CRP Breakout Session Hematologic Malignancies

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Conflicts of Interest

- No relevant disclosures

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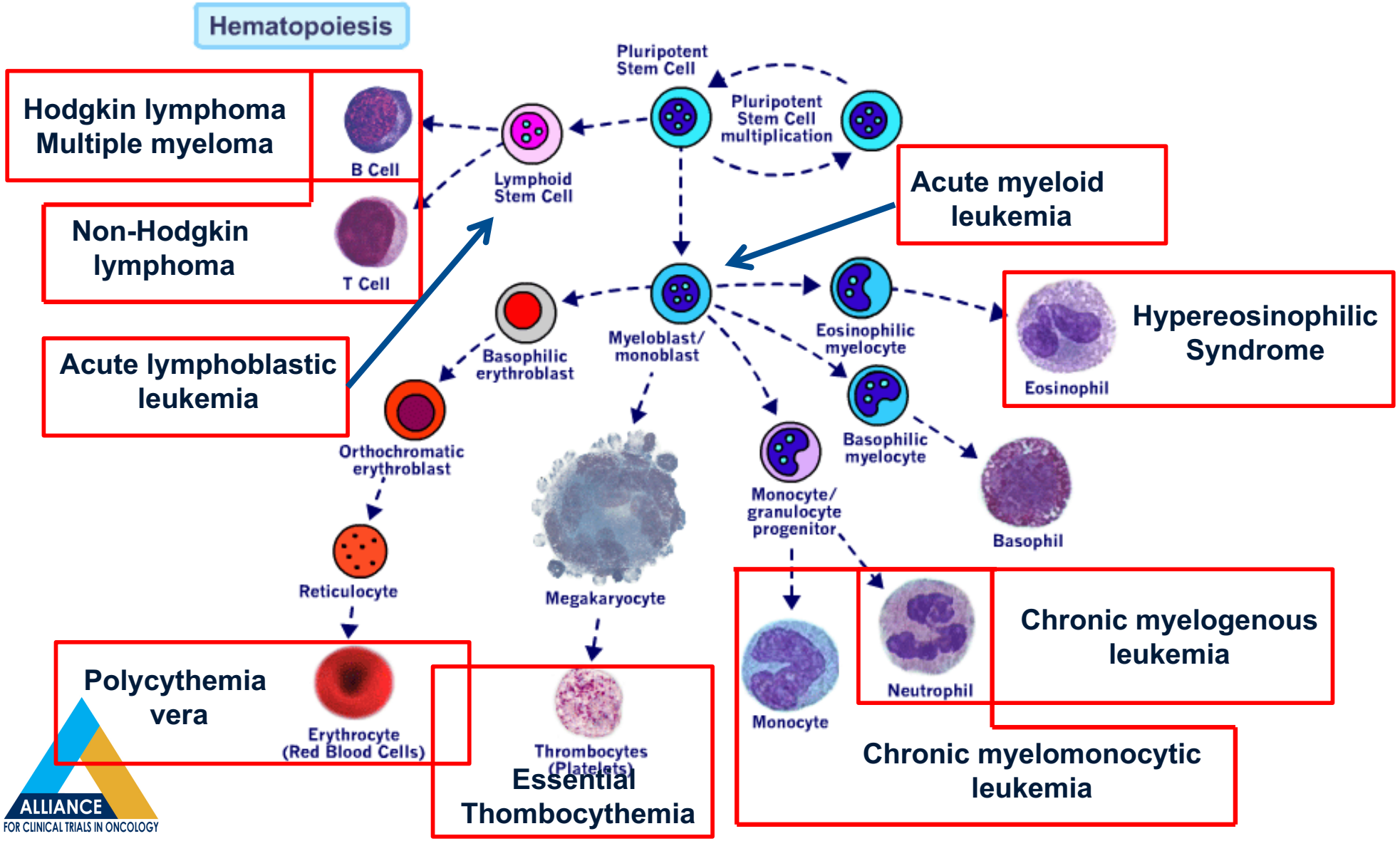
Overview of Hematopoiesis



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Major Heme malignancies

Myeloid malignancies

- Acute myeloid leukemia
- Chronic myeloid leukemia
- Myelodysplastic syndromes
- Myeloproliferative diseases
  - Polycythemia vera
  - Essential thrombocythemia
  - Myelofibrosis

Lymphoid malignancies

- Acute lymphoblastic leukemia
- Chronic lymphocytic leukemia
- Multiple myeloma
- Non-Hodgkin lymphoma
- Hodgkin lymphoma

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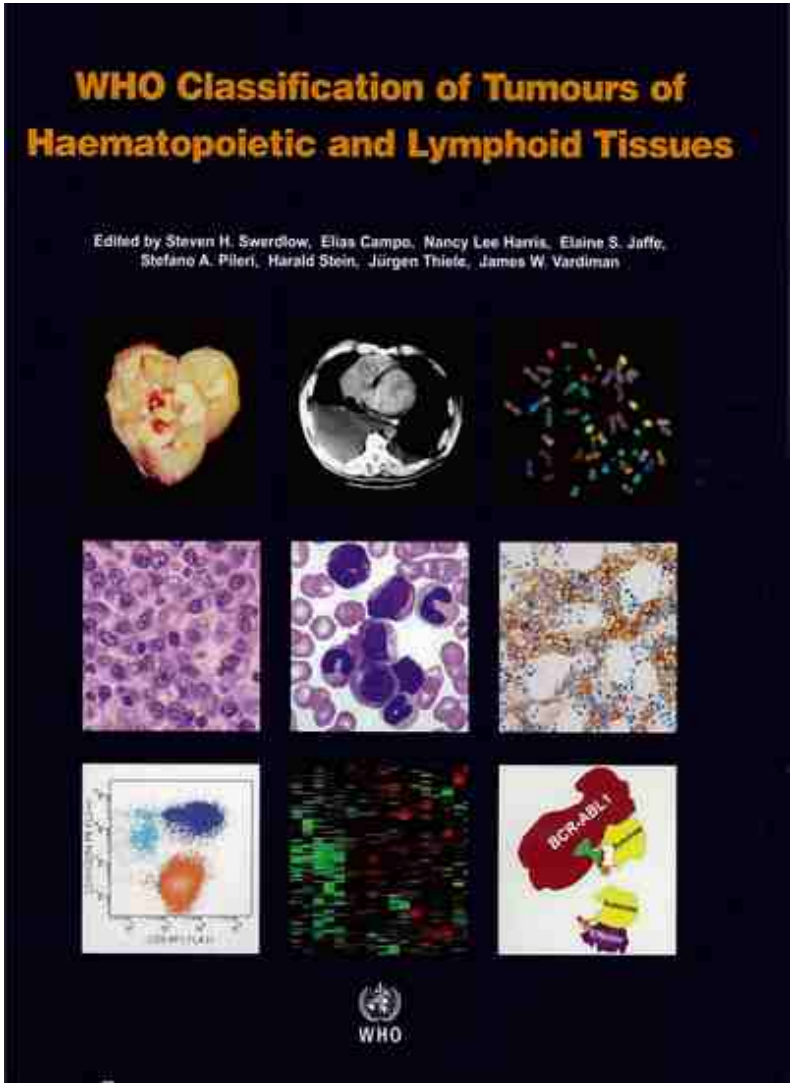
Why we classify heme malignancies?

