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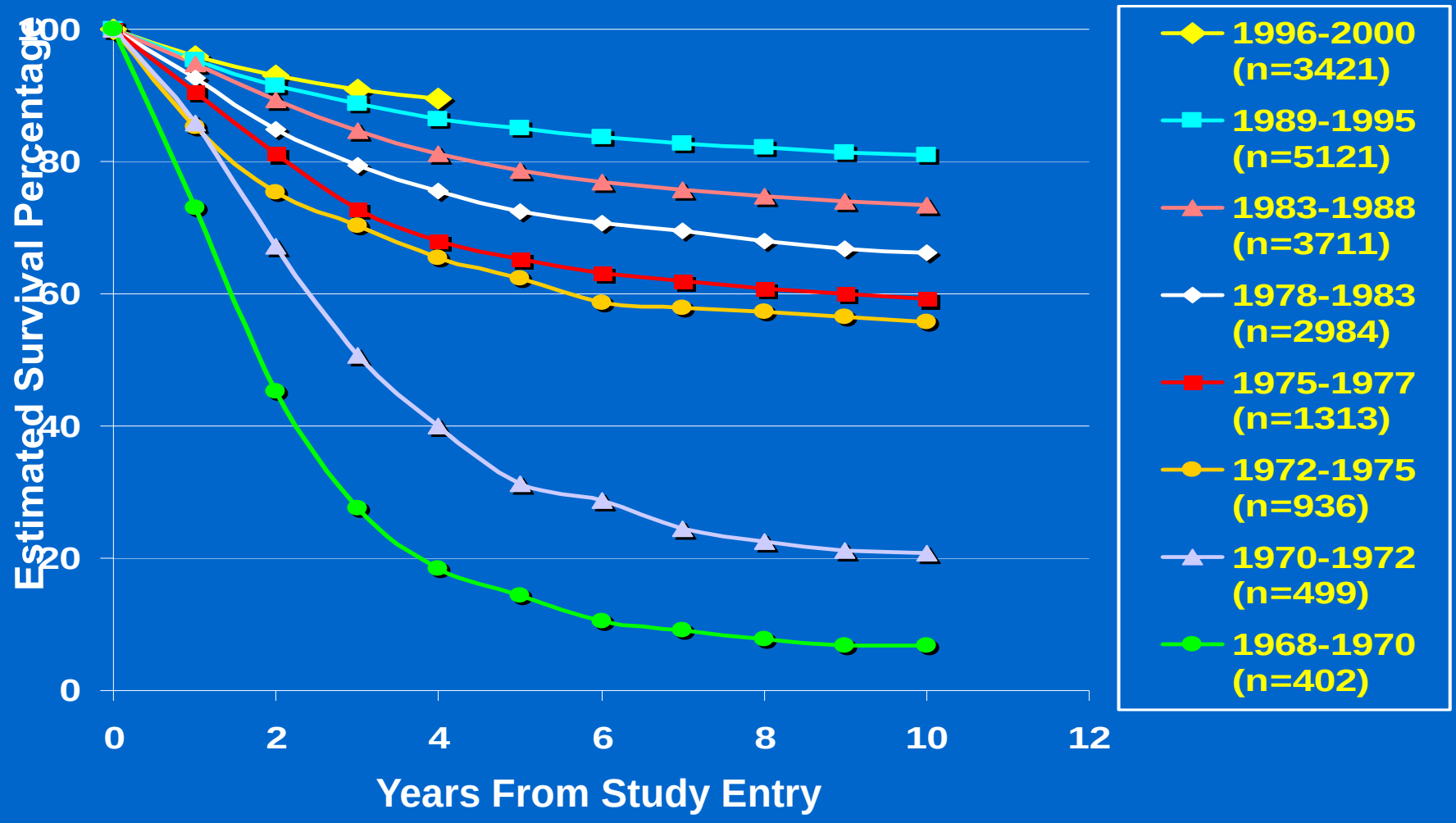
www.target.cancer.gov

Improved Survival in Childhood ALL
CCG ALL Trials 1968-2000

2000-2005: 5-yr Survival 90%



The Remaining Challenge for ALL

- ALL continues to be a major cause of cancer-related mortality in children and adolescents
- ALL treatment regimens have reached maximum tolerable intensity
- Only 1 new agent (imatinib) established to improve frontline therapy in past two decades
  - Relevant only for the 3% of pts with Ph+ ALL
- *Further improvements in outcome will require novel therapeutic approaches*

