Molecular Pathology as applies to Complex Malignant Hematology and Transition from Residency

Philip Berardi MD, PhD, FRCPC

Virtual Recording November 3, 2020

1PM – 2PM

Dr. Philip Berardi

I have the following financial relationships to disclose.

Consultant for:

Astellas Pharma (Advisory Board 2020)
Novartis Pharma Canada Inc. (Advisory Board 2020)
Janssen Pharmaceuticals (Advisory Board 2019)
Diaceutics (Consultant 2019)
Celgene (Advisory Board 2018)
Hoffmann-La ROCHE (Advisory Board 2018)

AND

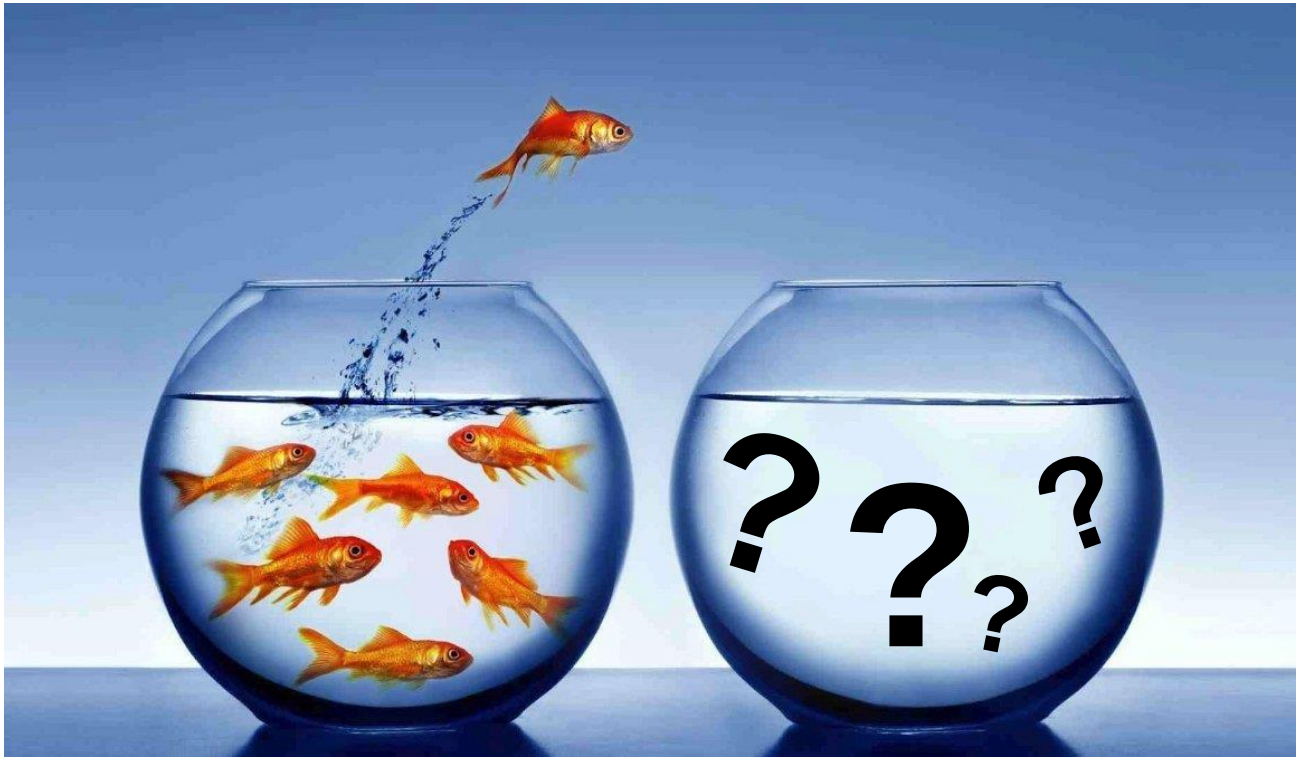
I will not discuss off label use and/or investigational use in my presentation.

Objectives

At the end of this session, participants will be able to:

1. Explain the principles of molecular diagnostic testing as applies to complex malignant hematology and recommend specimen requirements needed for accurate results.
2. Differentiate between clinically actionable mutations and predictive markers and describe how these can influence clinical decision-making in routine practice.
3. Discuss myeloid neoplasms arising in the setting of germline predisposition and adopting a strategy to managing these complex cases.
4. Outline a general approach to laboratory management and quality assurance as it pertains to molecular diagnostics.

