The role of pharmacogenetics in the new ESMO colorectal cancer guidelines

“...the introduction of recommendations on pharmacogenetics represents a big move forward.”

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In July 2016, an updated version of the ESMO guidelines on the management of metastatic colorectal cancer (mCRC) was released. Three working groups have been involved in the development of a consensus regarding the following: molecular pathology and biomarkers, local treatments and systemic treatment of colorectal cancer (CRC) patients. Moving from a systematic review of relevant literature, the groups reached an agreement on recommendations for each specific topic [1].

In our opinion, the introduction of recommendations on pharmacogenetics represents a big move forward. While in the 2014 edition, no data on SNPs were reported [2]; in the present version a statement on *DPD*, *ERCC1*, *UGT1A1* and thymidylate synthase (*TS*) has been included.

TS is the main target of 5-fluorouracil (FU). Retrospective data associated a high number of repeat in the promoter region of *TS* with high levels of *TS* expression and no response to fluoropyrimidine-based therapy [3–8]. However, translational studies deriving from big randomized clinical trials such as the FFCD 2000-05 and the FOCUS studies, failed to confirm those findings [9,10]. Based on such considerations, we totally agree with the ESMO Committee opinion not to recommend *TS* activity evaluation and *TS* promoter region genotyping for their use in clinical practice.

ERCC1 is involved in the nucleotide excision mechanism of DNA repair and has been related to the activity of oxaliplatin. In particu-

lar, *ERCC1* levels can be measured by protein or mRNA expression. *ERCC1* -118 T allele has been related with high expression of *ERCC1* and proposed as surrogate biomarker of resistance to oxaliplatin. Two pivotal studies performed on mCRC patients treated with oxaliplatin showed an association of the *ERCC1* -118 T allele with a worse outcome [11,12]. However, such promising data were only partially confirmed in the FFCD 2000-05 study and denied in the US Intergroup N9741 study [10,13]. More recently, an attempt of prospective validation for intratumoral mRNA levels of *ERCC1* as predictor of resistance to oxaliplatin was carried out with the Phase II MAVERICC trial. Untreated mCRC patients were randomized to mFOLFOX6-bevacizumab or FOLFIRI-bevacizumab stratifying by baseline tumoral *ERCC1* mRNA expression. The primary objectives were to assess *ERCC1* as a biomarker of progression-free survival (PFS) for oxaliplatin and bevacizumab-containing regimens in first-line mCRC, and within patients with high levels of *ERCC1* expression, to test whether FOLFIRI-bevacizumab was associated with a prolonged 1st-line PFS compared with mFOLFOX6-bevacizumab [14]. No differences were observed according to the chemotherapy backbone for PFS or OS in 376 randomized patients. *ERCC1* levels in patients treated with mFOLFOX6-bevacizumab did not predict resistance to oxaliplatin, median PFS was 9.9 versus 11.0 months in patients with high and low levels, respectively (hazard ratio [HR] = 0.94; 95% CI: 0.66–1.34;

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$p = 0.744$). Moreover, among patients with high ERCC1 levels ($n = 131$), no differences in terms of PFS were observed in the two treatment arms (median PFS 9.9 months with mFOLFOX6-bevacizumab vs 11.2 months with FOLFIRI-bevacizumab, HR = 0.84; 95% CI: 0.56–1.26; $p = 0.394$) [15]. These results support the ESMO guidelines' recommendation, according to which ERCC1 expression should not be considered in routine clinical practice for therapeutic decision on the use of oxaliplatin.

“...ESMO guidelines on pharmacogenetics raise the issue of the overall low level of evidence deriving from studies in the field of genotyping.”

