Review

Liver metastasis in uveal melanoma — treatment options and clinical outcome

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Abstract

Uveal melanoma (UM) is the most prevalent primary intraocular malignancy in adults with a stable incidence rate between five and seven cases per million in Europe and the United States. Although UM and melanoma from other sites have the same origin, UM has different epidemiological, biological, pathological and clinical features including characteristic metastatic hepatotropism. Despite improvements in the treatment of primary tumours, approximately 50% of patients with UM will develop metastases. In 90% of cases the liver is the first site of metastasis, however the mechanisms underlying this hepatic tropism have not been elucidated. Metastatic disease is associated with a very poor prognosis with a median overall survival of 6 to 12 months. Currently, there is no standard systemic treatment available for metastatic UM and once liver metastases have developed, prognosis is relatively poor. In order to prolong survival, close follow-up in all patients with UM is recommended for early detection and treatment. The treatment of metastatic UM includes systemic chemotherapy, immunotherapy and molecular targeted therapy. Liver-directed therapies, such as resection, radioembolization, chemoembolization, immunoembolization, isolated and percutaneous liver perfusion as well as thermal ablation represent available treatment options. However, to date a consensus regarding the optimal method of treatment is still lacking and the importance of setting guidelines in the treatment and management of metastatic UM is becoming a priority. Improvement in knowledge and a better insight into tumour biology, immunology and metastatic mechanism may improve current treatment methods and lead to the development of new strategies paving the way for a personalized approach.

Keywords: uveal melanoma; molecular characteristic; liver metastasis; chemotherapy; immunotherapy; liver-directed therapy; prognosis; review

1. Introduction

Uveal melanoma (UM) although relatively rare is the most common primary intraocular malignancy in adults representing approximately 3–5% of all melanoma and 80% of ocular melanoma cases [1–3]. The mean annual incidence is between five and seven cases per million in Europe and the United States, eight per million in Australia and has remained largely unchanged over the past 30 years [4–7]. The majority of ocular melanoma cases originate in the choroid (90%) followed by the ciliary body (7%) and the iris (3%) [8]. Predisposing factors for UM are ethnicity with incidence recorded as relatively low in Africa and Asia, age, sex with higher incidence seen in males, light eye colour and fair skin with sensitivity to sunburn. A higher incidence is also evident in individuals with cutaneous, iris and choroidal nevus, ocular or oculodermal melanocytosis (Nevus of Ota), dysplastic nevus syndrome and mutation of breast cancer 1 (BRCA1) associated protein 1 (BAP1) [2,4,9–17].

Cutaneous (CM) and UM share the same embryologic origin, however their epidemiology, prognostic features, molecular characteristics, clinical and biological behaviour differ and as such treatment of these tumours require different approaches [1,3,18,19]. Despite significant improvement in local treatment of the primary tumour, the 5-year survival rate of patients with UM is 50–70% and remains unchanged over the past decades [5,8,18–20]. Approximately 50% of the patients develop metastases within five years from initial diagnosis with median survival between 6 and 12 months due to the lack of efficient treatment options [9,21–25]. In patients with liver metastases, irrespective of the treatment method used the mortality rate is still approximately 80% after one year and 92% two years after diagnosis with only 1% of patients surviving over 5 years [26]. Better survival is seen in patients with metastases outside the liver or when the liver is not the first site [19]. Risk factors for systemic development include: large tumour size, epithelioid cell type, extra-scleral extension, loss

of chromosome 3 and chromosome 8q amplification [27]. To date there is no standard form of care for patients who develop metastasis. Potential treatment options are surgical resection or other liver directed therapy, chemotherapy, immunotherapy and targeted therapies [2,9,19,20,25–29].

Advancement in the understanding of UM biological behaviour, the molecular and immunobiological characteristics are central to the development of new therapeutic strategies which will enable new therapeutic goals and approaches to treatment bringing benefit to patients with metastatic UM.

2. Molecular and immunobiological features of uveal melanoma

2.1 Molecular features

It is established that the biological, clinical and pathological characteristics, prognosis and dissemination capacity of UM and CM notably differ, in fact CM has more in common with conjunctival melanoma than with UM [8,18, 21,30–47] (Table 1, Ref. [2,4–7,9–19,21,24–26,29,30,33–47]). Cutaneous and conjunctival melanomas have high tumour mutation burden which is attributed to the mutagenic effect of ultraviolet light, conversely UM shows a very low mutational burden, one of the lowest of all cancer types [33–35].

UM and CM activate the mitogen-activated protein kinase (MAPK) pathway which affects cell proliferation, however using different mechanisms. In CMs this pathway is activated by mutations in B-Raf Proto-Oncogene (BRAF) which is present in 52% of cases, RAS present in 10–25% of cases and loss of function in neurofibromin 1 (NF1) gene seen in 14% of cases [39,40]. Alternatively, UM is characterized by point mutations in the Guanine nucleotide-binding protein subunits α-Q (GNAQ) and Guanine nucleotide-binding protein subunits α-11 (GNA11) which are identified in approximately 80% of patients with UM and are mutually exclusive [41,42]. Mutations in GNAQ or GNA11 lead to YAP (yes-associate protein) over-activation which induces uncontrolled cell growth and proliferation, inhibits cell death and leads to the formation of malignant tumours [44,45].

