Plasma-derived cfDNA reveals potential biomarkers for response prediction and monitoring in NSCLC patients on immunotherapy

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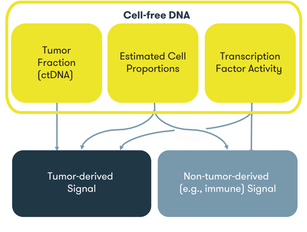
BACKGROUND

- Immune checkpoint inhibitors (ICI) have shown promising results in non-small cell lung cancer (NSCLC) with improved clinical responses (e.g., ORR, PFS, and OS) compared to chemotherapy[1]
- However, the clinical response rates can still be improved and a need for effective, predictive biomarkers remains
- Various molecular and cellular biomarkers, such as elevated levels of tumor-infiltrating cytotoxic T cells and Natural Killer (NK) cells at baseline, have been associated with ICI response[2,3]
- Accurate biomarkers in blood that predict response to ICI are needed; however, most blood tests rely on the detection of circulating tumor DNA (ctDNA), which represents only a very small fraction of total 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 associated with treatment response

OBJECTIVE

- The objective of this study was to investigate the potential of cfDNA biomarkers to predict response to the PD-1 immune checkpoint inhibitor nivolumab in patients with refractory metastatic non-small cell lung cancer (NSCLC)
- We explored the use of fragment size in total cfDNA to identify potential non-tumor-derived (e.g., immune) biomarkers associated with drug response and overall survival (**Figure 1**)

Figure 1. cfDNA reflects tumor- and non-tumor-derived (e.g., immune) signals



METHODS

- Plasma from stage IV NSCLC patients enrolled in ALCINA cohort 9 (NCT02866149) was collected before (baseline, BL, n=30) and at week 8 (on-treatment, WK8, n=17) of nivolumab therapy
- Response was determined using RECIST 1.1 (responders (PR) n=5; non-responders (SD+PD) n=25)
- Tumor fraction was assessed using ichorCNA[4] (**Figure 2**)
- Whole-genome sequencing was performed, and estimated cell proportions were determined by deconvolution of cfDNA co-fragmentation patterns[5] (**Figure 3**)
- Transcription factor activity for 504 transcription factors was estimated by measuring binding site accessibility across the genome[6] and normalized by tumor fraction (**Figure 4**)
- Statistical significance for cell proportions and binding site accessibility was estimated using Wilcoxon's rank sum test, and associated p-values are shown. Significance for tumor fraction analysis was estimated using T-test since it was normally distributed on a log10 scale. p-values are reported without false discovery rate correction due to the small sample size.
- Cox regression analysis was performed to assess the association between estimated cell proportions and overall survival, and is presented as hazard ratios

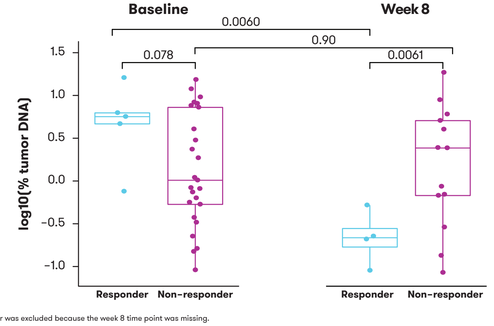
Table 1. Patient demographics

Patient Number	Timepoint Tested	Gender	Line of Therapy	Best Overall Response (BOR) RECIST 1.1	Response Status at Week 8	PFS Duration (mo)	OS Duration (mo)
01-002	BL	M	6	PD	Non-Responder	3.0	12.3
01-003	BL	F	2	SD	Non-Responder	5.8	37.9
01-005	BL & WK8	M	2	PD	Non-Responder	1.9	3.2
01-007*	WK 8	F	3	PD	Non-Responder	5.2	30.3
01-008	BL & WK8	F	6	PR	Responder	30.0	30.0
01-014	BL	M	2	PD	Non-Responder	1.9	4.6
01-015	BL	M	5	PD	Non-Responder	0.9	3.5
01-016	BL & WK8	F	2	PD	Non-Responder	1.8	5.7
01-017	BL & WK8	M	2	PD	Non-Responder	3.5	29.0
01-018	BL	F	6	PD	Non-Responder	3.2	5.8
01-019	BL	F	3	PD	Non-Responder	1.5	4.7
01-023	BL & WK8	F	2	PR	Responder	17.3	28.4
01-028	BL & WK8	M	4	PD	Non-Responder	1.9	6.3
01-029	BL	F	6	PR	Responder	27.4	27.4
01-037	BL & WK8	F	5	PD	Non-Responder	2.7	13.3
01-038	BL & WK8	M	5	PD	Non-Responder	6.2	13.5
01-045	BL & WK8	F	2	PD	Non-Responder	1.7	5.2
01-048	BL & WK8	F	4	PR	Responder	13.0	26.0
01-049	BL & WK8	M	3	PD	Non-Responder	2.8	4.9
01-065	BL & WK8	M	2	PD	Non-Responder	8.9	8.9
01-066	BL	M	2	PD	Non-Responder	1.8	2.1
01-068	BL & WK8	M	2	PD	Non-Responder	3.4	12.9
01-070	BL	M	3	PD	Non-Responder	1.9	6.8
01-071	BL	M	2	PD	Non-Responder	1.4	4.7
01-072	BL	F	6	PD	Non-Responder	1.4	3.1
01-073	BL & WK8	M	4	PD	Non-Responder	2.0	10.8
01-074	BL & WK8	F	2	PD	Non-Responder	1.0	2.2
01-076	BL	F	2	PD	Non-Responder	1.7	21.5
01-077	BL	M	2	PD	Non-Responder	2.1	2.1
01-079	BL	M	2	PD	Non-Responder	1.7	4.1
01-080	BL & WK8	F	5	PR	Responder	13.7	18.2

