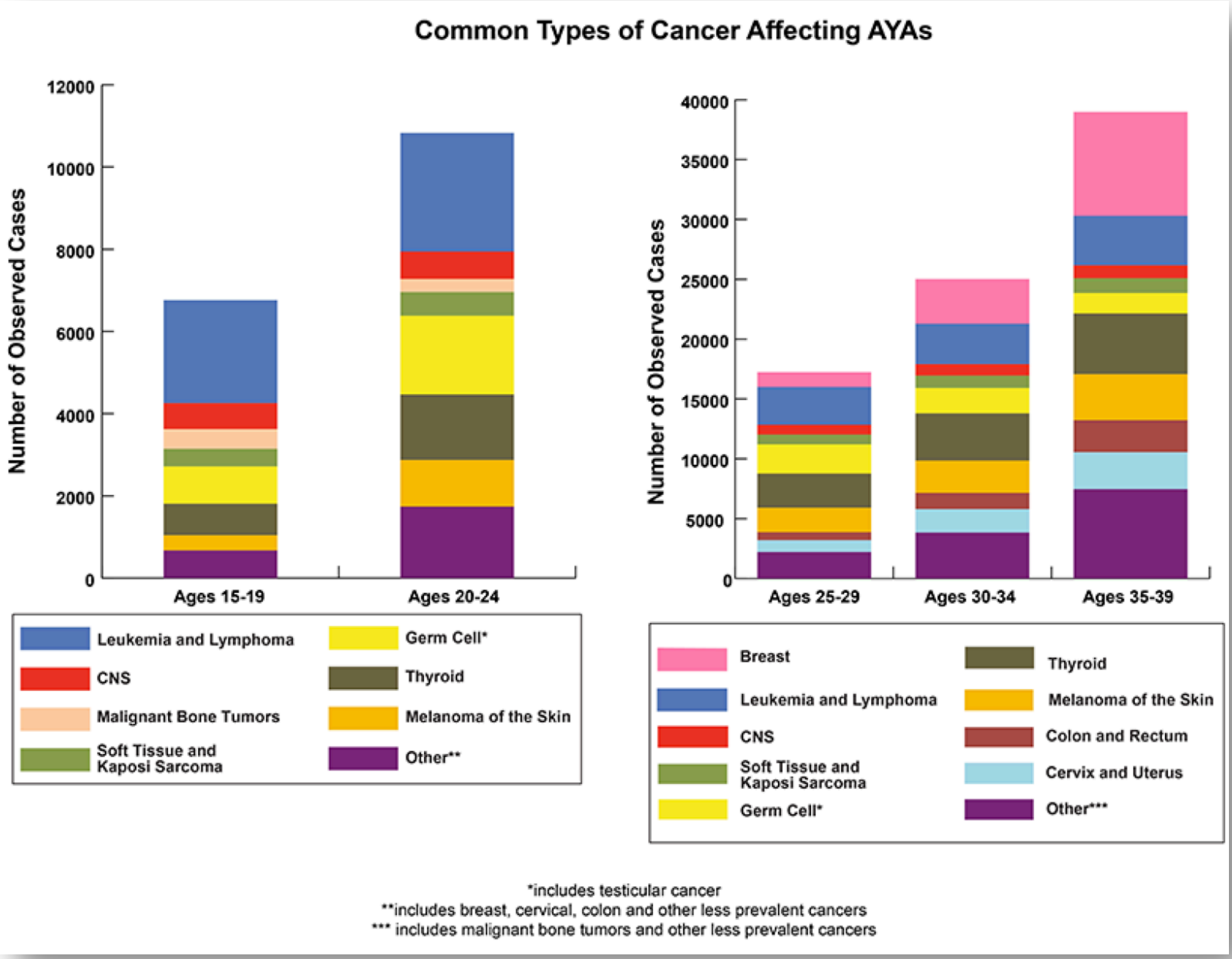
# Mixed lineage leukemia in AYA patients

# Lymphoma Tumor Board

# May 5, 2017




http://www.cancer.gov/types/aya

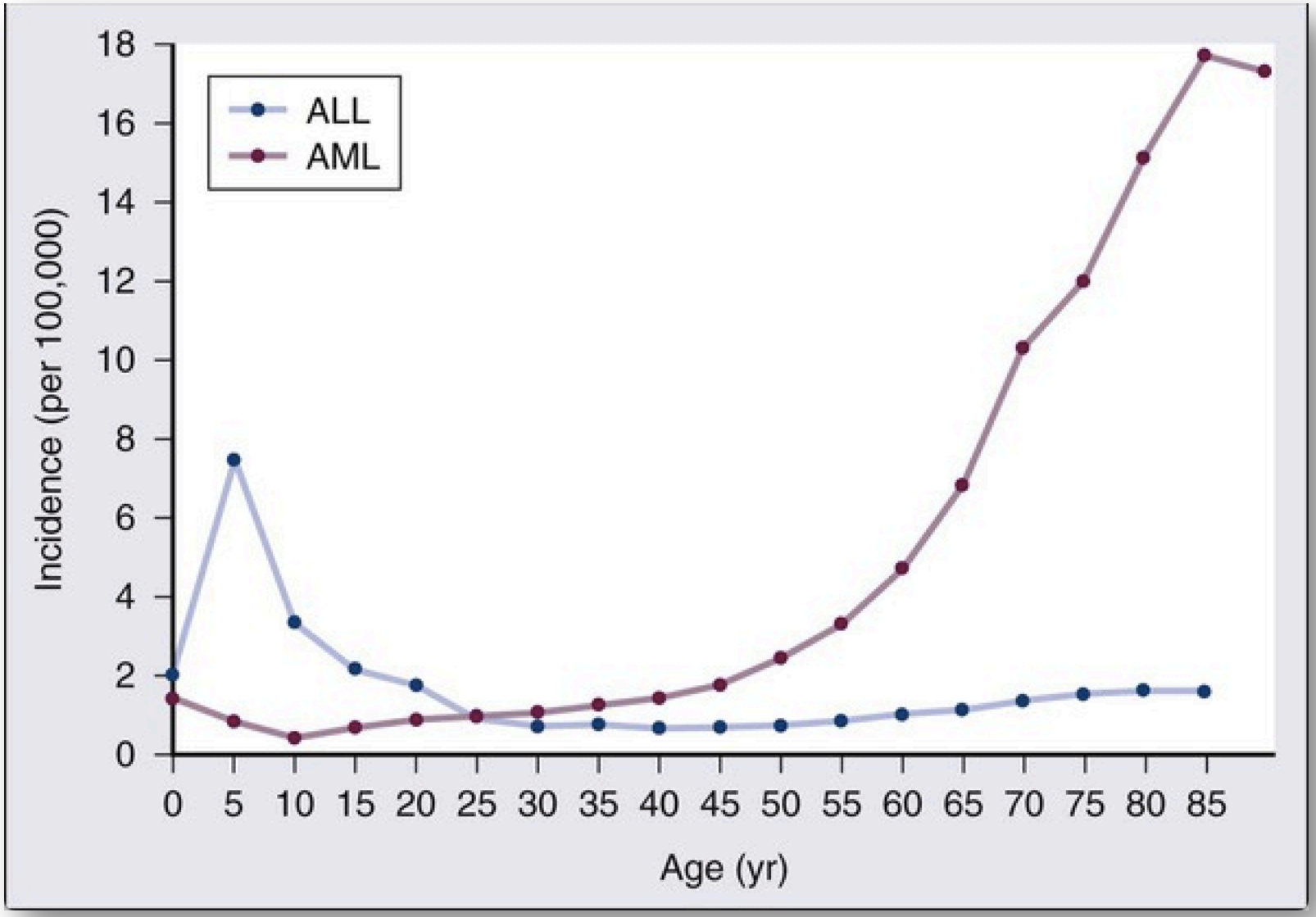
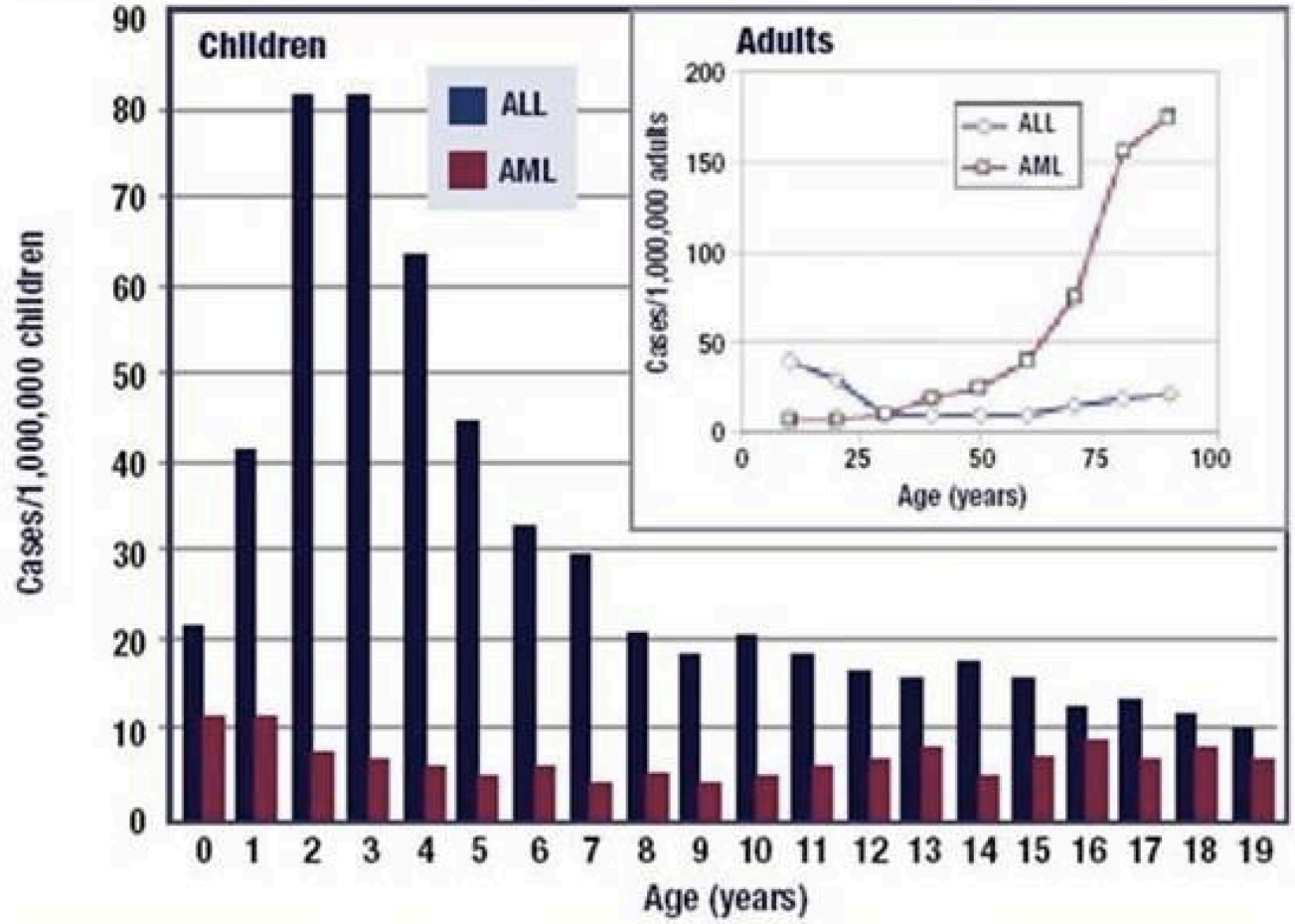