Childhood Cancer TARGET* Initiative: High Risk ALL Pilot Project

***Therapeutically Applicable Research to Generate Effective Treatments**

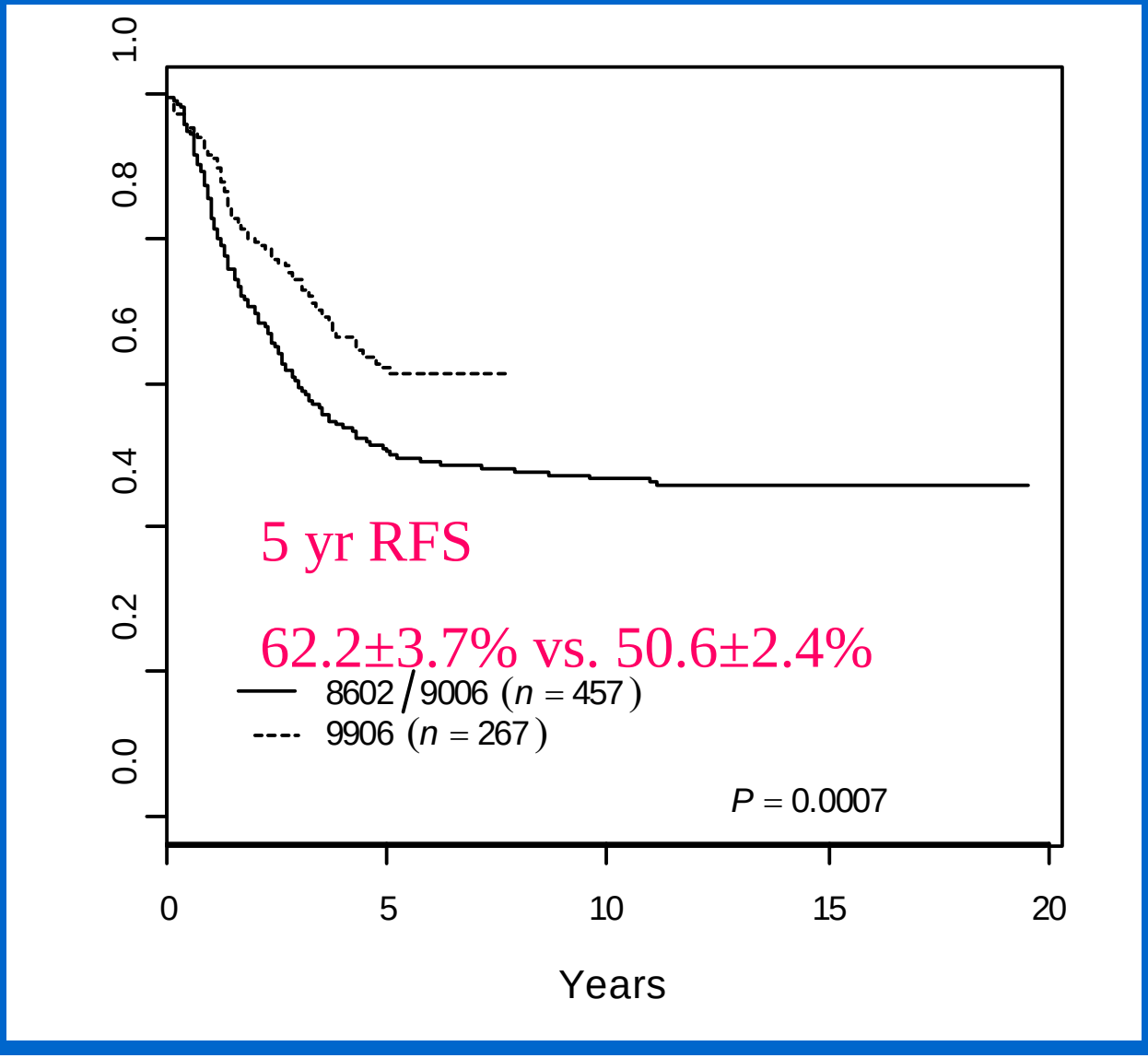
- Discover candidate therapeutic targets by identifying genes that are consistently mutated in lymphoblasts from patients with HR-ALL
- "Team science" approach to COG P9906 samples
  - COG: Stephen Hunger (Chair), William Carroll, Mini Devidas, Greg Reaman, Paul Bowman
  - Labs: Charles Mullighan, Jim Downing, Mary Relling, Cheryl Willman
  - NCI Office of Cancer Genomics: Daniela Gerhard
  - NCI Cancer Diagnosis Program: James Jacobson
  - NCI Cancer Therapy Evaluation Program: Malcolm Smith
  - NCI caBIG: Jinghui Zhang

High Risk Childhood ALL TARGET Initiative: COG P9906 Study Population

- Trial conducted by POG/COG from 3/00-4/03
- Identical therapy for all pts
- High risk pt population:
  - Higher WBC & older age
  - ~50% DFS on earlier trials
- No pts with Ph+, hypodiploid, or induction failure
- Few pts with “favorable” biological subtypes:
  - No trisomy 4/10 or TEL-AML1 unless CNS/testicular+
- 276 enrolled, 271 eligible

