em 80 meses foi de 8,7%. **Discussão:** Apesar da excelente sobrevida que a maioria dos pacientes com LMC apresenta com os ITQ, cerca de 30% apresentam falha ao tratamento. Um dos mecanismos mais frequentes de mutação são as mutações que ocorrem no domínio da tirosina quinase do ABL. A mutação T315I afeta o sitio de ligação do ATP e confere resistência a todos os ITQ aprovados, com exceção do ponatinibe. O asciminibe é um novo inibidor do sitio miristoil do ABL que também demonstrou eficácia contra essa mutação. Cerca de 41% de pacientes resistentes com mutação T315I obtiveram resposta molecular maior e 29% de pacientes com T315I tratados previamente com ponatinibe apresentaram resposta. No entanto, esses inibidores ainda não estão disponíveis no SUS, sendo a única alternativa para a mutação T315I o transplante de medula óssea, nem sempre viável dependendo da idade, comorbidades do paciente ou da disponibilidade do doador. Somente dois pacientes foram submetidos ao transplante e somente um obteve boa resposta. Em 50% dos casos houve progressão da doença e em 83,3% óbito, relacionado ou não à doença. **Conclusões:** A mutação T315I ainda é um desafio no tratamento da LMC no SUS, pela indisponibilidade de drogas mais eficazes, conferindo ao paciente um prognóstico desfavorável. O transplante de medula óssea permanece no momento como única alternativa de tratamento nessa situação.

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ANALYTICAL PERFORMANCE OF A DNA/RNA-BASED NEXT-GENERATION SEQUENCING (NGS) PANEL FOR MYELOID NEOPLASMS

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Myeloid neoplasms (MNs) are a heterogeneous group of neoplasms including acute myeloid leukemia, myelodysplastic syndrome, myeloproliferative neoplasms and myelodysplastic syndrome/myeloproliferative neoplasms. MNs show a complex genetic basis, and the number of alterations to be analyzed for risk diagnostic, stratification and management of patients increases continuously. Consequently, there is a demand for multiple molecular and cytogenetic techniques with the aim of detecting a wide variety of genetic abnormalities. Since conventional techniques are insufficient to study all such abnormalities, the NGS panel has become a useful tool for broad characterization of the spectrum of mutations in MNs. However, before NGS panels can be introduced in the laboratory routine, their performance should be evaluated. In this context, we describe the analytical performance of the Oncomine™Myeloid Research Assay (OMA) (Thermo Fisher), a DNA/RNA-based amplicon sequencing panel, that evaluated simultaneously 40 genes (17 full genes and 23 hotspots genes) and 29 fusions drivers genes (over 600 partners) known to be recurrently mutated in MNs. Commercial controls Seraseq Myeloid Mutation DNA and the Seraseq Myeloid Fusion RNA were used for sensitivity, specificity, accuracy, repeatability, reproducibility and limit of detection (LoD) testing. Prospective sequencing was performed for samples from 34 patients presenting with suspected or confirmed MN. Libraries was prepared by manual method and sequenced by the Ion S5 Sequencer. Data obtained were analysed on Ion Reporter 5.18 software. The OMA assay showed good performance in terms of depth of coverage, on-target reads, and uniformity. The mean reads lenght ranged from 223 to 231 bp for DNA sample and from 88 to 124 bp for RNA, within the expected standard for the panel. The panel achieved 98,8% and 100% concordance with DNA and RNA controls, reaching analytical sensitivity of 99% and 100% for DNA and RNA, respectively. Regarding the type of DNA mutation: single nucleotide variants (SNP), indels or indels hot spots, the sensitivity found was 100%, 96% and 95%, respectively. The accuracy and analytical specificity was 100% for DNA panel. The LoD was 5% variant allele fraction (VAF) for DNA (SNP and indel) and 1-log reduction from initial number of reads counts for RNA fusion genes. Repeatability and reproducibility were 98% for DNA and 100% for RNA. However, the coefficient of variation (CV) within-run and between-run in VAF was >20% for large indels in *CALR*, *FLT3* (ITD) and *SRSF2*. The vertical coverage of each sample was inspected with a coverage threshold of 100X. Some amplicons in the assay failed repeatedly (ASXL1_1.875, BCOR_8.202477, BRAF_18.30633, CEBPA_1.1.86676, PRPF8_12.173121, SH2B3_1.85663). However, the OMA is unable to detect specific variants in *ASXL1* (c.1900_1922del23 and c.1934_1935insG) and *CEBPA* (c.68_69insC). This is a known limitation of the Ion Torrent chemistry for accurate sequencing of large homopolymers and high GC content regions. Of the 34 patient samples, 28 (87.5%) had at least one reportable variant. A total of 58 DNA variants and six gene fusions were detected. Some variants were confirmed by orthogonal techniques. In conclusion, OMA is a highly accurate and reproducible NGS panel for the detection of common genetic abnormalities in MNs.

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RELATO DE CASO: LEUCEMIA MIELOIDE CRÔNICA RESISTENTE AO TRATAMENTO

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