Molecular Diagnostics in Hematopathology

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Learning Objectives

• Explain the principles of Molecular Diagnostics

• Describe methods used in Molecular Diagnostics

• Discuss applications of molecular diagnostics in the field of Hematopathology
Basic Genetic Concepts

46 chromosomes
- 22 pairs of autosomes and X/Y
Gene is basic unit of heredity
- Approximately 30,000 genes
- DNA sequences determine protein sequences
- all genes contributed equally by both parents except X/Y in males
A **genome** is all the DNA contained in an organism. The human genome consists of approximately 3.2 billion base pairs of DNA.

The genetic information is contained in structural units of DNA called **genes**.

The **gene** is the physical and functional unit of heredity that is passed from parent to offspring.
Allele

- An **allele** is one of two or more versions of a gene. An individual inherits two alleles for each gene, one from each parent.
- If the two alleles are the same, the individual is **homozygous** for that gene. If the alleles are different, the individual is **heterozygous**.
- A **locus** is the specific physical location of a
Central Dogma of Molecular Biology
Francis Crick, 1958

- DNA → Transcription → RNA → Translation → Protein
- Reverse Transcription
- Replication
The genetic code is said to be **degenerate** because in most cases, more than one codon specifies a given amino acid.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Symbols</th>
<th>Codons</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Ala</td>
<td>Alanine</td>
<td>GCA, GCC, GCG, GCU</td>
</tr>
<tr>
<td>C Cys</td>
<td>Cysteine</td>
<td>UGC, UGU</td>
</tr>
<tr>
<td>D Asp</td>
<td>Aspartic acid</td>
<td>GAC, GAU</td>
</tr>
<tr>
<td>E Glu</td>
<td>Glutamic acid</td>
<td>GAA, GAG</td>
</tr>
<tr>
<td>F Phe</td>
<td>Phenylalanine</td>
<td>UUC, UUU</td>
</tr>
<tr>
<td>G Gly</td>
<td>Glycine</td>
<td>GGA, GGC, GGG, GGU</td>
</tr>
<tr>
<td>H His</td>
<td>Histidine</td>
<td>CAC, CAU</td>
</tr>
<tr>
<td>I Ile</td>
<td>Isoleucine</td>
<td>AUA, AUC, AUU</td>
</tr>
<tr>
<td>K Lys</td>
<td>Lysine</td>
<td>AAA, AAG</td>
</tr>
<tr>
<td>L Leu</td>
<td>Leucine</td>
<td>UUA, UUG, CUA, CUC, CUG, CUU</td>
</tr>
<tr>
<td>M Met</td>
<td>Methionine</td>
<td>AUG</td>
</tr>
<tr>
<td>N Asn</td>
<td>Asparagin</td>
<td>AAC, AAU</td>
</tr>
<tr>
<td>P Pro</td>
<td>Proline</td>
<td>CCA, CCC, CCG, CCU</td>
</tr>
<tr>
<td>Q Gln</td>
<td>Glutamine</td>
<td>CAA, CAG</td>
</tr>
<tr>
<td>R Arg</td>
<td>Arginine</td>
<td>AGA, AGG, CGA, CGC, CGG, CGU</td>
</tr>
<tr>
<td>S Ser</td>
<td>Serine</td>
<td>AGC, AGU, UCA, UCC, UCG, UCU</td>
</tr>
<tr>
<td>T Thr</td>
<td>Threonine</td>
<td>ACA, ACC, ACG, ACU</td>
</tr>
<tr>
<td>V Val</td>
<td>Valine</td>
<td>GUA, GUC, GUG, GUU</td>
</tr>
<tr>
<td>W Trp</td>
<td>Tryptophan</td>
<td>UGG</td>
</tr>
<tr>
<td>Y Tyr</td>
<td>Tyrosine</td>
<td>UAC, UAU</td>
</tr>
</tbody>
</table>
Types of Genetic Variants

• Structural
  – Gain/loss of chromosome segments
  – Translocations
  – Rearrangements
  – Gene amplifications

• Molecular
  – Deletions/insertions
  – Nucleotide repeats (di-, tri-)
  – Point mutations
Before translocation

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Chromosome 20</th>
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</thead>
<tbody>
<tr>
<td></td>
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</table>

After translocation

<table>
<thead>
<tr>
<th>Derivative Chromosome 4</th>
<th>Derivative Chromosome 20</th>
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<tbody>
<tr>
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</table>

Mutations and their Effect on Gene Expression

Germ-line mutation: affects gametes and therefore can be transmitted to the next generation. An example of a germ-line mutation is hemophilia, which is an x-linked bleeding disorder.

