Tumor cell-free DNA copy number instability (CNI) to predict therapeutic response to immunotherapy prior to cycle 2

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Background

Tumor cell-free DNA (cfDNA) provides minimally invasive patient specific biomarkers to monitor tumor burden. Gains and losses of chromosomal regions have been detected in plasma as copy number aberrations (CNAs). Tregs are reported to be modulated by immunotherapy (immuno). We measured CNAs changes during treatment by computing a genomic copy number instability index (CNI) of cfDNA and Treg-specific demethylation region (TSDR) as measure for Tregs% of leukocytes compared to response.

Methods

In this prospective study, prior to treatment and before each cycle, extracted plasma-DNA was subjected to shallow whole genome sequencing with a post mapping (HG19) read coverage of 24,000-fold per 5.5Mbp bin. Read counts were transformed into (log2 ratio) Z-values, and a CNI-score was calculated. % of TSDR+ leukocytes (TSDR %) were quantified with digital PCR from PBMC DNA. Primary endpoint was best overall response by imaging (irRECIST and RECIST 1.1). Hypotheses were: a) response to immuno is reflected by CNI change vs. baseline during therapy an b) alters TSDR%. Outcome was unblinded for analysis.

Results

Of 27 enrolled patients (pts), 23 were assessable for response (Table 1): 4 advanced melanoma (MEL), 2 renal cell carcinoma (RCC), 5 gastrointestinal, 4 pulmonary, 3 breast, 1 ovarian cancers, 3 pancreatic adenocarcinomas, and 1 sarcoma. MEL and RCC received interleukin-2, while the rest received anti-PD-1 with chemotherapy on trials. Median age was 58 years, 12 were women. CNI was measured in 69 samples. Mean baseline cfDNA was 8,076 (CI^{90th}:2,894-13,258cp/mL) and CNI was 2,222 (CI^{90th}:1,162-3,282). Pts with partial response (PR) or stable disease (SD) (n = 12) showed a significant decrease in CNI before cycle 2 (C2) (Mean: -1,386 vs.+271; $p < 0.02$), before cycle 3 (C3) (-2,524 vs.+698, $p < 0.005$), and thereafter ($p < 0.002$) compared to pts with disease progression (PD). Figure 1 illustrates the CNI changes on immuno vs. pre-therapeutic values for early responders (PR +SD) vs. early PD. Total cfDNA was not correlated to CNI nor to response. TSDR% showed a decrease in all patients ($p < 0.02$; prior to C3) with immuno, independent of response (Figure 2). Figure 3 shows the prediction power of PR vs. PD by CNI over time course of treatment as ROC curves. Already after the first cycle of immuno 14 of17 patients are classified correctly, reaching an 88% accuracy before C3, which remained stable over the overviewed time course (up to 6 cycles).

Table 1. Clinical Characteristics with outcome and CNI Changes

Patient #	Age	Gender	Diagnosis	Type of Chemotherapy	Stage	Outcome	DCNI C2	DCNI C3	DCNI last Cycle
1	55	Female	PDAC	Gemcitabine/nab-paclitaxel/ pembrolizumab	4	PR	-1998	-2006	-2061
2	56	Female	OC	Liposomal doxorubicin/pembrolizumab	4	PR	-1702	-3922	-4992
3	59	Male	lung adenocarcinoma	Irinotecan/pembrolizumab	4	PR	625	-3251	-5051
4	51	Female	Melanoma	Interleukin-2	3B	PR	14	8	47
5	62	Male	PDAC	Gemcitabine/nab-paclitaxel/ pembrolizumab	4	PR	-5292	-5614	-5628
6	60	Male	SCLC	Irinotecan/pembrolizumab	4	PR	-3517	-6761	-1041
7	63	Male	PDAC	Gemcitabine/nab-paclitaxel/ pembrolizumab	4	SD	-3	38	38
8	56	Male	RCC	Interleukin-2	4	SD	-32	-442	-445
9	57	Female	Melanoma	Interleukin-2	4	SD	-4	-21	13
10	65	Male	RCC	Interleukin-2	4	SD	-36	-27	-16
11	72	Male	CRC	CAPIRI/nivolumab	4	SD	-4141	-4442	-4442
12	27	Female	Sarcoma	Gemcitabine/vinorelbine/pembrolizumab	4	SD	-545	-3847	-3847
13	40	Male	Gastric adeno	CAPIRI/nivolumab	4A	PD	76	67	-35
14	51	Female	SCLC	Irinotecan/pembrolizumab	4	PD	3007	-187	4669
15	55	Female	BC	Gemcitabine/vinorelbine/pembrolizumab	4	PD	-145	-187	3679
16	50	Male	CRC	CAPIRI/nivolumab	4	PD	-29	97	0
17	58	Female	BC	Liposomal doxorubicin/pembrolizumab	4	PD	3129	8667	1286
18	46	Female	lung adenocarcinoma	Investigational drug/pembrolizumab	4	PD	-513	-325	-1152
19	63	Male	GEJ adeno	PD-1/Radiotherapy	4	PD	-1795	-604	10082
20	60	Female	CRC	CAPIRI/nivolumab	4	PD	-439	-469	-396
21	66	Male	Melanoma	Interleukin-2	4	PD	0	-2133	4
22	62	Female	Melanoma	Interleukin-2	4	PD	-310	-314	-372
23	58	Female	BC	Gemcitabine/vinorelbine/pembrolizumab	4	PD	1	3067	3067

