

Next generation sequencing for identifying novel rare germline variants contributing to severe and life-threatening toxicity in patients treated with fluoropyrimidines

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Fluoropyrimidines (FL) (5-fluorouracil, tegafur and capecitabine) are frequently used in several solid tumors treatment. FL interferes with DNA and RNA synthesis by blocking the Thymidylate Synthetase (TS), thus leading to cell blockade in S-phase and, ultimately, to cell death. Unfortunately, about 26% of patient treated with FL will develop unpredictable severe to life-threatening toxicity (grade ≥ 3) (Twelves et al, 2005).

Known rare genetics variants and single nucleotide polymorphism (SNP) provide partial explanation to the overall adverse reactions (Toffoli et al, 2015). These latter are most commonly ascribed to the Dihydropyrimidine Dehydrogenase (DPYD) gene. Most variants affecting proteins phenotype in humans, including those responsible for drugs ADME, were reported to be novel and rare (Lek et al, 2016).

With this study, we aimed at analysing the genetic sequence of a set of 62 pharmacogenes and 3 promoters involved in the FL pharmacokinetics and pharmacodynamics, in the attempt to partly explain FL related severe toxicity.

A set of 603 patients treated with a FL-based chemotherapeutic regimen and meeting the inclusion criteria was selected from a prospective patients' collection of 5,126 clinical cases of the Experimental and Clinical Pharmacology Unit of Centro di Riferimento Oncologico (CRO)-National Cancer Institute, Aviano. Patients were considered eligible based on the following criteria: (i) histologically confirmed diagnosis of solid cancer; (ii) available peripheral blood biological sample; (iii) signed written informed consent approved by the local Ethical Committee; (iv) assumption of a FL-based treatment for at least three cycles unless interrupted due to a severe treatment-related toxicity occurrence

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Patients and respective biological specimens were previously recruited and stored at the Experimental and Clinical Pharmacology Unit (CRO-Aviano). Patients were considered eligible based on solid tumor diagnosis, FL-based treatment, available toxicity data related to the first three therapy cycles and absence of known DPYD risk variants (Toffoli et al.,2015). PharmGkb resources and literature data were used for genes selection in FL ADME pathway. Genomic DNA sequencing assessment will be performed by next generation sequencing (NGS) on MiSeq platform (Illumina).

Seventy-seven patients out of 603 were selected for NGS analysis based on grade ≥ 3 toxicity occurrence. A list of 60 genes was selected based on in silico data mining. A custom gene panel (SeqCap EZ, Roche) which covers the coding sequence and untranslated regions of target genes, including the flanked splice junctions regions was designed. An additional region of 3000bp downstream or upstream to the gene, based on gene orientation was included to address the promoter genetic variants for 3 genes with a pivotal role in FL pharmacodynamics (DPYD, MTHFR and TYMS). NGS analysis is now ongoing. Novel genetic variants will be validated by Sanger sequencing. Functional studies will be warranted to assess the effect of the variants highlighted by our study.

The identification by NGS of rare genetics variants related to high grade toxicity from FL holds the potentials to move further step towards personalized therapy in tumor patients (Kozyra et al., 2016).