The specific cytogenetic alterations monosomy 3, amplification of 8q and potential loss of chromosome 1 and 6q are characteristic for UM and are associated with risk of metastasis and poor survival [27,42,46,48]. It is reported that in 84% of metastatic and 38% of primary UM the BAP1 tumour suppressor gene mutation located on chromosome 3 (3p21.1) is present supporting the relevance of BAP1 mutations in the development of metastatic UM [17,21,43,47]. BAP1 acts as a tumour suppressor gene whereby its absence renders tumour cells more prone to metastasis. A more beneficial prognosis is associated with splicing factor 3b1 (SF3B1), serine/arginine-rich splicing factor 2 (SRSF2) and eukaryotic translation initiation factor 1A, X-linked (EIF1AX) mutations [3,49,50]. SF3B1 is

involved in pre-mRNA splicing and is mainly related with late-onset of metastasis whilst EIF1AX is involved in protein translation and is associated with low metastatic risk, however the carcinogenic mechanism of these mutations is still unclear [32,47,51,52]. Mutations in BAP1, SF3B1 and EIFlAX are mutually exclusive and represent possible prognostic markers for explaining and predicting the metastatic behaviour of UM [53].

In order to assess metastatic potential, UM can be classified using gene expression profiling (GEP) which determines the expression of 15 genes of the primary tumour. GEP divides UM into class 1 having low metastatic risk and class 2 with high metastatic risk. Class 2 is more strongly associated with the mutational inactivation of the tumour suppressor BAP1 and monosomy 3 and patients within this class of tumours have a five-year metastatic risk of 51% [17,54]. Despite having lower risk for metastases with a five-year risk of approximately 4%, as many as 15% of patients in class 1 also develop metastatic disease [38,47,55].

2.2 Immunobiological features

In recent times there has been a significant increase in understanding the immunobiology of UM, particularly regarding the cytogenetic and signal transduction pathways involved in carcinogenesis and metastatic growth [56].

The eye as an immune-privileged site of the body has various immunosuppressive mechanisms and defence capabilities which prevent uncontrolled inflammatory processes and an immune response which may cause impaired vision [19,57,58]. However, this immune privilege also reduces an immune response to melanoma tumour cells and may provide an escape mechanism for UM [19,59,60]. Restricting the access of inflammatory cells to the eye via the blood-eye barrier and suppressing T cell proliferation additionally reduces immune activity [19,58,61]. Further, inhibition of NK activity cells and NK cell-mediated tumour cell death is made possible since the aqueous humour of the eye contains various immunosuppressive and anti-inflammatory cytokines. This includes macrophage migration inhibitory factor (MIF), transforming growth factor-beta (TGF-β), α-melanocyte stimulating hormone (α-MSH), complementary regulatory protein (CRP), vasoactive intestinal peptide (VIP) and low expression of major histocompatibility complex (MHC) [56,57,59,61]. Another mechanism that enables unwanted immune responses is the anterior chamber associated immune deviation (ACAID) [19,62,63]. Immune responses to antigens are also modified via immunosuppressive mechanisms such as programmed cell death ligand-1 (PD-L1) and indoleamine 2,3-dioxygenase (IDO) in order to protect non-regenerating ocular tissues [19,58]. Retinal pigment epithelial cells express PDL1 and PD-L2 receptors, which inhibit T cell response and additionally provide immune privilege of the eye [61,64]. Some preclinical studies have shown the existence of an up-regulation of PD-L1 induced by interferon-gamma (IFN-γ) in UM which

Table 1. Characteristics of uveal, conjunctival and cutaneous melanoma.

	Uveal melanoma	Conjunctival melanoma	Cutaneous melanoma	References
Origin	Melanocytes from the stroma of the uveal layer of the eye	Melanocytes from the basal layer of the conjunctiva	Melanocytes from the basal layer of the epidermis of the skin	[33]
Relative incidence in all melanoma cases	5%	1%	90%	[29]
Trends in incidence	Stable	Rising	Rising	[4–7,30]
Etiological factors	Mostly unknown, oculodermal melanocytosis, 1% hereditary (BAP1)	Sun, primary acquired melanosis, conjunctival melanocytic nevi	Sun, melanocytic nevi, 2% hereditary (CDKN2A)	[2,4,9–17,29]
Mutation burden	Very low	High	High	[33–35]
Metastasizing	Hematogenous	Lymphogenous and hematogenous	Mostly lymphogenous	[24–26]
Most common sites of metastases	Liver (89%)	Lymph nodes (cervical, preauricular, parotid and submandibular)	Skin (13–38%)	[26,30,33,35]
	Lung (29%)	Lungs, liver, skin and brain	Distant lymph nodes (5–34%)	
	Bones (17%)		Distant subcutaneous tissues (32%)	
	Skin (12%)		Lung (18–36%)	
	Lymph nodes (11%)		Liver (14–20%)	
			CNS (2–20%)	
			Bone (4–17%)	
Five year survival	86.0%	81.6%	91.3%	[9]
Treatment of primary tumour	surgery (enucleation, endoresection, exoresection) radiotherapy (proton beam radiotherapy, gamma knife)	Mostly surgery with adjuvant therapy (cryotherapy, brachytherapy, topical chemotherapy)	Mostly surgery	[18,25,30,35]
Genetic mutations	GNA11 (55%)	BRAF (35%)	BRAF (40%)	[19,30,36–41]
	GNAQ (40%)	NRAS (20%)	NRAS (20%)	
	SF3B1 (25%)	NF1 (14%)	KIT (<5%)	
	EIF1AX (13%)	KIT (5%)	BAP1 (<1%)	
	SRSF2 (4%)		NF1	
	BAP1 (38% primary, 84% metastasizing UM)			
Chromosome anomalies	Amplification of 6p, 8q	Amplification of 1q, 3p, 7,17q	Amplification of 1q, 3p, 7,17q	[21,30,33,42–47]
	Loss of 3, 1p, 6q	Loss of 9p, 10, 11, 12q	Loss of 9p, 10, 11, 12q	

