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IOTECH IMMUNO-ONCOLOGY AND TECHNOLOGY

REVIEW

Immuno-oncology approaches in uveal melanoma: tebentafusp and beyond

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Available online 8 June 2023

Uveal melanoma (UM) is the most common ocular malignancy in adults, associated with the poorest prognosis, with metastatic disease occurring in up to 50% of patients. In contrast to metastatic cutaneous melanoma, the use of immune checkpoint inhibitors is associated with poor outcomes in metastatic uveal melanoma (mUM). Tebentafusp, a bispecific molecule, has recently become the first treatment in decades to improve overall survival for mUM. This review summarises the existing and emerging immuno-oncology approaches for the treatment of mUM, and biomarkers of response and resistance to the same. Finally, we propose future research directions that could maximise treatment benefit to a wider pool of patients with UM.

Key words: tebentafusp, bispecific molecule, metastatic uveal melanoma, immunotherapy, biomarkers

INTRODUCTION

Despite sharing the same cell of origin (i.e. melanocytes), melanoma subtypes are distinguished by their molecular profile, clinical behaviour and response to therapy. In the past decade, immune checkpoint inhibitors (ICIs) have transformed the prognosis of cutaneous melanoma (CM) both in the adjuvant and metastatic settings.[1,2] However, the success of ICIs has failed to translate to uveal melanoma (UM).[3] For decades, landmark overall survival (OS) in metastatic uveal melanoma (mUM) remained static, with only 8% of patients alive at 2 years after diagnosis.[4,5] Notably, mUM has a distinctive liver tropism, with liver metastases present in 90% of patients with mUM.

Today, tebentafusp is the first treatment to improve OS in patients with mUM who are human leukocyte antigen (HLA)-A2*02:01 positive, representing 50% of patients of European ancestry.[6,7] However, critical knowledge gaps remain regarding the mechanisms of response and resistance to tebentafusp, the optimal way to evaluate response, how tebentafusp synergises with other therapies and its likely effectiveness in the adjuvant setting.

In this review, we discuss the molecular landscape of UM, including canonical genetic, transcriptomic and tumour microenvironment (TME) features. We provide an overview of existing and emerging immuno-oncology approaches for the treatment of mUM and biomarkers of response and resistance. Finally, we propose future research directions that may maximise treatment benefit to a wider pool of patients.

THE MOLECULAR LANDSCAPE OF UVEAL MELANOMA

Almost all UMs harbour founder mutations in the mitogen-activated protein kinase (MAPK) signalling pathway. Mutations in *GNAQ* or *GNA11* genes, which encode the α-subunit of G-protein-coupled receptors,[8-11] are observed in 92% of all UMs with mutations in *CYSLTR2* or *PLCB4* accounting for the rest.[10,12] Secondary driver mutations in *BAP1* (45%), *SF3B1* (25%) and *EIFAX1* (15%) genes are observed in a mutually exclusive pattern[13] and associate with distinct clinical trajectories of metastatic disease.[14,15] Patients with tumours that harbour *EIF1AX* mutations rarely relapse with distant metastases. The presence of *SF3B1* mutations in primary tumours is associated with latent metastases; 64% of patients will develop metastatic disease within 10 years of primary diagnosis (median 8.2 years).[16] Mutations in *SF3B1* result in aberrant splicing that can generate tumour neoantigens, which have been observed to be shared across patients.[17] In a study of 13 *SF3B1*-mutant patients with mUM, peripheral memory CD8 T cells reactive to such shared neoantigens were detected in 20% of patients.[17] These observations may reconcile the more indolent behaviour of *SF3B1* UM. Primary UMs harbouring *BAP1* mutations present an aggressive phenotype with younger age at presentation, locally advanced tumours with ciliary body involvement and earlier metastases.[18,19] *BAP1* is a

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tumour suppressor gene located on chromosome 3p and plays a role in DNA repair, transcription, regulation of cell death and mitochondrial metabolism.[20] It is also implicated in other cancer types such as renal cell cancer and mesothelioma.[20] In UM, *BAP1* inactivation occurs via a two-hit process, loss of chromosome 3 (whole chromosome loss in almost all cases) and mutation of the remaining *BAP1* allele. Biallelic *BAP1* deletion is a less common mechanism of *BAP1* inactivation.[20,21]

Driver somatic copy number alterations (SCNAs) are frequent in UM, particularly involving losses in chromosomes 3, 1p, 8p, 6q and 16q, and gains in chromosomes 6p and 8q.[14,22] These SCNAs hold prognostic value. Primary UM tumours which are diploid for chromosome 3 and harbour 6p gain are associated with low metastatic risk and tend to co-occur with *EIF1AX* or *SF3B1* mutations. Loss of chromosome 3, found in ~55% of UMs, is linked with high metastatic risk and poor survival.[23] Accordingly, 83% of monosomy 3 (M3) UMs harbour BAP1 mutations.[14] Gains in 8q and losses in 1p, 8p and 16q further stratify patients with M3 into higher-risk categories (Figure 1).

Transcriptional differences can also distinguish metastatic risk, using a 15-gene signature; 'class 1' tumours are associated with low risk (5-year risk 4%), while 'class 2' tumours are associated with high risk (5-year risk 51%).[24,25] Tumours bearing a 'class 2' signature are associated with a dedifferentiated phenotype; they share features with neural/ectodermal stem cells and have reduced expression of melanocytic genes. Class 2 tumours correlate with M3 and BAP1 mutations.[14] Tumours with a 'class 1' signature are transcriptionally closer to normal uveal melanocytes.

Based on these findings, a classification system to predict metastatic risk, incorporating secondary driver mutations, key chromosomal aberrations and gene expression profiles (GEP), has been proposed (Figure 1).[14,15,26-28]

In contrast to other cancer types, the presence of tumour-infiltrating lymphocytes (TILs) in primary UM is associated with poorer prognosis.[29-32] Primary tumours with higher TILs had a higher expression of negative immune regulators such as *FOXP3*, *INDO*, *PD-1*, *CTLA-4* and *LAG3*. TILs are predominantly CD8+ and express HLA-E, suggesting that they may be CD8+ regulatory T cells (Tregs).[32] These observations may reconcile the negative prognostic value of TILs in primary UM. However, in UM metastases, a higher percentage of intratumoural CD8+ granzyme B+ T cells are associated with prolonged OS,[33,34] suggesting that the composition of the immune TME is not static during UM progression.