ALL
AML
Incidence (per 100,000)
0
2
4
6
8
10
12
14
16
18
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Age (yr)

FIGURE 3

## Age-specific incidence of ALL and AML, children and adults



# Background

- Mixed-phenotype acute leukemia (MPAL) is rare
- Heterogeneous group of rare leukemias for which assigning a single lineage of origin is not possible
- Defined by limited set of lineage-specific markers
- Multipotent progenitor cells can differentiate into both myeloid and lymphoid lineages
- Factors possibly related:
  - Viral infection
  - Hereditary factors
  - Radiation exposure
  - Chemical exposure
- Association with several mutations, most common being t(9;22) and *MLL* gene rearrangement at 11q23

# Adolescent and Young Adults with AML

- Acute Myeloid Leukemia (ALL) represents 33% of adolescent and 50% of adult leukemia.
- Diagnosis should be based on cytogenetic and molecular factors to avoid overtreatment.
- “Poorer prognosis of AYAs can be overcome with intensive pediatric protocols; whether a similar approach should be applied to AYAs with AML is not evidently provided.”
- Intensifying therapy or “one-size-fits-all” therapy does not improve survival rates.

# Adolescent and Young Adults with ALL

- Acute Lymphoblastic Leukemia (ALL) survival rate is close to 90% for most children.
- In older adolescents and young adults (AYA), event-free survival is only 30-45%.
- Improved outcome, with disease-free survival rates of 60-70% are observed when AYA patients are treated with pediatric-based approaches.
- National Cancer Institute has defined the AYA population as those between the ages of 15 and 39 years.

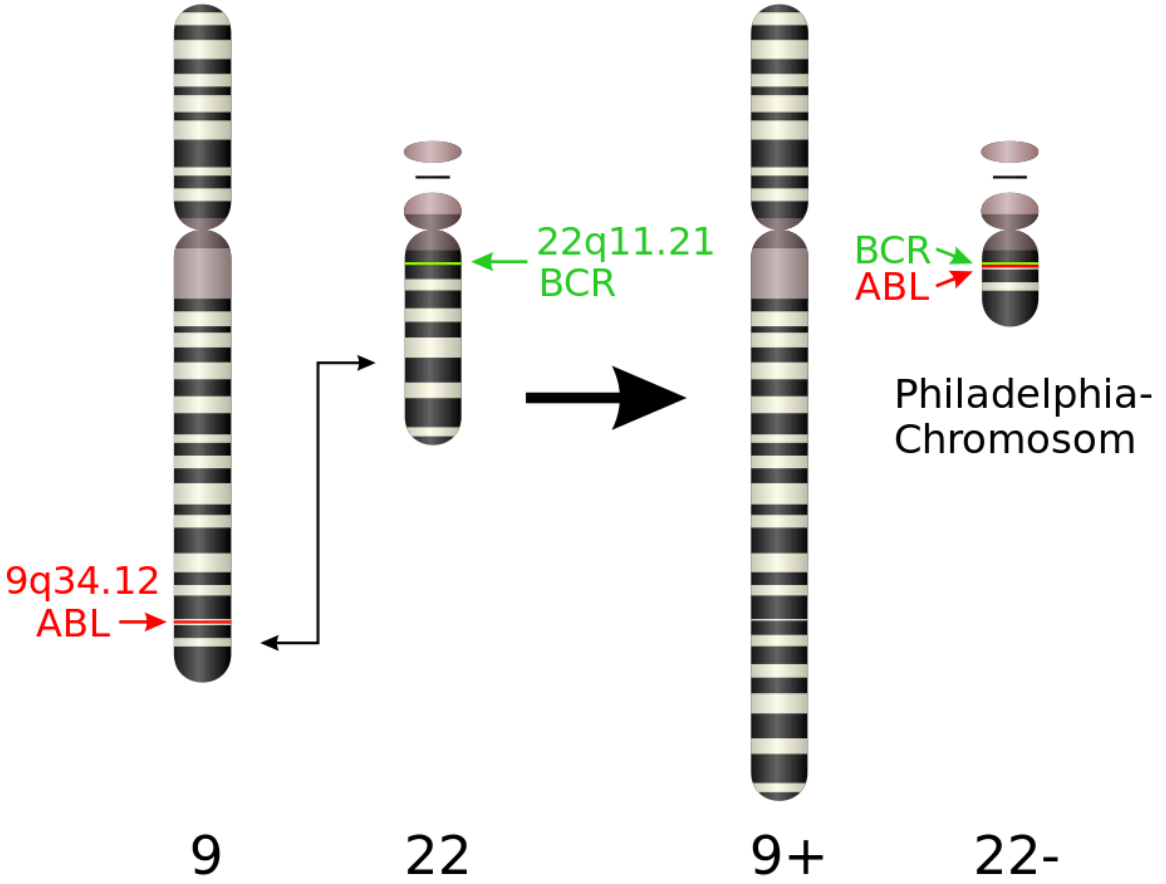



**Figure 1. T(9,22) translocation**

https://en.wikipedia.org/wiki/Biphenotypic_acute_leukemia

# Diagnosis

- Diagnosis requires >20% blasts in blood or marrow (or less in cases of chromosomal translocations and extramedullary presentation).
- "Sometimes the immature cells display cytochemical and/or immunophenotypic features of both lineages (**biphenotypic**) or there are different populations of leukemia cells (**bilineal**)."
- Symptoms due to bone marrow damage:
  - Bruising
  - Anemia
  - Persistent fever
  - Septicemia
- Symptoms due to leukemic cells infiltrating into tissues:
  - Lymphadenopathy
  - Joint pain
  - Swelling of the gums
  - Heptoslenomegaly
  - Headache/vomiting
  - Skin nodules or "lumps"
- Diagnosis **cannot** be based on morphology alone

# Diagnostic criteria for BAL and MPAL

A

| Points | B lineage | T lineage | Myeloid lineage |
|---|---|---|---|
| 2 | CD79a | CD3 (cyt/m) | Anti-MPO |
| | cyt IgM | anti-TCR α/β | |
| | Cyt CD22 | anti-TCR γ/δ | |
| 1 | CD19 | CD2 | CD13 |
| | CD10 | CD5 | CD33 |
| | CD20 | CD8 | CDw65 |
| | | CD10 | CD117 |
| 0.5 | TdT | TdT | CD14 |
| | CD24 | CD7 | CD15 |
| | | CD1a | CD64 |

[a]According to the EGIL criteria, biphenotypic leukemia (BAL) is diagnosed when scores are >2 for the myeloid and one of the lymphoid lineages. A marker is considered positive if more than 20% of cells stain positive with a monoclonal antibody; a lower threshold of 10% was set for MPO, CD3, CD79a and TdT.

Abbreviations: cyt, cytoplasmatic; m, membrane; MPO, myeloperoxidase; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

[b]The following lineage defining markers are required for assigning more than one lineage to a single blast population:

1) Myeloid lineage: myeloperoxidase (by flow cytometry, immunohistochemistry or cytochemistry) or monocytic differentiation (diffuse positivity for non-specific esterase or expression of at least 2 of the following: CD11c, CD14, CD36, CD64, lysozyme).

2) T-lineage: cytoplasmatic CD3 (flow cytometry with antibodies to CD3 epsilon chain; caution when using polyclonal anti-CD3 in immunohistochemistry as it may not be specific) or surface CD3.

3) B-lineage: strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmatic CD22, CD10 or weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmatic CD22, CD10.

