Exploring the prognostic and predictive impact of genomic Loss of Heterozygosity and Homologous Recombination Deficiency alterations in metastatic colorectal cancer patients

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LOH is prognostic and predictive of benefit from ICIs in MSS mCRC

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Abstract

Purpose

Genomic loss-of-heterozygosity (gLOH) consists in the loss of chromosomal regions and is associated with homologous recombination repair (HRR) system deficiency. We explored the role of gLOH and HRR-related gene alterations in metastatic colorectal cancer (mCRC).

Methods

FoundationOne®CDx assay was used to determine the percentage of gLOH and the presence of alterations in 27 HRR-related genes in archival chemo-naïve tumour tissues of mCRC patients treated with first-line oxaliplatin- or irinotecan-based doublets and triplet ± anti-PD-L1.

Results

Overall, 243 samples were analysed. None of 9 dMMR/MSI-H tumours were gLOH-high, while 16 (7%) of 234 pMMR/MSS tumours were gLOH-high. In the pMMR/MSS population, 6 (3%) and 18 (8%) had at least a biallelic or monoallelic HRR-related gene alteration, respectively. Among patients receiving FOLFOXIRI alone (N=68) or with an anti-PD-L1 (N=90), higher benefit from the addition of the ICI was observed in the gLOH-high subgroup (N=12), in terms of both PFS (p_{int}=0.02) and OS (p_{int}=0.03). No differences in PFS or OS were reported between patients treated with first-line oxaliplatin- (N=40) *versus* irinotecan-based doublets (N=25) or with the triplet FOLFOXIRI (N=68) *versus* doublets (N=65), according to the gLOH *status*. Among patients not receiving an anti-PD-L1, longer PFS was observed in the gLOH-low (N=138) *versus* the gLOH-high (N=6) group (5.1 versus 12.1 months; HR: 8.73, 95%CI: 3.64-20.9; p<0.001), and this was confirmed in the multivariate

analysis ($p<0.001$). No prognostic impact of mono- or biallelic HRR-related gene alterations was shown.

Conclusions

In pMMR/MSS mCRC, gLOH-high was associated with worse prognosis and higher benefit from the addition of anti-PD-L1 agents to chemotherapy. If confirmed in larger series, these results may inform the design of clinical trials.

Introduction

Loss-of-heterozygosity (LOH) is a common genetic event in the development of cancer and consists in the loss of one copy of a segment of DNA containing a gene or group of genes that typically include a wild-type allele of a tumour suppressor in presence of one (copy number losses LOH) or two mutated alleles (copy number neutral LOH).[1] A genome-wide accumulation of LOH events (gLOH), as well as other structural variations, like large-scale state transitions (LST) and telomeric allelic imbalance (TAI), also referred to as “signatures” or “scars”, are associated with tumours bearing a deficient of homologous recombination repair (HRR) system.[2–5] HRR is a high-fidelity system involved in repairing double-stranded DNA breaks (DSBs) and in the resolution of stalled replication forks during cell division.[6, 7] In case of HRR-deficiency (HRD), tumour cells attempt to repair the DSBs through error-prone repair systems like non-homologous end joining, microhomology-mediated end joining, and single-strand annealing, accumulating DNA alterations.[8]

As a consequence, HRD tumours are highly sensitive to platinum salts, that are able to induce DSBs, and to PARP-inhibitors, that lead to a synthetic lethal response.[9–12] Currently, PARP inhibitors have entered the clinical practice for the treatment of ovarian, breast, and more recently, pancreatic and prostate cancers, harbouring HRD or deleterious mutations in *BRCA1/2* genes, key components of the HRR pathway.[13–16] In addition, preclinical studies showed that HRD increases immunogenicity enhancing neoantigen loads, tumor-infiltrating lymphocytes, tumour mutational burden (TMB), and PD-L1 expression.[17–21] Recently, phase II and III studies showed promising outcomes with the combination of the PARP inhibitor olaparib and the anti-PD-L1 durvalumab in patients with *BRCA1/2*-mutated metastatic breast cancer and HRD metastatic ovarian cancer.[22–24]

