Next Generation Sequencing of Hematologic Neoplasms

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Learning Objectives:

1. List a few examples of the types of NGS tests

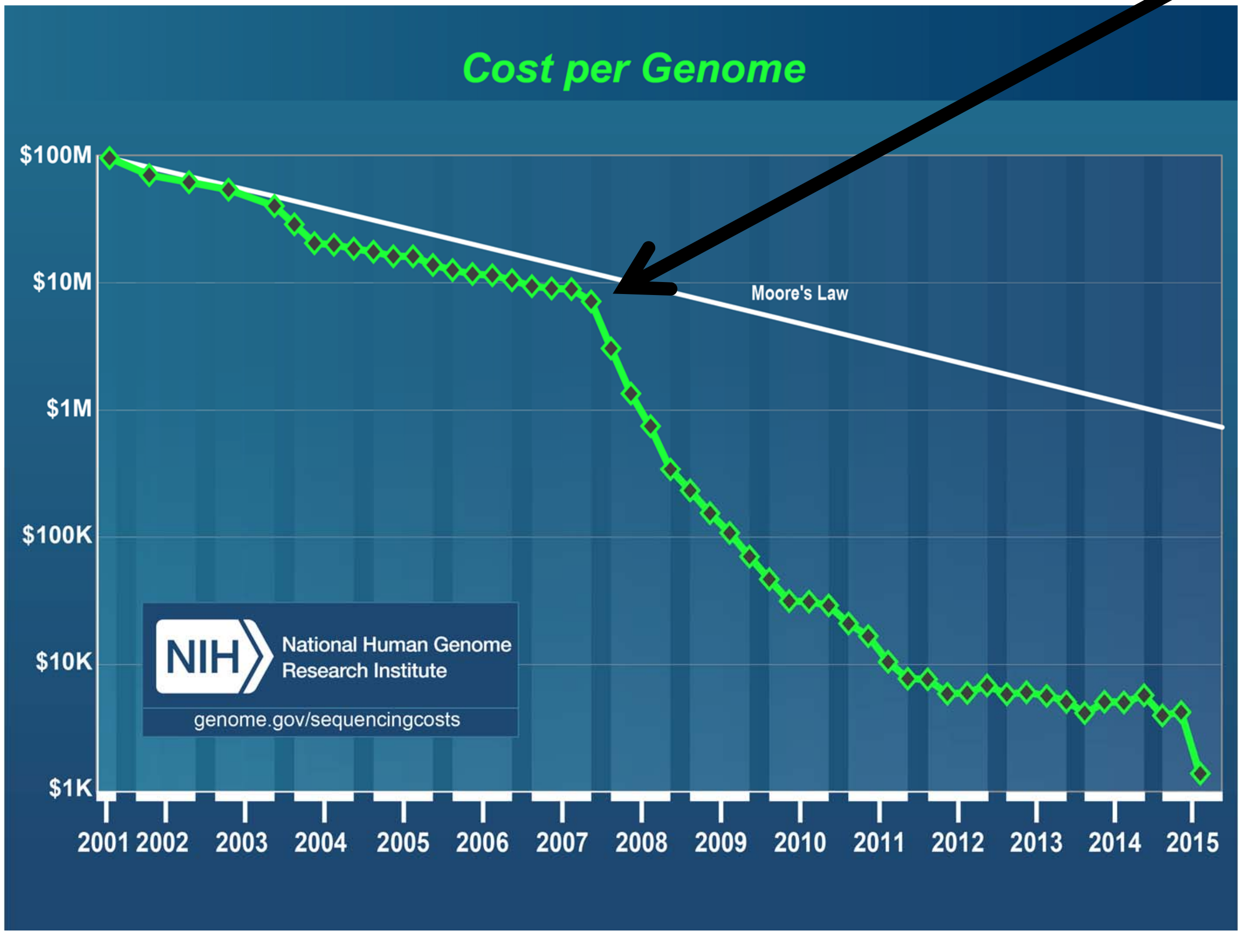
2. Describe the clinical utility of NGS technology in the context of testing of hematologic neoplasms

Outline

- NGS background
- Overview of types of clinical NGS tests
- NGS panels
- Single gene tests
  - Lymphoid clonality testing by NGS
  - *BCR-ABL1* kinase domain sequencing
- Conclusions

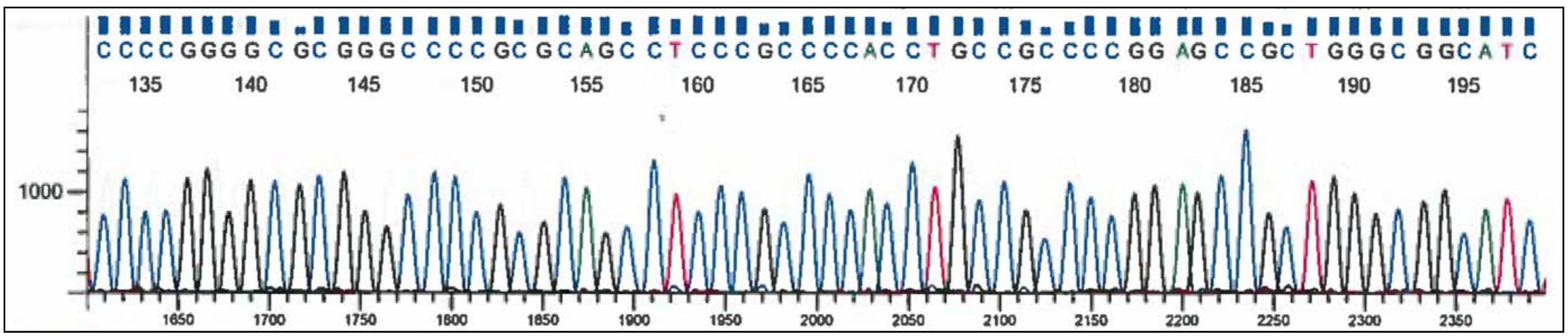
Next Generation Sequencing (NGS)

