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Abstract: Metastatic uveal melanoma (mUM) is a rare type of melanoma with poor outcomes. The first systemic treatment to significantly prolong overall survival (OS) in patients with mUM was tebentafusp, a bispecific protein that can redirect T-cells to gp-100 positive cells. However, the objective response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) may underestimate the clinical impact of tebentafusp. As metabolic response assessed by PET Response Criteria in Solid Tumors (PERCIST) has been reported to better correlate with clinical outcome, we here compared the patterns of radiological and morphological responses in HLA-A*02:01-positive patients with mUM treated with tebentafusp. In the 19 enrolled patients, RECIST showed an overall response rate (ORR) of 10%, median progression-free survival of 2.8 months (95% CI 2.5–8.4), and median OS (mOS) of 18.8 months. In 10 patients, where both RECIST and PERCIST evaluation was available, the ORR was 10% for both; however, the PFS was longer for PERCIST compared to RECIST, 3.1 and 2.4 months, respectively. A poor agreement between the criteria was observed at all assessments (Cohen's kappa ≤0), yet they differed significantly only at the first on-treatment imaging (P = 0.037). Elevated baseline LDH and age were associated with an increased risk for RECIST progression, while lymphocyte decrease after the first infusions correlated to reduced risk of RECIST progression. Detectable ctDNA at baseline did not correlate with progression. Early response to tebentafusp may be incompletely captured by conventional imaging, leading to a need to consider both tumor morphology and metabolism.

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Metastatic uveal melanoma (mUM) is a rare type of melanoma with poor outcomes. The first systemic treatment to significantly prolong overall survival (OS) in patients with mUM was tebentafusp, a bispecific protein that can redirect T-cells to gp-100 positive cells. However, the objective response rate according to Response Evaluation Criteria in Solid Tumors (RECIST) may underestimate the clinical impact of tebentafusp. As metabolic response assessed by PET Response Criteria in Solid Tumors (PERCIST) has been reported to better correlate with clinical outcome, we here compared the patterns of radiological and morphological responses in HLA-A*02:01-positive patients with mUM treated with tebentafusp. In the 19 enrolled patients, RECIST showed an overall response rate (ORR) of 10%, median progression-free survival of 2.8 months (95% CI 2.5–8.4), and median OS (mOS) of 18.8 months. In 10 patients, where both RECIST and PERCIST evaluation was available, the ORR was 10% for both; however, the PFS was longer for PERCIST compared to RECIST, 3.1 and 2.4 months, respectively. A poor agreement between the criteria was observed at all assessments (Cohen's kappa ≤0), yet they differed significantly only at the first on-treatment imaging ($P = 0.037$). Elevated baseline LDH and age were associated with an increased risk for RECIST progression, while lymphocyte decrease after the first infusions correlated to reduced risk of RECIST progression. Detectable ctDNA at baseline did not correlate with progression. Early response to tebentafusp may be incompletely captured by conventional imaging, leading to a need to consider both tumor morphology and metabolism. *Melanoma Res* XXX: XXXX–XXXX Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Uveal melanoma (UM) is a rare type of melanoma that accounts for approximately 3 to 5% of all melanoma cases, yet it is the most common primary intraocular malignancy in adults [1]. The incidence in Europe varies from 2 to 8 per million [2].

Treatment for the primary tumor include eye-sparing radio- and laser therapy that efficiently prevents local recurrences. Nevertheless, up to 50% of patients develop metastases, primarily in the liver [3,4]. The median overall survival (mOS) with liver-directed therapies ranges from 4 to 15 months [4], and systemic therapies had not proven to prolong the survival [5]. In contrast to cutaneous melanoma [6], only a minority of patients with metastatic UM (mUM) benefit from immune checkpoint inhibition (ICI) [7], likely due to low tumor mutational burden and an immunosuppressive microenvironment [8,9].

The first drug to significantly prolong mOS of HLA-A*02:01-positive patients with mUM is a first-in-class immune-mobilizing T-cell receptor (TCR) against cancer (ImmTAC), tebentafusp. Tebentafusp consists of HLA-A*02:01-restricted TCR for glycoprotein 100 (gp100), which is overexpressed on UM cells, and of an anti-CD3 variable fragment, binding of which leads to recruitment, activation, and antitumor effect of T-cells. In a phase III clinical trial, tebentafusp significantly increased the mOS compared to the investigator's choice (21.7 vs. 16.0 mo, HR of 0.51) [10]. In 2022, the US Food and Drug Administration [11] and European Medicines Agency [12] approved tebentafusp for treatment of unresectable mUM in HLA-A*02:01-positive adult patients. Despite significantly prolonged mOS, objective response rates were

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similarly modest in the tebentafusp and the control arm, which were 9% (95% CI: 2 to 13) and 5% (95% CI, 2 to 10), respectively [10]. In this phase III trial, objective response rate was measured using Response Evaluation Criteria in Solid Tumors (RECIST 1.1), which is a standardized guideline based on morphologic changes of target lesions [13–15]. However, RECIST may pose limitations, as it cannot always differentiate tumor tissue from fibrosis or scarring caused by the treatment [16].