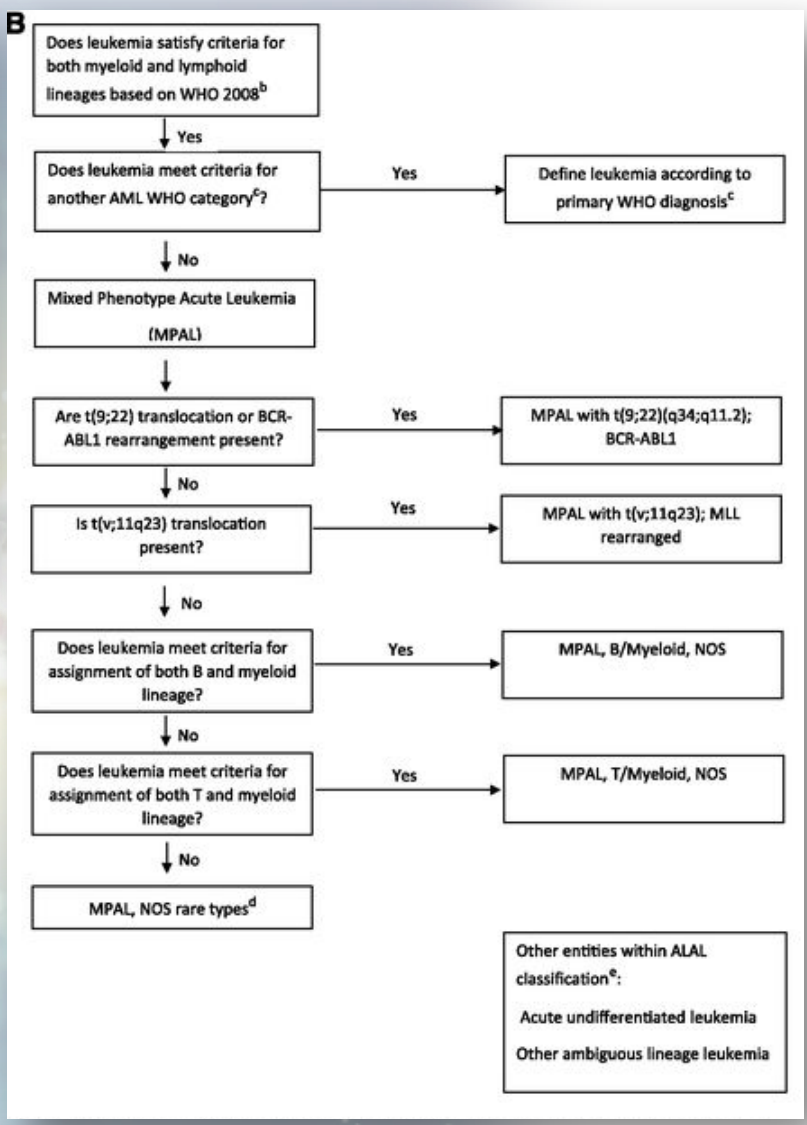
For bilineal MPAL the myeloid component can be recognized when there are 2 or more distinct populations of blasts, one of which would meet the criteria for AML (need not comprise >20% of nucleated cells).

[c]The following subgroups of leukemia should be defined primarily by their genetic or clinical features even if they satisfy the above requirements for mutil-lineage expression. A secondary notion regarding their mixed phenotype should be added: AML with recurrent cytogenetic abnormalities such as t(8;21), t(15;17) or inv(16), leukemia with FGFR1 mutations, chronic myeloid leukemia in blast crisis, myelodysplasia-related AML and therapy related AML.

[d]Include rare cases where leukemic blasts show evidence of both T and B lineage, trilineage T, B and myeloid commitment or other rare combinations; CD79a and CD10 should not be considered as evidence for B cell differentiation in this setting as they lack specifity.

[e]These diagnostic entities are associated with lack of definitive lineage commitment. 'Acute undifferentiated leukemia' include leukemias that express no lineage specific markers. 'Other ambiguous lineage leukemia' subgroup encompass the rare cases of leukemia that express combination of lineage associated markers that are suggestive but not sufficient for lineage assignment (so called 'acute unclassifiable leukemias'); Natural killer cell lymphoblastic leukemia/lymphoma is regarded as a provisional entity in this category.

B




**(A) EGIL criteria for the diagnosis of biphenotypic acute leukemia (B) 2008 WHO criteria**



Ofir Wolach, and Richard M. Stone Blood 2015;125:2477-2485

blood

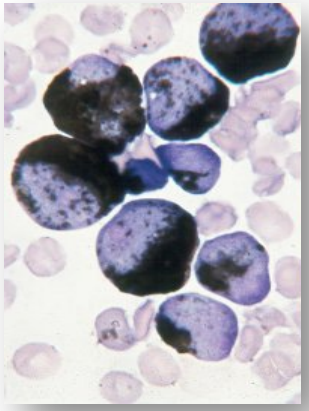
# Distinguishing between AML and ALL using cytochemical stains

| Cytochemical Reaction | Cellular Element Stained | Blasts Identified |
|---|---|---|
| Myeloperoxidase (MPO) | Neutrophil primary granules | Myeloblasts strong positive; monoblasts faint positive Lymphoblast Negative |
| Sudan Black B (SBB) | Phospholipids | Myeloblasts strong positive; monoblasts faint positive Lymphoblast Negative |
| Specific esterase | Cellular enzyme | Promyelocyte stage positive |
| Nonspecific esterase (NSE) | Cellular enzyme | Monoblasts strong positive Others Negative |
| Periodic acid-Schiff | Glycogen and related substances | lymphoblast's and pronormoblasts Negative to Positive . Myeloblasts usually negative. Metamyelocyte & PMN Strong +ve |

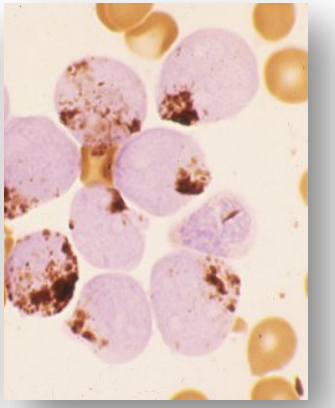
# Distinguishing between AML and ALL using cytochemical stains (2)

| | MPO | SBB | SPE | NSE | PAS |
|---|---|---|---|---|---|
| **Myeloblasts** | ++ | ++ | ++ | + | Diffuse |
| **Lymphoblasts** | - | - | - | -/+ | Block |
| **Monoblasts** | ++ | ++ | - | ++ | Diffuse |

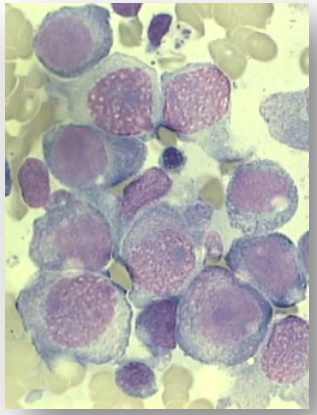
a. Myeloblast: neg for all, M1 and up + MPO
b. Lymphoblast: +PAS and acid phosphatase, +/- sudan black, neg for others
c. Monoblast: strong + NSE, Lysozyme; neg to weak for MPO



