Immunotherapy in advanced melanoma: crossing borders

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CHAPTER
9.2

Preliminary results phase I of the ACTME trial

Results

Patients and treatment

Between November 2018 and September 2019 all nine patients from cohort 1 started their treatment in the ACTME trial. Between January 2020 and May 2021 the nine patients in cohort 2 all started their treatment. In the first cohort, nine patients were treated with ACT and anti-PD-1. After every group of three patients, the data was presented to the DSMB. They concluded that the combination treatment of cohort 1 was safe and supported to start cohort 2. Hereafter, nine patients were treated with the final combination of ACT, anti-PD-1 and PEG-IFNa. Again after treating every 3 patients, the safety data was presented to the DSMB.

The baseline characteristics of all 18 patients are shown in Table 1. Four patients who signed the Patient Information Folder were eventually unable to start the trial due to fast progressive disease, and are therefore not included in Table 1. In one of these patients the fast disease progression followed after cessation of BRAF/MEK inhibition, and one patient already had fast disease progression during treatment with BRAF/MEK inhibition. Of the four excluded patients, three were men, the mean age was 62.8 years. The majority of the evaluable patients treated in the trial were men (72.5%), the mean age was 53.5 years, and one-third of patients had brain metastases at the time of inclusion. According to the inclusion criteria, all patients had progressed on anti-CTLA-4 and anti-PD-1 treatment.

Safety

None of the treated patients experienced TIL-related adverse events (Table 2). Seven patients (38.8%) experienced grade 1 adverse events, two patients (11.1%) had grade 2 adverse events, and one patient (5.5%) suffered from grade 3 diarrhea. All other 7 patients (38.8%) did not report any adverse events.

TABLE 1 Patient characteristics at baseline

Patient	Cohort	Age	Gender	WHO	LDH	CNS metastasis	Pre-treatments	Location metastasis	TIL culture location
2	1	48	Male	0	158	No	BRAF/MEK, aPD1, aCTLA4	LN	LN
4	1	47	Female	1	270	No	BRAF/MEK, aPD1&aCTLA4, TIL	Bone, lung, muscle, adrenal, liver	Bone (sternum)
5	1	54	Male	0	230	Yes	aCTLA4, aPD1	LN, lung	LN
6	1	60	Male	1	224	No	aPD1, aCTLA4, aPD1	Muscle, lung, liver, peritoneum	Liver
7	1	53	Female	0	197	No	aPD1, BRAF/MEK, aCTLA4	LN, liver	Liver
8	1	40	Male	0	217	Yes	aPD1&aCTLA4, BRAF/MEK, aPD1	Subcutaneous, Liver, gall bladder, cardiac	Subcutaneous
9	1	53	Male	1	241	No	aPD1, aCTLA4	LN, subcutaneous, lung, liver kidney, GI/peritoneum	Subcutaneous
10	1	75	Male	1	258	No	aCTLA4, aPD1	LN, liver	Liver
11	1	53	Male	0	220	No	aPD1&aCTLA4, BRAF/MEK	LN, liver, lung	LN
12	2	26	Female	1	243	No	aPD1, BRAF/MEK, aCTLA4	LN, fatty tissue leg,	Cutaneous
13	2	73	Female	0	374	Yes	anti-PD1, anti-CTLA4	LN, spleen, lung, pleura, brain	LN
16	2	42	Male	0	314	Yes	Dendr cells, aPD1, aCTLA4, aAXL	LN, spleen	LN
18	2	50	Male	0	356	No	aPD1, aCTLA4	LN, liver	liver
19	2	39	male	1	403	No	aCTLA4, aPD1, aPD1&aCTLA4	LN, bone, lung	bone
20	2	75	male	1	210	Yes	aPD1, aCTLA4	LN, adrenal gland, fatty tissue	LN
21	2	49	Male	1	174	No	aPD1, aCTLA4	Parotid gland, LN, (sub)cutaneous	LN
22	2	62	Male	1	201	No	aPD1, aCTLA4, ACTME	LN, lung, liver, muscle	Subcutaneous
23	2	64	Female	1	313	Yes	aPD1&aCTLA4, aPD1, imatinib	LN, adrenal gland, lung, omentum, bone	LN

WHO: World Health Organisation Performance Status, LDH: Lactate dehydrogenase, aPD1: anti-PD-1, aCTLA4: anti-CTLA-4, dendr cells: dendritic cell therapy, aAXL: anti-AXL, LN: lymph node, TIL: Tumour Infiltrating Lymphocytes