Impact of NGS



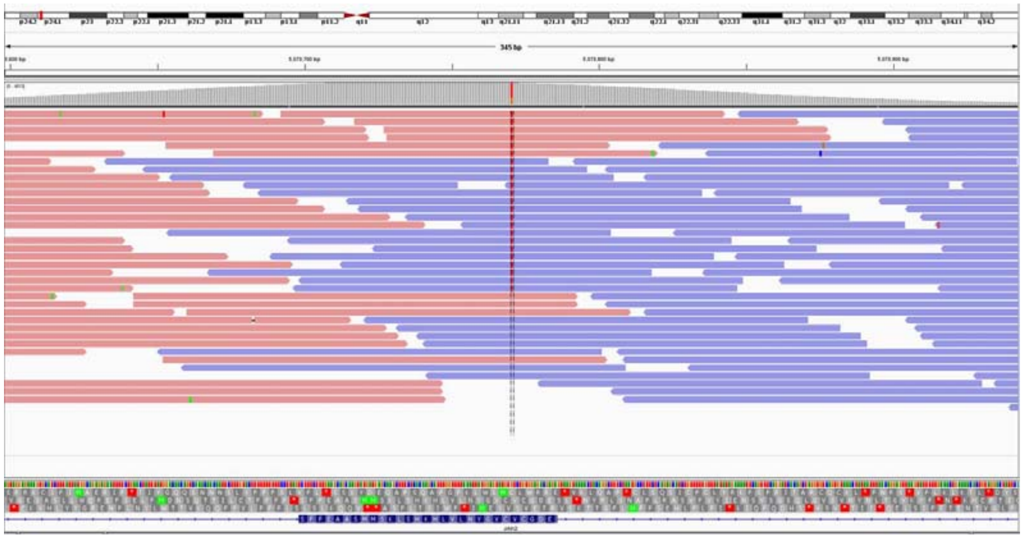
1st generation sequencing - Sanger sequencing

- utilizes chain terminating dideoxynucleotides
- slow and laborious, method has been relatively unchanged for ~30 years
- data = mixture of sequences
- sequence data can be reviewed manually
- poor sensitivity for detection of variants (~15-20%)
- relatively long contiguous sequence can be generated (>600bp)



NGS - also known as massively parallel sequencing

- parallel single molecule sequencing
- millions of small fragments of DNA are immobilized on a solid surface, amplified (copied), and sequenced simultaneously
- during sequencing a signal (light, pH change) is detected when a base is incorporated
- short contiguous sequences (reads) are generated
- reads are aligned to a reference sequence and analyzed
- analysis is computationally intense



Comparison of NGS applications

NGS Application	Cost/Time	Sensitivity (depth of coverage)	Portion of genome sequenced (breadth of coverage)	Suitable for MRD detection?
Whole genome sequencing	++++	+	++++	No
Whole exome sequencing	+++	++	+++	No
Mutation panels	++	+++	++	No
Single gene tests	+	++++	+	Yes

The power of NGS

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 30, 2013 VOL. 368 NO. 22

Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network

-Study performed by the Cancer Genome Atlas Research Network

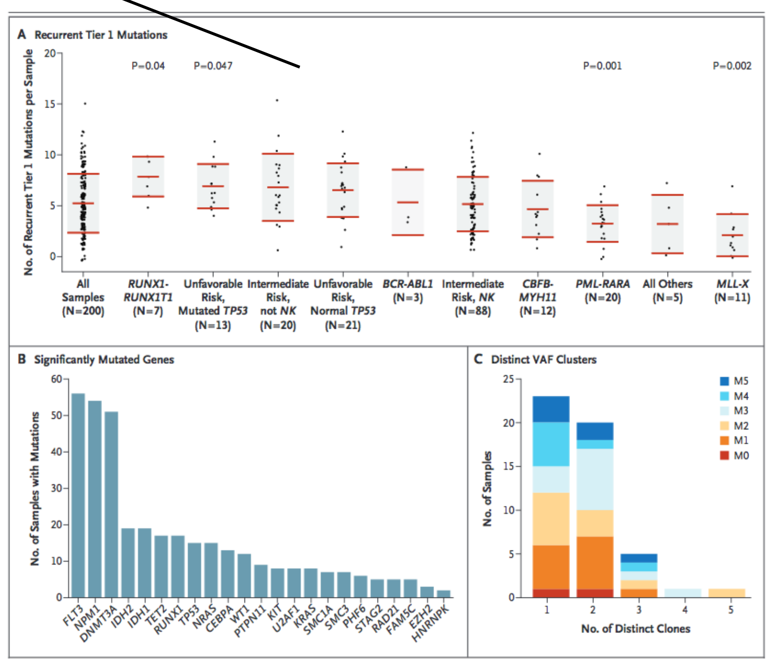
-200 cases of *de novo* adult AML subjected to whole genome (50) or whole exome (15) sequencing

