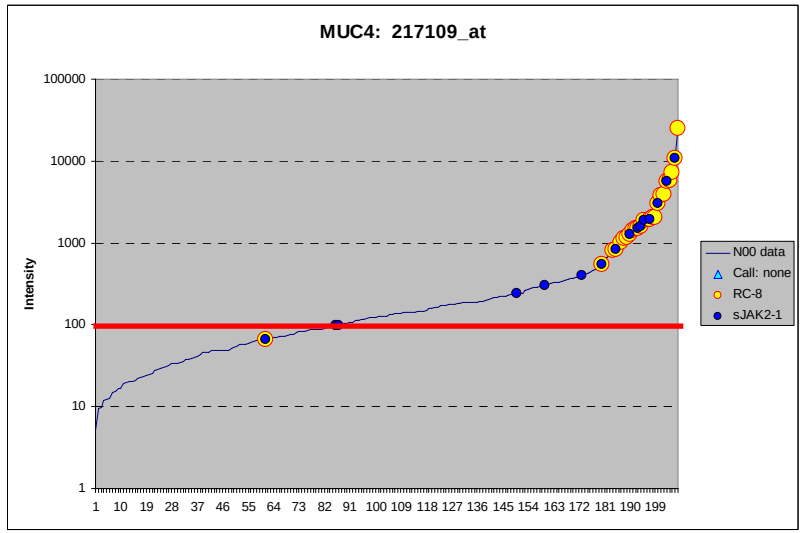
Intensity
10000
1000
100
10
1
1 10 19 28 37 46 55 64 73 82 91 100 109 118 127 136 145 154 163 172 181 190 199
N00 data
Call: none
RC-8
sJAK2-1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10000
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1
1 10 19 28 37 46 55 64 73 82 91 100 109 118 127 136 145 154 163 172 181 190 199
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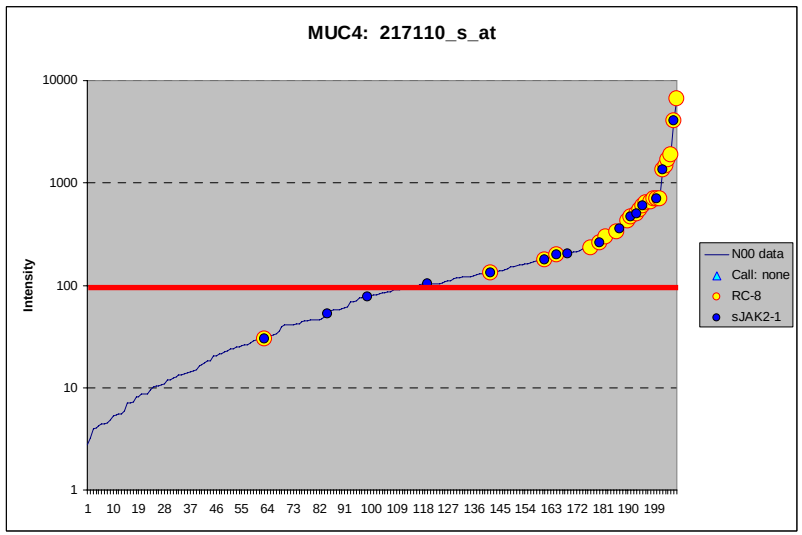
RC-8
sJAK2-1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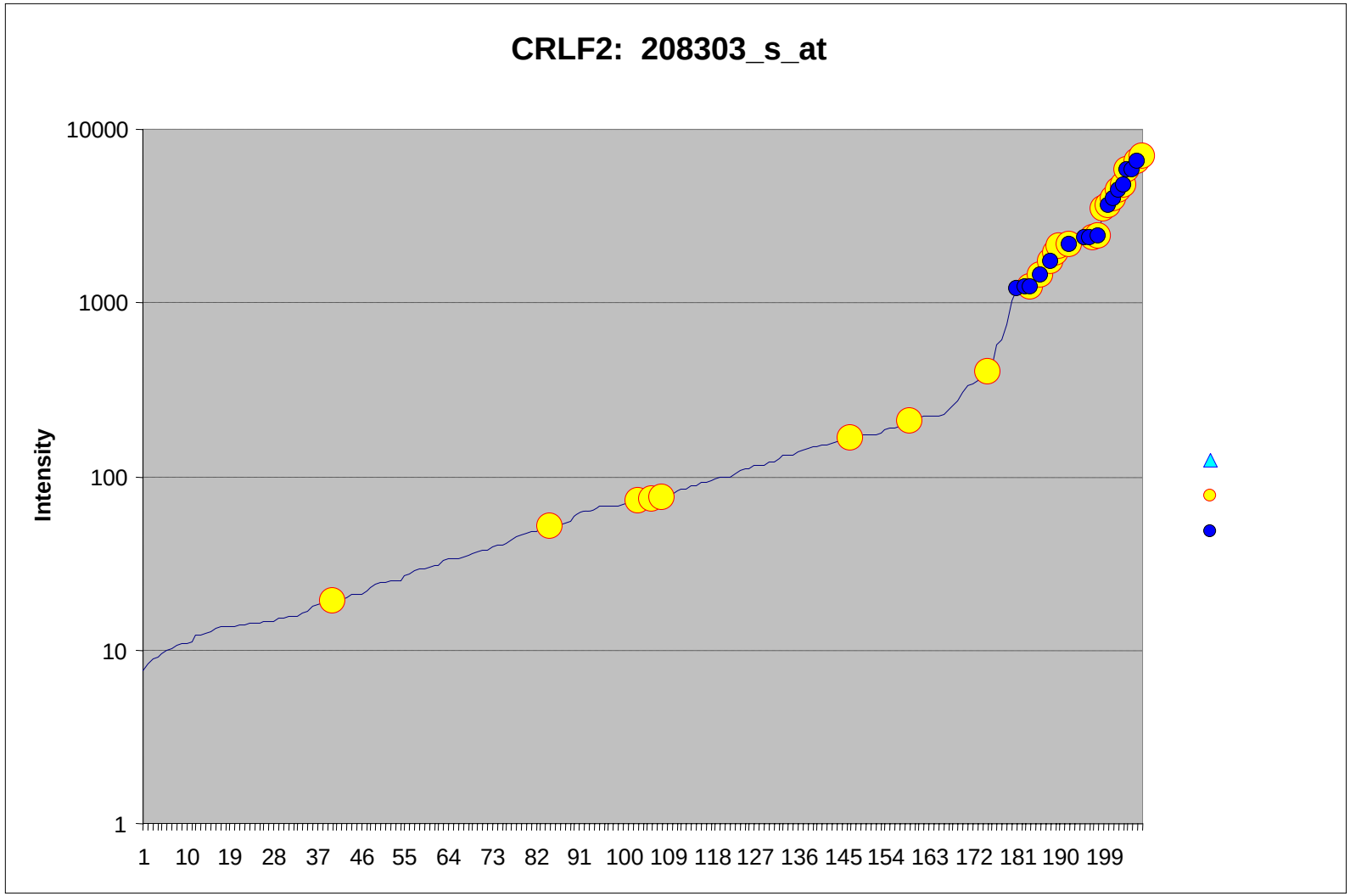
Call: none
RC-8
sJAK2-1