TABLE 2 Response to treatment

Patient	TIL-related toxicity (S)AE	Any treatment related (S)AE	Best overall response	Duration of response
2	None	None	PD (MR)	n.a.
4	None	None	PD (MR)	n.a.
5	None	Grade 1 headache	PD	n.a.
6	None	Grade 1 headache, grade 1 hypertension	**PR**	22 months
7	None	None	PD	n.a.
8	None	None	PD (MR)	n.a.
9	None	None	PD (MR)	n.a.
10	None	Grade 3 diarrhea	PD (MR)	n.a.
11	None	None	PD (MR)	n.a.
12	None	Grade 1 fever	PD	n.a.
13	None	None	**SD**	9 months
16	None	Grade 1 rash, grade 1 leukopenia	PD (**irRECIST: SD**)	n.a.
18	None	Grade 1 itch, grade 1 fatigue	**SD**	16 months
19	None	Grade 2 diarrhea, grade 1 rash, grade 1 leukopenia	PD	n.a.
20	None	Grade 1 Lymphopenia, grade 1 itch, grade 1 lethargy	PD	n.a.
21	None	Grade 1 thrombocytopenia, grade 1 itch	**ongoing PR**	11 months+
22	None	Grade 2 hepatitis, grade 1 fever, grade 1 fatigue	PD	n.a.
23	None	Grade 2 anemia	PD	n.a.

TIL: Tumor Infiltrating Lymphocytes, (S)AE: (serious) adverse event, PD: progressive disease, MR: mixed response, PR: partial response, SD: stable disease, irRECIST: immune-related response evaluation criteria in solid tumors, n.a.: not applicable

Clinical responses

In total, disease control was observed in 5 out of 18 patients (27,8%). In cohort 1, one out of nine patients (11,1%) responded and obtained a partial response. In cohort 2, four out of nine patients (44,4%) responded; two patients obtained a stable diseases and one a partial response according to RECIST1.1. In addition, one patient obtained a SD according to immune-related response criteria (irRC) (Table 2, Figure 1). The duration of the responses is shown in Table 2 and Figure 1. In Figure 1 also the duration of response to the previous treatments is depicted. Interestingly, patient 6, who had a partial response to treatment in cohort 1, initially responded but developed resistance to treatment with anti-PD-1 just before inclusion in our trial, while patient 21 with an ongoing partial response to the treatment in cohort 2, displayed primary resistance to previous treatments with anti-CTLA-4 and anti-PD-1 (Figure 1).

The size of the target lesions in cohort 1 (Figure 3) and cohort 2 (Figure 4) was followed in time. In patient 6 a long lasting partial response was observed. In patient 8 a relatively large metastasis disappeared over the course of the first treatment cycle. Multiple patients (# 2, 4, 8, 9, 10, 11) display some form of mixed response, as some lesions become smaller, while others grow (Figure 3).

The same pattern can be seen in the patients in cohort 2 (Figure 4). There, patient 21 has an ongoing partial response, and patient 13 had stable disease of the target lesions but was eventually defined as progressive because a new lesion appeared. Patient 16 displayed a mixed response when the target lesions were considered, but also developed new lesions resulting in progressive disease.

Translational studies

Although most translational studies including immunohistochemistry and serum/plasma marker tests are still ongoing, an initial test already showed that patient 13 with a stable disease for 9 months following treatment in cohort 2 still had an HLA class I proficient tumor, while non-responders 2, 5, and 7 all had lost their HLA class I before inclusion in the ACTME trial (Figure 1).

In contrast to what might have been expected based on our previous trial[1], only a trend in total leukocyte and neutrophil count reduction was observed in patients treated with anti-PD-1 and TIL in combination with PEG-IFNa in cohort 2. Monocyte (not shown) and lymphocyte counts as measured in the peripheral blood were not affected. Due to the small number of patients, it is not possible to draw any conclusions on the difference in peripheral blood count cell subtypes between patients with or without a clinical response (Figure 2).

