Madalena de Sousa Luís

Uveal Melanoma: Physipathology and New Situ-Specific Therapies

Monografia realizada no âmbito da unidade de Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pela Professora Doutora Eliana Maria Barbosa Souto e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

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ABSTRACT

Uveal melanoma is the most common primary intraocular tumour in adults. It can arise from melanocytes in the anterior (iris) or posterior uveal tract (choroid and ciliary body). Uveal melanoma has a particular molecular pathogenesis, being characterized by specific chromosome alterations and gene mutations (e.g. *GNAQ*/*GNA11*; *BAP1*), which constitute good targets for molecular therapy. Primary treatment of uveal melanoma includes radiotherapy (brachytherapy and charged-particle therapy), phototherapy (photocoagulation, transpupillary thermal therapy, and photodynamic therapy) and surgery (Local resection, enucleation and exenteration). Nevertheless, approximately half of patients with uveal melanoma will develop metastasis, especially to the liver. The treatment of metastatic uveal melanoma includes systemic chemotherapy, immunotherapy and molecular targeted therapy. Furthermore, liver-directed therapies, such as resection, chemoembolization, immunoembolization, radioembolization, isolated hepatic perfusion and percutaneous hepatic perfusion are also available to treat metastatic uveal melanoma. Several clinical trials are being developed in order to study new therapeutic options to treat uveal melanoma, mainly for those who present liver metastases. The present work discusses the physiopathology and new in-situ specific therapies for the treatment of uveal melanoma.

Key Words: Uveal melanoma, metastasis, radiotherapy, phototherapy, enucleation, exenteration, chemotherapy, immunotherapy, molecular target therapy, liver-directed therapies.

RESUMO

O melanoma uveal é o tumor intraocular primário mais comum nos adultos. Surge a partir dos melanócitos que se encontram, quer no tracto uveal anterior (íris) quer no tracto uveal posterior (coróide e corpo ciliar). O melanoma uveal apresenta uma patogénese molecular particular, sendo caracterizado por alterações cromossómicas e mutações génicas (Ex: GNAQ/GNA11; BAP1), que constituem alvos propensos para terapia molecular. O tratamento primário do melanoma uveal inclui radioterapia (braquiterapia e terapia com partículas carregadas), fototerapia (fotocoagulação, terapia térmica transpupilar e terapia fotodinâmica) e cirurgia (ressecação local e enucleação). Contudo, cerca de metade dos pacientes com melanoma uveal desenvolve metástases, principalmente para o fígado. O tratamento do melanoma uveal metastático inclui quimioterapia sistémica, imunoterapia e terapia molecular alvo. Para além disso, terapias diretas sobre o fígado, tais como ressecação, quimioembolização, imunoembolização, radioembolização, perfusão hepática isolada e perfusão hepática percutânea estão, igualmente, disponíveis para o tratamento do melanoma uveal metastático. Atualmente estão sendo desenvolvidos vários ensaios clínicos com o intuito de estudar novas opções terapêuticas para tratar o melanoma uveal, principalmente aqueles que apresentam metástases hepáticas. O presente trabalho discute a fisiopatologia e terapias específicas in-situ destinadas ao tratamento do melanoma uveal.

Palavras-Chave: Melanoma Uveal, metástases, radioterapia, fototerapia, enucleação, exenteração, quimioterapia, imunoterapia, terapia molecular alvo, terapias diretas sobre o fígado.

ABBREVIATIONS

α-MSH	α-melanocyte-stimulating hormone
AJCC	American Joint Committee on Cancer
BAP1	BRCA1 Associated Protein
BCUN	1,3-bis(2-chloroethyl)-1-nitrosourea
CGH	Comparative Genomic Hybridization
^{60}Co	Cobalt-60
COMS	Collaborative Ocular Melanoma Study
CRPs	Complement Regulatory Proteins
CT	Computed Tomography
CTLA-4	Cytotoxic T-lymphocyte associated protein-4
FDA	Food and Drug Administration
FDG-PET	Fluoro-2-deoxy-D-glucose Positron Emission/CT
CT	
FISH	Fluorescence In Situ Hybridization
GEP	Gene Expression Profiling
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GNA11	Guanine Nucleotide-binding Protein Subunit Alpha-11
GNAQ	Guanine Nucleotide-binding Protein G(q) Subunit Alpha
HDACis	Histone deacetylase inhibitors
HGF	Hepatocyte growth factor
^{125}I	Iodine-125
IFN-γ	Interferon-γ
IGF-1	Insulin-like Growth Factor-1
IGF-1R	IGF-1 Receptor
IHP	Isolated Hepatic Perfusion
MAPK	Mitogen-Activated Protein Kinase
MHC	Major Histocompatibility Complex
MLPA	Multiplex Ligation-dependent Probe Amplification
MRI	Magnetic Resonance Imaging
MSA	Microsatellite Analysis
MUM	Metastatic Uveal Melanoma
PBT	Proton Beam Therapy
^{103}Pd	Palladium-103