In metastatic colorectal cancer (mCRC), the role of HRR alterations is still widely unknown, and few data about their clinical impact are available.[25–27]

Based on these considerations, we evaluated the impact of gLOH and HRR-related gene alterations in predicting the efficacy of oxaliplatin-based and immune-checkpoint inhibitor-based treatments in mCRC patients included in a real-world registry of comprehensive molecular characterization and in two academic clinical trials, AtezoTRIBE and AVETRIC, investigating FOLFOXIRI (5-fluorouracil, oxaliplatin and irinotecan)/bevacizumab +/- atezolizumab and FOLFOXIRI/cetuximab plus avelumab, respectively.[28–31]

Methods

Study Population

AtezoTRIBE[28, 29] (NCT03721653) was a phase II randomized, open-label, multicenter trial where 218 patients with initially unresectable mCRC (18–70 years old with Eastern Cooperative Oncology Group performance status (ECOG-PS) of 2 or less or 71–75 years old with an ECOG-PS of 0) were randomized in a 1:2 ratio to receive FOLFOXIRI plus bevacizumab or the same regimen plus the anti-PD-L1 atezolizumab. All treatments were administered up to eight cycles, followed by 5-fluorouracil plus bevacizumab with or without atezolizumab, according to the randomization group, until disease progression, unacceptable adverse events, or consent withdrawal in both arms.

AVETRIC[30] (NCT04513951) was a phase II single-arm, multicentric trial where 62 patients with initially unresectable *RAS* and *BRAF* wild-type (wt) mCRC (18–70 years old with ECOG-PS of 2 or less or 71–75 years old with an ECOG-PS of 0) were treated with a modified schedule of FOLFOXIRI plus cetuximab and avelumab up to 12 cycles followed by maintenance with 5-fluorouracil plus cetuximab and avelumab until disease progression, unacceptable adverse events, or consent withdrawal.

Consecutive mCRC patients treated at Azienda Ospedaliero-Universitaria Pisana, undergoing comprehensive genomic profile as part of the molecular screening for the STAR-TRK2 protocol,[32] were included in a prospective registry.

In the present retrospective study, only patients with available clinical data of first-line treatments including clinical outcome and chemo-naïve archival tumour samples adequate for next-generation sequencing (NGS) analysis by means of FoundationOne®CDx were included.

Definition of genomic Loss of Heterozygosity

The percentage of gLOH was assessed as previously described,[33] and a cut-off of 14.08% was adopted to distinguish gLOH-high (gLOH score ≥ 14.08%) and gLOH-low (gLOH score < 14.08%) tumours, as already validated for colorectal cancer.[34] Further details are provided in the Supplementary Methods section.

HRR-related genes alterations

We focused on 27 genes involved in the HRR system[35] and included in the FoundationOne®CDx panel (*BARD1, BRCA1, BRCA2, BRIP1, MRE11A, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, RBBP8, XRCC2, ABL1, ATM, ATR, BAP1, CDK12, DNMT3A, ERCC4, FANCA, FANCC, FANCG, FANCL, PARP1*). Samples with no pathogenic or presumed pathogenic mutations, categorized according to the American College of Medical Genetics and Genomics standards, in any of the 27 HRR-related genes, were defined as wild-type, while the remaining samples as mono- or biallelic mutant tumours, based on allelic status. Monoallelic alteration was defined as an alteration documented in one gene copy with the other being wild-type, while biallelic mutations were called in the case of alterations in both gene copies or in only one gene copy with concurrent loss of the relative wild-type allele.[36]

Statistical analysis

Chi-square test, Fisher's exact test, or Mann-Whitney test were used when appropriate to compare clinical and molecular baseline characteristics among subgroups defined according to gLOH *status* and HRR-related gene alterations. Progression-free survival (PFS) and overall survival (OS) were defined as the time from the start of upfront chemotherapy to the first evidence of disease progression or death due to any cause, whichever occurred first, and as the time elapsed from the beginning of first-line

chemotherapy to death due to any cause, respectively. Survival curves were estimated by the Kaplan–Meier method and compared with the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CI) were estimated with a Cox proportional hazards model. Subgroup analyses according to gLOH status and treatments for PFS and OS were carried out using an interaction test. The impact of gLOH status and other prognostic factors on PFS and OS was firstly assessed in univariate analyses. Significantly prognostic covariates ($p \leq 0.10$) were included in a multivariable Cox proportional hazard model. Statistical significance was set at $p=0.05$ for a bilateral test. All analyses were performed with RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL http://www.posit.co/.