In contrast to morphological measurements, 18 F-fluorodeoxyglucose (18F-FDG) PET measures the metabolic activity of tumors and may provide a more dynamic approach to evaluate therapeutic response. This response can be assessed using PET Response Criteria in Solid Tumors (PERCIST 1.0), which was developed in 2009, and offers a standardized and structured assessment of treatment response using functional imaging methods [17]. Recent studies have demonstrated discrepancies in RECIST- and PERCIST-assessed objective response rate and progression-free survival (PFS) [18]. Moreover, metabolic response in PET/CT has been reported to correlate with early treatment outcomes in patients under immunotherapy [19].

Since the approval of tebentafusp, an increasing number of patients have been treated outside of clinical trials. In our center, we use PET/CT to monitor treatment response, and have noticed discrepancies between morphologic and metabolic changes in tumors during tebentafusp therapy. The aim of our project was to investigate the patterns of response using RECIST 1.1 and PERCIST 1.0 in patients with mUM treated with tebentafusp.

Materials and methods

Patients

With the approval of Cantonal Ethic Committee of Zurich (Project ID: 2021-02471), we collected retrospective data from electronic medical records (KISIM version 5.3.1.5) and our radiological image system. Patients were included if they were treated with tebentafusp for mUM at the University Hospital Zurich (USZ) between July 2018 and April 2022 and have undergone at least two imaging procedures (1 baseline PET/CT or CT and 1 on-treatment PET/CT or CT). In principle, imaging is performed at baseline and every 2 to 3 months thereafter.

We included patients treated in the phase III clinical trial IMCgp100-202 (NCT03070392), who were assessed using CTs and patients treated outside the trial, who were assessed with PET/CT. Out of 20 identified tebentafusp-treated patients, 19 fulfilled the imaging inclusion criteria.

Routine tumor molecular analysis was performed for some patients using the MelArray Dx Panel, Oncomine Focus Assay or FoundationONE CDx.

Response assessment according to RECIST 1.1 and PERCIST 1.0

CTs were analyzed according to RECIST 1.1 [13], and 18-FDG PETs according to PERCIST 1.0 [14,17] (Appendix A, Supplemental digital content 1, *http://links.lww.com/MR/A366*) by an experienced radiologist (13 years of experience in radiology and 7 in nuclear medicine) of the Radiology and Nuclear Medicine Department of the USZ. Images were imported from Picture Archiving and Communication System into Mint Lesion 3.7.2. (Mint Medical GmbH, Heidelberg, Germany). Tumor response evaluation according to RECIST 1.1 or PERCIST 1.0 was performed in Mint Lesion™ as follows, target and non-target lesions were determined by the physician, and the measurements of length (RECIST) or standardized uptake value normalized by lean body mass (SUL, PERCIST) were taken. Adherence to response criteria requirements were continuously monitored by built-in rules, and deviations were reported. The cumulative values and the response characteristics such as target response, non-target response or timepoint response were derived automatically and the corresponding results are represented in a table.

Response according to RECIST 1.1 was evaluated in all 19 patients, whereas comparison of RECIST 1.1 and PERSIST was conducted in 10 patients where both PET and CT were available.

Statistical analysis

To compare RECIST 1.1 and PERCIST 1.0, a 4-point scale was used where 1 represented CR or CMR, 2: PR or PMR, 3: SD or SMD and 4: PD or PMD. To determine the concordance between the two protocols, Cohen's κ coefficient was calculated, while the difference between the two protocols was determined by Wilcoxon signed-rank test.

PFS was analyzed with respect to various blood chemistry measurements, separately for RECIST and PERCIST, using Cox's proportional hazards. Cox models were fit individually for each blood measurement, and HRs and p-values were extracted. Then, again separately for RECIST and PERCIST, a multivariate model including all blood measurements was fit.