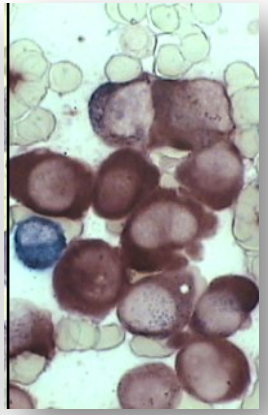
SBB



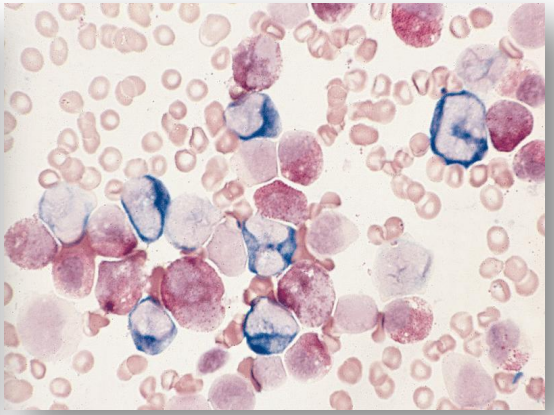
MPO



wright giemsa

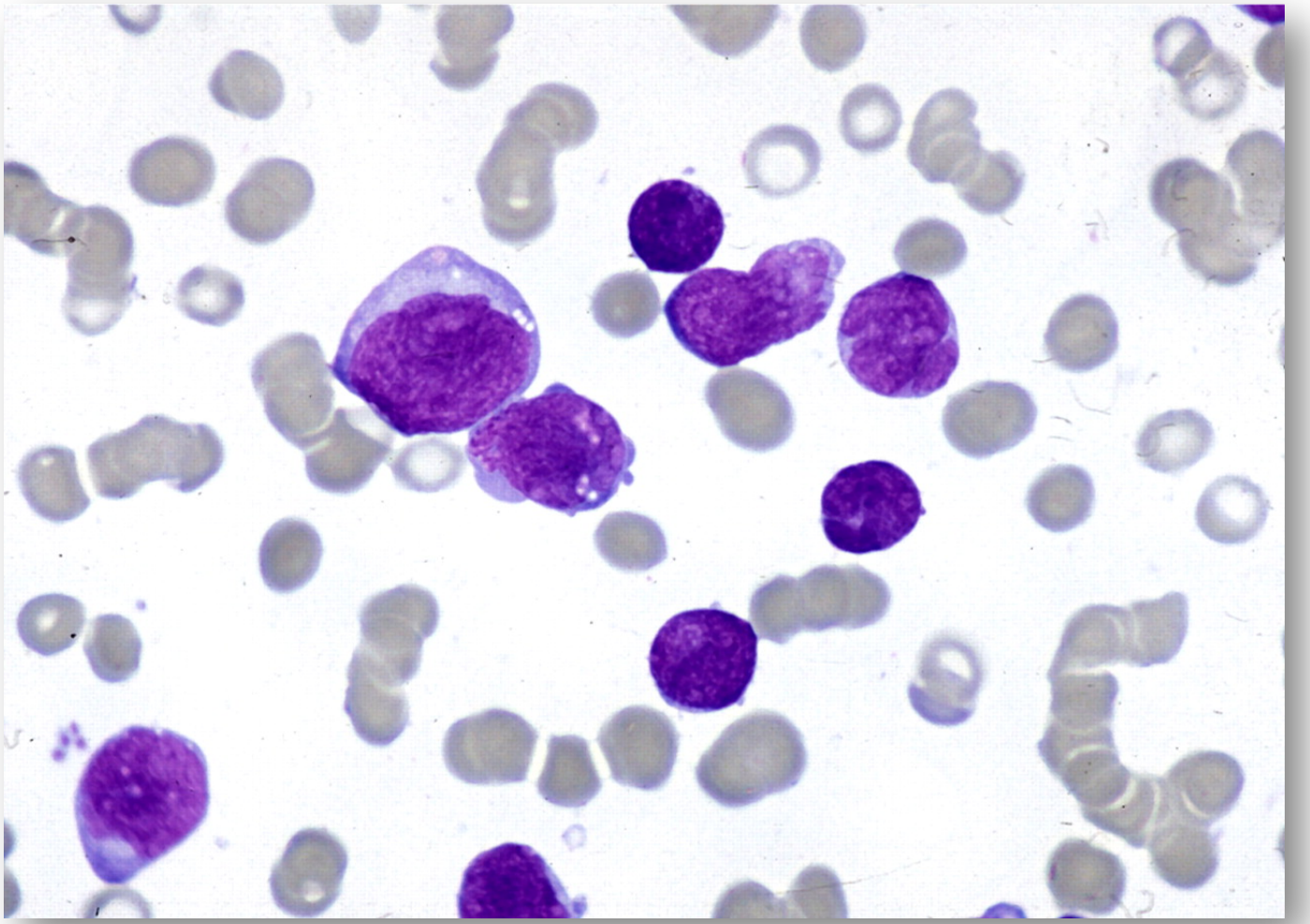


brown –
a-naphtyl acetal
esterase



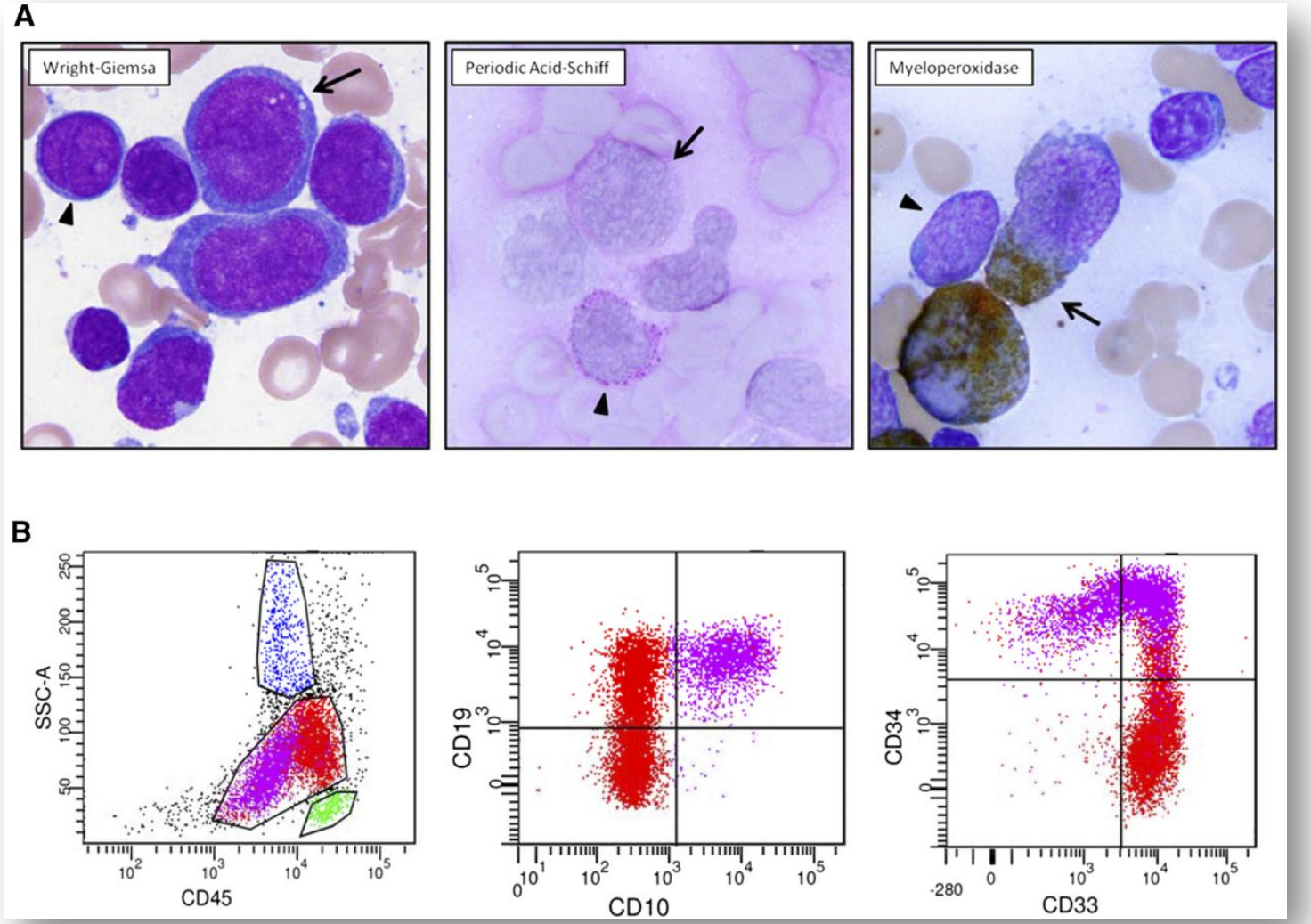
Blue is chloracetate esterase

# May-Grünwald-Giemsa–stained BM smear showing a mixed-cell population of large and small blasts



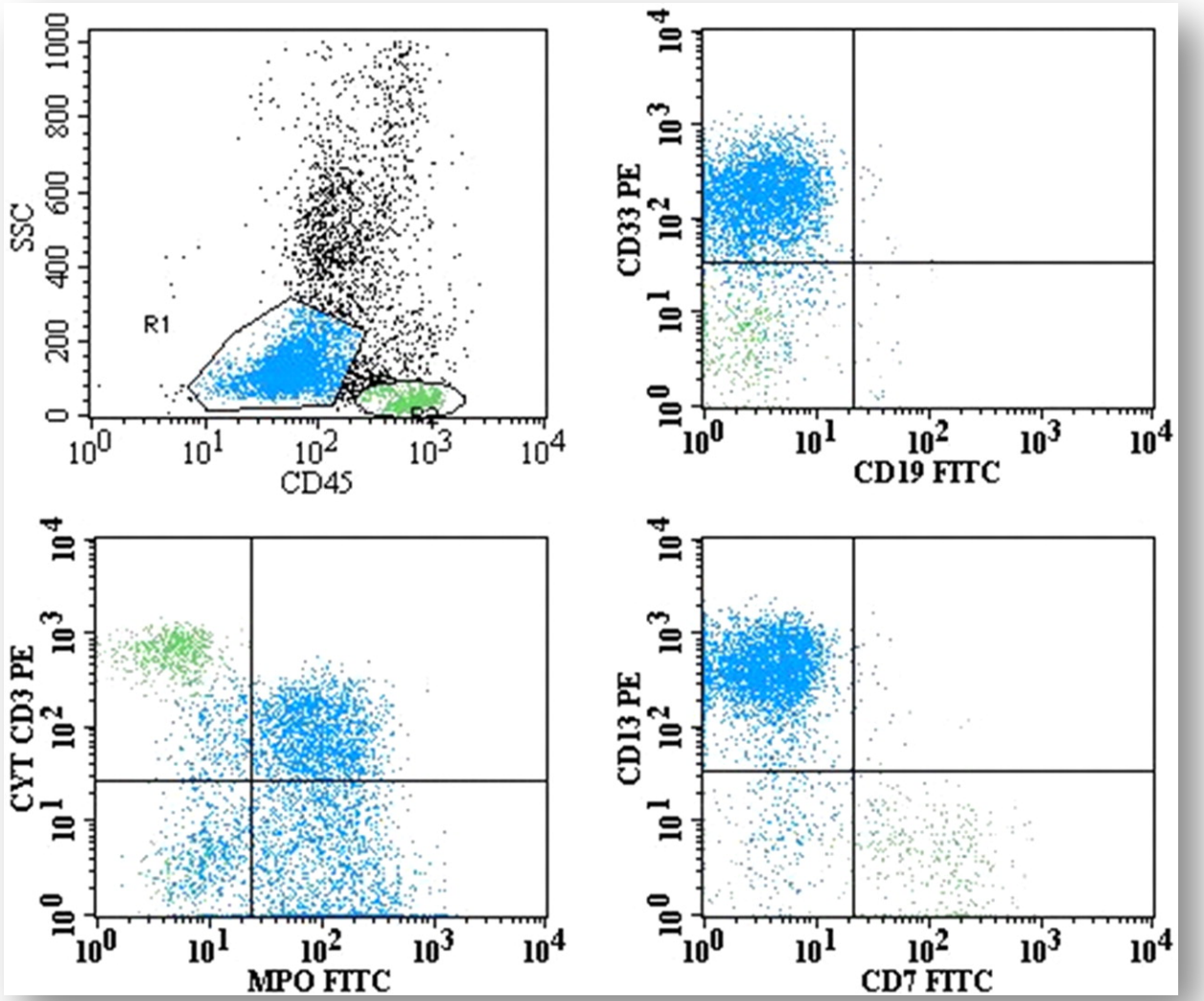
Estella Matutes et al. Blood 2011;117:3163-3171

