Myeloid Working Group
Revised Algorithms for Myeloid NGS Testing

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History of Mutational Profiling by Next General Sequencing (NGS) for Myeloid Neoplasms in Alberta

Up to late 2017 – sent out-of-province
2017 – NGS testing validated and piloted in Calgary
December 2017 – proposal for in-province testing approved, performed at CLS

Previous indications:
AML – newly diagnosed patients ≤ age 70, and selected older or relapsed/refractory pts on request
MDS – SF3B1 for RARS; others on request (e.g. diagnosis or prognosis)
MPN – On request for diagnosis or prognosis

Late 2019 – NGS testing introduced in Edmonton

Rationale for Revisions to Indications for NGS
Because many of the above indications were left to the discretion of the ordering hematologist, considerable variability in testing rates has evolved between Calgary and Edmonton, and between different physicians within each site. This is particularly the case for MPN and MDS.

In addition, there have been further developments over the past several years. The most notable is the continued increase in use of allogeneic HSCT in older patients, due to improvements in supportive care and an increase in donor availability. Prior to 2016 the upper age limit for eligibility was set at 65 years in Alberta, then increased to 70 years. Currently, selected fit patients in their early seventies are now being considered, and it is likely that this trend will continue to evolve in the coming years. NGS can provide valuable prognostic information in assessing transplant eligibility; therefore, restricting such testing to patients under age 70 is no longer appropriate.

There is increasing recognition of the prognostic value of molecular profiling for many myeloid malignancies. In MF, ASXL1, SRSF2 and U2AF1 mutations have been associated with more rapid time to progression and inferior survival and have been incorporated into several prognostic scoring systems. In MDS, EZH2, RUNX1 and TP53 mutations have similarly been associated with inferior survival. In AML, ASXL1, RUNX1, TP53 and DNMT3A mutations have been associated with higher release rates and inferior survival, either in specific subgroups or across multiple groups. For all of these diseases, the identification of poor prognosis mutations may influence the decision in favour of
earlier transplantation, before disease progression occurs. For AML, the identification of a TP53 mutation, particularly with high VAF, predicts for a uniformly poor prognosis and may influence a decision against proceeding with induction chemotherapy and/or transplant.

A third issue is the development of new targeted therapies. For MDS, luspatercept has now been approved in Canada for transfusion dependent low-risk MDS with SF3B1 mutations, which requires knowing this mutational status. For AML, gilteritinib has been approved for relapsed/refractory FLT3 mutated AML; this includes FLT3-TKD mutations which are not detected by our PCR assays. New targeted drugs for IDH and TP53 mutations are under investigation. The addition of venetoclax to azacitidine has demonstrated a survival benefit compared with azacitidine alone in previously untreated unfit AML patients, and has now been approved in Canada. Although not a specific mutation-targeted agent, the survival benefit is highly linked to mutated subtypes; in particular, IDH mutated patients appear to benefit the most from this combination.

There is also the group of patients with idiopathic cytopenias of undetermined significance (ICUS), in whom clonal hematopoiesis is demonstrable by NGS in approximately one-third of cases. For those ICUS cases, the definitive diagnosis of a myeloid neoplasm requires molecular profiling by NGS. This information can be extremely useful for counselling patients and may influence treatments decision (e.g. HSCT, luspatercept) for those with clinically significant cytopenias. Similarly, for patients with unexplained thrombocytosis or neutrophilia and lacking either a BCR-ABL, JAK2, CALR or MPL mutation, mutational profiling by NGS is needed to definitively establish a diagnosis of a myeloid neoplasm. This may have therapeutic implications (e.g. use of cytoreductive therapy).

Because of these recent developments in this rapidly changing field, it was felt to be necessary to update the guidelines for NGS testing, in order to more clearly delineate the indications. This will hopefully result in a harmonization of testing indications provincially, and provide clear guidance to both clinicians and pathologists as to when to order this test.