Circulating tumor DNA assessment

Cell-free DNA (cfDNA) was isolated from serum and plasma samples with the QIAamp circulating nucleic acid kit (Qiagen). The relative amount of GNAQ p.Q209P or GNA11 p.Q209L mutated molecules was quantified with droplet digital PCR using a protype primer-probe mix and proprietary software developed

by Oncobit AG. Additionally, sequencing libraries of cfDNA samples collected at baseline or progression of 10 patients were prepared using Oncobit LBM, a custom panel of oligonucleotides covering 64 melanoma-relevant genes and hotspots, together with the xGen cfDNA&FFPE DNA Library Prep MC Kit (IDT) and the xGen Hybridization Capture of DNA libraries (IDT).

Results

Demographics

Between July 2018 and April 2022, 19 patients with mUM were treated with tebentafusp at USZ and were included in this retrospective study. The median age at treatment start was 62 years. All patients had liver metastases at treatment start and 63% had additional extrahepatic metastases, including lung, bone, soft tissue, and brain metastases. One-third (37%) of the patients had prior systemic therapy (chemotherapy and/or immune checkpoint inhibitors). The patients received a median of 36 infusions, equivalent to an 8-month treatment (range, 8–49 infusions). Demographic and clinical characteristics of the patients are provided in Table 1.

Mutation analysis of tissue samples was available for 13 patients. The most commonly identified mutations were in the BAP1 and GNA11 genes (42% each), followed by GNAQ (26%). A detailed description of the mutations is shown in Appendix B, Supplemental digital content 1, *http://links.lww.com/MR/A366*.

Response assessment according to RECIST 1.1

CT scans from 19 patients were and evaluated according to RECIST 1.1. The best overall response rate (BOR) (Fig. 1) and PFS were 10% and 2.8 months (95% CI, 2.5–8.4 months), respectively.

At median follow-up of 11.6 months (range 3.1–18.8 months), 10 of the 19 treated patients have died, resulting in a mOS of 18.8 months (0.95 LCL: 11.7 months). Patients with a BOR of PR (n = 2) and SD (n = 3) showed a similar mOS of 11.6 months and 11.7 months, respectively. Fourteen patients (74%) had PD as BOR and had a mOS of 18.8 months. Patient cohorts with different BOR did not significantly differ in their OS ($P = 0.56$).

Table 1 Baseline demographic and clinical characteristics

Characteristic	Patients (N = 19)
Median age, yrs (range)	62 (24–76)
Sex, n (%)	
Male	12 (63)
Female	7 (37)
M1 stage, n (%)	
M1a	7 (37)
M1b	10 (52)
M1c	1 (5)
M1x	1 (5)
Localization of metastasis, n (%)	
Hepatic only	7 (37)
Hepatic and extrahepatic	12 (63)
Received prior systemic therapy, n (%)	7 (37)
Immunotherapy	7 (37)
Chemotherapy	4 (21)

M1x – data on size of the largest metastasis is unknown. M1 stage is based on the size of the largest metastasis, M1a ≤3.0 cm, M1b 3.1–8 cm, M1c ≥8 cm.
N.a., not available.

Comparison of morphologic and metabolic tumor response

Ten of the 19 patients were eligible for the comparative analysis of RECIST 1.1 and PERCIST 1.0. The overall response rate (ORR) was 10% considering both RECIST and PERCIST. PFS according to RECIST and PERCIST was 2.4 months and 3.1 months, respectively, and did not differ significantly between the groups (Kruskal–Wallis rank sum test $P > 0.05$). One patient achieved disease control (CR + PR + SD) at some time during the therapy according to RECIST; and five patients achieved disease control according to PERCIST at some time during the therapy.

Responses for each patient at the first three follow-ups are shown in Table 2. Table 2 also contains the results of response comparisons between the two response criteria. At each follow-up, Cohen's κ ranged between 0 and 0.13, corresponding to an interpretation of 'none to slight agreement' [20]. Similarly, in two of the three follow-ups, Wilcoxon signed-rank tests failed to detect significant differences between the rating criteria.

In the first follow-up, 9 of the 10 patients underwent a PET/CT scan in addition to CT. Four patients (44%) showed a progressive disease (PD, PMD) according to both RECIST and PERCIST (Table 2). Four patients (44%) showed morphologically progressive disease (PD) without metabolic progression (SMD) and 1 patient (11%) demonstrated a morphologically SD with partial metabolic response (PMR).

Out of 8 patients with available data for follow-up 2, five (63%) demonstrated progressive disease (PD, PMD) according to both response criteria. Two patients showed a morphologically PD without metabolic progression (SMD), and one patient had morphologically partial response (PR) yet progressive metabolic disease (PMD).

