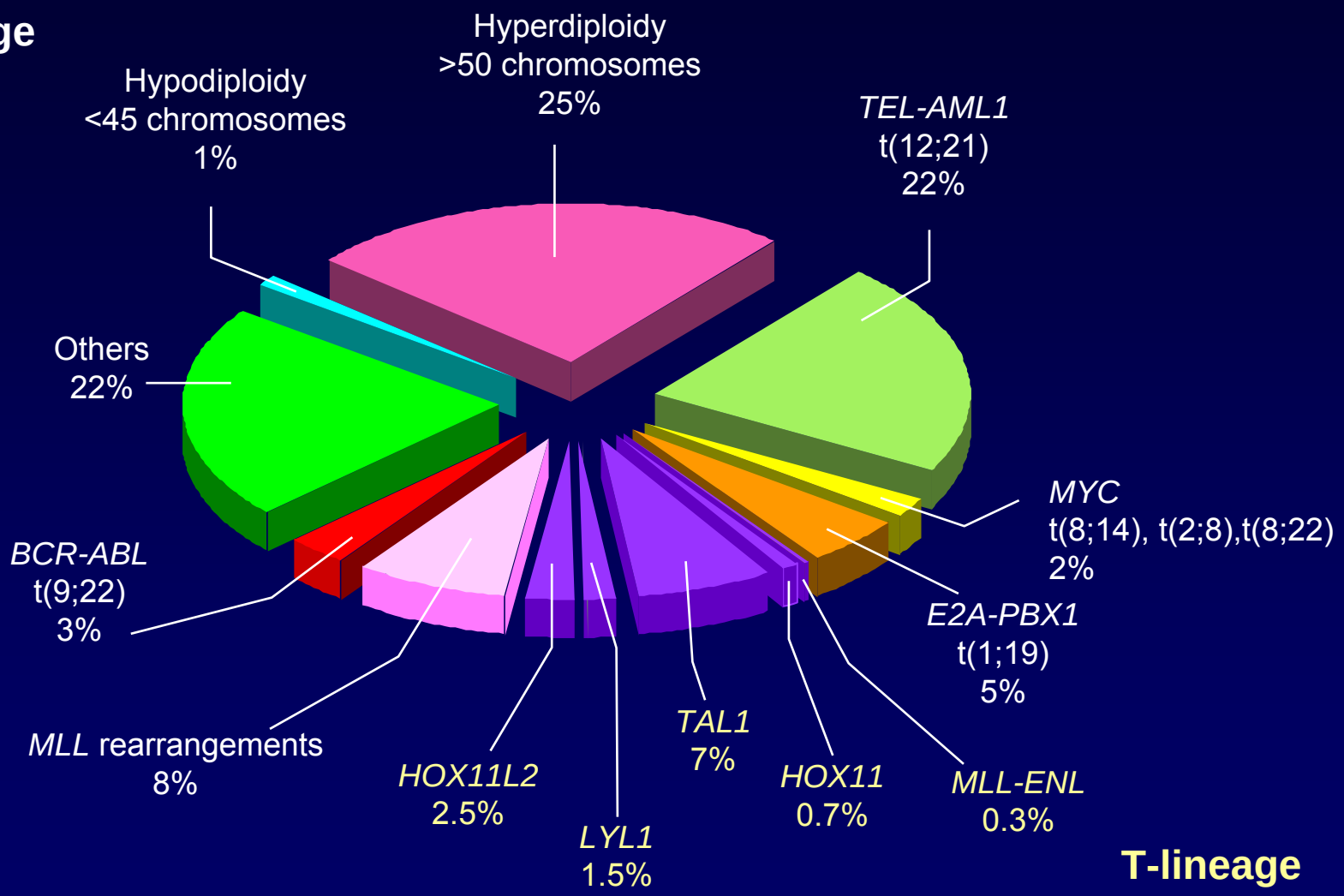
Genetic alterations in high risk ALL
Insights in to biology and therapeutic targets

Charles Mullighan
Pathology
St Jude Children's Research Hospital
Memphis

Cytogenetics of pediatric ALL



Pui N Engl J Med 2004;350:1535

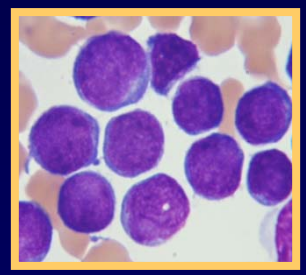
Genetics of ALL

- Known chromosomal alterations do not account for all the genetic lesions in ALL
- Frequency of recurring karyotypic alterations declines with increasing age
- Genetic basis of treatment failure poorly understood
  - Many relapses occur in cases that lack very high risk alterations (*MLL*, *BCR-ABL1*, hypodiploidy)
- Leverage genome-wide profiling of ALL
  - Lesions contributing to pathogenesis
  - Lesions determining relapse
  - Identify novel therapeutic targets

Dissecting the genetic basis of acute leukemia

Chromosomal Aberrations
- **Aneuploidy**
- **rearrangements**

DNA copy number abnormalities
- **Amplifications/Deletions**
- **Copy-neutral LOH**
- **Inherited copy number variants**



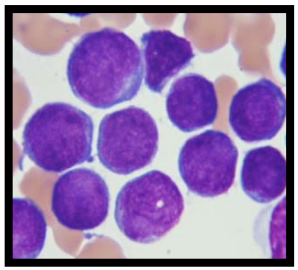
Sequence mutations
Sequence polymorphisms