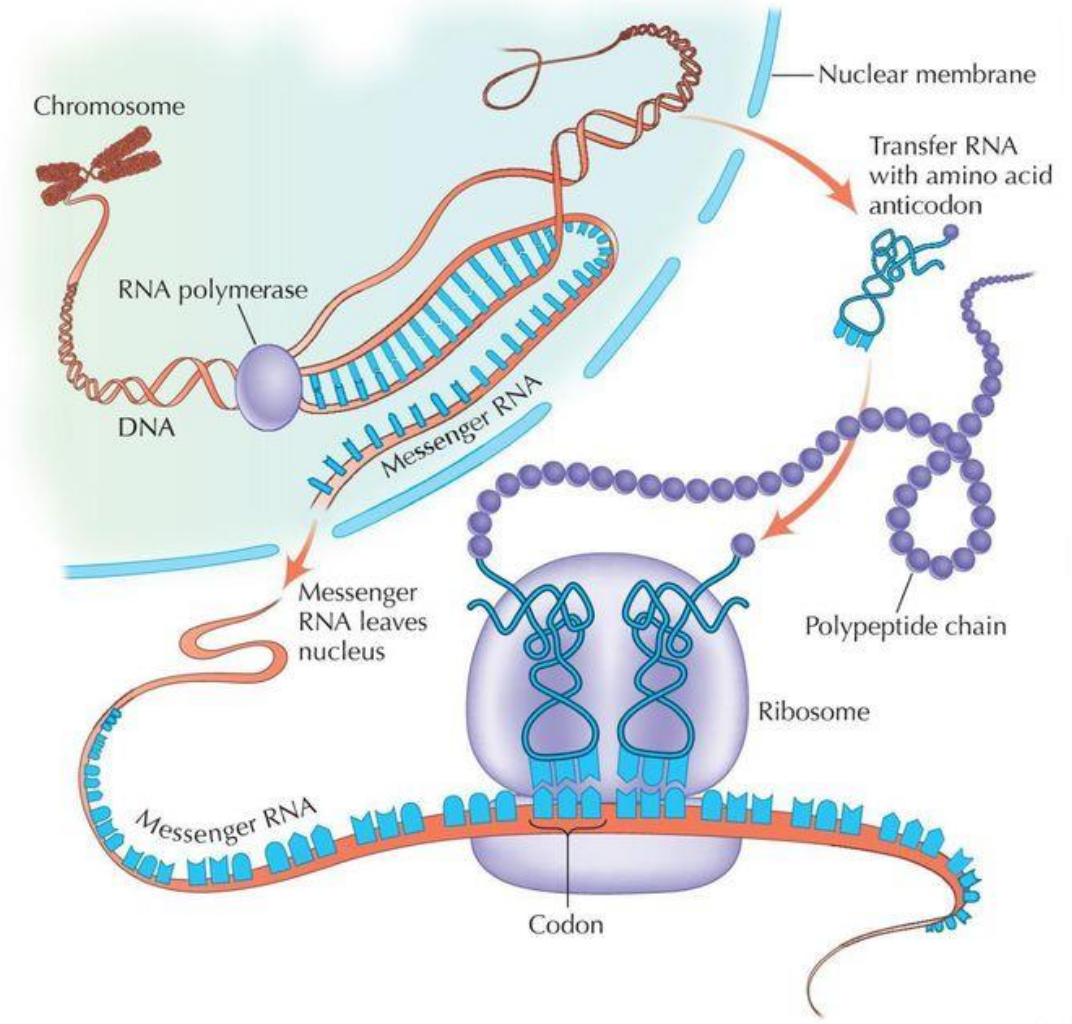
Transition to Practice



Residency

Independent Practice

DNA Synthesis & Transcription



DNA

↓

RNA

↓

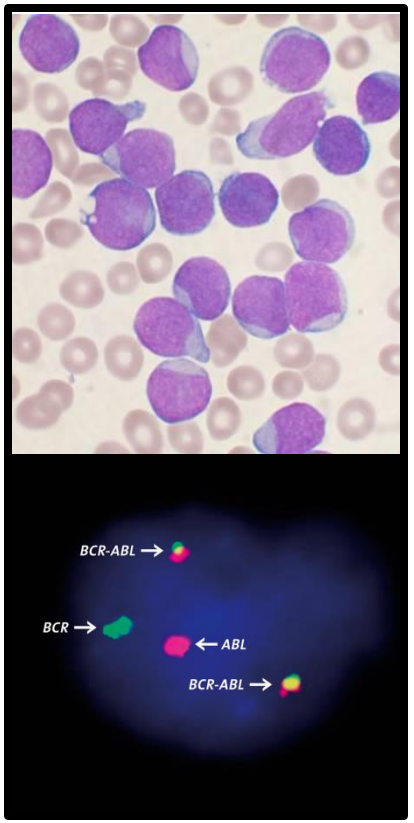
PROTEIN

23 chromosome pairs

~3 billion base pairs
~30,000 genes
~3 proteins/gene

Case 1: Ph+ ALL

- 28-year-old woman presented with **pre–B ALL (Ph+)** with more than 50% bone marrow blasts and a negative CSF examination at ToD.
- Treated with chemo/imatinib, including intrathecal prophylaxis [MTX] and subsequent allo-SCT.
- Now presents with ‘CNS’ symptoms and possible relapse.
- Clinical team requesting PCR for BCR-Abl1 on CSF to rule out CNS relapse.