PD-L1	Programmed death ligand-1
PDT	Photodynamic therapy
PFS	Progression-free survival
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein Kinase C
^{106}Ru	Ruthenium-106
SNPs	Single Nucleotides Polymorphisms
TGF- β	Transforming growth factor β
TTT	Transpupillary Thermal Therapy
UM	Uveal Melanoma
US	Ultrasound
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
VIP	Vasoactive Intestinal Peptide
YAP	Yes-activated protein

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1. INTRODUCTION

Ocular melanoma is a rare type of melanoma, representing 3% to 5% of all melanoma cases, which can arise from melanocytes anywhere within the eye, including the uveal tract, conjunctiva and orbit. Uveal melanoma, which is the most common primary intraocular tumour in adults, can arise from the anterior (iris) or posterior uveal tract (choroid and ciliary body). Despite advances in the understanding of the disease and new therapeutic options, the incidence of uveal melanoma has remained unchanged. Uveal melanoma has a particular molecular pathogenesis, being characterized by specific chromosome alterations and gene mutations, which constitute good targets for molecular therapy. These cytogenetic features are important in the diagnosis and prognosis of this pathology. UM is genetically characterized by mutually exclusive mutations in guanine nucleotide-binding protein G(q) subunit alpha (GNAQ) and guanine nucleotide-binding protein subunit alpha-11(GNA11). Furthermore, 47% of patients with primary uveal melanoma present mutations in BRCA1 associated protein (BAP1). The diagnosis is usually made on ophthalmoscopy and ultrasound (US). Fluorescein angiography is also a useful technique. Cross-sectional imaging may be necessary in certain cases and this include magnetic resonance imaging (MRI), computed tomography (CT) and fluoro-2-deoxy-D-glucose positron emission/CT (FDG-PET CT). Primary treatment of uveal melanoma includes radiotherapy (brachytherapy and charged-particle therapy), phototherapy (photocoagulation, transpupillary thermal therapy, and photodynamic therapy) and surgery (Local resection, enucleation and exenteration). All these techniques have showed good local control of the tumour. Since the results of the Collaborative Ocular Melanoma Study (COMS) study suggested no difference in mortality for patients with medium-sized melanomas treated with either brachytherapy or enucleation, eye-conserving therapies have been used in the majority of patients. Nevertheless, approximately half of patients with primary uveal melanoma will develop metastases, especially to the liver. The treatment of metastatic uveal melanoma includes systemic chemotherapy, immunotherapy and molecular targeted therapy. Response rates of metastatic uveal melanoma to systemic chemotherapy are poor and overall survival is questionable. Liver-directed therapies are also used to control metastatic uveal melanoma and present higher response rates and median survival. These include resection, chemoembolization, immunoembolization, radioembolization, Isolated Hepatic Perfusion and Percutaneous Hepatic Perfusion (PHP).

1.1 Eye Anatomy and Physiology

The eye is made up of 3 main parts: eyeball, orbit and accessory (adnexal) structures. The eyeball is also called the globe and is rich in blood vessels. Its interior is filled with vitreous humour which fills the posterior part of the eye and helps to support the internal structures and maintain the shape of the eye. The outer part of the eyeball is called the wall of the eye and can be divided into 3 layers (or tunics): an outer, middle and inner layer (from the outside to the inside of the eye) (Bethesda e Institute, 2016; Society, 2016).

The outer layer includes the sclera and cornea. The sclera is the white portion of the eye and acts as the protective covering. Blood vessels and optic nerve pass through the sclera, and muscles that control the eye movement attach to this structure. The cornea covers the pupil and the iris and does not contain any blood vessels (Bethesda e Institute., 2016; Society, 2016).