Epigenetic changes
- **CpG methylation**
- **Histone acetylation**

Data Acquisition

Data Analysis

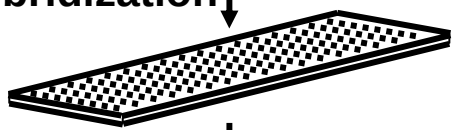
Leukemic Blasts



Extract DNA

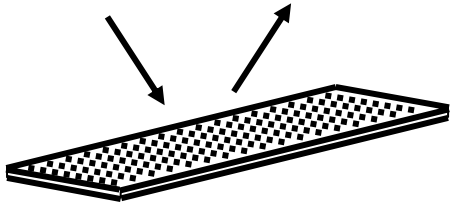
leukaemia and normal cells

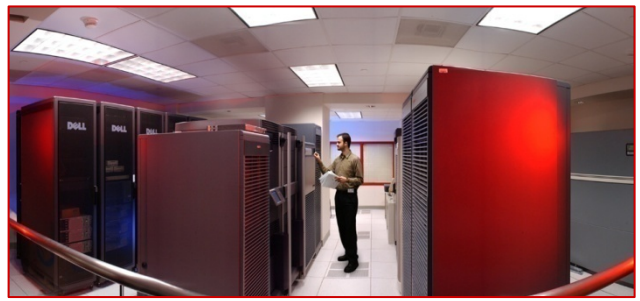
Label and Hybridization

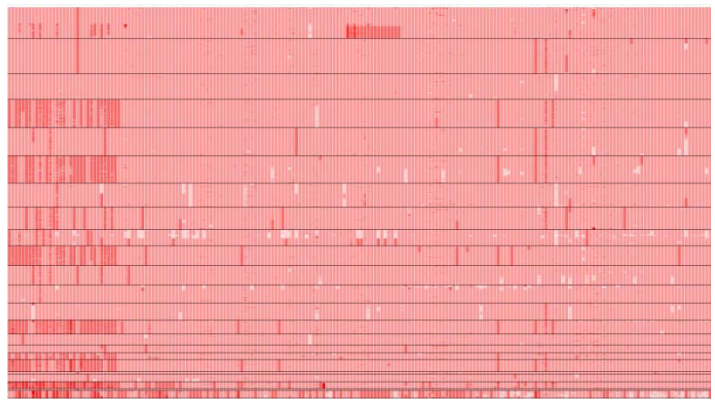


Laser Excitation

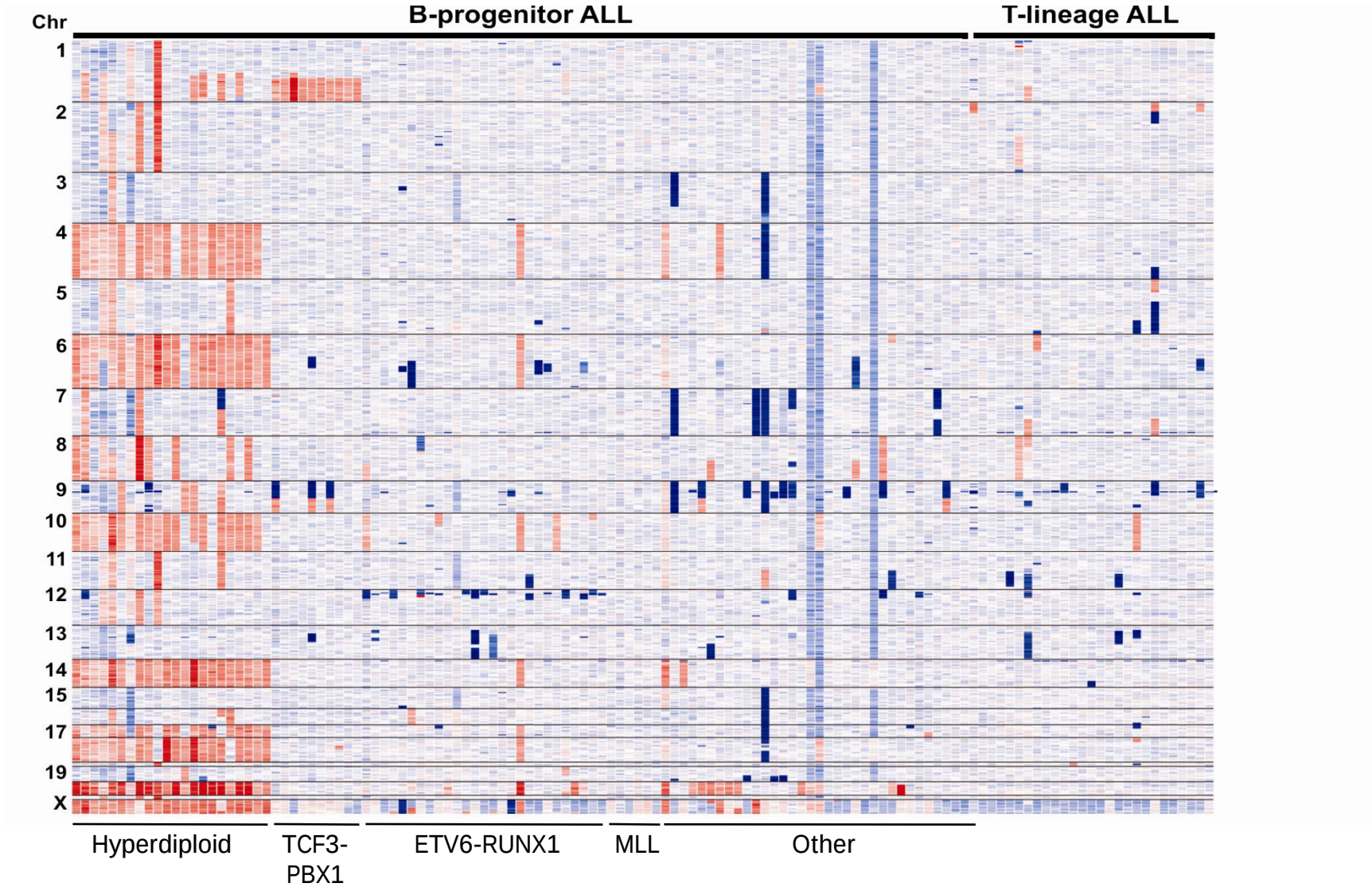
Signal Acquisition







Genetic alterations in diagnosis ALL samples

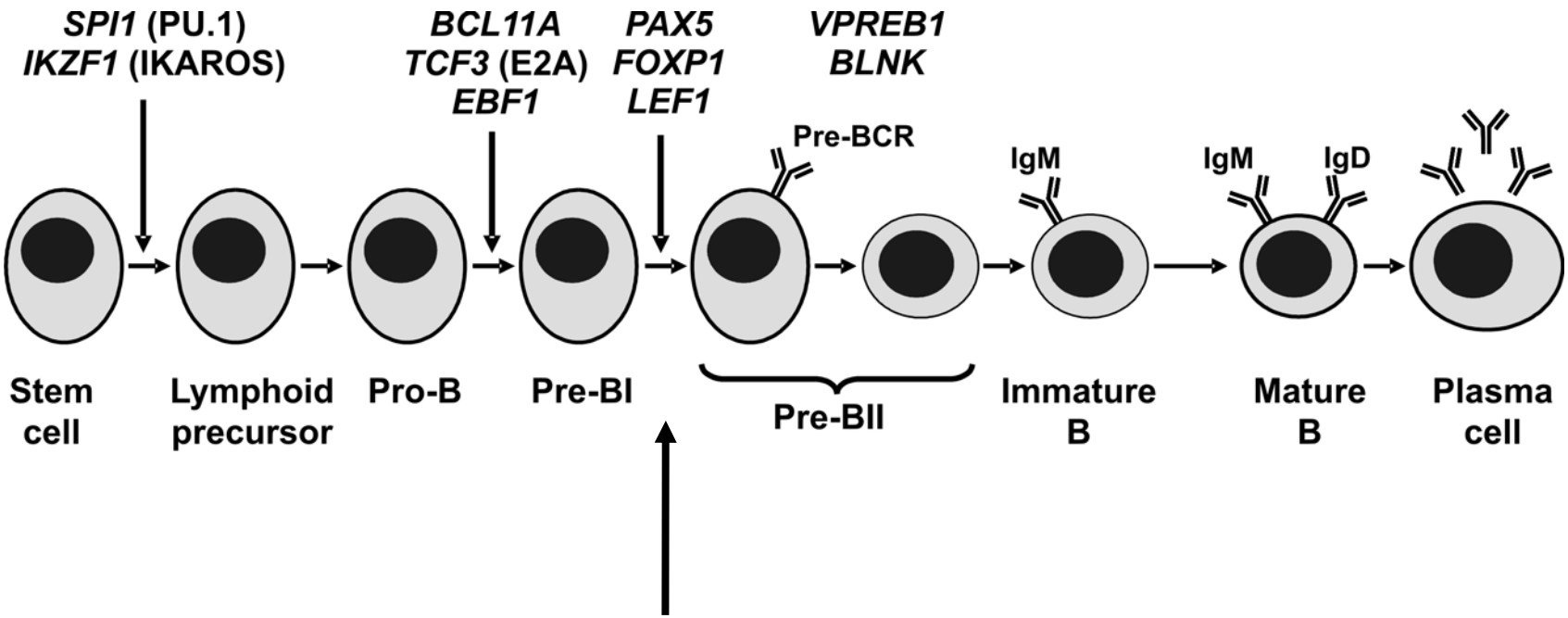


SNP array analysis of diagnosis ALL samples

- 242 B-progenitor and T-lineage ALL cases
- Lack of genomic instability - ~6 lesions per case
- >50 recurring regions of deletion/gain. Most focal (<1Mb)
- Significant variation in lesion type and frequency between ALL subtypes:
  - *MLL*: <1 lesion/case
  - *BCR-ABL1, ETV6-RUNX1:* >8 lesions/case
- Lesions target key cellular pathways:
  - Lymphoid development: *PAX5, EBF1, IKZF1:* over 60% of cases

Nature 2007;446:758

Genes regulating B lymphoid development are mutated in ~65% B-progenitor ALL



Block in maturation in B-ALL

SNP array analysis of diagnosis ALL samples

- 242 B-progenitor and T-lineage ALL cases
- Lack of genomic instability - ~6 lesions per case
- >50 recurring regions of deletion/gain. Most focal (<1Mb)
- Significant variation in lesion type and frequency between ALL subtypes:
  - *MLL*: <1 lesion/case
  - *BCR-ABL1, ETV6-RUNX1:* >8 lesions/case
- Lesions target key cellular pathways
  - Lymphoid development: *PAX5, EBF1, IKZF1*
  - Tumor suppressors: *CDKN2A*/B, *RB1, ATM, NF1*
  - Lymphoid signaling pathways: *BTLA, CD200, CRLF2*
  - Oncogene amplification: *MYB* (T-ALL)
  - DNA structure: Histone genes
  - Apoptosis regulation: *BTG1*
  - Drug response: *NR3C1* (glucocorticoid receptor)
- Role in treatment response?