"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."



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Diagnostic techniques

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

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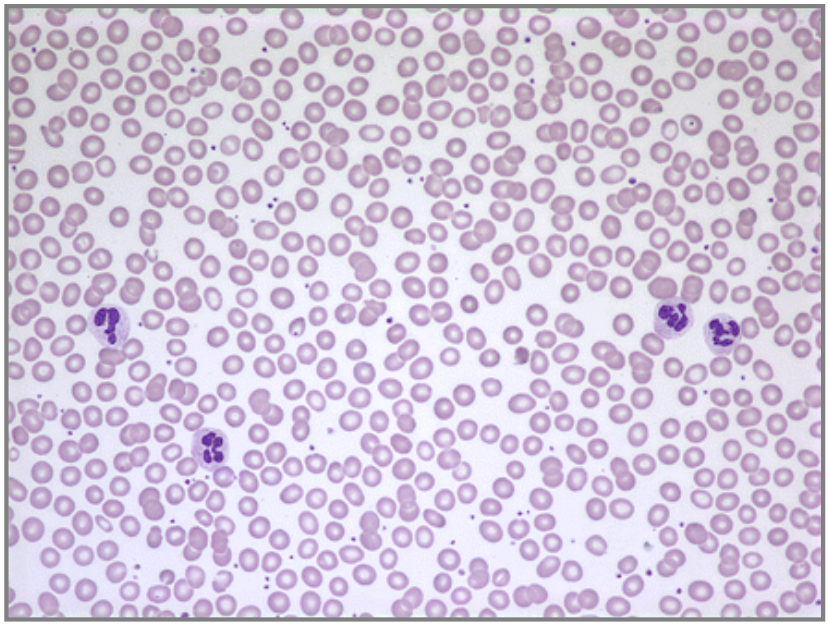
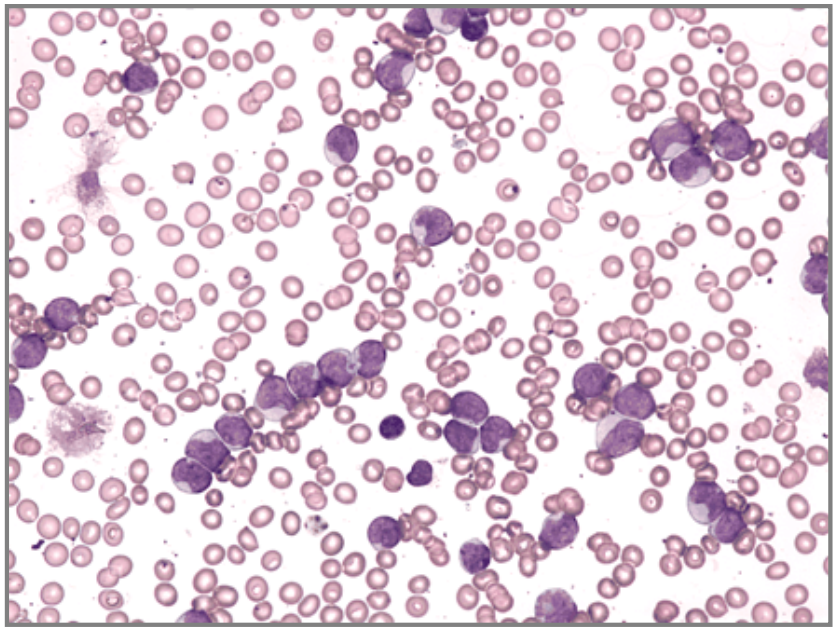
Morphology

- 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

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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

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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