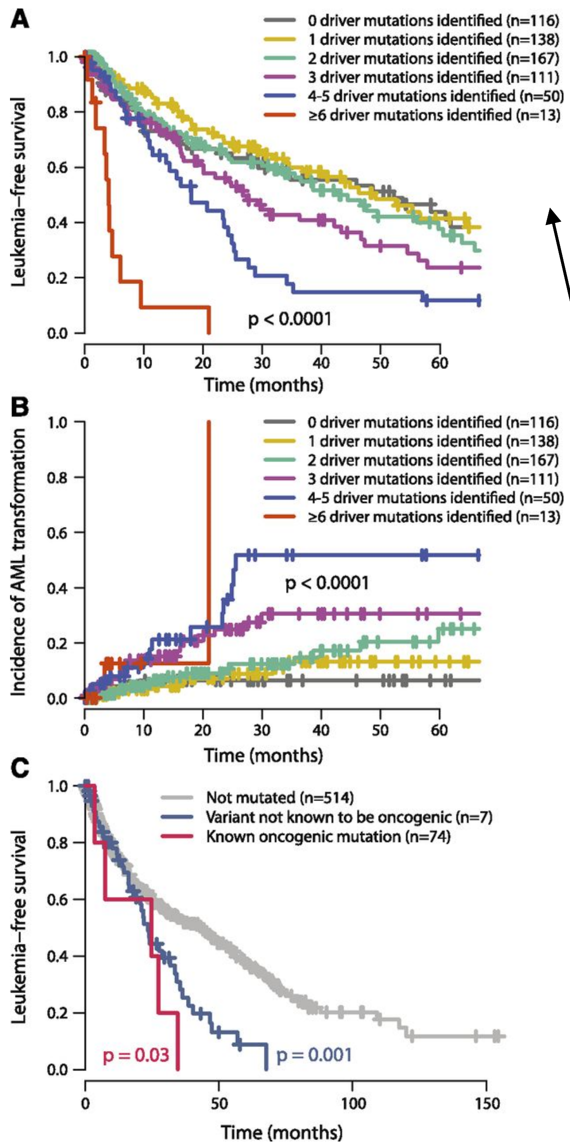
-Tier 1 – coding changes or splice sites

-average of 13 overall (all tiers) mutations per case

-23 genes significantly mutated (>5% of cases)

-majority of cases demonstrated more than 1 clone based on distinct clusters of variant allele frequencies (VAFs)





Clinical impact of somatic mutations

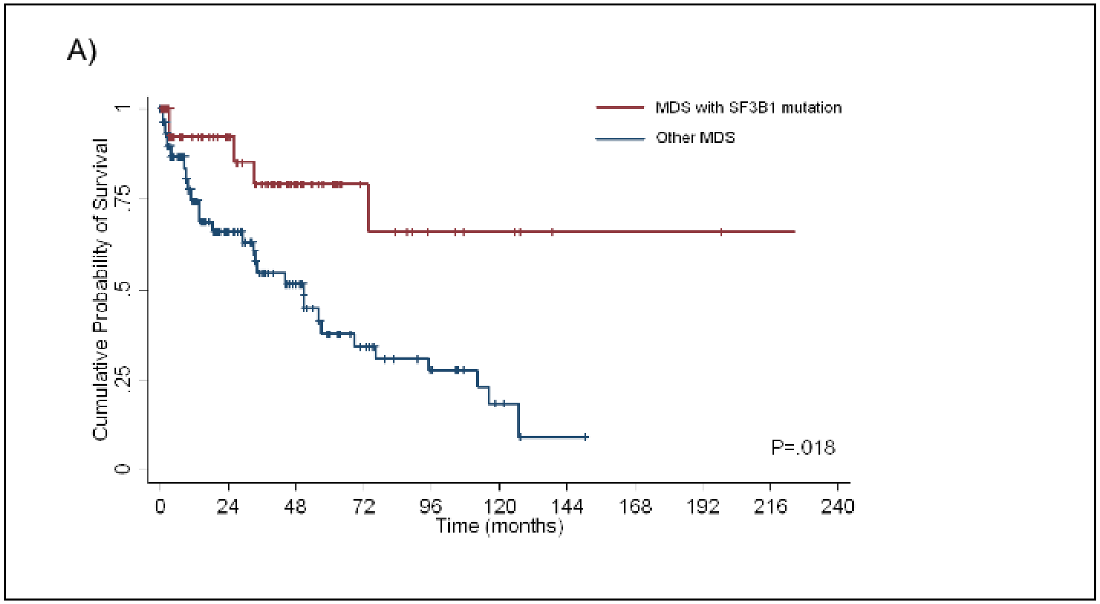
-738 patients with MDS, MDS-MPN
-111 cancer associated genes were sequenced by NGS (gene panel)
-78% of patients had 1 or more oncogenic mutations
-<u>No systematic differences between DNA derived from bone marrow or peripheral blood</u>