COG P9906: Improved Outcome vs. Historical Controls

Bowman WP et al., Submitted



High Risk Childhood ALL TARGET Initiative: Approach

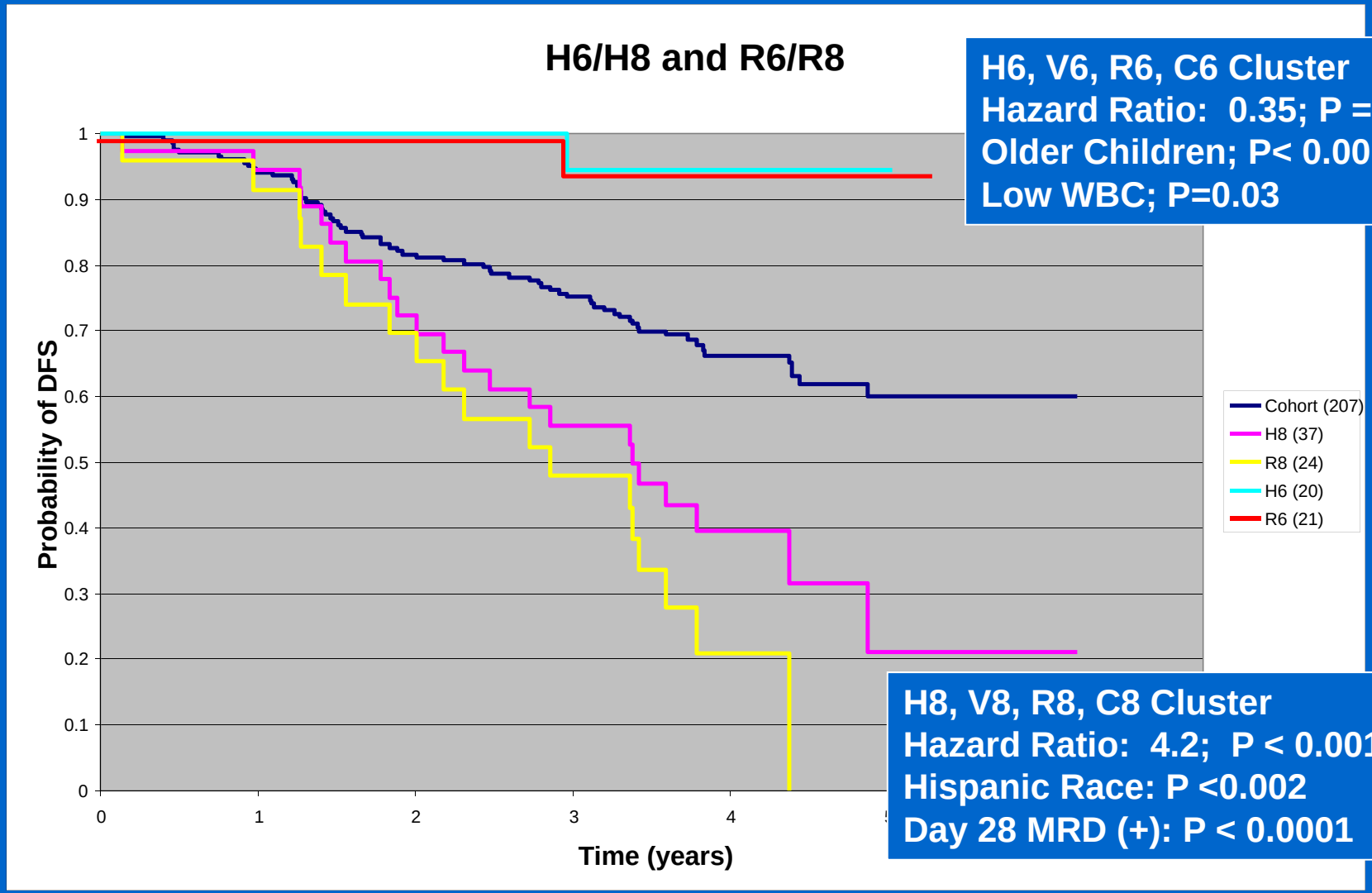
- Willman laboratory: Gene expression profiling
  - Affymetrix U133 Plus 2.0
- Mullighan + Downing laboratories: CNA and LOH
  - Affymetrix 500k + 100k SNP chip analyses on paired leukemia/germline specimens
- Use data to select genes for complete resequencing in leukemia samples
  - Sequence germline DNA for all identified alterations