# Characteristic morphology




Ofir Wolach, and Richard M. Stone Blood 2015;125:2477-2485

# Dot plots with the blast population highlighted in blue (R1) and lymphocyte population in green (R2)

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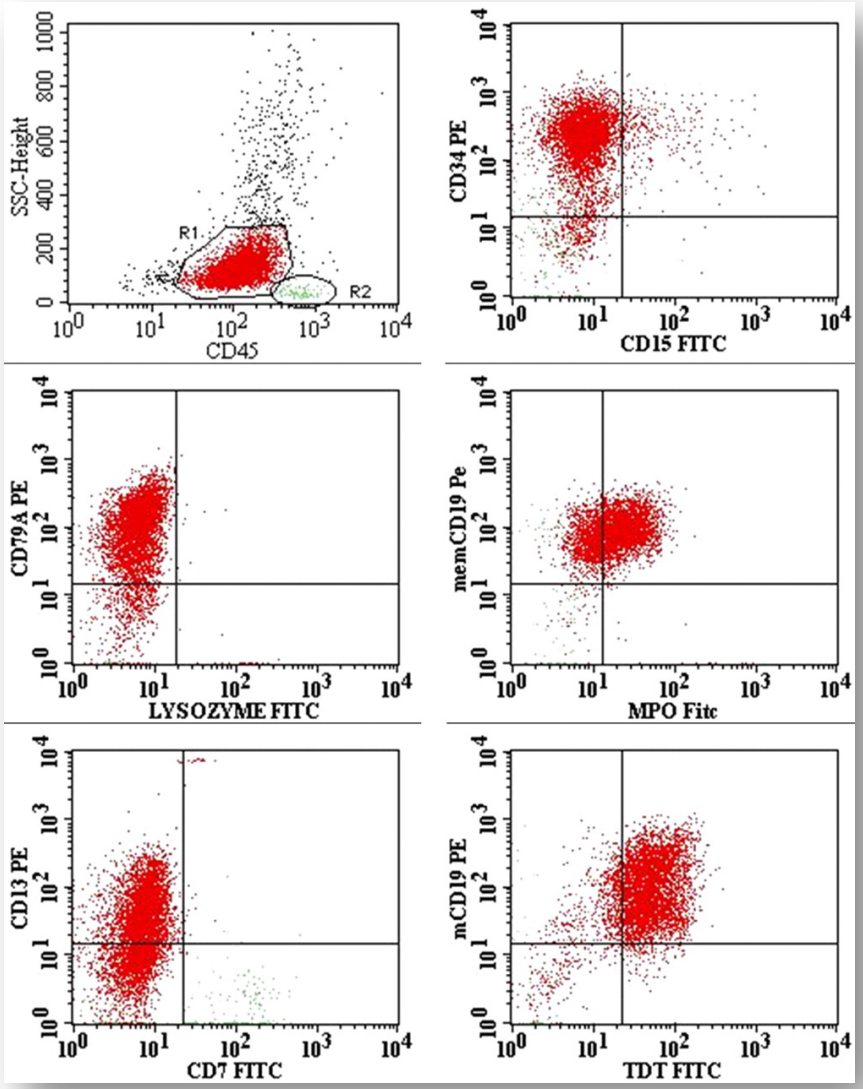
# Dot plots with the blast population highlighted in red (R1) and lymphocyte population in green (R2)

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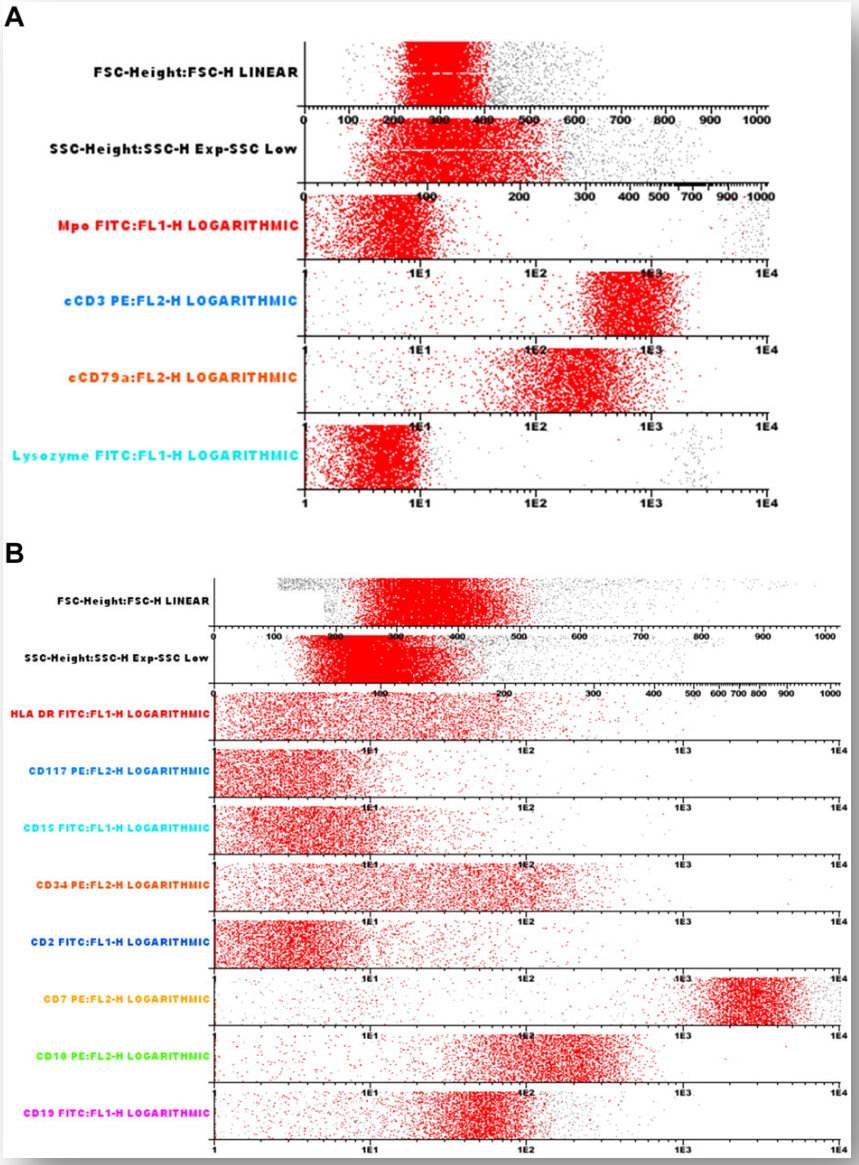
# Infinicyte band representation

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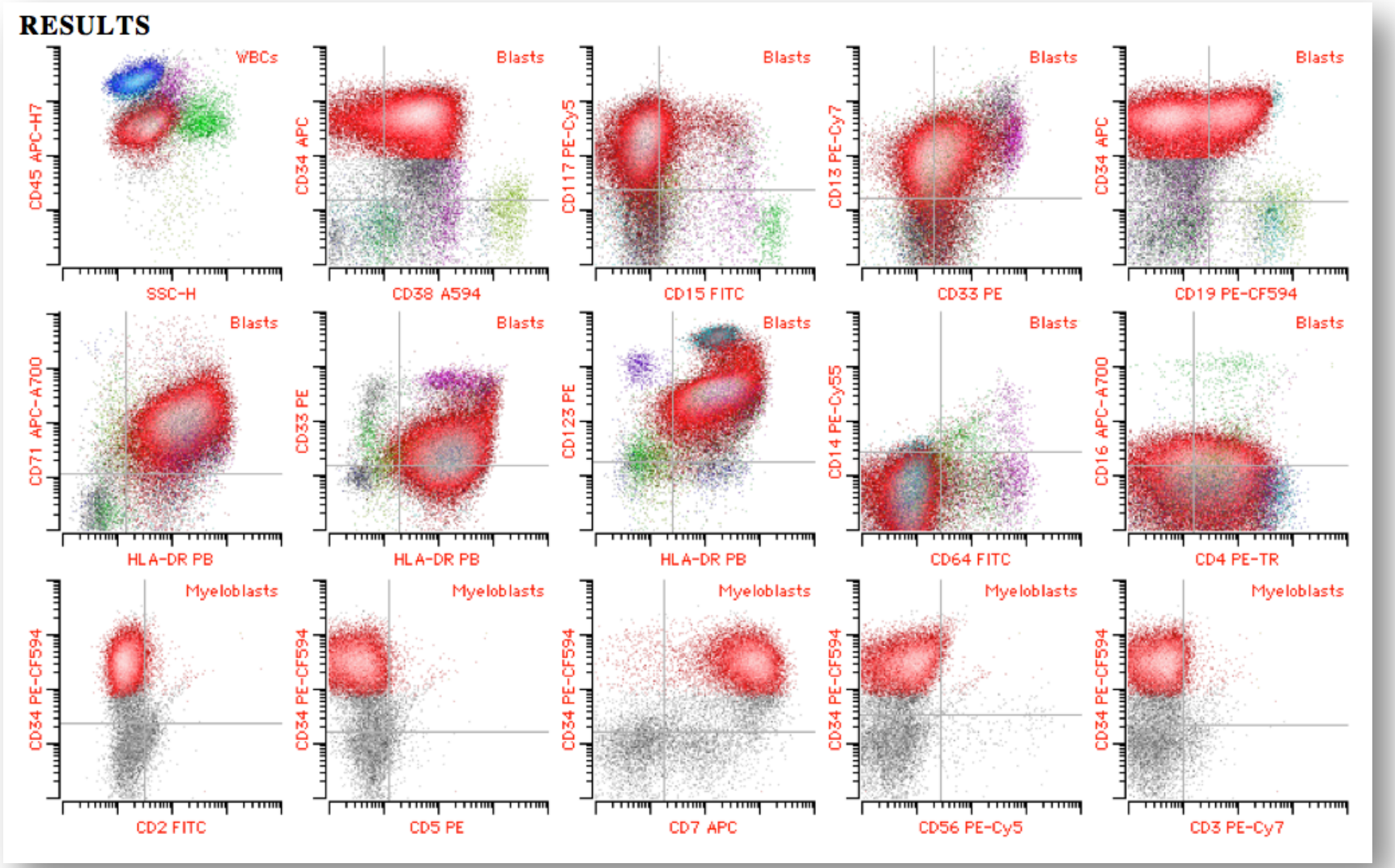
# MPAL case study (FHCRC): Bone marrow aspirate