IMMUNE CHECKPOINT INHIBITORS IN UM

Before tebentafusp, no therapies had been shown to improve OS in mUM. In a large meta-analysis, chemotherapy was associated with an OS of 10 months.[35] Selumetinib, a MEK inhibitor, is the only tyrosine kinase inhibitor tested in a phase III trial to date but failed to demonstrate benefit.[36] Only a small percentage of patients benefit from anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and anti-programmed cell death protein 1 (PD-1) agents, and we review this evidence below.

Clinical evidence

In early-phase trials of patients with mUM, ipilimumab was associated with an overall response rate (ORR) of 0%-6.5%.[37] Reported ORRs with anti-PD-1 in case series are between 0% and 12%.[38-40] Recently, anti-PD-1 has acted as the predominant control treatment in first-line (1L) phase III trials in mUM, and was associated with an ORR of ~5% and 58% survival at 1 year.[6]

Two single-arm phase II trials with ipilimumab and nivolumab combination (IpiNivo) in mUM have been reported to date. The GEM-1402 trial included 52 treatment-naive patients with favourable clinical features, such as M1a disease (64%), normal lactate dehydrogenase (LDH) (68%)

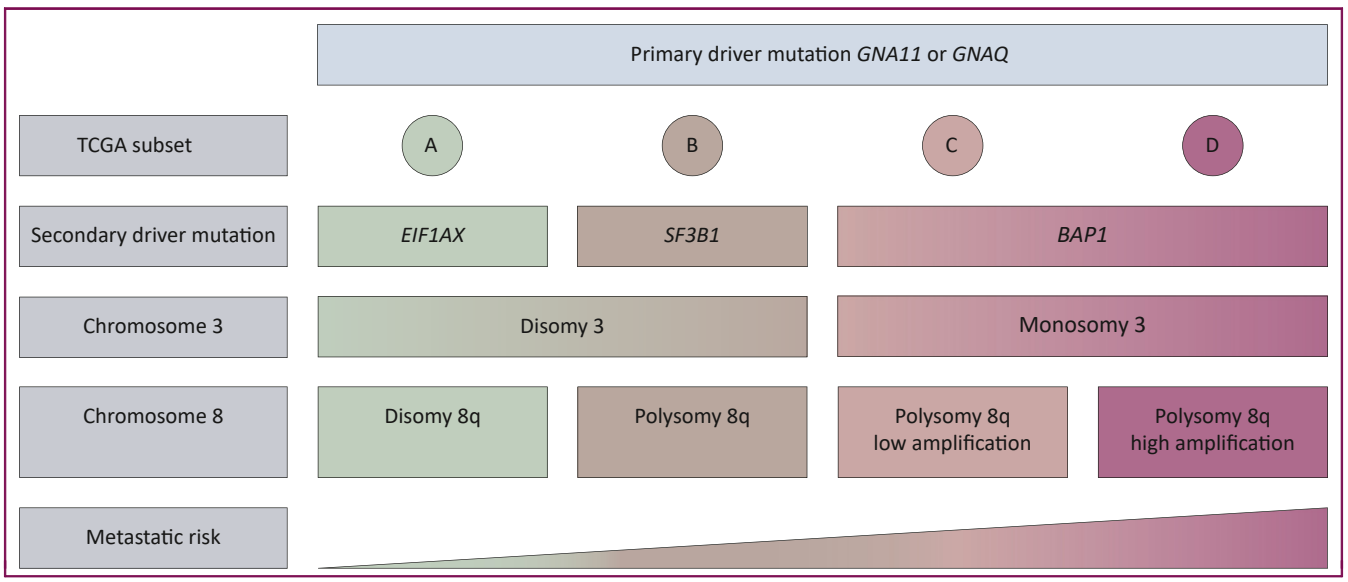

Figure 1. Classification of metastatic risk. TCGA, The Cancer Genome Atlas. Adapted from Smit et al.,[26] Robertson et al., Cell 2018,[14] Royer-Bertrand et al.,[15] Jager et al.,[27] and Vichitvejpaisal et al.[28]

and extrahepatic metastases (58%).[41] The ORR was 11%, median progression-free survival (mPFS) 3.0 months and median OS (mOS) 12.7 months. Another IpiNivo trial of 35 treatment-naive and previously treated patients reported an ORR of 18% and an mOS of 18 months, despite being a higher-risk population (M1a 49%) compared to GEM-1402. A retrospective report of 89 patients treated with IpiNivo showed an ORR of 11%,[42] with durable responses up to 22 months.[43] Taken together, responses to IpiNivo, whilst observed in patients with mUM, are infrequent.

No trial has directly compared IpiNivo and single-agent anti-PD-1 in mUM. Acknowledging the limitations of cross-trial comparisons, IpiNivo does not appear to provide benefit beyond single-agent anti-PD-1 (1-year survival 56% versus 58%, respectively), although longer follow-up is needed to assess durability of response.[6,44] Critically, IpiNivo has a higher toxicity risk compared to anti-PD-1 monotherapy (grade 3/4 toxicity 69% versus 43%).[45] If IpiNivo does provide additional benefit to a subset of patients, biomarkers are urgently required to identify them to minimise excessive toxicity in all comers.

Predictive biomarkers of ICI response and resistance

Few predictive biomarkers for ICIs have been identified prospectively in mUM. Retrospective efforts have been conducted in small, heterogeneous populations which largely lacked statistical power, highlighting the need for internationally collaborative approaches in rare cancers.