Nature 2007;446:758

Children's Oncology Group P9906 study

- Childhood B-progenitor ALL
- High risk based on age, sex, leukocyte count (median age 13.3)
- Augmented Berlin-Frankfurt-Münster regimen; 2000-2003
- *BCR-ABL1* positive, hypodiploid, or induction failures excluded
- No trisomy 4/10 or *ETV6-RUNX1* unless CNS/testicular disease
- 221 cases studied
- 170 cases lacked a sentinel chromosomal lesion
- Genomic analysis (NCI TARGET* project)
  - Affymetrix 500K SNP array: all cases
  - Affymetrix U133 Plus 2.0 gene expression profiling: 198 cases
  - Candidate gene resequencing: *PAX5*, *IKZF1*, *EBF1*

*Therapeutically Applicable Research to Generate Effective Treatments
http://target.cancer.gov

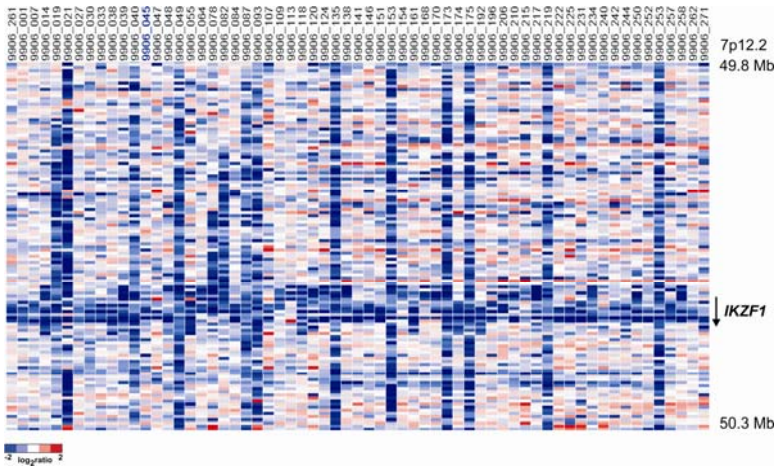
N Engl J Med. 2009 Jan 7. [Epub]

Copy number alterations (CNA)

Deletion	COG 9906 (N=221)	St Jude B-ALL (N=258)
CDKN2A	101 (46)	87 (34)
PAX5 CNA	70 (32)	79 (31)
PAX5 CNA or mutation	81 (37)	83 (32)
IKZF1	63 (29)	48 (19)
IKZF1 CNA or mutation	67 (30)	48 (19)
ETV6	28 (13)	63 (24)
RB1	25 (11)	15 (6)
BTG1	23 (10)	18 (7)
13q14.2 (miRNA)	21 (10)	16 (6)
C20orf94	19 (9)	20 (8)
EBF	17 (8)	12 (5)
IL3RA/CSF2RA	15 (7)	18 (7)
DMD	15 (7)	11 (4)
B cell development pathway	147 (67)	137 (53)

IKZF1 (Ikaros) alterations in high risk ALL

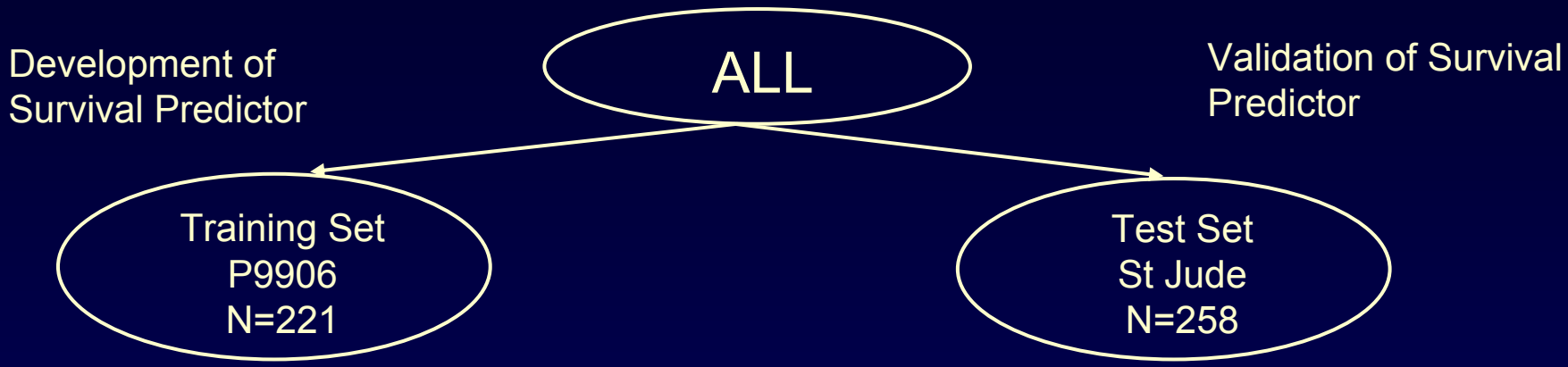
Deletion	COG 9906 (N=221)	St Jude B-ALL (N=258)
CDKN2A	46%	34%
PAX5 CNA or mutation	37%	32%
IKZF1 CNA or mutation	30%	19%
B cell development pathway	67%	53%



IKZF1 deletions

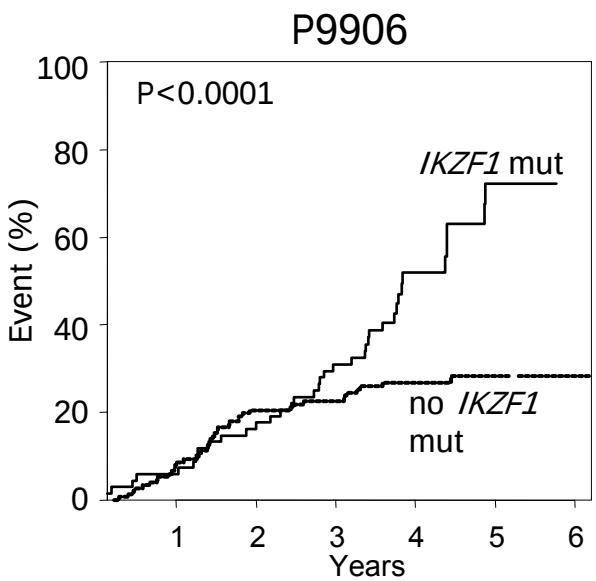
IKZF1 mutations

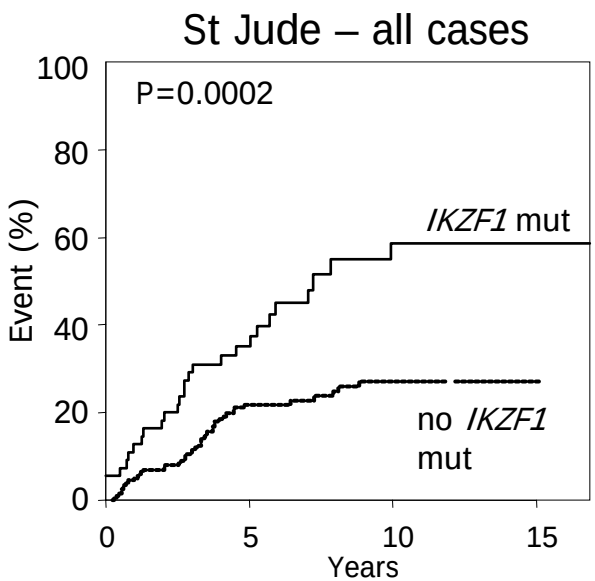
Predicting outcome using genome-wide copy number data