Somatic mutations affect somatic cells. Cancer very often involves a somatic mutation.
Sources of RNA and DNA

Fresh tissue
Blood – whole, cells, spots (no heparin)
Body fluids & buccal swabs
Formalin fixed Paraffin embedded tissue
Preparation of Nucleic Acids

- **Degree of purity**  Presence of contaminants
- **Integrity**  High molecular weight versus low molecular weight
- **Yield**

![Image: High Quality DNA: Single band close to origin](Degraded DNA)
Gel Electrophoresis

DNA Electrophoresis

Electrophoresis is a technique used to separate DNA fragments by their size.
An electrophoretic apparatus is used consisting of a chamber to hold the buffer, a casting tray to hold an electrophoresis gel, and positive and negative electrodes are connected to a power source.
Drawing of a stained DNA gel with bands and a standard. Note that the bands are migrating to the anode (+), and the smaller bands move faster in the gel and therefore are located towards the bottom of the gel.
Capillary Electrophoresis
Fragment Analysis by Capillary Electrophoresis
Hybridization

- Reversible annealing or base pairing of complementary DNA or RNA.
  - DNA-DNA
  - DNA-RNA
  - RNA-RNA
- “Target” is a "unique sequence" used to identify a microorganism or gene

- A probe (also called an oligonucleotide or oligo) is a known short sequence of DNA or RNA (15-45 bases) that is labeled in order to detect and identify a target nucleic acid sequence
Hybridization Techniques

- Southern Blot
- Northern Blot
- Dot Blot and Allele Specific Oligonucleotide (ASO) Hybridization
- Reverse Dot Blot and Line Probe Assay
- Fluorescent In-Situ Hybridization (FISH)
- Microarrays
**Probes**

- **Probe** is a nucleic acid that
  - will hybridize to another nucleic acid on the basis of base complementarity
- **Types of labels**
  - Radioactive ($^{32}\text{P}$, $^{35}\text{S}$, $^{14}\text{C}$, $^{3}\text{H}$)
  - Fluorescent
    - FISH: fluorescent in situ hybridization
      - chromosomes
  - Biotinylated (avidin-streptavidin)
  - Enzyme (Alkaline Phosphatase, Horseradish peroxidase)
Southern Blot for Mutation Detection

Normal Gene (N)

Mutant Gene (M) – deleted restriction site

Digest with Restriction Enzyme

Electrophoresis

N  M

Denature, Transfer to Membrane, Incubate with Probe, Wash, Develop

N  M

↑ = mutation site

△ = restriction enzyme site

● = probe binding site
Allele Specific Oligonucleotide (ASO) Hybridization

Patient DNA is spotted on two membranes (replicate spots). In this example, there are eight patients, numbered 1 through 8.

Labeled normal probes hybridized to top membrane; Labeled mutant probes hybridized to bottom membrane.

Wash, add substrate, develop

Interpretation: Normal homozygous – wild type, no mutation: Patient 1, 5, 6
Homozygous Mutant: Patient 2, 4, 7
Heterozygous Mutant: Patient 3 and 8
FISH (fluorescence in situ hybridization) is a cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to the chromosomes.
### Fluorescence In Situ Hybridization (FISH) for Translocations