Middle layer, where intraocular melanoma forms, is also called the uvea or vascular tunic and has three main parts: iris, choroid and ciliary body. Iris is the coloured part of the eye and is located between the cornea and the lens. The pupil is in the centre of the iris and changes its size to let more or less light to enter in the eye. Intraocular melanoma of the iris is, usually, a small tumour that grows slowly and rarely spreads to other part of the body. The choroid is a layer of blood vessels that bring oxygen and nutrients to the eye. This structure is rich in pigment-producing cells called melanocytes. Thus, the choroid is a part of the eye where most intraocular melanomas begin and they are often larger and more probable to spread to other parts of the body. Lastly, the ciliary body is a ring of tissue with muscle fibres that helps the eye focus since it changes the shape of the lens. It is located behind the iris and extends forward from the choroid. The ciliary body contains cells that make aqueous humour, a clear fluid that fills the space between the cornea and the iris. Intraocular melanoma of the ciliary body is larger and more likely to spread to other parts of the body than intraocular melanoma of iris. Thus, in the uvea we can find endothelial cells, immune cells and melanocytes (Bethesda e Institute., 2016; Society, 2016).

Finally, the inner layer is composed by retina or neural tunic, which is a layer of cells at the back of eyeball. It also includes nerve cells that are connected to the brain by optic nerve, which sends information from the eye to the brain and allows human beings to see.

The lens, which is a transparent structure in the inner part of the eye, is responsible for focus light rays on the retina (Society, 2016).

Orbit is a bowl-shaped cavity made up of bone formed from the skull that contains the eyeball and the connective tissues surrounding the eyeball, which are responsible for cushioning and protecting the eye. Muscles attached to the eyeball make it move in different directions. Furthermore, these small muscles attach to the sclera near the front of the eye and to the bones of the orbit at the back. The orbit also contains nerves, fat, blood vessels and a variety of connective tissues (Society, [s.d.]).

The three layers of the eye, along with the lens, function as boundaries for the three chambers within the eye. They are anterior chamber, which is the space between the cornea and the iris, posterior chamber, which is the space between the iris and the lens and vitreous chamber, which is the space between the lens and the retina. The eye can be further divided into anterior segment, which is formed by the cornea and both anterior and posterior chambers and posterior segment which contains the vitreous chamber, the retina, retinal pigment epithelium, posterior sclera and the uvea (Centre, [s.d.]).

Accessory structures include the eyelids, conjunctiva, caruncle and lacrimal glands. Conjunctiva is a clear mucous membrane that lines the inner surface of the eyelids that secretes mucus to lubricate the eyeball and keep it humid (Society, [s.d.]).