BAP1, mutation of breast cancer 1 (BRCA1) associated protein 1; GNA11, Guanine nucleotide-binding protein subunits α-11; GNAQ, Guanine nucleotide-binding protein subunits α-Q; SF3B1, splicing factor 3b1; EIF1AX, eukaryotic translation initiation factor 1A, X-linked; SRSF2, serine/arginine-rich splicing factor 2; NF1, neurofibromin 1.

consequently down-regulates immune response and T cell activation. All these processes lead to an immunosuppressive effect on the tumour microenvironment (TME) [58]. A lymphocyte-rich TME for different types of cancer usually implicates favourable outcome. On the other hand a distinguishing feature of UM in relation to other tumours is that an unfavourable prognosis is associated with tumour-infiltrating lymphocytes (TILs) as well as high infiltration by tumour associated macrophages [19,31,65]. The genetic background of UM determines this inflammatory environment, and it is proposed that gain of chromosome 8q may activate macrophage infiltration whilst sequential loss of BAP1 expression may be associated with T cell infiltration [41,66]. Uveal melanoma tumour cells have adapted and used anti-inflammatory environment in the eye to avoid immune recognition. This immunological privilege, together with the mechanisms of carcinogenesis of UM, has resulted in the ineffectiveness of immunotherapy [60,63,67]. Additional mechanism that could protect metastatic UM cells from systematic immune surveillance is the immune-modulating microenvironment in the liver [65].

3. Uveal melanoma and the liver

Circulating tumour cells are cancer cells which after separating from the primary tumour spread throughout the body via the bloodstream [68]. The interaction between the liver immune system and cancer cells creates unique and complex TME. New immune treatment strategies, such as blockade of immune checkpoints have significantly improved the survival of patients with CM without liver metastases; however, the response to this form of therapy in patients with hepatic metastases is less pronounced. Similarly, UM patients with liver metastases failed to reap benefit from these forms of immunotherapy [65].

The liver as a frequent site of metastases creates an immune-protected site for tumour growth due to its ability to locally mediate in T-cell inactivation, immune tolerance and cell destruction. All this can provide additional protection and a stimulus for the growth of UM cells [69]. Immunosuppressive TME as found in the liver may limit immunotherapy activity in advanced UM. Due to the role of the liver in filtering toxins from the body it can be assumed that the liver increases tolerance to tumour antigens compared to other organs [70]. Recent research shows an association between metastatic site and progression-free survival (PFS), overall survival (OS) and response rate to immunosuppressive treatment of melanoma. The response to immunotherapy has been shown to be more favourable in the case of metastases in the lungs and skin compared to metastases in other organs, particularly in the liver [19,36,60,70–72].

3.1 Liver metastasis of uveal melanoma

The mechanisms involved in pronounced hepatotropism of UM and metastatic spread to the liver are still not fully understood, however it is considered that multiple factors are involved. One of the proposed factors refers to slow flow in the liver sinusoids which enable a greater contact between hepatic cells and foreign molecules allowing UM cells to be captured in the liver [73,74]. This confinement of tumour cells may be additionally increased by vascular cell adhesion molecule-1 present on sinusoidal endothelial cells [73,75]. Further, the high concentration of chemokines in the liver may attract UM cells causing an interaction with the chemokine receptors located on the surface of tumour cells. Alternatively, chemokine-related liver tropism can be due to the loss of chemokine receptors once melanoma cells enter the liver [65,76]. It has also been hypothesized that high incidence of UM hepatic metastases may be caused by increased expression of cMET, a tyrosine kinase inhibitor activated by binding to the hepatic growth factor (HGF) receptor produced in the liver which is elevated in primary UMs [21,77]. Another proposed reason for hepatotropism is the presence of growth factors in the liver such as the insulin-like growth factor-1 (IGF-1) which plays a key role in tumour genesis, stimulation of cell growth, prevention of apoptosis and the maintenance of malignant phenotype. IGF-1 is mainly produced in the liver and a high expression of IGF-1 receptor (IGF-1R) has been identified in samples of UM hepatic metastasis [78]. Further, the presence of interleukin-8 and vascular endothelial growth factor in the liver could promote the angiogenesis of tumour in the liver immune-modulatory microenvironment and consequently tumour growth [79,80]. Specific chromosomal and genetic abnormalities related to BAP1 mutation as well as monosomy 3 and polysomy 8q may be an additional cause of liver tropism. However, this expression is not present in all hepatic metastasis of UM and as such these abnormalities need further research [62,74,81].

3.2 Immunomodulatory mechanisms in the liver

The liver is the most common site of metastasis for various types of malignancies, namely gastrointestinal cancers, breast and prostate carcinomas, melanomas including UM, neuroendocrine tumours and sarcomas [82]. The immunomodulatory nature of the liver is determined by its continuous exposure to exogenous food antigens and allergens as well as gut derived endotoxins. The characteristic anatomy of the liver encourages both direct and indirect influx of lymphocytes and through its ability to induce antigen-specific tolerance it can modulate the immune response to pathogenic and tumour cells [65]. The liver microenvironment is composed of resident non-immune and immune cells, such as hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), T, NK, and NKT cells that regulate the balance between tolerance and the defence against pathogens. LSECs are capable of receptor-mediated phagocytosis and can present blood-derived antigens to CD4+ T and CD8+ T cells. Upon stimulation, LSECs also produce the chemokines, CXCL9 and

CXCL10 that recruit T lymphocytes. Alternatively, LSECs may express the inhibitory immune checkpoint PD-L1, regulating T cell activation. KCs, the most represented tissue macrophages, located in the sinusoidal vascular space can recognize microorganisms and tumour cells via the C-type lectin receptor Dectin-2 [83]. They may induce a down regulation of MHC class II expression and the costimulatory molecules, CD80 and CD86, on LSECs, inhibiting antigen presentation to helper T cells by producing soluble factors, such as IL-10 and prostaglandin E2 [65,83].