High Risk Childhood ALL TARGET Initiative: Accomplishments I

- Supervised analysis of gene expression profiles identifies robust molecular risk classifiers that predict outcome and MRD
  - Kang, Willman et al, submitted
- Unsupervised analysis of gene expression profiles identifies intrinsic clusters linked to sentinel genetic lesions (known and previously unknown) and outcome
  - Harvey, Willman et al, submitted

Disease-Free Survival: Clusters H6/R6 and H8/R8

Harvey et al, Submitted

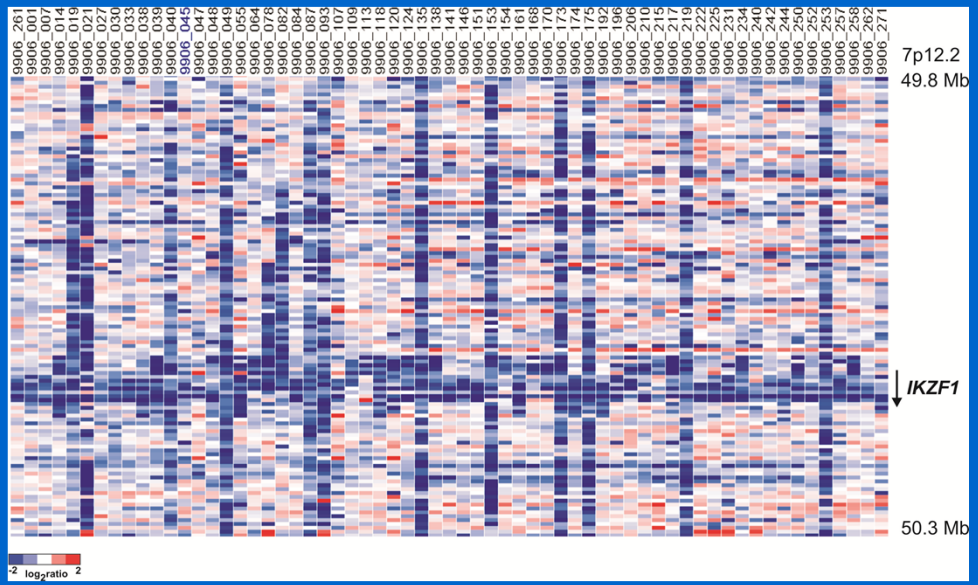


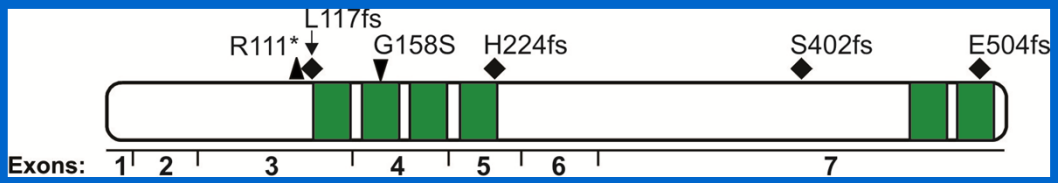
Association of H8/R8 with poor outcome confirmed in CCG 1961 cohort

High Risk Childhood ALL TARGET Initiative: Accomplishments II

- 67% cases have lesions in B-cell development pathway genes
- IKZF1 (IKAROS) alterations in ~ 30% of cases
  - Strong predictor of poor response and adverse outcome
  - Bcr/Abl-like gene signature (*suggests there may be kinase gene mutations*)