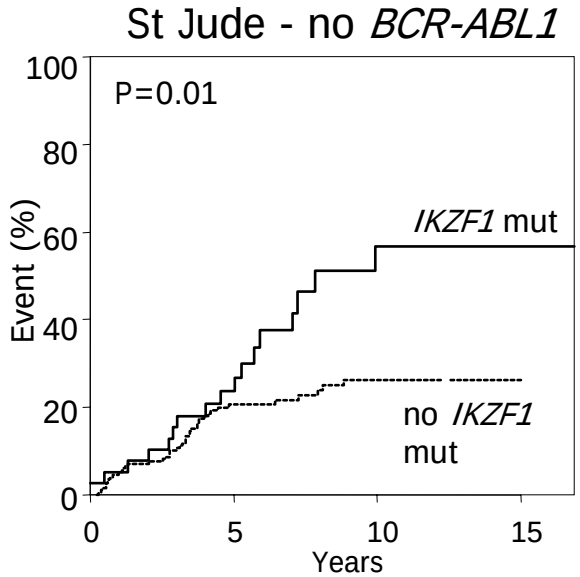


	P9906 (N)	St Jude (N)
TCF3-PBX1	25	17
ETV6-RUNX1	3	**50**
High hyperdiploid	4	**44**
Hypodiploid	0	**10**
MLL-rearranged	19	**24**
BCR-ABL1	**0**	**21**
Other	**170**	92

IKZF1 alteration and poor outcome in ALL







Multivariable analysis: relapse

Factor	Hazard Ratio (95% CI)	P
Age	0.79 (0.4-1.6)	0.46
Subtype	1.10 (0.4-3.2)	0.86
WBC	1.21 (0.7-2.0)	0.47
D29 minimal residual disease	2.55 (1.3-4.9)	0.0044
IKZF1 alteration	2.40 (1.38-4.2)	0.0021

IKZF1 (Ikaros) is deleted in 83.7% *BCR-ABL1* ALL

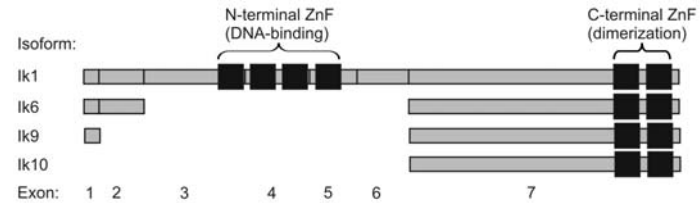
ALL subtype (N)	*IKZF1* (Ikaros) (%)	*CDKN2A* (%)	*PAX5* (%)
BCR-ABL1 de novo ALL (43)	84	53	51
- childhood (21)	76	62	57
- adult (22)	91	45	45
Chronic myeloid leukemia			
- chronic phase (N=60)	0	0	0
- lymphoid blast crisis (N=10)	70	70	50
- myeloid blast crisis (N=28)	18 (7p-/-7)	18	14

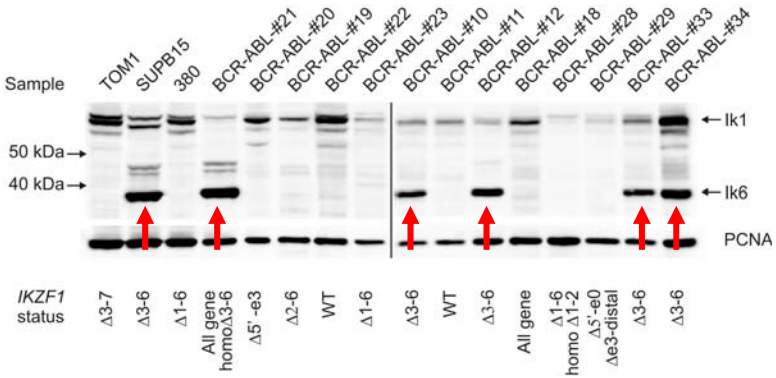
- Similar genetic abnormalities in *de novo* BCR-ABL1 ALL and lymphoid blast crisis CML
- Genomic lesions are critical determinants of the lineage of BCR-ABL1 leukemia

Nature 2008;453:110

IKAROS and ALL

- Zinc finger transcription factor required for lymphoid development
- Ikaros null mice lack all lymphoid lineages
- Aberrant IKZF1 isoforms in ALL
- Internal *IKZF1* deletions results in dominant negative isoforms
- Mice heterozygous for a dominant negative Ikaros mutation develop aggressive lymphoproliferative disease