CRLF2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100
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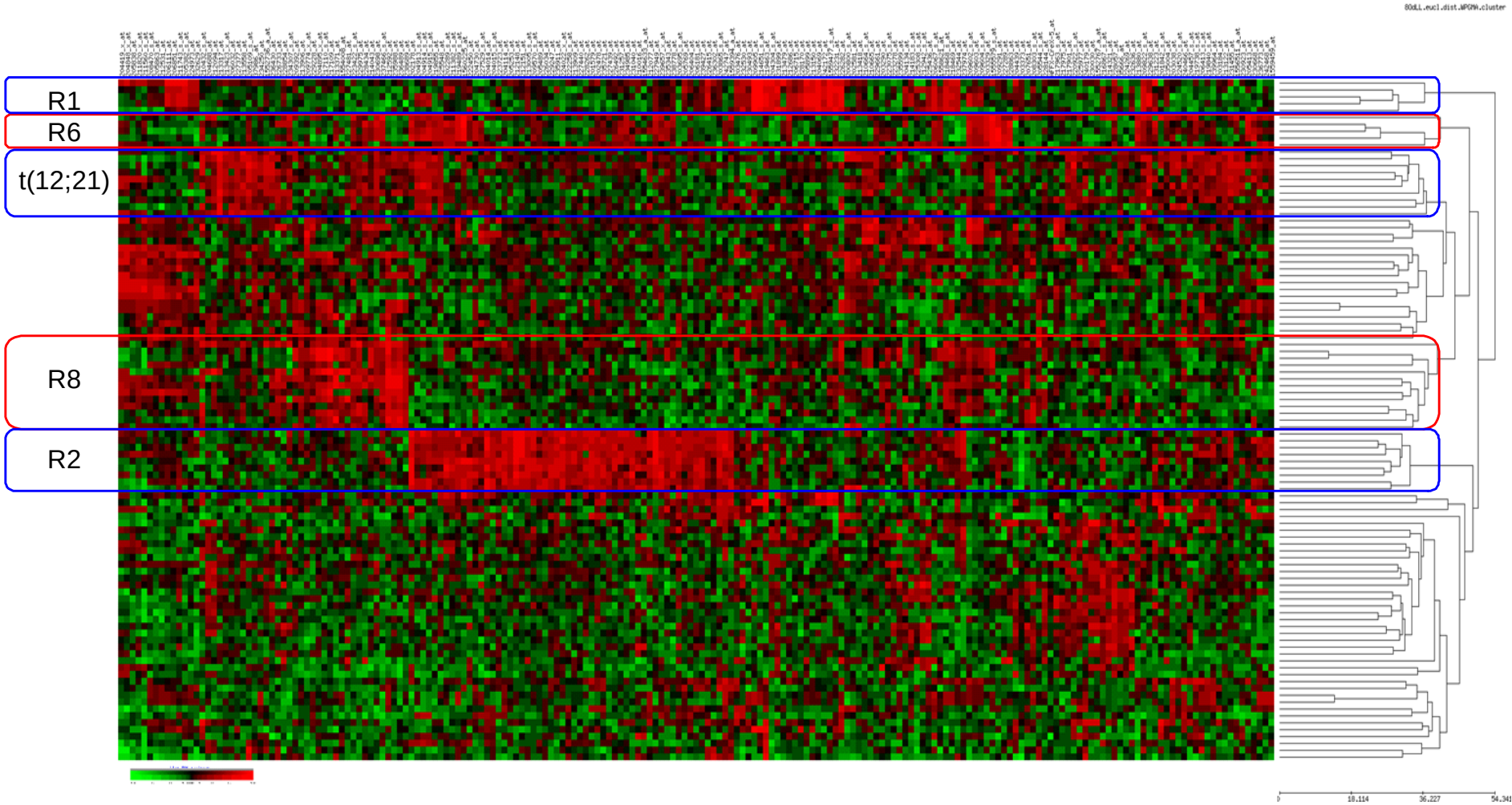
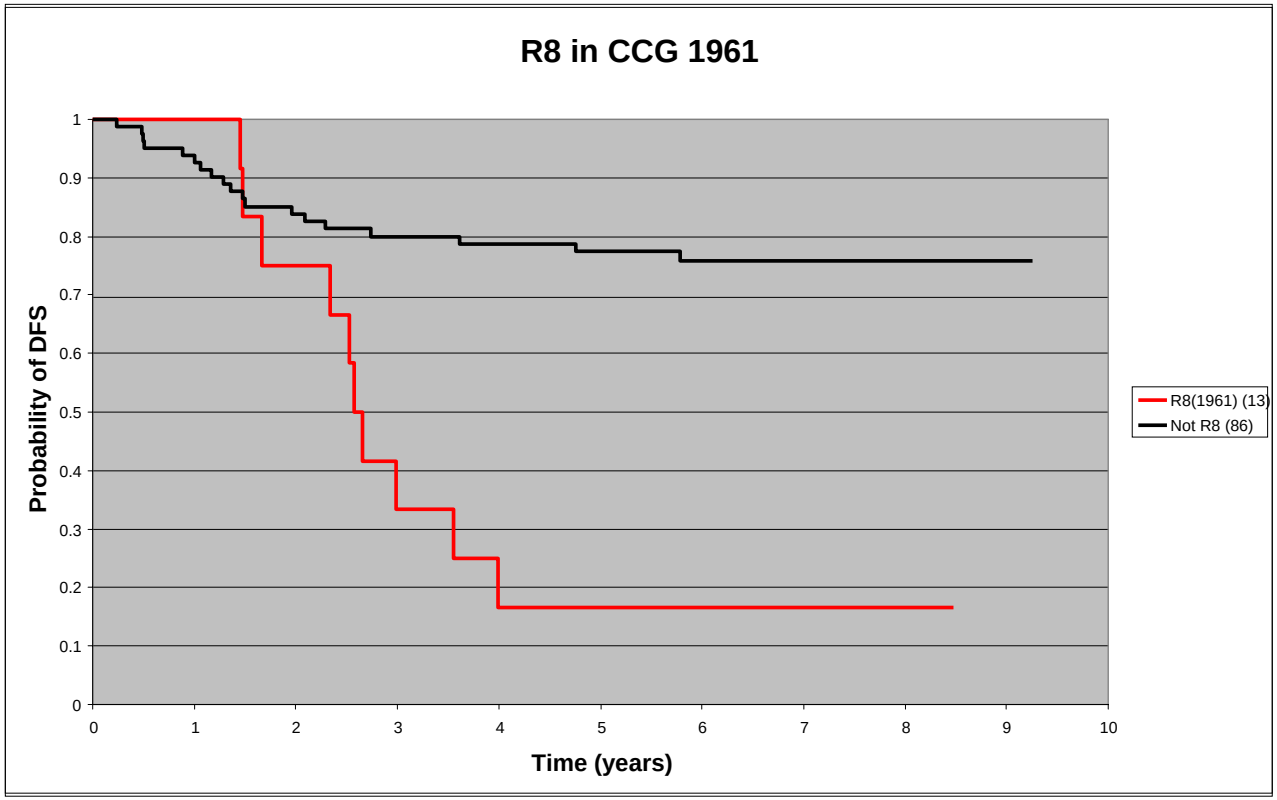
1
1 10 19 28 37 46 55 64 73 82 91 100 109 118 127 136 145 154 163 172 181 190 199

ROSE Clustering: COG CCG 1961



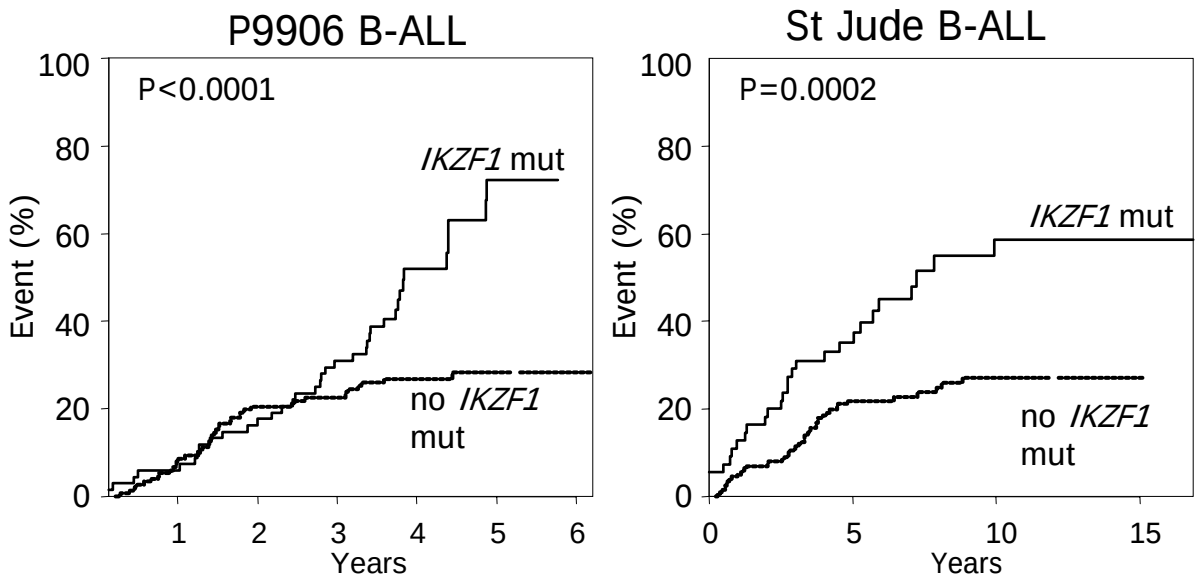
Relapse -Free Survival of R8 Cluster in CCG1961