In the GEM-1402 study, LDH $<$2.5-fold of the upper limit of normal was associated with superior PFS following IpiNivo.[41] Presence of liver metastases is another potential clinical biomarker. Pan-cancer studies have shown that patients with liver metastases respond poorly to ICIs, independent of other factors.[46] Patients with liver metastases have lower levels of peripheral and intratumoural T cells, with impaired efficacy.[46] In mice, liver metastases diminished the number of circulating activated CD8 T cells, while immunosuppressive hepatic macrophages promoted CD8 T-cell apoptosis, fostering systemic resistance to ICIs.[46] Thus, mUM's liver tropism may partly explain its poor response to ICIs: 90% of patients with mUM will develop liver metastases, and 50% will have liver metastases only.[5,47] A pooled analysis compared patients with mUM treated with IpiNivo with matched historical controls.[48] In patients with extrahepatic disease only, IpiNivo was associated with survival $>$3.2 times longer than historical treatments, suggesting that this subgroup derived benefit from IpiNivo.[48] However, in patients with hepatic disease, there was no difference in survival between treatments. Another retrospective cohort of 178 ICI-treated patients supported these findings; patients with extrahepatic metastases trended to longer OS than patients with hepatic metastases only (18.2 versus 6.1 months, $P = 0.07$).[49] In the broader population, 10% of patients with mUM have extrahepatic disease only,[50] but are overrepresented in the mUM IpiNivo trials (20%);[49] thus we speculate that these trials may overestimate survival outcomes post-IpiNivo.

A high tumour mutation burden (TMB) is associated with ICI response across multiple cancers, and TMB $>$10 mutations/Mb has received Food and Drug Administration approval as a tumour-agnostic indication for pembrolizumab.[51] UM has the lowest TMB (average 0.5 mutation/Mb) amongst solid tumours, which translates to a low number of tumour neoantigens derived from somatic mutations (such as single nucleotide variants, and insertions and deletions).[52] This translates to limited subtrate to generate antitumour immunity, with notable exceptions. Patients with *SF3B1*-mutated tumours may associate with increased number of neoantigens generated by alternative splicing.[53] A case series of 58 patients with SF3B1-mutated tumours reported a trend to superior mOS (20 months) and 1-year survival (74%) post-ICI compared to historical controls.[54] Outlier ICI responses have been described in patients with *MBD4* loss-of-function germline and somatic mutations.[55] *MBD4* encodes a glycosylase involved in DNA damage repair;[56] mutations in *MBD4* result in hypermutated tumours, and by extension a higher likelihood of ICI response.[57,58]

Other markers, such as interferon-γ (IFN-γ) signatures, TIL count and decreasing circulating tumour DNA (ctDNA) on therapy, have been reported to associate with a numerically higher ICI response rate, albeit in small patient numbers.[57,59] Programmed death-ligand 1 (PD-L1) expression correlates with anti-PD-1 response in various cancer types.[60] mUM is associated with a lower rate of PD-L1 expression compared to metastatic CM (mCM, 5% versus 26%).[34] This may also partly explain its poorer response to anti-PD-1, although to our knowledge no studies have compared PD-L1 expression in mUM responders with non-responders.

The presence of intratumoural CD8+ T cells is a prognostic and predictive marker of ICI response in CM and other cancers.[60] However, in UM liver metastases, CD8+ T cells are few in number, which may contribute to ICI non-response. Digital spatial profiling studies have identified peritumoural fibrotic areas that facilitate T-cell exclusion in mUM.[61] Conversely, CD4+ T cells and CD163+ tumour-associated macrophages (TAMs) are more prevalent, contributing to an immunosuppressive TME.[61]

Emerging immune checkpoints and other rational combinations

Intratumoural T cells express immune checkpoints indicative of exhaustion that may be leveraged as therapeutic targets (Figure 2). In mUM, lymphocyte-activation gene 3 (LAG3) is the most expressed immune checkpoint.[62-64] Mechanistically, LAG3 expression inhibits T cells by lowering the pH around the T-cell immune synapse, which disrupts CD8 and CD4 signalling and activation.[65] LAG3 expression is enriched in high-risk UM with epithelioid cell type, M3, BAP1 loss[88] and higher T-cell infiltrate and TAMs.[62] Therefore, targeting LAG3 may synergise with anti-PD-1 inhibition. In 1L mCM, treatment with relatlimab (anti-LAG3) with nivolumab was superior to nivolumab alone (PFS 10 versus 4 months).[66] However, in a single-arm phase