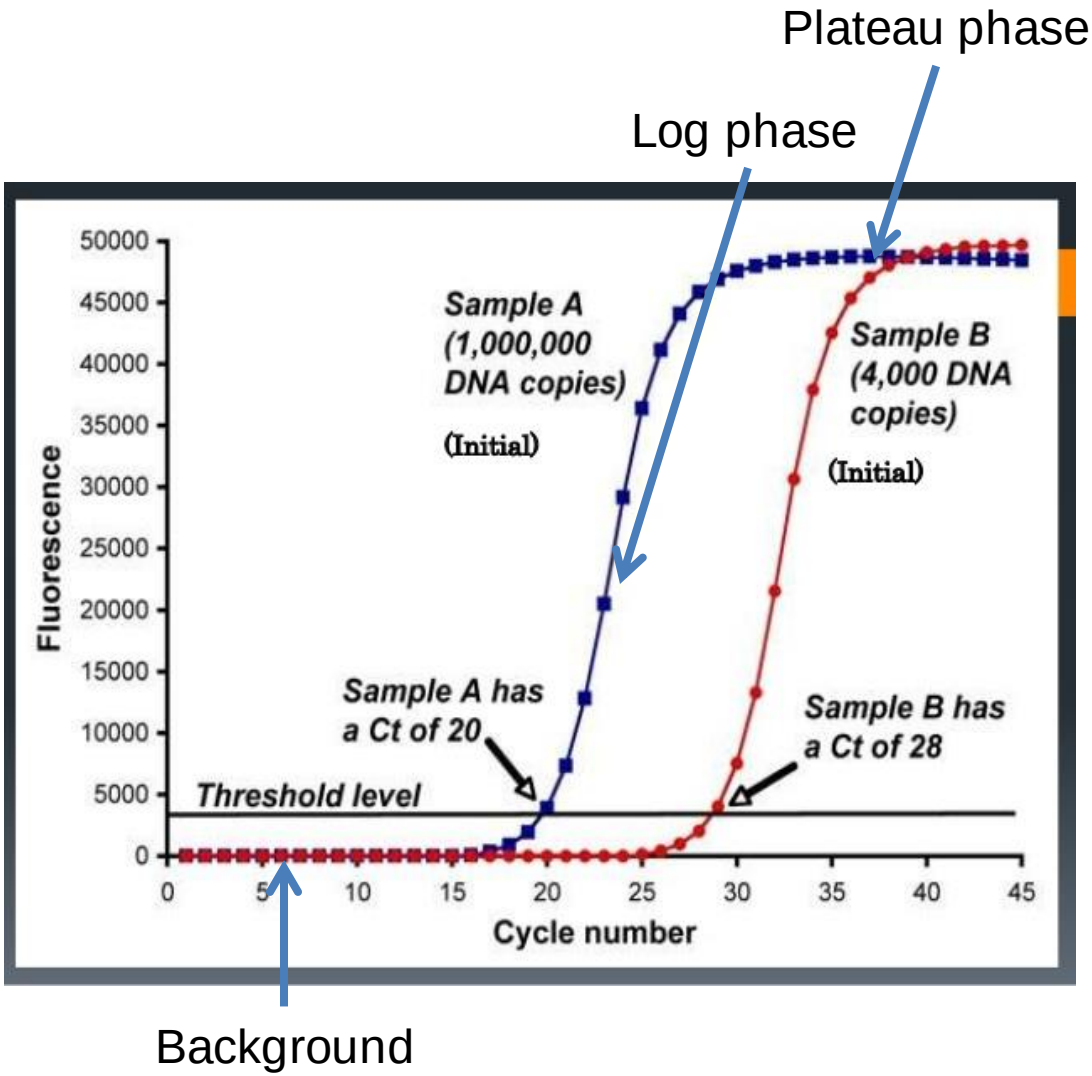
Case 1 Discussion

- Is this an '*acceptable*' approach to assess for CNS relapse?
- What are some considerations before accepting the sample, reporting the results, etc.
- If there is adequate amplification of your housekeeping target, can you assume the assay performed as expected?

Case 1 Discussion

- Detection of BCR-ABL1 fusion gene in CSF by PCR in relapsed Ph+ALL is *possible* when rigorously **validated**.
- The acceptable specimen type should be clearly indicated on the lab's SOP (i.e. **establish criteria for sample rejection**).
- Opportunity for laboratory physician to discuss the best available options for diagnostic tools with the clinical team.
- For example, use interphase FISH to assess for BCR-ABL1 fusion gene on cells in CSF instead of PCR.

Quantitative Detection of Gene Fusions



Childhood Ph-like ALL

Provisional Entity 2016 WHO

Table 1. Repertoire of kinase rearrangements in Ph-like ALL along with their partner genes and potential therapeutic targets

Kinases	5′ partner genes (number of patients)	Potential TKI	Clinical trials
ABL1	*ETV6* (3), *NUP214* (6), *RCSD1* (1), *RANBP2* (1), *SNX2* (1), *ZMIZ1* (2)	Dasatinib	AALL1131
ABL2	*PAG1* (1), *RCSD1* (4), *ZC3HAV1* (2)	Dasatinib	AALL1131
PDGFRB	*EBF1* (6), *SSBP2* (1), *TNIP1* (1), *ZEB2* (1)	Dasatinib	AALL1131
CSF1R	*SSBP2* (4)	Dasatinib	AALL1131
CRLF2	*IGH* (19), *P2RY8* (11)	Ruxolitinib	AALL1521
JAK2	*ATF7IP* (1), *BCR* (2), *EBF1* (1), *ETV6* (2), *PAX5* (7), *PPFIBP1* (1), *SSBP2* (2), *STRN3* (1), *TERF2* (1), *TPR* (1)	Ruxolitinib	AALL1521
EPOR	*IGH* (7), *IGK* (2)	Ruxolitinib	AALL1521
TSLP	*IQGAP2* (1)	Ruxolitinib	AALL1521
IL2RB	*MYH9* (1)	JAK1/JAK3 inhibitor	N/A
TYK2	*MYB* (1)	TYK2 inhibitor	N/A
NTRK3	*ETV6* (1)	Crizotinib	N/A
PTK2B	*KDM6A* (1), *STAG2* (1)	FAK inhibitor	N/A
DGKH	*ZFAND3* (1)	Unknown	N/A

Blood. 2017 Nov 9;130(19):2064-2072. doi: 10.1182/blood-2017-06-743252. Epub 2017 Oct 2.

Leukemogenesis: a two-hit theory

- In AL, *somatic* genetic changes are thought to contribute to leukemogenesis through a "two-hit" process.

1. **CLASS I mutation:** hematopoietic cell **proliferation**
   (i.e. FLT3-ITD, FLT3-TKD, c-KIT, etc.)
2. **CLASS II mutation:** Hematopoietic cell **maturation**
   (i.e. PML-RARA, RUNX1-RUNX1T1, etc.)

- Some of these regulatory pathways may be affected in other myeloid neoplasms including **MDS**, **MPN** and **MDS/MPN** and are often involved in transformation.

- Targeted therapies approved by Health Canada and/or the FDA are becoming more widely available in Hematology and require testing for some of these important molecular biomarkers.