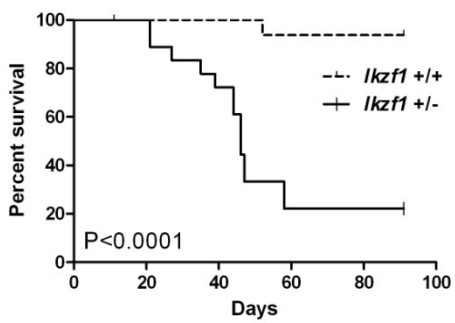
Ikzf1$^{+/+}$ or *Ikzf1*$^{+/-}$ marrow

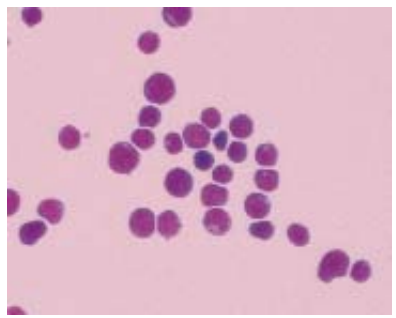
↓

MSCV-GFP-IRES-p185 BCR-ABL

↓

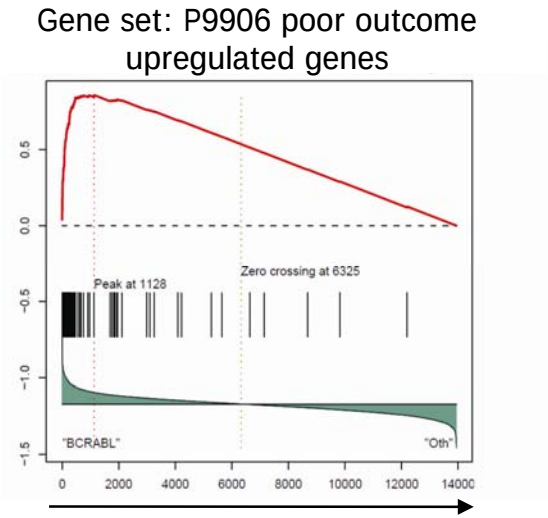
Transplant into irradiated recipients

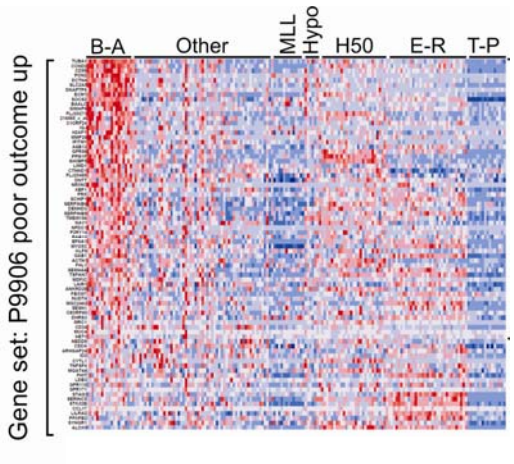




Racquel Collins-Underwood

A novel subtype of “BCR-ABL1-like” high risk ALL

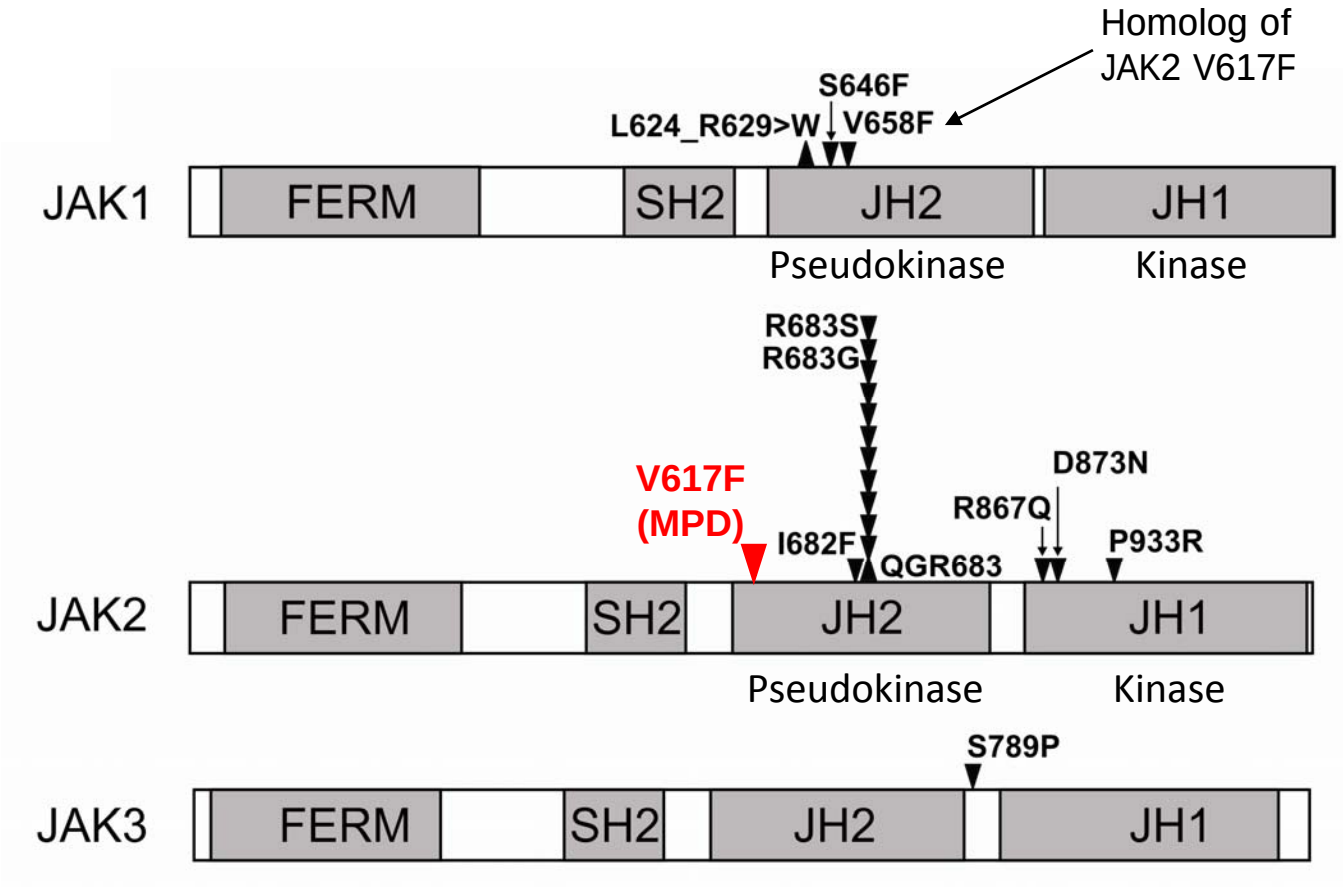




- Gene set enrichment analysis shows similarity of signatures of Ph+ (*IKZF1* deleted) and Ph- (*IKZF1* deleted) ALL
- Implications of the “BCR-ABL1-like” subtype of ALL
  - *IKZF1* alteration central to BCR-ABL1 positive and negative ALL
  - Unidentified kinase activating lesions?

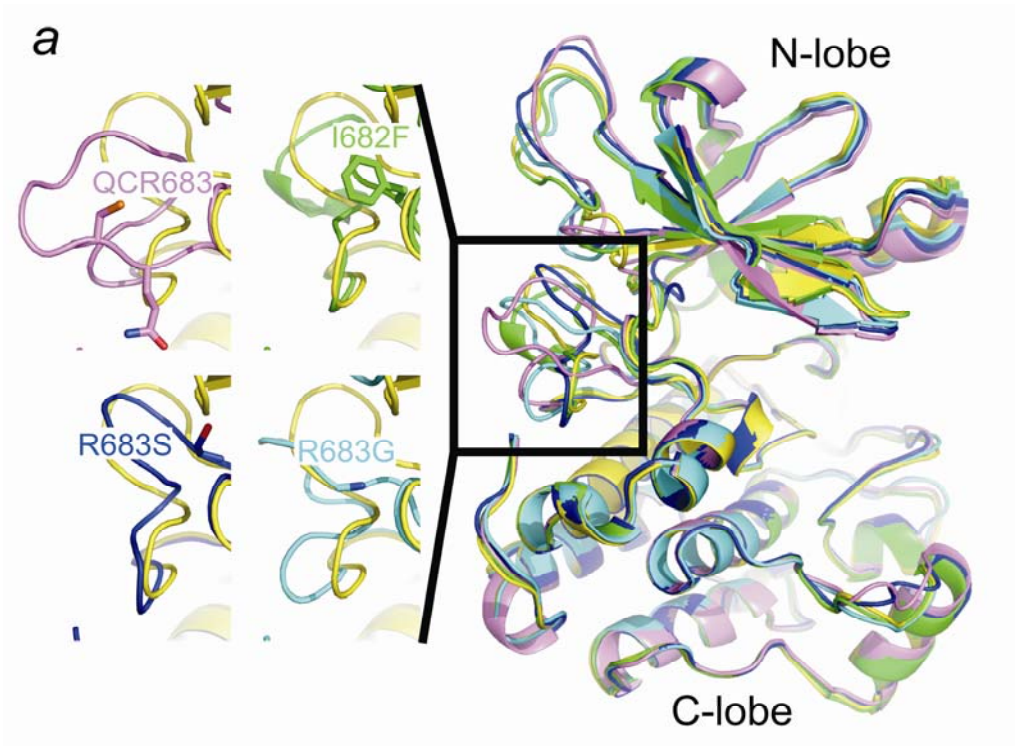
Mullighan et al. N Engl J Med. 2009;360:470
also: den Boer et al Lancet Oncol 2009;10:125

JAK mutations in high-risk B-ALL

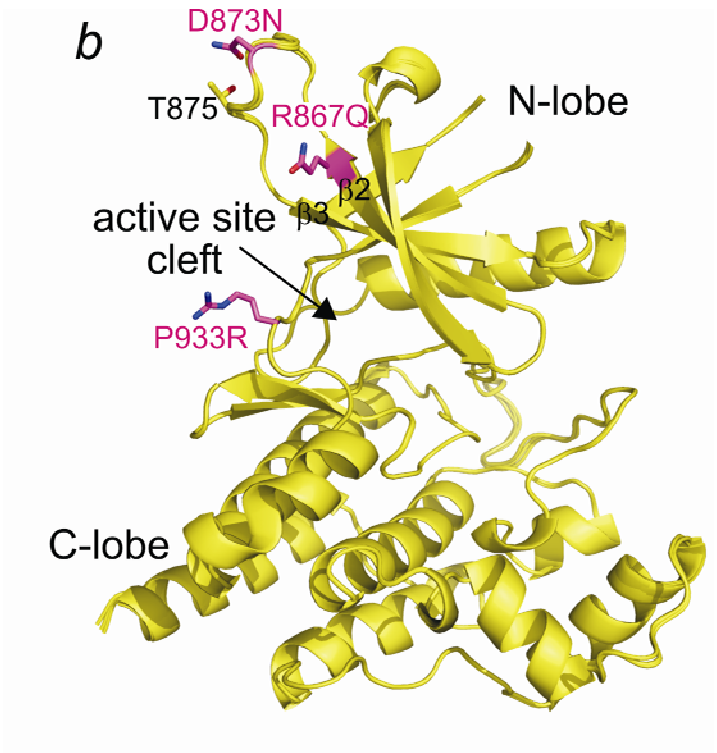


PNAS 2009;May 22 [Epub]

