CRP Breakout Session
Hematologic Malignancies

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Conflicts of Interest

- No relevant disclosures
Overview of Hematopoiesis

- Hodgkin lymphoma
- Multiple myeloma
- Non-Hodgkin lymphoma
- Acute lymphoblastic leukemia
- Polycythemia vera
- Essential thrombocythemia
- Acute myeloid leukemia
- Chronic myelogenous leukemia
- Chronic myelomonocytic leukemia
- Hypereosinophilic Syndrome
- Multiple myeloma
- Non-Hodgkin lymphoma
Major Heme malignancies

Myeloid malignancies
- Acute myeloid leukemia
- Chronic myeloid leukemia
- Myelodysplastic syndromes
- Myeloproliferative diseases
  - Polycythemia vera
  - Essential thrombocytopenia
  - Myelofibrosis

Lymphoid malignancies
- Acute lymphoblastic leukemia
- Chronic lymphocytic leukemia
- Multiple myeloma
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
Why we classify heme malignancies?

“Classification is the language of medicine: diseases must be described, defined and named before they can be diagnosed, treated and studied.

Furthermore, a consensus on definitions and terminology is essential for both clinical practice and investigation.”
Diagnostic techniques

- Morphology
- Cytochemistry / special stains
- Immunophenotyping / flow cytometry
- Cytogenetics / FISH
- Molecular Diagnostics
- Imaging Studies
  - PET/CT
Morphology

- Many diseases have characteristics or even diagnostic appearances
- Peripheral smear, bone marrow biopsy aspirate/biopsy, lymph node biopsy
Cytochemical / Special Stains

- Myeloperoxidase: stains myeloid blasts / promyelocytes; helpful for distinguishing AML from ALL
- Non-specific esterase: marker of monocytic differentiation
Immunophenotyping

- Can grow and isolate monoclonal antibodies which recognize specific antigen / epitope on the cell surface (or inside cell if you permeabilize cell membrane)
- Immunohistochemistry – tissue sections
- Flow cytometry – cell suspensions

- Useful for identifying cell types / frequencies
Flow Cytometry

- Technique of measuring physical and chemical characteristics of INDIVIDUAL PARTICLES as they pass single file in a fluid stream

- Clinically combined with fluorescently labeled monoclonal antibodies for the immunophenotyping of neoplastic and nonneoplastic hematopoietic cells
Schematic of Flow Cytometer

- **Fluidics**
  - Sample
  - Sheath
  - Flow Chamber

- **Optics**
  - Laser Optics
  - Laser Beam

- **Electronics**
  - Detectors
  - Photomultiplier tubes
What We Measure

- Physical Parameters
  - Forward Scatter: measures how large a cell is
  - Side Scatter: measure of internal complexity

Figure 3-2  Cell subpopulations based on FSC vs SSC
What We Measure

- **Optical Parameters**
  - Use panel of monoclonal antibodies typically to cell surface markers expressed on cells
  - Antibodies are conjugated to fluorescent molecule

Anti-CD3 FITC  Anti-CD19 PE

B-Cell
B-cell fluoresces red when excited by laser

T-Cell
T-cell fluoresces green when excited by laser
What is Measured?

- Typical clinical flow cytometer has 1-3 lasers
- Typically measure 500-5,000 cells per second
- Analyze 10,000 – 50,000 cells

- Use multiple antibody combinations per sample
- Measure multiple parameters for EACH INDIVIDUAL CELL
Representation of Data

Histograms

2D Representations:
Dot Plots
Density Plots
Gating

CD19+, CD3- B-cells

CD19-, CD3+ T-cells
“Blast Gate” in AML

CD45 dim, Side Scatter low
Application to Hematologic Malignancies

Analysis is all about pattern recognition

- Certain malignancies have specific expression patterns of cell surface markers which are unique
  - i.e. pre B ALL: CD10+, CD19+, TdT+
  - CLL: CD5+, CD23+, CD20 dim

- Certain malignancies look like normal cells but are present in abnormal percentages
  - i.e. B-cell lymphomas: CD19+, CD20+, skewing of kappa, lambda ratios

- Certain malignancies can aberrantly express markers not found in normal cells
  - i.e. AML expressing lymphoid markers
What flow can and can’t do

- Helpful for identifying and subclassifying heme malignancies
- Can be used to detect disease relapse / minimal residual disease
- Helpful for determining potential to respond to certain therapies (CD20 for Rituximab, CD30 for Brentuximab)
- Can’t tell you anything about morphology (but can give you clues)
- Can’t always distinguish between malignant and reactive processes
  - CML vs. leukemoid reaction
Cytogenetics

- Normally have 46 chromosomes, each has a designated short (p) and long arm (q) divided by the centromere.