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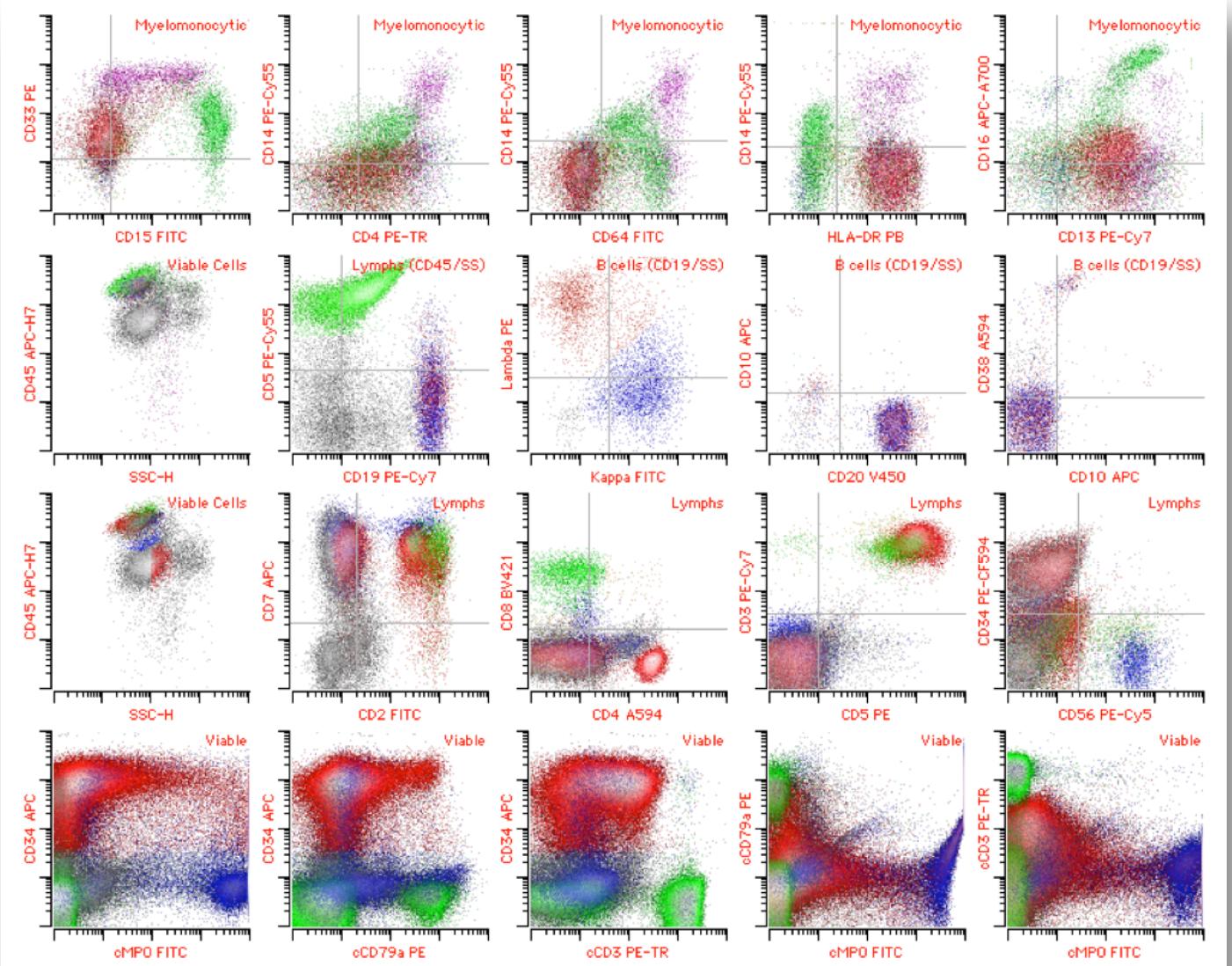
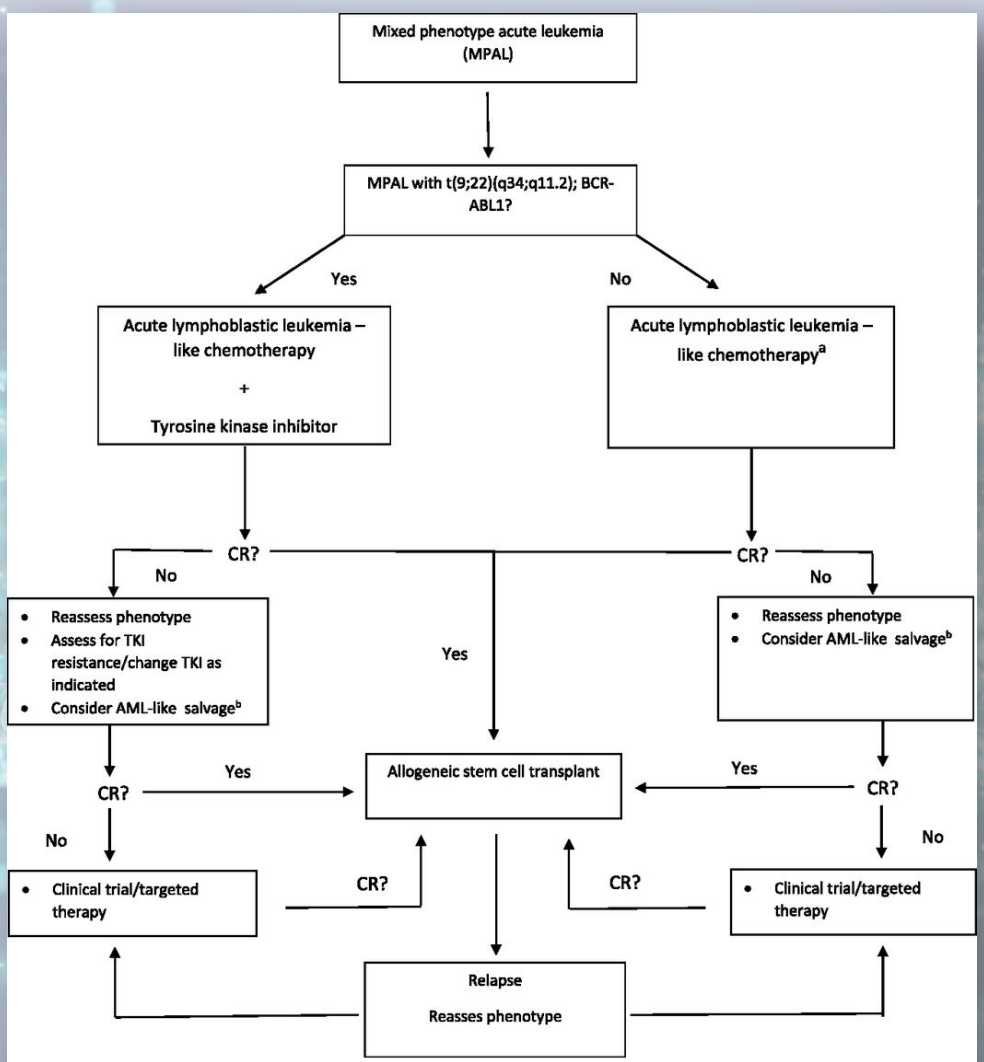



Immunophenotyping by flow cytometry after lysis of the erythroid cells reveals that the white blood cells consist of 51 % blasts (CD34+), 6.8 % maturing neutrophilic forms, 4.2 % monocytes, and 30.5 % lymphocytes. The lymphocytes consist of 20.0 % B cells (CD19+), 63.5 % T cells (CD3+) having a CD4:CD8 ratio of 3.3, and 16.5 % NK cells (CD3-, CD7+).

# Treatment

- Optimal treatment is still undefined as MPAL is quite rare
- Treatment based on:
  - Patient age
  - Medical history
  - Comorbidities
  - Blast morphology
  - Cytogenetics
  - Immunophenotype
  - Molecular studies
- Patients with 11q23 are considered separate entities
- Critical to define the $Ph^+$ patients so TKI can be added
- Most patients get either AML or ALL treatment
- AML induction:  cytarabine, anthracycline
- ALL induction:  prednisolone, dexamethasone, vincristine, asparaginase, daunorubicin
- Using both may be associated with superior outcome

# Therapeutic approach in patients with MPAL




[a]Consider pediatric inspired protocol if <40 years old (in which case transplant in first remission not generally indicated)

[b]For example High-dose cytarabine and mitoxantrone (HAM).

Ofir Wolach, and Richard M. Stone Blood 2015;125:2477-2485

# Preventive/Supportive Care and Monitoring

- Allopurinol is recommended for the first 10 days of induction therapy to prevent hyperuricemia.
- Antimicrobial prophylaxis, antiviral and *Pneumocystis jiroveci* pneumonia prophylaxis throughout treatment.
- Fungal prophylaxis should include mold coverage throughout induction therapy.
  - Broader spectrum azole antifungals cannot be used with vincristine.
- Asparaginase-related toxicities
  - Asparaginase-related hypersensitivity reactions can occur in 20% of children and adults.

