Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma[1]

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Presented by Dominik Yang and Diana Lin

Diffuse Large B Cell Lymphoma (DLBCL)

- Most common type of non-Hodgkin lymphoma (NHL)[2]
- Under a microscope, lymphoma cells appear larger than normal lymphocytes[2]
- Most of the time, starts in the lymph nodes but it can also start in organs and tissues outside the lymph nodes[2]
- 30-40% of cases are diagnosed in stage 1 or 2 (localized)[2]
- Rest of the cases have spread to lymph nodes above and below the diaphragm or to liver, spleen, or bone marrow[2]
- Treated with R-CHOP chemotherapy[3]
  - **R**ituximab
  - **C**yclophosphamide
  - **H**ydroxydaunomycin
  - **O**ncovin
  - **P**rednisolone

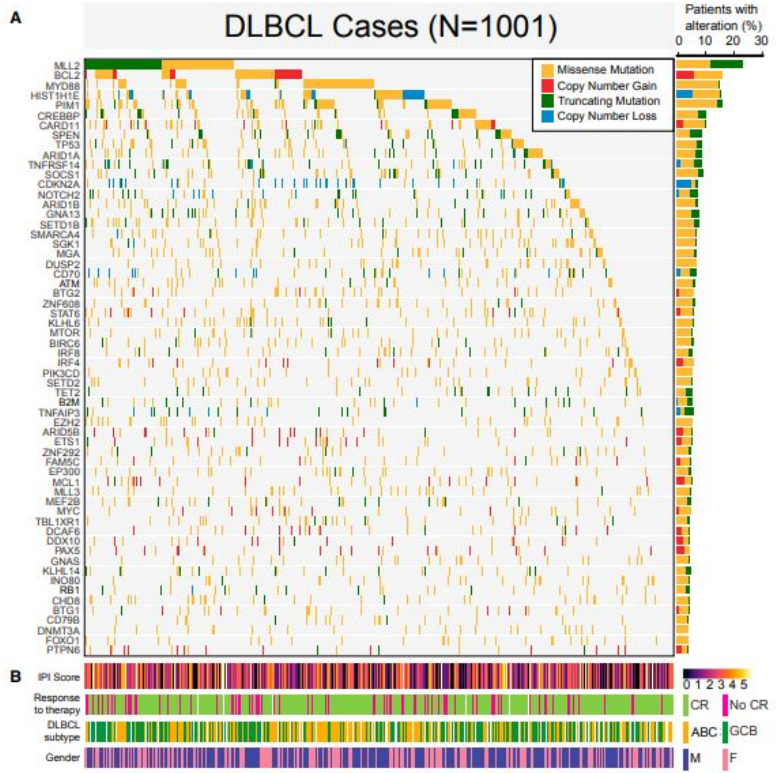
Background

- Most common hematologic malignancy (annual incidence of over 100,000 cases worldwide)
- **Striking molecular and clinical heterogeneity makes it difficult to study this disease**
- Requires a large sample size to discover rare but important mutations

Goals

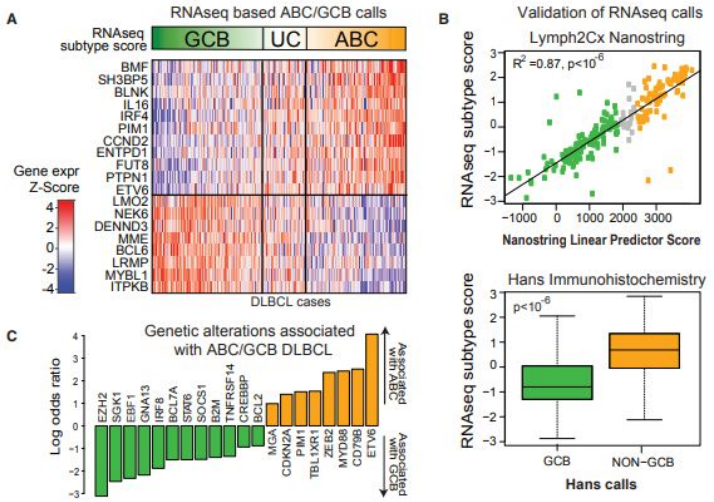
- Define the link between the functional impact of these genetic mutations to the growth of lymphoma cells
- Ultimately be able to create therapeutics that target specific genetic drivers of DLBCL

Discovery of Genetic Drivers



- Whole-exome sequencing of 1001 DLBCLs
- 150 possible driver genes identified
- Mean of 7.75 mutations per DLBCL patient
- Driver genes express common mutation patterns:
- Missense mutation and copy number gains - oncogene (BCL2, CARD11, IRF4)
- Truncating mutations and copy number losses - tumor suppressor (SPEN, CDKN2A, TNFAIP3)