Modeling of JAK mutations



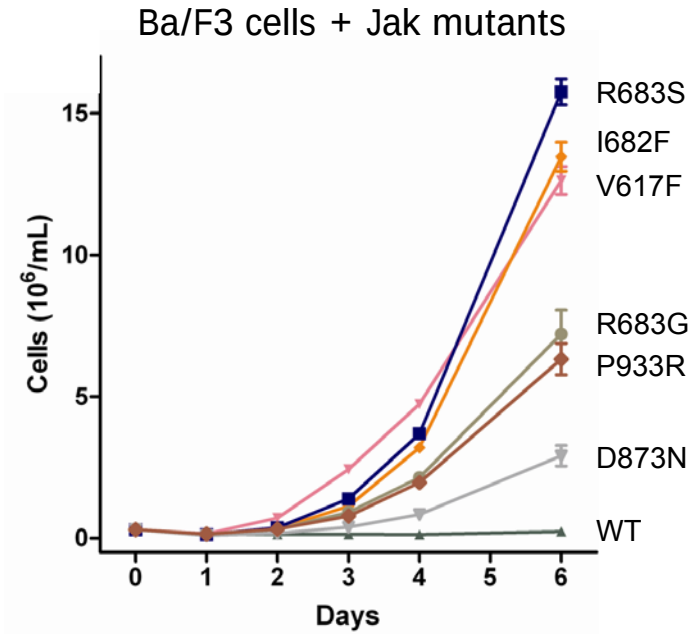
Pseudokinase

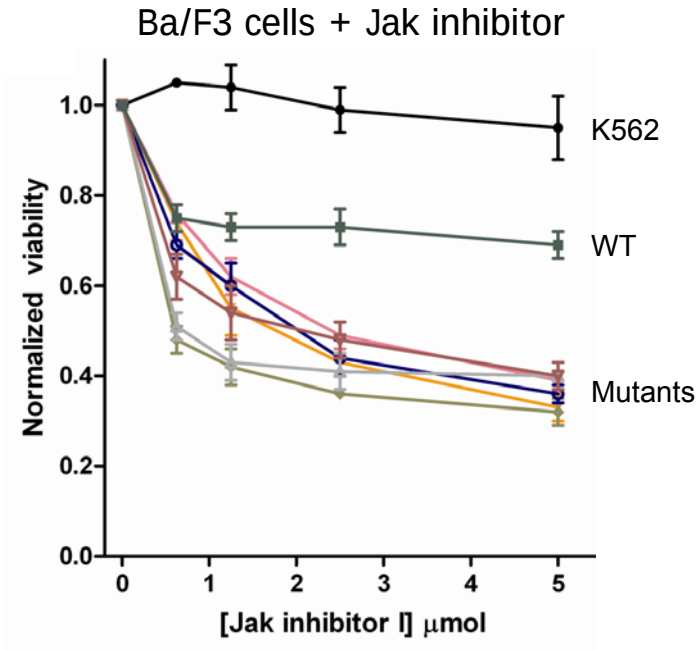


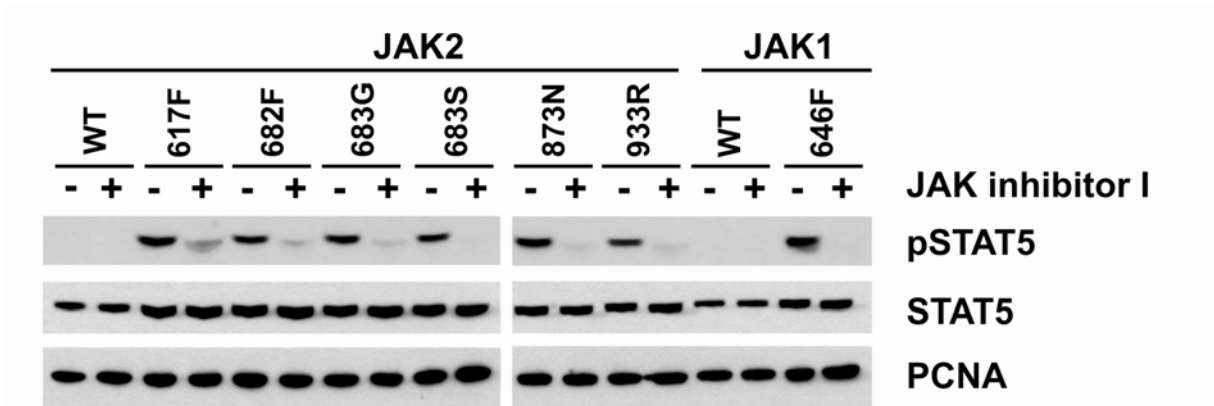
Kinase

Brenda Schulman

JAK mutations are transforming and sensitive to JAK inhibitors





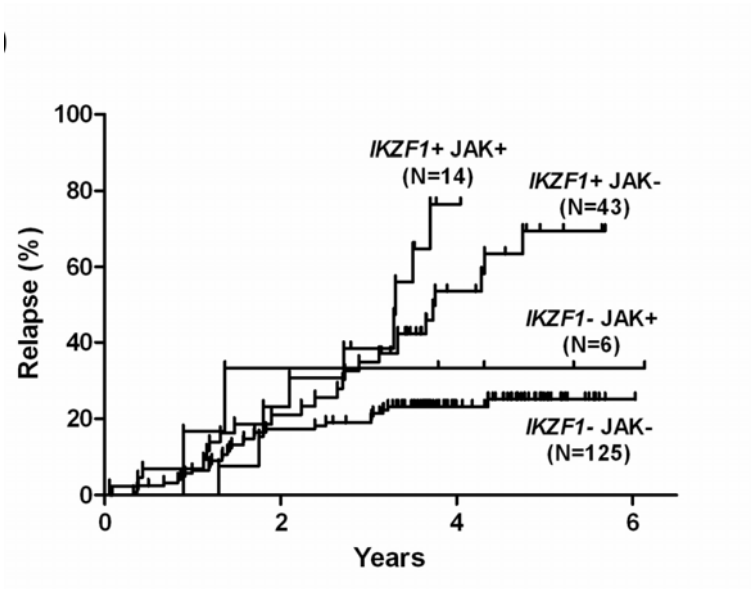


JAK mutations in ALL – other data

- JAK2 mutations in up to 28% Down syndrome ALL
  - Izraeli, Kearney, Rabin groups
- JAK1 mutations in (adult) T-lineage ALL (uncommon)
- No JAK mutations in non-DS B-progenitor ALL
- *BUT:*
  - Not comprehensive screening of all exons of all JAKs
  - Possible cohort dependence (Izraeli study enriched for low risk)
- Follow-up studies
  - SJ: JAK mutations in DS and non-DS ALL
  - Other COG HR and SR cohorts
  - AYA ALL

JAK, IKZF1 and outcome

Mutations	4 yr Relapse Risk
JAK + IKZF1	78% (p=0.0002)
IKZF1 only	54%
JAK only	33%
Neither	24%