Key: PDAC-pancreatic ductal adenocarcinoma, OC-ovarian cancer, SCLC-small cell lung cancer, RCC-renal cell carcinoma, CRC-colorectal adenocarcinoma, Gastric adeno-gastric adenocarcinoma, BC-breast cancer, GEJ adeno-gastro-esophageal junction adenocarcinoma, CAPIRI-capecitabine/irinotecan, DNCI-absolute difference in CNI to baseline measured prior to: Cycle 2(C2), Cycle 3(C3) or last cycle

Figure 1: Early Responders vs. Early Disease Progression after initiation of immunotherapy

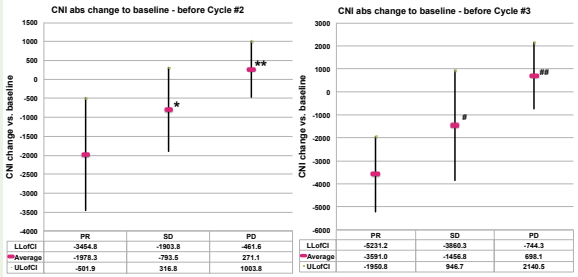
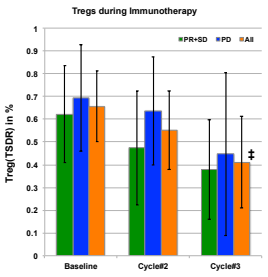
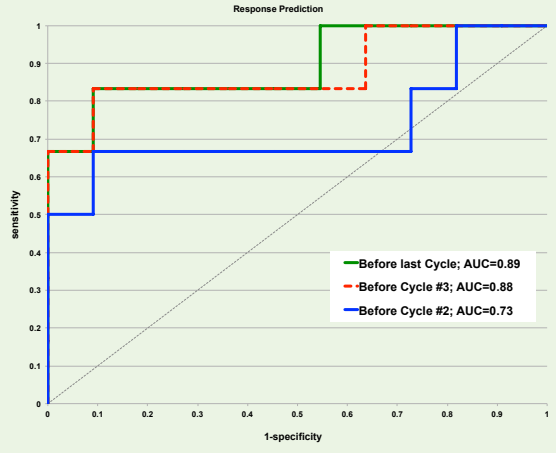


Figure 2: TSDR time course



Averages with 5% confidence limit (LLofCI) and 95% confidence limit (ULofCI) are given for patients with PR (n=6), SD (N=6), and PD (n=11); Significance vs Early Responders: *) p=0.1; **) p=0.03; #) p=0.05; ##)p=0.0035. ‡) Significance of entire group vs. baseline: p<0.02. Abs-absolute

Figure 3: Prediction of Immunotherapy Response by CNI



When PR patients were compared to PD prior to C3, 15|17 patients are classified into the right outcome category by CNI.

Conclusions

CNI change vs. baseline predicted response before C2 approximately 3-12 weeks prior to scan results, and therefore may serve as early predictor of therapeutic response to immuno. Immuno lowers Tregs % in blood, which might reflect T-cell activation, but does not differ between outcomes.

Relevant Reference and Acknowledgments

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