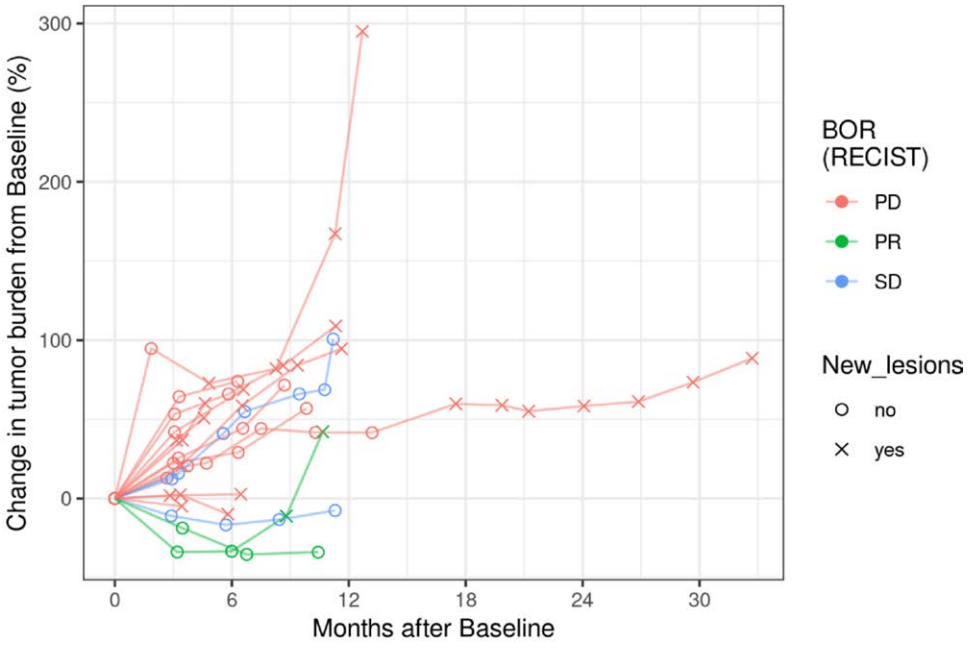
Of 5 patients with available third on-treatment imaging, 3 showed morphologic and metabolic progression (PD, PMD), 1 showed morphologic PD but stable metabolic disease in PERCIST and 1 patient showed a morphologic partial response yet PMD.

BOR could be calculated for 10 patients. Four patients had a BOR of SMD according to PERCIST, but a BOR of PD according to RECIST. In 4 patients, BOR was both PD and PMD.

Response and progression timelines

A timeline of response and progression during the treatment is shown in Fig. 2.

Fig. 1



NA=not available; PD= Progressive disease; PR=Partial response.

Best overall response (BOR) according to RECIST 1.1 in 19 patients treated with tebentafusp. NA, not available; PD, progressive disease; PR, partial response.

Table 2 ORR according to RECIST 1.1 and PERCIST 1.0 at follow-up 1, 2 and 3; n = 10

	Follow-up 1*		Follow-up 2		Follow-up 3		BOR	
Pat_no	RECIST 1.1	PERCIST 1.0	RECIST 1.1	PERCIST 1.0	RECIST 1.1	PERCIST	RECIST 1.1	PERCIST 1.0
10	PD	SMD	PD	SMD	PD	PMD	PD	SMD
11	PD	NA	PD	PMD	PD	PMD	PD	PMD
12	PD	PMD	NA	NA	NA	NA	PD	PMD
13	PD	SMD	PD	PMD	NA	NA	PD	SMD
14	PD	PMD	PD	PMD	NA	NA	PD	PMD
15	PD	PMD	PD	PMD	NA	NA	PD	PMD
16	PD	PMD	PD	PMD	PD	PMD	PD	PMD
17	SD	PMR	PR	PMD	PR	PMD	PR	PMR
18	PD	SMD	PD	SMD	PD	SMD	PD	SMD
19	PD	SMD	PD	NA	NA	NA	PD	SMD
				Comparisons				
n		9		8		5		10
Cohen's κ		0		0.091		0.111		0.134
P-value		0.037		1		1		1