Different populations of resident and transit lymphocytes can be found in the liver differing significantly from those observed in other tissues and in the circulatory system. Nearly half of the liver lymphocytes comprise NK cells. Liver-resident NK cells are composed of CD49+ and Eomeshi NK cells where Eomeshi NK cells located in the sinusoidal space account for 50% of human liver NK cells. These cells respond to a variety of cell-surface ligands expressed by infected damaged or tumoral cells exerting direct cytotoxicity by releasing cytotoxic granules containing perforin and granzymes. NKT cells represent an important immunomodulatory population of the liver, have a restricted TCR activity and are capable of reacting to lipid antigens. However, NKT cells based on the type of activating signal may encourage inflammatory and anti-inflammatory responses, producing cytokines, namely IFN-γ, IL-4, and IL-17. NK cells are presumed to control metastatic growth of UM, whilst NK T cells are capable of suppressing the cytotoxicity of NK cells via bone marrow-derived cells [83].

There is evidence that the liver represents a permissive microenvironment that sustains the survival and subsequent outgrowth of circulating tumour cells and may be crucial in facilitating cancer liver metastasis [83,84]. Cancer cells entering the liver depend on interaction with the liver immune microenvironment for arrest, immune evasion, colonization, migration and proliferation. Parenchymal and nonparenchymal liver cells, as well as recruited inflammatory and immune cells, participate in the response to the invading tumour cells and may impede or promote their progression [85]. Considering its physiological role the liver is continually exposed to antigens and is generated by immunosuppressive mechanisms such as T cells anergy, induction of regulatory T cells and deletion of antigen specific T cells [86]. UM cells that exit from the eye find additional protection within the immune-modulatory microenvironment of the liver. They may share similar mechanisms with the tropism of various cancer metastasis via regulation of the liver immune microenvironment which may favour metastatic growth, protecting cancer cells from cytotoxic immune responses [74,83]. Metastatic cells can trigger a liver specific tolerance mechanism to suppress systemic anti-tumour T cell immunity. This could explain why patients with liver metastases have a worse response to immunotherapy and poorer outcome making liver metastasis the major contributor in tumour-related death [29,87].

4. Systemic metastasis of uveal melanoma

Circulating tumour cells can be found in UM patients before clinical signs of metastatic disease [29]. There is a correlation between metastatic risk and the size of the tumour, suggesting that metastatic seeding might occur throughout tumour growth [27,88]. Analysis of a large group of patients stressed that early treatment of UM may prevent metastatic spread in some patients which is in accordance with this hypothesis [89]. In only 2% of patients metastatic disease is confirmed at the time of diagnosis implicating the presence of subclinical micro-metastases prior to actual diagnosis [90]. Eskelin *et al*. [91] estimated that micro-metastases from UM could develop as early as five years before the treatment of the primary tumour. The evidence indicates that limited immune surveillance in the immune-privileged eye environment enables the UM tumour to be dormant and may be the explanation for the appearance of metastasis or recurrence after more than five years of a recurrence-free period [92].

Up to 50% of patients with UM develop metastases within 15 years of diagnosis [2,20,21,23,25,58]. Due to the lack of lymphatic drainage from the eye primary UM spreads predominately haematogenously [19,93]. Approximately half of all patients with primary UM ultimately develop metastasis with spread of tumour cells to the liver (89%), lung (29%), bone (17%), skin (12%) and lymph nodes (11%) [94]. Despite successful treatment of the primary tumour using radiotherapy or surgical resection, survival has remained relatively unchanged [9,21–25,58]. The 10-year metastasis rate varies among UM patients depending on the tissue of origin: 33% for ciliary body melanoma, 25% for choroidal melanoma and 7% for iris melanoma [8,92]. Hepatic metastasis is an important determinant of clinical course and survival rate. After their development, the median survival of patients is 6 to 12 months with a slightly better prognosis in patients receiving treatment for metastasis [9,21–25,95]. The latency period for the onset of metastatic disease could be even more than 25 years, as such the patients require thorough monitoring over a long period of time [21].

Numerous clinical and histopathological features have been investigated in order to predict prognosis of patients with UM. Clinical factors associated with poor outcome include advanced patient age at time of diagnosis, large tumour size measured in diameter and thickness, extrascleral extension of the tumour, involvement of the ciliary body and presence of subretinal fluid or intraocular haemorrhage. Some pathologic features including epithelioid cell type, increased mitotic activity, extracellular matrix patterns, immune cell infiltration, genetic background and incomplete local control after primary tumour treatment are also associated with unfavourable prognosis [2,8,18,25,30]. The genetic analysis of melanocyte lesions has identified that extraocular invasion is related to the inactivation of the tumour suppressor gene, BAP1 and monosomy 3, as the main

risk factors for disease spread and strongly correlates with decreased survival [33,96]. The three-year OS rate among patients with monosomy 3 is 60%, whereas patients with disomy 3 have a three-year OS rate of 95–100% [97,98]. Classification of UM using the GEP system may estimate metastatic potential however it cannot determine the time of metastasis occurrence. Nonetheless it may help in planning patient follow-up strategies based on an individualized approach and the selection of patients who could benefit from intensive and long term follow up and potential adjuvant therapy [21,55].

There is no established criteria regarding the type nor the time and frequency of screening for systemic metastasis in UM mostly due to the lack of randomized studies [99–101]. Recommendations for follow-up after primary tumour treatment is within three to six months for two years and subsequently extended to a 6–12 months interval including local and systemic surveillance [21,25]. The aim of ophthalmological examinations is the early detection of treatment-related complications as well as possible local tumour recurrence. Every follow-up should include a full ocular examination and local tumour assessment [102].