Cell-of-Origin Effects



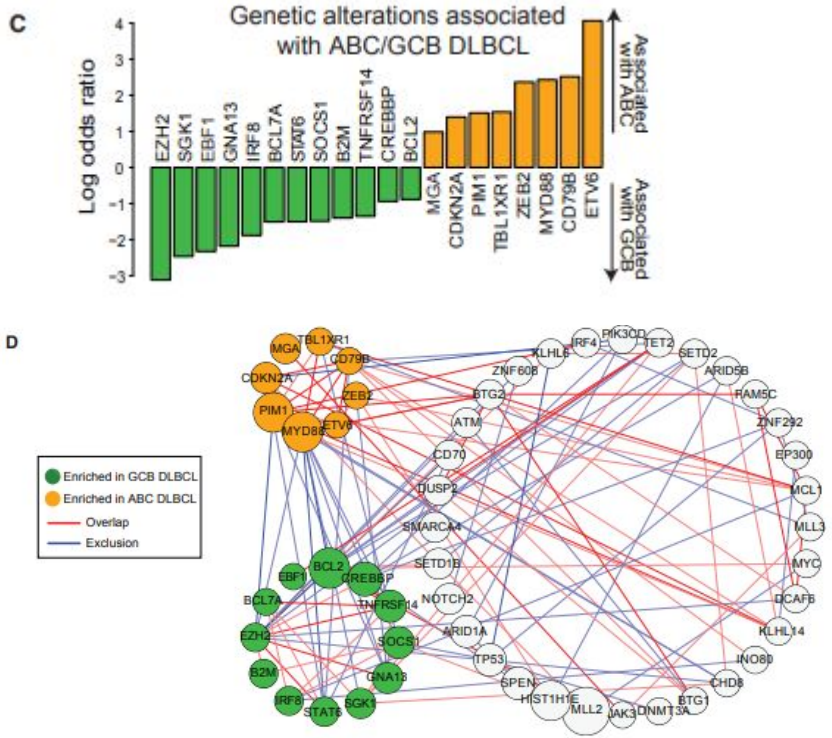
- RNA sequencing of gene expression in 775 patients to determine tumor cell of origin (A)
  - 313 activated B cell-like (ABC)
  - 331 germinal Center B cell-like (GCB)
  - 131 unclassified
- Validated by 2 complementary methods (B)
  - Nanostring assays
  - Immunohistochemistry-based Hans algorithm
- ABC DLBCLs had significantly worse overall survival than GCB DLBCLs

Cell-of-Origin Effects

Question:

Why do ABC DLBCLs have worse survival than GCB DLBCLs in response to standard therapy?

Connectivity of Driver Genes

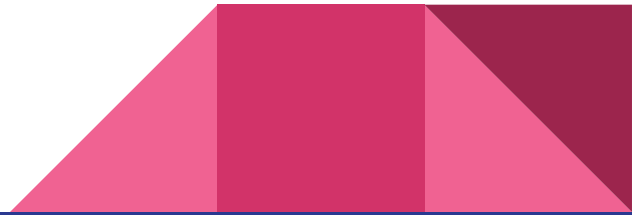


- ABC and GCB DLBCLs share most driver genes
- 20 genes had significantly different mutations (C)
- Of the 150 driver genes, 61 were significantly related to other driver genes (D)
  - MLL2 mutations mostly exclusive with MYC
  - TP53 mutations mutually exclusive with KLHL6

Functional Genomics via CRISPR Screening

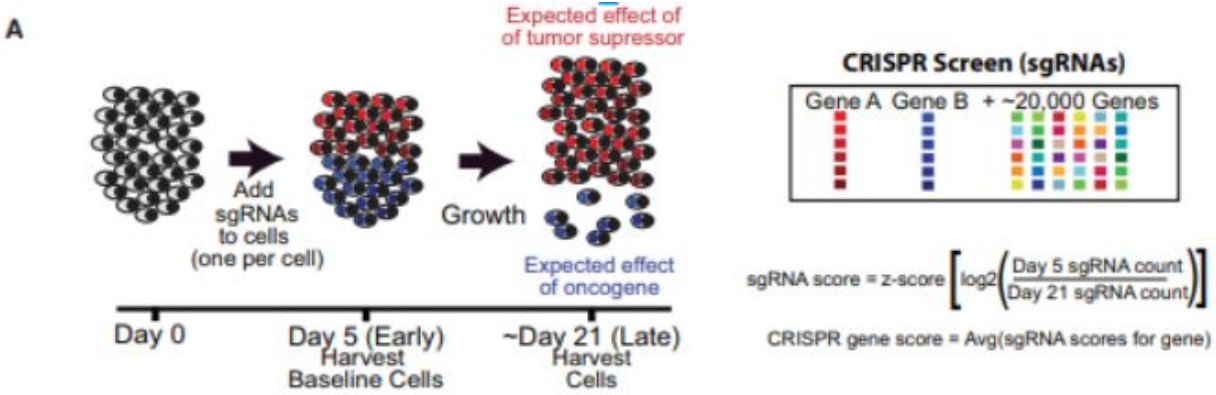
- 3 ABC, 2 GCB DLBCL, 1 BJAB (similar to GCB DLBCL)
- Selectively knock-out each gene in the DLBCLs to observe the effect on cell growth

QUESTION:
What did the authors expect to see from the effect of oncogenes vs tumor-suppressor genes?