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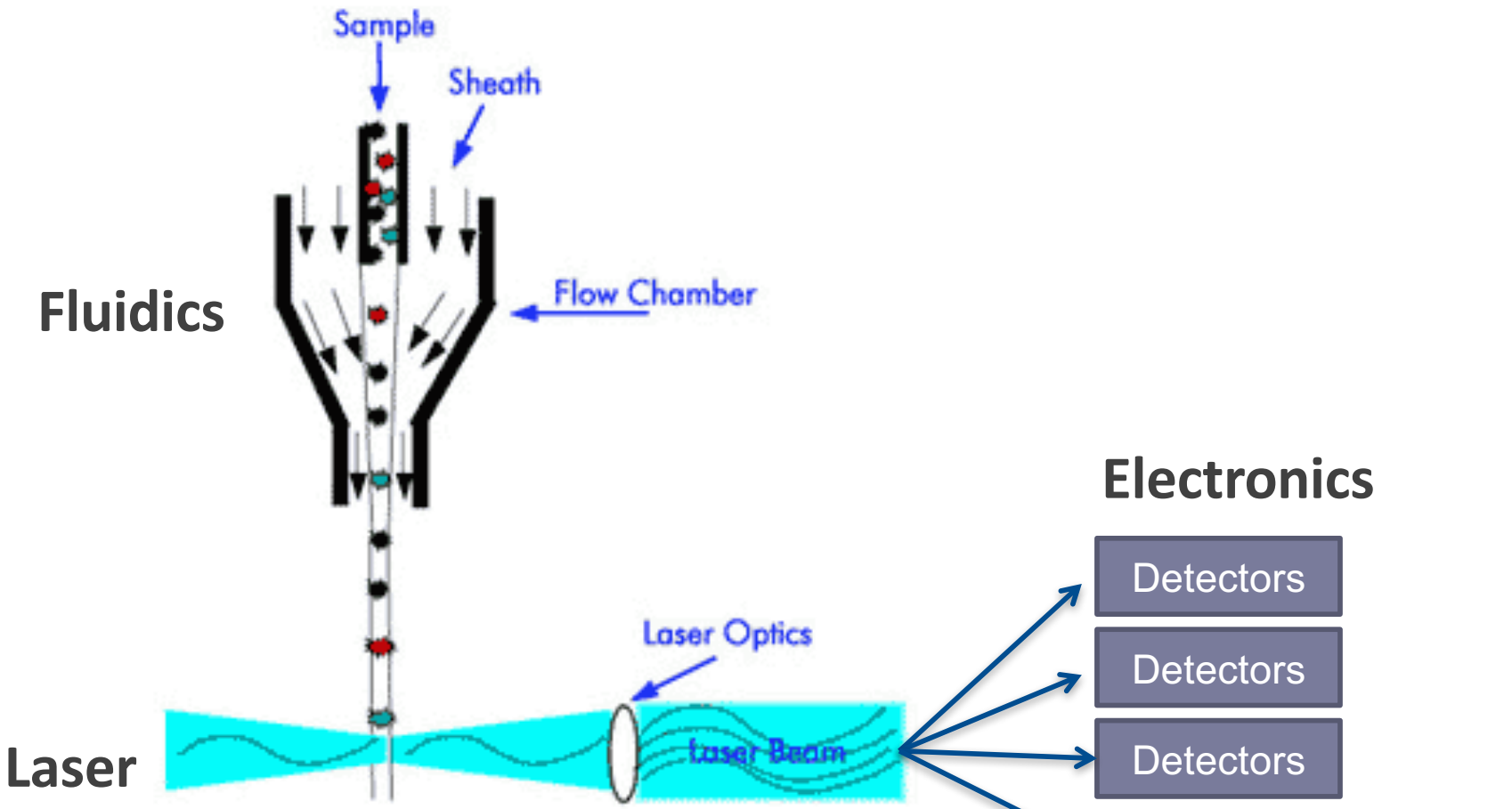
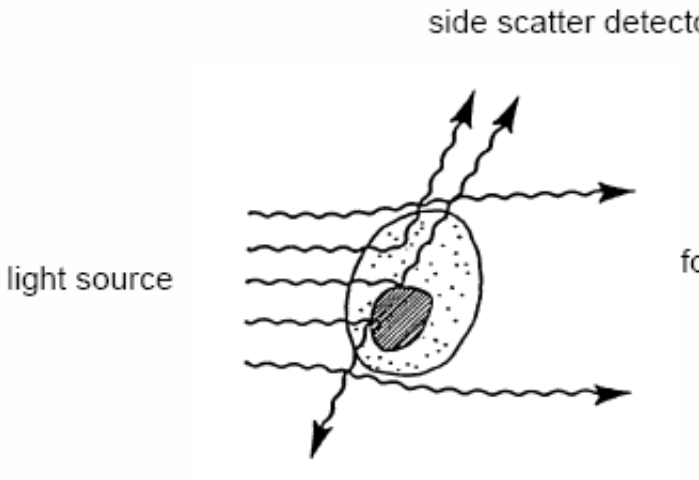
Schematic of Flow Cytometer