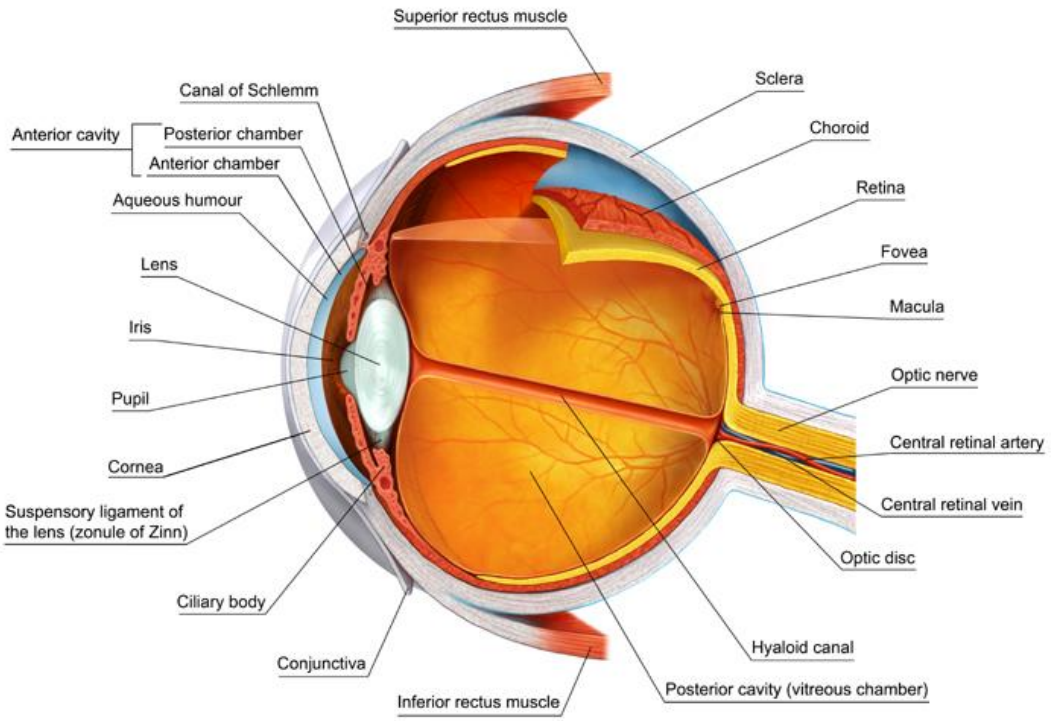

Figure 1 - Eye anatomy; In http://www.myvmc.com/anatomy/the-eye-and-vision/

1.2 Uveal Melanoma: Physiopathology

The majority of melanomas arise from melanocytes within the skin; however, they can less commonly arise at other sites, such as the eye. Ocular melanoma is a rare type of melanoma, representing 3% to 5% of all melanoma cases, which can arise from melanocytes anywhere within the eye, including the uveal tract, conjunctiva and orbit (Blum *et al.*, 2016).

Uveal melanoma (UM) can arise in the anterior (iris) or posterior uveal tract (ciliary body and choroid). The majority of cases, approximately 85%, occur in the choroid with the remaining cases arising in the ciliary body or iris. Melanomas of posterior uveal tract are generally more malignant, detected later and metastasize more frequently than iris melanomas. Thus, melanomas of the ciliary body have the least favourable prognosis and iris melanomas the best prognosis (Bethesda e Institute., 2016).

Uveal melanomas present as discrete masses between the thick, fibrous sclera and the retina, often pushing the retina into the vitreous space. UM usually remains confined to the globe, but larger tumours may develop extra-scleral extension. Other pathways of extraocular spread include the optic nerve and the lumen of vortex veins (Kapoor *et al.*, 2015). The highly vascular nature of uvea provides a good mean for the spread of UM cells to distant organs through the bloodstream (Onken, Li e Cooper, 2014). The lymphatic structures in the eye are rare (confined to extraocular conjunctiva and limbus) and too small for the passage of the cells, so regional spread of UM is extremely rare. Instead, UMs metastasize by haematogenous dissemination (Harbour, 2012; Onken *et al.*, 2014; Kapoor *et al.*, 2015). The most common sites of involvement include liver (93%), lung (24%), and bone (16%), with the overwhelming majority presenting initially in the liver (Harbour, 2012).

Most uveal melanomas are initially completely asymptomatic. Nevertheless, as the tumour enlarge it may cause distortion of the pupil (iris melanoma), blurred vision (ciliary body melanoma), or markedly decreased visual acuity caused by secondary retinal detachment (choroidal melanoma). Serous detachment of the retina can also occur and if extensive detachment occurs, secondary angle-closure glaucoma occasionally develops (Bethesda e Institute., 2016).

1.2.1 Epidemiology

Melanoma of the uveal tract is the most common primary intraocular tumour in adults, with an annual incidence of six cases per million in Caucasians and 5.1 per million worldwide. This rate appears to remain stable over the time despite advances in the understanding of the disease and new therapeutic options (Oliva, Rullan e Piulats, 2016). In Europe uveal melanoma incidence shows the north-south gradient, decreasing from over 8 per million in northern to less than 2 per million in southern countries (Jovanovic *et al.*, 2013).

Most patients with uveal melanoma are age between 50 and 80 years, with a peak in seventies, and the mean age at diagnosis is 58 years. Iris melanoma is more common among young patients (<20 years) and represents 21% of all uveal melanoma among them, compared to 4 and 2% in age groups 20-60 and >60, respectively (Jovanovic *et al.*, 2013).

Up to 50% of patients develop metastases within a median time of 2.4 years and the median survival with metastases ranges from 3 to 12 months because of the lack of effective treatment options (Nabil *et al.*, 2015).

1.2.2 Molecular Pathology of Uveal Melanoma

The molecular pathogenesis of uveal melanoma is distinct from that of cutaneous melanoma, being characterized by specific chromosome alterations and gene mutations.