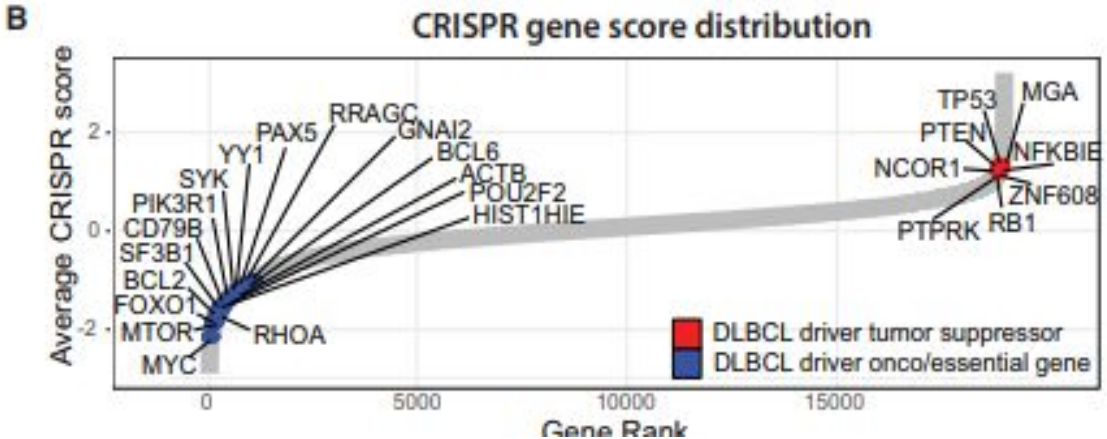
Functional Genomics via CRISPR Screening

- Expected outcome: cells with oncogene-targeting sgRNAs would be depleted, cells expressing tumor-suppressor targeting sgRNA would be enriched

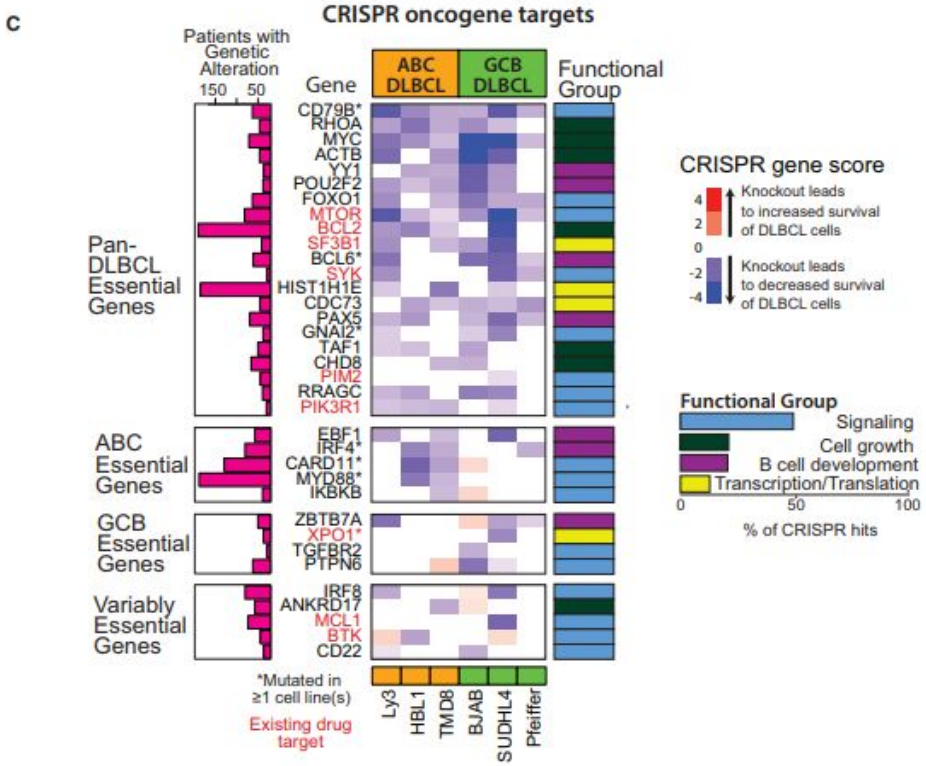


Functional Genomics via CRISPR Screening

- 1956 essential genes with significant role on cell fitness
- CRISPR score: degree of alteration in each gene as a function of altered abundance of sgRNAs targeting the gene



