Objectives

• Refresh your basic understanding of blood, bone marrow, stem cells and “Mutations”
• Review the MDS diagnosis, distinguish it from CCUS and AML
• Discuss how or why MDS develops
• Compare and contrast germline and somatic mutations
• Consider how genetic mutations may lead to cancer
• Outline the treatment approach to MDS
• Identify “targetable” mutations in MDS
How Does a Factory Making 310,000,000,000 cells Daily Function for 80+ Years??????

1. **Needs Ingredients**: proteins, iron, oxygen, B12, Folate, copper, etc.

2. **Needs Stimulus**:
   - Erythropoietin – from the kidneys stimulates Red Cell Production
   - GCSF – from blood vessel lining, immune cells stimulates WBC Production
   - Thrombopoietin – from the liver stimulates Platelet Production

3. **Needs Source**:  
   
   *STEM CELLS*
What is a stem cell?

A single cell that can replicate itself, or...

differentiate into many cell types.
What Is Myelodysplastic Syndrome (MDS)?

- **MYELO** = Greek myelos = marrow

- **DYSPLASIA** = dys (ABNORMAL) + plasia (GROWTH or DEVELOPMENT)
MDS Leads to Abnormal Blood Cell Production

ANEMIA

Low Platelets

Low White Cells

Normal Amount of Red Blood Cells

Red Blood Cell

Platelet

White Blood Cell

Anemic Amount of Red Blood Cells

ANEMIA
DNA/Gene Mutations in Blood-Producing Precursors Cause MDS

WHY????

• Exposure to gene-damaging agents
  • Chemotherapy
  • Chemicals
  • Radiation

• Spontaneous Mutations in DNA

• Inherited Predisposition Genes or Mutations

• Environmental exposure Causing New Mutations in DNA
REROUTING.... What Are Mutations???
Mutations = DNA Changes That Affect the Protein Genes Produce
Mutations Can Be Congenital or Acquired During Life

**CONGENITAL (Germline)** – present at birth, inherited or acquired during development, *mutation is in every cell*

Examples:
- Sickle Cell Anemia
- Hemophilia
- Down's Syndrome
- Familial Cancer Syndromes (BRCA, Polyposis, etc)

**ACQUIRED (Somatic)** - happen after birth, spontaneous or due to genotoxic exposure or immunodeficiency, *mutation is only in certain cell types*

Examples:
- MDS & AML
- Most Solid Tumors
- AIDS-related Cancers
- Post-transplant lymphoproliferative disorders
## Testing For Mutations

<table>
<thead>
<tr>
<th>KARYTOPE</th>
<th>FISH</th>
<th>PCR (&quot;NGS&quot;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal = 46 XX, XY</td>
<td>Can Detect 1/200 Cells Carrying a Specific Mutation</td>
<td>Can Detect 1/10,000+ Cells Carrying a Small, Specific DNA Change</td>
</tr>
<tr>
<td>Picks up 1/20 Cells with a structural mutation</td>
<td>Examples: 5q-</td>
<td>Examples: See next slide</td>
</tr>
<tr>
<td>Examples: 5q-</td>
<td>Monosomy 7</td>
<td></td>
</tr>
<tr>
<td>Monosomy 7</td>
<td>Deletion 20</td>
<td></td>
</tr>
<tr>
<td>Deletion 20</td>
<td>Translocations</td>
<td></td>
</tr>
<tr>
<td>Translocations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- FISH can detect 1/20 cells with a structural mutation. Examples include 5q-, Monosomy 7, Deletion 20, and Translocations.
- PCR ("NGS") can detect 1/10,000+ cells carrying a small, specific DNA change. Examples include 5q-, Monosomy 7, Deletion 20, and Translocations.
Back to Mutations and MDS
MDS Molecular Mutations Categorized

- Splicing Factors (~50%)
  - SF3B1 (18%)
  - U2AF1 (12%)
  - SRSF2 (12%)
  - ZRSR2 (5%)
  - Others (5%)
  Rarely co-occur with each other

- Epigenetic Regulators (~45%)
  - TET2 (20%)
  - ASXL1 (15%)
  - DNMT3A (12%)
  - EZH2 (5%)
  - IDH1/2 (5%)
  - Others (5%)
  Often co-occur except for TET2 and IDH

- No Common Abnormality (~5%)
- Karyotype Abnormality Only (~5%)

- Mutations in Other Genes Only (~15%)
  - Transcription Factors
    - RUNX1, ETV6, PHF6, GATA2, ...
  - Kinase Signalling
    - NRAS, KRAS, JAK2, CBL, ...
  - Cohesins
    - STAG2, SMC3, RAD21, ...
  - DNA Repair

- Both Splicing Factors (SF) & Epigenetic Regulators (ER) Overlap (25%)
- TP53 and no SF or ER (~5%)
  Often complex karyotypes with frequent del(5q), abnormal chromosome 7, and monosomies
  Other mutations less frequent

- Others (5%)
Epigenetic Genes Influence Other Genes: Turn on/off, Increase/Decrease Protein Production
Mutation of normal stem cells leads to aberrant epigenetic programs, resulting in normal, trilineage differentiation.