Results

Genomic Loss of Heterozygosity

Overall, 243 mCRCs were assessed for gLOH status. 234 (96%) and 9 (4%) tumours were proficient mismatch repair (pMMR)/microsatellite stable (MSS) and deficient mismatch repair (dMMR)/ (microsatellite instability high) MSI-H tumours, respectively (Supplementary Figure 1). The median gLOH score was 6.1% (interquartile range: 3.8 – 9.8%) (Figure 1A). All dMMR/MSI-H tumours were gLOH-low, whereas 16 (7%) out of 234 pMMR/MSS tumours were found gLOH-high. Considering the absence of gLOH-high cases among dMMR/MSI-H patients and the different biological characteristics between MSI-H/dMMR and MSS/pMMR tumours, the analyses were exclusively conducted in the pMMR/MSS group. The distribution of gLOH-high and gLOH-low patients across cohorts is reported in Supplementary Table 1, without evidence of a statistically significant difference between groups (p = 0.20). Baseline characteristics are listed in Table 1. As compared to patients with gLOH-low tumours, those with gLOH-high tumours had a higher percentage of primary tumour resected (100% *versus* 77%, p=0.03). In addition, gLOH-high tumours were more frequently *BRAF*^{V600E} mutated (25% *versus* 5%) with a lower percentage of *RAS* mutation (19% *versus* 54%) (p=0.003), Immunoscore-IC high (57% *versus* 18%, p=0.03), and had a higher occurrence of biallelic HRD alterations (21% *versus* 1%, p=0.004) as compared to gLOH-low tumours. Tumor Mutational Burden (TMB) distribution was superimposable between gLOH-low and gLOH-high tumors, both as a continuous (median: 3.8 *versus* 3.8 mutations/Megabase (Mb), respectively, p>0.99) and a dichotomous variable (TMB ≥ 10 mut/Mb: 8% *versus* 6%, respectively, p>0.99).

Overall, no differences were observed between gLOH-high and gLOH-low tumours in terms of both PFS (p=0.78) and OS (0.80) (Supplementary Figure 2A and B).

Among patients treated with doublet chemotherapy ± biologic agent (N=65), no different efficacy was observed between oxaliplatin- (N=40) and irinotecan- (N=25) based treatments, neither in the gLOH-high subgroup (N=4; p for PFS=1.0; p for OS=0.81) or in the gLOH-low one (N=61; p for PFS=0.39; p for OS=0.40) (Supplementary Figure 3A and B).

Similarly, no differences were reported between patients receiving triplet (N=68, including 2 gLOH-high tumours) versus doublets (N=65, including 4 gLOH-high tumours) chemotherapy backbone ± biologic agent based on gLOH *status* in terms of both PFS (p=0.34 for gLOH-high and p=0.08 for gLOH-low) and OS (p=0.15 for gLOH-high and p=0.95 for gLOH-low) (Supplementary Figure 4A and B).

On the other hand, a heterogeneous magnitude of benefit from the anti-PD-L1 administration seemed evident across subgroups based on gLOH *status*. In particular, among patients with gLOH-high tumours, although recognising the small size of this subgroup (N=12), longer median PFS (14.4 *versus* 3.6 months; HR: 0.16, 95%CI: 0.02-1.14; p=0.07) and OS (not reached *versus* 6.7 months; HR: 0.08, 95%CI: 0.01-0.95; p=0.045) were shown with the addition of the anti-PD-L1 to FOLFOXIRI ± biologic agent, while no difference was reported among those with gLOH-low tumours (N=146) (median PFS: 13.1 *versus 12.6* months; HR: 0.82, 95%CI: 0.58-1.16; p=0.26; median OS: 35.6 *versus* 33.2 months; HR: 0.98, 95%CI: 0.93-1.52; p=0.92) with a significant interaction between gLOH *status* and immune-checkpoint inhibitor administration in terms of both PFS (p=0.02) and OS (p=0.03) (Figure 2A and B).