What We Measure

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



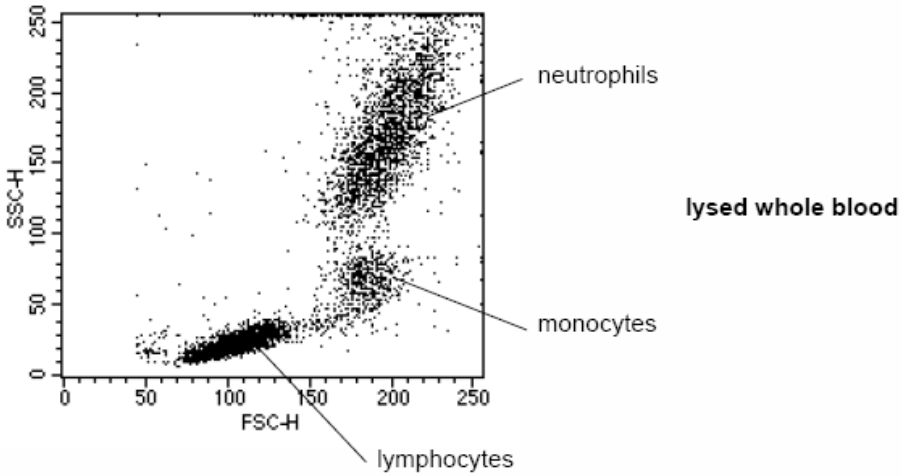


Figure 3-2 Cell subpopulations based on FSC vs SSC

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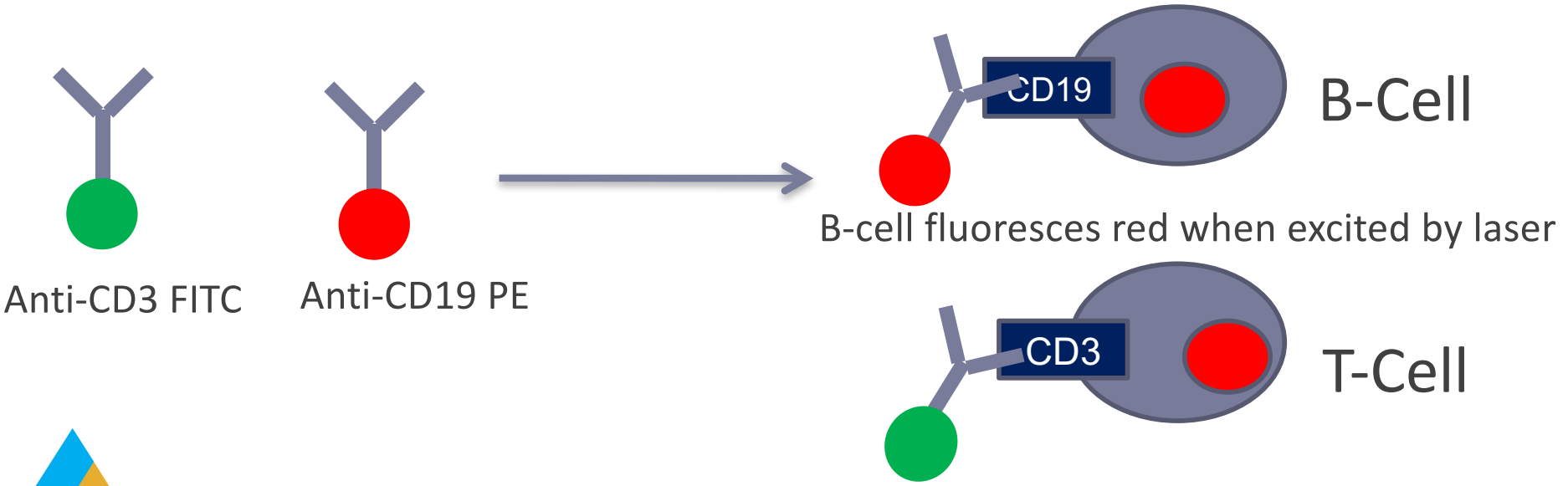
What We Measure

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



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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

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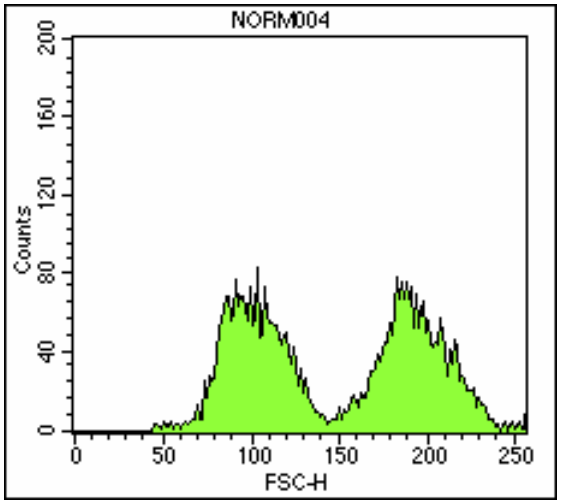
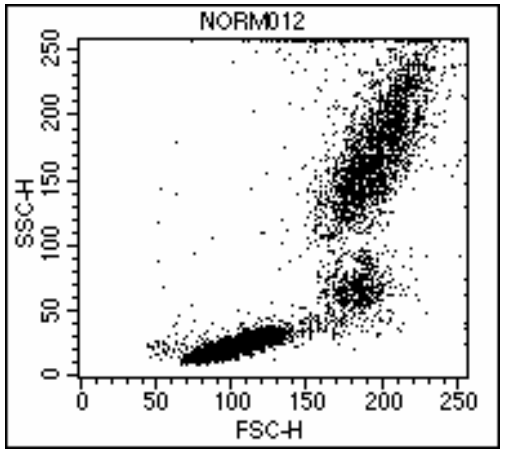
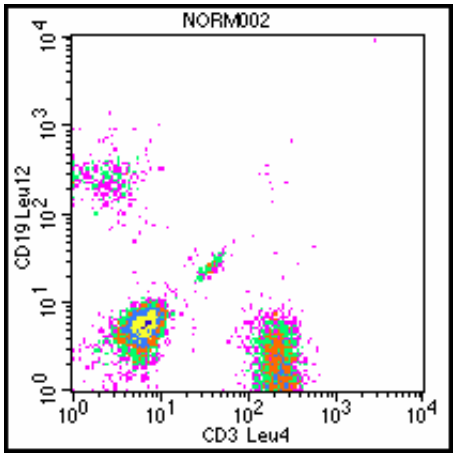
Representation of Data



Histograms





2D Representations:
Dot Plots
Density Plots

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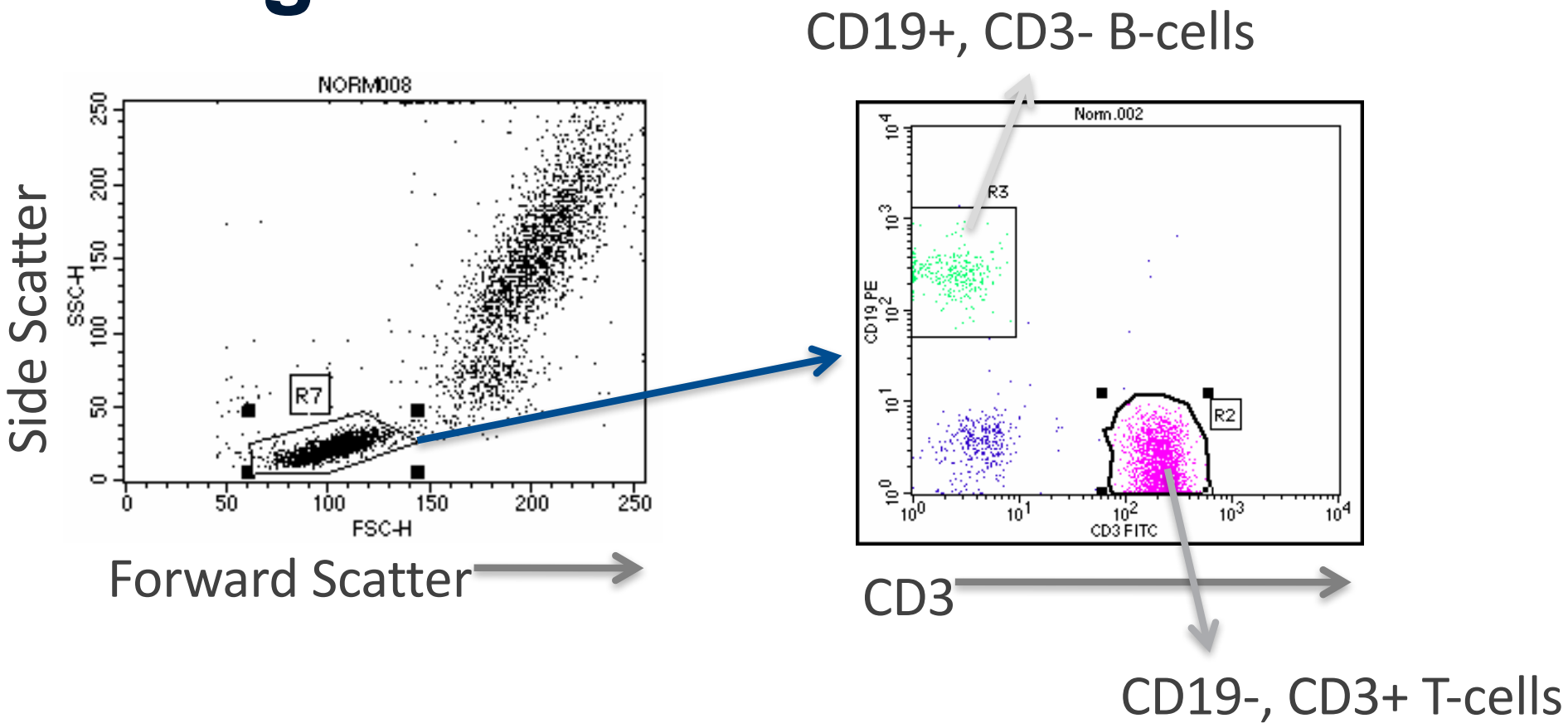
Gating