Further mutation of MDS stem cells results in aberrant growth signals and reduced apoptosis, leading to trilineage dysplasia, compensatory stem cell expansion and increased apoptosis.

More mutations in MDS stem cells can lead to AML, characterized by inhibited differentiation and uncontrolled blast cell proliferation.
RUNX1, DDX41, GATA2, TERT, TP53 Mutations Can be Acquired or Inherited in Families with MDS
MDS Mutations Can Co-Occur in the Same Person AND They Are Found in Other Blood Cancers...
Mutations Precede MDS
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full-form</th>
<th>Operational definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH/ARCH</td>
<td>Clonal hematopoiesis/Age-related clonal hematopoiesis</td>
<td>This is an all-encompassing term to describe detection of somatic pathogenic variants identified in the hematopoietic compartment (originating in the hematopoietic stem and progenitor cells), typically present at higher frequencies in aging individuals.</td>
</tr>
<tr>
<td>CHIP</td>
<td>Clonal hematopoiesis of indeterminate potential</td>
<td>Somatic pathogenic variants detected at a variant allele fraction (VAF) of ≥2%, and in the absence of cytopenias, cytosis, or bone marrow dysplasia. The 2% threshold was chosen as it is the lower limit of detection for most sequencing assays.</td>
</tr>
<tr>
<td>CCUS</td>
<td>Clonal cytopenia of undetermined significance</td>
<td>Somatic pathogenic variants in the presence of cytopenias typically at higher VAF (≥10%-20%) in the absence of MDS diagnostic criteria (≥10% dysplasia cells from any hematopoietic cell lineage on bone marrow evaluation, absence of excess blasts, and absence of MDS-defining cytogenetic abnormalities).</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic syndromes</td>
<td>Persistent cytopenia(s) in one or more peripheral blood lineages, and morphologic dysplasia (≥10% dysplastic cells) in one or more bone marrow blood cell lineages OR one of MDS-defining cytogenetic abnormalities.</td>
</tr>
<tr>
<td>CMUS</td>
<td>Clonal monocytosis of undetermined significance</td>
<td>CH plus monocytosis defined as absolute monocyte count &gt;0.5 × 10⁹/L and &gt; 10% of white blood cells (WBCs) in the absence of bone marrow (BM) findings suggestive of CMML.</td>
</tr>
<tr>
<td>CCMUS</td>
<td>Clonal cytopenia and monocytosis of undetermined significance</td>
<td>CH plus cytopenia plus monocytosis (defined as above).</td>
</tr>
<tr>
<td>CMML</td>
<td>Chronic myelomonocytic leukemia</td>
<td>Myeloid neoplasms with absolute (≥0.5 × 10⁹/L) and relative (≥10%) peripheral blood monocytosis with blasts &lt;20% of cells in peripheral blood and bone marrow, and with characteristic bone marrow findings.</td>
</tr>
<tr>
<td>CCsUS</td>
<td>Clonal cytosis of undetermined significance</td>
<td>Elevated peripheral cell counts (other than monocytosis) in the absence of bone marrow morphological features diagnostic of a myeloid neoplasm.</td>
</tr>
<tr>
<td>Mosaic</td>
<td>chromosomal abnormality</td>
<td>Clonal, structural (deletions, duplications or copy number neutral loss of heterozygosity), somatic alterations identified in hematopoietic stem and progenitor cells.</td>
</tr>
</tbody>
</table>
Aging, exposure to genotoxic stress (cytotoxic therapy, radiation)

Clonal hematopoiesis of indeterminate potential

Smaller clone size
- Slower clonal growth rate
- Absence of cytopenia in patient
- Mutations in genes associated with DNA damage response (similar to age-related declines in function)

Lower risk of malignant disease

Larger clone size (clones w/ higher VAFs)
- More rapid rate of clonal growth
- Cytopenia in patient
- Multiple driver mutations
- Mutations in splicing factor genes
- Inflammation

Higher risk of malignant disease
The features of HSCs in the context of aging and MDS are shown.
Prevalence of Mutations Increases With Age

Jaiswal et al. NEJM 2014.
WTC First Responders Developed CHIP

![Graph showing frequency of CHIP development in different age groups for WTC FDNY and Controls.]