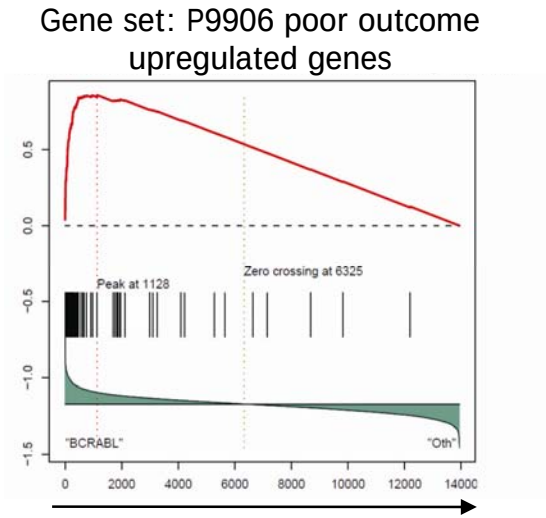
Correlation of Genome-Wide DNA Copy Number Abnormalities and ROSE Gene-Expression Cluster Groups

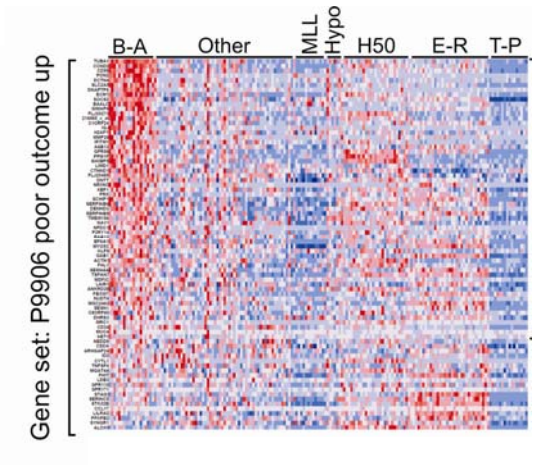
Rose Cluster Group	R1	R2	R2A	R5	R6	R8	No Cluster	P-Value
# Cases / Cluster	20	22	11	11	21	24	89	
DNA Copy Number Abnormality								
1q gain	0	14	0	1	0	0	2	<0.0001
EBF1	0	0	0	0	0	9	4	<0.0001
IKZF1	1	0	0	2	6	20	26	<0.0001
CDKN2A-B	4	9	10	2	5	15	51	<0.0001
TCF3	0	14	0	2	2	0	2	<0.0001
ERG	0	0	0	0	8	0	1	<0.0001
VPREB1	0	0	0	1	8	14	28	<0.0001
B cell pathway**	5	17	5	4	12	23	66	<0.0001
B cell pathway including VPREB1**	5	17	5	5	14	24	68	<0.0001
TBL1XR1	0	0	3	1	1	0	0	0.0002
PAX5 CNA	1	9	4	0	3	7	39	0.0005
RAG1-2	1	0	1	0	0	5	0	0.0005
NUP160-PTPRJ	0	0	0	0	0	4	0	0.0014
ETV6	1	0	3	4	1	0	15	0.0031
DMD	0	5	1	2	3	0	3	0.0059
IL3RA-CSF2RA	0	0	1	1	0	7	6	0.0061
C20orf94	0	0	0	1	0	7	8	0.0073
ADD3	0	1	0	0	0	7	9	0.0144
NF1	1	1	0	2	0	1	0	0.0188
ARMC2-SESN1	0	2	0	2	0	5	4	0.0291

IKZF1 (IKAROS) Alterations are Associated with a BCR-ABL "Activated Kinase"-like Gene Expression Signature



Gene set enrichment: Similarity of signatures of Ph+ (*IKZF1* deleted) and Ph- (*IKZF1* deleted) ALL





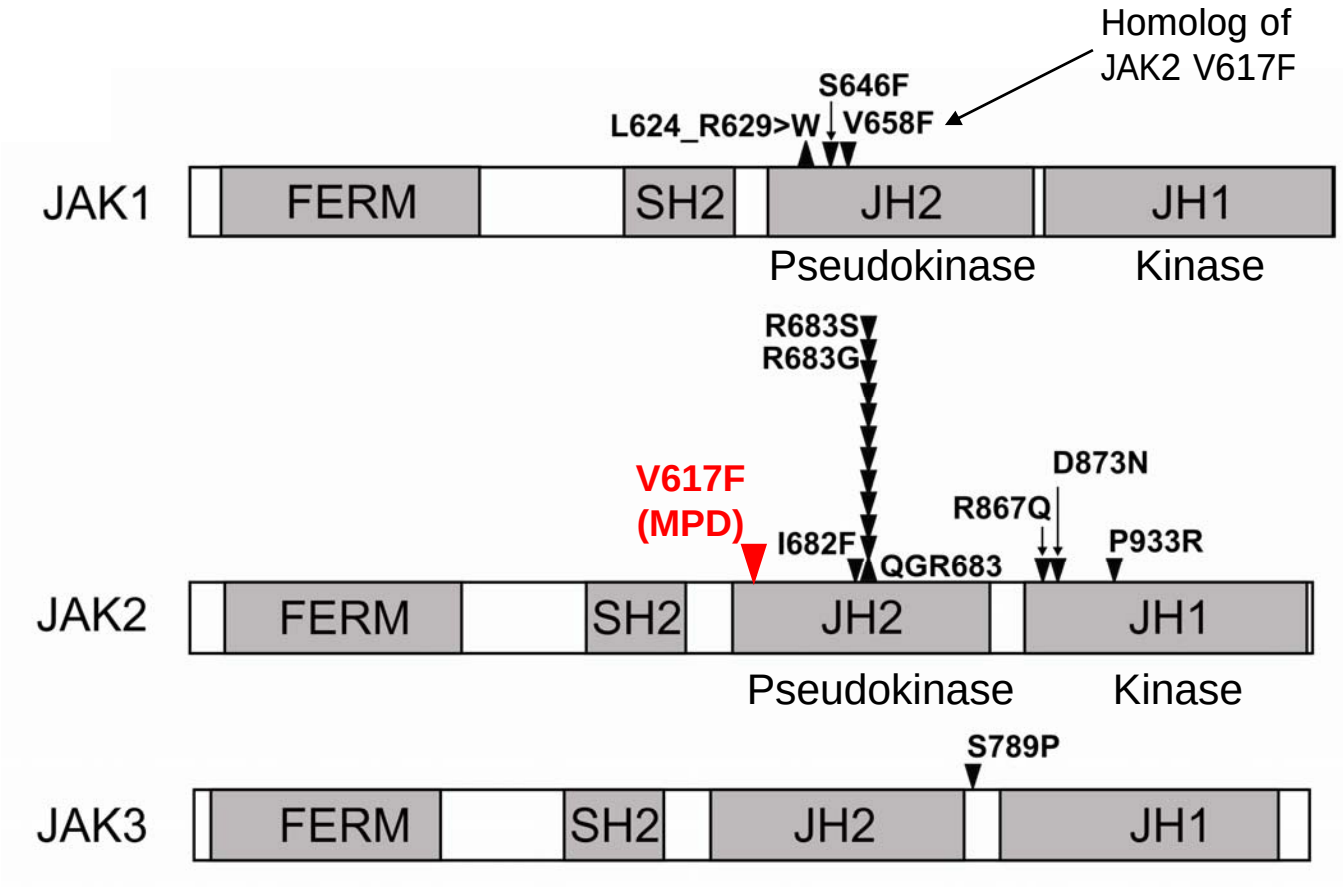
NEJM 2009;360:470

TARGET Kinase Targeted Sequencing

- JAK family sequenced in 187 cases with available material
- *JAK1*, *JAK2*, *JAK3*, *TYK2*
- 20 (10.7%) cases harbored somatic heterozygous *JAK1*, *JAK2*, *JAK3* mutations

PNAS 2009; in press.

