Peer Review File

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Review Comments

Comment 1: The authors of this paper provided an overview of the toxicity profiles of most of the anti-cancer agents used in breast cancer for the purposes of focusing on a personalized approach to treatment. Although the authors did a great job with highlighting the toxicities associated with the use of these agents, the authors did not provide their insights into how to individualize treatment.

Reply 1: Thank you for your comments. We provided some suggestions all along the manuscript on the potential ways to personalize treatment according to the biomarkers and factors analysed, based on the quality of evidences and recommendations available to date. We aimed to present the current evidence on the topic of precision oncology focused on toxicity and to provide some advices for the readers, but leaving them free to reflect on them without influencing them in an excessive way. Nonetheless, we added further insights on treatment individualisation according to our view in the Conclusion section (See Page 21, lines 549-553 – track-change version)

Changes in the text: See Page 21, lines 549-553 – track-change version: “Currently, the upcoming application of these findings in clinical practice is urgently needed to reach this objective, but further research is needed. We believe that these factors, if confirmed in the near future and in further studies, might be helpful in the individualisation of treatment; on one hand, they would allow a better selection of patients; on the other hand, they will permit to tailor the patients’ monitoring for toxicities in the perspective of an individualised I and improvement of clinical outcome.”

Comment 2: These are our comments to the authors: Fluoropyrimidines: the guidelines (including CPIC) recommend DPYD testing prior to initiating therapy with fluoropyrimidines for patients with colorectal cancers. Not quite sure this is applicable to the use of 5-FU and its derivatives in the breast cancer space.

Reply 2: Thank you for your comment. We believe that the DYPD testing should become clinical practice in the management of breast cancer patients who are candidates to fluoropyrimidines treatment in order to minimize potential toxicities and optimize the selection of patients. Indeed, DYPD testing would allow to adapt fluoropyrimidines dosing according to the DYPD variant identified, thus preventing unnecessary and potentially serious adverse events related to fluoropyrimidines administration in BC patients. Recent

studies have shown significant clinical and financial benefits of routine DPYD genotype screening application also in the BC setting. For these reasons, we decided to discuss this topic in our review. This will become even more important with the routine administration of adjuvant capecitabine at similar doses as those administered for colorectal cancer management, where DYPD testing is recommended. We added some data (see Page 5, lines 126-130 and Page 6, lines 131-132 – track-change version).

Changes in the text: See Page 5, lines 126-130 and Page 6, lines 131-132 – track change version: “For several years routine DPYD genotype screening prior to fluoropyrimidine administration has not been the standard of care for BC management. However, recent studies have shown significant clinical and financial benefits of routine DPYD genotype screening application also in this malignancy (21-26). Notably, this has increasing importance with the introduction of adjuvant capecitabine at similar doses as those for colorectal cancer in human epidermal growth factor receptor 2 (HER2)-negative, stage I–IIIB BC without complete pathologic response or with a complete response with positive lymph nodes after neoadjuvant chemotherapy and surgery (27).”

Comment 3: There are other agents used in treating breast cancer which the authors did not include in their review and there are genetic polymorphisms that have been interrogated for toxicity i.e. anthracyclines and aromatase inhibitors. I think these should be included in the paper. Although the evidence is not compelling enough for clinical implementation; it does set the framework for further investigation.

Reply 3: Thank you for your suggestion. We included a section on anthracyclines and the role of genetic polymorphisms in predicting adverse events, in particular cardiotoxicity, haematological and gastrointestinal toxicity (see Page 4, lines 84-104 and Page 5, lines 105-116 – track-change version). Moreover, we implemented the section “ENDOCRINE TREATMENT” with more data on aromatase inhibitors and genetic polymorphisms assessed for toxicity development predisposition (See Page 18, lines 451-461 – track-change version). References have been updated accordingly in the main text and in the reference list. We updated also table 2 (See page 41, Anthracyclines and 43, endocrine therapy- AI – track change version)

Changes in the text:

- Page 4, lines 84-104 and Page 5, lines 105-116 – track-change version:

ANTHRACYCLINES - Anthracyclines represent a cornerstone of BC treatment (7). Cardiotoxicity, myelosuppression, nausea and vomiting are the main AEs, for which various studies assessed potential predisposing factors. An analysis by Chen et al. in 211 BC patients treated with epirubicin-cyclophosphamide-docetaxel chemotherapy reported a significant correlation between fibroblast growth factor receptor 2 (FGFR2) rs2420946 CC genotype and higher AEs occurrence vs TT ($p=0.038$) and CT/TT genotypes ($p=0.019$); similar results were found for FGFR2 rs2981578 AG genotype vs GG genotype ($p<0.0001$)

(8).

