

Síndromes Linfoproliferativos

Jose A. Martínez Climent

Área de Oncología

Centro de Investigación Médica Aplicada

Universidad de Navarra, Pamplona

BIOLOGY OF LYMPHOPROLIFERATIVE SINDROMES

The Evolution and Impact of Subclonal Mutations in Chronic Lymphocytic Leukemia

Dan-Avi Landau, Catherine J. Wu; Yale University, New Haven, CT; Dana Farber CI, Boston, MA
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Davide Rossi, Gianluca Gaidano; University of Eastern Piedmont, Novara, Italy

Whole Genome/Exome Sequencing in Sequential Biopsies Reveals the Genetic Evolution of Follicular Lymphoma

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Michael Green, Ash Alizadeh; Division of Oncology, Stanford University, Stanford, CA

ID3 Is a Novel Tumor Suppressor Gene in Burkitt Lymphoma

Cassandra Love, Sandeep S. Dave; Department of Medicine, Duke University, Durham, NC

High Incidence of EZH2 Mutations in Follicular Lymphoma and Its Consequences for EZH2 Targeted Therapy

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Kian-Huat Lim, Louis M. Staudt; Center for Cancer Research, NCI/NIHealth, Bethesda, MD, USA

The Evolution and Impact of Subclonal Mutations in Chronic Lymphocytic Leukemia

Dan-Avi Landau, Catherine J. Wu

Yale University, New Haven, CT; Medical Oncology, Dana Farber Cancer Institute, Boston, MA
(Plenary Scientific Session, abstract #5)

Aim 1: To determine the impact of clonal heterogeneity on cancer progression in chronic lymphocytic leukemia (CLL).

Hypothesis: The evolutionary dynamics of subclonal mutations contribute to the variations in disease tempo and response to therapy that characterize CLL.

Methods: Whole exome sequencing (WES) and SNP arrays performed to analyze subclonal and clonal point mutations and copy-number alterations in 149 CLLs. A novel computational approach was used, which integrates purity and local ploidy information, to infer the cancer cell fraction of each mutation from WES data → to classify mutations as clonal or subclonal.

Results: Subclonal mutations were detected in 146/149 CLLs. Higher numbers were associated with prior anti-leukemia therapy ($P=0.017$), suggesting that a strong extrinsic selection pressure, such as cytotoxic treatment, promotes the expansion of fitter subclones.

Of the 149 samples, only 3 drivers (*MYD88*, trisomy 12, and *del(13q)*) that were clonal in 80-100% of samples harboring these alterations were found, suggesting that they arise earlier in CLL development. Other drivers (e.g., *ATM*, *TP53* and *SF3B1*) were often observed at subclonal frequencies, indicating that they arise later in leukemic development.

The Evolution and Impact of Subclonal Mutations in Chronic Lymphocytic Leukemia

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Yale University, New Haven, CT; Medical Oncology, Dana Farber Cancer Institute, Boston, MA;

Aim 2: To assess the evolution of somatic mutations in 18 patients, in which data from two distant timepoints were available.

Results: Clonal evolution was observed in 11 of 18 patients (10 of 12 who received intervening treatment, but only 1 of 6 without intervening treatment, $P=0.012$) and confirmed that subclonal mutations (del(11q), *SF3B1* and *TP53*) shifted towards clonality over time.

If treatment-associated genetic evolution leads to expansion of a fitter subclone, a shorter time to relapse can be predicted in these individuals. In the 149 samples, CLLs with subclonal driver mutations were associated with shorter times from diagnosis to first therapy ($P=0.001$). Regression models adjusting for CLL prognostic factors (*IGHV* status, prior therapy and high risk cytogenetics) demonstrated that the presence of a subclonal driver was an independent risk factor for earlier retreatment (adjusted hazard ratio of 4.61 (CI 1.59-13.34), $P=0.005$).

Thus, the detection of *subclonal drivers* (indicative of an active evolutionary process) is associated with earlier treatment and shorter duration of remission.