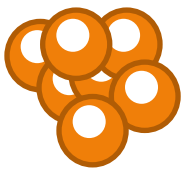
JAK mutations in high-risk B-ALL



PNAS 2009; in press.

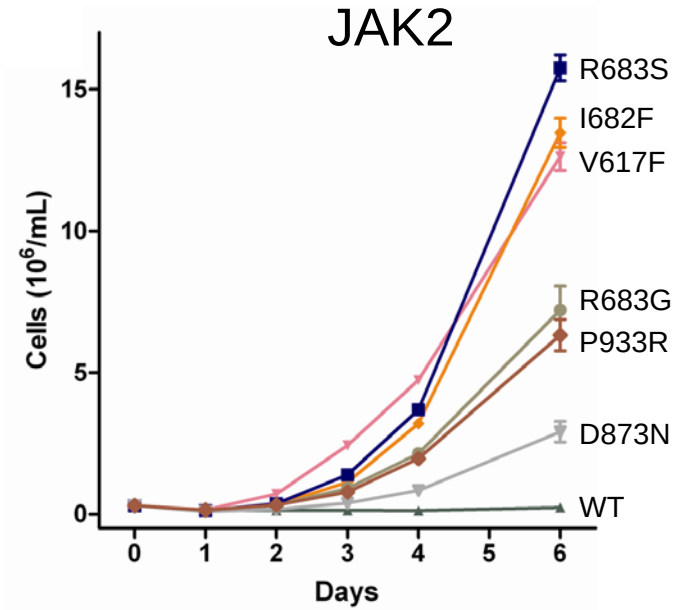
JAK Mutations Confer Factor Independence in Ba/F3 EPO Cells

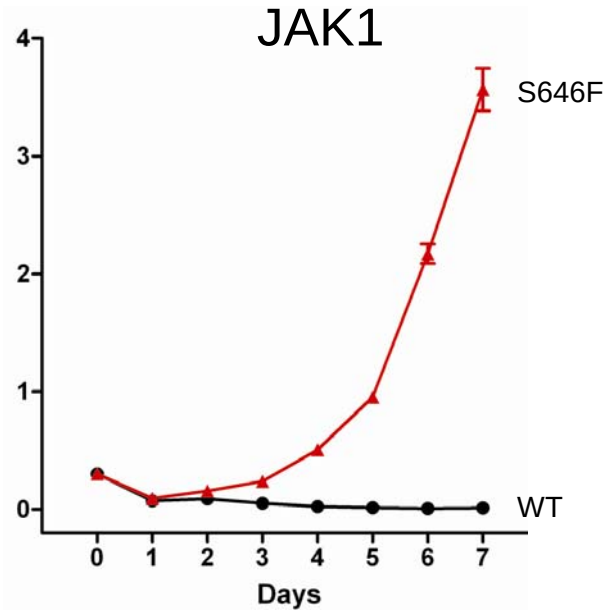
MSCV-mJak1/2-IRES-GFP
(WT and mutant)

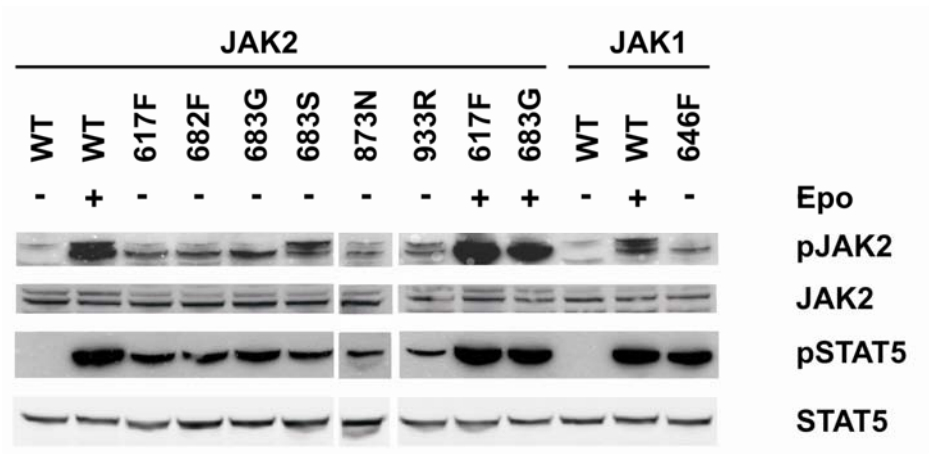


Ba/F3-EpoR
(mouse Pro-B)

GFP sort
Wash
Withdraw cytokine
Monitor growth
Phosphosignaling
Treat with JAK inhibitors