- Recurring cytogenetic abnormalities are a hallmark of hematologic malignancies / can define malignancy. 
  - t(8;14) Burkitt’s lymphoma, t(9;22) CML

- Structural abnormalities
  - Gains and losses of part or whole chromosomes
    - Addition (add), deletion (del), trisomy (+), monosomy (-)
  - Chromosomal rearrangements
    - Inversions (inv), translocations (t)
Conventional Cytogenetics

Grow cells in culture and arrest in metaphase with colchicine

Chromosomal banding
• Giemsa-banding
• G-banding regions AT-rich and gene poor.

Analyze ~20 cells (~5% sensitivity)

Good for large gains, losses, & translocations
Not sensitive for small lesions
Reading a cytogenetics report

46,XY,t(9;22)(q34;q11.2)[9]/46,XY[11]

# of chromosomes, Sex chromosomes
Which Chromosomes involved
Breakpoints
# of abnormal chromosomes seen

44~45,XX,del(4)(q2?4q2?6),del(5)(q13q33),+8, der(8)?t(8;11)(p11.2;q12),der(8)?t(8;21)(p11.2;q11.2),i(8)(p10),-17,-18,-21,+mar[cp21]
FISH (Fluorescent *in situ* Hybridization)

Dual color, dual fusion probe

- Sensitivity 1:200-1:500
- Can detect very small gains, losses and translocations
- Need to know what you are looking for
Reading a FISH report

nuc ish(ABL1,BCR)x3(ABL1 con BCRx2)[138/200]

Rearrangement was observed in 138/200 nuclei, which exceeds the normal range (up to 1%) established for these probes in the Cytogenetics Laboratory at WUSM.
Molecular diagnostics

- DNA/RNA based tests: usually sequencing or PCR based tests
- Establish clonality / malignancies
  - IGH gene rearrangement – for B-cell malignancies
  - TCR gene rearrangement – for T-cell malignancies
- Establish diagnosis
  - JAK2 V617F – myeloproliferative diseases
  - BCR-ABL – CML
  - BCL2- non-Hodgkin lymphomas
  - PML-RARA – AML (acute promyelocytic leukemia)
- Establish prognosis
  - FLT3, NPM1 – AML
  - IgHv mutation - CLL
- Disease monitoring
  - BCR- ABL - CML
qRT-PCR (Quantitative Real-Time PCR)
qRT-PCR Assay

• Make a cDNA library from the mRNA specimen
• Design quantitative real-time PCR to amplify area of interest (may span fusion breakpoints)
• Detect specific transcript based on use of allele specific probe
• Rate of change in fluorescence proportional to number of molecules in sample

• Quantify relative expression to control gene, (B2-microglobulin or Abl)
qRT-PCR Report

Results: Positive: Major breakpoint (0.504%)

INTERPRETATION: This result is consistent with ALL.

BLOOD: BCR-ABL TRANSCRIPT

Quantitative units: BCR-ABL transcript levels are reported as a ratio of fusion gene transcript to β-2-microglobulin reference gene transcript.

COMMENT:
FISH results are pending for this specimen. This test can detect the BCR-ABL translocations e13a2, e14a2, e1a2 to a sensitivity level of > 1 in 100,000 transcripts (0.001%). Alternative nomenclature is p210 for the major breakpoints (or b2a2 and b3a2 or e13a2 and e14a2, respectively) and p190 for the minor breakpoint (e1a2). All results should be considered in the context of the clinical status, as well as cytogenetic, histopathologic and immunophenotypic findings.

Method: RNA is isolated from the sample provided and converted to cDNA using reverse transcriptase. The cDNA is amplified by real-time polymerase chain reaction (PCR) for the major and minor BCR-ABL fusion genes.
Reporting of Bone Marrow

- % Blasts
  - Assess on BM aspirate, based on morphology, not flow or cytochemistry
  - If BM aspirate is hemodilute, dry tap, can estimate based on core by IHC

- Cellularity:
  - “Normal” cellularity decreases with age
  - Assessed on core rather than aspirate
  - Acellular, reduced normal increased, markedly increased

