AZIENDA CERTIFICATA
UNI EN ISO 9001, UNI EN ISO 13485

IDENTIFICATION OF 3 POLYMORPHISMS OF THE GENE DIHYDROPYRIMIDINE DEHYDROGENASE (DPYD): DPYD * 2A; DPYD * 13; DPYD * 2846; DPYD * 1236, DPYD * 2194. Toxicity to fluoropyrimidines

Ampli- DPYD Real-Time Cat. 2.027RT

Dihydropyrimidine dehydrogenase (DPYD) is the key enzyme involved in the catabolism of 5-fluorouracil (5-FU), the most widely used chemotherapy in the treatment of solid tumors, and of compounds with a similar chemical structure, being responsible for the degradation of this pyrimidine analogue. to its inactive metabolite dihydrofluorouracil. 80-90% of the 5-FU administered is degraded by the enzyme dihydropyrimidine dehydrogenase (DPYD) mainly in the liver. However, there is a great individual heterogeneity of enzymatic activity, which can vary up to 8-21 times. Patients with low enzymatic activity for DPYD are unable to efficiently inactivate 5-FU resulting in increased drug bioavailability which can cause severe haematological, neurological and gastrointestinal toxicities. Many single nucleotide polymorphisms have been highlighted in the complex structure of the DPYD gene responsible for an inefficient metabolism of the drug with a consequent increase in the risk of serious and potentially fatal toxicity; among them the most frequent is the point mutation G → A in a splice site of exon 14 (IVS14 + 1G> A), which appears to be involved in the correct splicing of exon 14. If present, this nucleotide substitution involves the loss of the exon itself and the formation of an incomplete protein product devoid of enzymatic activity. Although a high percentage of the 5-FU toxicities observed may be due to enzyme deficiencies distinct from DPYD and there are numerous mutations in the DPYD gene that can lead to the total absence of enzyme activity, the presence of this allelic variant (also known as allele DPYD * 2A) is present in approximately 1% of the general population and accounts for approximately 50% of all DPYD deficiencies. Furthermore, to cause enzymatic insufficiency and 5-FU toxicity, the presence of the substitution on only one of the possible alleles is sufficient. For these reasons, screening for this polymorphism in subjects requiring 5-FU-based chemotherapy may be of particular interest in clinical oncological practice. The presence of the homozygous DPYD * 2A genotype causes a complete lack of the DPYD enzyme, while the heterozygous genotype results in a partial deficiency of the enzyme itself. Two less common mutations, namely the I560S in position 1679T> G (also known as the DPYD * 13 allele) and the variant c.2846 A> T are associated with decreased DPYD activity and therefore with an increase in toxicity due to the 5 -FU, found in about 2% of patients treated with this chemotherapy. Isoleucine in position 560 is conserved both in humans and in other species (mouse, rat, bovine and pig), suggesting its importance in maintaining the enzymatic activity of DPYD. The DPYD * 13 mutation was not detected in individuals with normal DPYD enzyme activity.
Furthermore, according to today's guidelines on personalized pharmacological therapy, the investigation of the polymorphisms c.1236 G> A (rs56038477) and c.2194 G> A (1801160) is important in order to fully evaluate the risk / benefit ratio with the 5-fluorouracil therapy. The Ampli - DPYD Real-Time kit allows the search for the following polymorphisms by Real Time PCR technique with specific primers and probes: DPYD * 2A rs 3918290; DPYD * 13 rs 55886062; DPYD * 2846 rs 67376798; DPYD * 1236 rs 56038477, DPYD * 2194 rs 1801160.
The aforementioned mutations are recommended by AIOM-SIAPEC for patients in both pre and post therapy with fluoropyrimidines.

Principle of the method: A) genomic DNA extraction B) amplification C) detection using the real-time PCR device
Applicability: On genomic DNA extracted and purified from Whole Blood samples.
Number of tests: 25.
Stability: over 12 months if properly stored

The analysis of the results will be carried out by the specific program
(ALLELIC DISCRIMINATION) of the Real-Time tool
PCR previously set. In any case, however, it is also useful to analyze the AMPLIFICATION graphs PLOT, to make sure that the reaction took place in mode correct.
Heterozigous

Wild Type

CONTENUTO DEL KIT E SUA CONSERVAZIONE

AMPLIFICAZIONE	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X DPYD*2A	-20°C
Primer-probe mix 20X DPYD*13	-20°C
Primer-probe mix 20X D949V	-20°C
Primer-Probe mix DPYD*1236	-20°C
Primer-Probe mix DPYP*2194	20°C
Control W.T.	-20°C
Control Heterozygous of DPYD*2A	-20°C
Control Heterozygous of DPYD*13	-20°C
Control Heterozygous of D949V	-20°C
Control Wilde Type DPYD*1236	+4°C
Control Wilde Type DPYD*2194	+4°C
Control Eterozigote DPYP*2194	+4°C

Bibliografia:

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