The purpose of systemic surveillance is early detection of metastatic disease and particular attention should be taken in the presence of high-risk factors namely class 2 primary tumours, monosomy 3 or tumours greater than 8 mm in apical dimension [46,102]. Since the most frequent site of metastasis is the liver, surveillance of UM patients should include liver function tests and specific liver imaging [2,18,23,25]. Some studies imply that lactate dehydrogenase (LDH) and GGT are sensitive liver function tests for UM and are most often elevated with advanced hepatic involvement [103]. Imaging modalities used in the staging of UM at baseline and follow-up include liver ultrasound, computed tomography (CT) of the head, chest, abdomen and pelvis, whole body positron-emission tomography (PET)-CT or magnetic resonance imaging (MRI) [102]. CT and MRI represent more effective methods than ultrasound [104] where MRI shows the best sensitivity in detecting small liver lesions and is also useful for the detection of lesions in the lungs and retroperitoneal nodes, bones and the brain [99]. Although PET/CT is not currently used as a routine method for detection of metastasis, it can be used as an alternative for patients with contraindications to MRI [104].

5. Treatment of primary uveal melanoma

Local treatment depends on the size and location of the tumour, patient preferences and present comorbidities. The most effective treatment is enucleation, however it highly impacts patient's quality of life. Other surgical options are tumour exoresection or endoresection which can be combined with local radiotherapy to prevent recurrence [2,9,18,24,25]. Currently, the most common local treatment for UM is plaque brachytherapy [18,25]. Other treat-

ment modalities frequently used are proton beam radiotherapy and gamma knife radiotherapy, both very useful since they offer an eye preserving option with good local control, especially in the posterior pole. Possible complications of these therapies include the risk of increased intraocular pressure, cataract formation and optic neuropathy [2,9,18,24,88]. Additional local treatment options that are usually combined with radiation techniques in order to reduce the risk of metastasis are transpupillary thermotherapy, photocoagulation and photodynamic therapy [9,18,25,88,89,92].

6. Treatment of metastatic uveal melanoma

Despite conducted research in which various treatment modalities of disseminated UM were evaluated definite systemic standard treatment for metastatic disease has not yet been established. A meta-analysis of 29 metastatic UM trials conducted between 2000 and 2016 showed the median PFS of 3.29 months and median OS of 10.2 months. In the same analysis the six-month PFS was recorded in 27% of patients and the one-year OS in 43% of patients [100]. Failure of metastatic UM treatment can be due to the specific gene expression profile, somatic mutations, the lack of effective treatment approaches and absence of clinical evidence-based guidelines [36].

6.1 Adjuvant therapy

Local treatment of a primary UM is effective in preventing local recurrence in over 95% of cases. However, circulating UM cells have been detected at diagnosis in patients with no detectable metastases and nearly 50% of patients will develop metastatic disease in a median time of five years [25,105]. Since metastatic UM is resistant to treatment with no evidence that current treatment can extend survival the efficacy of systemic treatment could be improved with adjuvant therapies that target micrometastases and the identification of patients at high risk [23]. A number of adjuvant therapy trials conducted prior to the introduction of molecular methods of UM prognosis failed to show benefit in improving survival rate [106]. Currently, a number of clinical trials with novel classes of molecules namely c-Met, c-Kit and HDAC inhibitors, estrogen receptor modulator, alkylating agents, immunotherapy with dendritic cell vaccination, ICI and anti-LAG3 are underway with promising results. However, in the absence of clear evidence adjuvant therapy for UM cannot be recommended as a standard treatment option at this time [101,106].

6.2 Liver-directed therapies

The hepatotropism of UM contributes to the development of liver-directed therapy methods [105–123] (Table 2, Ref. [28,95,108–110,112,113,115,118–123]). In certain cases, surgical removal of metastatic nodules can offer long-term survival benefit. Surgical resection is the preferred treatment for patients who are medically fit, although

Table 2. Liver directed therapy methods used in the treatment of metastatic uveal melanoma.

Therapy	Characteristics	Clinical outcome	References
Hepatic metastasectomy	● Surgical resection ● Limited indication: good physical condition for general anaesthesia; <10% patients with liver uveal metastasis ● Could be combined with other local treatment	● Median OS of 14 months, extended to 27 months after microscopically complete resection ● Common local relapse	[115]
Radiofrequency ablation	● Alternative for poor surgical candidates with a small number of liver lesions ● Minimally invasive – spares the hepatic parenchyma ● Without anaesthesia ● Minimal morbidity and mortality	● No difference in survival time and DFS in regard to surgical resection ● OS in patients with less than 6 metastatic liver lesions – 19.3 months	[28,109,110]
Laser induced interstitial thermo-therapy	● Novel ablative technique ● Under NMR guidance ● Well tolerated, no mortality, no morbidity reported	● Median survival up to 3 years	[121]
Hepatic arterial infusion of chemotherapy	● Option for patients with liver predominant disease ● High concentrations of chemotherapeutic agent in the liver ● Mitigating systemic side effects	● Significantly longer PFS compared to intravenous administration of chemotherapy ● OS ranging from 10 to 24 months	[112,113,118]
Isolated hepatic perfusion (IHP)	● Open surgical technique ● Liver – surgically isolated and perfused with high doses of chemotherapeutic agent ● Minimum systemic drug exposure ● Not repeatable ● Long procedure time duration, extensive hospitalization ● High morbidity rate	● Response rate ranging from 37.5–68% ● Treatment related death 27% ● Median PFS 8–9 months ● Median OS 11–12 months ● Potential survival benefit up to 1 year	[119]
Percutaneous hepatic perfusion (PHP)	● Similar procedure to IHP, simpler to perform ● Minimally invasive ● Repeatable procedure	● Response rate 36.4% ● Significantly improved median PFS compared with alternative treatment care ● Overall PFS 5.4 months ● Hepatic PFS 7.0 months	[108]
Transarterial embolization	● Particulate or liquid embolic agents ● Complications: PES, liver abscess, liver biloma, liver failure	● Median OS 17 months	[95]

Table 2. Continued.