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





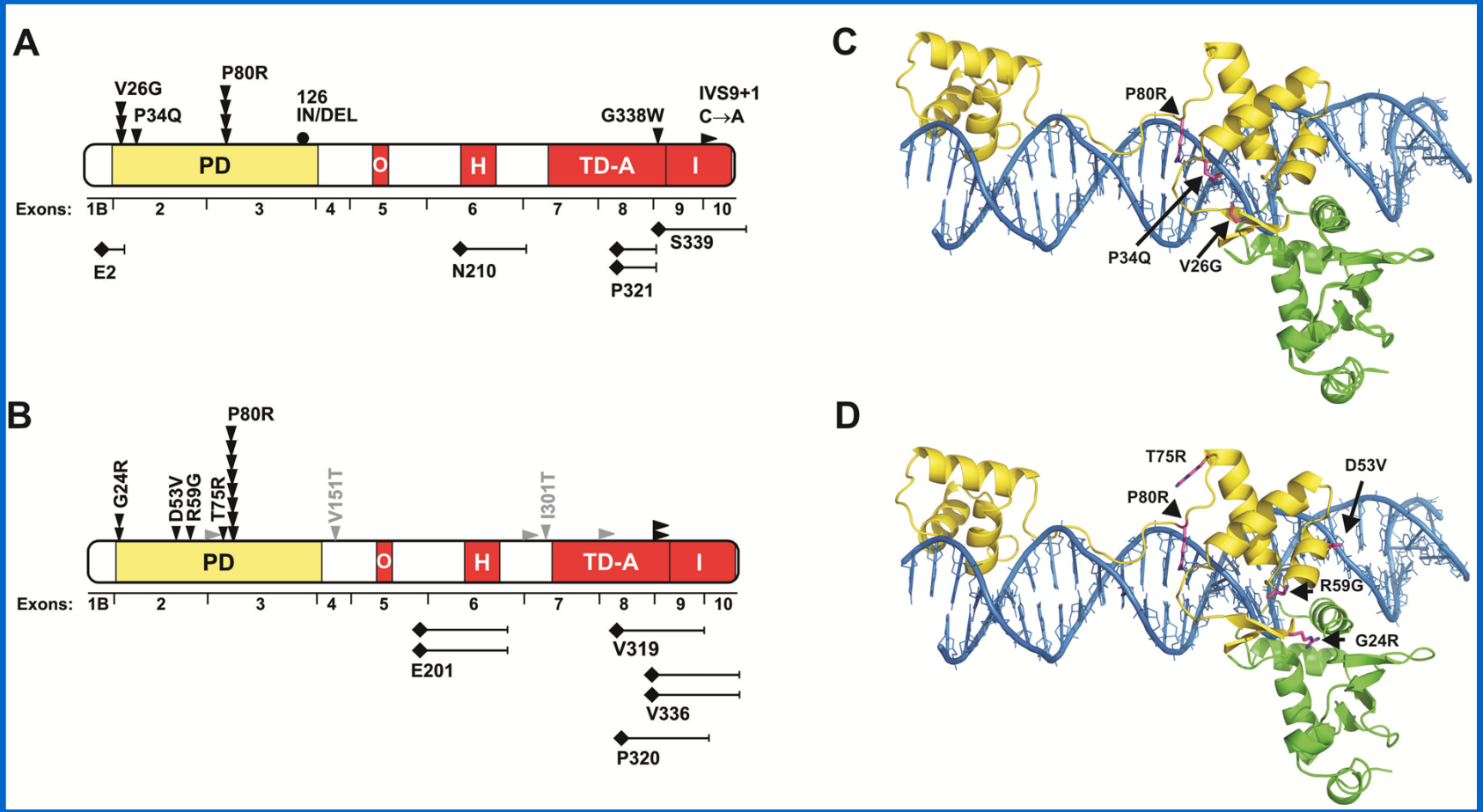
Mullighan et al, N Engl J Med. 360:470, 2009

Gene Resequencing Results

- 125 genes selected for resequencing in 187 ALL samples based on:
  - Genes located in regions of CNA
  - Known cancer genes
  - Genes with expression that characterized intrinsic clusters with poor outcome (R7/H7, R8/H8)
- Resequencing of 1st 100 genes complete in leukemia DNA

Gene Resequencing Results

- Confirmed previously identified mutations:
  - PAX5 mutation spectrum similar to SJCRH cohort



Mullighan et al, N Engl J Med. 360:470, 2009

Janus Family Kinases (JAK)

- Includes JAK1, JAK2, JAK3, and TYK2
- Key mediators of signal transduction
- JAK2 mutated in P. vera and other MPD
  - V617F pseudokinase domain mutation
  - JAK2 inhibitors in clinical trials
- JAK2 R683G mutation recently reported in ~20% of Down syndrome-ALL cases
  - JAK mutations detected previously in <<1% of other ALL cases