Higher overall number of oncogenic mutations correlated with worse outcome

Papaemmanuil E et al. Blood 2013;122:3616-3627

Clinically important information is derived from large scale genetic analysis by NGS: The example of MDS

- *SF3B1* mutations are associated with favorable outcome



Malcovati L et al. Blood 2014;124:1513-1521

308 pts w/ myeloid neoplasms
  MDS: 245
  MDS/MPN: 34
  AML-MDS: 29

111 gene mutation panel

*Almost all patients with RARS (refractory anemia with ring sideroblasts) had an *SF3B1* mutation

Clinical applications of NGS in hematology

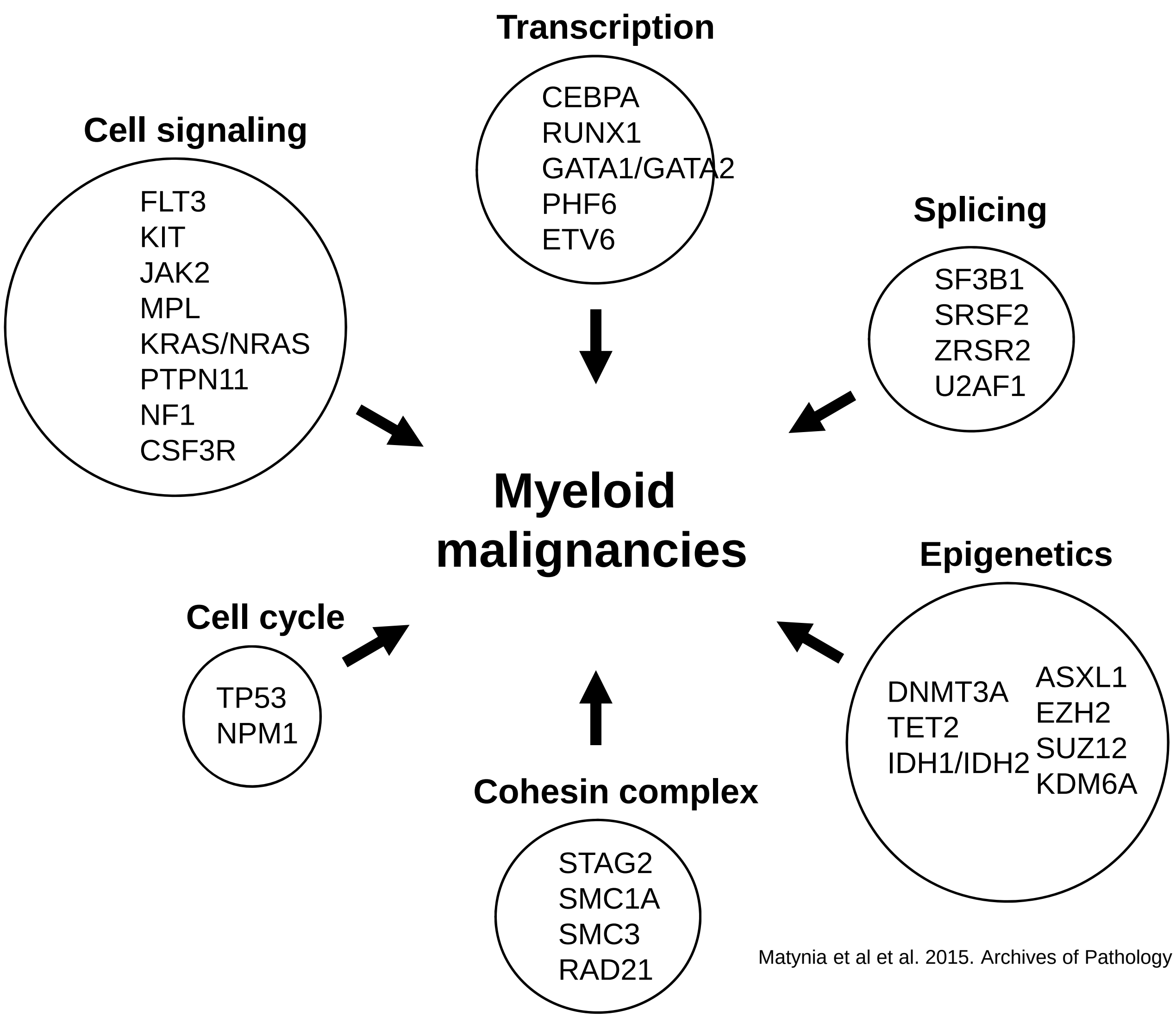
- Clinical applications:
  - Whole genome sequencing (entire genome - ~3B base pairs)
  - Whole exome sequencing (~30M base pairs)
    - Sequencing limited to protein coding regions representing ~1% of genome
  - Mutation panels
    - Myeloid
      - AML prognostic markers – *FLT3, NPM1, CEBPA, ASXL1, IDH1/2*
      - Myelodysplastic syndromes (MDS) – cohesin and spliceosome genes frequently mutated
      - Myeloproliferative neoplasms (MPNs) – *JAK2, CALR, MPL, ASXL1*
      - Pan myeloid panels
    - Lymphoblastic leukemia and mature lymphoid neoplasms
      - Ph-like lymphoblastic leukemia
      - Diffuse large B cell lymphoma (BCR pathway mutations)
      - Mutations associated with T cell lymphoproliferative disorders (JAK-STAT pathway mutations)
      - Pan lymphoid panels
    - Congenital disorders – bone marrow failure syndromes, congenital hemolytic anemias
  - Detection of complex genomic abnormalities - copy number variants (CNVs) and translocations
  - Analysis of single genes with high complexity
    - Ex. lymphoid clonality and *IGH* or *TRG*/*TRB* genes