BCR-ABL1 → Imatinib, nilotinib, ponatinib, dasatinib, bosutinib
FLT3 Type I Inhibitors → Sunitinib, midostaurin, lestaurtinib, crenolanib, gilteritinib
FLT3 Type II Inhibitors (ITD) → Sorafenib, ponatinib, quizartinib
IDH1 Inhibitors → Ivosidenib
IDH2 Inhibitors → Enasidenib

Mutations in Routine HP Practice

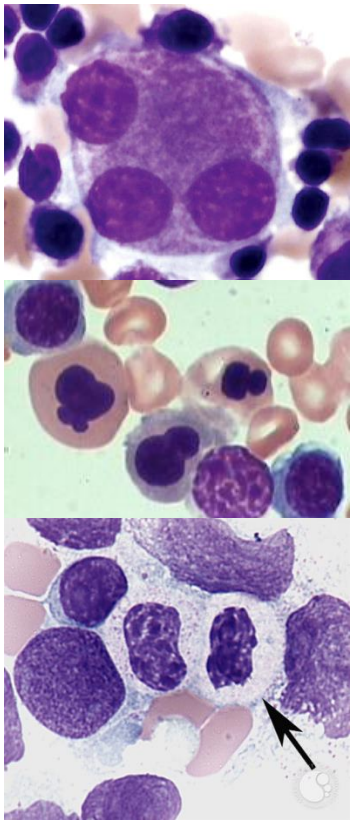
- *Risk assessment*
- **Diagnostic**
- *Prognostic*
- **Therapy selection**
- **Monitoring**

- Silent
- Missense (LOF, GOF)
- Nonsense
- Frameshift
- Splice site

Translocations/Gene Rearrangements

Case 2: High Risk MDS

- 58-year-old woman with ***pancytopenia***.
- Previously healthy with no reported exposure to alkylating agent chemo or radiation therapy.
- Hgb 80 g/L, Plt 60 x 10^9/L, ANC 0.6.
- Hypercellular bone marrow with prominent dysplasia and 6% blasts (no Auer rods).
- Cytogenetic karyotyping shows isolated del (7q).
- **IPSS-R → 6 points (High Risk)**

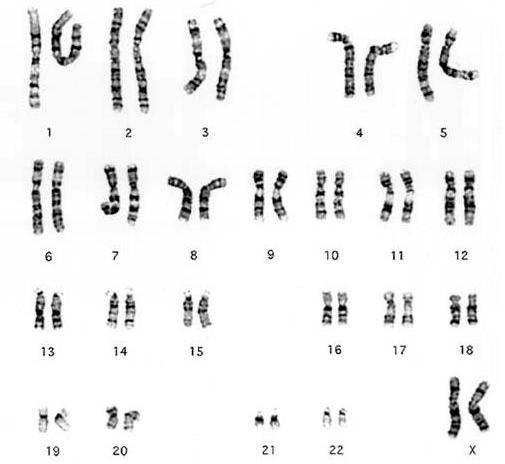


Case 2 Discussion

- Owing to good performance status, high risk IPSS-R and matched sibling donor, an allo-BMT was performed.
- Initially responded well but within 6 months had progressive cytopenias.
- Repeat BMB was performed and showed mild dyspoietic change but no diagnostic evidence of MDS (blasts 2-3%)
- Repeat cytogenetic karyotyping was normal.

Case 2 Discussion

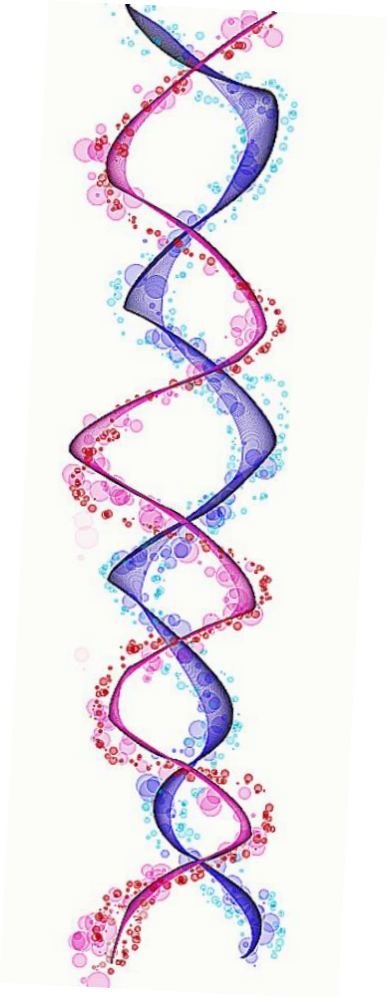
- Are these findings consistent with disease remission?
- The repeat cytogenetic karyotyping shows a normal karyotype, the clinical team wants to know the significance of this finding.
- How many metaphases are typically examined for routine G-band cytogenetic karyotyping?
- Is this approach intended for identifying refractory disease/relapse?



Normal Karyotype

Actionable Mutations and Predictive Markers

- **Somatic** and/or **germline** DNA mutations are found in *all* cancers.
- Somatic mutations are **acquired** (sporadic) and germline mutations are **inherited** (familial).
- Mutations are referred to as "**actionable**" based on evidence from clinical trials that the presence or absence of mutations can be used to inform clinical management.
- A **prognostic marker** identifies outcome in patients regardless of treatment whereas a **predictive marker** is one that predicts whether the "next" therapy will work.



Common "**Actionable**" Mutations in AML

Recurrent translocations

- t(15;17)
- t(8;21)
- t(16;16) or Inv(16)

Somatic mutations

- NPM1
- FLT3
- CEBPA (somatic/GL)
- IDH1, IDH2
- DNMT3A
- KIT/JAK2
- RUNX1 (somatic/GL)