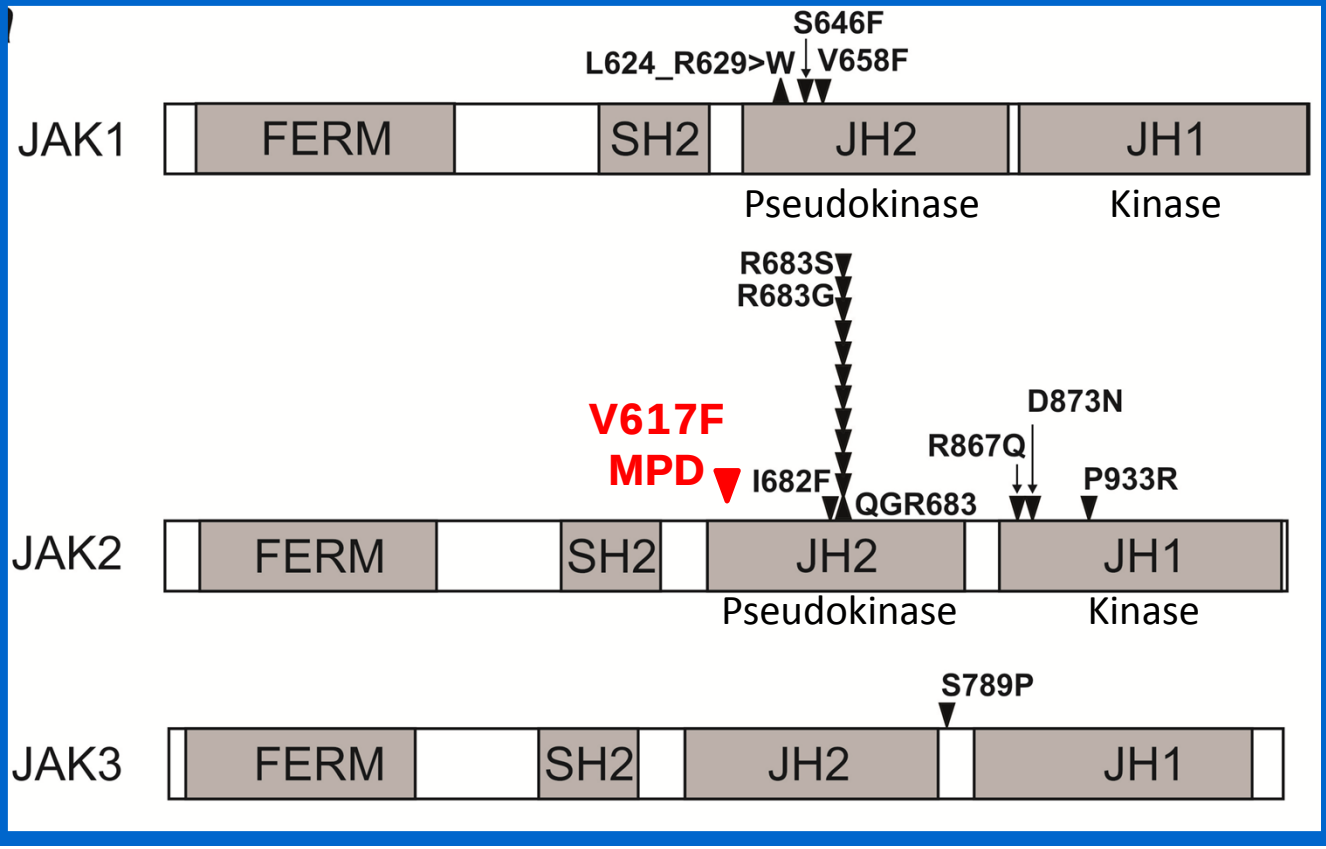
JAK Mutations in High Risk ALL

Mullighan et al, Submitted

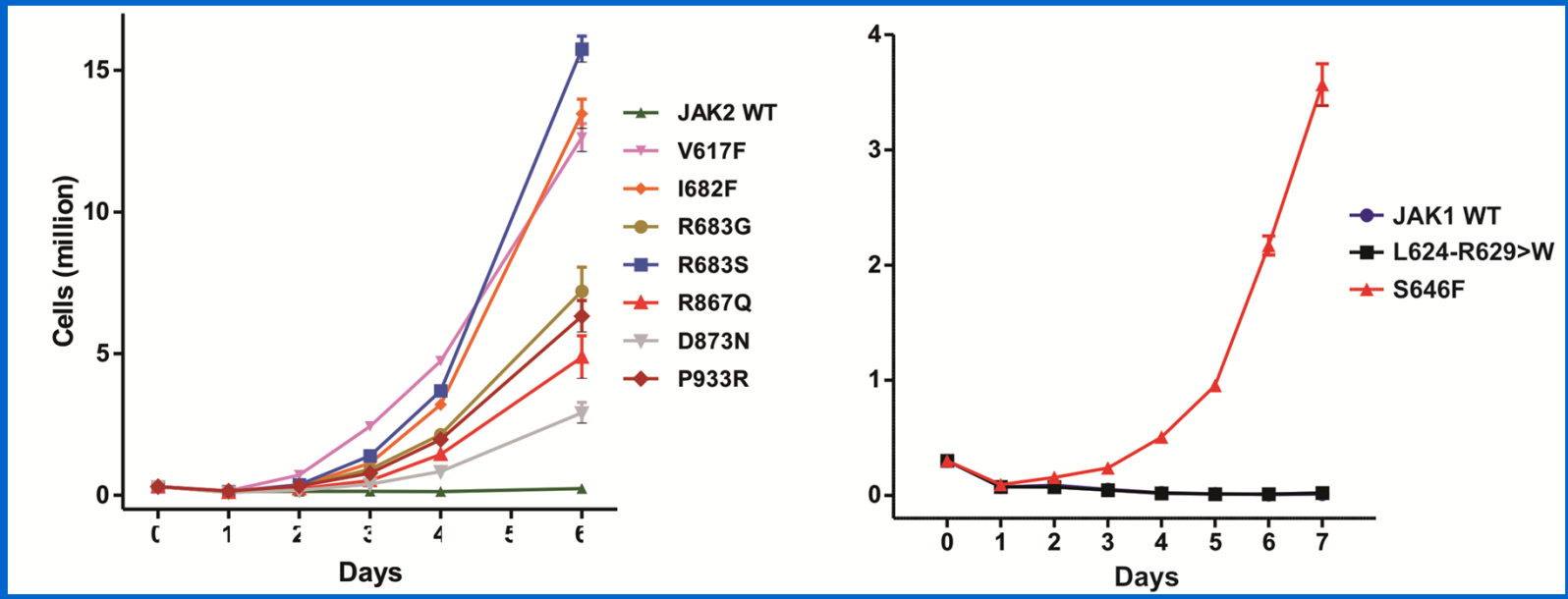
- Heterozygous somatic non-silent JAK mutations present in 20/187 P9906 cases
  - 18/178 non-DS (10.1%) and 2/9 DS
- All lacked common known translocations
- JAK mutations highly correlated with IKZF1 deletions and the R8 poor outcome cluster