Considering the impact of gLOH status on clinical outcomes with anti-PD-L1 agents, the prognostic effect of gLOH was assessed in the group of patients not treated with an immune-checkpoint inhibitor. In this cohort (N=144), patients with gLOH-high tumours (N=6) achieved shorter PFS (5.1 *versus* 12.1 months; HR: 8.73, 95%CI: 3.64-20.9; p<0.0001) than those with gLOH-low tumours (N=138) (Figure 3, panel A). This finding was confirmed at

the multivariable model (p<0.0001) (Supplementary Table 2). No difference was observed in terms of OS between gLOH-high and gLOH-low tumours (p=0.91) (Figure 3, panel B).

HRR-related genes alterations

Among pMMR/MSS tumours (N=234), 31 (13%) tumours harboured at least one HRR-related gene alteration, the most common being in *ATM* gene, detected in the 42% of cases, followed by *DNMT3A* (19%), *FANCA* (9%), *ATR* (9%), and *RAD54L* (6%). Alterations in *XRCC2*, *RAD41D*, *NBN*, *FANCL*, *BRCA2*, and ABL genes were found in the 3% of tumours each (Figure 1B). Overall, 18 and 6 (5 *ATM* and 1 *BRCA2* mutation) tumours had mono and biallelic HRR-related gene alterations, respectively. As expected, tumours with biallelic HRR-related genes alterations were more frequently LOH-high (p=0.004) (Table 2). No difference was observed among wild-type, monoallelic mutant and biallelic mutant patients in terms of PFS (p=0.86) and OS (p=0.25) (Supplementary Figure 5A-B).

Discussion

The identification of HRD and, in particular, *BRCA1/2* mutations as predictive biomarkers of efficacy of PARP inhibitors and platinum compounds, have enlarged the treatment armamentarium and improved outcomes in several tumours, especially of ovarian, breast, prostate and pancreatic origin. In addition, preliminary reports showed a correlation between HRD and ICI efficacy.[13–16, 22–24]

In mCRC, a recent retrospective study conducted by our group[27] using a large dataset of more than 9300 CRCs analysed by means of a next-generation sequencing assay[37] showed that alterations in HRR-related genes accounted for approximately 14% of tumours and were associate with gLOH-high in MSS/pMMR tumours. In addition, mCRCs bearing HRR-related gene alterations were more frequently TMB-high and PD-L1 positive and showed better prognosis than those with no alterations in genes of the HRR pathway. However, an important limitation of our study consisted in the lack of distinction between mono- and biallelic alterations of HRR-related genes as well as in the choice of a threshold value for the definition of gLOH-high (16%), that was translated from experiences in ovarian cancer. Indeed, a previous pan-cancer analysis revealed that biallelic pathogenic alterations in HRR-related genes were more often associated with the genomic features of HRD than mono-allelic mutations and that the cut-off values for the definition of gLOH-high varied across different cancer types.[34–36]

In the present study, we assessed the role of gLOH and alterations of HRR system genes in pMMR/MSS mCRC. Consistently with previous evidence by our research group, only the pMMR/MSS subgroup was enriched in LOH-high tumours, while no LOH-high cancers were found in the dMMR/MSI-H subgroup.[27] For the definition of gLOH-high, we adopted the cut-off of 14.08% previously defined for colon cancer,[34] while for HRR-system genes, we moved from the widest list of genes published by Riaz et al.[35] -including 102 genes- focusing on the

27 genes assessed by the FoundationCDx panel,[38] differentiating bi-allelic and mono-allelic pathogenic alterations of HRR-related genes.