Table 1: Categories of Actionable Mutations

Actionable Categories	Mutated Genes	Clinical Significance
Diagnostic	"MDS" genes: *TET2, DNMT3A, TP53, SF3B1, SRSF2, U2AF1, ZRSR2, ASXL1, RUNX1, EZH2, NRAS,* etc.	Markers of clonal hematopoiesis: to distinguish MDS from other benign causes of cytopenias
	CSF3R	Diagnostic marker for chronic neutrophilic leukemia (and more rarely atypical CML)
	MYD88 L265P	Diagnostic marker for lymphoplasmacytic lymphoma/ Waldenstrom macroglobulinemia
	BRAF V600E	Diagnostic marker for hairy cell leukemia
	JAK2 (V617F or exon 12), *CALR* (exon 9), *MPL*	Distinguish clonal myeloproliferative neoplasm from benign mimics
Prognostic	*NPM1, CEBPA*	Favorable risk in AML (without a *FLT3* mutation)
	RUNX1	Independently associated with a poor prognosis in MDS and AML
	TP53	Independently associated with a poor prognosis in MDS and AML
	DNMT3A	Adverse effect on outcome in cytogenetically normal AML
	ASXL1	Independently associated with a poor prognosis in MDS and CMML
	KIT	Poor prognosis in CBF AML
	U2AF1	Associated with a poor prognosis in MDS
	ZRSR2	Associated with a poor prognosis in MDS
Therapeutic	*FLT3*	Tyrosine kinase inhibitors in phase I/II clinical trials for AML (midostaurin, sorafenib, gilteritinib, crenolanib, quizartinib, etc.)
	IDH1/IDH2	IDH1 and IDH2 anti-metabolite inhibitors in clinical trials for AML (AG-120, AG-221)
	JAK2, MPL, CALR	Ruxolitinib
	BRAF	Vemurafinib
	KIT	Tyrosine kinase inhibitors in clinical trials for AML and systemic mastocytosis (midostaurin)
Minimal Residual Disease	Any driver mutations identified in the diagnostic specimen	Mutation persistence after therapy predicts future relapse[16,17]

Abbreviations: AML, acute myeloid leukemia; CBF, core binding factor; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome.

Yang F, Press R. Next-Generation Sequencing Multi-Gene Mutation Panels in Myeloid Malignancies (Mini-Review). American Society of Hematology: The Hematologist. April 2016, Volume 13, Issue 3.

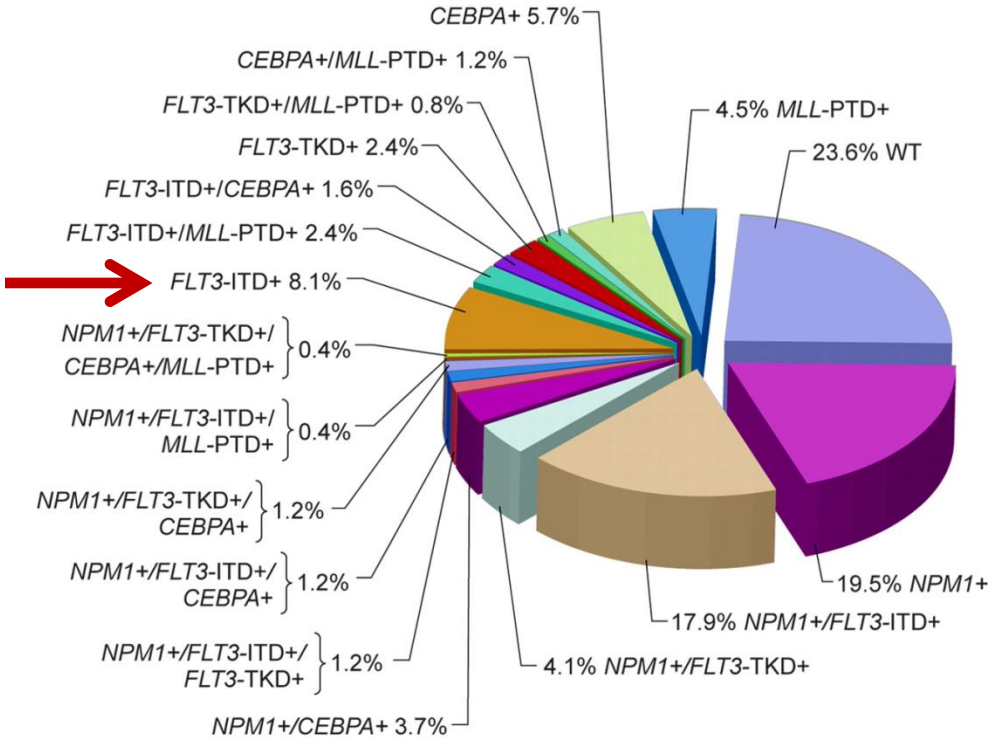
Detecting Gene Mutations

- Multiple methods for detecting gene mutations, most often using genomic DNA or RNA.

- **IHC** – mutated NPM1 cytoplasmic localization (WHO endorsed).
- **FISH** – various probe types (gene fusion or break-apart probes) .
- **NGS** – massive parallel sequencing.
- **Microarray/TMA** – detection of known point mutations.
- **Q-PCR** – single gene assays for rapid TAT.
- **Sanger sequencing** – cumbersome and suboptimal sensitivity but useful for orthogonal validation of results.

