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INVESTIGATING MYELOID NEOPLASMS IN THE ERA OF PRECISION ONCOLOGY

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INTRODUCING THE ONCOMINE™ MYELOID ASSAY GX

Myeloid neoplasms have traditionally been regarded as morphologically and aetiologically distinct groups of disorders. With the advent of next-generation sequencing (NGS) and tumour genomic profiling, it has however become apparent that significant mutational overlap exists between these disorders, and that many of them might, in fact, be viewed as different evolutionary phases of the same disease.

The 2016 World Health Organization (WHO) classification clearly recognises the importance of genetic testing in myeloid malignancies.[1] Increasingly, these cancers are now being diagnosed, classified and risk-stratified, based on their unique genomic profile. The outcome of many myeloid neoplasms has been drastically improved through the use of molecular targeted therapies such as imatinib. The independent prognostic value of specific mutations is also recognised in prognostication models such as the 2017 European Leukaemia Net (ELN) Guidelines for risk-stratification in acute myeloid leukemia (AML).[2]

Ampath is one of the leading providers of comprehensive oncology genetic testing in South Africa. The Oncomine™ Myeloid Assay GX (also known and referred to hereafter by the mnemonic OMAGEN) is the latest in a string of state-of-the-art technologies being offered that allows for the simultaneous investigation of various myeloid malignancies in one approach.

WHICH CONDITIONS CAN BE DIAGNOSED USING THE ONCOMINE™ MYELOID ASSAY GX?

ACUTE MYELOID LEUKAEMIA

Testing for recurrent genetic abnormalities is incorporated into the National Comprehensive Cancer Network (NCCN), as well as other international guidelines used in the work-up and management of AML.[3] Many of these abnormalities have therapeutic, as well as prognostic significance.

All WHO defined molecular subtypes of AML can be diagnosed and monitored using the OMAGEN panel. These include:

- AML with t(8;21)(q22q22.1); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- APL with *PML-RARA*
- AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
- AML with t(6;9)(p23;q34.1); *DEK-NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*
- AML with mutated *NPM1*
- AML with biallelic mutation of *CEBPA*
- Provisional entity: AML with *BCR-ABL1*
- Provisional entity: AML with mutated *RUNX1*

AML risk-stratification can also be done using the OMAGEN panel, as indicated in Table 1.

TABLE 1: RISK-STRATIFICATION AS DEFINED BY THE 2017 ELN GUIDELINES[2]

Risk category	Genetic abnormality
Favourable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD* low
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3-ITD* high
	Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD* low (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favourable or adverse*
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)*
	Complex karyotype or monosomal karyotype*
	Wild-type *NPM1* and *FLT3-ITD* high
	Mutated *RUNX1*
	Mutated *ASXL1*
	Mutated *TP53*

*Requires additional chromosome analysis/karyotyping and/or FISH

CHRONIC MYELOID LEUKAEMIA

Detection of a *BCR-ABL1* rearrangement is required for the diagnosis of chronic myeloid leukaemia (CML).[1] The OMAGEN panel can detect and differentiate between 18 different *BCR-ABL1* fusions, including the most common p210, p190 and p230 fusion transcripts. Sequencing of the *ABL1* gene is also included in this assay, which allows for the detection of tyrosine kinase resistance mutations in CML patients on treatment. Resistance mutations that can be detected in CML include (but are not limited to) those indicated in Table 2 below.

TABLE 2: 2022 NCCN GUIDELINES FOR TYROSINE KINASE RESISTANCE MUTATIONS IN CML[3]

ABL1 mutation	Confers resistance/reduced sensitivity to:
T315I	Bosutinib, Dasatinib, Nilotinib
T315A	Dasatinib
V299L	Bosutinib, Dasatinib
G250E	Bosutinib
F317L	Bosutinib, Dasatinib
F317V/I/C	Dasatinib
Y253H	Nilotinib
E255K/V	Nilotinib
F359V/C/I	Nilotinib

MYELOPROLIFERATIVE NEOPLASMS (MPNs)

Confirming the presence of a driver mutation in *JAK2/MPL/* Calreticulin is a WHO-defined criterion for the diagnosis of polycythaemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythaemia (ET). Exclusion of a *BCR/ABL1* rearrangement is an additional requirement in the case of PMF and ET.[1] While this testing is typically embarked on in a sequential manner to save costs, this approach often results in a delay in diagnosis. In cases where no driver mutation is detected (so-called triple negative MPNs), further genetic testing needs to be requested to detect other mutations, which may provide evidence for clonality. The OMAGEN panel enables simultaneous assessment for all the MPN driver mutations, the *BCR/ABL1* rearrangement, and any additional myeloid-associated mutations, which may be significant either diagnostically and/or prognostically.

CLONAL EOSINOPHILIAS

The OMAGEN panel covers all rearrangements typically associated with clonal eosinophilias, including *PDGFRA, PDGFRB, FGFR1* and *JAK2* rearrangements. Other myeloid malignancies which can present with secondary eosinophilia, including AML with *CBFB-MYH11*, systemic mastocytosis and CML, can also be diagnosed or excluded using this panel.

CLONAL CYTOPENIAS AND MYELODYSPLASTIC SYNDROMES

The OMAGEN panel can reliably exclude pathogenic variants/mutations in more than 50 genes and at an allele frequency of >5%, allowing for the diagnosis of clonal cytopenias of uncertain significance (CCUS) and clonal haematopoeisis of indeterminate potential (CHIP). Copy number variation and structural chromosomal changes such as monosomies and large structural deletions can, however, not be detected using the OMAGEN panel. These will still require additional conventional cytogenetic and/or targeted FISH testing.

OTHER MYELOID DISORDERS

The category of myeloid neoplasms with germline predisposition was added to the WHO's classification of haematopoietic and lymphoid tumours in 2016.[1] The OMAGEN panel enables the detection of several of these variants, including *CEBPA, DDX41, RUNX1, ANKRD26, ETV6, GATA2* and *TP53*.

CONCLUSION

Providing comprehensive, timeous and cost-effective cancer genetic testing within the rapidly-evolving field of precision oncology is a challenge faced by diagnostic genetic laboratories across the world. The Oncomine™ Myeloid Assay GX offers a unique solution to this challenge by using targeted NGS technology to detect all clinically relevant myeloid genetic biomarkers, mutations and fusions, all in one assay.

For more information, contact the NGS laboratory at ngs@ampath.co.za. Genetic counselling enquiries or appointment bookings can be made on 012 678 0645 or via email at geneticsclinic@ampath.co.za.

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REFERENCES

1. Swerdlow, S.H., (2017). WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer. Available from: http://publications.iarc.fr/Book-And-Report-Series/Who-Iarc-Classification-Of-Tumours/Who-Classification-Of-Tumours-Of-Haematopoietic-And-Lymphoid-Tissues-2017.
2. Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F.R., Büchner, T., Dombret, H., Ebert, B.L., Fenaux, P., Larson, R.A. and Levine, R.L., (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood, The Journal of the American Society of Hematology*, 129(4), pp. 424–447.
3. National Comprehensive Cancer Network, (2022). NCCN guidelines: Treatment by cancer type. Available from: https://www.nccn.org/guidelines/category_1 [accessed 7 April 2022].