Functional Genomics via CRISPR Screening

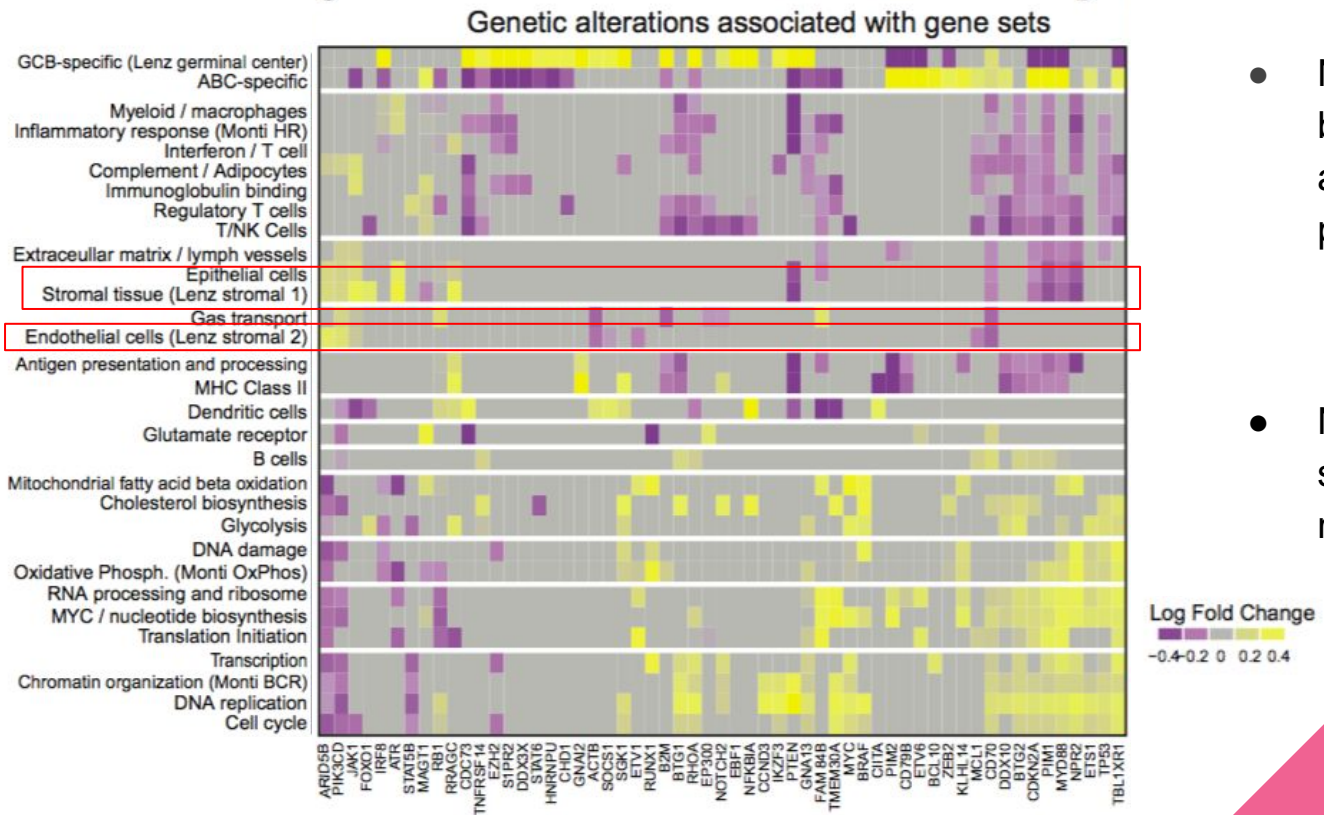


- 35 driver genes identified as oncogenes
  - 9 sub-type specific ABC or GCB
- Four functional groups:
  - Signalling
  - Cell growth
  - B cell development
  - Transcription and translation
- Lethal effects of gene knockdown also affects non-mutated cells

Gene Expression Signature Associations with Genetic Alterations and Outcome

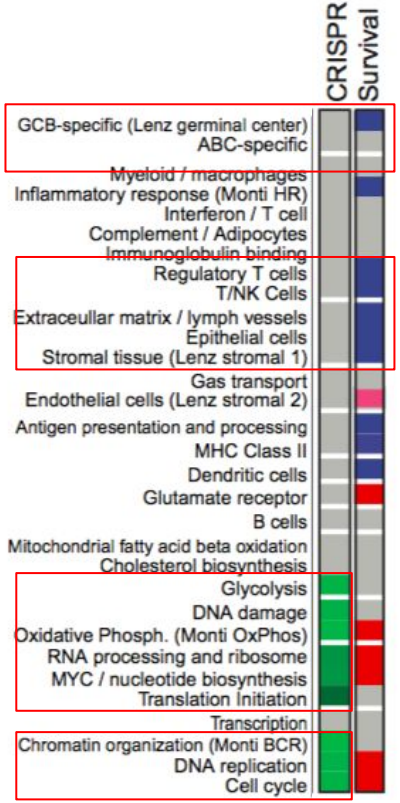
- Gene expression signatures with significant correlation fall into two classes:
  - Immune cell types (regulatory T cells, myeloid cells, NK cells)
  - Stromal connective tissue (epithelial and endothelial cells)
- *RHOA* and *MYC* mutations associated with proliferation-related signatures