Therapy	Characteristics	Clinical outcome	References
Transarterial chemoembolization	• Combines hepatic artery embolization with infusion of chemotherapeutic agents • Well tolerated, decreases systemic side effects and limits washout of chemotherapy • Administration of particulate or liquid embolic agents into hepatic arteries • Repeatable procedure • Complications: PES, hepatic decompensation, renal injury, biliary injury, infection, and non-target embolization	• Response rate 20% • Patients with low tumour burden (<20% liver involvement) — significantly improved OS • Patients with high tumour burden (<75% liver involvement) — poor clinical response and numerous complications • OS ranged 5–29 months, average 10 months	[95,120]
Transarterial immuno-embolization	• Infusion of GM-CFS an immune-stimulating agent into hepatic artery, followed by embolization	• Median OS up to 21 months	[122]
Transarterial radioembolization	• Liver directed approach using yttrium-90 (^{90}Y) radiospheres • Complications: Radioembolization-induced liver disease, post-radiation syndrome	• Response rate up to 62% patient • Median OS 7.1 months (range, 1.2–32.3 months), and the median PFS 5.0 months (range, 1.1–32.3 months)	[123]

OS, overall survival; DFS, disease-free survival; PFS, progression free-survival; NMR, nuclear magnetic resonance; IHP, isolated hepatic perfusion; PHP, percutaneous hepatic perfusion; PES, postembolization syndrome; GM-CFS, granulocyte-macrophage-colony-stimulating factor.

this is rarely possible in patients with UM, due to diffuse pattern of the disease. Surgical treatment also depends on experience and techniques used at the particular medical centre. Current hepatic metastasectomy in patients with margin-free resectable tumours in combination with systemic therapy had demonstrated best long-term survival results [107]. Liver surgery in highly selected cases may prolong survival which is correlated with the marginal status and the number of lesions [114–116]. Surgery could be combined with other local treatment such as transarterial embolization, selective internal radiotherapy, isolated hepatic perfusion (IHP), hepatic artery infusion, and immune-embolization which can additionally prolong survival however only in selected patients [95]. A retrospective analysis of surgical treatment of UM liver metastasis showed an OS of 14 months which was extended to 27 months in the case of microscopically complete, R0 resection. Prolonged survival is correlated with successful surgical resection, the presence of 4 or less resected metastases, absence of miliary disease and an interval longer than 24 months between primary tumour diagnosis and occurrence of liver metastasis [115]. Alternative approaches to surgical treatment particularly in patients who are not candidates for surgery include radiofrequency ablation, cryotherapy and stereotactic radiotherapy [24,25,33,48,95]. Other liver-directed therapies take advantage of the dual blood supply in the liver which allows more direct treatment of the metastases via the hepatic artery. Hepatic artery branches vascularize the melanoma, whereas the portal circulation delivers the majority of blood to the normal liver tissue. Intrahepatic therapeutic methods include bland embolization, intra-arterial administration of chemotherapies, intra-arterial hepatic chemoembolization, radioembolization, immune embolization and intra-arterial hepatic perfusion. Intra-arterial hepatic perfusion can be done by IHP which is an open surgical technique that cannot be repeated or by percutaneous IHP (PHP) which is minimally invasive and repeatable [95,105,107,117–119]. Liver-directed therapies enable regional delivery of high doses of medications while minimizing systemic toxicity, meaning that they ensure comparable oncologic treatment effect while minimizing morbidity and can be repeated during the treatment period [117–119]. Prospective data regarding the efficacy of liver-targeted therapies are insufficient, however conducted studies show some clinical benefit. A meta-analysis of data from 912 patients with metastatic UM, showed that 6-month PFS was significantly higher in liver-focused therapy compared with chemotherapy, immunotherapy and targeted therapy, even after adjustment for prognostic factors [100].

6.3 Systemic chemotherapy

A large number of patients are not suitable candidates for locoregional treatments particularly those with multiple metastatic sites and as such the use of systemic therapy has been investigated as an alternative option. Systemic chemotherapy adopted from CM has shown to be relatively ineffective in metastatic UM with a response rate ranging from 0% to 15% [124,125]. Once UM metastasizes to distant organs, particularly the liver the disease becomes resistant to current conventional chemotherapies and their use has had no significant effect on metastasis-free survival or OS of patients [9,27,29,105,107].

6.4 Systemic immunotherapy

Recent advances in immunotherapy have considerably improved survival of patients with metastatic CM although the clinical benefit in UM is more limited. A possible explanation could be differences in expression of neoantigens by the tumour, low immunogenicity and low mutational burden of UM as well as immunosuppressive TME [19,36,62,63,70,98,105,126,127]. Additional reasons arise from immune privilege of the eye, meaning an adaptation to reduce immune-mediated injury in organs that have limited capacity for regeneration such as the eye and brain. This immune privilege may be due to the absence of lymphatic vessels in the choroid and alymphatic barrier of the sclera, which protect primary UM from the immune system and allows its spreading almost exclusively via the hematological route [19,93,97].