Whole genome sequencing

- Many of the biomarkers we now know to be important were discovered in whole genome sequencing studies (ie. *DNMT3A*, *IDH1/2*, etc)
- Not routinely performed in the clinical lab
  - Would need paired normal tissue for tumors
  - Time consuming
  - Expensive
  - Yields relatively low coverage (~30X) so results may be difficult to interpret, especially with low tumor burden
- Benefit: Not limited to selected targets

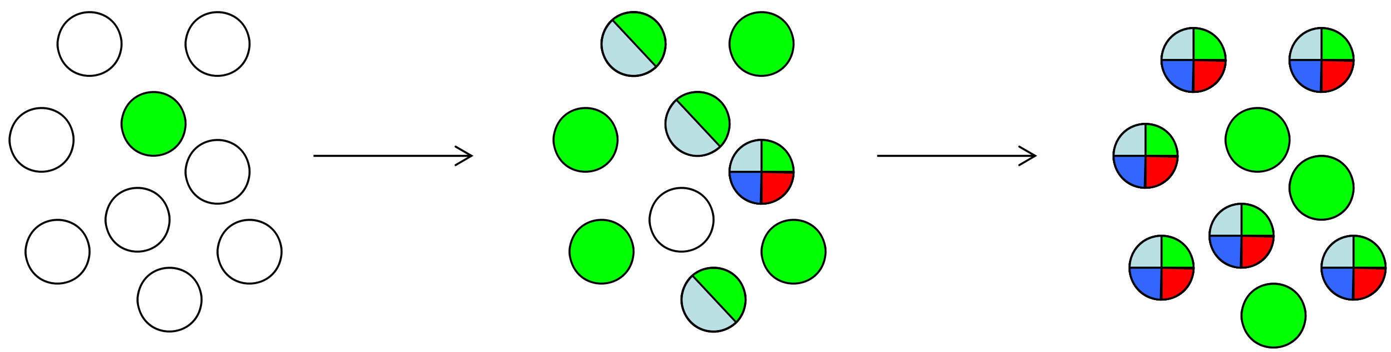
Spectrum of mutations in myeloid malignancies

AML, MDS, MPN and MDS/MPN overlap disorders



Matynia et al et al. 2015. Archives of Pathology and Laboratory Medicine.

There is often a complex subclonal architecture in myeloid malignancies



Pre diagnosis | Diagnosis | Relapse

Ex. clonal hematopoiesis of uncertain significance (CHIP)

Matynia et al et al. 2015. Archives of Pathology and Laboratory Medicine.

Variant Associations

Gene	MPN	MDS	MDS/MPN	De novo AML	Secondary AML	Effect *
JAK2	++	-	+	-	-	Gain
MPL	+	-	-	-	-	Gain
CALR	++	-	+	-	-	Gain
FLT3	-	-	-	++	-	Gain
NPM1	-	-	+	++	-	Gain
CEBPA	-	-	-	+	-	Loss
RUNX1	-	+	++	+	-	Loss
KIT	+	-	-	+	-	Gain
CSF3R	+	-	+	-	-	Gain
DNMT3A	+	+	+	++	-	Loss
TET2	+	++	++	++	+	Loss
IDH1/2	+	+	+	++	+	Gain
SF3B1	-	+	+	-	+	Unknown
SRSF2	-	+	++	+	++	Unknown
STAG2	-	+	-	-	++	Loss
ASXL1	++	++	++	+	++	Unknown
EZH2	+	+	+	-	++	Loss
TP53	+	+	+	+	+	Loss

From: Tietz textbook of Clinical Chemistry and Molecular Diagnostics, 6th Edition

Mutation panels: Variant reporting

- ***Tiered strategy***
  - ***A variety of systems are in use and this area currently lacks a uniform standard***

Higher tiers – more likely to be pathogenic or actionable

NRAS c.37G>C, p.Gly13Arg

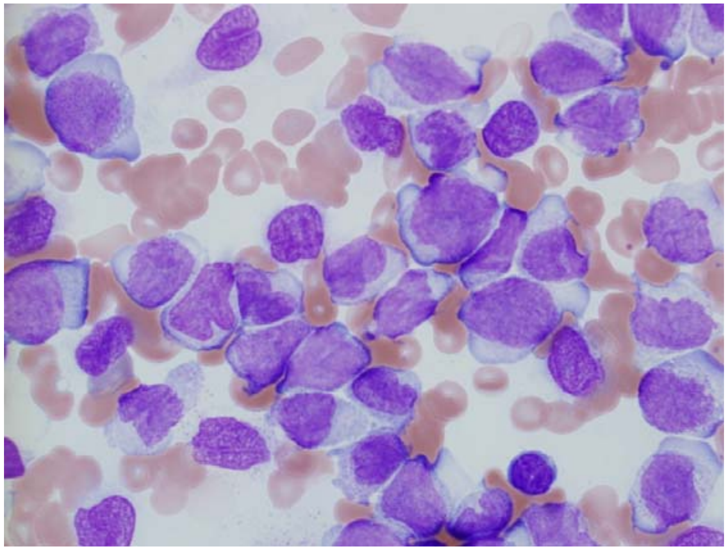
Variants of unknown significance (VUSs)