Gene Expression Signature Associations with Genetic Alterations and Outcome



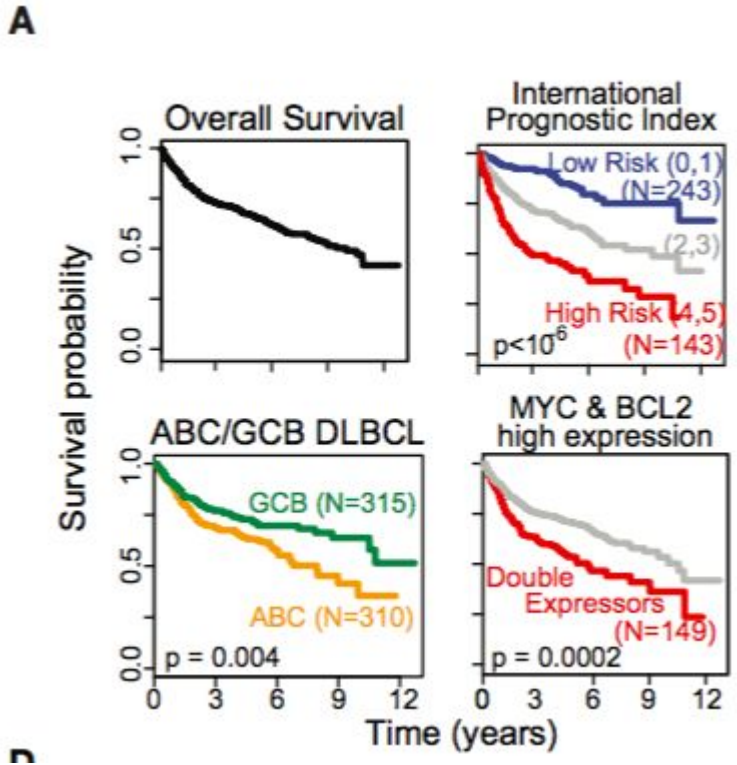
- No reliable association between signalling pathways and mutations in those pathways
  - Non-malignant cells may have affected effective measurements
- No association between stromal signatures and mutations (insignificant)

Gene Expression Signature Associations with Genetic Alterations and Outcome



- Gene sets related to cancer-related processes contained significantly more CRISPR-identified driver genes
  - DNA damage, oxidative phosphorylation, DNA replication, cell-cycle, RNA processing
- GCB DLBCL associated with better survival rate
- Stromal and immune signatures also associated with better survival rate
  - Regulatory T cells
- Proliferation signatures associated with worse survival rates
  - MYC/Nucleotide biosynthesis

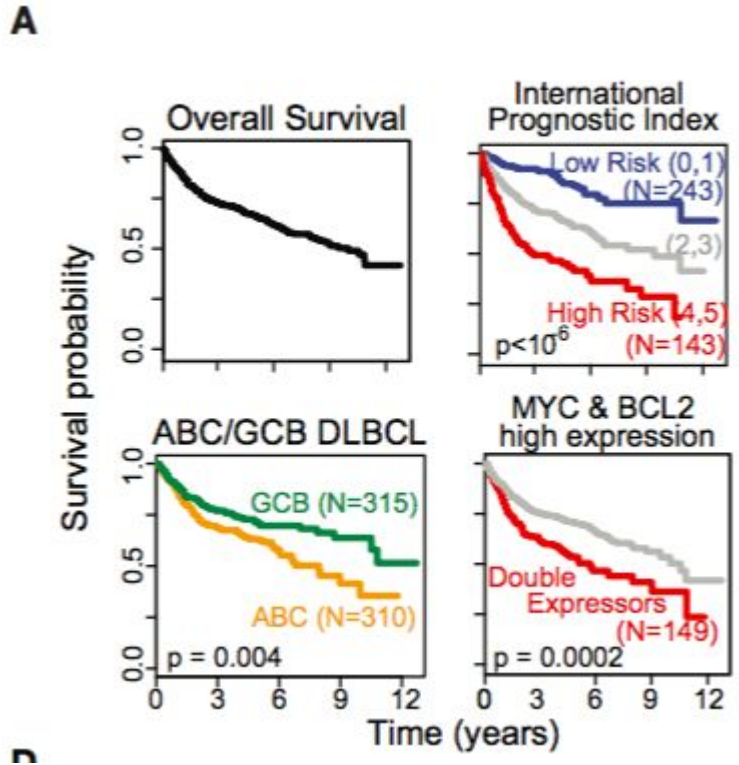
Clinical Characteristics of DLBCL Driver Genes



- GCB DLBCL patients have a better survival probability than ABC DLBCL
- High expression of *MYC* & *BCL2* are associated with a worse prognosis and lower survival probability

