Whole exome sequencing of plasma circulating tumor DNA identifies dynamic mutational changes to guide targeted therapies in colon cancer patients

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Background

Despite the use of standard adjuvant treatment (ACT) based on fluoropyrimidines plus oxaliplatin, up to 80% of patients with localized colorectal cancer (CC) presents detectable circulating tumor DNA (ctDNA) related with relapse3. No biomarkers of response and resistance to ACT have been clarified and the molecular mechanisms of relapse remain unknown. Our aim is to identify the dynamic molecular changes at relapse, intratumoral heterogeneity (ITH) and potential novel therapeutic approaches to eradicate residual disease.

Methods

Whole exome sequencing (WES) of matched primary tumor, plasma ctDNA at relapse and white blood cells of 25 CC patients was performed. From 12 of them, plasma ctDNA at diagnosis was also available. Oncogenic variants were annotated according to COSMIC, OncoKB and an in-house database.

Functional enrichment analyses have been done by GSEA and cancer hallmark selecting processes with FDR < 0.05.

Results

❖ 33.9% of variants were exclusively observed in plasma at relapse (Fig 2), suggesting that ctDNA could provide a more extensive molecular information than the one coming from a single biopsy, offering a more complete picture of it and identifying new and valuable actionable mutations.

❖ At time of relapse a correlation between the dN/dS ratio and TMB values was found (p = 0.023), while this was not seen at baseline (Fig 4A). Parallel evolution in antigen processing and presentation pathways (HLA mutations) was observed as a distinct pattern of relapse. 269 genes showed a significant increase in the number of mutations per gene (Fig 4B).

❖ The targetable mutations were matched with precision drugs by OncoKB. 75% (9/12) and 80% (20/25) of these patients were found to harbor 1 or more pathogenic somatic variants at baseline and at relapse, respectively (Fig 5).

❖ Drug sensitivity assays demonstrated that our PDO model was more sensitive to molecular-matched therapies versus 5FU +/- oxaliplatin. PDO growth inhibition was observed with very low concentrations of Alpelisib and with the combination of PARP and MEK inhibitors and Adavosertib (Fig 6).

Conclusions

cDNA genomic profiling in CC patients

(1) identifies mechanisms of progression based on immune evasion and evolutionary selection of mutation load, showing the potential role for immunotherapy and (2) detects targeted alterations with therapeutic value which could eliminate residual disease more efficiently compared to ACT. PDOs may help in personalizing ACT.

Figure 1: Study design. The tumor type and the number of patients for both baseline and relapse stages are indicated, as well as for the PDOs.

Figure 2: Molecular landscape showing the oncogenic mutations and CNVs in 12 paired tissue and plasma baseline CC patients.

Figure 3: Venn diagram representing the number of mutations found at baseline (in green) and at relapse (in purple). Functional enrichment analysis by Hallmarks shows enriched pathways in mutated genes at baseline and relapse with FDR<5%. The fish plot represents the mutational events in the evolution of the disease.

Figure 4: A) Spearman correlation between TMB and dN/dS plasma samples at baseline (red) and at relapse (blue). B) Functional enrichment analysis of all significant genes with higher number of mutations at relapse in comparison baseline.

Figure 5: Potential targetable mutations detected in plasma. The treatment indicated by the OncoKb database for each mutation is shown on the right side of the table.

Figure 6: Dose-response curves of the PDOs models. Cell viability of the CT065 PDO according to increasing doses of the ACT and targeted agents.