6.4.1 The role of immune checkpoint inhibitors

Cancer immunotherapy has gained increasing importance in recent years, partly due to the clinical antitumour effect observed with immune checkpoint inhibitors (ICI) such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and their ligands B7 and PD-L1 [19,57]. ICIs are antibodies, which bind to checkpoints, suppress them and lead to activation and proliferation of T cells, which assist in lysis and degradation of cancer cells [19]. Immune checkpoint blockade has become the basis for clinical research of anti-tumour activity for the most currently approved immuno-oncology agents targeting anti-CTLA-4 (ipilumimab, tremelimumab) and anti-PD-1 (nivolumab, pembrolizumab) or anti-PD-L1 (atezolizumab, durvalumab, and avelumab) [126–129]. Anti CTLA-4 and PD-1 antibodies have shown significant clinical activity in advanced CM [19,58,60,72,98,128]. In several CM treatment trials individual cases of UM have been included however these trials have failed to confirm the beneficial effect of immunotherapy in its treatment [19,72]. The best results in immunotherapy are obtained by combining several different immunotherapies, such as ipilimumab with pembrolizumab or nivolumab with ipilimumab, with median OS of 18.4 months and 19.1 months respectively [48]. Treatment with ICI in patients with metastatic UM has not achieved satisfactory results with response to single-agent immunotherapy with ICI being below 5% [19,98,117,129,130]. The attempt to treat with a combination of CTLA-4 and PD-1

blockade (ipilimumab and nivolumab) failed to show significant clinical benefit in UM; however, it was shown to be associated with the considerable side effects [130]. Studies have shown no association between prior treatments with ipilimumab or liver directed therapy and PFS or OS [73].

6.4.2 Novel immune-based therapy

Despite unsatisfactory results with the application of ICI, several new forms of immunotherapy are being explored in search of new treatment options for metastatic UM [19,56,57]. One potential new therapeutic approach is the use of IMCgp100 [19,57] which is presented by the human leukocyte antigen (HLA)-A*02 and only patients having this genotype may benefit from this treatment [57]. *In vitro*, IMCgp100 redirects a potent T cell-mediated immune response toward gp100 positive melanoma cells and alters the tumour immune profile, making tumour cells more responsive to ICIs [19].

Other immunotherapeutic approaches in UM treatment include the use of glembatumumab vedotin (CDX-011), preferentially expressed antigen in melanoma (PRAME), the infusion of autologous TILs and lymphocyte-activation gene 3 (LAG3) [19,57,131]. Glembatumumab vedotin, (CDX-011) is a fully human monoclonal antibody against glycoprotein nonmetastatic B (GPNMB), a transmembrane protein which is highly expressed in multiple tumour types, including UM. Glembatumumab vedotin has been assessed in a single-arm Phase II study showing some benefit in metastatic UM treatment being well-tolerated in the metastatic UM patient population [19,132]. Another interesting immunotherapeutic method in UM treatment is the infusion of autologous TILs [19,57]. The relatively long latency period between initial diagnosis and metastatic recurrence in UM implicates some degree of immune surveillance, which may be used for therapeutic purposes. A single-arm, phase II study was carried out to determine whether the transfer of reactive TILs could induce tumour regression in patients with metastatic UM suggesting that adoptive transfer of TILs with threshold production of INF-gamma could promote objective tumour regression [133]. These results support the need for further research into the application of immunotherapy in the treatment of UM. Improving T-cell therapy may be the key to improving the frequency of clinical responses and the overall suitability of this type of treatment. Many primary and metastatic UM express PRAME which is closely related to an increased risk of metastasis in both class 1 and class 2 UM and is a possible indicator of UM metastatic risk [134,135]. Considering its lack of expression on normal cells, it has been proposed as a potential immunotherapeutic target in primary and metastatic UM [131,136]. LAG3 has recently been recognized as an immune checkpoint whereby a high expression of LAG3 and its ligands Galectin-3 and HLA class II molecules was found in UM with high-risk tumour

parameters, such as epithelioid or mixed cell type and chromosome 3/BAP1 loss. Their expression correlates with the presence of infiltrating immune cells and this distribution suggests a potential benefit of monoclonal antibodies against LAG3 or Galectin-3 as adjuvant treatment in patients with high-risk UM [137].

6.4.3 Targeted therapy

Targeted therapy uses drugs designed for blocking a specific molecules pathway. However, results of current research of application of these form of therapy in UM treatment have been disappointing with response rates less than 10% [19,138]. The majority of primary and metastatic UM are characterised by mutations of oncogenes GNAQ or GNA11 that activate the MAPK pathway and reduce the activity of effectors including the MEK [44]. Highly selective inhibitors of MEK, selumetinib and trametinib, have been evaluated in monotherapy and combined therapy without relevant results [19,138].

6.4.5 Epigenetic therapy

It has been shown that epigenetic dysregulation plays an important role in the pathogenesis of UM and that down regulations of genes which encode epigenetic modifiers in UM are connected with high metastatic risk [139]. Analysis of 80 UMs determined that DNA methylation and histone modification are involved in the initiation and progression of UM and are associated with the poor-prognosis subtype characterized by monosomy 3 and BAP1 mutations [47]. Histone deacetylase (HDAC) inhibitors are drugs which affect these processes, induce dormancy of micro-metastatic disease through differentiation of UM cells and modification of UM cells from Class 2 to the Class 1 form. They have shown to be effective on UM cell lines growth *in vitro* and *in vivo* [140]. Some investigations indicate that epigenetic drugs may enhance low efficiency of ICIs and combining these therapies could enable more precise targeting and increase the effect of immunotherapy [19,141].

6.4.5 Oncolytic virus therapy

Oncolytic viruses are a unique type of agent whose application is based on the ability of viruses to infect and selectively replicate in tumour cells, leading to oncolysis and the release of new viruses generating an immune response to the tumour [48,142]. Adenovirus was the most commonly used in clinical trials, however with limited results in UM treatment after systemic administration [143].

