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
- Many predictive and prognostic blood tests in oncology rely on the detection of circulating tumor DNA (ctDNA), which represents only a fraction of all cell-free DNA (cfDNA)
- The majority of cfDNA originates from the immune system and, together with ctDNA, offers a unique opportunity to identify tumor- and non-tumor-derived biomarkers of predictive and prognostic value

OBJECTIVE

- The objective of this study was to identify biomarkers from cfDNA that may be associated with clinical outcomes in patients with metastatic colorectal cancer (mCRC) receiving andecaliximab (800mg Q2W IV)/ mFOLFOX/bevacizumab
- We explored the use of epigenetic signatures in total cfDNA to identify potential non-tumor-derived biomarkers associated with either disease progression or drug response (**Figure 1**)

METHODS

Sample collection

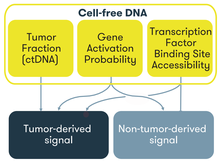
- Plasma samples were collected longitudinally from stage IV CRC patients enrolled in NCT01803282, in which 45 previously untreated metastatic CRC patients were treated with andecaliximab and standard doses of mFOLFOX6/bevacizumab. The overall response rate was 62%, median PFS was 10 months and OS was not reached. Andecaliximab is no longer being developed as an anti-cancer therapeutic.[1]
- Twelve patients were analyzed pre-therapy (baseline) and longitudinally during treatment (92 samples) (**Table 1**)
- Tumor assessments by CT or MRI were obtained after every 2 cycles of therapy (dosed day 1 and 15 of 28 days)

Table 1. Patient demographics

PT ID	Age	Sex	Best Objective Response (RECIST 1.1)	Response Status	Progression Status*	# of Samples Tested
104	75	F	CR	Responder	Non-Progressor	5
114	70	M	CR	Responder	Non-Progressor	3
117	52	M	CR	Responder	Non-Progressor	12
103	74	M	PR	Responder	Progressor	10
115	74	F	PR	Responder	Non-Progressor	10
116	53	F	PR	Responder	Progressor	7
124	54	F	PR	Responder	Non-Progressor	9
100	48	F	SD	Non-Responder	Progressor	8
102	62	F	SD	Non-Responder	Progressor	8
127	58	M	SD	Non-Responder	Progressor	6
131	56	M	SD	Non-Responder	Progressor	8
142	77	M	SD	Non-Responder	Non-Progressor	6

*Progression was defined by increased tumor size at the time of the clinical scan
CR = complete responder; PR = partial responder; SD = stable disease

Figure 1. cfDNA captures tumor- and non-tumor-derived signals

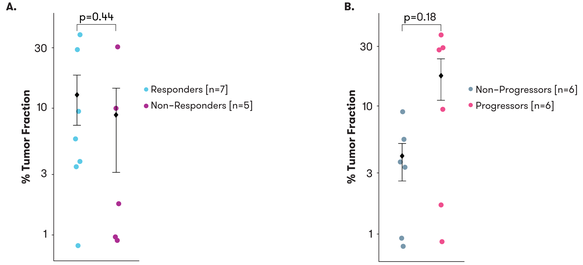


cfDNA analyses

- **Tumor fraction** was estimated using ichorCNA, which leverages somatic copy number alterations[2] (**Figure 2**)
- Whole-genome sequencing was performed and the **probability of gene activation** across each gene in the transcriptome was inferred from cfDNA fragment length and counts around transcription start sites[3] (**Figures 3 & 5**)
  - 59 genes curated from the literature were assessed when comparing baseline to time of first RECIST response (**Figure 3**)
- Transcription factor activity for 504 transcription factors was estimated by measuring **binding site accessibility** across the genome[4] (**Figure 4**)
- Statistical significance was estimated using Wilcoxon's rank sum test and associated p values are shown
- Significance across multiple time points over each patient group was assessed using a repeated measure ANCOVA
- Multiple hypothesis testing correction was applied by using FDR

RESULTS

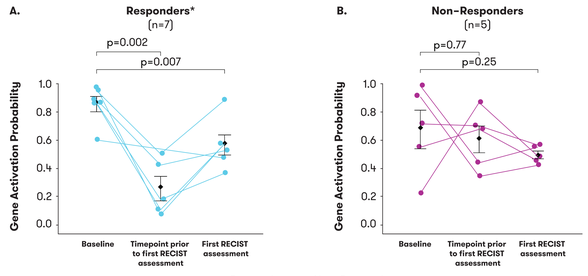
Figure 2. Tumor fraction at baseline does not predict response or progression