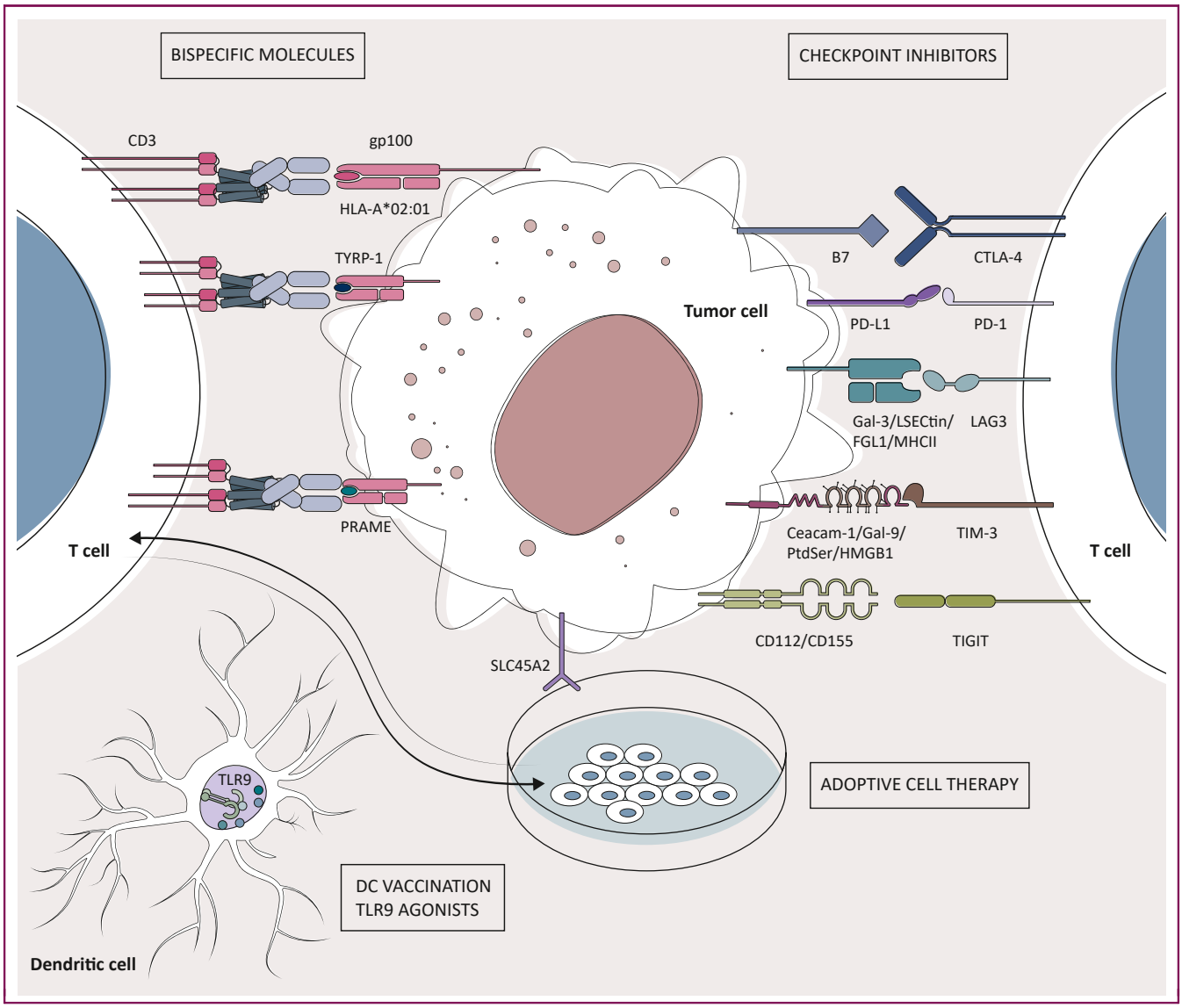

Figure 2. Molecular targets in early-phase immuno-oncology trials in metastatic uveal melanoma (mUM).
ctDNA, circulating tumour DNA; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; LAG3, lymphocyte-activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TME, tumour microenvironment.

II study in mUM, combination relatlimab and nivolumab resulted in an ORR of 10% with an OS of 11.2 months, and no durable responses.[67,68] Further biomarker and translational work is underway.

Other T-cell exhaustion markers such as T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) are also expressed by intratumoural T cells in mUM.[63,64,69] Early trials targeting these checkpoints [IMCAGN02390 (NCT03652077) and tiragolumab (NCT05483400), respectively] are underway across various tumour types (including mUM).[70] Table 1 highlights key early-phase immuno-oncology trials in progress in mUM.

Although primary UM and liver metastases do not express indoleamine 2,3-dioxygenase (IDO, an immune checkpoint) at baseline, treatment with IFN-γ in UM cell lines up-regulated IDO.[71] This may be another mechanism of immune escape; although IDO inhibitors have not been trialled in mUM, and mCM, they have failed to demonstrate benefit.[72]

Combination pembrolizumab and entinostat [histone deacetylase inhibitors (HDACi)] has been investigated in mUM. HDAC co-regulates gene expression by repressing transcription. HDACi open chromatin, increase tumour antigen and HLA-I expression and can reduce myeloid-derived stem cell function and deplete Tregs in the TME.[73,74] In a phase II trial of entinostat and pembrolizumab in 29 patients with mUM and favourable prognostic features (M1a 59%), ORR was 14% (4/29 patients), mPFS 2.1 months and mOS 13.4 months, similar to previous anti-PD-1 monotherapy cohorts.[75] Therefore, it is unclear whether these responses were driven by anti-PD-1 therapy alone, or if HDACi provided any additional benefit. Once again, understanding of biology and biomarkers to stratify patients is urgently needed.

Table 1. Key early-phase immuno-oncology clinical trials in progress in mUM

Clinical trial title (NCT)	Mode of action/drugs involved	UM cohort or basket trial	Status	Molecular inclusion criteria
A study of RO7293583 in participants with unresectable metastatic tyrosinase related protein 1 (TYRP1)-positive melanomas (NCT04551352)	Bispecific molecule targeting TYRP-1 ± obinutuzumab pretreatment	UM cohort	Active, not recruiting	TYRP-1-positive melanoma. HLA agnostic
Safety and efficacy of IMC-F106C as a single agent and in combination With checkpoint inhibitors (NCT04262466)	Bispecific molecule targeting PRAME ± anti-PD-1	UM cohort	Recruiting	HLA-A2*02:01
FS118 first in human study in patients with advanced malignancies (NCT03440437)	Bispecific molecule targeting LAG3 and PD-L1	Basket	Recruiting	NA
Nivolumab plus relatlimab in patients with metastatic uveal melanoma (NCT4552223)	Anti-LAG3 and anti-PD-1	UM cohort	Recruiting	NA
A safety and tolerability study of INCAGN02390 in select advanced malignancies (NCT03652077)	Anti-TIM-3	Basket	Recruitment completed	NA
Autologous CD8+ SLC45A2-specific T lymphocytes with cyclophosphamide, aldesleukin, and ipilimumab in treating patients with metastatic uveal melanoma (NCT03068624)	Autologous CD8+ SLC45A2 T cells with cyclophosphamide, aldesleukin and ipilimumab	UM	Recruiting	HLA-A2*02:01 or HLA-A*24:02
RTX-240 monotherapy and in combination with pembrolizumab (NCT04372706)	Red cells expressing 4-1BB and IL-15TP ± anti-PD-1	Basket	Recruiting	NA
IKKb-matured, RNA-loaded dendritic cells for metastasised uveal melanoma (NCT04335890)	IKKb-matured, RNA-loaded dendritic cells	UM	Active, not recruiting	NA
Intrahepatic delivery of SD-101 by pressure-enabled regional immuno-oncology (PERIO), with checkpoint blockade in adults with metastatic uveal melanoma (NCT04935229)	TLR9 agonist	UM	Recruiting	NA

LAG3, lymphocyte-activation gene 3; NA, not applicable; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; UM, uveal melanoma.

BISPECIFIC MOLECULES—TEBENTAFUSP AND EMERGING AGENTS

Clinical evidence

Tebentafusp is the first drug to demonstrate OS benefit in mUM. Its use is restricted to HLA-A2*02:01-positive patients, representing 50% of people from European ancestry.[7,76] It is a bispecific molecule, consisting of a T-cell receptor (TCR) targeting a gp100 peptide presented by HLA-A2*02:01, and an anti-CD3 T-cell-engaging domain.[6] Gp100 is a melanocytic lineage-specific glycoprotein widely expressed by UM cells, up to 100% in primary UM.[77] Tebentafusp acts by redirecting T cells to tumour cells expressing gp100.

