REPORT
25th SOCIAL RETURN OF THE RESEARCH
CANCER

IMPLEMENTATION OF LIQUID BIOPSY BEYOND THE CURRENT APPLICATIONS: PROSPECTIVE STUDY OF THE PROGNOSTIC AND PREDICTIVE VALUE OF CIRCULATING TUMOR DNA IN METASTATIC COLORECTAL CANCER

Dr Elena Élez Fernández

VHIO Vall d'Hebron Institut d'Investigació Oncològica

Dra. Cristina Santos Vivas

Institut Català d'Oncologia - IDIBELL Institut d'Investigació Biomèdica de Bellvitge

1. Summary of the project

Despite significant advances in the treatment of metastatic colorectal cancer (mCRC), 5-year survival rates remain distressing. Better prognostic and predictive biomarkers of response are needed to identify patients at highest risk and implement personalized treatments.

Circulating free DNA (cfDNA) in blood plasma is a mixture of normal and tumor-derived DNA fragments (circulating tumor DNA, ctDNA). The process by which tumor cells release ctDNA into the bloodstream is known as shedding. The amount of cfDNA is substantially higher in cancer patients than in healthy individuals; the proportion of ctDNA to cfDNA is deduced from the mutated allele fraction (MAF). This can be analyzed using a liquid biopsy. Our group has shown that the MAF value of the *RAS* mutation (*RAS*-MAF) at the beginning of treatment significantly correlates with overall survival (OS). Patients with low MAF have better OS and tend to have better progression-free survival (PFS), so *RAS*-MAF in plasma would be a prognostic factor. An exploratory analysis demonstrated that patients with elevated *RAS*-MAF treated with antiangiogenic drugs and chemotherapy had a longer time to disease progression. Therefore, plasma *RAS*-MAF could depend on the quality of tumor vascularization. This project proposes to study the prognostic value of ctDNA in patients with mCRC treated in a first line of treatment and later; its correlation with tumor vascularization through histomorphological evaluation of the vasculature; as well as the predictive value of early changes in it due to the effect of antiangiogenic therapy using a state-of-the-art non-invasive technique in real time: liquid biopsy. We believe that the results obtained will directly impact the life expectancy and standard of care of patients with mCRC, and will allow us to identify other areas of research in tumors that could benefit from antiangiogenic treatments.

2. Results

The aim of this project is to study the prognostic and predictive value of response to antiangiogenic therapies of ctDNA in a prospective population of patients with mCRC; its linkage with the tumor vascularization status, and the predictive value of tumor vascular imaging for antiangiogenic therapy.

For this reason, an initial cohort of 185 mCRC patients treated in the first line with chemotherapy +/- antiangiogenics was included. Once the project started, it was decided to explore the cohort of patients treated with chemotherapy +/- antiangiogenics in second or later lines. From this second cohort, a total of 59 patients were included.

After analyzing the MAF values in the two proposed cohorts, the results conclude that MAF values serve as prognostic indicators for both patients treated in the first line with chemotherapy +/- antiangiogenics, and those treated in second or later lines. In both cases, overall survival is significantly higher in patients with a low MAF value compared to those with a high MAF value.

Regarding the objective of studying ctDNA as a predictive biomarker of response to first-line treatment with antiangiogenics, PFS was evaluated based on the treatment received (chemotherapy +/- antiangiogenics), and it was concluded that patients with a high MAF had better PFS when treated with chemotherapy + antiangiogenics compared to the group treated only with chemotherapy. On the other hand, patients with a low MAF showed no significant differences in PFS value based on treatment with or without antiangiogenics. Therefore, it can be concluded that MAF values have predictive value for the response to antiangiogenic drugs in the first line.

During the project execution, the correlation of MAF value with tumor vascularization was also evaluated through histomorphological evaluation of tumor vasculature. Out of the 185 patients included, the most extreme cases have been analyzed to date: 8 patients with the highest MAF value and 5 with the lowest value. The vasculature in these 13 samples showed significant variability in the structure and density of blood vessels compared to samples from patients analyzed during the first year of the project. This can be easily explained by dealing with extreme cases of MAF values compared to samples from patients with much more intermediate phenotypes. Additionally, within the project framework, a new technique has been developed to assess the permeability of blood vessels. Permeability could be a relevant factor in vessel structure since it could be an important factor in the extravasation of tumor DNA into the bloodstream, thus increasing the MAF signal. Methodologically, this technique is based on identifying vessel permeability by detecting extravasated red blood cells.

The initial statistical evaluation of "extreme" cases using this new technique has yielded promising results, which need to be confirmed and validated in the complete series of 185 patients, a validation that is underway.