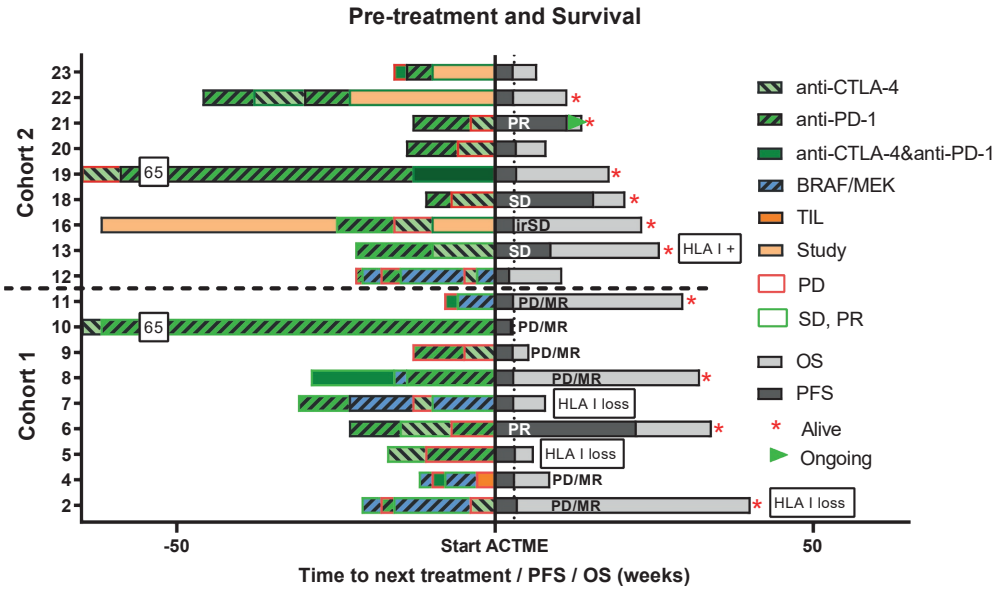

FIGURE 1 Duration and response to pre-treatment, and survival following treatment in ACTME. Treatments received before start of treatment in ACTME trial are depicted for every individual patient in the left part, followed by their Progression Free Survival (PFS, dark grey) and Overall Survival (OS, light grey) in weeks in the right part. Patients below the dotted line received Tumor Infiltrating Lymphocytes (TIL) and anti-PD-1 (cohort 1), while patients depicted above the dotted line received TIL, anti-PD-1 and pegylated-interferon-alpha (PEG-IFNa) (cohort 2). The response according to RECIST 1.1 is shown for responding patients; partial response (PR) or stable disease (SD). One patient in cohort 2 had an immune-related stable disease (irSD) according to the immune-related response criteria. Several patients with progressive disease (PR) had a mixed response (MR), where at least one tumor lesion was reduced in size. Human leukocyte antigen type I (HLA type I) genotyping was performed on patient's PBMC followed by flowcytometric evaluation of the surface expression on the tumor cell lines using specific antibodies. Cell lines were either HLA type I proficient (HLA I +), or HLA deficient (HLA I loss).