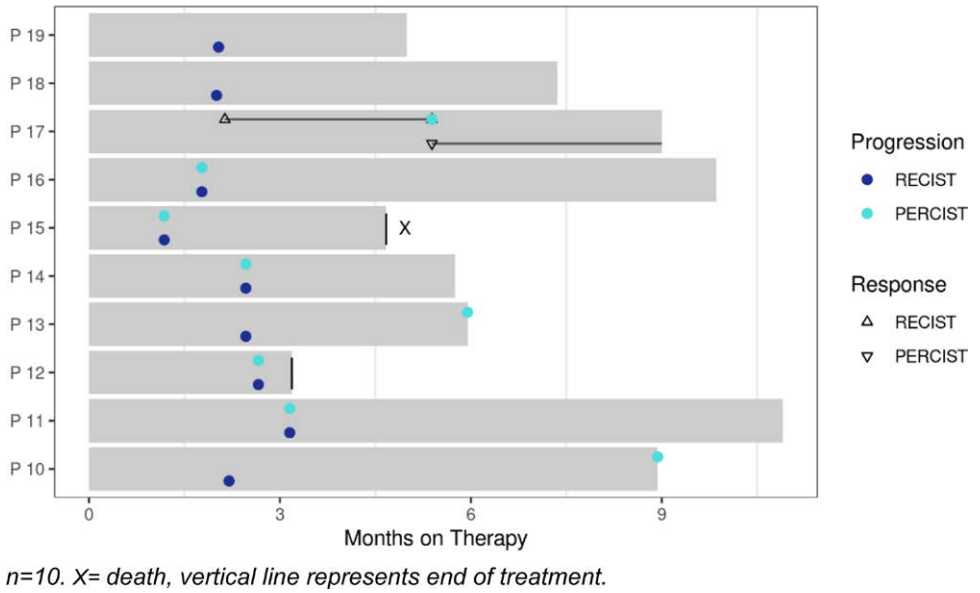
Follow-up 1 corresponded to around 3 months after the treatment start, follow-up 2–6 months and follow-up 3–9 months after treatment start. BOR – best overall response. Cohen's k and Wilcoxon signed-rank tests were calculated to determine agreements and differences between the two criteria protocols.
NA, not available; PD, progressive disease; PMD, progressive metabolic disease; PMR, partial metabolic response; PR, partial response; SD, stable disease; SMD, stable metabolic disease.

In five cases, progression occurred simultaneously considering the two response criteria. In four cases, progression was detected at different times according to RECIST and PERCIST. In the one remaining case, initial metabolic response (PMR with morphologic SD at follow-up 1), was followed by a morphologic response (PR at follow-up 2 and 3) with PMD.

Histopathological analysis of liver metastases in relation to radiological imaging

In 6 of the 8 patients who showed morphologic progression in the PET/CT, a core needle biopsy of a liver metastasis was performed to exclude pseudoprogression. In 3 cases, immunohistochemistry revealed a dense intra- and peritumoral lymphocyte infiltrates

Fig. 2



n=10. X= death, vertical line represents end of treatment.

Tumor response according to RECIST and PERCIST during the tebentafusp treatment. n = 10. X = death, vertical line represents end of treatment.

Table 3 Multivariate Cox-model for morphologic and metabolic PFS evaluating routine blood values and clinical features

	HR [1] (95% CI)		*P*-value	
Progression-free survival	RECIST 1.1 (n = 19)	PERCIST 1.0 (n = 10)	RECIST 1.1 (n = 19)	PERCIST 1.0 (n = 10)
Age at therapy start	1.11 (1.027–1.20)	0.98 (0.86–1.1)	0.009	0.826
Baseline LDH	5.04 (1.488–17.07)	0.83 (0.21–3.20)	0.009	0.784
Drop in lymphocytes count [2]	0.05 (0.007–0.34)	3.11 (0.15–62.7)	0.002	0.459
Prior immunotherapy	2.20 (0.492–9.88)	NA	0.302	NA

Hazard ratios were calculated as Cox proportional hazards [1]. Decreased lymphocytes count after first or second tebentafusp-infusion [2].
HR, hazard ratio; NA, not available.

composed largely of CD8 + T-cells (Fig. 3). In the other 3 patients, only slight inflammation was present intra- and peri-tumorally.

Disease monitoring using circulating tumor DNA

We analyzed circulating tumor DNA (ctDNA) on the serum and plasma samples of the 10 patients who underwent PET/CTs during the treatment. The samples were taken at un-predetermined timepoints during the tebentafusp-therapy. ctDNA was detectable in at least one timepoint of all 10 patients (Appendix D, Supplemental digital content 1, *http://links.lww.com/MR/A366* shows two patients as an example). Compared to baseline, new oncogenic mutations near the time of progression were found in 7 of 10 evaluable patients including the following mutations: RASA2, RASA3 p.Q266P; RASA3 p.H832Q; RASA3 p.D215_F216del BAP1 p.K529T; RET p.I803L; CBL p.M55R; AKT2 p.N2T; GNA11 p.Q209L (Appendix B, Supplemental digital content 1, *http://links.lww.com/MR/A366*).