- Dysplasia: refers to abnormal morphology / maturation
Bone marrow dysplasia

- Alterations in morphology of BM precursor cells: can occur in one or more lineages
- Important in MDS, AML
- Dyserythropoiesis
  - Nuclear budding, internuclear bridging, karyorrhesis, multinuclearity, nuclear hyperlobation, megaloblastic changes
  - Ring diseroblasts, vacuolization, Periodic acid-Schiff positivity
- Dysgranulopoiesis
  - Small or unusually large, Nuclear hypolobation, pseudo- Pelger-Huet, irregular hypersegmentations. Pseudo Chediak-Higashi granules, Auer rods
- Dysmegakaryocytopoiesis
  - Micromegakaryocytes, nuclear hypolobation, multinucleation
Response evaluation in heme malignancies

- Includes assessment from multiple sources
  - Bone marrow
  - Peripheral blood
  - Imaging studies: CT / PET
  - Other studies: SPEP/UPEP, Free-lite assay MM

- Criteria tend to be both disease and protocol specific
Response Criteria in AML / ALL (IWG)

- **Morphologic Complete Remission (CR)**
  - < 5% blasts in BM, no auer rods
  - ANC ≥ 1,000 uL, Plt count ≥ 100K/uL
  - RBC transfusion independent
  - No residual extramedullary leukemia

- **Morphologic Complete Remission with incomplete blood count recovery (CRi)**
  - All criteria for CR except for low ANC and/or Plts
  - Requirement for RBC transfusion independence variable in some protocols

- Residual disease detected by flow, cytogenetics or molecular techniques does not matter for CR
Relapse in AML / ALL

- Reappearance of leukemic blasts in PB or > 5% in BM not attributable to other cause

- Can have other types of relapse which may or may not be counted as an event / relapse
  - Cytogenetic
  - Molecular
Response Criteria in CLL

- Complete remission (must be sustained)
  - Absence of constitutional symptoms
  - No LN > 1.5 cm in diameter
  - No HSM on PE
  - Normal / near normal CBC
    - ALC < 4,000 / uL
    - ANC > 1,500 / uL
    - Plt count > 100,000 / uL
    - Hgb > 11g/dL untransfused
Response Criteria in CLL

- Partial remission
  - $\geq 50\%$ decrease in lymphocyte count from baseline $\geq 50\%$ reduction in lymphadenopathy.
  - $\geq 50\%$ reduction in size of liver and/or spleen
  - CBC
    - Leukocytes $\geq 1500/\mu l$ or $50\%$ improvement over baseline.
    - Platelets $> 100,000/\mu l$ or $50\%$ improvement over baseline.
    - Hemoglobin $> 11.0$ gm/dl or $50\%$ improvement over baseline without transfusions.

- Progressive disease
  - $\geq 50\%$ increase in lymphocyte count
  - $\geq 50\%$ increase in sum of products of LN or
  - $\geq 50\%$ increase in size of liver or spleen
Assessing Response / Progression in CLL

- Tumor flare
  - Development of increase in pain / LAD +/- fever, rash prior to response
  - Seen with lenalidomide

- Tumor mobilization
  - Decrease in LAD with increase in circulating lymphocytes
  - Frequently seen with ibrutinib
  - Measure nodal response / nodal PR
PET-CT in response assessment

- Patients with lymphoma can frequently have residual masses following therapy
  - May or may not represent disease
- Fluorodeoxyglucose ($^{18}$F)-PET: measures metabolic activity of tumors by glucose uptake
- Standard for response in most FDG-avid lymphomas
- Some low grade lymphomas have less or variable FDG-uptake (CLL/SLL, marginal zone)
## Classification for FDG-PET Response (Deauville Criteria)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No uptake above background</td>
</tr>
<tr>
<td>2</td>
<td>Uptake &gt; mediastinum, but ≤ liver</td>
</tr>
<tr>
<td>3</td>
<td>Uptake moderately &gt; liver</td>
</tr>
<tr>
<td>4</td>
<td>Uptake markedly higher than liver and/or new lesions</td>
</tr>
<tr>
<td>5</td>
<td>Uptake markedly higher than liver and/or new lesions</td>
</tr>
<tr>
<td>X</td>
<td>New areas of uptake unlikely to be related to lymphoma</td>
</tr>
</tbody>
</table>

5 point semiquantitative scale to quantify intensity of signal

1-2 Complete Metabolic Response
3 Can vary by study
4-5 Residual disease
CT interpretation in lymphoma

- Differs from RECIST criteria
- RECIST
  - Sum of diameters of target lesions
- Lymphoma / CLL
  - SPD: Sum of the product of diameters
  - Measure longest diameter and longest diameter perpendicular to longest overall diameter
Conclusions

- Being a CRA for heme malignancy trials is not easy
- Different methodologies than solid tumors
  - Disease classification
  - Measurement of response and progression
- Often tumor and protocol specific and even drug specific