Tebentafusp is administered intravenously on a weekly basis, with an initial dose escalation regimen of over three doses.[78] Patients are monitored for up to 16 h following the first three doses, to manage cytokine release syndrome (CRS), which may manifest as hypoxia, hypotension and pyrexia. CRS is common (89% of patients), but rarely severe (grade ≥3, 1%). Most adverse events occurred during the early inpatient phase of the treatment and seldom led to treatment cessation (2%).[6]

The phase II single-arm trial included patients with previously treated mUM [second line and beyond (2L+)]. Two-year survival rate was 37%, compared to 24% in historical controls treated with anti-PD-1 monotherapy.[3,79,80] The phase III trial investigated tebentafusp in the 1L setting. Compared to investigator's choice of treatment [comprised mostly pembrolizumab (82%), ipilimumab or dacarbazine], tebentafusp significantly improved mOS [21.7 versus 16 months, hazard ratio (HR) 0.51] and 1-year OS (73% versus 59%).[6] The response rate was 9%, but disease control rate was higher (46% versus 27%). Notably, IpiNivo was not an option in the control arm, although it is unknown if IpiNivo is superior to anti-PD-1 alone in mUM (see section Immune Checkpoint Inhibitors in UM - Clinical Evidence).[41,44]

Strikingly, tebentafusp improved OS regardless of response. In patients who had progressive disease (PD) as best overall response (BOR), the tebentafusp group had a superior mOS (15.3 versus 6.5 months, HR 0.43). This suggests that radiological assessment is insensitive to OS benefit from tebentafusp, and that benefit may extend beyond PD, as it may reshape tumour biology to a more indolent course. Notably, 43% of tebentafusp-treated patients received treatment beyond progression; the potential benefit of this approach is currently under investigation.[81]

Predictive biomarkers of tebentafusp benefit

The tebentafusp trials were accompanied by extensive multiomic efforts to investigate mechanisms of response and predictive biomarkers (Figure 3). The development of an early rash was hypothesised to be an on-target adverse effect that may predict tebentafusp efficacy.[82] Although the presence of a rash during the first week was associated with prolonged OS, it lost statistical significance following adjustment for other prognostic factors. The absence of rash also did not exclude clinical benefit.[6]

A normal LDH and alkaline phosphatase at baseline and female sex were not significantly associated for tebentafusp

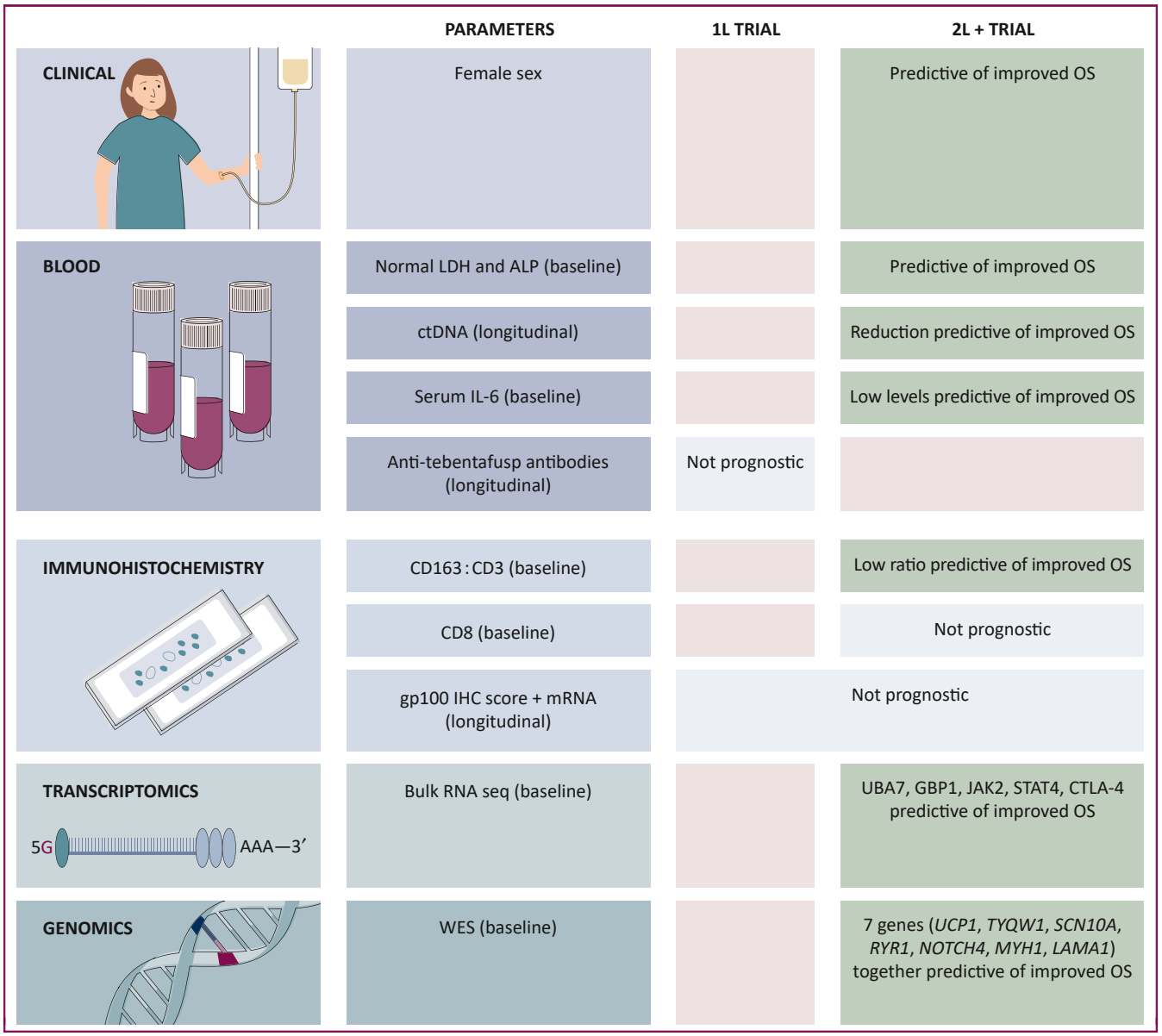

Figure 3. Multiomic determinants of survival following tebentafusp.
1L, first line; 2L+, second line and beyond; ALP, alkaline phosphatase; CD163, M2 macrophage; CD8, cytotoxic T cell; CD3, T cell; ctDNA, circulating tumour DNA; IHC, immunohistochemistry; IL-6, interleukin-6; LDH, lactate dehydrogenase; mRNA, messenger RNA; NA, no data available to date; OS, overall survival; WES, whole exome sequencing.