7. Future development and prospects

Uveal melanoma is a rare but serious disease with a poor survival rate and a large number of patients developing metastasis. The OS of affected patients has remained unaltered and there is still no effective treatment for metastatic disease available. Recently, increasing attention has focused on the molecular mechanisms involved

in UM carcinogenesis and progression which could allow for the identification of valuable diagnostic and prognostic biomarkers as well as novel therapeutic targets [33]. Immune-modulating agents and ICIs as new forms of therapy have significantly improved treatment of CM, however due to differences in molecular biology, molecular profiles and cytogenetic alterations these methods have not shown to be effective in UM treatment. A better insight into the biological behaviour and genomic alterations of the UM is essential for the identification of new therapeutic targets and developing new therapeutic strategies [39,105].

Substantial obstacles in UM treatment are caused by the fact that histological, cytological, immunological and genetic analysis is rarely performed in treatment of primary tumours [19,144]. In recent studies emphasis has been placed on genetic and epigenetic characteristics of tumours considering their role in carcinogenesis and the fact that they can clarify tumour behaviour [145]. Analysis of tumour tissue and determination of the immune and genetic profile at the time of diagnosis will help establish the potential risks and selection of the most effective systemic therapy. In this way planning treatment and follow up can be individualized [88,145]. Additional problems associated with UM include limited published studies, insufficient knowledge, high risk for distant metastases, absence of effective systemic treatment and management outside expert centres. Further, in up to 30% of cases patients with UM can be asymptomatic particularly those with tumours located outside the macula where vision is not affected and this can result in misdiagnosis or late diagnosis of the primary tumour [146].

Inappropriate management at the early stage may result in increased risk of metastasis with possible fatal outcome [23]. Given that the occurrence of systemic disease is associated with increased mortality and adverse outcome it is important to identify high risk patients. Strong prognostic genetic biomarkers predicting the development of metastasis include chromosomal aberrations, DNA mutations and RNA profiles [38]. A possible direction for future research could be identifying biomarkers that enable the selection of patients who have a higher risk of developing metastasis as well as those who would respond best to treatment [19,144,145,147,148].

Current research has given useful insights into the biological behaviour of UM enabling the development of targeted therapies [33,36,145,148]. Understanding the mechanisms involved in resistance and adverse drug reactions would greatly improve therapeutic strategies for metastatic UM and the setting of guidelines for new treatments [36, 149]. Achieving improvement should involve intensive biomarker research, exploring tumour immunogenicity and studying the mechanisms involved in immune escape. To achieve this goal, future UM investigations should anticipate biological analyses by collecting samples and sharing the obtained data. In addition, we need to improve our understanding of the aetiology and tumorigenesis of UM in order to have the opportunity to develop therapeutic strategies that alter clinical outcomes. The observation regarding the biological, clinical and genetic differences emphasises the need for therapy specifically designed and adopted for UM patients. Specific efforts are required to accelerate fundamental and clinical research, expand access to clinical trials and raise awareness about UM particularly its metastatic form [38]. Current data suggest that several immunological mechanisms partake in the development, growth and spread of UM and thus may be potential targets for immunotherapy [33,57,58,72,98]. It is crucial to identify the presence of metastasis in the early phase in order to initiate timely treatment. Intensive research and understanding of UM behaviour will enable the advancement of treatment methods that will affect specific stages of tumour development with an individual approach applied for every specific case [21,32,33,44,76,114].

New drug discovery and innovative surgical methods such as IHP or PHP provide a localized approach to treatment. Current treatment of metastatic disease should focus on live directed therapy due to the lack of success of systemic chemotherapy and immunotherapy. Despite the relative improvement in survival with hepatic perfusion, novel therapy is required to improve outcome in these patients. Newer approaches are focused on studying the success of combined therapies including targeted and immunotherapies together with local liver-directed therapy. Future research should evaluate how the tumour cells evade the immune system with the aim of developing new therapies directed at these pathways. Novel therapies also include dendritic cell vaccination and biospecific molecules. Molecular therapies with RNA vaccines focusing on the genomic mutations are currently being investigated in cases of CM and have shown objective response in small studies [56,57,60,98]. These results may be extrapolated to cases of UM offering personalized treatment based on the patient's genetic mutations [60].

Advancement in treating UM patients may also be achieved with the knowledge of whether systemic adjuvant therapy at the time of primary tumour management improves prognosis and prolongs survival outcome. To date this form of treatment has not proven to be effective in reducing the risk of metastasis [150]. Further, the benefit and potential side effects of monotherapy versus combined therapies to obtain the optimal treatment outcome should also be clarified. In order to explain a number of issues related to the treatment of UM patients, it is necessary to conduct well-planned prospective studies [19].

8. Conclusions

Uveal melanoma is a rare and life threatening disease which shows notably different biological behaviour compared to other forms of melanoma and as such requires specific treatment strategies. Regardless of improvements

in local treatment OS has not increased and therefore new treatment options in patients with metastatic disease are essential. Uveal melanoma has a predisposition to metastasize to the liver and many different loco regional techniques have been developed however with modest results. Even though there has been significant progress in understanding the biology of this type of melanoma leading to new targeted therapies and approaches to immunotherapy, standardized treatment for metastatic UM still does not exist. Available treatment options for metastatic UM have not been able to prolong the survival rate of affected patients. Additional studies are essential to comprehend and enhance the efficacy of targeted therapy and immunotherapy as well as liver directed therapies. Decisions regarding treatment options and the best clinical approach are crucial in providing individualized patient care.

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