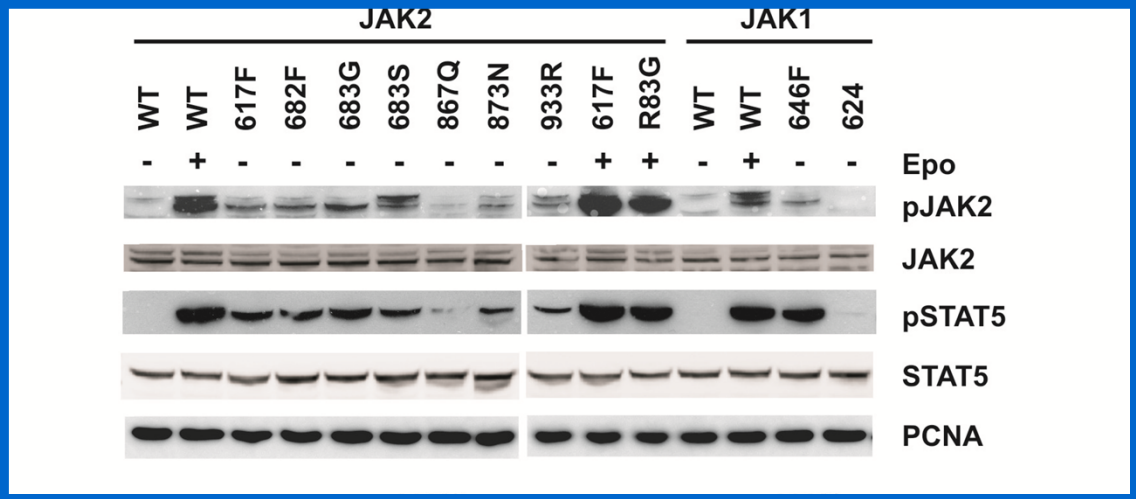
JAK mutations in “BCR-ABL1-like” ALL

- JAK2 (n=16): 10 R683G; 3 non-R683G pseudokinase domain; 3 kinase domain
- JAK1 (n=3): 3 pseudokinase domain
- JAK3 (n=1): uncertain functional consequences

