CIRCULATING TUMOR DNA SEQUENCING COUPLED WITH THERAPEUTIC DRUG MONITORING AS AN INNOVATIVE STRATEGY FOR THE PHARMACOLOGICAL MONITORING OF GIST PATIENTS - A CASE REPORT

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Introduction: The standard of care for the first line treatment of advanced gastrointestinal stromal tumor (GIST) is represented by imatinib, which is administered daily at a standard dosage until tumor progression. Secondary resistance to imatinib commonly occurs through the clonal selection of genetic mutations in the tumor DNA, and an increase in imatinib dosage was demonstrated to be efficacious as a second line treatment at tumor progression. A molecular and pharmacokinetic monitoring of GIST patients under imatinib treatment could help an early detection of tumor progression and a tailoring of the drug dosage. We present here the case report of a male patient affected by GIST, who underwent an experimental protocol of circulating tumor DNA (ctDNA) sequencing and therapeutic drug monitoring in course of imatinib.

Materials and methods: Serial blood samples of patients suffering from GIST was collected during imatinib therapy. Total cfDNA was extracted, quantified trough fluorescence, and sequenced by exploiting a deep next-generation sequencing barcode-aware approach for the identification and the dynamic monitoring of tumor-derived DNA. The Actionable Solid Tumor Panel DNA (Qiagen) was used to sequence genes of pivotal relevance in human cancer. For the validation of somatic mutations detected in the blood, custom digital droplet PCR assays was used, and genomic DNA extracted from buffy-coat was sequenced with the same gene panel to exclude germline variants from the count of somatic mutations detected in cfDNA. In parallel, imatinib and nor-imatinib (active metabolite) plasma levels was measured by means of a LC-MS/MS method. Clinical records concerning disease status was assessed according to RECIST criteria.

Results: We identified in two serial ctDNA samples, belonging to the same patient, a sharp increase in the allele frequency of a never-described TP53mutation (c.560-7_560-2delCTCTTAinsT) located in a splice acceptor site and suspected to lead to the exon 6skipping, according to bioinformatic predictive tools. This finding was consistent with a clinical progression of the disease that, despite the administration of an increased imatinib dose, led to patient death. The plasmatic level of the drug was within the reported therapeutic range. The same TP53mutation was retrospectively identified at a very high allelic frequency in the metastatic hepatic lesion and the presence of few copies of DNA with the same mutation was also confirmed in the primary tumor by digital droplet PCR.

Conclusions and discussion: This case report demonstrates that a combined pharmacokinetic and molecular monitoring approach is feasible in the clinical practice and could provide useful information for the patient management. The use of next-generation sequencing in this context allows not only the identification of known resistance associated mutations but also the discovery of new potential genetic markers.