Case 3: FLT3 ITD Mutated AML

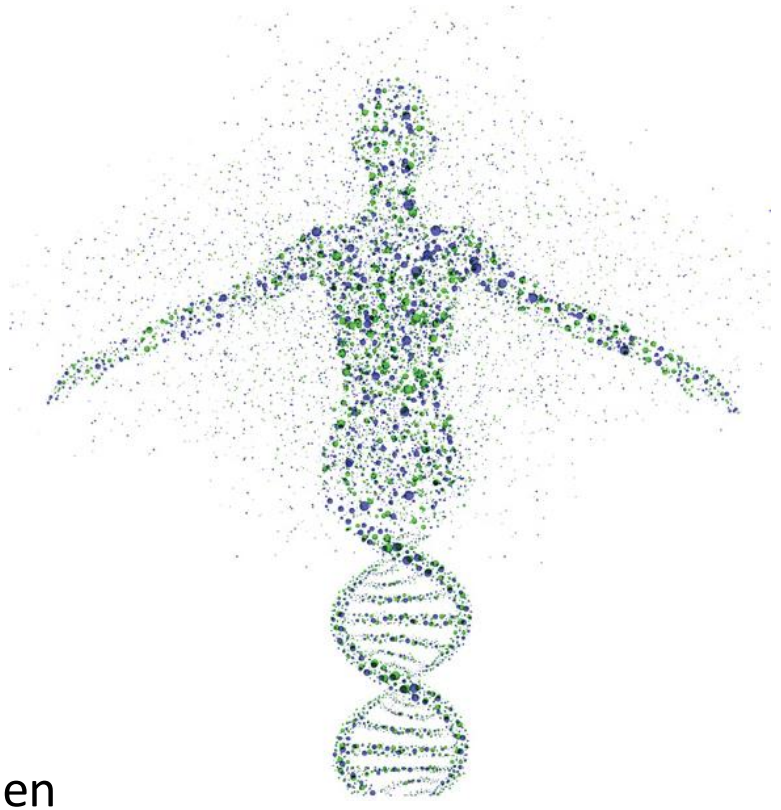
- 65-year-old man with new diagnosis of AML.
- Recurrent translocation testing is negative (no translocations).
- Traditional cytogenetic karyotyping is ‘normal.’
- Molecular testing shows wild type/normal NPM1 and FLT3-ITD mutation.



Mrózek K et al. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007 Jan 15;109(2):431-48.

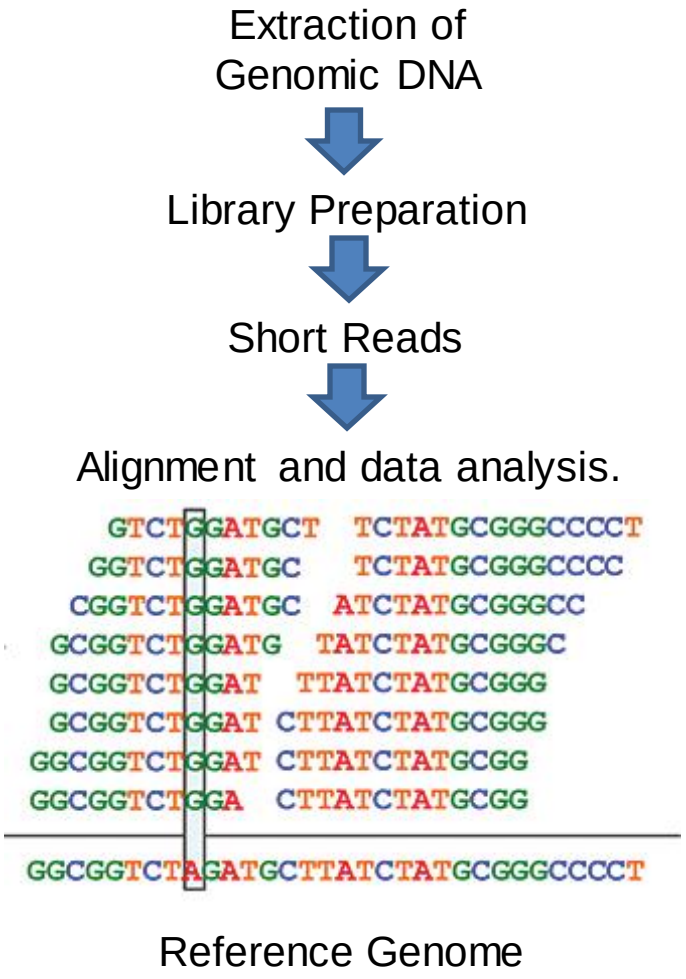
Case 3 Discussion

- What is the clinical significance of this finding?
- How can the FLT3-ITD insertion size affect the NGS variant calling?
- What steps can be taken to minimize the risk of inaccurate variant calling?
- Does the current evidence support reporting the **size of the FLT3-ITD** and **mutant allele ratio/burden** (>50%)?
- How might the insertion size be useful when setting up an analysis pipeline?



Case 3 Discussion

- Large insertions/deletions can be challenging to detect by NGS leading to **false negative** results.
- FLT3-ITD in the juxtamembrane domain can have 12-240 nucleotides (mean = 40-50 nt).
- Validation of NGS assays must encompass the complete end-to-end process, including the wet-laboratory steps & data analysis pipeline.



Molecular Monitoring in AML

Abnormal fusion genes: ~20-30 percent.

- t(8;21)/RUNX1-RUNX1T1
- Inv(16)/CBFβ-MYH11
- t(15;17)/PML-RARα
- t(7;11)/NUP98-HOXA9
- t(11;v)/MLL-partner gene

Mutations that are usually stable during disease progression.

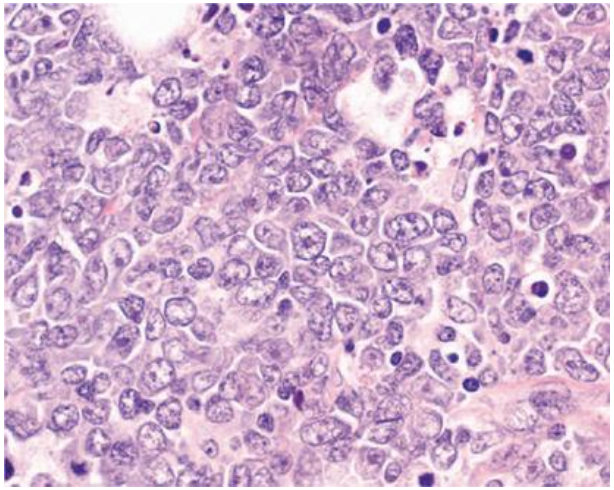
- NPM1
- IDH1/2
- DNMT3A
- CEBPA
- MLL-PTD

Gene overexpression.