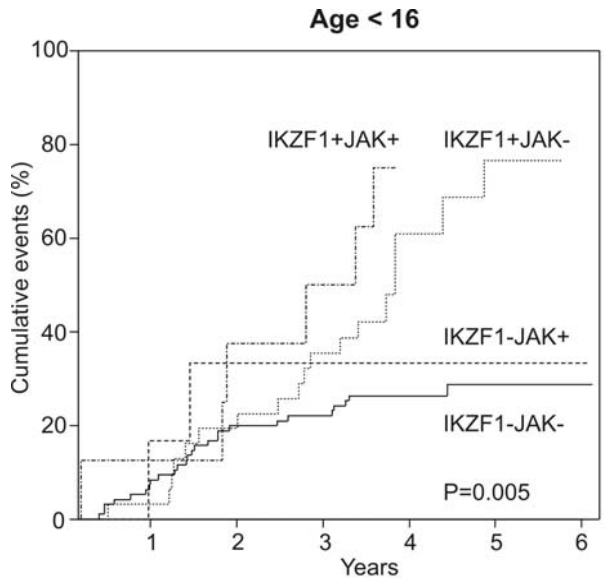
Unanswered questions

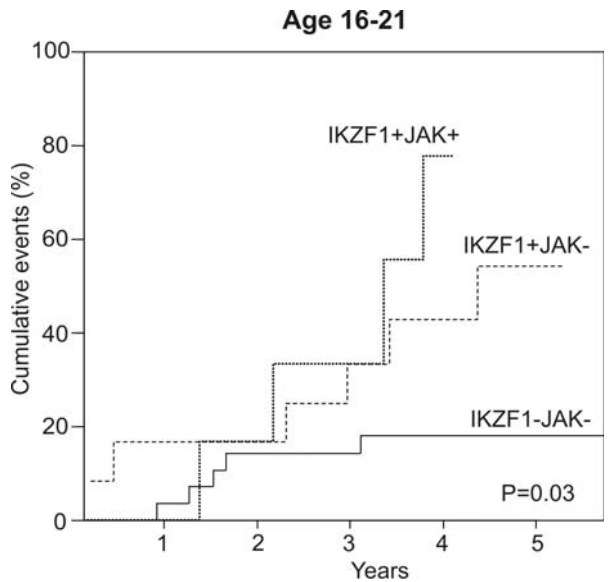
- What additional kinase alterations are present in “BCR-ABL1-like” high risk ALL
- Is JAK inhibition a useful therapeutic target, and if so, in which patients? Only those with JAK mutations, or all those with JAK-STAT activation?
- Why does *IKZF1* connote such poor prognosis?
- Which alterations should be explored as diagnostic markers?
- Validation in other cohorts/studies
- Biology
  - Leukemogenesis
  - Responsiveness to JAK inhibitors

Genomic analysis of AYA ALL

- Dearth of specific data
- Limited data in adult population (Paulsson PNAS 2008)
- P9906 cohort
  - 58 patients age 16-21, 50 lacked a sentinel chromosomal alteration

Mutations	All pts	Age 16-21
JAK + IKZF1	78%	78
IKZF1 only	54%	43
JAK only	33%	-
Neither	24%	18





Conclusions

- Genome-wide profiling has provided important insights into the genetic basis of ALL
- Many lesions submicroscopic
- Genetic lesions determine risk of disease recurrence
- Specific lesions have roles in outcome in addition to pathogensis
- Integrated genetic analysis can identify novel targets for therapy

The future

- Current data mostly copy number and gene expression
- genome wide profiling of CNA, expression, epigenetics
- Arrays → next generation sequencing
- Interrogation of older, and adult cohorts

Acknowledgements

St Jude - Pathology
Letha Phillips
Racquel Collins-Kneebone
Chris Miller
Ina Radtke
Zhongling Cai
James Dalton
Xiaoping Su
Sheila Shurtleff
Jim Downing

Pharmaceutical Sciences
Mary Relling
Wenjian Yang

Oncology
Ching-Hon Pui
Dario Campana
Elaine-Coustan Smith

Hartwell Center
Jing Ma
John Morris
Emily Walker

NCI/NIH
Jinghui Zhang
Daniela Gerhard
Malcolm Smith

Children's Oncology Group
Steve Hunger

University of New Mexico
Cheryl Willman
Rick Harvey

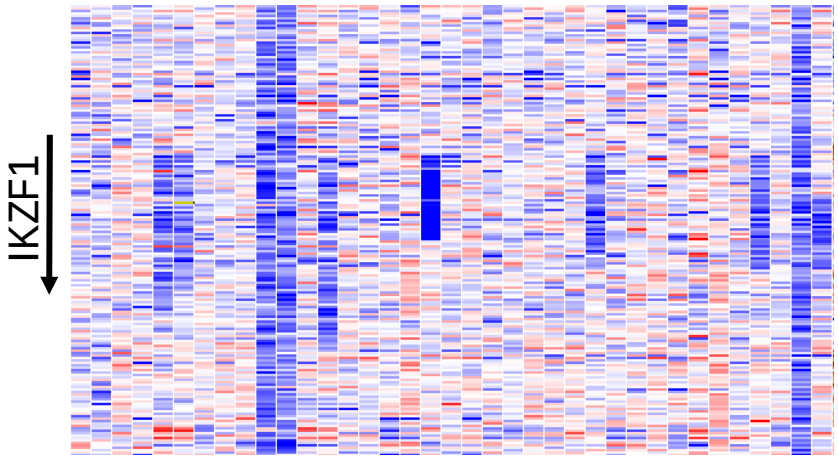
UCSF
Sarah Tasian
Mignon Loh

Australia
Timothy Hughes (IMVS, Adelaide)

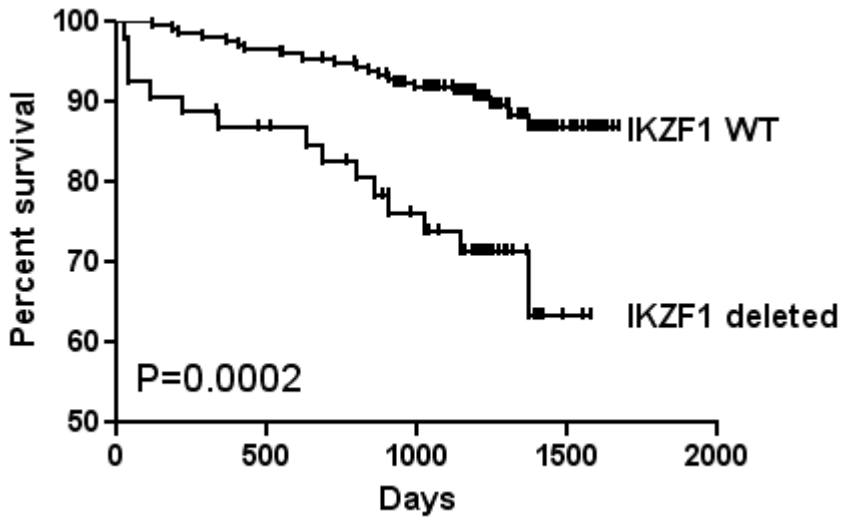
Funding
St Jude, ALSAC,
NH&MRC (Australia)
Children's Oncology Group
NCI, AACR, ASH

IKZF1 and outcome (ALL0232)

Subtype	N	*IKZF1*
ETV6-RUNX1	20	1
Hyperdiploid	28	2
MLL	6	0
TCF3-PBX1	17	1
BCR-ABL1	11	8
Other	167	41