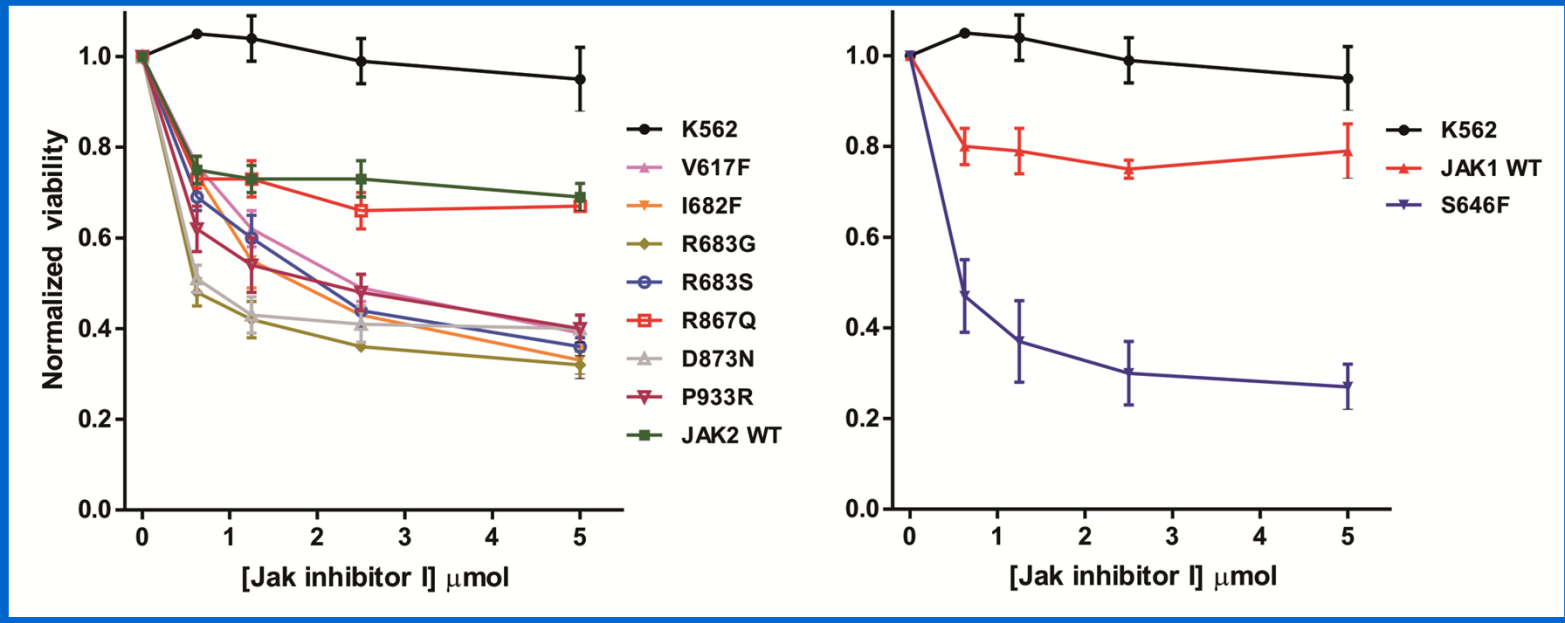


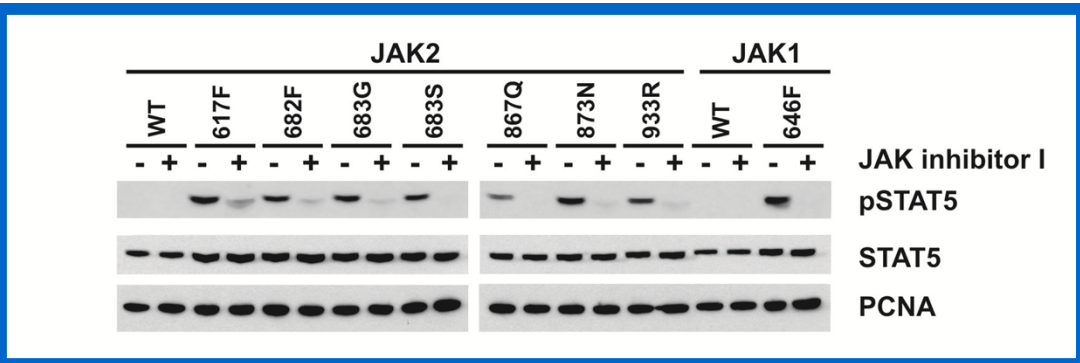
Cytokine Withdrawal Confirms Factor Independence of JAK-Transduced BaF3-EpoR Cells





JAK-Transduced BaF3-EpoR Cells are Sensitive to Pharmacologic JAK Inhibition





COG P9906 Relapse Risk: Effect of JAK and IKZF1

Mullighan et al, Submitted

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

Use of JAK Inhibitors in ALL: Rationale and Next Steps

- JAK mutations present in ~10% of COG P9906 cases
  - Confer factor independence
  - Factor dependence restored with JAK inhibitor therapy
- Will agents developed for V617F P. vera pseudokinase domain mutation also inhibit ALL mutations?
  - Test in BaF3-EpoR cells transduced with various mutants
- Will JAK inhibitors be effective in this subset of ALL?
  - Test in xenografts established from ALL with JAK mutations
  - Plan for pediatric clinical trials

ALL TARGET Project: Next Steps

- Complete analysis of leukemia and germline sequencing results for initial 125 gene dataset
- Complete studies of IgH-CRLF2 translocations
  - Highly correlated with JAK mutations
  - May be another way to activate JAK pathway suggesting JAK inhibition may be effective in cases lacking JAK mutations
- Are there other kinase mutations in BCR-ABL like cases that lack JAK mutations?
  - Sequence tyrosine kinome in these cases
- Determine if this new genomic knowledge can be integrated into next generation COG ALL trials