At the same time, there is a need to balance the clinical requirements for testing with resource utilization. Unrestricted NGS testing in all patients risks overwhelming the molecular pathology labs, thereby increasing turnaround times and costs. This document is an attempt to provide a rational approach to NGS testing for patients that need it, either for diagnostic, prognostic or therapeutic purposes, and to avoid unnecessary testing for those whose management is unlikely to be impacted by the result.

As NGS provides much more extensive and definitive information than single point mutation testing, it has been decided to replace CALR and MPL molecular tests with NGS, for patients with suspected MPNs. Bone marrow is preferred for NGS, but it can be done on peripheral blood (PB); however, other lower VAF mutations could be missed.
Revised Indications for Myeloid Panel by NGS (see flow diagrams):

1. **MPN:**
   a. Suspected ET (any age) (see algorithm A):
      - PCR-based testing: JAK2 → if negative, then NGS to confirm diagnosis (on request by hematologist)
   b. Myelofibrosis (see algorithm B):
      - All patients age 74 or under (automatic order by pathology)
      - Patients age ≥ 75 if JAK2-V617F negative (automatic order by pathology)
   c. PRV – NGS rarely needed (see algorithm C)
      - If JAK2 V16F and Exon12 negative (on request by hematologist)

2. **MDS** (see algorithm D):
   a. Diagnostic:
      - SF3B1 for MDS-RS (automatic order by pathology)
      - Idiopathic cytopenia of undetermined significance (ICUS) (on request by hematologist)
   b. Prognostic/therapeutic:
      - All pts age 74 and under (automatic order by pathology)
      - age ≥ 75, if eligible for treatment based on result (on request by hematologist)

3. **AML** (see algorithm E):
   a. New diagnosis:
      - All pts age 74 or under (automatic order by pathology)
      - Age 75 and older: if eligible for treatment based on result (on request by hematologist)
   b. Relapse/Refractory
      - If treatment eligible based on result (on request by hematologist)

4. **Other Myeloid neoplasms:**
   - CMML – same as for MDS
   - MDS/MPN overlap – same as for MDS

**N.B.** NGS requests should be restricted to hematologists, to reduce risk of inappropriate testing.
A. Diagnostic algorithm for suspected essential thrombocytemia:

- **JAK-2 V617F, BCR-ABL on PB**
  - **Negative**
  - **Positive**

On request by Hematologist:

- **NGS – BM (preferred) or PB**
B. Diagnostic/prognostic algorithm for suspected myelofibrosis (MF):

BM consistent with MF

< age 75

- JAK-2 V617F + or

- BM for NGS

Ordered by Pathologist

≥ age 75

- JAK-2 V617F +

- BM for NGS

Ordered by Pathologist

- JAK-2 V617F -
C. Diagnostic algorithm for PRV:

- **JAK-2 V617F**
  - **Negative**
    - **JAK-2 EXON12**
      - **Negative**
        - **BM for NGS**
      - **Positive**
        - **Consider other etiology (e.g. hereditary)**
  - **Positive**

*On request by Hematologist*
D. Diagnostic algorithm for MDS, (including CMML, MDS/MPN overlap syndromes):

Suspected MDS

Bone marrow with cytogenetics

Nondiagnostic (ICUS)

Clinically significant*

NGS for diagnosis

On request by Hematologist

Diagnostic for MDS

< age 75, or MDS-RS (any age)

NGS for Classif. Prognosis, Rx

Ordered by Pathologist

≥ age 75

NGS for possible Rx

On request by Hematologist

* Moderate-severe cytopenias
(e.g. Hgb <100, Plts <100, ANC <1)
E. Diagnostic algorithm for AML:

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AML

Age < 75
   | Ordered by Pathologist
   | NGS

Age ≥ 75 or Relapsed/Refract.
   | Molec needed for prognosis/Rx?
   | No
   |   | On request by Hematologist
   |   | NGS
   | Yes
      | NGS
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