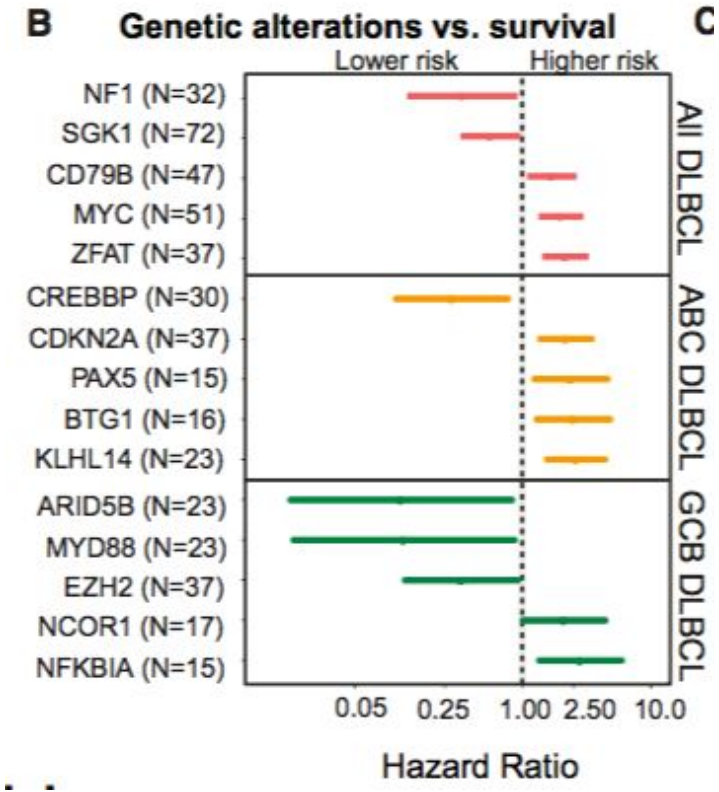
Question:
What are the proteins that *MYC* & *BCL2* encode involved in?

Clinical Characteristics of DLBCL Driver Genes



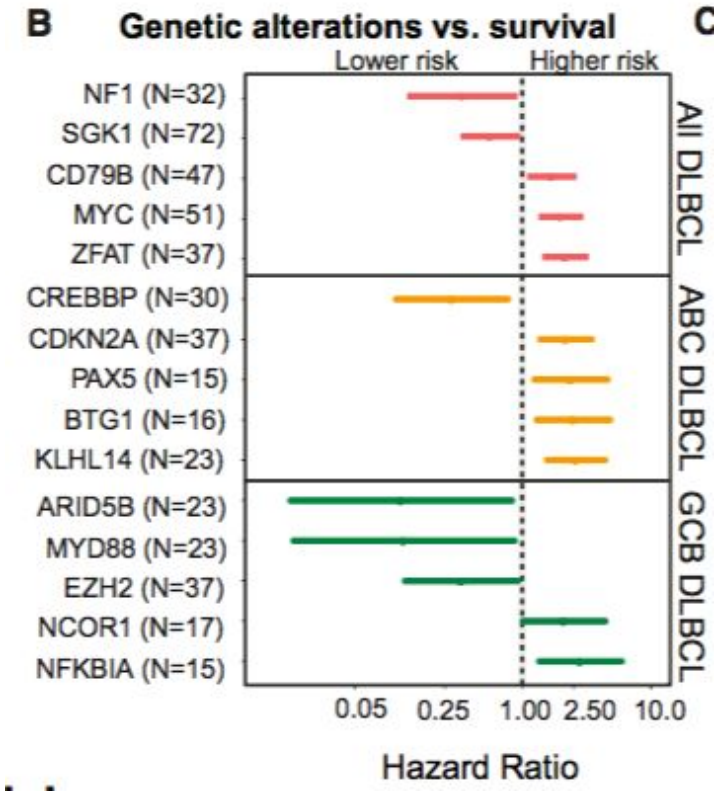
- GCB DLBCL patients have a better survival probability than ABC DLBCL
- High expression of *MYC* & *BCL2* are associated with a worse prognosis and lower survival probability
  - *MYC:* codes for a transcription factor involved in processes of the cell cycle, **apoptosis**, etc.[4]
  - *BCL2:* codes Bcl-2 family of regulator proteins involved in the regulation of **apoptosis**[4]

Clinical Characteristics of DLBCL Driver Genes



- Alterations in *NF1* and *SGK1* were associated with more favourable survival
- Alterations in *MYC*, *CD79B* and *ZFAT* associated with poor survival

Clinical Characteristics of DLBCL Driver Genes



- Within ABC DLBCL...
  - Alterations in *KLHL14*, *BTG1*, *PAX5*, *CDKN2A* associated with significantly poorer survival
  - Alterations in CREBBP associated with favourable survival
- Within GCB DLBCL...
  - Alterations in NCOR1 and NFKBIA are associated with poorer prognosis
  - Alterations in EZH2, MYD88, and ARID5B are associated with a significantly better prognosis

Clinical Characteristics of DLBCL Driver Genes

Question:

What do a lot of these genes have in common?