These data challenge us to therapeutically address not only genetic targets but also their dynamic evolutionary landscape

Integrated Mutational and Cytogenetic Analysis Identifies New Prognostic Subgroups in Chronic Lymphocytic Leukemia

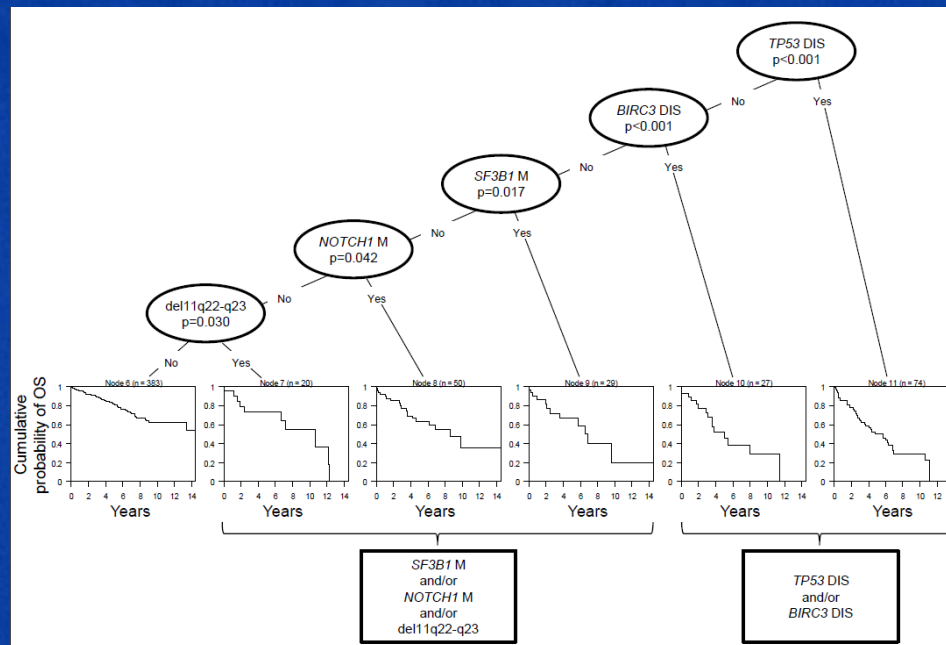
Davide Rossi, Gianluca Gaidano

Division of Hematology, University of Eastern Piedmont, Novara, Italy

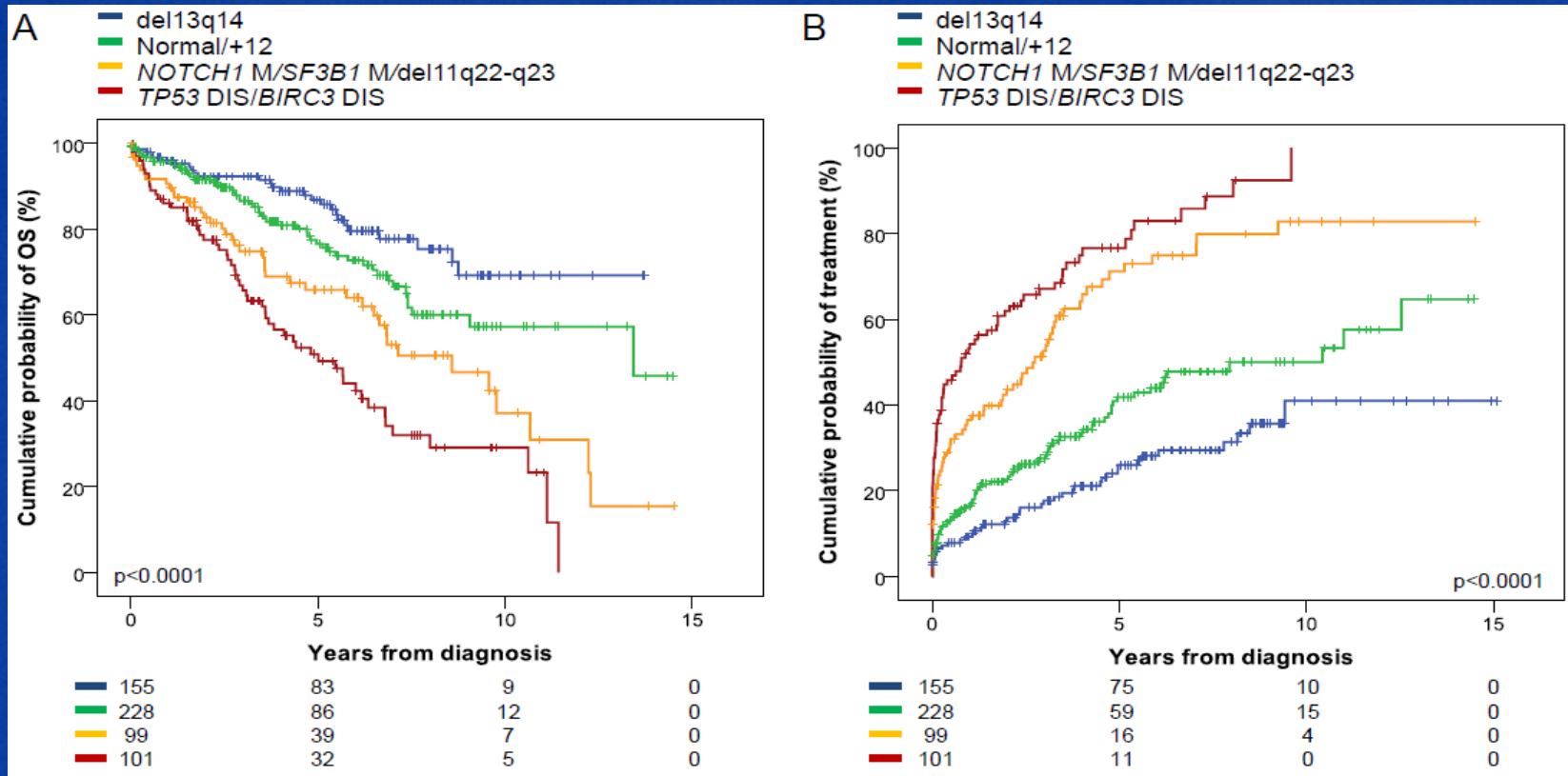
Abstract #709

Aim: To establish a comprehensive and dynamic prognostic algorithm in chronic lymphocytic leukemia (CLL) including the new and already known gene mutations, chromosomal abnormalities, and their changes during clonal evolution.

Methods: Integrative mutational and cytogenetic analyses in 1274 CLL samples using both a training-validation and a time-dependent design.



Integrated Mutational and Cytogenetic Analysis Identifies New Prognostic Subgroups in Chronic Lymphocytic Leukemia



Results: Four CLL subgroups were hierarchically classified:

This integrated mutational and cytogenetic model independently predicted survival and improved CLL prognostication accuracy

This model may have implications for the design of clinical trials aimed at assessing the use of mutational profiling to inform therapeutic decisions significantly differ from a matched general population.

Whole Genome/Exome Sequencing in Sequential Biopsies Reveals the Genetic Evolution of Follicular Lymphoma

Jessica Okosun J, Jude Fitzgibbon

Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK
Abstract #145

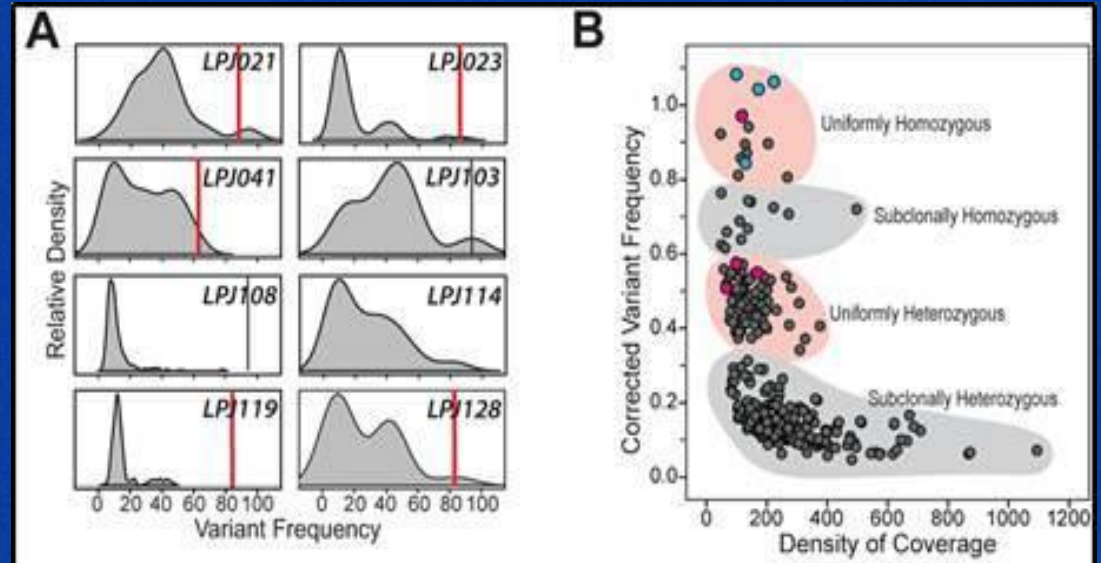
Michael Green, Ash Alizadeh

Division of Oncology, and Center for Cancer Systems Biology, Stanford University, Stanford, CA
Abstract #149

Hypothesis: Novel tailored therapies towards actionable somatic mutations in Follicular Lymphoma (FL) are being developed. However, for these therapies to be most effective, the mutations must be uniformly present in tumor cells and in self-renewing tumor cell precursors.