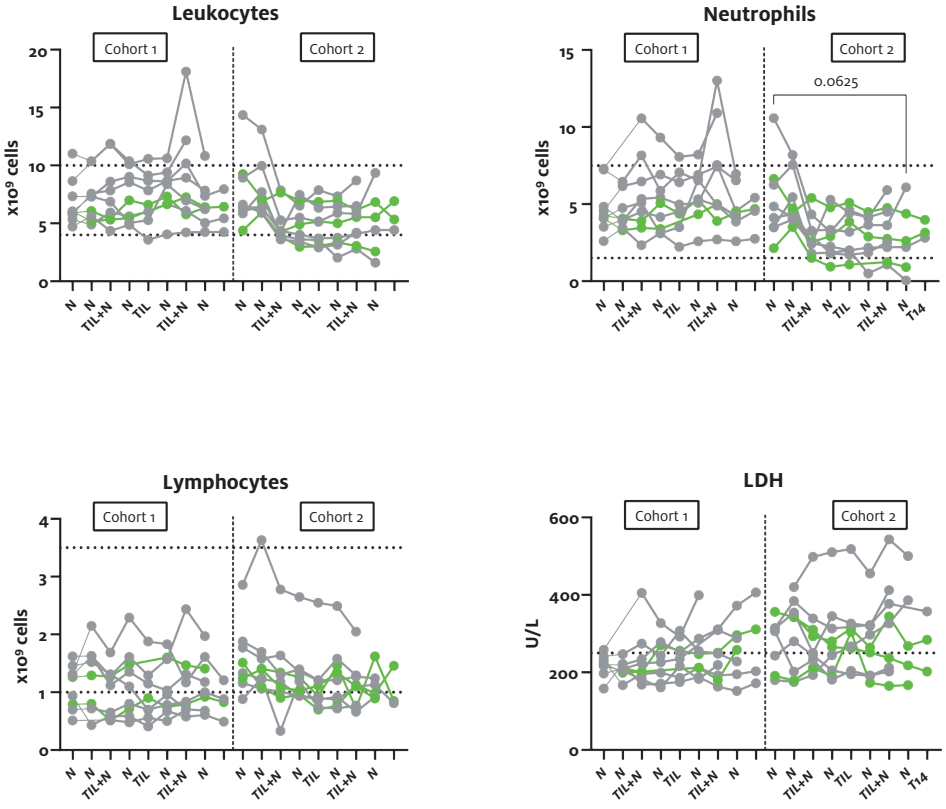

FIGURE 2 Treatment effect on peripheral blood counts.

Absolute blood counts and LDH plasma concentrations were determined in peripheral blood collected at different time points: before start of the first and every subsequent gift of Nivolumab (N), and at the moment just before each infusion of Tumor Infiltrating Lymphocytes (TIL).
In green the values of patients with a clinical response are shown.
Differences within patients were calculated using the Wilcoxon signed rank test, data differences between response groups were calculated using a Mann-Whitney U test.