Predictors for progression

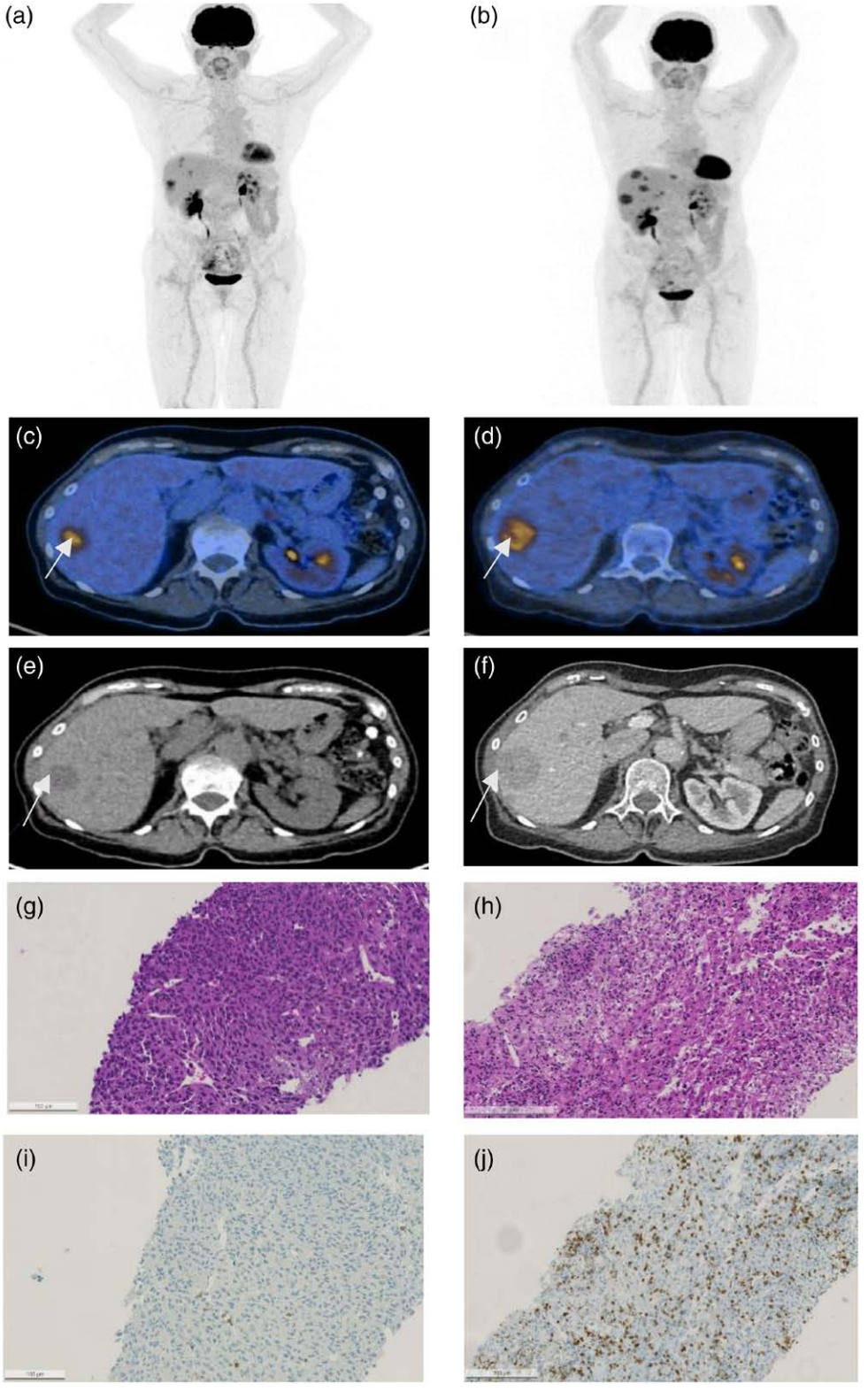
In the multivariate Cox-model, elevated baseline LDH and age at therapy-start were associated with an increased risk of morphologic progression, whereas a lymphocyte decrease after one of the first two tebentafusp infusions was related to a reduced risk for PD. No significant associations were found in relation to metabolic progression (Table 3).

Univariate analysis showed an association of reduction of the peripheral lymphocytes count after the first or second tebentafusp-infusion with a longer morphologic PFS *(P = 0.0231)*. The detection of serum ctDNA at baseline did not show significant correlation with PFS (Appendix C, Supplemental digital content 1, *http://links.lww.com/MR/A366*).