1.2.2.1 Chromosome alterations

Most UMs exhibit relatively low degree of genomic instability and aneuploidy compared with many other cancer types. The most common chromosomal abnormalities include loss on 1p, 3, 6q, and 8p and gain on 1q, 6p and 8q. These cytogenetic features are important in the prognosis of uveal melanoma. These were initially identified by standard karyotypic analysis, but have been confirmed by fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), spectral karyotyping, microsatellite analysis (MSA), multiplex ligation-dependent probe amplification (MLPA) and single nucleotides polymorphisms (SNPs)(Harbour, 2012).

Loss of part of chromosome 1p occurs in about a quarter of UMs and more often occurs in the context of monosomy 3. Although concomitant loss of chromosome 1p and 3 has a stronger correlation with melanoma-related metastases, this is one of the few chromosomal

abnormalities which are independent of chromosome 3 status, with its presence leading to decreased disease-free survival (Kaliki, Shields e Shields, 2015;Harbour, 2012).

Gain of chromosome 6p and loss of 6q occurs, equally, in about a quarter of UMs. While 6q loss is associated with poor prognosis, 6p gain is associated with better prognosis since it tends to occur in the absence of monosomy 3. The coexistence of 6p gain and monosomy 3 occurs only in 4% cases of uveal melanoma (Harbour, 2012;Kaliki *et al.*, 2015). This led some investigators to speculate about its "protective" effect against metastases (Harbour, 2012).

8p loss occurs in about a quarter and 8q gain in almost 40% of UMs. 8q gain is statistically associated with metastases given that the smallest region of common gain on 8q has been narrowed to the large region 8q23-24 →qter, which contains many potential oncogenes. However, no pathogenic significance has been established and no specific oncogenic mutations on 8q have been reported in UM. 8q gain is a poor prognosis factor (more frequently associated with metastases), mainly when it occurs in the context of 8p loss, suggesting the formation of an isochromosome 8q. This frequently occurs in tumours that have lost a copy of chromosome 3, and it is considered an independent prognostic factor of progressive disease(Harbour, 2012;Correa, 2016). Gain of chromosome 8 or acquisition of an isochromosome 8q may be a later event in the setting of uveal melanoma and is seen in both low and high-risk uveal melanoma (Correa, 2016).

Loss of copy of chromosome 3 (monosomy 3) occurs in almost half of UMs, being the most prognostically significant chromosomal marker in UM. Monosomy 3 is strongly associated with clinical and histopathologic prognostic factors (larger tumour diameter, ciliary body tumour location, epithelioid cell type, high mitotic rate, vascular loops, and extraocular extension) and with metastatic death (Harbour, 2012;Kaliki, Shields e Shields, 2015). In order to search candidate genes on chromosome 3 and to identify specific mutations needed to established pathogenic relevance, Harbour *et al.* (2010) conducted a study of two UMs who were known to be monosomic to chromosome 3 and to have given rise for metastases. They found that BAP1, located at chromosome 3p21.1 was the only gene on chromosome 3 that was mutated in both tumours. Furthermore, they found inactivating mutations in BAP1 in 27 of 57 UMs (47%). These mutations occurred almost exclusively in metastasizing tumours that had also lost the other copy of chromosome 3.

1.2.2.2 Mutations

Uveal melanoma is genetically characterized by frequent, mutually exclusive mutations in guanine nucleotide-binding protein G(q) subunit alpha (*GNAQ*) and guanine nucleotide-binding protein subunit alpha-11 (*GNA11*), two closely related large GTPases of Gαq family. (Blum *et al.*, 2016; Luke *et al.*, 2016). These mutations occur at either arginine 183 (R 183) or glutamine 209 (Q209) and are considered driver mutations in uveal melanoma since they convert the G-proteins into a constitutively active form by blocking intrinsic GTPase activity and, consequently, activate downstream pathways (Zhao *et al.*, 2015; Patel *et al.*, 2016). Therefore, *GNAQ* and *GNA11* mutations lead to the activation of three pathways which are known to promote cell growth and proliferation. They include mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase (PI3K)/Akt pathways and Yes-activated protein (YAP) pathway. These appear to be major contributors to the development of uveal melanomas (Blum *et al.*, 2016; Xu *et al.*, 2014). These three pathways are known to to promote cell growth. Thus, Gα activates phospholipase C (PLC-β), which leads to protein Kinase C and Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway activation. These mutations can also lead to YAP pathway activation through Trio-Rha/Rac signalling circuit, which promotes the polymerization of globular actin to filamentous actin. This then binds to the cytoskeletal protein angiomotin, releasing YAP, and allowing it to enter the