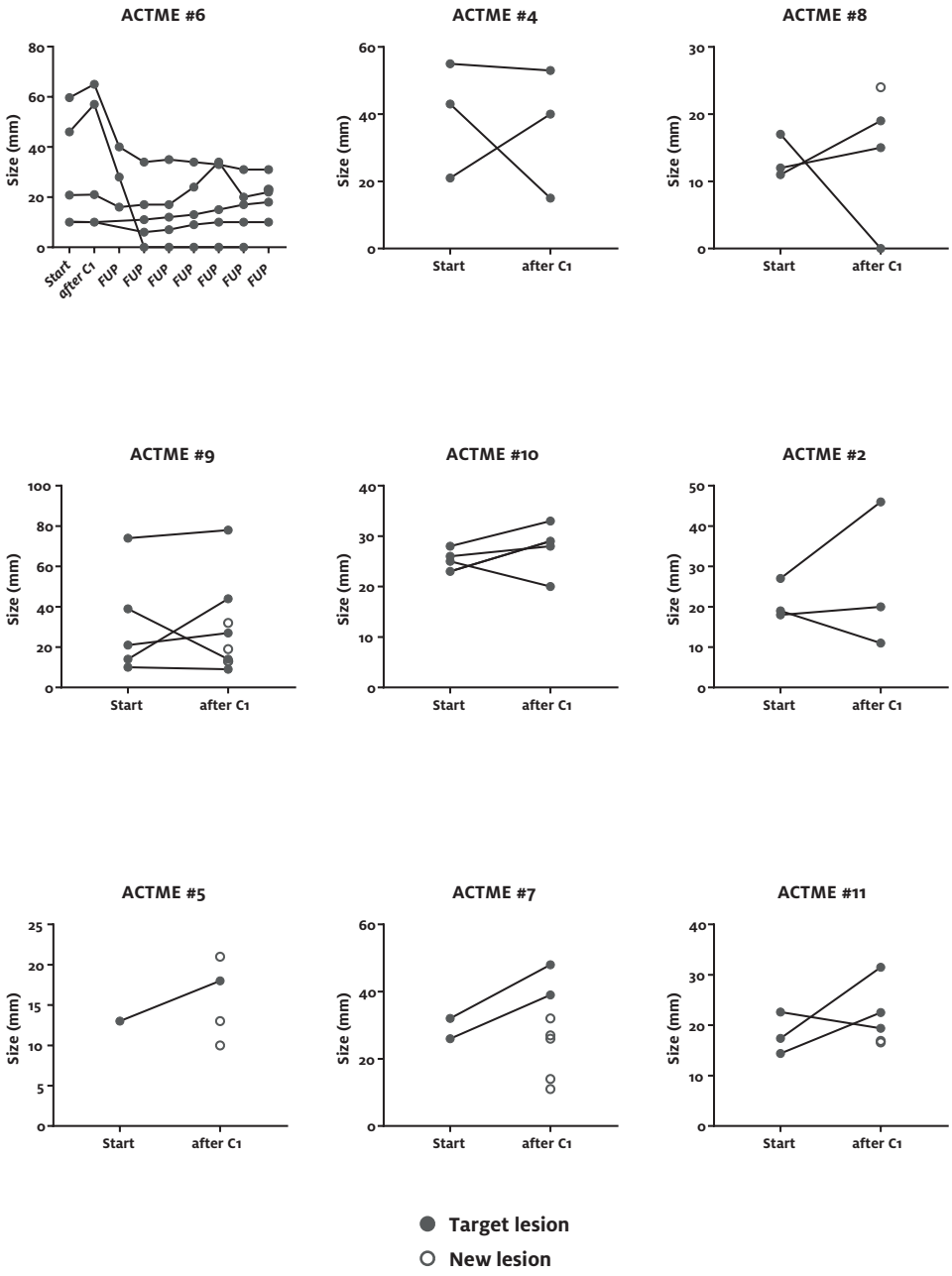

FIGURE 3 Change in lesion sizes in patients treated in cohort 1. The target lesion sizes of individual patients treated in cohort 1 are shown prior to (start) and after TIL infusions (C1). The best overall response of patients #6 is partial response, patients #2, 4, 8, 9, 10, and 11 have progressive disease with a mixed response according to RECIST1.1. In patient #8 one target lesion even disappears under treatment.