Discussion

In this retrospective analysis, we summarize data of 19 patients with mUM treated with tebentafusp. To our knowledge, this is the first study to report the

Fig. 3



Histopathological analysis of one liver metastasis in relation to PET/CT images of patient 19. Compared to baseline (a, c, and e), the patient demonstrated PD with SMD at follow-up 1 (b, d, and f). Although the liver metastasis (white arrow) increased in size, its metabolic activity was stable. Histopathologically, a dense intra- and peritumoral lymphocyte infiltrates are shown in the on-treatment biopsy (h,j) compared to baseline (g, i). These infiltrates consist mainly of CD 8 positive cells (j). g and h stained with hematoxylin and eosin; i and j stained for CD8.

metabolic responses and compare them to the morphologic responses in this patient population. Despite no difference in the morphologic and metabolic response rates or PFS, we identified disagreements in response at the first on-treatment imaging with morphologic metabolic progression but metabolic stability.

Treatment with tebentafusp has changed the outlook of HLA-A*02:01 positive patients with mUM by for the first time, showing a prolonged mOS in a phase III clinical trial [10]. Tebentafusp is an ImmTAC, which is a fusion protein consisting of a soluble, affinity-enhanced TCR that binds to a peptide derived from the melanocyte-lineage antigen gp100, presented through HLA-A*0201, and an anti-CD3 single-chain variable fragment (scFv), that redirects and activates T-cells [21]. It is a novel drug, distinct from ICIs, which block checkpoints on the T-cells, thus preventing binding to their ligands and thus inhibition [22]. Tumor cells are expected to lyse, leading to a decrease of tumors size and prolonged survival. However, tebentafusp did not show a high objective response rate despite prolonging the mOS with the unprecedented HR for death of 0.51 [10]. Moreover, patients who received tebentafusp and had disease progression as BOR, still showed a longer OS than patients with progression as BOR in the control group (15.3 mo vs. 6.5 mo, HR for death of 0.43) [10]. This data indicates that conventional morphologic imaging may provide an erroneous assessment of the clinical benefits of tebentafusp in patients with mUM. Another imaging technique, 18FDG-PET, uses a positron emitting glucose analogue and reflects tissue metabolism based on the 18FDG uptake, which may provide a more dynamic information about tumor response than tumor size.

Similar to the randomized phase III clinical trial (NCT03070392) [10], we observed a low response according to RECIST 1.1 in the patients with mUM treated with tebentafusp (10% ORR, PFS 2.8mo (95% CI 2.5–8.4mo)). However, comparison of responses in CT and PET/CT revealed discrepancies in early tumor response. In the first follow-up, 4 (40%) of the patients showed stable metabolic disease despite a morphologic progression and one patient (pt17) show a decreased metabolic activity (PMR), without change in the tumor burden (SD). Two of the four patients with PD continued to show PD and SMD, while one showed a PD and PMD upon next imaging and one did not undergo a PET/CT. Pt17 developed a morphological tumor regression upon the next imaging however with PMD. The relevance of PMD in this setting is unclear: in the on-treatment tumor samples from 3 of the 6 patients with PMD, we saw an increase of CD8 + T-cells, and the increased glucose uptake may be either by the immune cells as in pseudo-progression [23], or the tumor cells as in actual tumor progression.

Poor agreement and significant differences were observed between the morphologic and metabolic responses in the first scan after therapy start (Cohen K = 0; $P = 0.037$). Follow-up 2 and 3 continued to show a low agreement between the two criteria guidelines, however, these differences were NS. BOR according to RECIST 1.1 vs. PERCIST 1.0 also showed no agreement according to Cohen's K, but the Wilcox test revealed no significant differences.

A retrospective analysis of 104 patients with metastatic melanoma treated with anti-PD1-based immunotherapy demonstrated that 18-FDG PET is useful in predicting long-term clinical benefit as patients with CMR at 1 year since treatment started had 90% PFS and 96% OS at year 5. In patients with a partial morphologic response at 12 months, PFS was higher in those who also had a CMR compared to those without a CMR (88% vs. 59%) [24]. 18F-FDG PET scans also appear to play an important role in predicting early efficacy of therapy. In a clinical trial including 24 patients with non-small cell lung cancer who had undergone imaging at 1 month since start of ICI, 18-FDG PETs was significantly superior to CTs in predictive probability of response (100% compared to 29%, $P = 0.021$) and progression (100% vs. 22.2%, $P = 0.002$) [19]. Due to different mechanism of action than ICI, tebentafusp may present other challenges in measuring treatment response. Therefore, investigation of biomarkers that can complement imaging methods, is highly important.