Cardiotoxicity

Vaitiekus et al. identified a significant association between HFE gene H63D single nucleotide polymorphism (SNP) and subclinical cardiac damage in 81 BC patients treated with doxorubicin-based chemotherapy ($p<0.005$) (7). 18 SNPs of NFKBIL1, TNF-a, ATP6V1G2-DDX39B, MSH5, MICA, LTA, BAT1, and NOTCH4 were suggested as potentially related to doxorubicin-induced cardiotoxicity (9). A genome-wide association study in 3431 patients of three phase III adjuvant BC trials found an association of rs28714259 SNP with congestive heart failure (CHF) induced by anthracyclines (10). Vulsteke et al. identified 6 cycles of 5-fluorouracil, epirubicin and cyclophosphamide vs 3 cycles (OR 1.3, 95%CI: 1.1–1.4, $p<0.001$) and heterozygous status for ABCC1 rs246221 T-allele vs homozygous (OR 1.6, 95%CI:1.1–2.3, $p=0.02$) as significantly related to left ventricular ejection fraction (LVEF) reduction > 10 % in early BC (EBC) (11). Another study detected UGT2B7 -161 T allele as a potential independent biomarker of low occurrence of cardiotoxicity during adjuvant epirubicin-cyclophosphamide-docetaxel chemotherapy ($p=0.004$) (12).

Haematological toxicity

In a study by Cui et al. CBR1 rs20572 (C>T), ABCG2 rs2231142 (G>T) SNPs involved in anthracyclines pharmacokinetics or the combination of two polymorphic alleles were significantly associated to reduced risk of leukopenia (OR 0.412, 95%CI: 0.187–0.905, $p=0.025$) and neutropenia (OR 0.354, 95%CI: 0.148–0.846, $p=0.018$) in 194 BC patients receiving adjuvant anthracyclines. Moreover, patients carrying polymorphic allele T of CBR1 rs20572, or polymorphic allele C of AKR1A1 rs2088102 combined with ABCG2 rs2231142(G>T) plus SLC22A16 rs6907567(A>G) mutations showed an extremely low risk of grade 3-4 anaemia (OR 0.058, 95%CI: 0.006–0.554, $p=0.008$; OR 0.065, 95%CI: 0.006–0.689, $p=0.022$; OR 0.037, 95%CI: 0.004–0.36, $p=0.015$, respectively). Thus, these SNPs might be useful to identify which patients who are less likely to develop haematological AEs (13).

Gastrointestinal toxicity: nausea and vomiting

A study conducted in 110 BC patients treated with epirubicin +/- cyclophosphamide exploring the role of 5-hydroxytryptamine receptor 3 (HTR3C) genes for chemotherapy-induced nausea and vomiting (CINV), the variant genotype of K163N (HTR3C) was associated with vomiting ($p=0.009$) (14). Tsuji et al. suggested that TACR1 1323TT SNP, involving the gene encoding the neurokinin 1 receptor, might be a genetic risk factor for the development of delayed CINV (OR, 2.57; $P = 0.014$) (15).

- Page 18, lines 451-461 – track-change version)

AI administration might be associated with hot flushes and musculoskeletal AEs affecting quality of life. rs10046 variant T/T of CYP19A1 seemed to be associated to lower occurrence of hot flashes/sweating with exemestane and ovarian function suppression in premenopausal patients enrolled in the TEXT trial, thus improving patients' compliance to AI

treatment (146). Borrie et al. found that BC patients with higher body mass index (p=0.001) and those receiving letrozole vs anastrozole (p=0.018) were more likely to develop arthralgia and subsequently discontinue AI. Moreover, the Authors found that CYP19A1 rs4775936 and ESR1 rs9322336, rs2234693, rs9340799 SNPs were associated with occurrence of arthralgia (p=0.016, 0.018, 0.017, 0.047) and that CYP19A1 rs4775936 SNP was related to AI discontinuation for intolerable arthralgia (147). rs2073618 SNP in osteoprotegerin gene was found to be related with higher risk of muscoloskeletal symptoms and pain in 254 AI-treated (148). In a nested case-control correlative study by Niravath et al. in BC patients enrolled in the MA.27 trial, VDR Fok-I variant genotype was associated to lower incidence of arthralgia after 6 months of AI vs wild type VDR (p<0.0001) (149).

- Table 2: See page 41, Anthracyclines and 43, endocrine therapy- AI – track-change version

Comment 4: As for monoclonal antibodies such as trastuzumab and pertuzumab, have the authors considered adding some data on FC gamma receptor polymorphisms

Reply 4: Thank you for your suggestion. We added some data on the potential role of FC gamma receptor polymorphisms in predicting cardiotoxicity, which is the topic for which there is more evidence though limited (See Page 8, lines 200-206 – track-change version). References have been updated accordingly in the text and in the reference list.

Changes in the text: See Page 8, lines 200-206 – track-change version: As for the potential influence of FC gamma receptor (FCGR) polymorphisms, most studies focused on anti-HER2 efficacy and provided contrasting findings on FCGR2A and FCGR3A role (67,68). Limited data are available on FCGR SNP and toxicity. In a study by Cresti et al. in 101 HER2 positive EBC patients receiving trastuzumab every 3 weeks after adjuvant chemotherapy, FCGR2A His131Arg SNP was significantly related to trastuzumab-related cardiotoxicity occurrence (69). Roca et al. found a significant association between cardiotoxicity after trastuzumab and HER2–I655V genotype (p=0.025), but not with FCGR2A–H131R and FCGR3A–V158F SNPs (70). Though of interest, these findings require more extensive research to be confirmed.