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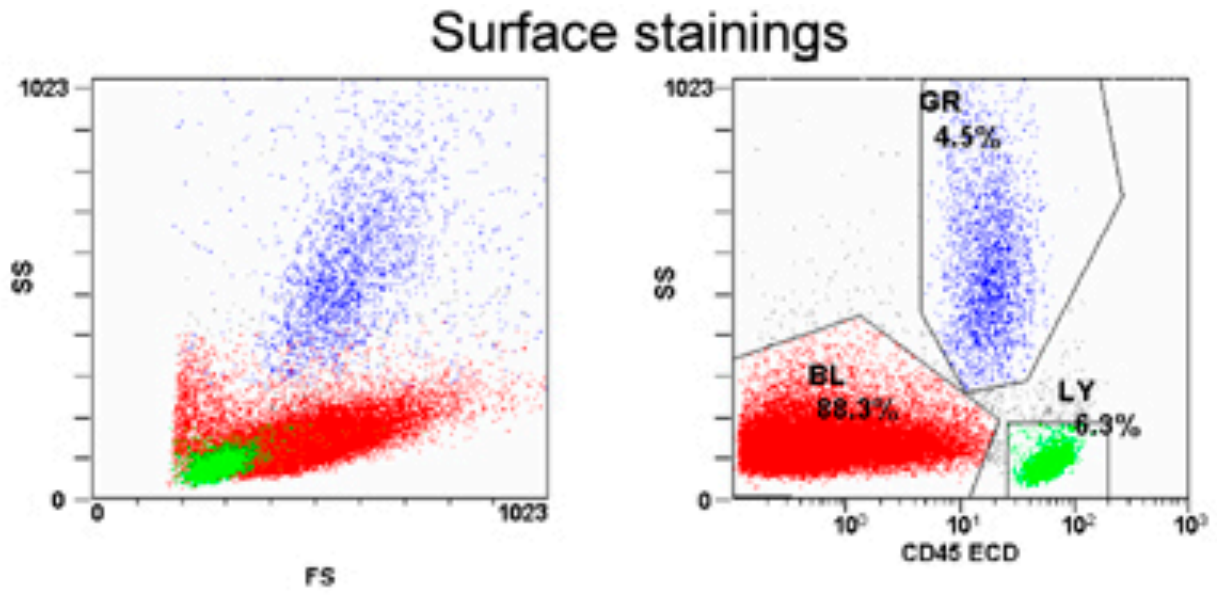
“Blast Gate” in AML



CD45

CD45 dim, Side Scatter low

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Application to Hematologic Malignancies

Analysis is all about pattern recognition

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

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

- Certain malignancies can aberrantly express markers not found in normal cells
  - ie. AML expressing lymphoid markers

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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

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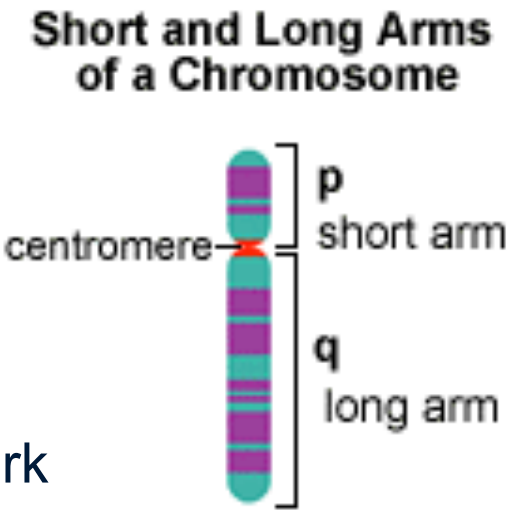
Cytogenetics



- 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
  ie. 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)