UGT1A1 is an enzyme of the glucuronidation pathway that transforms the active metabolite of irinotecan (SN-38) in an excretable metabolite. *UGT1A1* gene polymorphisms have been associated with an increased risk for neutropenia in patients receiving irinotecan. Among *UGT* polymorphisms, the most studied is a common TA repeat variant in the promoter region, so-called *UGT1A1**28. This genetic variant is associated with a significant decrease of SN-38 glucuronidation and reduced metabolism of irinotecan [16,17]. The ESMO guidelines suggest *UGT* genotyping as an option in the following situations: patients with a suspicion of UGT1A1 deficiency and patients where a dose of irinotecan >180 mg/sqm is planned. Similarly, the National Comprehensive Cancer Network guidelines version 2.2016 states that irinotecan should be used with caution and at a decreased dose in patients with Gilbert syndrome, but routine testing of *UGT* SNPs is not recommended and dose modifications according to genotyping is not a standard practice [18]. However, recently, a note was added to the irinotecan FDA label suggesting an initial dose reduction for patients carrying *UGT1A1**28 homozygous allele [19]. In the Phase III TRIBE trial, investigating FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab, the association of adverse events and *UGT1A1**28 was analyzed. Among 443 evaluable patients, those carrying the *UGT1A1**28/28 homozygous variant were at higher risk of developing grade 3/4 neutropenia compared with 1*/28 and 1*/1 carriers (59 vs 35% $p = 0.003$) irrespectively from the treatment arm [20]. Those data confirm the association of *UGT1A1**28 and neutropenia also when a reduced dose of irinotecan is administered (in both arms irinotecan dosage was <180 mg/sqm).

The role of *DPYD* genotyping is a highly debated issue; this enzyme is responsible for the first step of 5-FU inactivation. Deficiency in DPD activity leads to severe toxicity from 5-FU that may cause death [21].

Several assessment methods/techniques have been proposed to detect DPD deficiency or as indirect markers of altered DPD activity [22]. In our opinion, data so far available support the use of genotyping tests as the most reliable, low cost and easily reproducible technique. In the Caucasian population, the most common SNP of *DPYD* is IVS14+1G>A, also known as *DPYD**2, c.1905+1G>A or rs39182901905 G>A. This allelic variant is responsible for the lack of exon 14 in the mRNA transcript, resulting in the production of a truncated mRNA with virtually absent enzyme activity [23] and therefore posing at very high risk of severe toxicity from treatment with fluoropyrimidines [24]. The incidence of such variation is low (1% of patients carry the heterozygous variation). Thus, data deriving from several randomized trials where its predictive role was retrospectively investigated are underpowered for a formal validation despite their relatively large sample size [9,10,20,25]. ESMO recommendations regarding the use of DPD are partially conflicting. DPD activity is defined as a predictive biomarker of potential toxicity for fluoropyrimidine-based treatment. Moreover, a dosage adaptation is suggested for patients with known partial DPD deficiency and a different treatment option is recommended in case of complete DPD deficiency. Despite this strong statement, authors conclude that since there is no recommended standardized assessment technique, none of the current strategies are adequate to mandate routine DPD testing before starting fluoropyrimidine-based therapy and testing for DPD deficiency remains an option. We can assume that consensus regarding this issue was hardly reached leading to set IIc and IID as level of evidence for DPD testing in two different paragraphs.

Recently, new data emerged regarding the role of *DPYD* SNPs as predictive factors for toxicities in patients treated with fluoropyrimidines. Most importantly, a meta-analysis carried out by Meulendijks *et al.* including 7365 patients, demonstrated the clinical validity of four *DPYD* variants (*DPYD**2A, c.2846A>T, c.1679T>G and c.1236G>A/haplotype B3) as risk factors for fluoropyrimidine-associated toxicity [26]. Moreover, a large prospective study demonstrated the feasibility of upfront DPD screening in the daily practice. A total of 2038 patients were prospectively screened for *DPYD**2A and patients carrying the heterozygous *DPYD**2A variant received a 50% reduced fluoropyrimidine starting dose. *DPYD**2A genotype-guided dosing improved safety of fluoropyrimidine therapy for patients, reducing the incidence of severe toxicity in *DPYD**2A carriers from 73 (in historical controls) to 28% ($p < 0.001$), and the incidence of fatal toxicity from 10 to 0%. In addition, the cost analysis showed that DPD genotype-guided dosing was cost saving [27].

Moving from the above-specified reports, we believe that the level of evidence and grade of recommendation might be increased for *DPYD* genotyping. This was also underlined in two letters published by other experts in the field in reply to the ESMO guidelines [28,29].

From a more general perspective, the ESMO guidelines on pharmacogenetics raise the issue of the overall low level of evidence deriving from studies in the field of genotyping. Although many data are available concerning possible prognostic or predictive SNPs in mCRC patients, due to the large number of contrasting results and to the absence of confirmatory trials, genotyping is optional or not recommended in routine clinical practice. This is an important warning for clinicians and researchers involved in pharmacogenetics,

that are requested to make bigger efforts for increasing the level of evidence of their studies and to network in order to conduct prospective and well-designed pharmacogenetic-driven trials.

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