Specifications Sanquin Molecular diagnostics for AML (P042)

Design AML NGS Panel

The AML Panel (AML.20141205) exists of 327 amplicons and is covering 100% of submitted areas (all coding regions (exons)) and is able to analyze variants in 19 genes implicated in AML. Indicated exons (Table 1) include flanking intronic regions based on 5 base exon padding. For some genes the 5 base exon padding is not achieved or only a hotspot location is covered. See Table 2 for detailed coverage information about these aberrant regions.

Table 1. Design AML panel

Gene	Chromosome	NCBI Transcript	Exon	Covergae %
ASXL1	chr20	NM_015338.5	12	100
BRAF	chr7	NM_004333.4	15	100
CBL	chr11	NM_005188.3	8, 9	100
CEBPA	chr19	NM_004364.3	1	100
DNMT3A	chr2	NM_022552.4	2 to 23 (full)	100
FLT3	chr13	NM_004119.2	16, 20, 21	100
GATA2	chr3	NM_032638.4	3 to 7 (full)	100
IDH1	chr2	NM_005896.3	4	100
IDH2	chr15	NM_002168	4	100
JAK2	chr9	NM_004972.3	14	100
KIT	chr4	NM_000222.3	8, 10, 11, 17	100
KRAS	chr12	NM_033360.3	2, 3	100
NPM1	chr5	NM_002520.6	11	100
NRAS	chr1	NM_002524.4	2, 3	100
PTPN11	chr12	NM_002834.4	3, 13	100
RUNX1	chr21	NM_001754.4	3 to 8	100
TET2	chr4	NM_001127208.2	3 to 11 (full)	100
TP53	chr17	NM_000546.5	2 to 11	100
TP53β	chr17	NM 001126114.2	alt 10	100
TP53γ	chr17	NM 001126113.2	alt 10	100
WT1	chr11	NM_024426.4	7, 10	100

Table 2. Aberrant covered regions AML panel

Gene	Exon	Coding region
BRAF	15	c.1763 - c.1852
FLT3	16	c.1995 - c.2053+5
FLT3	20	c.2430 - c.2541+5
FLT3	21	c.2542-5 -c.2653+2
TP53	2	c.-5 - c.74+2
TP53	3	c.75-5 - c.96+2

Coverage of the NGS AML Panel

Coverage is the number of times a base is sequenced. The deeper the coverage of each base the greater the reliability and sensitivity of the sequencing assay. The minimum depth of coverage required for detection of somatic variants with the AML AmpliSeq Panel is 500X. The percentage of Target Base coverage (%Base500x) is the percentage of target bases in a panel that is covered at least 500 times.

The percentage of target bases that is covered at least 500 times (%Base500x) is at least 99.38% at 2.500.000 Mapped Reads. With this acceptance criteria two amplicons failed to yield >500 times coverage, specific locations are listed in Table 3. One amplicon in DNMT3A results in a too low coverage of 80bp at the 3'side of exon 4. For exon 4 one variant c.226C>T (p.P76S) is described in the COSMIC database. The mutation was encountered in 1 out of 125 (0.8%) examined samples and was related to an ovary tumor. Besides DNMT3a, one amplicon in RUNX1 has less than 500x coverage of 72bp in exon 4 and 5bp in intron 3. There are several AML related variants described in this region.

Table 3. Information failed amplicons AML panel

Chr.	GRCh37/hg19 coordinates		Gene	Bases failing Coverage 500X	Exon	Missing COSMIC
	Start	End				
Chr2	25505496	25505600	DNMT3A	80	4	c.226C>T (p.P76S)
Chr21	36259322	36259413	RUNX1	77	4	Several

Design AML Fusion Transcript Panel

The AML-Fusion Transcript Panel (IAD84643) is able to detect fusion transcripts BCR::ABL1, CBFB::MYH11, PML::RARA and RUNX1::RUNX1T1 (AML1::ETO).
Fusion of genes may result in the usage of different donor and/or acceptor exons. Consequently, the corresponding fusion gene transcripts will have different exon compositions. In Table 4, a summation is listed with the number of fusion forms that are detected by the AML Fusion Transcript Panel.

Table 4. Overview fusion transcripts in AML Fusion Transcript Panel

Fusion Gene	Transcript Variants
BCR::ABL1	18
CBFB::MYH11	11
PML::RARA	5
RUNX1::RUNX1T1	5

Reporting: addition hematological malignancies variants

This test does not distinguish between somatic and germ line alterations in analyzed gene regions, particularly when variant allele frequencies (VAF) are near 50% or 100%. If nucleotide alterations in genes associated with germline mutation syndromes are present and there is also a strong clinical suspicion or family history of malignant disease predisposition, appropriate genetic counselling may be indicated.

Variants detected between 5% and 10%. Variant Allele Frequency may indicate subclonal tumor populations. However, the clinical significance of these findings may not always be distinct. It is demonstrated that in blood DNA samples from individuals with advancing age and who do not have a hematologic neoplasm, a low incidence of gene variants that are associated with myeloid neoplasms can be detected. This phenomenon of clonal hematopoiesis of indeterminate potential (CHIP) may not be clearly distinguishable from tumor-associated mutations, especially if detected as a sole abnormality (DOI: 10.1182/blood-2015-03-631747).

Correlation with clinical, histopathologic and additional laboratory findings is required for final interpretation of the results. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician.

NGS data are interpreted with the current knowledge concerning variants in relation to disease or as explanation of a phenotype. For reporting variants, the following guidelines will be followed: 'Best Practice Guidelines for Reporting Molecular Genetics results' written by R.J.L. Treacy and D.O. Robinson. The authorization of the results is done by a recognized Clinical molecular geneticist. All variants are annotated and reported as designated by the Human Genome Variation Society (HGVS) nomenclature, as described at their website.