TET2 c.5284A>G, p.Ile1762Val

Lower tiers – less likely to be pathogenic or likely or known germline polymorphism

Clinical Scenario #1

- 52 year-old female presented with easy bruising and fatigue
  - CBC: WBC – 33 K/uL, Hgb – 9.6 g/dL, Platelets – 12,000 K/uL
  - Flow cytometry on BM aspirate: large CD34 negative atypical myeloid blast population (48% of leukocytes)
  - BM morphology – Acute myeloid leukemia
  - Cytogenetics/FISH – normal karyotype



Clinical scenario #1 -mutations

Mutation panel testing by NGS:

Tier 1 variants:

1. NPM1 c.860_863dup, p.Trp288fs

-Variant frequency 35.5%

-Associated with good prognosis except when a FLT3-internal tandem duplication mutation is present.

2. FLT3 c.1802_1803ins45, p.Leu601_Lys602ins15

-Variant frequency 30.0%

-Associated with early relapse and poor overall survival.

3. DNMT3A c. 2645G>A, p.Arg882His

-Variant frequency 41.2%

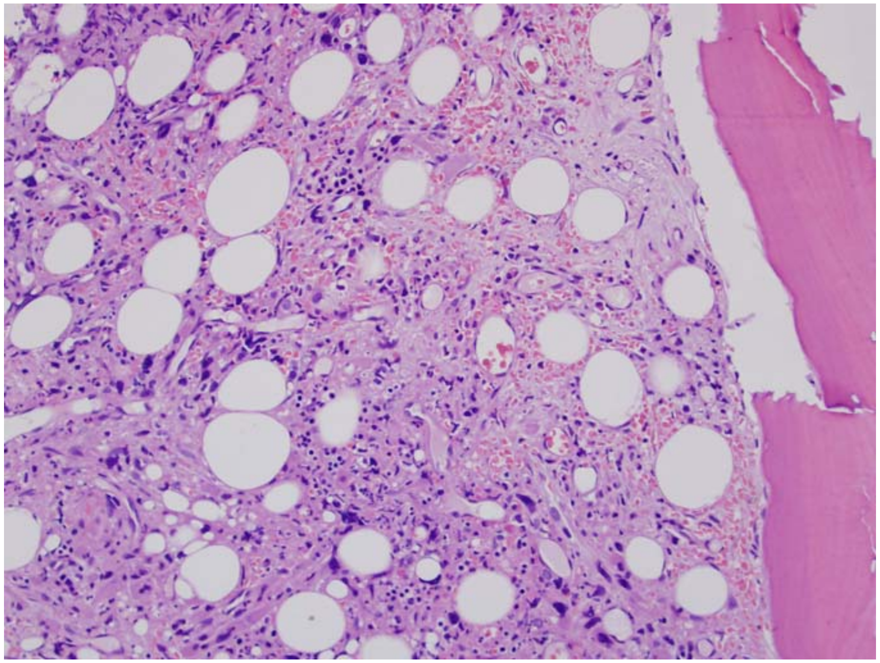
-Commonly seen with NPM1 mutations in patients with CN-AML

-DNMT3A R882 mutations are associated with poor outcome when compared to NPM1 mutated AML patients without DNMT3A mutations

Conclusion – Poor prognosis; patient should proceed to BM transplant

Clinical scenario #2

- 75 y/o male with complaint of fatigue and history of primary myelofibrosis
- CBC:
  - WBC: 40.05 k/uL
  - Hgb: 14.9 g/dL
  - MCV: 76.5 fL
  - Plts: 205 k/uL
- Cytogenetics: 46, XY, inv(12)



