«TableStart:Samples»

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| --- | --- | --- |
| To: PETER MAC CANCER CENTRE  305 GRATTAN STREET  MELBOURNE  VIC 3000 | **Patient**: «patient»  **URN**: «urn»  **DOB**: «dob»  **SEX**: «sex»  **Location**: «location»  **Requester**: «requester» | Sample: «sample»  Ext Ref: «extref»  Collected: «collect\_date»  Received: «rcvd\_date»  Specimen:  Block ID: |
|  |  |  |

**INTEGRATED MOLECULAR REPORT** «isdraft»

|  |  |
| --- | --- |
| **MUTATIONS DETECTED** | |
| **Gene** | **Mutation** |
| **«TableStart:Variants»«gene»** | «refseq»: «hgvsc»;«hgvsp»  «TableEnd:Variants» |
|  | |
| **SUMMARY** | |
|  | |

**Clinical indication –**

**Sample type –**

**Histological features –**

|  |  |
| --- | --- |
| **ASSAY** | **MYELOID NGS PANEL or MYELOPROLIFERATIVE NGS PANEL** |
| **Genes** | JAK2 (exon 12, 14(V617F)), MPL (exon 10), CALR (exon 9), c-KIT (exon 17), SF3B1 (exon 14-16), CSF3R (exon 14, 17), ASXL1 (exon 12)  ASXL1 (exon 12), BRAF (exon 15), CALR (exon 9), CBL (exon 8, 9), CSF3R (exon 14, 17), DNMT3A (exon 23), EZH2 (exon 2-20), FLT3 (exon 14, 15, 20), GATA2 (exon 4, 5), IDH1 (exon 4), IDH2 (exon 4), JAK2 (exon 12, 14, 16), JAK3 (exon 13), KIT (exon 8, 10, 11, 17), KRAS (exon 2, 3, 4), MPL (exon 10), NPM1 (exon 11), NRAS (exon 2, 3, 4), RUNX1 (exon 4-9), SETBP1 (exon 4), SF3B1 (exon 14,15,16), SRSF2 (exon 1), TET2 (exon 2-11), TP53 (exon 2-11), U2AF1 (exon 2, 6), WT1 (exon 7, 8, 9) |
| **CEBPA** | NOT PERFORMED |
| **FLT3-ITD** | NOT PERFORMED |

**Individual Variant Analysis**

«TableStart:Variants»**«gene»:** «mut»«TableEnd:Variants»

**Method**

DNA is analysed using a custom-designed myeloid amplicon gene panel (Myeloid v5.4). Samples are uniquely indexed, pooled and sequenced on the Illumina MiSeq using MiSeq v2 chemistry at 2x151bp reads. Alignment, variant calling and annotation are performed using an amplicon-optimised pipeline. Only plausible pathogenic variants passing multiple functional and quality filters and that are present in the “*Genes analysed*” list above are reported. Amplicons with less than 100 aligned reads are not analysed. The technology employed here is not suitable for detecting loss of heterozygosity, copy number variations, gross structural rearrangements, or aneuploidies. At 1000x coverage, this assay has a detection limit of approximately 5%. For the variants JAK2 V617F and c-KIT D816V the sensitivity of the assay is approximately 1%. Please note, systemic mastocytosis and related disorders frequently have allelic burdens of <1% and therefore will not be reliably detected with this assay.

NGS assays do not detect all FLT3-ITD mutations. For Myeloid NGS samples, FLT3-ITDs are also tested for by fragment analysis using capillary electrophoresis. The results of this assay are included in the above results table. The FLT3 allelic ratio is calculated by peak height ITD/peak height WT. The sensitivity of FLT-ITD detection is approximately 1%. The most common ASXL1 mutation (c.1934dupG;p.Gly646Trpfs\*12) is not adequately detected by NGS. All samples undergo fragment analysis of a part of exon 12 of ASXL1 in order to detect the presence of any +1bp frameshift mutation, including c.1934dupG;p.Gly646Trpfs\*12. The sensitivity of ASXL1 detection is approximately 5%. CEBPA analysis comprises DNA (Sanger) sequencing of the CEBPA gene using two overlapping PCR fragments. The sensitivity of Sanger sequencing is approximately 20%. Mutations detected by CEBPA sequencing (if performed) are included in the table above.

DNA extraction produced sufficient good quality material for myeloid amplicon gene panel testing. Sample processing passed all expected QC metrics and high quality sequence with high coverage («ampReads» mean aligned reads/amplicon) and uniformity («ampPct»% amplicons >0.2 mean aligned reads) was obtained.

Please contact the laboratory on 03 8559 8402 if you wish to discuss this report further.

**Reported by: Dr. Piers Blombery (Consultant Haematologist)**

**Authorised by: Ms. Michelle McBean**

**Reported:**

*«TableEnd:Samples»*