- WT1

Case 4: Large B-cell Lymphoma

- 66-year-old man with new onset diffuse lymphadenopathy (> 5cm).
- Right axillary LN excisional biopsy shows diffuse infiltrate of intermediate to large cells.
- IHC shows abnormal cells are BCL2 positive and show 40 percent of cells express c-MYC.
- The case is suspected to be a HGBCL with possible double hit pathogenesis.



Positive	Negative
CD20	MUM1
CD10	TdT
BCL2	CD30
BCL6	Cyclin D1
Ki67: 60%	EBER
c-MYC: 40%	

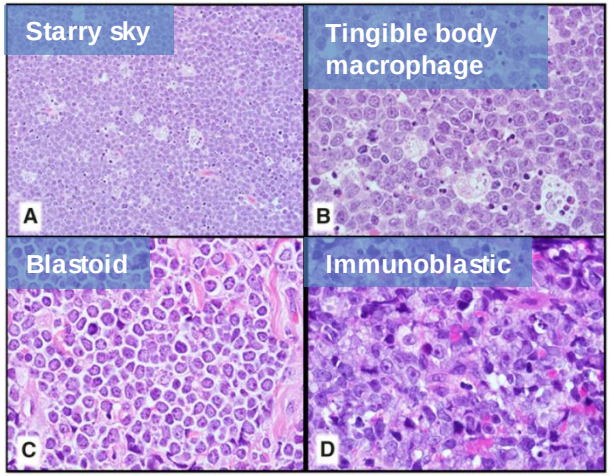
Case 4 Discussion

- What are some considerations prior to requesting additional molecular investigations?
- FISH is a common method for detecting chromosome abnormalities on FFPE tissue, what probe set would you recommend?
- Overexpression of MYC by IHC is sometimes the result of t(8;14) MYC-IGH translocation, what are some other causes of MYC overexpression?
- Many of these tumors do not carry MYC/BCL2 chromosomal alterations and have been named “double-expressor lymphoma.” What is the relevance of this finding?

Case 4 Discussion

- HGBCL-NOS, together with the new category for the “double-/triple-hit” lymphomas, replaces the 2008 category of BCLU, with features intermediate between DLBCL and Burkitt lymphoma.

- Includes blastoid-appearing large B-cell lymphomas and cases lacking MYC and BCL2 or BCL6 translocations that would formerly have been called BCLU.



Cytologic spectrum of HGBL, with MYC and BCL2 and/or BCL6 rearrangements.

BLOOD, 19 MAY 2016, VOLUME 127, NUMBER 20

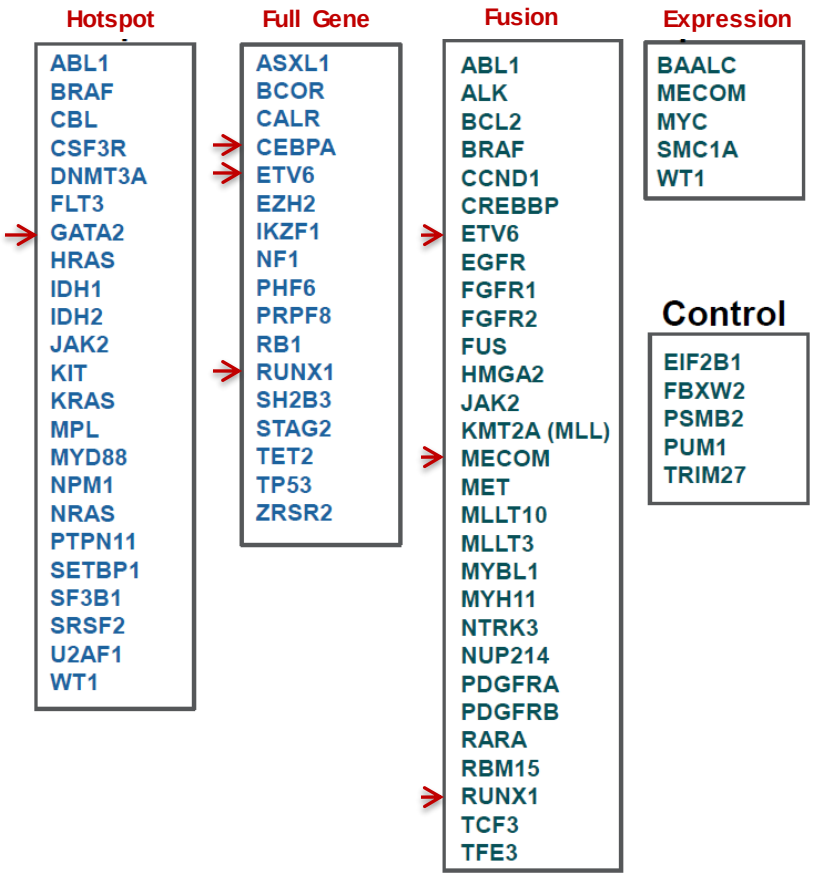
Myeloid Neoplasms with Germ Line Predisposition

- Hematologic malignancies have been at the vanguard among cancers in the use of *genetic analyses* for:
  - **Diagnosis**
  - **Classification**
  - **Prognostication**
  - **Therapeutic decision-making**
- The link between *germline alterations* and predisposition to myeloid neoplasms has led to establishment of a WHO category of "**myeloid neoplasms with germ line predisposition.**"
- Germline lesions in **CEBPA**, **RUNX1**, **ETV6**, **MECOM** and **GATA2** are among a host of mutations that can lead to bone marrow (BM) failure syndromes, telomere dysfunction and activated RAS signaling.

Hereditary Myeloid Malignancy Syndromes

- Working classification of the inherited myeloid malignancies is rapidly evolving.
- The current approach suggested by the ***University of Chicago***:
  - **Germline mutations characterized by thrombocytopenia, functional platelet defects and increased risk of MDS/AL (i.e. RUNX1).**
  - **Increased risk of MDS/AL but NO thrombocytopenia or other organ manifestations (i.e. CEBPA).**
  - **Increased risk of MDS/AL and associated organ manifestations (i.e. GATA2).**

Myeloid mutations covered (AML, MDS, MPN)



- Molecular profiling of myeloid neoplasms using the *Oncomine Myeloid Assay*.
- Can identify genetically-defined Hereditary Myeloid Malignancy Syndromes (HMMSs).
- Discussion with the clinical teams may be valuable in determining if the mutation is suspected to be germline vs. somatic.