response; however, they were strongly predictive for survival ≥2 years in multivariate analysis.[83]

In the 2L+ trial, whole exome sequencing was carried out at baseline in 63 tebentafusp patients. Patients with tumours harbouring somatic missense mutations in seven specific genes (including NOTCH4 and UCP1) had longer OS (HR 0.2, 95% confidence interval 0.1-0.47) when grouped together; however, the biological significance of these genes is incompletely characterised.[84] When grouped by secondary driver mutations, no associations with response or survival benefit were observed.

Tumour bulk RNA sequencing, multiplex immunohistochemistry (IHC) and serum were analysed at both baseline and on-treatment timepoints (post-third dose) in the 2L+ trial.[84] Longitudinally, tebentafusp resulted in up-regulation of antigen presentation gene expression (e.g. *HLA-I*, *TAP1*, *TAP2*, *PSMB8*), IFN signatures and GZMB messenger RNA (mRNA). Patients with high baseline expression of UBA7 and GBP1, which are inducible by IFNs, had improved OS following tebentafusp.[84]

As the molecular target of tebentafusp, gp100 expression was investigated as a predictive marker. gp100 mRNA and IHC scores were assessed at baseline and following three doses. Patients were divided into gp100-low (lowest quartile at baseline) and gp100-high (the remainder) subgroups. Tumours with high gp100 mRNA had increased CD3 and CD8 infiltration after treatment, and increased T-cell activation (increased IFN-γ, GZMB and PRF1 expression), whereas gp100-low tumours had minimal change.[85] However, there was no difference in tumour shrinkage or OS between gp100-high and -low groups, showing a disconnect between immune infiltration and clinical benefit. It is likely

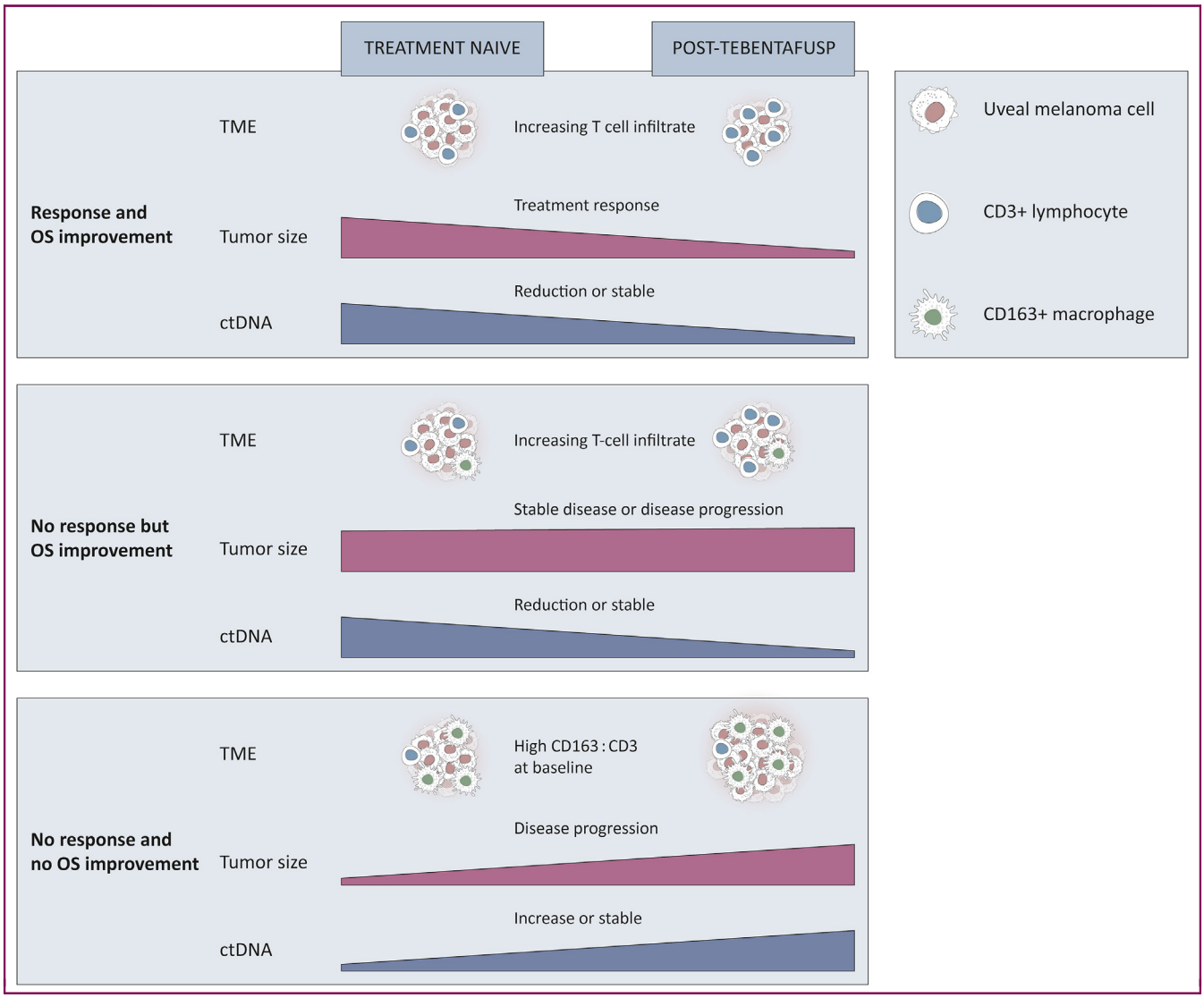

Figure 4. Hypothesised circulating tumour DNA (ctDNA) and tumour microenvironment (TME) dynamics following tebentafusp.
OS, overall survival.

that not all T cells trafficked into the TME by tebentafusp are tumour reactive; longitudinal characterisation of intratumoural TCRs and phenotypes on-treatment is needed. Notably, a partial response was observed in one patient with very low gp100, suggesting that low gp100 expression can still generate a response. By IHC, tebentafusp increased CD3 and CD8 infiltration by two- to threefold compared to baseline in all patients.[86] Separately, in a post-mortem study, metastases from tebentafusp-treated patients had a higher T-cell infiltration compared to non-tebentafusp-treated patients.[87] This suggests that increased infiltration may persist beyond tebentafusp treatment, which may contribute to tebentafusp benefit beyond radiological progression.

