Exploratory longitudinal analysis of cfDNA reveals potential biomarkers of mCRC progression and treatment response

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BACKGROUND

• Accurate biomarkers to predict disease progression and therapeutic response in cancer patients are needed
• Reliable and prognostic blood-based tests are essential for the detection of circulating tumor DNA (cfDNA), which represents in full of all solid tumor RNA
• The majority of cfDNA studies have been performed on tumor samples or tissue biopsies, whose technical limitations together with cfDNA analysis offers a unique opportunity in identifying, better tumor and non-tumor derived biomarkers and prediction of prognosis and survival

OBJECTIVE

• The objective of this study was to identify biomarkers of cfDNA that may be associated with clinical outcomes, treatment with metastatic colorectal cancer (mCRC) receiving bevacizumab as either primary therapy or after chemotherapy failures

METHODS

Sample collection

• Plasma samples were collected longitudinally from stage IV CRC patients enrolled in FOCUS2 (NCT02655476) in which 80 metastatic patients with mCRC were treated by chemotherapy and/or chemotherapy plus bevacizumab. The median time of first response was 66 days (ranging from 11 to 214 days).

• Patients were stratified into progressors, non-progressors, and non-responders. Progression was defined as an increase in tumor burden of >20% (based on the worst tumor size at baseline). Non-progressors were defined as those who did not develop new lesions. Non-responders were defined as those who experienced an objective response but whose tumor burden did not decrease by >20%.

• Twelve patients were stratified per group (3 progressors, 3 non-progressors, 3 non-responders)

• Tumor fraction was inferred from cfDNA fragment length and counts around transcription start sites

Statistical analysis

• Statistical significance was estimated using Wilcoxon’s rank sum test and associated p values are shown

• Timepoint prior to CT scan was ~2 months +/- 1 month (mean 59 days, SD 36 days)

• Timing of CT scan was ~4 months +/- 2 months (mean 112 days, SD 36 days)

RESULTS

Exploratory longitudinal analysis of cfDNA reveals potential biomarkers of mCRC progression and treatment response

Table 1. Patient demographics

<table>
<thead>
<tr>
<th>Status</th>
<th>Non-Progressors</th>
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<th>Non-Responders</th>
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Figure 1. cfDNA captures tumor- and non-tumor-derived signals

Figure 2. Tumor fraction at baseline does not predict response or progression

• Baseline tumor fraction did not differ significantly between non-responses or progression or non-progressors

Figure 3. BMPR1A activation probabilities decreases significantly in responders

• BMPR1A activation is significantly lower in progressors over time

Figure 4. SMAD1 binding sites are more accessible in responders during treatment

• Estimation of SMAD1 binding sites might be the most important target during treatment

Figure 5. KIR2DL1 activation is significantly higher in progressors over time

• KIR2DL1 expression is significantly higher in progressors over time

Figure 6. Baseline KIR2DL1 activation may be associated with progression

CONCLUSIONS

• In this exploratory longitudinal study, we demonstrated the ability of our unique cfDNA platform to interrogate multiple features to reveal genes associated with metastatic CRC, drug response and their underlying mechanisms

• Even cfDNA were identified biomarkers associated with progression and response

• Decreased BMPR1A gene activation in responders

• Increased SMAD1 binding site accessibility in responders

• Increased KIR2DL1 gene expression in progressors

• These genes are involved in NK cell maturation, indicating a possible relationship between the obliteration of NK cell subpopulations and immunosuppressive response

• This work highlights the potential of cfDNA to provide biological insights beyond tumor fraction and that identification of non-tumor derived signals may benefit biomarker discovery and drug target identification

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