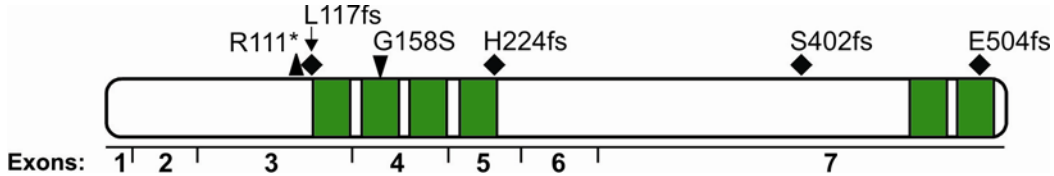


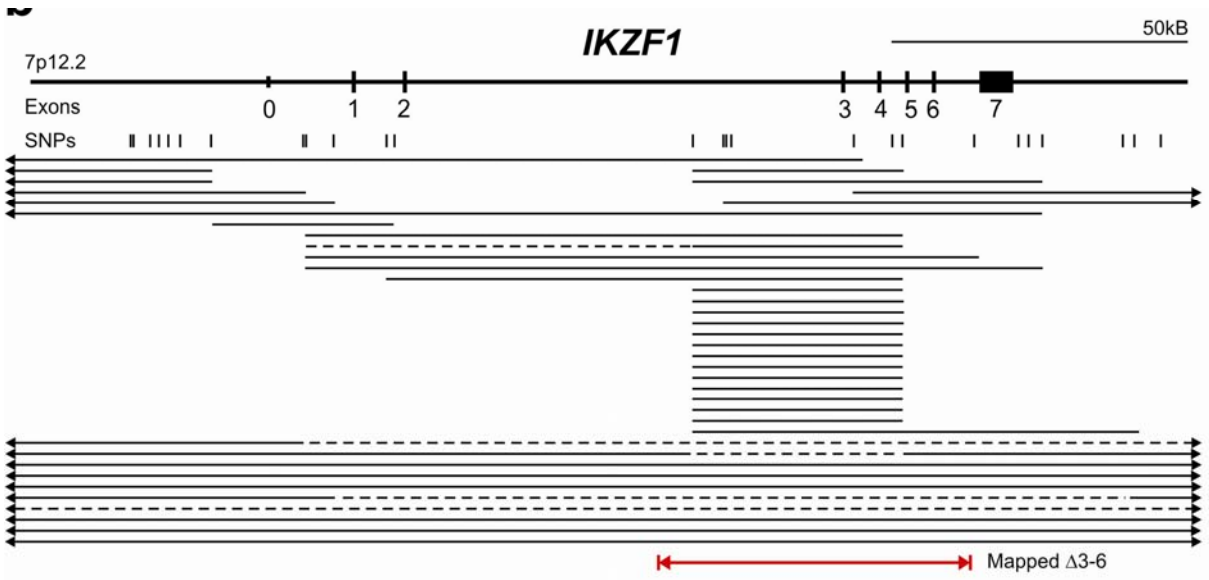
- Follow-up: median 3.2 years (0.08-4.6)
- IKZF1 associated with MRD
- IKZF1 associated with EFS in multivariable analysis incorporating MRD



Testing: *IKZF1*

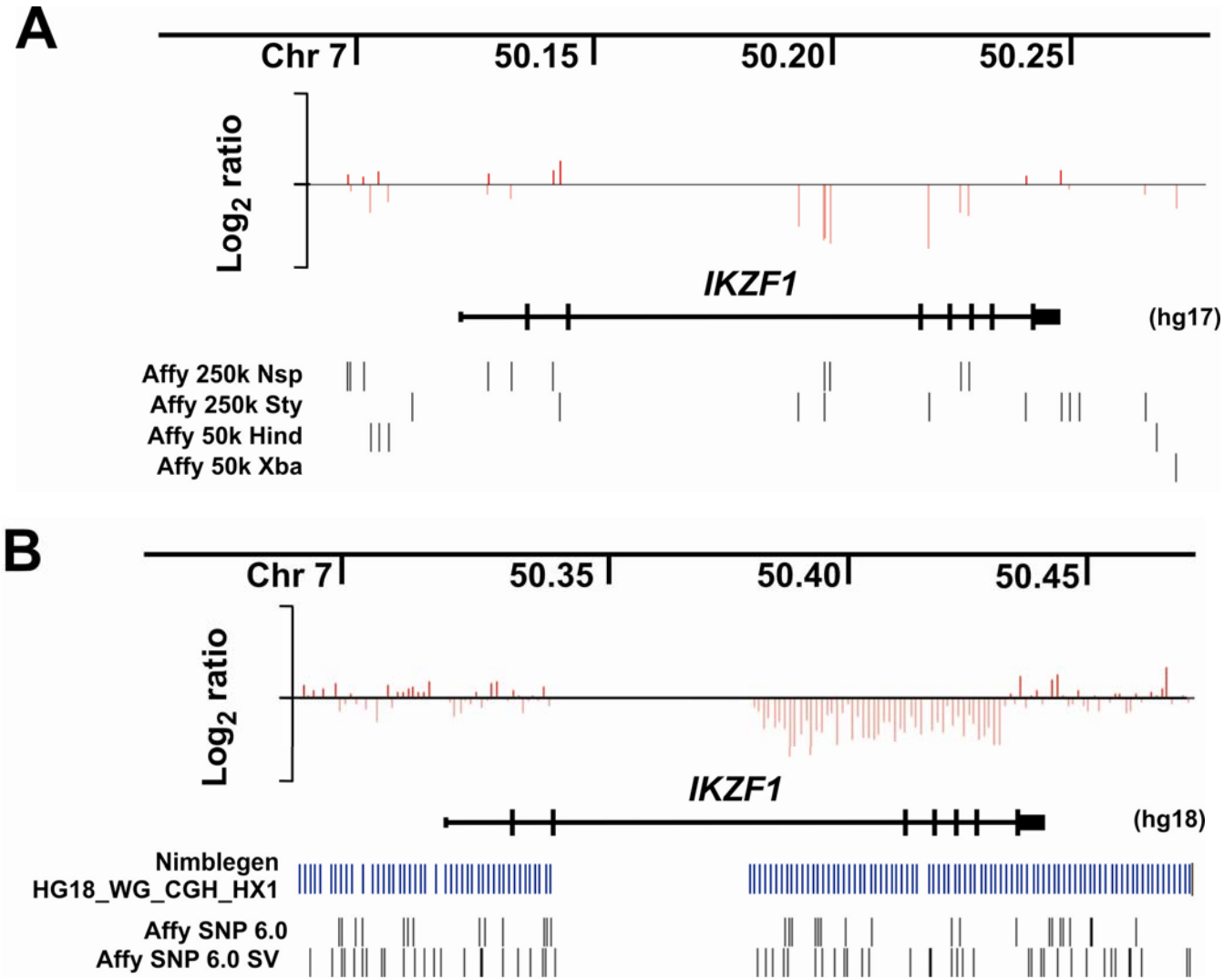
	MLL	Other
Deletion	1	62
All gene	0	15
Focal	1	47
Δ3-6 (Ik6)	1	20
Sequence mutation	0	6





Testing – *IKZF1*

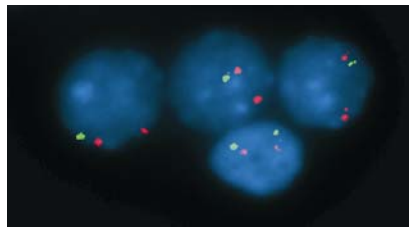
- Microarray: Genome wide (SNP 6.0) or targeted (*IKZF1*, other lesions)?



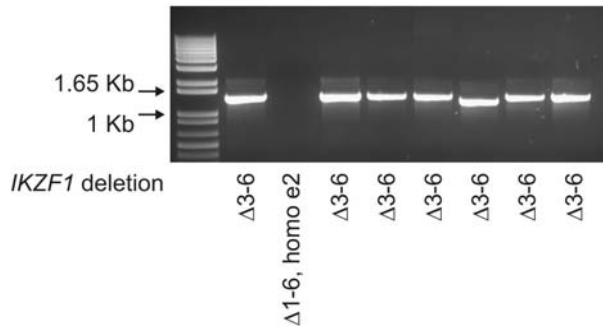
Testing – *IKZF1* and JAK

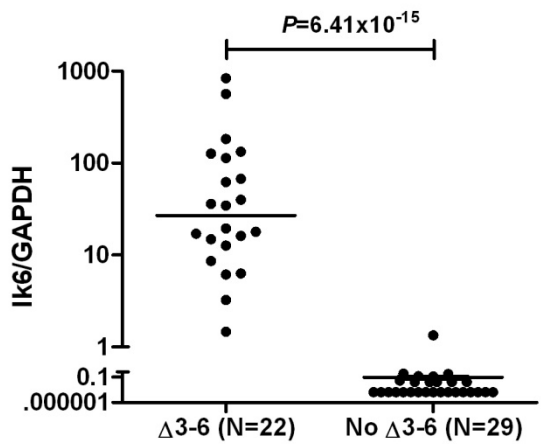
- FISH
- Exon specific qPCR
  - Robust, validated
- DNA PCR for *IKZF1* D3-6
  - Extremely conserved breakpoints
  - Qualitative – robust
  - Quantitative - ?role
- RNA PCR for *IKZF1* D3-6
- JAK mutation detection
  - Sanger sequencing
  - Screening – HRM, DHPLC (WAVE)

FISH



IKZF1
Del 3-6
DNA PCR





IKZF1
Del 3-6
RNA qPCR