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Conventional Cytogenetics

Grow cells in culture and arrest in metaphase with colchicine

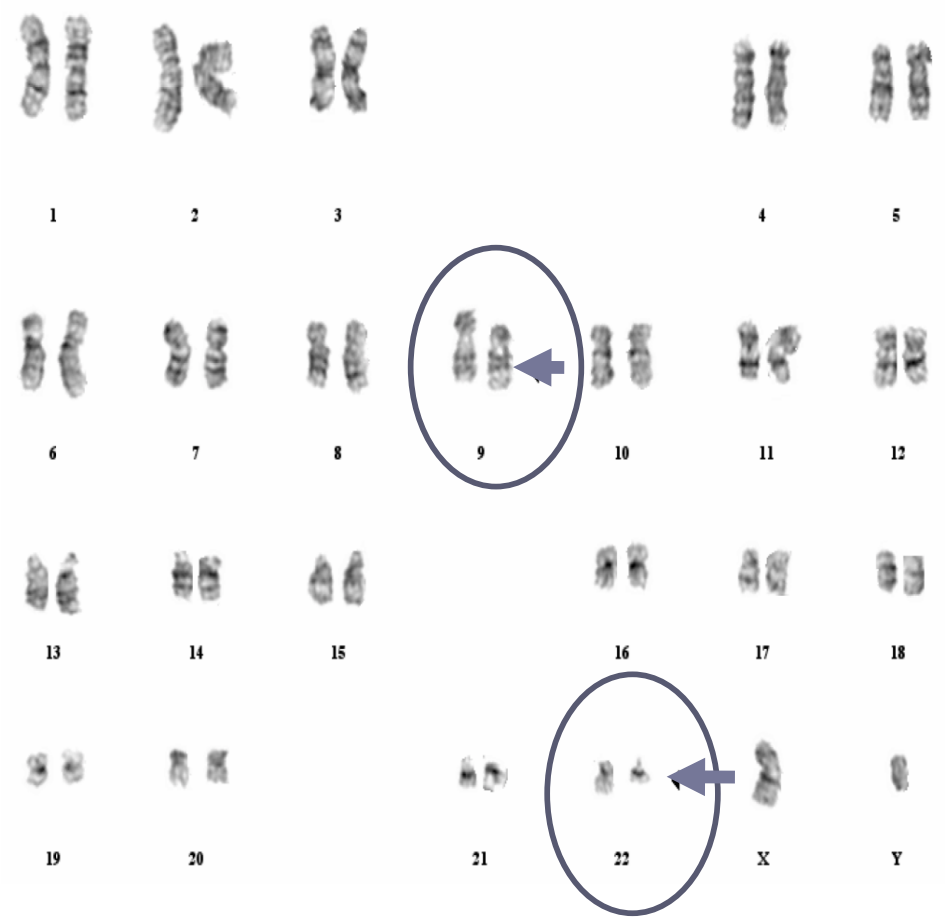
Chromosomal banding

- 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



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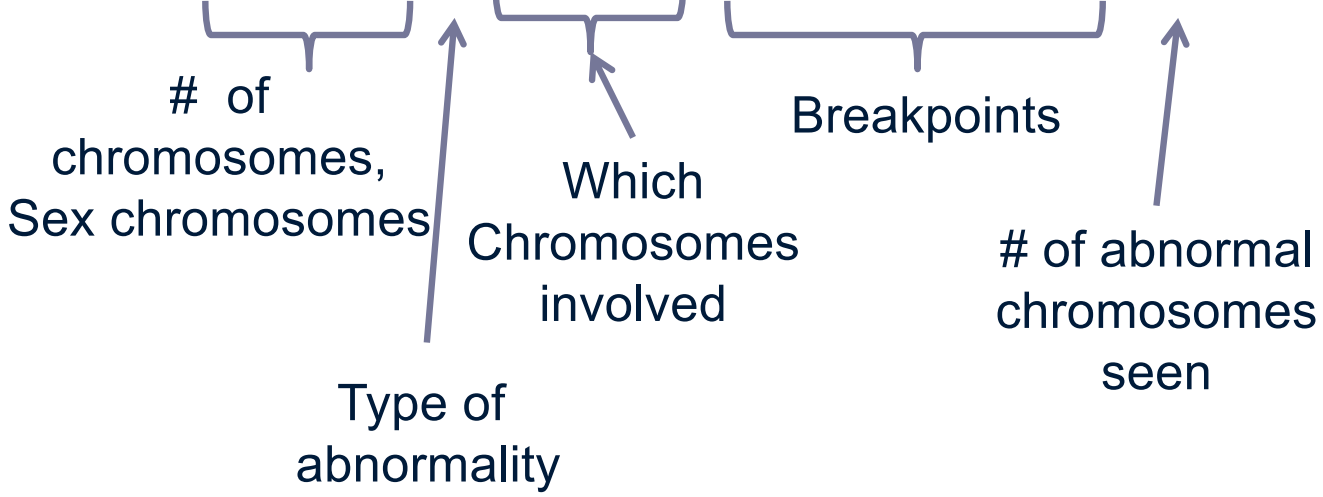
Reading a cytogenetics report

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



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]

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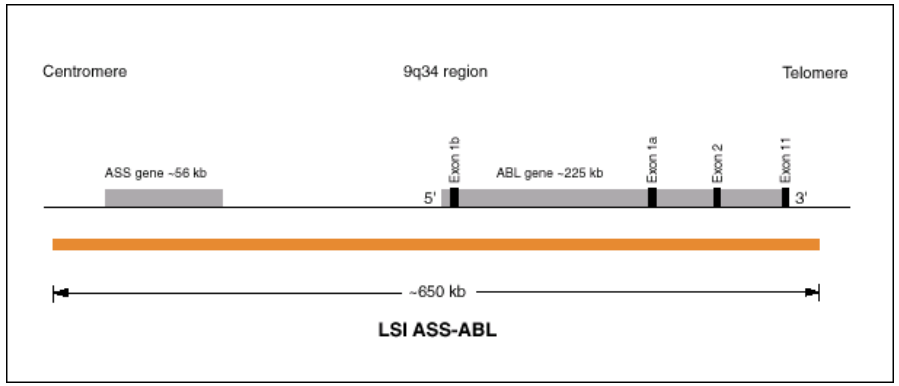
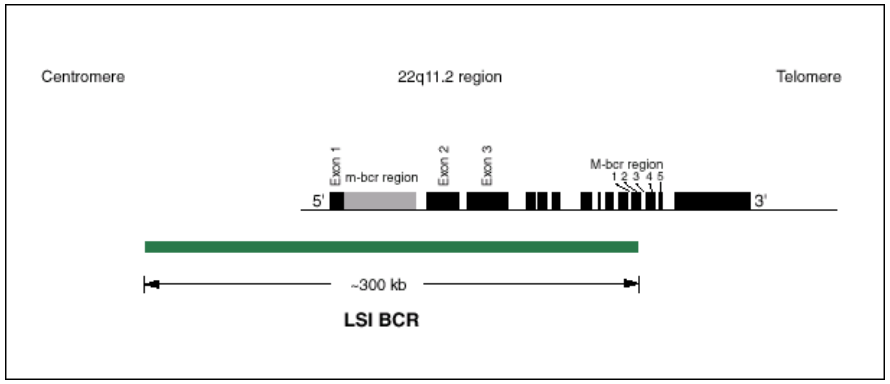
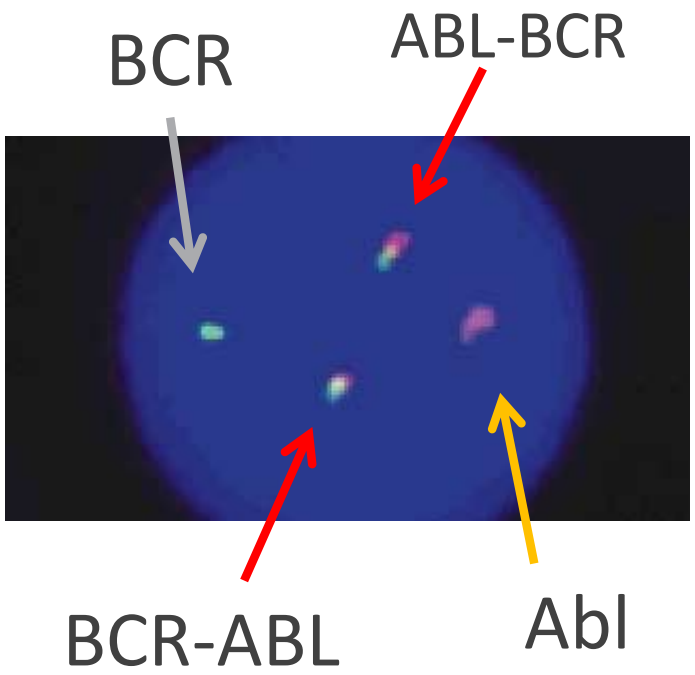
FISH (Fluorescent *in situ* Hybridization)