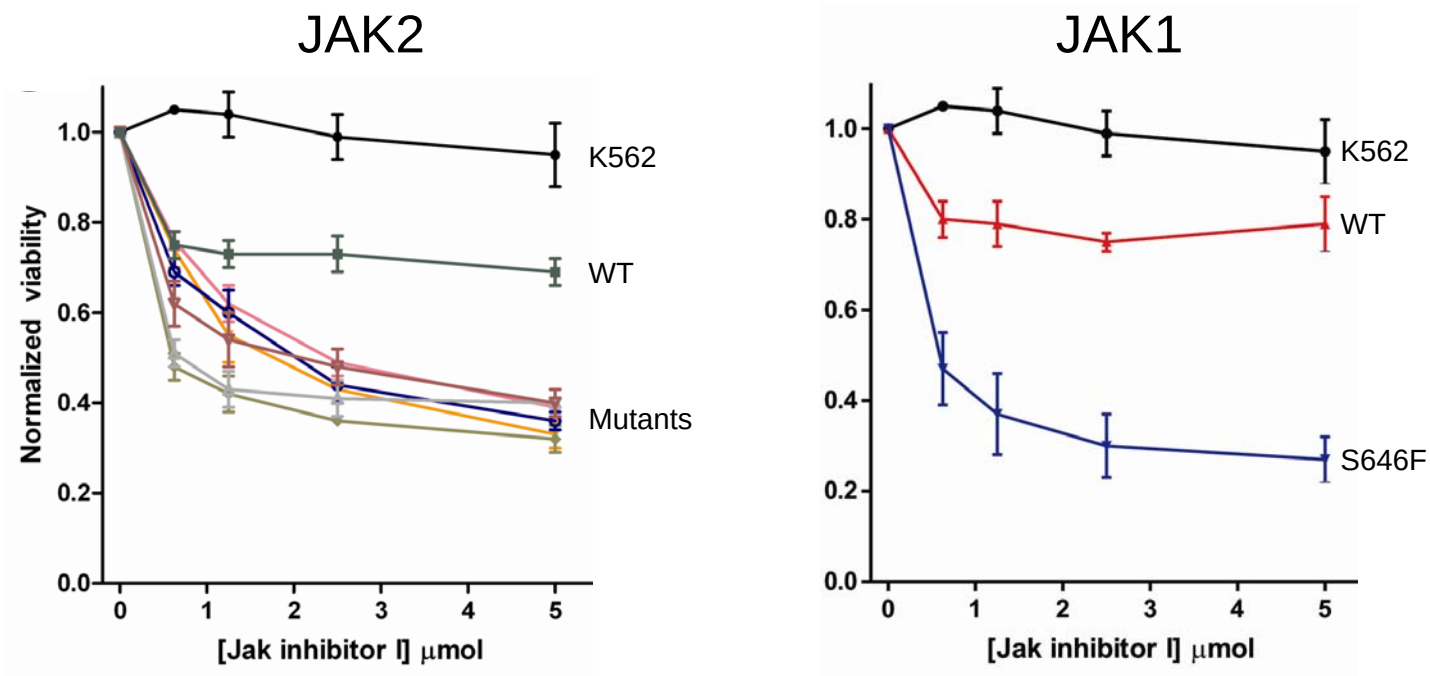


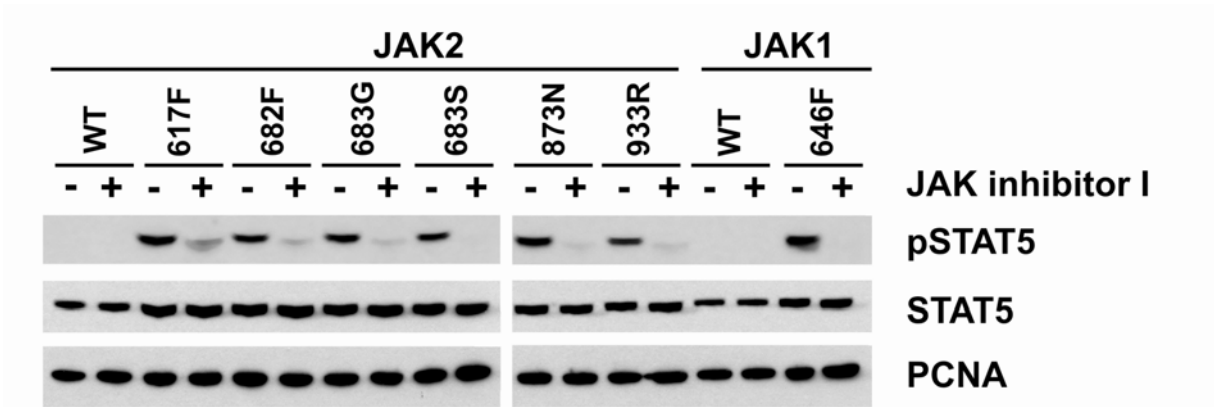




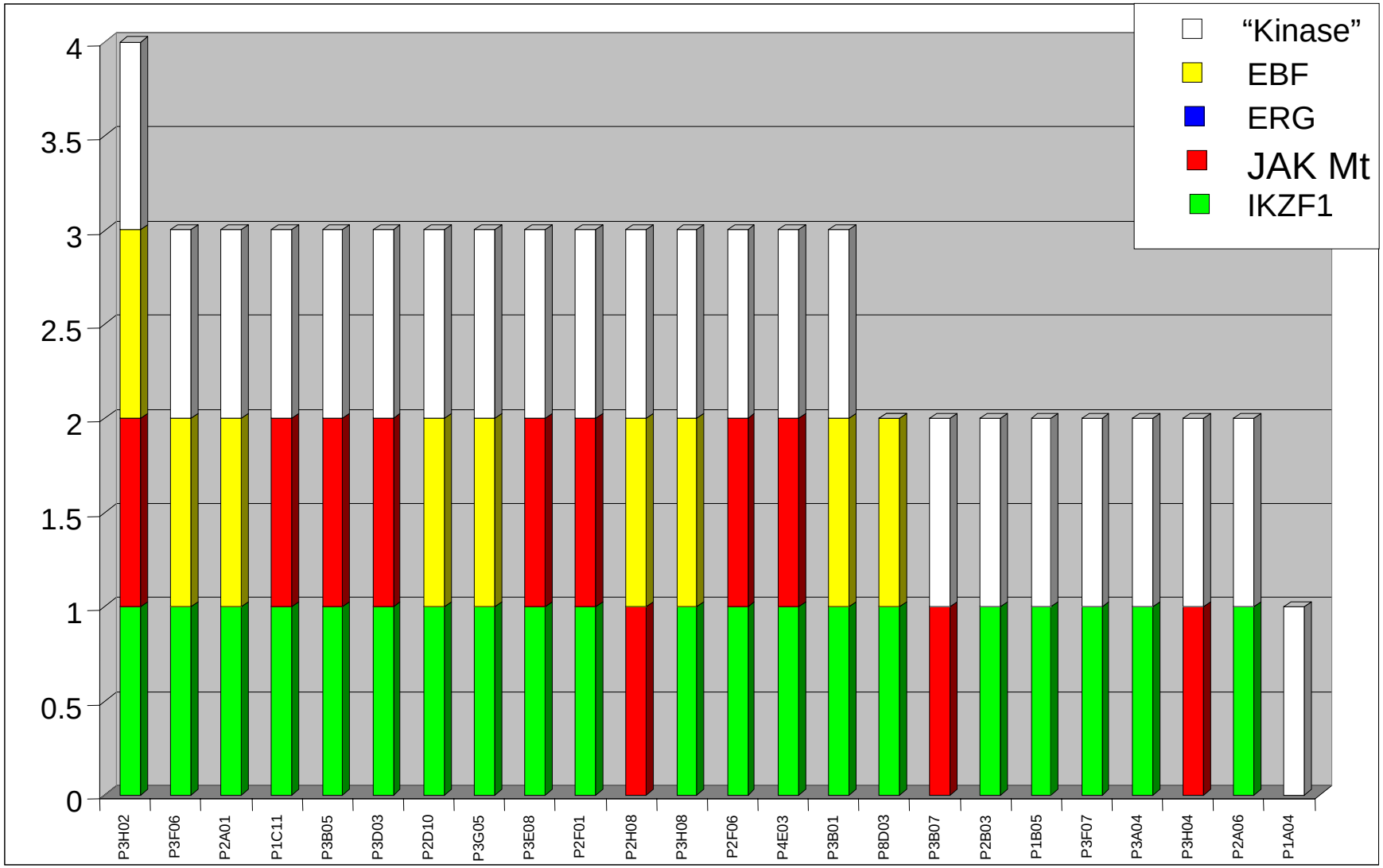
PNAS 2009; in press.

Mutant Jak-Transformed Ba/F3-EpoR cells are Sensitive to Pharmacologic Jak Inhibition