Overall, about 7% of pMMR/MSS mCRC are gLOH-high according to the adopted definition, being slightly lower than the percentage reported in our previous work (9.6%),[27] although in the latter a higher threshold of 16% was adopted for the definition of gLOH-high, which should have intuitively resulted in a lower gLOH-high percentage. However, study populations were not comparable since also early-stages CRCs were included in the previous study.[27] Also, we acknowledge that the gLOH cut-off adopted based on a previous work on CRC (14.08%),[34] shows the highest mismatch between specificity (the highest: 91.46%) and sensitivity (the lowest: 49.49%), as compared to other solid tumours. We speculate that gLOH profiling in a homogenous cohorts including only mCRC may improve accuracy of gLOH threshold.

As expected based on both the biological background[17–21] and preliminary clinical reports,[22–24, 39, 40] gLOH-high[41] seems to predict the efficacy of adding an anti-PD-L1 to the triplet ± biologic agent with a significant p for interaction for both PFS and OS, although the small number of gLOH-high cases requires a cautious interpretation of results and subsequent conclusions. Similarly, considering the small sample size, we are not able to distinguish the predictive impact of gLOH and Immunoscore-IC, a biomarker of immune activation and a predictor of the benefit of the anti-PD-L1 addition to chemotherapy,[28, 29] nor to estimate the added value of gLOH on top of Immunoscore-IC .

After excluding patients receiving anti-PD-L1 combination treatments, whose survival was affected by gLOH status, gLOH-high was associated with poor prognosis, at least for PFS, as reported among patients with HRD breast and prostate cancers not treated with platinum compounds, PARP inhibitors or immunotherapy.[42, 43] Even if gLOH-high tumours were associated with adverse features, including *BRAF*^{V600E} mutation, the prognostic value of gLOH was confirmed at the multivariate analysis.

Unexpectedly,[25] in the gLOH-high group, higher efficacy of oxaliplatin-based *versus* irinotecan-based doublets was not reported. At the same time, when patients receiving anti-PD-L1-based treatments were excluded, no better outcome was observed for triplet *versus* doublet therapy in patients with gLOH-high tumours. To this regard, preclinical studies reported that also topoisomerase I inhibitors, as well as platinum compound, are able to induce DSBs and are more active in HRD tumours,[44, 45] while oxaliplatin results in fewer DSBs than other platinum agents with a relative lack of efficacy in HRD cells.[46] Even if in a retrospective analysis in metastatic pancreatic cancer, a HRD signature predicted the efficacy of first-line FOLFIRINOX *versus* gemcitabine plus paclitaxel, it is not possible to distinguish whether this increased benefit of the triplet in HRD tumours was due to oxaliplatin or irinotecan.[47] Moreover, in another retrospective study of mCRC patients treated with upfront 5-Fluorouracil or FOLFOX (5-Fluorouracil and oxaliplatin), no incremental benefit was observed with the addition of oxaliplatin based on a damage immune response signature that was associated with HRD in breast cancer.[48]

Differently from our previous report, HRR-related genes mutations showed no prognostic value. However, in the earlier study, we were not able to differentiate monoallelic and biallelic mutations and the panel of HRR genes was larger.[27] In addition, it should be acknowledged that mutations in different genes of the HRR system may have different effects and that HRR-related genes may not be of equal importance in terms of susceptibility or treatment response.[49] Indeed, in the present study, although an association between biallelic alterations of HRR genes pathway and LOH-high was observed, only 20% of LOH-high tumours harboured biallelic HRR-related genes mutations, and only the 50% of tumours with biallelic alterations of HRR genes were gLOH-high.

Unfortunately, the limited number of tumours harbouring biallelic HRR genes alterations did not allow assessing the predictive value of this molecular feature. A recent study showed no

different outcome in patients with biliary tract cancer treated with a platinum-based therapy according to HRR-related genes alterations not distinguished by allelic status.[50]