<table>
<thead>
<tr>
<th>Age Group</th>
<th>WTC FDNY</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39 yrs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40-49 yrs</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>50-59 yrs</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>60-69 yrs</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>70-79 yrs</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>80-99 yrs</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Age Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>215</td>
</tr>
<tr>
<td>115</td>
</tr>
<tr>
<td>32</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>481</td>
</tr>
</tbody>
</table>
Are There Treatments For CHIP?

- Clinical Trials Exploring:
  - Anti-inflammatories
  - Vitamin C
  - Curcumin
  - Low-risk MDS: oral azacitidine causes more harm than benefit
Can We Target the Mutations To Treat MDS?
DRIVER MUTATIONS = CONTINUE CAUSING DISEASE
CURRENT TREATMENT ALGORITHM NOT GENE-SPECIFIC

**LOWER RISK**
(IPSS low, INT-1)  
(IPSS-R VL, L, INT)  
(BM blasts < 10%)

Any age

Iron chelation  
Growth factors  
Luspatercept  
HMAs  
Lenalidomide (5q-)  
Immune modulation  
Clinical trial

**HIGHER RISK**
(IPSS INT-2, high)  
(IPSS-R INT, H, VH)  
(BM blasts ≥ 10%)

Age <70-75

Intensive chemotherapy  
HMA (5-AZA/decitabine)  
Clinical trial

AGE >70-75

HMA (5-AZA/decitabine)  
Clinical trial  
Intensive chemotherapy

ALLO SCT
Lenalidomide Significantly Reduces Transfusion Requirements in MDS with "5q-minus Syndrome"
Luspatercept Significantly Improves Anemia in MDS with Sideroblastic Anemia

**Rates of red blood cell transfusion independence**

- Luspatercept: 58.5%
- Epoetin alfa: 31.2%

**SF3B1** particularly Associated with Sideroblastic Anemia
Epigenetic Therapies Work Across Genetic Subtypes Because Myeloid Cancers Are Highly Methylated

2 FDA-approved Hypomethylating Agents:

- **VIDAZA (5-azacytidine)**
- **DACOGEN (decitabine)**

**Figure 1** — Epigenetic silencing of gene expression. DNA methyl-transferases carry out the methylation of CpG dinucleotides, which triggers the process of gene silencing by recruitment of methyl binding domain (MBD) and Histone deacetylases (HDAC) to bind to the methylated DNA. This results in histone deacetylation and chromatin condensation leading to loss of transcription factor binding and subsequent repression of transcription.
Assessing Response Based on Genes is COMPLEX – Might AI Help US?

Aziz Nazha et al. JCO 2019
AI Program Identifies Mutations in MDS with Poor Response to Epigenetic Therapies

**TABLE 3.** Genomic Biomarkers Defined by the Recommender System Association Rules for Resistance to HMAs

<table>
<thead>
<tr>
<th>Genomic Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1, NF1</td>
</tr>
<tr>
<td>ASXL1, EZH2, TET2</td>
</tr>
<tr>
<td>ASXL1, EZH2, RUNX1</td>
</tr>
<tr>
<td>EZH2, SRSF2, TET2</td>
</tr>
<tr>
<td>ASXL1, EZH2, SRSF2</td>
</tr>
<tr>
<td>ASXL1, RUNX1, SRSF2</td>
</tr>
<tr>
<td>ASXL1, TET2, SRSF2</td>
</tr>
<tr>
<td>ASXL1, BCOR, RUNX1</td>
</tr>
</tbody>
</table>
ASXL1 (frameshift) Mutations Adverse in MDS

Fig 1. Kaplan-Meier curves for overall survival (OS) and time to acute myeloid leukemia (AML) transformation. (A) Point and frameshift mutations considered: OS in patients with myelodysplastic syndrome (MDS) with mutated (n = 54) and unmutated ASXL1 (n = 120). (B) Only frameshift mutations considered: OS in patients with MDS with mutated (n = 24) and unmutated ASXL1 (n = 130). Genes in patients with ASXL1 point mutations are considered wild type (WT) in this analysis. (C) Point and frameshift mutations considered: time to AML transformation in patients with MDS mutated (n = 94) and unmutated ASXL1 in n = 114. (D) Only frameshift mutations considered: time to AML transformation for patients with MDS with mutated (n = 24) and unmutated ASXL1 in n = 124. Genes in patients with ASXL1 point mutations are considered WT in this analysis.

