COMPARISON OF FOUR DIFFERENT ASSAY METHODS FOR UGT1A1*28 GENOTYPING

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Introduction:

Irinotecan is widely used in the treatment of colon and other gastrointestinal cancers. It is an inhibitor of the Topoisomerase I enzyme, a ubiquitous enzyme responsible for managing the DNA supercoils, allowing its replication, transcription and cell mitosis. The inactivation of the cytotoxic metabolite of irinotecan, SN-38, involves a phase II enzyme, UDP-glucuronosyltransferase (UGT) which converts it to the inactive glucuronide metabolite. The most involved isoforms are UGT1A1, UGT1A7 and UGT1A9. Variations in this metabolic pathway can result in changes in the therapeutic activity or adverse effects of irinotecan. The most important genetic variant which affects this pathway is UGT1A1*28: patients with homozygote genotype UGT1A1*28/*28 show a significant reduction in enzymatic activity which results in higher incidence of side effects (mainly neutropenia and diarrhea). Thus, either drug regulatory agencies, and scientific associations, for instance the Clinical Pharmacogenetics Implementation Consortium and the Italian Society of Pharmacology in collaboration with the Italian Association of Medical Oncology, recommend UGT1A1 genetic testing and the subsequent adjustment of drug doses on the basis of the UGT1A1*28 genotype, especially in patients in which irinotecan is used at high doses.

Aim:

This work has a dual aim:

1. To evaluate the results obtained after a 3-years use period of the home-made analytical protocol for the individuation of UGT1A1*28;

2. To evaluate the implementation and the analytical performances of three CE-IVD methods which allow a higher throughput than sequencing.

Methods:

Concerning the first point of this work, the protocol consisted of the following steps:

1. Genomic DNA (gDNA) automatic extraction from blood samples of patients who underwent the UGT1A1 genetic test pre-treatment at the Azienda Ospedaliero Universitaria Careggi, Florence, ITALY;

2. PCR amplification of the gDNA and its subsequent analysis by HRMA for a qualitative evaluation;

3. UGT1A1 genotyping by Sanger Sequencing.

Concerning the second point, the following 3 different CE-IVD methods have been applied to the genotyping of ten DNA samples with known genotype:

1. Easy® UGT1A1 kit (Diatec Pharmacogenetics) by Real-Time PCR

2. Therascreen UGT1A1 Pyro® kit (Qiagen) by Pyrosequencing;

3. Miriapod® ADMET UGT1A1*28 TSER 28bp VNTR Amplification Reagents (Diatech Pharmacogenetics) by the dedicated agarose gel based approach.

Results:

The Sanger-sequencing polymorphism analysis for the TA repetitions evidenced the following distribution in 385 patients (collected from 2014 to 2017):

• 44,15% of (TA)6/6 (UGT1A1*1/*1) – 170 patients;

• 43,12% of (TA)6/7 (UGT1A1*1/*28) – 166 patients;

• 12,47% of (TA)7/7 (UGT1A1*28/*28) – 48 patients;

• 0,26% of (TA)5/7 (UGT1A1*38/*28) – 1 patient;

The three CE-IVD methods showed a variable concordance with Sanger sequencing. Even if all the CE/IVD kits are specific for the identification of genotype *1/*28 (as declared by the manufacturer), both kits from Diatech allowed evidencing the presence of a heterozygote in samples characterized by a (TA)5/7 genotype.

Conclusions:

The homemade automatic sequencing, once optimized, appears to be reliable and suitable for a routine diagnostics purpose, since it is affordable and capable of identifying also unknown variants; it has the drawbacks of being time consuming and not CE-IVD certified.

Among the three CE-IVD evaluated kits, the Real-Time PCR showed high specificity, rapidity in preparation and execution of the analysis, good affordability. On the other hand, Pyrosequencing despite the higher throughput, requires a more expensive effort and specialized operator to complete the test. Finally, the agarose gel based approach provides a simple-to use protocol, but the gel preparation and interpretation represent a limiting step to a routine analysis also affecting a general reproducibility of the data.