Aim: Understanding of the clonal dynamics during disease the progression and transformation of FL to DLBCL (tFL).

Methods: Whole-exomes and transcriptomes of 26 tumor-derived subpopulations sorted based on expression of CD20 in 10 lymphomas derived from 8 patients, including pairs at diagnosis and first disease relapse. qPCR of invariant BCL2 and VDJ recombination was used to quantitate tumor purity in subpopulations.



Whole Genome Sequencing in Sequential Biopsies Reveals the Genetic Evolution of Follicular Lymphoma

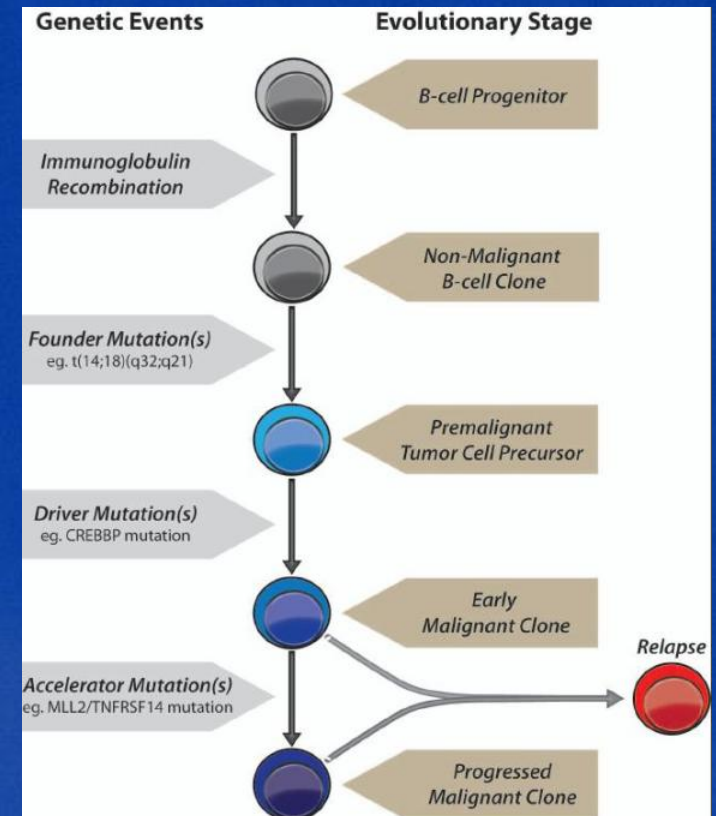
Results: Each tumor 72 somatic mutations, higher in tFL genomes (mean: 83.6 vs. 64.8). Mutations in genes involved in histone methylation (*MLL2*, *EZH2*), histone acetylation (*CREBBP*, *MEF2B*), NF- κ B and BCR signaling, DNA repair and apoptosis regulation

A single mutational event that was restricted to the tFL 'branch' and could immediately be linked with transformation was not found.

Striking intratumoral clonal diversity within FL tumors in the representation of mutations was found in the majority of genes, capturing a clonal hierarchy with respect to IG somatic mutations and *BCL2* translocations.

BCL2 translocations and *CREBBP* mutations are early events, while *MLL2* and *TNFRSF14* (CD258) mutations likely represent late events during disease evolution.