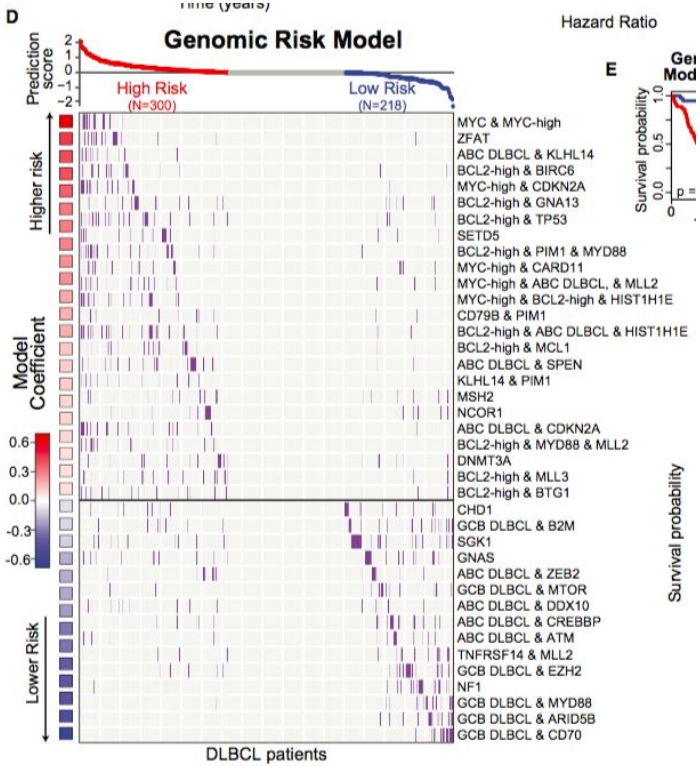
Clinical Characteristics of DLBCL Driver Genes

- *NF1:* codes for protein neurofibromin which acts as a **tumour suppressor** protein[5]
- *SGK1:* codes for Serine/Threonine Kinase, involved in regulating **cell proliferation** and **apoptosis**[6]
- *CD79B:* codes for Igβ protein of **B cell** antigen[7]
- *ZFAT:* codes a zinc finger protein[8]
- *KLHL14:* codes for adapter protein for certain **ubiquitin** ligases[9]
- *BTG1:* codes for **anti-proliferative** protein involved in regulating cell growth and **proliferation**[10]
- *PAX5:* codes for **B-cell** lineage specific activator protein[11]
- *CDKN2A:* codes for a protein that stabilizes **tumour suppressor** protein p53[12]
- *CREBBP:* encodes CREB binding protein that is involved in growth control and **chromatin remodeling**
- *NCOR1:* codes for protein that promotes **chromatin condensation**[13]
- *NFKBIA:* codes for **NFκB** inhibitor[14]
- *EZH2:* codes for histone methyltransferase involved in **transcriptional repression**[15]
- *MYD88:* codes for adaptor protein involved in **immune** cell signaling
- *ARID5B:* encodes a DNA binding protein that is involved in **transcription regulation**[16]

Comparison of Clinical vs Genomic Risk Model

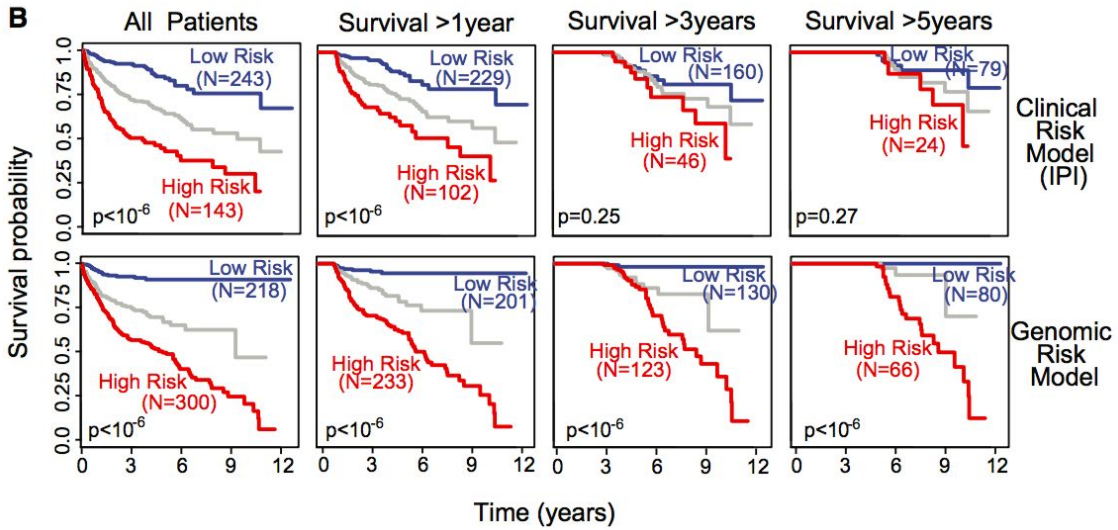
- Clinical Risk Model
  - Based on the International Prognostic Index (IPI) score[18]
    - Age > 60 (1 pt)
    - Stage III or IV disease (1pt)
    - Elevated serum LDH (1 pt)
      - High levels of LDH (lactate dehydrogenase) indicate tissue damage and cellular destruction
    - WHO score of 2, 3 or 4 (1 pt)[18]
      - 2: symptomatic; <50% in bed during the day
      - 3: symptomatic; >50% in bed during the day but not bedbound
      - 4: bedbound
    - > 1 extranodal site (1 pt)