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Fusion Probes</strong></td>
<td><img src="image" alt="Normal Single Fusion Probes" /></td>
<td><img src="image" alt="Translocation Single Fusion Probes" /></td>
</tr>
<tr>
<td><em>Red probe to gene on one chromosome, green probe to gene on other chromosome</em></td>
<td><img src="image" alt="Normal Single Fusion Probes" /></td>
<td><img src="image" alt="Translocation Single Fusion Probes" /></td>
</tr>
</tbody>
</table>

| **Dual Fusion Probes**  | ![Normal Dual Fusion Probes](image)        | ![Translocation Dual Fusion Probes](image) |
| *Dual red probes to gene on one chromosome, dual green probes to gene on other chromosome* | ![Normal Dual Fusion Probes](image)        | ![Translocation Dual Fusion Probes](image) |

| **Breakaway Probes**    | ![Normal Breakaway Probes](image)         | ![Translocation Breakaway Probes](image) |
| *Red and green probes to gene on same chromosome; cannot identify fusion partner* | ![Normal Breakaway Probes](image)         | ![Translocation Breakaway Probes](image) |

**Probes are designed to hybridize adjacent to breakpoint regions**

When red probe and green probe are together on the same chromosome, there is yellow fluorescence **+ =**
Microarray Technology

Source: www.nhgri.nih.gov/DIR/VIP/Glossary/Illustration/microarray_technology.html
Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction consists of the following components:

• DNA template to be amplified - called the target DNA
• Two primers that are complementary to ends of DNA segment to be amplified.

The primers determine the specificity of the reaction.

• dATP, dCTP, dGTP, dTTP (collectively called deoxynucleotide triphosphates or dNTPs)
• DNA polymerase that is thermostable – Taq polymerase
• MgCl₂
• Tris-HCl-KCl buffer
Polymerase Chain Reaction
<table>
<thead>
<tr>
<th># Cycles</th>
<th>Copies of Target</th>
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<tbody>
<tr>
<td>0</td>
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<td>33,554,432</td>
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<tr>
<td>30</td>
<td>1,073,741,824</td>
</tr>
</tbody>
</table>
Real-time PCR or QPCR

Qiagen.com

Affymetrix.com
Figure 44: Melting Curve Analysis - Normal
2 Linear Probes + 2 Fluorophores

Normal:

5' GACTA
CTGAT

3' TATCT
ATAGA

Fluorescence

5' D
GACTA

3' A
TATCT

No Fluorescence

CTGAT
ATAGA

Normal gene is exactly complementary with both probes

Needs higher temperature to dissociate probes

The association of the probe with the normal gene is strong due to the exact complementarity.
Figure 46: Melting Curve Analysis
2 Linear Probes + 2 Fluorophores

\[ \Delta F/\Delta t = \text{change in fluorescence over time} \]
Reverse Transcriptase PCR for amplification of RNA targets

mRNA to cDNA using Reverse transcriptase

Amplification of cDNA using DNA polymerase
Dideoxy (Sanger) sequencing

Principle:
Gel electrophoresis: discrimination of 1 bp below ~1000 bp
Synthesis: starts with a DNA oligo, stops after incorporating a (marked) ddNTP
First ~ 60 bp uncertain (high relative mass of the fluo. dye)

Radiolabeling:
4 reactions

Dye-termination:
4 fluorescent dyes, one reaction
Sanger Sequencing
Schematic of 1 form of NGS. The process starts by randomly cutting genomic DNA (or cDNA) into short fragments (a few hundred base pairs in length).
Ion Torrent workflow

a. Prepare Genomic Library → Prepare Template on Bead → Sequence on Ion Chip → Signal Processing and Base Calling

b. DNA → Fragmented Size-Selected DNA → Adapted Library DNA

c. Thermocycle PCR

Jerry is *REALLY* old... Apparently he once actually *poured* and *ran* a sequencing gel.
<table>
<thead>
<tr>
<th>Alteration Type</th>
<th>Hematologic Condition(s)</th>
<th>Methods to Detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large DNA deletions</td>
<td>Thalassemias</td>
<td>Southern blot, genomic assays</td>
</tr>
<tr>
<td>DNA repeats/insertions</td>
<td>Inherited cytopenias, drug response in leukemia</td>
<td>Southern blot, genomic arrays, PCR</td>
</tr>
<tr>
<td>DNA point mutations</td>
<td>Acute and chronic leukemias, lymphomas</td>
<td>AS-PCR, DNA sequencing, arrays</td>
</tr>
<tr>
<td>Epigenetic regulation</td>
<td>Bone marrow failure, myelodysplasia</td>
<td>Methylation sequencing, methylation-sensitive PCR, pyrosequencing</td>
</tr>
<tr>
<td>Ribosomal biogenesis</td>
<td>Diamond Blackfan anemia</td>
<td>Transcript profiling, protein expression</td>
</tr>
<tr>
<td>Alternate mRNA species</td>
<td>Coagulopathy</td>
<td>Transcript profiling, protein expression</td>
</tr>
</tbody>
</table>
Molecular Methods with Hematopathology applications