These data provide insight into which of the genetic lesions represent suitable candidates for targeted therapies



ID3 Is a Novel Tumor Suppressor Gene in Burkitt Lymphoma

Cassandra Love, Sandeep S. Dave

Department of Medicine, Duke University, Durham, NC

Abstract #898

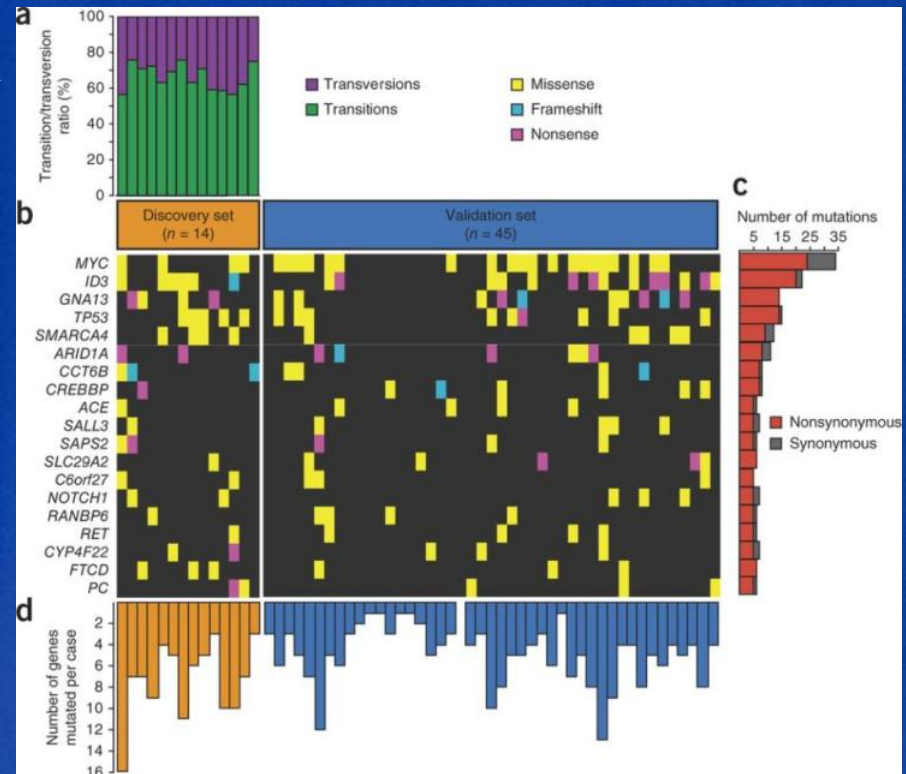
Burkitt lymphoma is characterized by deregulation of MYC, but the contribution of other genetic mutations to the disease is largely unknown.

Aim: To identify gene mutations that cooperate with MYC using high-throughput sequencing analysis of Burkitt lymphoma samples.

Methods: Whole genome sequence (WGS) of a Burkitt lymphoma tumor and germline DNA from the same affected individual. Whole exome sequencing (WES) of 59 Burkitt lymphoma tumors and comparison with the sequenced exomes from 94 DLBCLs.

Results: Seventy genes that were recurrently mutated in Burkitt lymphomas, including ID3, GNA13, RET, PIK3R1 and the SWI/SNF genes ARID1A and SMARCA4. Other novel genes included CCT6B, SALL3, and FTCD. ID3 mutations occurred in 34% of Burkitt lymphomas and not in DLBCLs.

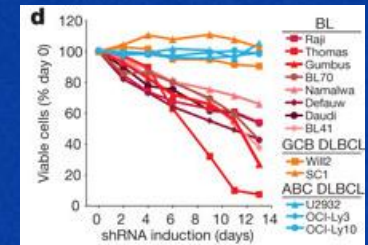
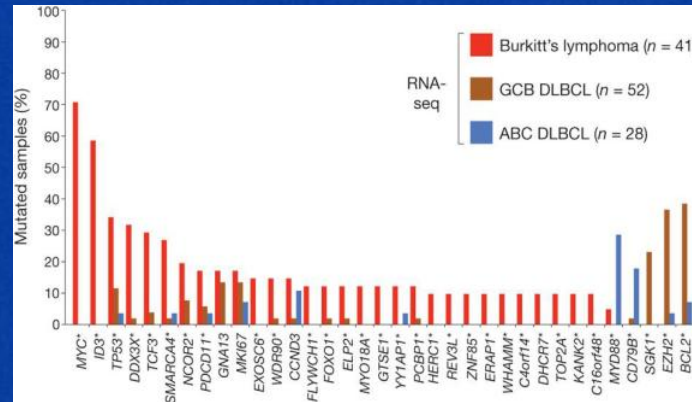
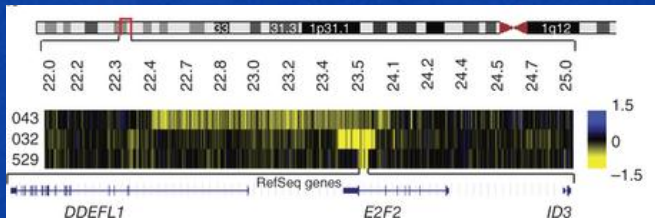
Love C, et al, Nature Genetics Dec 2012