Comparison of Clinical vs Genomic Risk Model



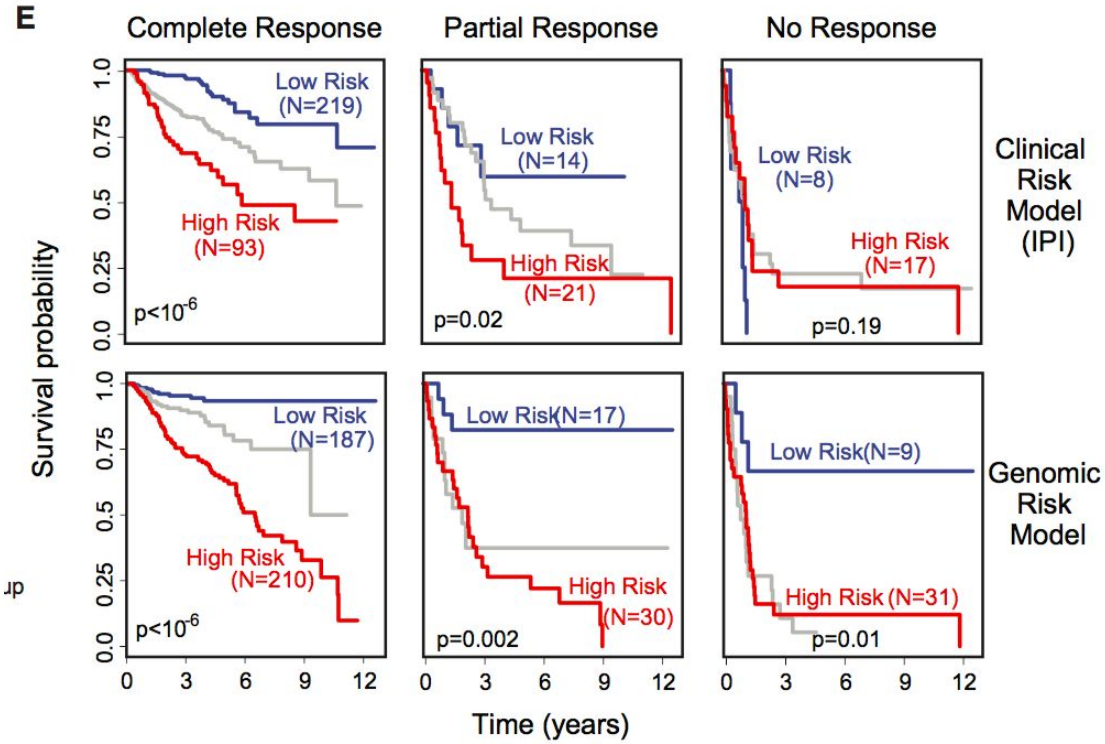
- Genomic Risk Model
  - Based on genetic and molecular features
    - Known expression subgroups
      - ABC, GCB subgroups
      - *MYC, BCL2* high expression
    - Mutations and copy-number events in the 150 DLBCL associated genes identified in this study

Comparison of Clinical vs Genomic Risk Model



- Clinical Risk Model is highly prognostic in early stages (early mortality)
- Genomic Risk Model **better** at predicting long-term mortality (early and late mortality)

Comparison of Clinical vs Genomic Risk Model



- Early mortality of those that completely respond to treatment can be predicted by the Genomic Risk Model
- Indicates that genomic profile of a patient strongly influences their prognostic risk

Summary

- DLBCL is the most prevalent blood cancer in the world
- Diverse number of mutations causing DLBCL make it difficult to understand the impact of the mutations for therapeutic reasons
- 150 driver genes were identified, including 27 newly identified genes
- Functional screening showed many possible therapeutically targetable genes
- Can now distinguish which DLBCL patients would likely not benefit well from standard therapy, and can pursue new targeted therapies
- Genomic risk model can be used clinically: measure cell of origin, BCL2 and MYC expression, targeted sequencing of driver genes

Future Outlook

- Better understanding of the combined effects of the gene combinations and potential to aid in developing new therapeutic approaches
- Apply genomic methodologies to large cohorts and uncover patterns in a variety of other cancers with genetic heterogeneity that would not be discovered in small sample sizes
- Clinical and functional drivers of DLBCL identified → first step in improving outcomes for DLBCL

Strengths and limitations

- Pros:
  - Large sample size
  - Lots of statistical verification for their results
  - Provides novel and valuable information that can be translated into a clinical setting
- Cons:
  - A lot of their starting data is reliant on other researchers' data
  - There were many cases where the authors did not explain why they chose to do certain things
  - Requires a lot of statistical educational background to fully understand some tests they conducted
  - Requires a deep understanding of biological methodology and techniques to fully understand the paper on another level

Questions?

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