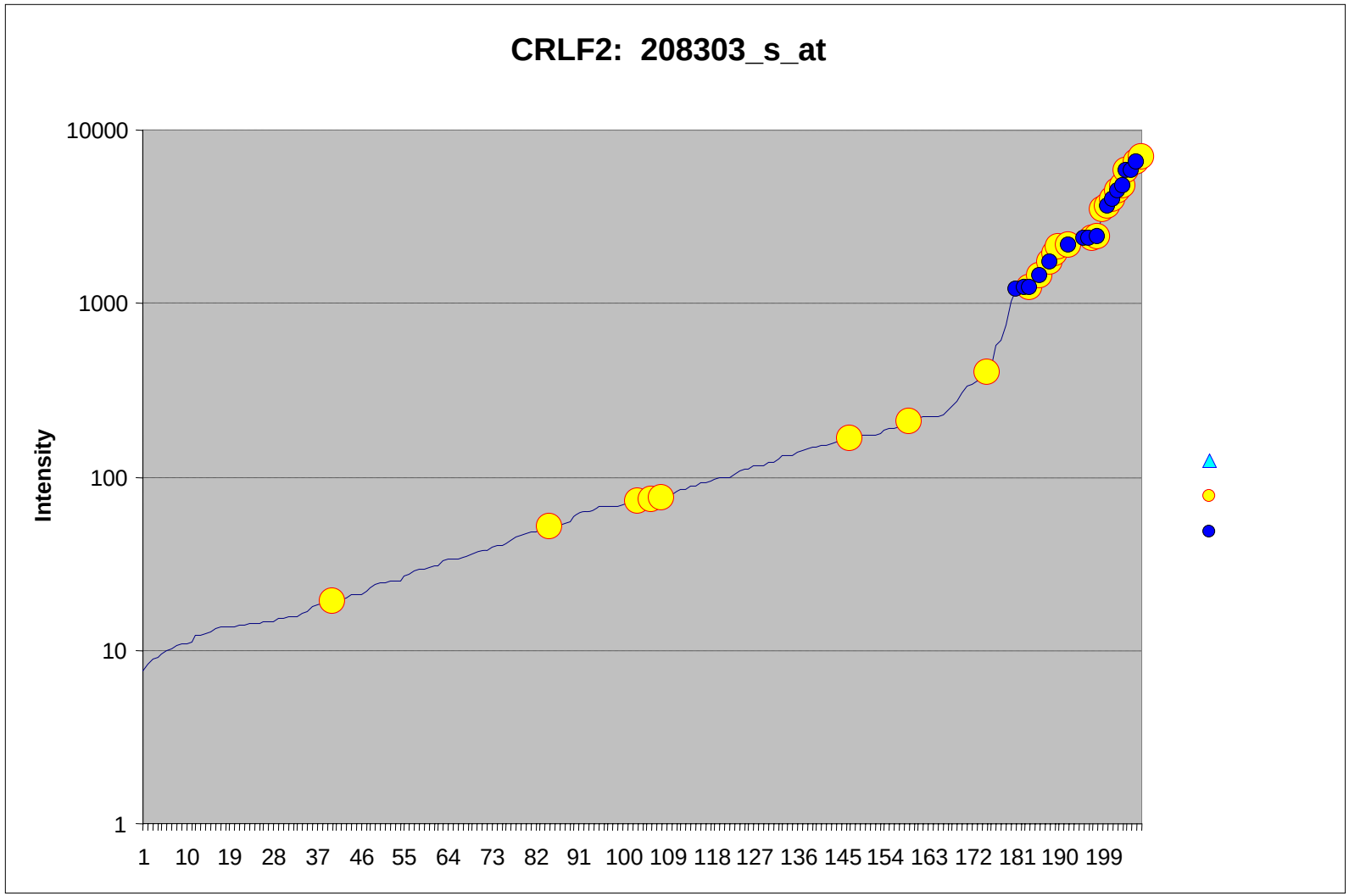




R8 Cluster: Association with Copy Number Abnormalities and an Activated Kinase Gene Expression Signature

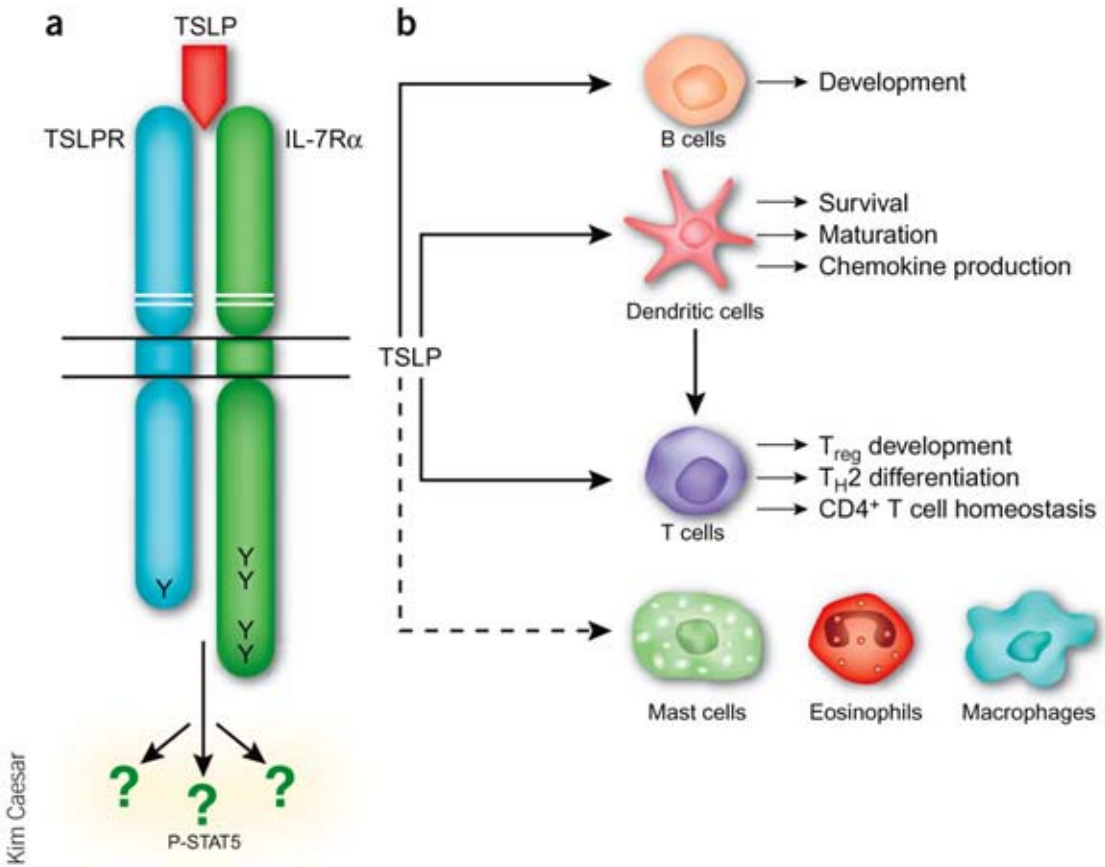


High CRLF2 Defines R8 / Subset of R7 Cases
Correlation of High CRLF2 Expression (Yellow) and JAK Mutations (Blue)