The balance of pro-inflammatory and immunosuppressive influences within the TME may impact tebentafusp efficacy. *In vitro*, immunosuppressive CD163+ macrophages inhibited bispecific molecule-mediated T-cell killing of CM cell lines. In patients with mUM, a low CD163 : CD3 (macrophage/T-cell) ratio in pretreatment tumour biopsy was associated with improved OS. In the 1L study, patients in the lowest CD163 : CD3 quartile had a superior 1-year OS of 80%, compared to 65% in the rest.[88] Across the cohort, tebentafusp increased serum IFN-γ and pro-inflammatory chemokines CXCL9/10 after each dose.[89] In the 2L+ trial, patients with low pre-treatment serum IL-6, a pro-angiogenic cytokine involved in tumour proliferation, also had improved OS.

Liquid biopsy technologies, such as ctDNA measured longitudinally at baseline and on-treatment, has demonstrated utility as prognostic and predictive markers across multiple tumour types, including mCM.[51] Within mUM, ctDNA is detectable in 92% of patients.[90] Higher ctDNA levels correlate with higher mUM tumour burden, inferior OS and PFS.[90-93]

In the 2L+ trial, ctDNA was evaluated at baseline, week 5, 9 and 25.[90] Tebentafusp reduced ctDNA in 70% of all patients, and in 65% of patients with PD as BOR.[90,94] Notably, ctDNA levels did not decrease in two out of four responders, suggesting that ctDNA reduction is not mandatory for response. The magnitude of ctDNA reduction correlated with OS

improvement; for each 0.1 log reduction in ctDNA, OS improved by an HR of 0.8. Patients who achieved ctDNA clearance had markedly superior OS with an HR of 0.1. ctDNA clearance was achieved in 14% of the cohort; most of these patients had PD or stable disease as BOR.

Pilot radiomic studies have studied patients who achieve ctDNA reduction despite PD as best response.[95] Through unsupervised machine learning, a volumetric signature classified whether PD patients had any ctDNA reduction, with 81% sensitivity and 63% specificity (area under the curve 0.71). This may have utility as radiomics is likely more accessible than ctDNA, or could be used in combination with ctDNA.

We hypothesise that ctDNA reduction may be a superior measure indicator of survival benefit than radiological response alone, for multiple reasons (Figure 4). Firstly, tebentafusp-mediated immune infiltration of metastases may mask low-volume tumour shrinkage, but result in ctDNA reduction. In patients who live longer despite PD as BOR, tebentafusp may alter the TME and slow tumour growth without tumour shrinkage, thereby reducing ctDNA.

Markers beyond radiological response are urgently needed to identify non-responders who are likely to derive survival benefit from tebentafusp. Furthermore, it is unknown if patients benefit from continuing tebentafusp beyond progression; further research to determine the optimal time to stop therapy for maximal benefit and minimal toxicity is required.

Future approaches and emerging agents

As tebentafusp increases tumour T-cell trafficking,[87] and these T cells may up-regulate immune checkpoints, it may synergise with ICIs.[96] The optimal combination or sequencing of tebentafusp with ICIs is unknown but their potential combined toxicity must be considered. Combination tebentafusp, durvalumab ± tremelimumab was recently reported in an early 2L+ mCM study.[97] Tumour shrinkage occurred in 49% of patients with primary anti-PD-1 resistance compared with 28% in patients with acquired anti-PD-1 resistance. Thus, tebentafusp may sensitise tumours with primary ICI resistance, but less effectively in acquired ICI resistance, suggesting a possible shared mechanism of resistance in that setting. As most mUMs are ICI refractory, tebentafusp with anti-PD-(L)1 should be investigated in the 1L, while triplet anti-PD-(L)1, anti-CTLA-4 and tebentafusp should be evaluated carefully for toxicity relative to incremental benefit.

We hope that adjuvant tebentafusp trials are on the horizon. We advocate that such trials should prioritise high-risk tumours (such as M3, BAP1 loss, more advanced-stage tumours or class II GEP) and be stratified by risk groups, to identify patients who are likely to benefit.[4,98] Although not all patients will have tumour tissue available for molecular profiling, as biopsy is not mandatory for diagnosis, larger tumours usually undergo enucleation and will likely capture most high-risk tumours. In future, peripheral blood may also yield the molecular profile, as liquid biopsy methods become increasingly sensitive.

There is a critical inequity for non-HLA-A2*02:01 patients who are ineligible for tebentafusp. To address this, bispecific molecules that are HLA agnostic or cater to broader HLA subtypes are in development. RO7293583 is an HLA-agnostic bispecific molecule targeting TYRP1, a melanosomal enzyme, currently in early trials for patients with TYRP1-positive melanomas (NCT04551352). TYRP1 is expressed in 61% of primary UMs, and is associated with BAP-1 loss and inferior metastasis-free survival.[99] IMC-F106C is an HLA-A2*02:01 bispecific molecule that targets PRAME (melanoma antigen expressed in 69% of mUMs[100]) and is also in early trials (NCT04262466). Other bispecific molecules targeting key checkpoints such as LAG3 and PD-(L)1 are in development, but are not yet recruiting patients with melanoma.[101]