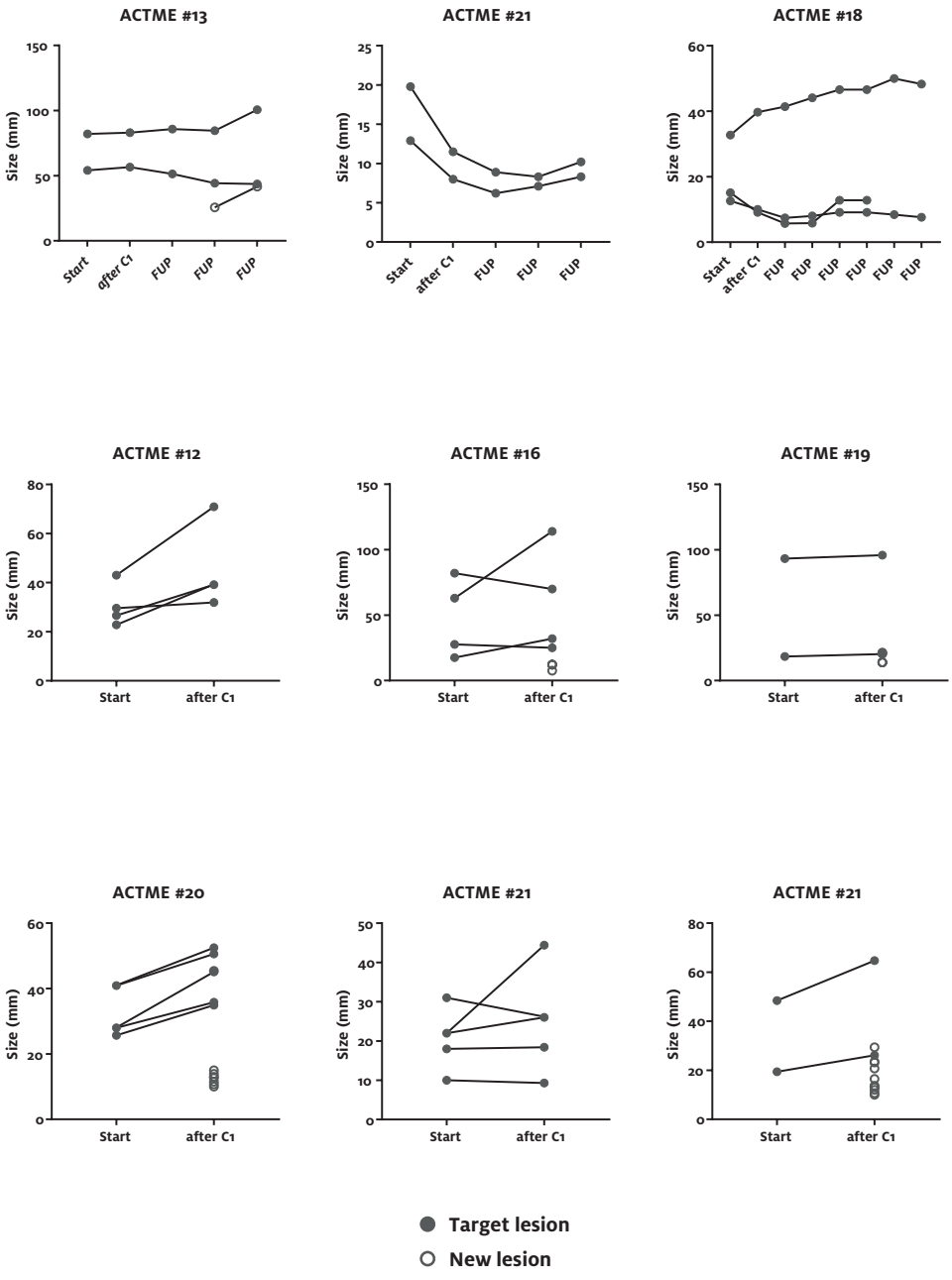

FIGURE 4 Change in lesion sizes in patients treated in cohort 2. The target lesion sizes of individual patients treated in cohort 2 are shown prior to (start) and after TIL infusions (C1). The best overall response of patients #13 and #18 is stable disease, patient #21 has a partial response according to RECIST1.1 and patient #16 has stable disease according to irRECIST.

Discussion

These preliminary data of phase I of the ACTME trial show that the combination of ACT, anti-PD-1 and PEG-IFNa can be safely given to patients with metastatic melanoma, and causes relatively few (serious) adverse events.

At the time of writing the phase II part of the ACTME trial is still ongoing, this will evaluate the efficacy of the treatment combination. So far, the phase I part already gives an indication that the treatment is safe and can result in clinical responses in patients with metastatic melanoma refractory to standard immunotherapy options.

A possible explanation for the difference in treatment effect on peripheral blood counts when comparing our current data with the data from our previous phase I/II trial with IFNa and TIL, is the fact that in the ACTME trial PEG-IFNa is used. Leukopenia has more frequently been reported as a side-effect of IFNa (>10%) when compared to PEG-IFNa (incidental)[2]. However, no head-to-head comparison has been made so far. Although we have previously shown that leukopenia is correlated with clinical response[1], it remains challenging to see if this phenomenon is crucial for the clinical outcome since in the currently ongoing trial clinical responses were obtained in patients who did not experience such leukopenia.

An additional objective of the study is to investigate if some markers in either the infusion product, serum or tumor of the treated patients correlate with clinical results. In this respect, our preliminary data showed that 3 non-responders had HLA I deficient tumors, while the tumor of 1 responder was HLA I proficient. HLA type I loss is described as a very effective immune evasion mechanism of tumor cells[3-9] and may be triggered by T cell mediated therapy including our combination treatment, thus explaining the unresponsiveness to treatment. If HLA class I expression is already absent before treatment, this will hamper further effectiveness of treatment relying on reinforcement of anti-tumor T cell immunity.

Therefore, examination of the HLA class I expression on tumors in the additional patients treated in our trial will reveal whether HLA type I deficiency should be added as an exclusion criteria.

So far, we do not know exactly why some lesions within one patient do respond to treatment while others do not (intra-patient heterogeneity). It is possible that certain tumor characteristics, like the already mentioned HLA class I loss or the presence of specific mutation-derived neoantigens, are not present in all metastases. Additionally, (stromal) immunosuppressive factors could vary depending on the location and the perfusion of the specific tumor.

Further research will be needed to study the influence of the characteristics of the infusion product on the response to treatment. This includes phenotype of the T cells, and the effect of these markers on the persistence of the T cells. Furthermore, the specificity of the T cell product will have to be studied, including the broadness of the tumor-reactivity. As a broad tumor-reactivity could less easily result in the development of antigen escape variants of the tumor, it would be interesting to see whether mixing T cells from multiple lesions of one patient will lead to a better and longer lasting tumor control.

The fact that patients with primary resistance to anti-CTLA-4 and anti-PD-1 immunotherapy can still respond to our new treatment combination is interesting and suggests that a lack of sufficient numbers of tumor-reactive T cells is one of the underlying mechanisms hampering the effect of the checkpoint inhibitors. Potentially, the combined ACT treatment may overcome this by providing the required numbers of tumor-specific T cells that are subsequently unleashed by anti-PD-1 to lyse the tumor cells.

In conclusion, these promising preliminary data warrant full evaluation of the safety and clinical efficacy of the combination treatment after completion of phase II of the ACTME trial.

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