One of the sub-objectives of the project was to evaluate parameters derived from multiparametric magnetic resonance imaging (mpMRI) in mCRC as predictive biomarkers of response to antiangiogenic therapies. To achieve this sub-objective, a prospective study was proposed with 20 patients who would undergo MRI for this evaluation. Unfortunately, the inclusion of prospective patients was not possible, and it was decided to study the retrospective CT scan images of the project cohort. Sixty-three patients from those included in the project, with hepatic metastases treated with chemotherapy +/- anti-angiogenics, were selected. The results indicate that phenotypes based on CT radiology can provide valid information on tumor characteristics, including vasculature, which is associated with the response to bevacizumab and higher levels of ctDNA MAF.

Finally, during the second year of the project, a new line of research based on animal models derived from patient-derived xenografts (PDX) was initiated to evaluate the possible concordance between tumor vascularization status and MAF value. In this regard, work has been done on developing PDX models capable of releasing ctDNA into blood. Two PDX models have been generated, one with the ability to release ctDNA into blood (G13D) and a second model without that capacity (G12D). This new tool will allow us to obtain more information about shedding and to evaluate, through a different approach, the prognostic value of *RAS*-MAF. Currently, a series of functional experiments are being conducted with these models treated with chemotherapy +/- anti-angiogenics.

3. Relevance with possible future implications

The formation of new blood vessels or angiogenesis plays a crucial role in cancer progression, making the inhibition of this process a promising therapeutic pathway. The importance of inhibiting angiogenesis in stopping tumor progression has been demonstrated, with various antiangiogenic targets identified in cancer treatment. However, implementation of these therapies presents clinical challenges, including side

effects and financial costs. Therefore, there is an urgent need to identify biomarkers capable of predicting patient responses to antiangiogenic therapy, thereby optimizing treatment outcomes and resource allocation.

Previous studies have demonstrated a correlation between *RAS*-MAF in circulating tumor DNA and prognosis in patients with mCRC. Based on that, our study aimed to extend these findings to the *RASwt* population. The results demonstrated that MAF levels of driver genes in ctDNA could serve as prognostic biomarkers for mCRC patients, potentially independently of the tumor mutational profile. This observation could help oncologists select patients with a more favorable prognosis and provide additional prognostic tools for daily clinical practice.

Furthermore, these results have been observed in patients treated both in the first line and subsequently, acting as a reliable biomarker throughout the patient's oncological history.

In this study, MAF analysis of ctDNA provides valuable predictive information. The results indicate that patients with high levels of MAF tend to have better PFS with the antiangiogenic drug bevacizumab, compared to those with high FAM treated without bevacizumab. In contrast, patients with low MAF showed no differences in PFS regardless of bevacizumab treatment. Although more prospective data are needed, incorporating ctDNA analysis into routine clinical practice allows clinicians to make more informed decisions about whether or not to select patients for treatment with bevacizumab.

In summary, our study underlines the clinical relevance of MAF as a prognostic biomarker in mCRC, highlighting its potential role as a predictive biomarker for antiangiogenic treatment. These findings have implications for personalized medicine and may pave the way for randomized controlled trials to validate these results.

In addition, the development of new tools, such as the one generated to evaluate the permeability of blood vessels; or models with the capacity to release ctDNA, will allow us to obtain more information about the shedding process, and evaluate the prognostic value of FMAF-*KRAS* using a different approach.

4. Generated scientific bibliography

Publications in scientific conferences

- Poster presentation at ASCO (American Society of Clinical Oncology) 2022; Chicago 3-7 June

Impact of circulating tumor DNA (ctDNA) mutant allele fraction in response to anti-angiogenic therapy in RAS-mutant metastatic colorectal cancer (mCRC): Clinical data in the first-line setting and correlation in patient-derived xenograft (PDX) models.

Authors: Nadia Saoudi Gonzalez, Javier Ros Montañá, Giulia Martini, Raquel Comas, Ariadna Garcia, Iosune Baraibar, Francesc Salva, Cabellos Laia, Gloria Castillo, Victor Navarro Garces, Raquel Perez-Lopez, Hector G. Palmer, Ana Vivancos, Josep Tabernero, and Elena Élez.

Publication: Journal of Clinical Oncology Volume 40, Number 16_suppl https://doi.org/10.1200/JCO.2022.40.16_suppl.3560

- Poster presentation at ISREC-SCCL SYMPOSIUM 2023; LAUSANNE, CH 21-24/08/2023

Authors: Felix Peix, Roser Pons, Mar Martínez, Oriol Casanovas