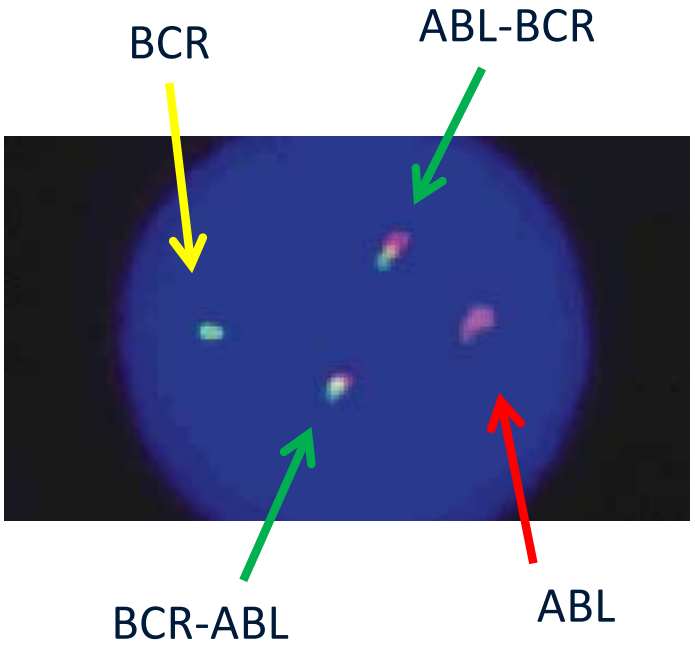
Dual color, dual fusion probe



- 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.

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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

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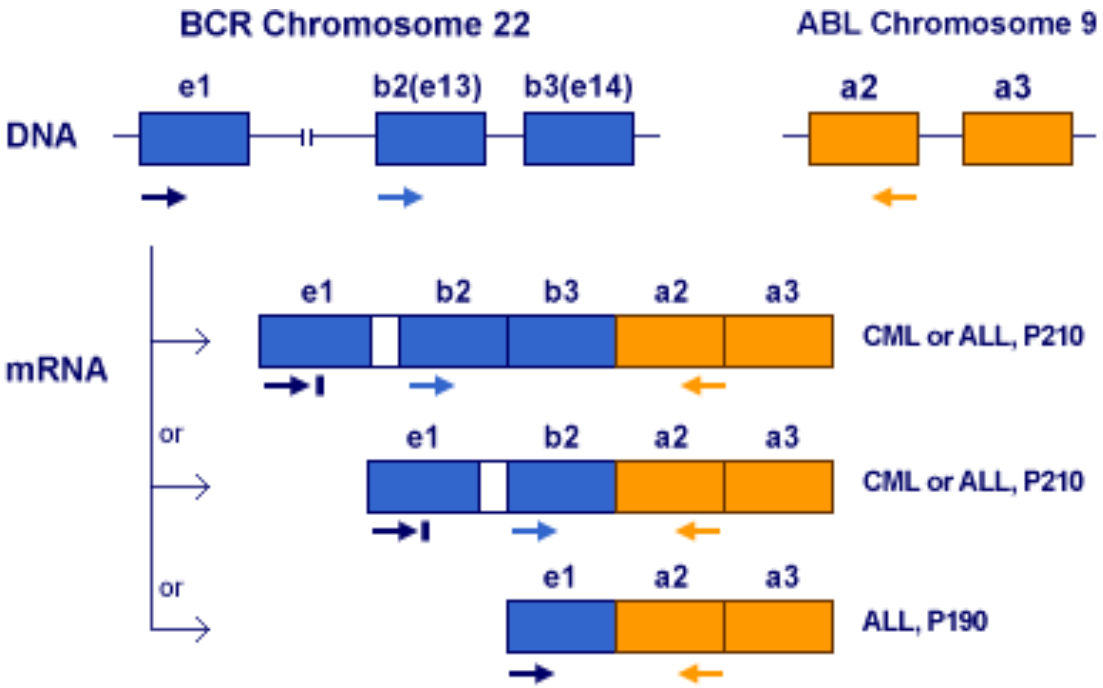
qRT-PCR (Quantitative Real-Time PCR)