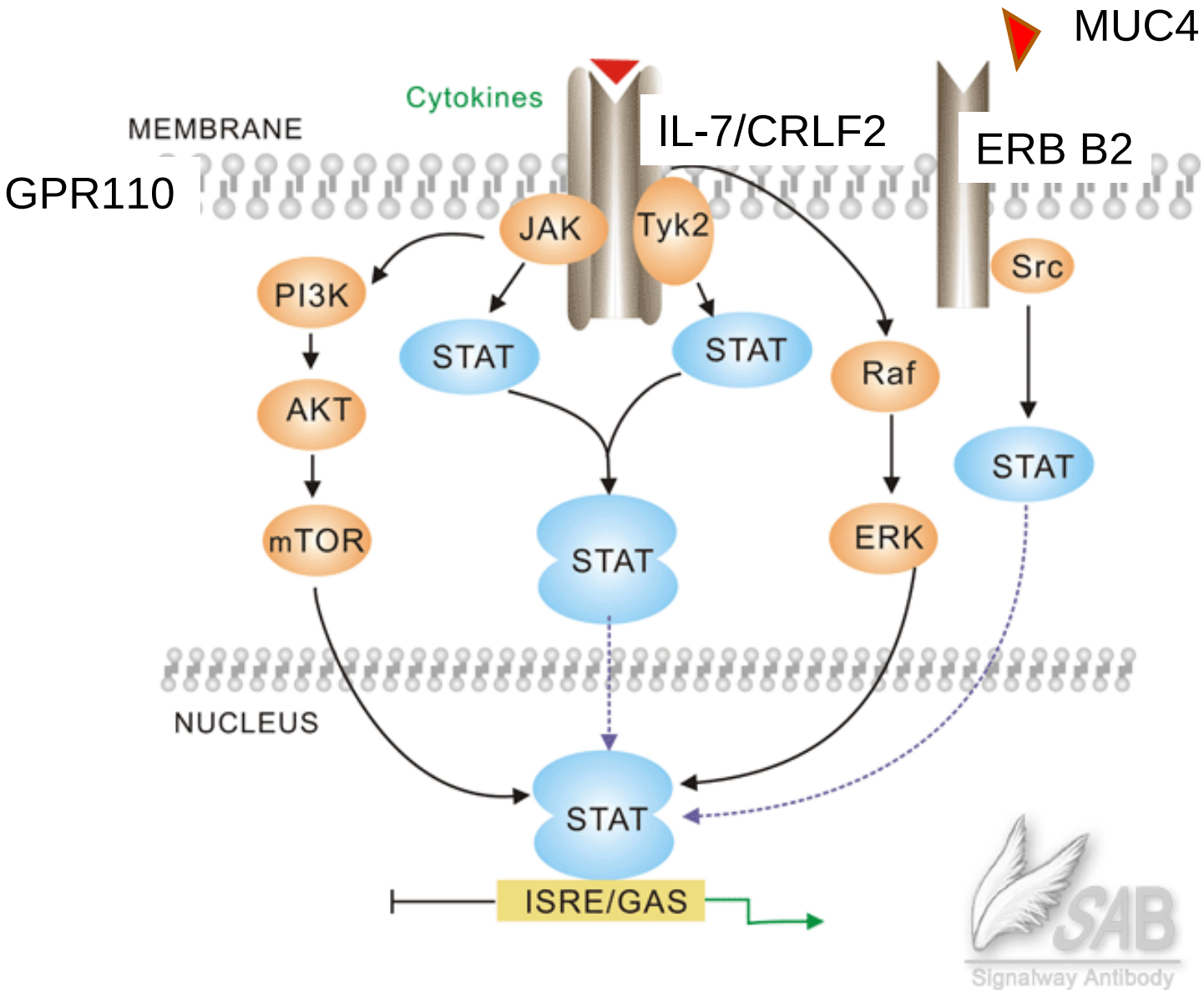


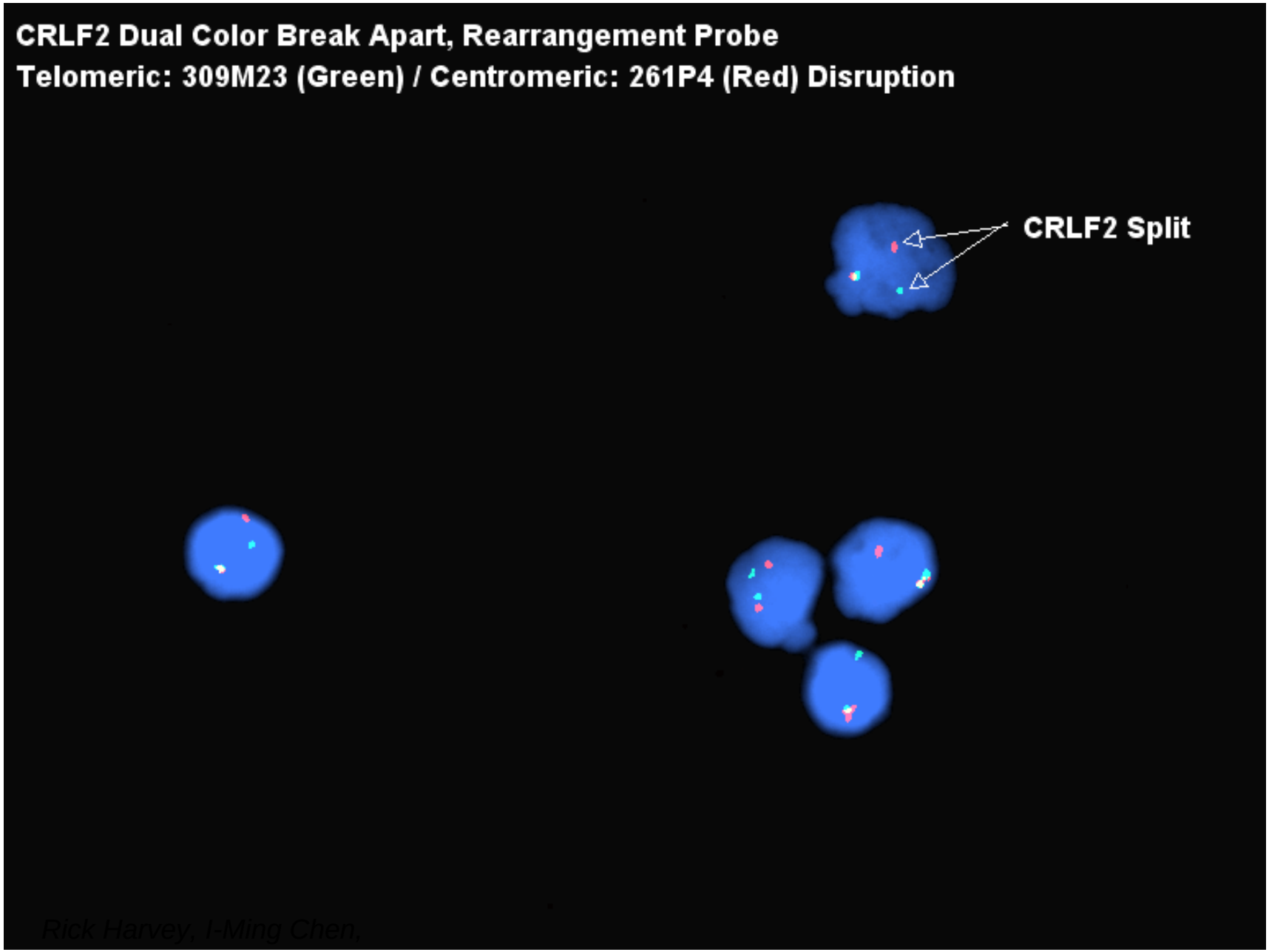
CRLF2

- TSLP R is a heterodimer of CRLF2 and IL-7RA
- TSLP R is a type 1 cytokine receptor and contains the conserved 'box1' sequence found in other cytokine receptors, but lacks the conserved 'box2'.
- TSLP R, like IL-7R, induces STAT5a and STAT5b phosphorylation, but unlike IL-7R is not thought to activate any of the known JAK kinases.



Ziegler & Liu, Nature Immunol 7, 709-714 (2006)


MUC4
Cytokines
MEMBRANE
IL-7/CRLF2
ERB B2
GPR110
JAK
Tyk2
Src
PI3K
STAT
STAT
Raf
AKT
STAT
mTOR
STAT
ERK
NUCLEUS
STAT
ISRE/GAS
SAB
Signalway Antibody