Mean and standard error are shown

- Baseline tumor fraction levels did not distinguish responders from non-responders or progressors from non-progressors

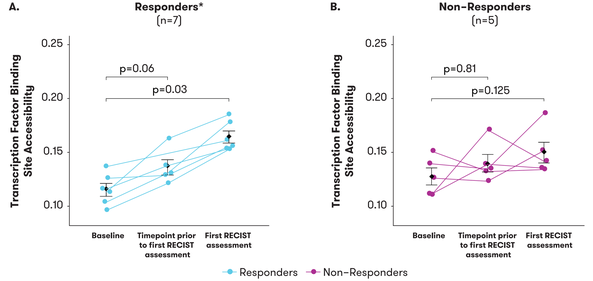
Figure 3. *BMPR1A* activation probabilities decrease significantly in responders



*One responder (PT ID 104) was excluded from this analysis because the time of response (CT scan) was missing
Timing of blood draw 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 48 days); Mean and standard error are shown; *BMPR1A* = bone morphogenetic protein receptor 1A

- Estimated *BMPR1A* activation probability decreased in responders following therapy administration ($p < 0.05$)
- This decrease was observed prior to the first RECIST response
- BMPR1A is a receptor for ligands of BMP2, which is a member of the TGF-ß superfamily known to be involved in cancer growth
- Germline mutations in *BMPR1A* cause CRC in patients with hereditary mixed polyposis syndrome[5]

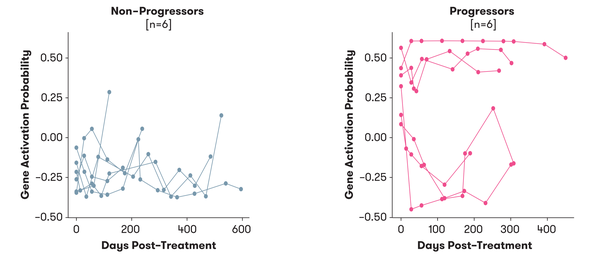
Figure 4. SMAD1 binding sites are more accessible in responders during treatment



*One responder (#104) was excluded from this analysis because the time of response (CT scan) was missing
BMP = bone morphogenetic proteins

- The DNA-binding activity of SMAD1 increased in responders post-therapy ($p < 0.05$) but did not increase in non-responders
- SMAD1 functions directly downstream of BMPR1A in the BMP2 pathway
- Activation of the BMP2 pathway induces NK-cell activity and inhibits the development of CRC[6,7]

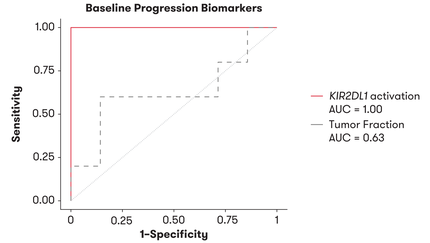
Figure 5. *KIR2DL1* activation is significantly higher in progressors over time



*One responder (#104) was excluded from this analysis because the time of first response was missing

- Gene activation probabilities were normalized by tumor fraction and compared over time between progression groups
- Patients with elevated activation of *KIR2DL1* progressed ($p < 0.0001$)
- KIR2DL1 inhibits cytotoxic activity in NK cells, suggesting a potential mechanism of progression involving immune suppression
- Levels of KIR2DL1 have been previously identified as a negative prognostic marker for survival[8,9]

Figure 6. Baseline *KIR2DL1* activation may be associated with progression



Disease characteristics were not included in this model

- Baseline *KIR2DL1* activation levels distinguished progressors from non-progressor with high accuracy
- Discriminatory power of *KIR2DL1* activation (compared to that of tumor fraction) was measured by the area under the ROC curve

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
- From cfDNA we identified biomarkers associated with progression and response:
  - decreased *BMPR1A* estimated gene activation in responders
  - increased SMAD1 binding site accessibility in responders
  - increased *KIR2DL1* estimated gene activation in progressors
- These genes are involved in NK cell maturation, indicating a possible relationship between the distribution of NK cell subpopulations and therapeutic 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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