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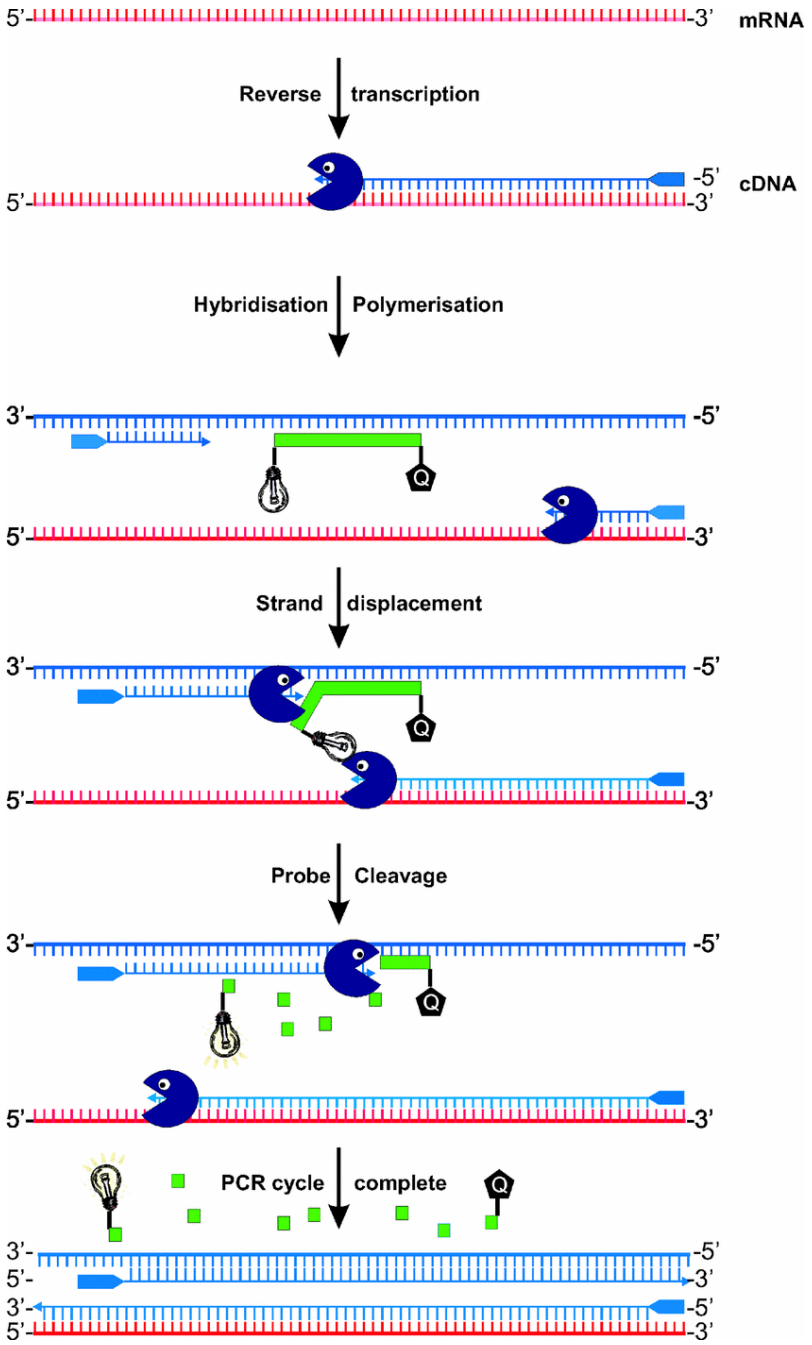
qRT-PCR Assay

- Make a cDNA library from the mRNA specimen
- Design quantititive 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)**



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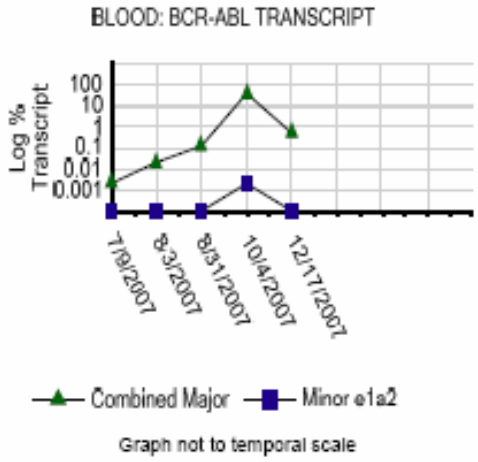
qRT-PCR Report

Results: Positive: Major breakpoint (0.504%)

INTERPRETATION: This result is consistent with ALL.



BLOOD: BCR-ABL TRANSCRIPT

Collection Date	Specimen #	Major e13a2	Major e14a2	Major combined	Minor e1a2
12/17/2007	12640114	0.504%	not detected	0.504%	not detected
10/4/2007	60910910	37.840%	not detected	37.840%	0.002%
8/31/2007	12367420	0.123%	not detected	0.123%	not detected
8/3/2007	12298424	0.020%	not detected	0.020%	not detected
7/9/2007	12236918	0.002%	not detected	0.002%	not detected

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.

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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 abnomal morphology / maturation

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Bone marrow dysplasia

- Alterations in morphology of BM precursor cells: can occur in one or more lineages
- Important in MDS, AML
- Dyserythopoiesis
  - 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

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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

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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

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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

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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

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Response Criteria in CLL

- Partial remission
  - ≥ 50% decrease in lymphocyte count from baseline ≥ 50% reduction in lymphadenopathy.
  - ≥ 50% reduction in size of liver and/or spleen
  - CBC
    - Leukocytes ≥ 1500/µl or 50% improvement over baseline.
    - Platelets > 100,000/µl or 50% improvement over baseline.
    - Hemoglobin > 11.0 gm/dl or 50% improvement over baseline without transfusions.
- Progressive disease
  - ≥ 50% increase in lymphocyte count
  - ≥ 50% increase in sum of products of LN or
  - ≥ 50% increase in size of liver or spleen

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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

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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)

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Classification for FDG-PET Response (Deauville Criteria)

Score	
1	No uptake above background
2	Uptake > mediastinum, but ≤ liver
3	Uptake moderately > liver
4	Uptake markedly higher than liver and/or new lesions
5	Uptake markedly higher than liver and/or new lesions
X	New areas of uptake unlikely to be related to lymphoma

5 point semiquantitative scale to quantify intensity of signal

1-2 Complete Metabolic Response

3 Can vary by study

4-5 Residual disease

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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

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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

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