Responder=PR; Non-Responder=SD + PD; PR=partial response; SD=stable disease; PD=progressive disease; BL=baseline; WK8=week 8 on therapy; OS=overall survival; PFS=progression-free survival

RESULTS

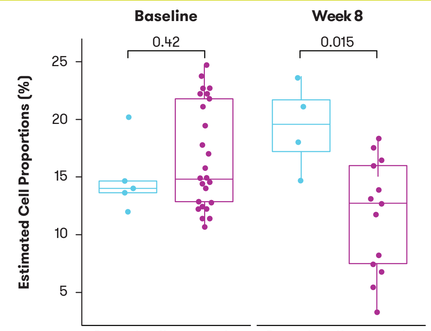
Figure 2. Baseline tumor fraction does not predict response



One responder was excluded because the week 8 time point was missing.

- Tumor fraction does not distinguish responders from non-responders at BL (p=0.078) but does at WK8 (p=0.0061)
- As expected, a significant decrease in tumor fraction was observed from BL to WK8 in responders (p=0.0060) but not in non-responders (p=0.90)

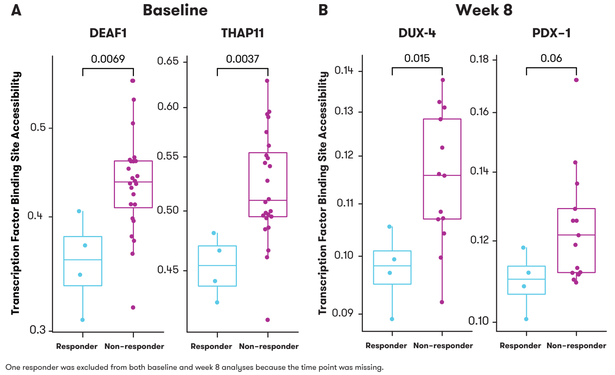
Figure 3. Elevated levels of monocytes at week 8 of therapy are associated with response



Though preliminary results reported in the abstract indicated estimated NK cell levels differed between responders and non-responders at BL, subsequent analyses demonstrated that this result was not significant. One responder was excluded because the week 8 time point was missing.

- Cell-mixture deconvolution from cfDNA revealed different immune cell proportions in responders compared to non-responders
- At week 8 of nivolumab therapy, estimated monocyte levels were strongly associated with response (p=0.0034); no difference was observed at baseline

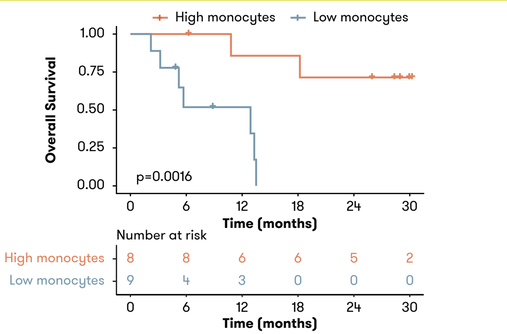
Figure 4. Immune-related transcription factors have lower accessibility in responders at baseline and week 8 of nivolumab therapy



One responder was excluded from both baseline and week 8 analyses because the time point was missing.

- In responders, lower accessibility was observed for DEAF1 (p=0.0069) and THAP11 (p=0.0037) at baseline, and for DUX-4 (p=0.015) and PDX-1 (p=0.06) at week 8
- Prior expression analyses have shown DEAF1 is preferentially expressed in T-cells and macrophages, and THAP 11 is preferentially expressed in T-cells, myeloid dendritic cells and basophils[7]
- DUX-4 is preferentially expressed in B-cells and interacts with P53[7,8]
- PDX-1 is preferentially expressed in plasmacytoid dendritic cells[7]

Figure 5. Estimated monocyte levels at week 8 are associated with overall survival



- Subjects were divided into high and low monocyte groups based on median value for survival analysis
- In subjects with high monocytes, an overall survival benefit was observed (HR=15.02, p=0.0016)

CONCLUSIONS

- In this study, our cfDNA platform identified cell-free biomarkers associated with nivolumab response in stage IV NSCLC patients
- The cellular proportion of monocytes at week 8 of nivolumab therapy was associated with response
- In responders, decreased DEAF1 and THAP11 accessibility was observed at baseline and decreased DUX-4 and PDX-1 accessibility at week 8 of nivolumab therapy
- Higher levels of monocytes at week 8 were associated with more favorable overall survival
- Plasma cfDNA signatures may be useful for response prediction and monitoring in NSCLC patients on immunotherapy
- Changes in the immune system, as reflected by cellular composition and transcriptional activity inferred from cfDNA, may provide biological insights beyond tumor fraction alone that may benefit biomarker discovery and drug target identification

ACKNOWLEDGMENTS

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