Nucleic acid isolation - DNA and RNA

Amplification of nucleic acids - PCR and RT-PCR

Detection of nucleic acids
- Electrophoresis
- RFLP
- Nucleic acid hybridization and Southern blotting
- Signal amplification techniques - RNA and DNA
- DNA sequencing - Sanger and Next generation
- Real-time PCR - qualitative and quantitative
- Fluorescence in situ hybridization (FISH)
Acute Myeloid Leukemia

- NPM1 Mutation
- FLT3 Mutation
- CEBPA Mutation
- IDH1 and IDH2 Mutations
- KIT D816V Mutation
- KIT Mutation for AML - Exons 8, 17
- PML/RARA t(15;17) Translocation, Qualitative

Myeloproliferative Neoplasm

- JAK2 V617F Mutation
- JAK2 Exon 12 Mutation
- CALR Mutation
- MPL Mutation
- KIT D816V Mutation
- BCR/ABL1 Analysis, Quantitative
- BCR/ABL1 Kinase Domain Mutation
Lymphoproliferative Disorders

- B Cell Clonality (IGH and IGK Gene Rearrangement)
- B Cell Clonality (IGK Gene Rearrangement)
- B Cell Clonality (IGH Gene Rearrangement)
- T Cell Clonality (TRG & TRB Gene Rearrangement)
- T Cell Clonality (TRG Gene Rearrangement)
- T Cell Clonality (TRB Gene Rearrangement)
- IGH/BCL2 t(14;18) Translocation by PCR
- IGH/BCL2 t(14;18) Translocation by FISH
- BCL6 (3q27) Rearrangement by FISH
- MYC (8q24) Rearrangement by FISH
- MALT1 (18q21) Rearrangement by FISH
- MYD88 (L265P) Mutation
- BRAF V600E/V600K Mutations
Applications of Molecular Testing in Inherited Disorders

- **Diagnostic Testing**: testing for a gene mutation in symptomatic individuals as a diagnostic aid.

- **Newborn screening**: screen populations to identify prevalent genetic mutations in asymptomatic infants.

- **Carrier Screening**: testing for a gene mutation in an autosomal recessive disorder in asymptomatic individuals.

- **Presymptomatic Testing**: testing for a gene mutation in asymptomatic individuals.

- **Prenatal Diagnosis**: This application involves testing fetal cells or tissues for mutations to determine if a fetus is affected.
A single gene disorder is one in which a disease results from the mutation in one gene. An example is Sickle Cell Anemia or Cystic Fibrosis.

Polygenetic disorders may be due to the inheritance of a certain combination or pattern of genes along with the influence of environmental factors (Coronary Artery Disease) and the influence of smoking.
Known Gene Sequence - Only One Known Mutation

- PCR-based methods such as RFLP (if the mutation creates or destroys a restriction enzyme site)
- Real time PCR based methods such as allele specific PCR and melt-curve analysis
- DNA sequencing may also be performed
Inherited Thrombophilia: Genetic Risk Factors

(Carrier screen or presymptomatic testing)

Factor V Leiden (G1691A) mutation

Prothrombin mutation (G20210A)

MTHFR mutation (C677T)

homozygosity
Sickle Cell Anemia - One Consistent Point
Mutation in One Gene

The 5th, 6th and 7th codon in the normal beta chain of hemoglobin is: CCT GAG GAG pro glu glu
The 5th, 6th and 7th codon in the sickle cell mutation is:
CCT GTG GAG pro val glu
PCR-RFLP for the Sickle Cell Mutation