Clinical scenario #2 - Mutations

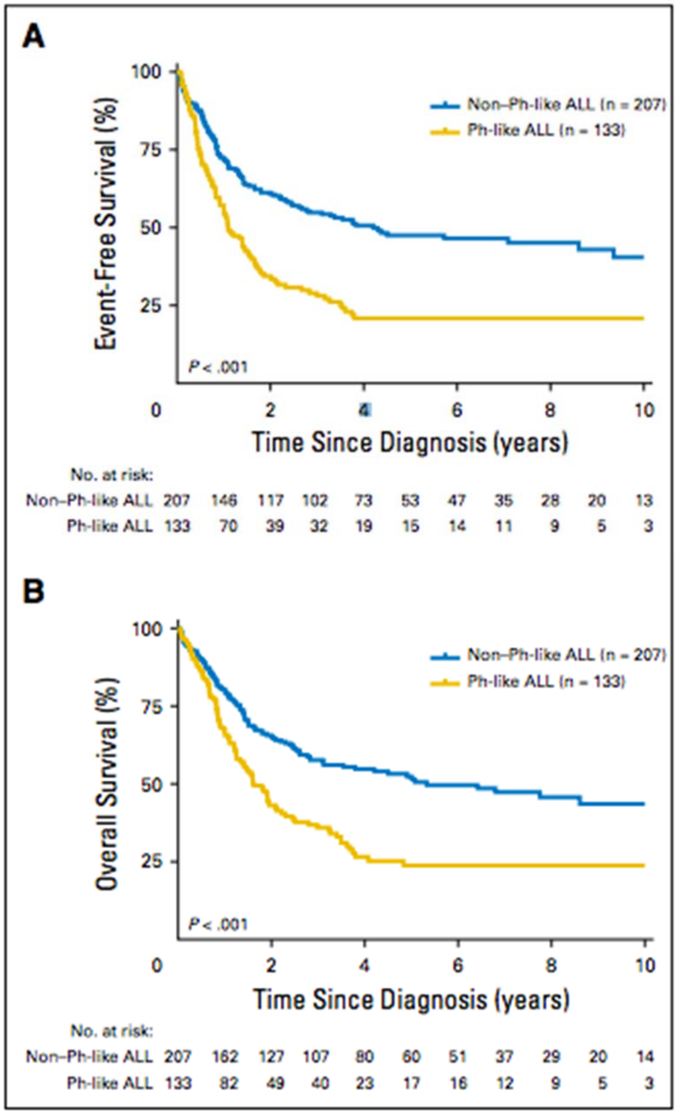
1. *JAK2* c.1849G>T, p.Val617Phe
   – Variant frequency: 92.4% ← Dominant clone, VAF implies LOH @ 9p
2. *NRAS* c.37G>C, p.Gly13Arg
   – Variant frequency: 16.5% ←
3. *NRAS* c.183A>C, p.Gln61His
   – Variant frequency: 8.6% ←
4. *ASXL1* c.2275_2284del, p.Gln760fs
   – Variant frequency: 8.3% ←