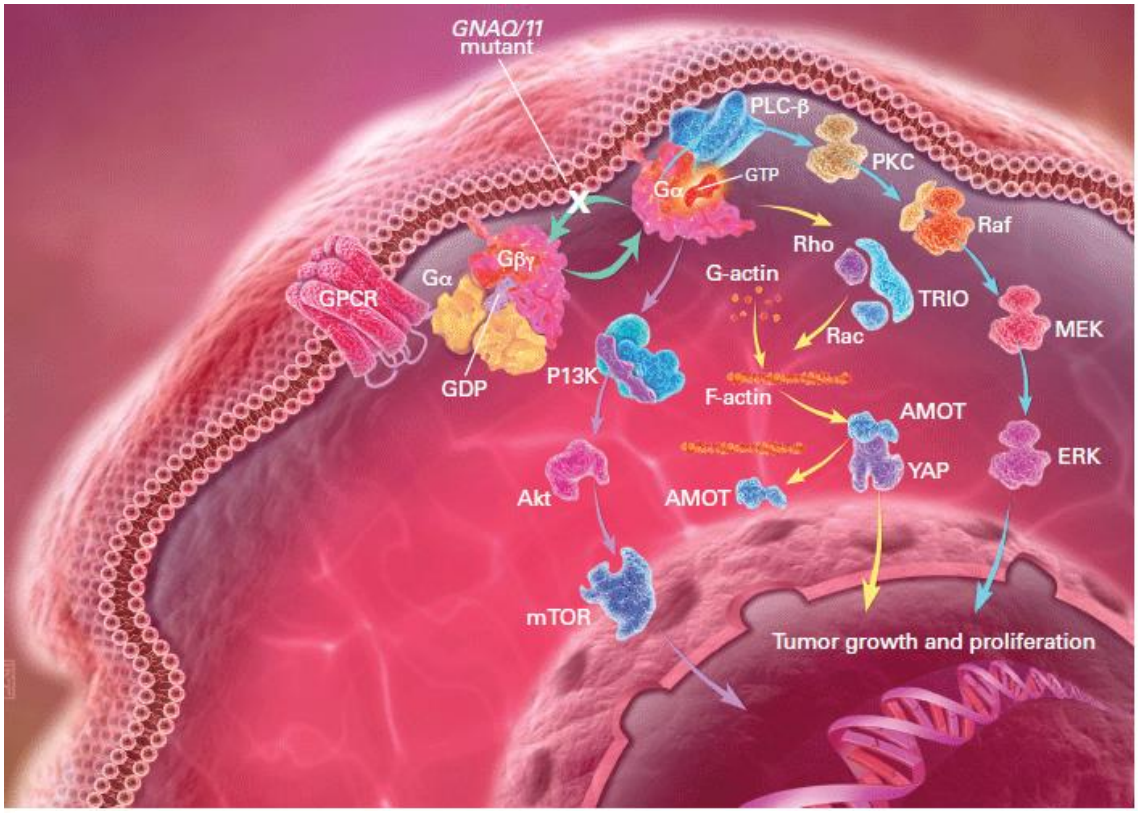

Figure 2 - Gα signaling in Uveal melanoma; In BLUM, E. S. et al. - Clinical Management of Uveal and Conjunctival Melanoma. Oncology Journal. ISSN 08909091 , 30(1), 29–32, 34–43, 48.

nucleus and initiate transcription of genes involved in proliferation, anti-apoptosis and cell survival (Figure 2).

BRCA1 *associated protein* (*BAP1*) is a gene that is mutated in approximately 47% of primary UM lesions. The presence of *BAP1* mutations in UM is associated with a high likelihood of metastases. Potential functions of *BAP1* include cell cycle regulation and maintenance of cell integrity (Luke *et al.*, 2016). BAP1 is an enzyme that removes ubiquitin molecules from specific proteins to regulate their function. Genes involved in melanocyte differentiation and function are affected by BAP1 loss. Thus, loss of BAP1 leads to the reversion of melanocytes to a de-differentiated, stem-cell like state that possibly contributes to their pro-metastatic behaviour (Field e Harbour, 2015).