# Treatment Regimens (1)

- Adult Regimens:
  - Intensive use of myelosuppressive agents:
    - Duanorubicin
    - Cytarabine
    - Cyclophosphaminde
    - Allogeneic stem cell transplantation (SCT)
- Pediatric Regimens:
  - Berlin-Frankfurt-Munster (BFM) backbone:
    - Glucocorticoids
    - Vincristine
    - Asparaginase
    - Early and Frequent CNS prophylaxis and prolonged maintenance therapy

# Treatment Regimens (2)

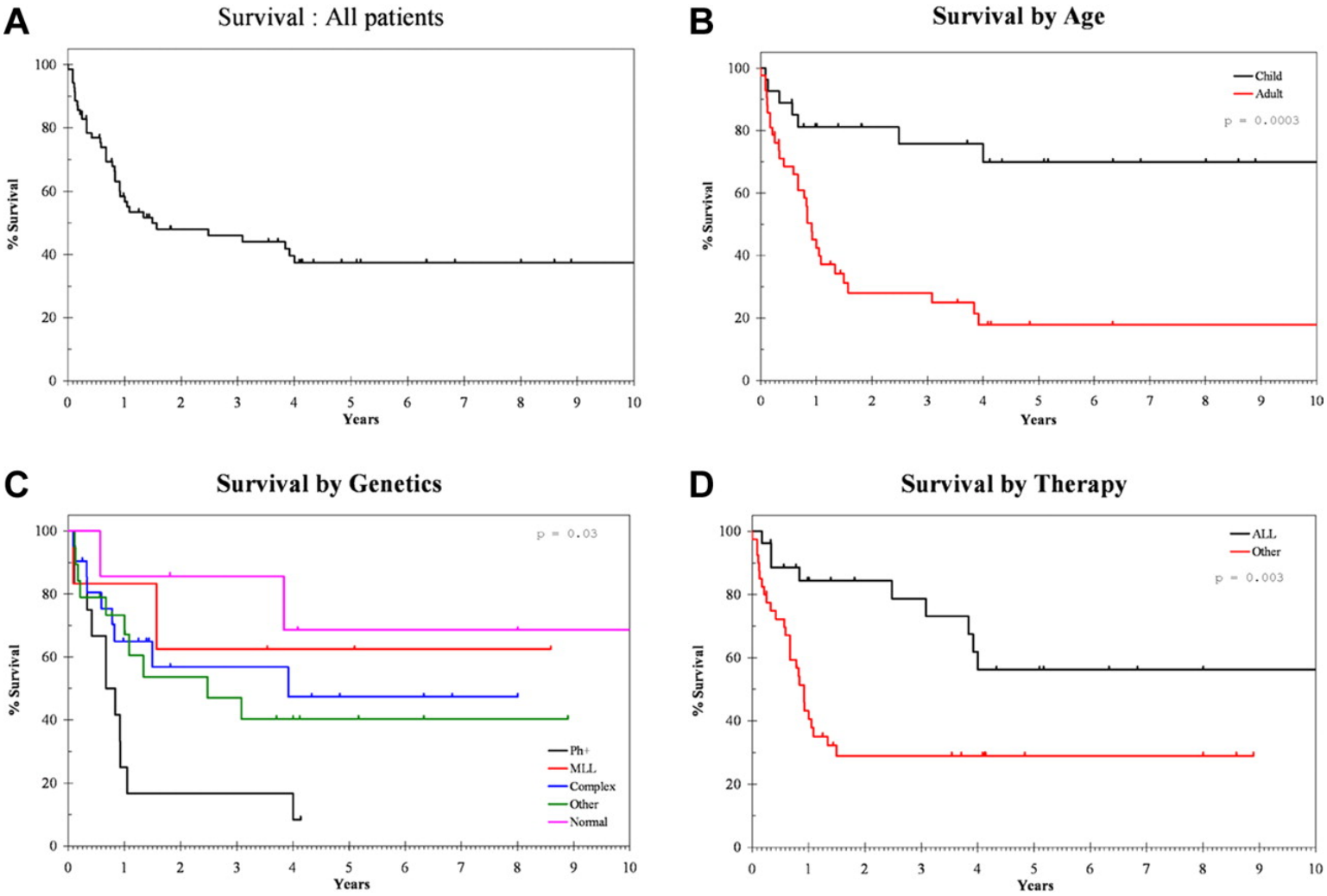
- "3+7" continues to be the backbone of induction therapy.
  - (daunorubicin 60–90 mg/$m^2$/day idarubicin 10–12 mg/$m^2$/day or mitoxantrone 10–12 mg/$m^2$/day) and seven days of cytarabine (100–200 mg/$m^2$/day)

- AYA patients usually receive one or two cycles of induction therapy.

- Additional CNS therapy is routine in most pediatric protocols.

- Bone marrow assessment on the $7^{th}$ or $10^{th}$ day after completion of induction treatment.

# Overall survival of MPAL patients

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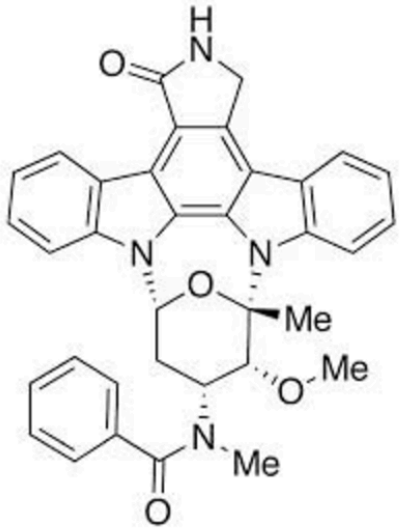
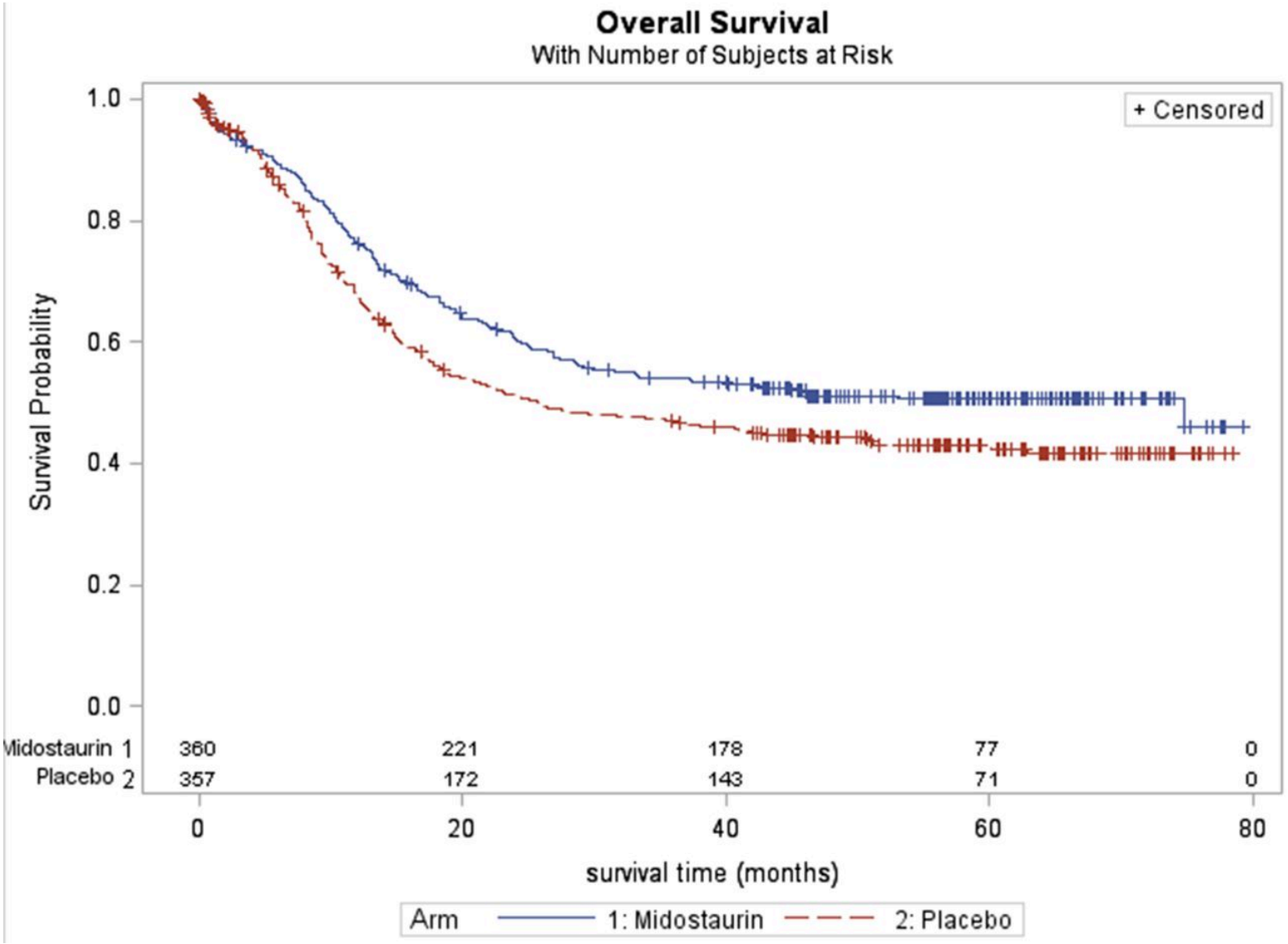
# FDA Approval of midostaurin (RYDAPT®)

- Approved on April 28, 2017 by U.S. Food and Drug Administration
- For treatment of AML patients who are *FLT3* mutation-positive
- LeukoStrat CDx *FLT3* Mutation Assay was also approved to be used in conjunction with midostaurin to test patients with AML
- Based on randomized trial of 717 patients with previously untreated *FLT3*+ AML
- Most common serious adverse reaction was febrile neutropenia occurring in 16% of patients
- Recommended dose of midostaurin in AML is 50mg twice daily with food on days 8 to 21 of each cycle of induction and consolidation chemotherapy followed by 50mg with food as a single agent for up to 12 months