Thol et al. JCO 2011;39:2499-2506.
Hypothesis: AZA Resistance Due to T cell Silencing (via PD-1) in MDS

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>PD-1 demethylation</th>
<th>No PD-1 demethylation</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (months) from diagnosis until 5-aza-start median (range)</td>
<td>4 (0-37)</td>
<td>6.5 (0-11)</td>
<td>3 (0-37)</td>
<td>.83</td>
</tr>
<tr>
<td>Median no. of cycles of 5-aza</td>
<td>5 (2-14)</td>
<td>4 (2-13)</td>
<td>5 (3-14)</td>
<td>.40</td>
</tr>
<tr>
<td>IWG 2006 Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>3 (11%)</td>
<td>1 (8%)</td>
<td>2 (13%)</td>
<td>1</td>
</tr>
<tr>
<td>Overall response</td>
<td>10 (37%)</td>
<td>1 (8%)</td>
<td>9 (60%)</td>
<td>.014</td>
</tr>
<tr>
<td>Overall survival (months) (median)</td>
<td>10.2</td>
<td>7.1</td>
<td>11.0</td>
<td>.11 (log rank)</td>
</tr>
</tbody>
</table>
VARI/SU2C Phase I/II Multicenter Trial: Guadecitabine + Atezolizumab in R/R MDS

Casey O’Connell, MD

Kirsten Grønbæk, MD
Association of Overall Survival with AXSL1 Mutation

- **Myeloid Mutation Only (n=7)**: Median (95% CI) 9.5 (8.6-NE), Likelihood-Ratio P-value 0.001
- **No Mutation (n=15)**: Median (95% CI) 16.4 (9.8-NE)
- **Shared Mutation (n=5)**: NE (NE-NE)

Months since Treatment Start

Estimated Probability of Survival
New Targeted Strategies in MDS

- **HMA + ** **IDH1/2 Inhibitor**
  - Already approved in AML
  - FDA to fast track review of IDH1 inhibitor Ivosidenib (8/28/23)
  - Enasidenib for IDH2-mutant MDS effective in relapsed/refractory setting in combination with HMA or alone

- Splicing Factor Targeted Therapy in Clinical Trials
What About P53 Mutations in MDS?

HAVING 2 COPIES OF P53 MUTATION SIGNIFICANTLY IMPACTS SURVIVAL BUT 1 COPY OF THE MUTATION DOES NOT
Azacitidine is Effective in P53-Mutated MDS

<table>
<thead>
<tr>
<th>N</th>
<th>p53 negative patients</th>
<th>p53 positive patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Aza cycles</td>
<td>n=65</td>
<td>n=35</td>
<td>0.926⁺</td>
</tr>
<tr>
<td>Median (range)</td>
<td>4 (1-29)</td>
<td>5 (1-22)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORR (CR, PR, SD with HI)</td>
<td>16 (25%)</td>
<td>16 (46%)</td>
<td>0.033*</td>
</tr>
<tr>
<td>SD without HI</td>
<td>23/32 (72%)</td>
<td>9/32 (28%)</td>
<td>0.020*</td>
</tr>
<tr>
<td>MDS</td>
<td>n=29</td>
<td>n=24</td>
<td></td>
</tr>
<tr>
<td>ORR (CR, PR, SD with HI)</td>
<td>4 (14%)</td>
<td>11 (46%)</td>
<td>0.008*</td>
</tr>
<tr>
<td>sAML</td>
<td>n=30</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>ORR (CR, PR, SD with HI)</td>
<td>11 (37%)</td>
<td>3 (33%)</td>
<td></td>
</tr>
<tr>
<td>Very poor cytogenetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORR (CR, PR, SD with HI)</td>
<td>3/9 (33%)</td>
<td>7/21 (33%)</td>
<td></td>
</tr>
<tr>
<td>+ abnormal Chr. 5</td>
<td>2/6 (33%)</td>
<td>7/17 (41%)</td>
<td></td>
</tr>
<tr>
<td>+ monosomal KT</td>
<td>1/4 (25%)</td>
<td>5/14 (36%)</td>
<td></td>
</tr>
</tbody>
</table>

For comparison between patients with and without TP53 mutations a Fisher’s exact (*) or χ² (+) or Mann-Whitney-U test (⁺) was used. ORR: overall response rate; CR: complete remission; PR: partial remission; SD: stable disease; HI: hematologic improvement.
APR-246 Not Successful in Targeting P53
Conclusions

• Genetic Mutations are Ubiquitous in MDS
• Mutations are Usually Acquired in MDS
• Analyzing the impact of mutations is a complex task as they are rarely found alone
• Targeting the mutations is also complex, must hit the DRIVER to stop the disease
• Enormous research efforts focused on this area
• Clinical trials and dedicated analysis required to personalize MDS care