PCR

- 772 bp

Bsu 361 digestion

- Normal Bsu 361 site
- Mutant – loss of restriction site

228 → 202
430

PCR-RFLP for Sickle Cell Mutation

<table>
<thead>
<tr>
<th>Normal (AA)</th>
<th>Mutant (SS)</th>
<th>Heterozygous (AS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>430 bp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>228 bp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>202 bp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Known Gene Sequence - Multiple Mutations

Multiplex PCR reactions are used to amplify different regions of the same gene or several genes at the same time. Mutations can then be interrogated by using various post-PCR techniques including several microchip/microbead arrays such as Luminex technology, the Hologic Invader, the Nanosphere microarray.
Known Gene Sequence - Unknown Mutation or Numerous Multiple Mutations

- PCR-Single Strand Conformational Analysis
- PCR-Heteroduplex analysis, or melting curve analysis
- DNA sequencing has become quite popular in the clinical laboratory setting.
Applications of Molecular Testing in Oncology

• Early detection of cancers
• Evaluation of the risk of predisposition to cancers
• Tumor subtyping
• Predicting prognosis
• Detection of minimal residual disease
• Early detection of relapse of cancers
• Monitoring effectiveness of therapy
• Predicting response to therapy
• Determining risk of adverse reactions to therapy
With some notable exceptions, more than one type of mutation is often involved in a given leukemia.

- Translocations are the most common type of mutation seen in hematologic malignancies.
- Gain or loss of chromosomes: trisomies, monosomies
- Gene deletions resulting in the absence of the protein product
- Point mutations resulting in an abnormal protein product or a truncated or shortened protein
- Gene amplification causing overexpression of a gene product
- Dysregulation of epigenetic mechanisms affecting the expression of critical genes involved in hematopopiesis.
# Types of Molecular Monitoring Assays for Leukemias and Lymphomas

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Examples</th>
<th>Disease Types (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion transcript RT-PCR (RNA)</td>
<td><em>BCR-ABL1, PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, NPM1-ALK, BIRC3-MALT1</em></td>
<td>CML, AML</td>
</tr>
<tr>
<td>Translocation detection by PCR of DNA</td>
<td><em>IGH@-BCL2</em></td>
<td>ALL/LBL</td>
</tr>
<tr>
<td>Leukemia-associated quantitative mutation detection</td>
<td><em>NPM1, FLT3</em></td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Leukemia-associated elevated gene expression</td>
<td><em>BAALC, WT1</em></td>
<td>Follicular lymphoma$^{52}$</td>
</tr>
<tr>
<td>Clone-specific IGH PCR</td>
<td><em>IGH@VDJ/FR3</em> custom-designed primers</td>
<td>AML$^{56,57}$</td>
</tr>
<tr>
<td>Surrogate markers</td>
<td><em>EBV and HHV8 viral load</em></td>
<td>AML$^{56}$</td>
</tr>
</tbody>
</table>

Dan Jones, Mol. Diag. Hematology, in Wintrobe's Clinical Hematology
Mutations in Hematologic Malignancies

• Integral part of diagnostic work-up for myeloid neoplasms (AML, MDS, MPN, MDS/MPN)

• Diagnostic, prognostic and predictive value

• Growing list of mutated genes with clinical utility: NPM1, FLT3, RAS (KRAS, NRAS), KIT, CEBPA, WT1, IDH1, IDH2, DNMT3A, EZH2, JAK2, MPL, several new genes in lymphoid tumors

• New associations of known mutations: BRAF in hairy cell leukemia
Myeloid Malignancies

• The majority of the mutations are chromosomal translocations involving the rearrangement of a protooncogene.

• Myeloid malignancies are further divided into: acute myelogenous leukemia, myeloproliferative neoplasms (including chronic myelogenous leukemia), and myelodysplastic
Diagnosis of Promyelocytic leukemia by RT-PCR

Formal name: \([PML-RARA \ t(15;17)]\)
The Philadelphia Chromosome and Chronic Myelogenous Leukemia (CML)

Normal Chromosomes

Translocated Chromosomes

The translocated *abl* gene inserts into the *bcr* gene. The two genes fuse. The altered *abl* gene functions improperly, resulting in CML.