Midostaurin Structure

# An International Prospective Randomized (rand) P-Controlled Double-Blind Trial (CALGB 10603/RATIFY [Alliance])

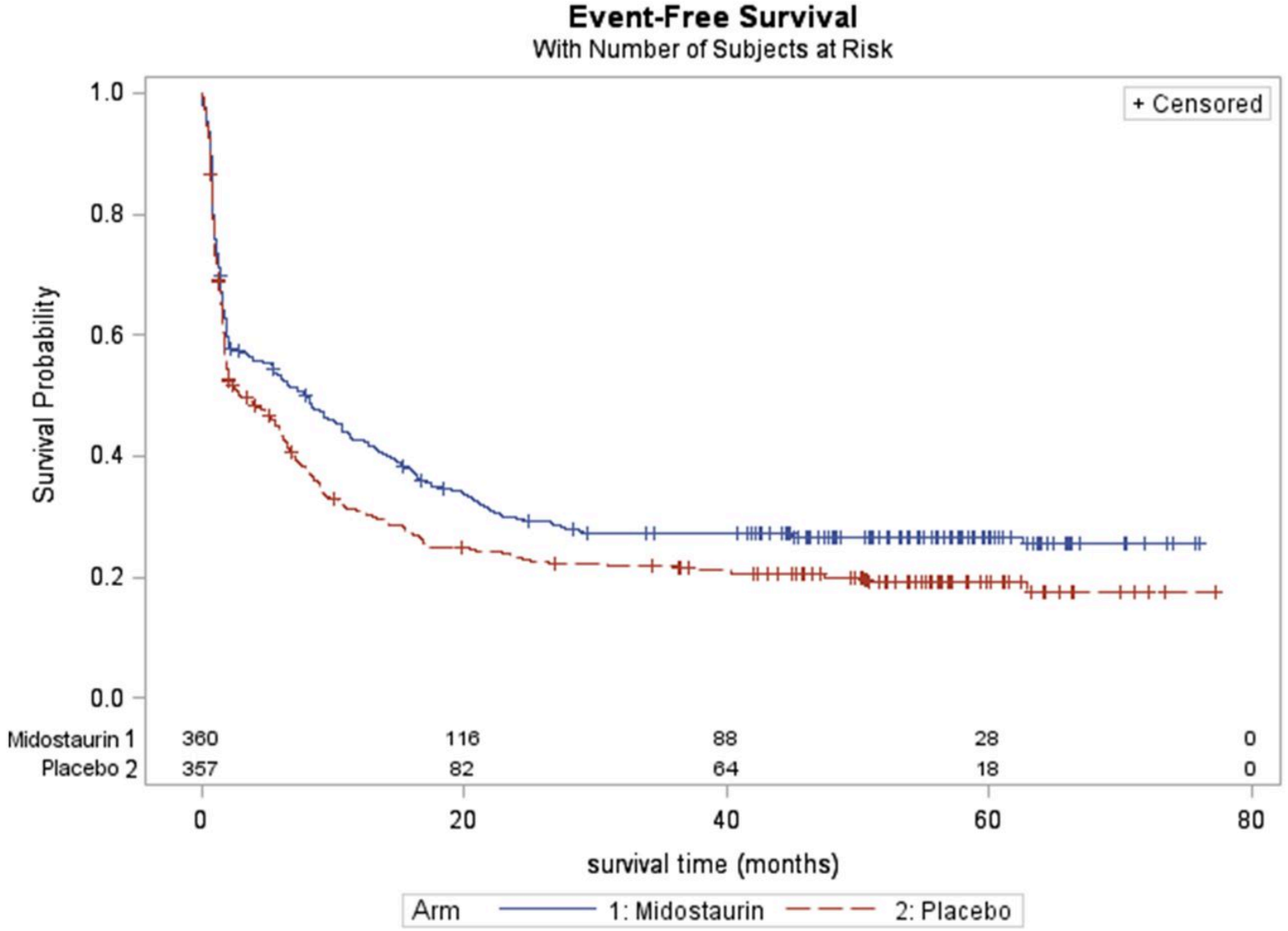



Richard M. Stone et al. Blood 2015;126:6



blood

# An International Prospective Randomized (rand) P-Controlled Double-Blind Trial (CALGB 10603/RATIFY [Alliance])

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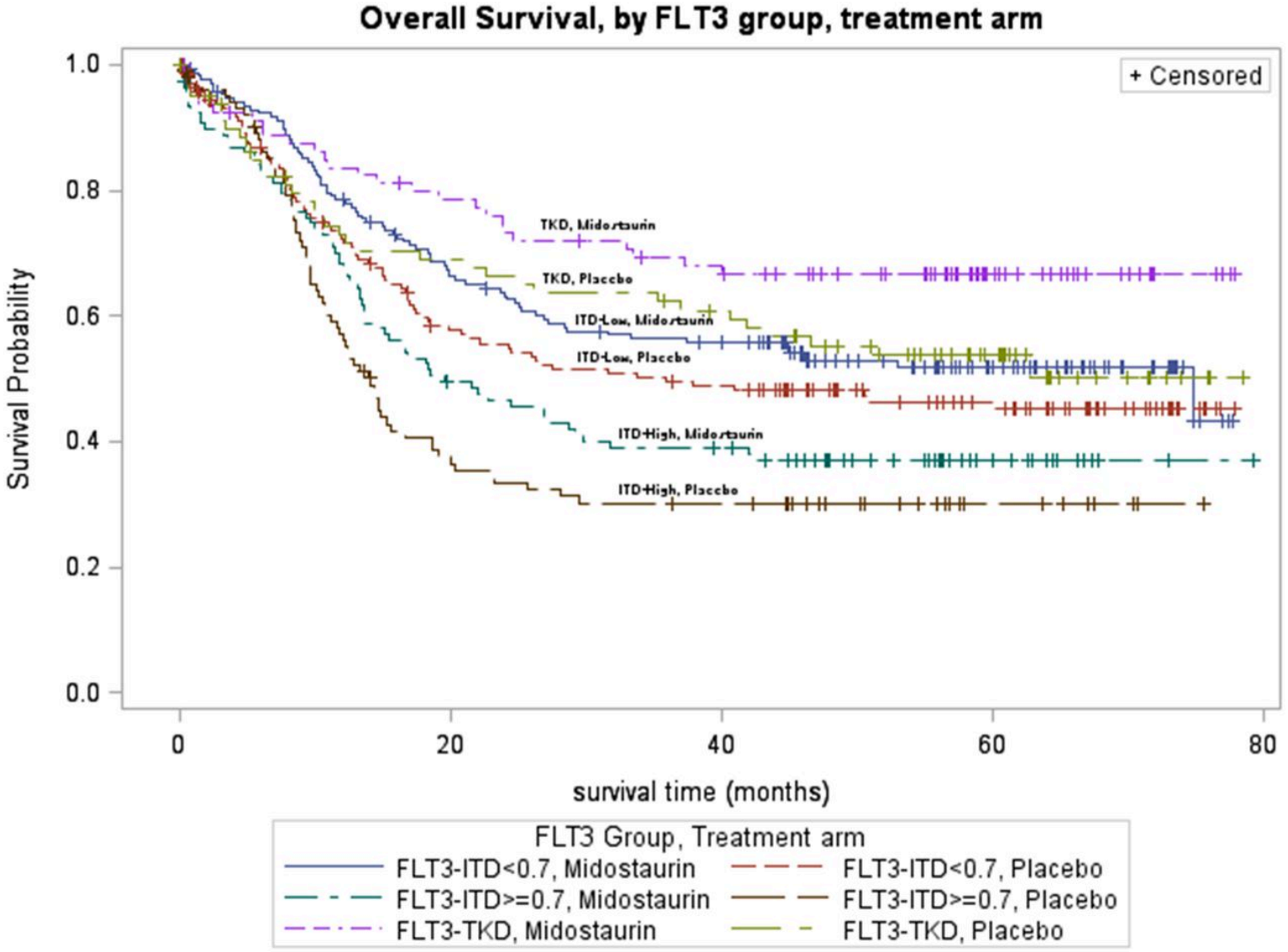




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# References


- Wolach, O., & Stone, R. M. (2015). How I treat mixed-phenotype acute leukemia. Blood, 125(16), 2477-2485. Accessed September 28, 2016. http://dx.doi.org/10.1182/blood-2014-10-551465.
- Matutes, E., Pickl, W. F., van't Veer, M., Morilla, R., Swansbury, J., Strobl, H., Attarbaschi, A., Hopfinger, G., Ashley, S., Bene, M. C., Porwit, A., Orfao, A., Lemez, P., Schabath, R., & Ludwig, W. (2011). Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood, 117(11), 3163-3171. Accessed September 28, 2016. http://dx.doi.org/10.1182/blood-2010-10-314682.
- https://en.wikipedia.org/wiki/Biphenotype_acute_leukemia
- Background photo: https://www.oriel.nhs.uk/Web/Content/images/sg2.jpg
- Jonathan Fromm, MD, PhD – University of Washington/Seattle Cancer Care Alliance
- http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4470138/
- http://www.cancer.gov/types/aya
- *Curran, E., & Stock, W. (2015). How I treat acute lymphoblastic leukemia in older adolescents and young adults. Blood, 125(24), 3702-3710. Accessed June 14, 2016. http://dx.doi.org/10.1182/blood-2014-11-551481.*
- http://news.feinberg.northwestern.edu/2017/01/halting-lethal-childhood-leukemia/
- Stone, R. M., Mandrekar, S., Sanford, B. L., Geyer, S., Bloomfield, C. D., Dohner, K., Thiede, C., Marcucci, G., Lo-Coco, F., Klisovic, R. B., Wei, A., Sierra, J., Sanz, M. A., Brandwein, J. M., de Witte, T., Niederwieser, D., Appelbaum, F. R., Medeiros, B. C., Tallman, M. S., Krauter, J., Schlenk, R. F., Ganser, A., Serve, H., Ehninger, G., Amadori, S., Larson, R. A., & Dohner, H. (2015). The Multi-Kinase Inhibitor Midostaurin (M) Prolongs Survival Compared with Placebo (P) in Combination with Daunorubicin (D)/Cytarabine (C) Induction (ind), High-Dose C Consolidation (consol), and As Maintenance (maint) Therapy in Newly Diagnosed Acute Myeloid Leukemia (AML) Patients (pts) Age 18-60 with FLT3 Mutations (muts): An International Prospective Randomized (rand) P-Controlled Double-Blind Trial (CALGB 10603/RATIFY [Alliance]). Blood, 126(23), 6. Accessed May 03, 2017. Retrieved from http://www.bloodjournal.org/content/126/23/6.