In conclusion, gLOH-high identifies a distinct subgroup of pMMR/pMMR mCRC with specific molecular characteristics and prognostic implications. Though acknowledging the limitations of a *post-hoc* analysis of two randomized trials and the incorporation of a not homogeneous cohort of patients treated as per clinical practice at a single hospital, the promising efficacy of the addition of immune checkpoint inhibitors to chemotherapy in this subgroup suggested by the present study deserves further investigation. The potential sensitivity of gLOH-high tumours to agents targeting the HRR system is worthy of clinical investigation especially considering that platinum-sensitivity does not predict efficacy to PARP inhibitors in unselected mCRC differently than ovarian cancers unselected for HRD.[51] Indeed, contrarily from preclinical data,[25] a recent a phase III study comparing first-line maintenance olaparib, alone or in combination with bevacizumab, *versus* bevacizumab plus a fluoropyrimidine in molecularly unselected mCRC not progressed after induction with first-line bevacizumab plus oxaliplatin-based doublet was prematurely closed for futility.[52] *Therefore, an appealing investigation may be a proof-of-concept trial to assess the efficacy of immunotherapy/PARP-inhibitors or their combination with chemotherapy in pMMR/MSS gLOH-high mCRC patients (i.e. induction chemotherapy plus immunotherapy followed by maintenance with PARP inhibitor plus immunotherapy).* It also remains to be defined whether other genomic tests may expand the number of HRD tumours compared with the gLOH assessment. However, HRD tests are developed and validated mainly in ovarian and breast cancers with a lack of harmonization across cohorts, cancer types,[34] treatments, and cut-off values, thus preventing to reach an overall *consensus* for HRD definition.[53] Although recent studies evaluating the harmonization of HRD tests showed feasibility of different laboratory-developed techniques and commercial assays to assess HRD in ovarian cancer,[54–59] further efforts are needed in mCRC.

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Figure Legends

Figure 1: distribution of gLOH scores (A) and type of HRR-related gene alterations (B)

gLOH: genomic loss of heterozygosity; HRR: homologous recombination repair

Figure 2: Progression-free survival (A) and overall survival (B) according to LOH score in patients treated with triplet ± biologic agent or triplet ± biologic agents + anti-PD-L1

Biol: biologic agent; CI: confidence interval; gLOH: genomic loss-of-heterozygosity; HR: hazard ratio; mOS: median overall survival; mPFS: median progression-free survival; NA: not assessable; NR: not reached; pop: population

Figure 3: Progression-free survival (A) and overall survival (B) according to LOH score upon exclusion of patients treated with anti-PD-L1

CI: confidence interval; gLOH: genomic loss-of-heterozygosity; HR: hazard ratio; mOS: median overall survival; mPFS: median progression-free survival; pop: population

Supplementary Figure Legends

Supplementary Figure 1: Consort diagram

dMMR/MSI-H: deficient mismatch repair/microsatellite instable; gLOH: genomic loss-of-heterozygosity; HRD: homologous recombination deficiency; NGS: next generation sequencing; pMMR/MSS: proficient mismatch repair/microsatellite stable

Supplementary Figure 2: Progression-free survival (A) and overall survival (B) according to LOH score in the overall population

CI: confidence interval; gLOH: genomic loss-of-heterozygosity; HR: hazard ratio; mOS: median overall survival; mPFS: median progression-free survival; pop: population

Supplementary Figure 3: Progression-free survival (A) and overall survival (B) according to LOH score in patients treated with irinotecan or oxaliplatin-based doublets ± biologic agents

Biol: biologic agent; CI: confidence interval; gLOH: genomic loss-of-heterozygosity; iri: irinotecan; HR: hazard ratio; mOS: median overall survival; mPFS: median progression-free survival; NA: not assessable; oxa: oxaliplatin; pop: population

Supplementary Figure 4: Progression-free survival (A) and overall survival (B) according to LOH score in patients treated with triplet or doublet ± biologic agents

Biol: biologic agent; CI: confidence interval; gLOH: genomic loss-of-heterozygosity; HR: hazard ratio; pop: population; mOS: median overall survival; mPFS: median progression-free survival; NA: not assessable; pop: population

Supplementary Figure 5: Progression-free survival (A) and overall survival (B) according to HRD *status*

CI: confidence interval; HR: hazard ratio; HRR: homologous recombination repair; mOS: median overall survival; mPFS: median progression-free survival; mut: mutation; pop: population