ID3 Is a Novel Tumor Suppressor Gene in Burkitt Lymphoma

Richter J, et al; Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nature Genetics* Dec 2012

Whole-genome, whole-exome and transcriptome sequencing of four BLs. Seven recurrently mutated genes were found, including ID3, mapped to a region of focal homozygous loss. In an extended cohort, 36 of 53 molecularly defined Burkitt lymphomas (68%) carried potentially damaging mutations of ID3.



Schmitz R, et al; Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* 2012

High-throughput RNA sequencing and RNA interference screening to discover essential regulatory pathways in BL that cooperate with MYC. In 70% of sporadic BL cases, mutations affecting the transcription factor TCF3 (E2A) or its negative regulator ID3 were identified. TCF3 activated the pro-survival PI3K pathway in BL, in part by augmenting tonic B-cell receptor signalling.

These studies implicate TCF or its negative regulator ID3 as a common deregulated pathway cooperating with MYC in BL by activating PI3K signalling

High Incidence of EZH2 Mutations in Follicular Lymphoma and Its Consequences for EZH2 Targeted Therapy

Csaba Bödör, Jude Fitzgibbon

Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK
Abstract #545

Aim: As EZH2 inhibitors are currently in development, *EZH2* mutation status were assessed in 367 FL cases (238 diagnosis, 129 relapse).

Methods: Next-generation sequencing (NGS) was initially performed for all 27 *EZH2* exons of 50 FL cases and this confirmed mutations at the 3 codons Y646, A682, and A692. Targeted resequencing was therefore restricted to these 3 codons and performed on whole tumor DNA from 367 FL cases by conventional Sanger and high-throughput sequencing.

Results: Sixty-three *EZH2* mutations (17%) were detected using both approaches at a mean mutation load of 29.8% (range: 4-61%). Critically, deep-sequencing enabled detection of an additional 43 mutations with an average mutational load of 10.2% (range: 2-31%) increasing the overall mutation rate to 29%.

This study demonstrate higher prevalence of EZH2 mutations in FL then previously reported, suggesting the use of the newly developed EZH2 inhibitors in this lymphoma subtype

- McCabe HT, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* 2012
- Knutson SK, et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat Chem Biol.* 2012

IRAK4 Kinase As A Novel Therapeutic Target in the ABC Subtype of Diffuse Large B Cell Lymphoma