BCR-ABL FISH

A cell that is BCR-ABL positive by FISH (yellow fusion signal). The yellow signal shows the fusion of the green probe (the BCR portion of chromosome 22) with the red probe (the ABL1 portion of chromosome 9).

Courtesy of Mary Lowery Nordberg, PhD
Acute lympholastic leukemia (ALL) is the result of a malignant transformation of a lymphocytic precursor cell at a specific stage in its development. ALLs are classified as B lymphoblastic and T lymphoblastic. Disorders affecting the bone marrow and peripheral blood are called leukemias, whereas diseases predominantly affecting lymph nodes and other non-marrow or extramedullary sites are called lymphomas.

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease characterized by the accumulation of mature, activated B lymphocytes in the peripheral blood and bone marrow.
Detection of Clonality in Lymphocyte Populations - B and T Cell Gene Rearrangement

Normal B and T lymphocytes are “polyclonal” because millions of clones occur in normal lymphocyte development as a result of genetic rearrangements.

Malignant lymphocytes are usually “monoclonal” and are the result of uncontrolled proliferation of a single clone with all cells in the clone having the same genetic rearrangement.
a. TCRG gene complex (#7p14)

b. 

Vγ

Vγ family primers

Jγ

Jγ family primers

IgH Gene

Chromosome 14
T and B Cell Clonality Assay Workflow

Amplify the rearranged V and J regions of the Ig gene or the gamma chain gene of the TCR of interest using fluorescently labeled primers

Capillary Electrophoresis

Data Analysis
IGH-Rearrangement

Clonal Control

Polyclonal Control

Specimen
TCRG-Rearrangement

Clonal Control

Polyclonal Control

Specimen
Next Generation sequencing

<table>
<thead>
<tr>
<th>ABL1</th>
<th>CEBPA</th>
<th>HRAS</th>
<th>MYD88</th>
<th>SF3B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>CSF3R</td>
<td>IDH1</td>
<td>NOTCH1</td>
<td>SMC1A</td>
</tr>
<tr>
<td>ATRX</td>
<td>CUX1</td>
<td>IDH2</td>
<td>NPM1</td>
<td>SMC3</td>
</tr>
<tr>
<td>BCOR</td>
<td>DNMT3A</td>
<td>IKZF1</td>
<td>NRAS</td>
<td>SRSF2</td>
</tr>
<tr>
<td>BCORL1</td>
<td>ETV6/TEL</td>
<td>JAK2</td>
<td>PDGFRA</td>
<td>STAG2</td>
</tr>
<tr>
<td>BRAF</td>
<td>EZH2</td>
<td>JAK3</td>
<td>PHF6</td>
<td>TET2</td>
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<tr>
<td>CALR</td>
<td>FBXW7</td>
<td>KDM6A</td>
<td>PTEN</td>
<td>TP53</td>
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<tr>
<td>CBL</td>
<td>FLT3</td>
<td>KIT</td>
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<td>U2AF1</td>
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<tr>
<td>CBLB</td>
<td>GATA1</td>
<td>KRAS</td>
<td>RAD21</td>
<td>WT1</td>
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<tr>
<td>CBLC</td>
<td>GATA2</td>
<td>MLL</td>
<td>RUNX1</td>
<td>ZRSR2</td>
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<tr>
<td>CDKN2A</td>
<td>GNAS</td>
<td>MPL</td>
<td>SETBP1</td>
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</table>
In total, 220 genes were selected based on frequency of mutation, theranostic, diagnostic, and prognostic associations as well as their potential to interact with known dysregulated pathways in B-cell lymphoma.

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**ARCHITECTURE OF THE FOCUS::LYMPHOMA™ NGS PANEL**

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
<th>Gene 4</th>
<th>Gene 5</th>
<th>Gene 6</th>
<th>Gene 7</th>
<th>Gene 8</th>
<th>Gene 9</th>
<th>Gene 10</th>
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<td>HIST1H1C</td>
<td>JAK2</td>
<td>NFKB1B</td>
<td>PIK3CG</td>
<td>RAF1</td>
<td>SYK</td>
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**Clinical Targets**

**Associated with BCR/PI3K/WNT pathway**

**Outcome/Cell of Origin**

**Located within aberration (DLBCL only)**
Thank you