OTHER IMMUNO-ONCOLOGY APPROACHES

Adoptive cell therapy

Adoptive cell therapy (ACT) is one of the first treatments that demonstrated durable response in mUM, although the benefit was limited to a small number of patients.[102] The trial included 21 patients who received unselected (as opposed to tumour-reactive) autologous T cells.[102] This reflected a moderate-risk cohort; 86% were M1b/1c, 38% BAP1 mutated and 85% had extrahepatic disease. The ORR was 35% with three ongoing responses [up to 21 months complete response (CR)]. This suggests that tumour-reactive T cells were present in the TME before ACT but were insufficient to overcome the tumour.[102]

Compared to non-responders, responder TIL products had a higher percentage (9.4% versus 0.6%) and absolute number of tumour-reactive T cells (8.1×10^9 versus 0.5×10^9), and higher levels of IFN-γ release after exposure of T cells to tumour cells *in vitro*. In responders, disease progression (4/7) occurred only via new metastases; responding lesions did not later progress. We hypothesise that new clones may arise following immunoediting and immune escape mechanisms such as alterations in antigen presentation machinery. New lesions may also arise from a tumour clone that was not present in the metastases used to manufacture the TIL product, and thus corresponding tumour-reactive T cells were not represented. Therefore, harvesting a single metastasis for ACT may be limited by intertumoural heterogeneity. The organ of origin of tumour harvest may also impact ACT efficacy; for example, the immunosuppressive TME of liver metastases may yield more exhausted T cells compared to other sites.[61]

Novel approaches hypothesise that a more tumour-specific TIL product will be superior to an unselected T-cell product.[103] Other ACT trials are targeting shared antigens such as SLC45A2, a highly UM-specific melanosomal transport protein in patients with HLA-A2*02:01 or HLA-A*24:02[104,105] (NCT03068624). However, such approaches targeting a single HLA-restricted antigen may lead to

immunoediting, with selective pressure potentially leading to emergence of other subclones.

Vaccines

Early-phase cancer vaccine trials in mUM have utilised dendritic cells (DCs) loaded with neoantigens derived from autologous tumour RNA. A pilot trial with 5 patients with M3 mUM recently reported an mOS of 36 months.[106] In one patient, the vaccine antigen was adjusted following each episode of disease progression, resulting in disease regression and maintained CR at 65 months.

Next-generation vaccines are now trialling inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)-activated RNA-transfected DCs.[107] IKKβ activates the nuclear factor-κB pathway, which increases DC expression of co-stimulatory molecules, improves memory cytotoxic T-cell response and activates natural killer cells.[108,109] In this approach, the DCs are loaded with commonly expressed UM antigens, RNAs derived from common UM driver mutations and autologous tumour RNA, and given with PD-1 or IpiNivo. DC vaccinations may be a novel personalised treatment that can be adapted as acquired resistance develops.

TLR9 agonists

Toll-like receptor 9 (TLR-9) receptors are highly expressed on plasmacytoid dendritic cells (pDCs). When TLR-9 is engaged, pDCs release IFN-α and present antigens more effectively, strengthening the antitumour response.[110] SD-101 is a TLR-9 agonist currently in early trials in mUM, administered alone or in combination with ICIs (NCT04935229). It is delivered by pressure-enabled hepatic artery infusion and improves drug delivery by overcoming poor perfusion pressure due to abnormal tumour vascularity.

Protein kinase C and MET inhibitors

Combination darovasertib (protein C kinase inhibitor, downstream of GNAQ and GNA-11) and crizotinib (cMET inhibitor) presents a novel synergistic approach that converges on the MAPK pathway.[111] It is hypothesised that hepatocyte growth factor signalling may limit darovasertib activity, but this may be overcome by combination with MET inhibition. The darovasertib crizotinib phase I trial included 35 patients in any line, with an ORR of 31%.[112] A phase II/III trial of this combination is underway. Single-agent darovasertib is under investigation in the neo-adjuvant setting, following observations of primary tumour shrinkage in pilot studies.[113]

CONCLUSION

mUM is characterised by a low TMB and limited neo-antigens within an immunosuppressive TME, rendering it suboptimal for immuno-oncology approaches. However, antitumour immune responses do occur, evidenced by responses to ICIs and ACT.

Tebentafusp is the first therapy in decades to improve OS in mUM. Critically, tebentafusp challenges the traditional response-survival paradigm, improving survival even in non-responders. Predictive markers beyond RECIST are urgently needed; ctDNA may be suitable, but is not yet widely available in the clinic. Trials involving bispecific molecules that benefit broader HLA subtypes must continue, so that the inequity for non-HLA-A2*02:01 patients may soon be abolished.

Moreover, there are a significant number of immuno-oncology trials in progress in mUM, raising the promise of further advances. Tebentafusp may synergise with ICIs; optimal combinations and sequencing await investigation. Highly personalised treatments such as ACT and cancer vaccines have also demonstrated potential for durable response in pilot studies.

Given the broad range of survival outcomes in UM, future clinical trials should stratify patients by prognostic subgroups. The rarity of UM has meant that scientific discovery is limited by poor statistical power; international efforts can overcome this. Clinical trials should be paired with multiomic studies to characterise mechanisms of response and resistance. Collectively, we must collaborate to maximise the potential of durable responses to successfully bring UM to the forefront of the immuno-oncology era.

FUNDING

This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (CRUK) (CC2044), the UK Medical Research Council (CC2044), and the Wellcome Trust (CC2044). For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

DISCLOSURE

ST is funded by CRUK [grant number C50947/A18176), the NIHR]Biomedical Research Centre at the Royal Marsden Hospital and the Institute of Cancer Research [grant number A109], the Kidney and Melanoma Cancer Fund of the Royal Marsden Cancer Charity, the Rosetrees Trust [grant number A2204], Ventana Medical Systems [grant numbers 10467, 10530), the National Institutes of Health (US) and the Melanoma Research Alliance. ST has received speaker fees from Roche, Astra Zeneca, Novartis and Ipsen. ST has patents filed Indel mutations as a therapeutic target and predictive biomarker PCTGB2018/051892 and PCTGB2018/051893; Clear Cell Renal Cell Carcinoma Biomarkers P113326GB. PN has received funding from AZ, BMS, Immunocore, Ipsen, MSD, Merck, Novartis, Pfizer, 4SC. All other authors have declared no conflicts of interest.

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