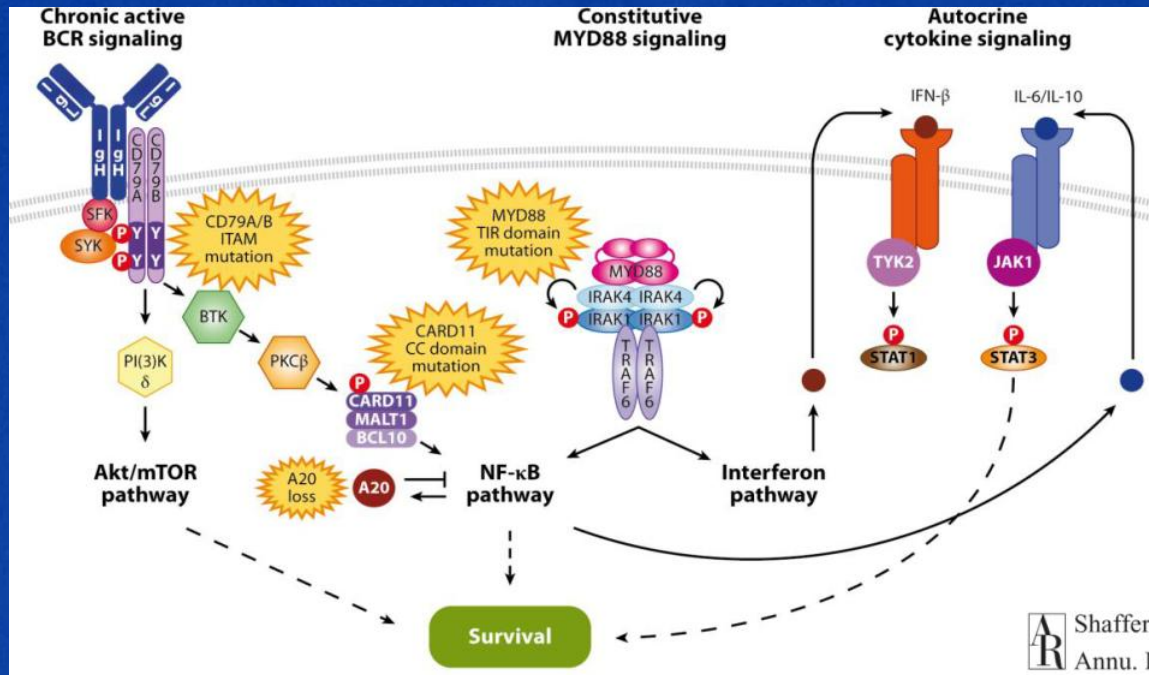
Kian-Huat Lim, Louis M. Staudt

Center for Cancer Research, NCI/NIHealth, Bethesda, MD, USA

Abstract #62

Background: Activating mutations of MyD88, particularly L265P, occur in about 30% of ABC-DLBCLs. Oncogenic signal of mutant MyD88 is transduced through IRAK4 and IRAK1, resulting in constitutive NF- κ B signaling. The kinase activity of IRAK4, but not IRAK1, is required for the oncogenic effect of mutant MyD88 in ABC-DLBCL (Ngo et al, Nature 2011).

Aim: To screen for inhibitors of IRAK4 kinase activity that could have a therapeutic impact in lymphomas with MyD88 mutations.



IRAK4 Kinase As A Novel Therapeutic Target in the ABC Subtype of Diffuse Large B Cell Lymphoma

Kian-Huat Lim, Louis M. Staudt

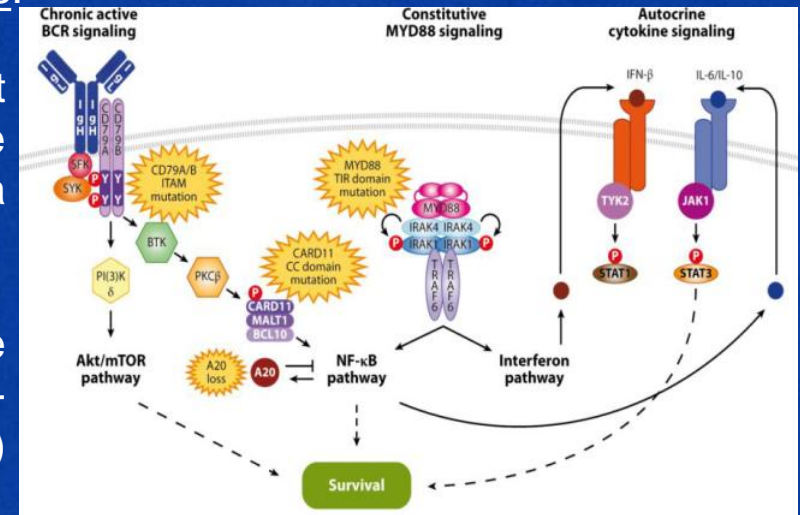
Center for Cancer Research, NCI/NIHealth, Bethesda, MD, USA

Results: Two potent small molecules, highly selective IRAK4 inhibitors, ND-2158 and ND-2110, were identified across a panel of >300 kinases.

Both inhibitors are toxic towards ABC-DLBCL but not GCB DLBCL cell lines. The molecules demonstrate good pharmacologic drug-like properties and have a suitable safety profile for clinical evaluation.

Mechanistically, both inhibitors potently abrogate IRAK4-mediated phosphorylation of IRAK1 and NF- κ B activity resulting from the MyD88 (L265P) mutation in ABC DLBCL.

Results: A second, parallel survival pathway in ABC DLBCL is engaged by “chronic active” B cell receptor signaling, which can be blocked by inhibiting Bruton's tyrosine kinase (BTK) either genetically or pharmacologically. Notably, the IRAK4 inhibitors strongly synergized with BTK knockdown in killing multiple ABC DLBCL cell lines.



These results provide a rationale for the further development of IRAK4 inhibitors for the therapy of ABC DLBCL, and suggest that simultaneous inhibition of BCR signaling may provide superior clinical responses