Clinical features at baseline, blood markers and the immune cell landscape of metastases may play an important role in predicting the therapy benefit. In our cohort, elevated pretreatment LDH values correlated with a shorter morphologic PFS (multivariate Cox model $P = 0.009$). Similar results are reported in the phase III clinical trial NCT03070392, which displayed the association of increased LDH at baseline with reduced OS [10]. On the other hand, we found decreased peripheral lymphocyte count after first or second tebentafusp infusion to be associated with reduced risk of morphologic progression in both multiple variate and univariate tests. In a multicenter phase I/II clinical trial evaluating the mechanism of action of tebentafusp in patients with metastatic melanoma, decline of circulating CD8+ CXCR3 + T cells was observed along with an increase of intratumoral T-cells indicating a redirection of the T cells towards the malignant cells [25]. There is evidence that supports an association between the immune microenvironment of metastases and overall survival (OS) in patients with mUM. Cases of SD evaluated by RECIST 1.1 criteria have been observed to have a higher ratio of $CD8^+$ and Granzyme B cells both within and around the tumor and a greater concentration of cytotoxic T-cells in the tumor compared to progressive mUM cases [26]. However, patients enrolled in this study received intraarterial chemoembolization concurrent with conventional systemic therapies available at that time, not including tebentafusp.

Another potential biomarker that has been recently widely researched is ctDNA. Studies show a correlation of pretreatment ctDNA detectability with reduced clinical benefits and abrupt PFS in patients with advanced melanoma [27]. Patients with detectable ctDNA during treatment also tended to exhibit poorer clinical benefits and OS [28[29]. In a recent multicenter, single-arm open-label phase II clinical trial analyzing the objective response to tebentafusp according to RECIST in patients with mUM, an exploratory analysis was performed using plasma from 118 patients. Baseline ctDNA levels correlated with radiological tumor burden and rapid reduction of ctDNA levels during the treatment was associated with increased survival [30]

In our study, no significant correlation between serum ctDNA detectability before treatment and PFS was found. However, serum ctDNA was detected at least at one-time point throughout therapy in 10/10 evaluable patients who showed progression by any of the criteria (RECIST 1.1 or PERCIST 1.0). Since limited plasma samples were available for ctDNA analysis, serum samples were primarily utilized. Serum contains increased levels of non-tumor DNA as a result of the leukocyte lysis involved in serum preparation. Dilution of ctDNA by leukocyte cfDNA could compromise detection of the ctDNA, leading to false-negative results [31].

Currently used detection techniques for ctDNA in liquid biopsies include ddPCR and NGS. ddPCR allows the detection of previously identified specific mutations while NGS is an untargeted approach that allows the revelation of new molecular alterations [32,33]. In this study, ddPCR was performed on 8 evaluable patients and NGS on 10. NGS was applied on baseline samples and samples taken around the time of radiological progression. Seven out of 10 evaluable patients showed new genetic alterations not previously identified prior to treatment including mutations in the BAP1, RET, CBL, AK1 and RASA3 genes. Larger prospective studies evaluating the dynamic of the mutations in the tumor in liquid biopsies are still needed to identify de novo mutations related with resistance or response.

Conclusion

In our retrospective study, we observed a statistically significant discrepancy between early morphologic and metabolic responses in patients with mUM treated with tebentafusp. Our results suggest that response assessment with tools other than RECIST criteria may better capture the treatment effect of tebentafusp. The interpretation of our results is limited by the small patient cohort and small number of events, however, the observed differences grant further research with larger cohorts to evaluate the value of metabolic response in the monitoring of tebentafusp treatment. Moreover, liquid biopsies and ctDNA measurement should be further evaluated in determination of disease response or pseudoprogression, and potentially integrated in standard response assessment.

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Institutional review board statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Cantonal Ethic Committee of Zurich (BASEC-Nr.2021-02471) in January 2022.

Conflicts of interest

E.B. is currently employed at Oncobit AG, which develops diagnostic tests that are the basis of those discussed in this manuscript. P.T. is a shareholder and former employee of Oncobit. MPL has received unrelated research support from Novartis, Roche, Molecular Partners, Oncobit, and Scailyte. R.D. has intermittent, project focused consulting and/or advisory relationships with Novartis, Merck Sharp & Dhome (MSD), Bristol-Myers Squibb (BMS), Roche, Amgen, Takeda, Pierre Fabre, Sun Pharma, Sanofi, Catalym, Immunocore, outside the submitted work. E.R. has intermittent, project focused consulting and/or advisory relationships or has received travel or research grants from Sanofi, Pierre Fabre, Bristol-Myers Squibb (BMS), Amgen, Galderma, Takeda, SunPharma, Novartis, Merck Sharp & Dhome (MSD), Leo-Pharma, outside the submitted work. For the remaining authors, there are no conflicts of interest.

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