Rick Harvey, I-Ming Chen,
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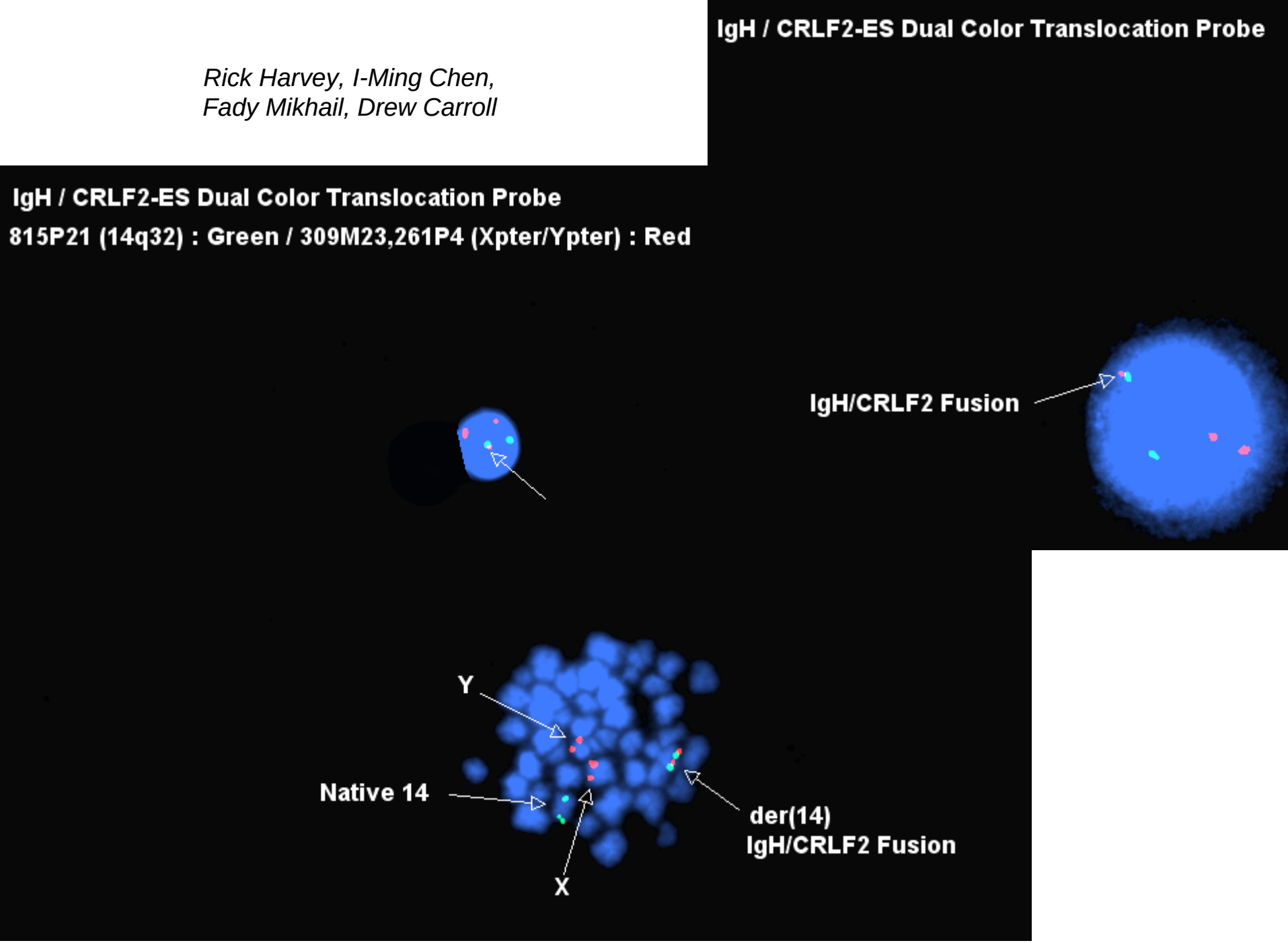
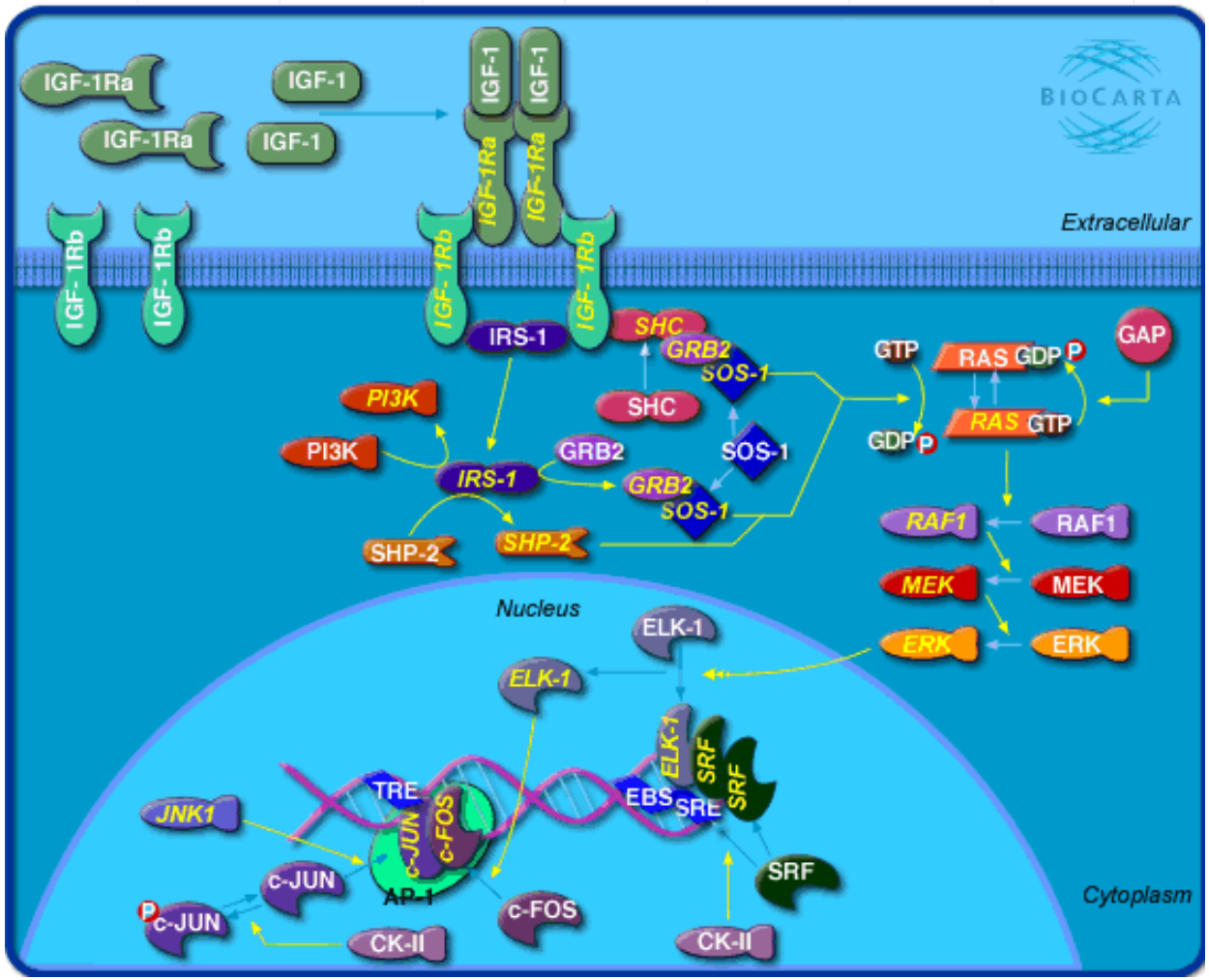


TARGET Sequencing Update
897,707 Traces Analyzed To Date



11% of Samples have JAK Kinase Mutations / High CRLF2

25% of Samples have RAS Family Mutations (N-RAS, K-RAS)

42% of Samples have Mutations in the FLT3 / RAS Pathway (RAS, FLT3, NF1, PTPN11)

Conclusions

- Discovered novel cluster groups in high risk ALL, associated with distinct outlier genes and DNA copy number abnormalities – new targets for improved diagnosis, risk classification, and therapy
- Discovering new underlying genetic abnormalities in high risk ALL through comprehensive molecular analyses: gene expression profiling and whole genome DNA copy number changes, both of which are informative to direct targeted gene sequencing
- Developing animal models for further study of novel genetic lesions and pathways
- Extending TARGET to a cohort of 400 AYA ALL cases (CALGB / ECOG) and to a new cohort of 200 standard/high risk pediatric ALL cases that have relapsed
- Designing early phase clinical trials for JAK inhibitors
- Public Datasets: caArray (https://array.nci.nih.gov/caarray/) or GEO (http://www.ncbi.nlm.nih.gov/geo/)

Acknowledgements

UNM / Willman
Richard Harvey
I-Ming Chen
Huining Kang
Susan Atlas
Xuefei Wang
Ed Bedrick
Kip Wharton

St. Jude
Jim Downing
Charles Mullighan
Mary Relling
Cheng Cheng
Ina Radtke
JJ Yang

NCI
Malcolm Smith
Kevin Dobbin
Daniela Gerhardt
Jinghui Zhang
Jim Jacobson
Tracey Lively
Richard Locke

NYU
William Carroll
Elizabeth Raetz
Deepa Bhowjani

COG
Steven Hunger
Mike Borowitz
Mini Devidas
Drew Carroll

Gregory Reaman
Bruce Camitta
Mignon Loh

CHOP / U PENN: Stephen Grupp