Subclone(s) implied by VAFs

Variant frequencies illustrate complex underlying clonal architecture

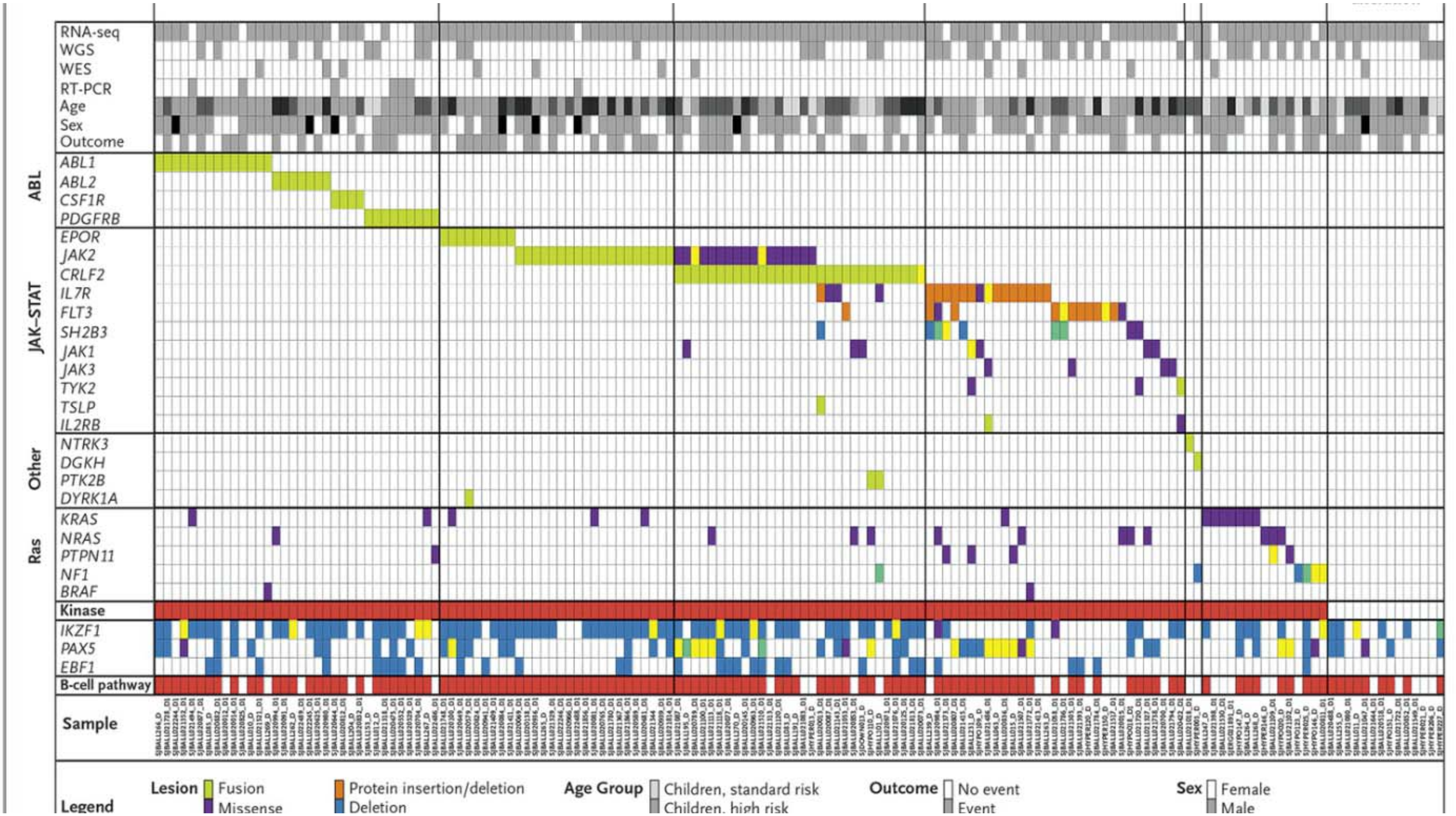
Clinical scenario #3

- Ph-like lymphoblastic leukemia
  - Gene expression profile similar to Ph+ (BCR-ABL1+) lymphoblastic leukemia but do not have t(9;22);*BCR-ABL1*.
  - Affects children (10% with standard risk ALL) and adults (~20%).
  - Variety of molecular abnormalities that activate tyrosine kinase signaling pathways including rearrangements (CRLF2, ABL1, ABL2, etc) as well as mutations involving FLT3, IL7R, SH2B3, etc)
  - Worse outcome compared to non-Ph-like ALL
  - Benefit from tyrosine kinase inhibitor therapy
  - A properly designed NGS panel can assess for all of the potential molecular genetic abnormalities using a single test



Roberts KG et al 2016 J Clin Oncol

Ph-like lymphoblastic leukemia



Roberts et al, N Engl J Med 2014;371:1005-15

Targetable kinase activating abnormalities in Ph-like ALL

Table 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

Kinase Gene	Tyrosine Kinase Inhibitor	Fusion Partners	Patients	5′ Genes
		number		
ABL1	Dasatinib	6	14	*ETV6,*[11] *NUP214,*[11] *RCSD1,*[11] *RANBP2,*[11] *SNX2,*[19] *ZMIZ1*[20]
ABL2	Dasatinib	3	7	*PAG1,* RCSD1,* ZC3HAV1**
CSF1R	Dasatinib	1	4	*SSBP2**
PDGFRB	Dasatinib	4	11	*EBF1,*[11-13] *SSBP2,* TNIP1,* ZEB2**
CRLF2	JAK2 inhibitor	2	30	*IGH,*[21] *P2RY8*[22]
JAK2	JAK2 inhibitor	10	19	*ATF7IP,* BCR,*[11] *EBF1,* ETV6,*[23] *PAX5,*[11] *PPFIBP1,* SSBP2,*[24] *STRN3,*[11] *TERF2,* TPR**
EPOR	JAK2 inhibitor	2	9	*IGH,*[11] *IGK**
DGKH	Unknown	1	1	*ZFAND3**
IL2RB	JAK1 inhibitor, JAK3 inhibitor, or both	1	1	*MYH9**
NTRK3	Crizotinib	1	1	*ETV6*[25-27]†
PTK2B	FAK inhibitor	2	1	*KDM6A,* STAG2**
TSLP	JAK2 inhibitor	1	1	*IQGAP2**
TYK2	TYK2 inhibitor	1	1	*MYB**

* The gene is a previously unreported fusion partner.

† *ETV6–NTRK3* has been reported in multiple cancers, including congenital fibrosarcoma[25,26] and secretory breast carcinoma,[27] but it has not previously been described in acute lymphoblastic leukemia.[28,29]

Roberts KG et al. 2014. N Engl J Med 371;11

Clinical scenario #3

- 29 year old male with relapsed B-lymphoblastic leukemia
  - Initial diagnosis 2012
    - Negative for t(9;22);*BCR-ABL1*
  - Tested at relapse using a single NGS panel (Foundation Medicine)
    - *IGH-CRLF2* rearrangement
    - *IKZF1* deletion
    - *PAX5* missense mutation
  - Findings indicate “Ph-like” ALL
  - Awaiting transplant, may benefit from kinase inhibitor therapy

Panel-based NGS testing

Mutation panel testing by NGS

Pros

1. Variants are reported together, at the same time, on a single report
2. Interpretation takes into account all variants identified
3. Cost is less compared to multiple single gene tests
4. Variant frequencies provide information on subclonal structure
5. Pattern and identity of mutations facilitates accurate subclassification and prognostication
6. Detection of certain variants allows for the use of targeted therapies

Cons

1. May not be reimbursed by payers
2. Variants of unknown significance – what to do?
3. Some of the information is not currently actionable

Conclusions

- NGS is revolutionizing pathology and laboratory medicine
- Allows for <u>true personalized medicine</u>
- Facilitates use of targeted therapeutic strategies
- Costs are rapidly decreasing while the technology continues to improve
- Challenges remain
  - Cost and reimbursement
  - Data analysis
  - Variant interpretation
  - Other aspects of testing (ie. PCR) can affect the results!
- Today – panels and genetically complex single gene analysis; detection of targeted structural variants
- Future – routine comprehensive whole genome analysis of tumors