Indications for Genetic Counseling & Germline Testing

FAMILY HISTORY

- Family history of myeloid neoplasm
- Early onset hematologic malignancy
- Multiple close relatives with cancer

PERSONAL HISTORY

- History of bleeding episodes or preexisting platelet disorders
- Lymphedema/monocytopenia/atypical infection
- Skin pigmentation/nail abnormalities
- Leukopenia/pulmonary fibrosis
- Bone marrow failure syndrome

MUTATION DETECTED IN MYELOID NEOPLASM

- Biallelic CEBPA
- Deleterious GATA2 mutation
- Deleterious RUNX1 mutation
- Frameshift DDX41 mutation

A Canadian guideline on the use of next-generation sequencing in oncology[a]

S. Yip MD PhD,*A. Christofides MSc RD,† S. Banerji MD,‡ M.R. Downes MB BCh BAO MD,§ I. Izevbaye MD PhD,‖ B. Lo MD PhD,# A. MacMillan MS CCGC CGC,** J. McCuaig MSc CCGC CGC,†† T. Stockley PhD,‡‡ G.M. Yousef MD PhD,§§ and A. Spatz MD‖‖

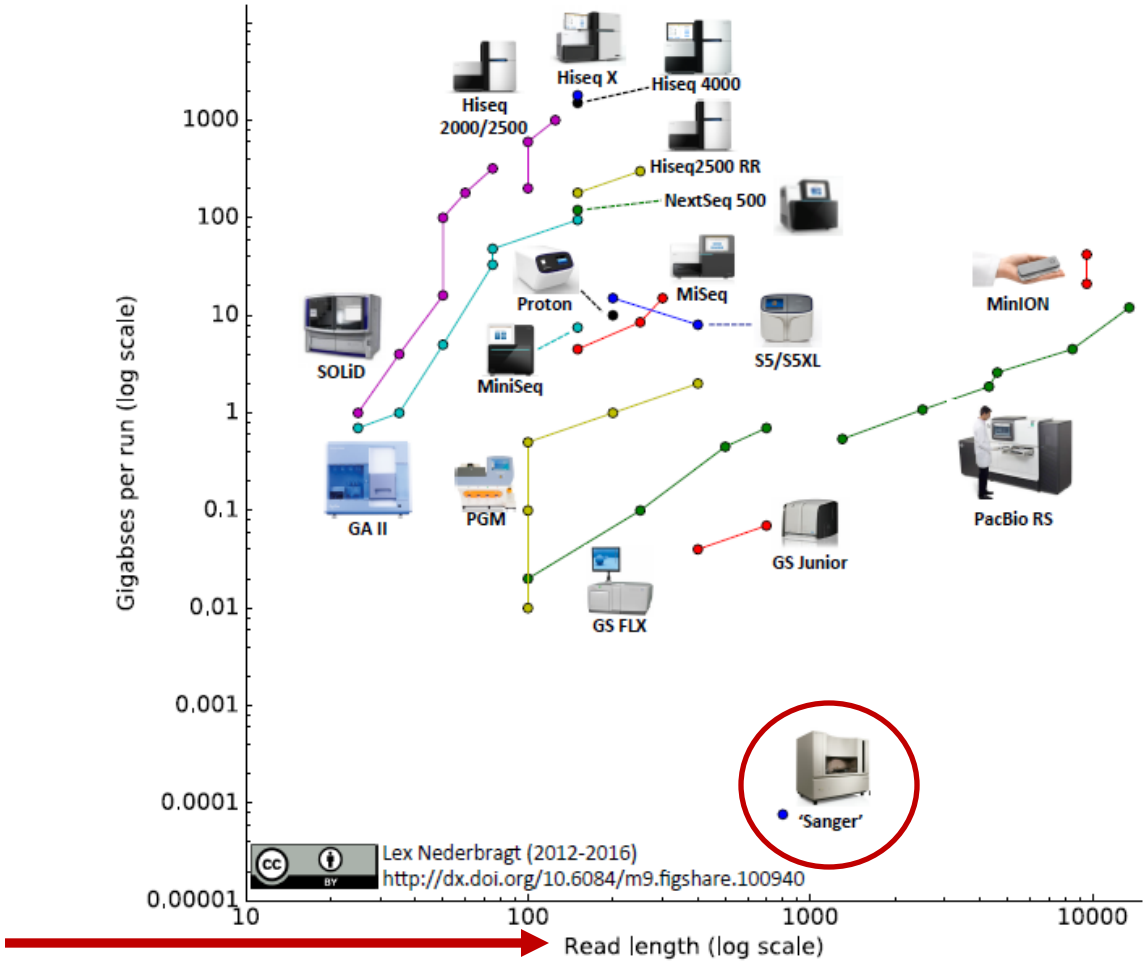
Curr Oncol. 2019 April;26(2):e241-e254

CCMG practice guideline: laboratory guidelines for next-generation sequencing

Stacey Hume,[1] Tanya N Nelson,[2,3,4] Marsha Speevak,[5] Elizabeth McCready,[6] Ron Agatep,[7,8] Harriet Feilotter,[9] Jillian Parboosingh,[10,11] Dimitri J Stavropoulos,[12,13] Sherryl Taylor,[1] Tracy L Stockley,[13,14] On behalf of Canadian College of Medical Geneticists (CCMG)

J Med Genet 2019;**0**:1–9. doi:10.1136/jmedgenet-2019-106152

Development in high-throughput sequencing

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