The *BAP1* gene maps to chromosome 3p21 and *BAP1* mutations in UMs are accompanied by primarily somatic complete or partial loss of chromosome 3. Approximately 1-3% of patients with UM are likely to harbour a predisposing germline mutation in *BAP1*, although tumour development will also depend on loss of wild type *BAP1*. Although rare, *BAP1* mutations are also associated to predisposition to a variety of other cancers (Luke *et al.*, 2016). According to Woodman (2012) *BAP1* mutations were highly correlated with class 2 tumour status, chromosome 3 loss in primary tumours and the ultimate emergence of metastatic disease in the patients.

GNAQ/11 mutations are not sufficient for full malignant transformation to melanoma. This would seem to place *GNAQ/11* mutations as early or perhaps initiating events in UM progression. On the other hand, *BAP1* mutations are seen almost exclusively in metastasizing class 2 tumours with monosomy 3, suggesting that this mutation occurs relatively late in the primary tumour and may represent a rate-limiting step in metastases. Either *BAP1* mutation or loss of chromosome 3 can occur first, but both events appear to be necessary for the tumour to acquire the metastasizing class 2 phenotype (Harbour, 2012).

Significant effort has been made in order to better elucidate the biological mechanisms of distant spread to the liver. A variety of growth factors, such hepatocyte growth factor (HGF) which is produced in the liver and its corresponding tyrosine kinase receptor c-Met, have been considered to be involved in metastatic progression of uveal melanoma. Activated c-met seems to play an important role in proliferation, survival and cell migration and appears to be highly expressed in primary uveal melanoma cells (Woodman, 2012).

Another hormone highly secreted by the liver is insulin-like growth factor 1 (IGF-1). IGF-1 binding to its receptor, IGF-1 receptor (IGF-1R), results in cell proliferation, survival and migration. Like c-met, IGF-1R has been shown to be expressed in primary uveal melanoma (Woodman, 2012).

2. RISK FACTORS, DETECTION AND DIAGNOSIS OF UVEAL MELANOMA

There are several risk factors for the developing of uveal melanoma. Among host and environmental risk factors are the presence of related ocular or cutaneous melanocytic lesions, fair complexion and light iris (Rishi P, Koundanya VV, 2015). Regarding sunlight exposure, the relationship between ultraviolet (UV) light exposure and uveal melanoma risk has not been clearly established. According to Shah *et al.* (2005) chronic UV exposure , including sunlight exposure and geographic latitude was not significantly related with uveal melanoma. On the other side, Mainster e Turner (2010) concluded that UV-B radiation exposure and lack of use of sunglasses have been associated with uveal melanoma risk.

The diagnosis is usually made on ophthalmoscopy and ultrasound (US) due to the superficial location of the globe and its cystic nature. Ultrasound with Doppler enables non-invasive and cost-effectiveness follow-up. The technique is well-tolerated easy to perform, and has a high accuracy for the characterisation of vascular lesions (Purohit *et al.*, 2016). US is also a valuable tool for the follow-up in patients submitted to conservative treatments (Kapoor *et al.*, 2015).

Fluorescein angiography is also a useful technique given that in patients with melanoma causes irregular hyperfluorescence in the early phase while staging of the tumour is observed in the late phase. Furthermore, it provides an accurate assessment and follow up of the basal dimension of the lesion primarily and post-treatment (Kapoor *et al.*, 2015).

Cross-sectional imaging may be necessary in case of opaque lens or significant subretinal effusion. The appropriate imaging modality depends on clinical presentation and patient's age. Cross-sectional imaging plays an important role in the diagnosis and management of these lesions. However, a biopsy may be needed to provide tissue diagnosis. Magnetic resonance imaging (MRI), computed tomography (CT) and fluoro-2-deoxy-D-glucose positron emission/CT (FDG-PET CT) are, equally, useful imaging techniques. MRI is the imaging technique of choice, whereas CT is reserved for cases with suspected bony pathology and

whenever MRI cannot be performed (Purohit *et al.*, 2016). MRI can be used to provide intraocular enhancement of the lesion, helping to predict the degree of malignancy and for monitoring the response to treatment (Kapoor *et al.*, 2015). PET CT is able to detect metabolic activity of choroidal melanomas. Moreover, it plays a valuable role in detecting regional and distant metastases from choroidal melanomas. However, it is unable to differentiate between small melanomas and suspicious choroidal nevus (Purohit *et al.*, 2016).

Histopathological risk factors play an important role in the diagnosis of ocular melanoma and differentiating it from a nevus and include cell type, location, growth pattern, mitotic figures, pigmentation, necrosis and inflammatory components and vascularity (Rishi P, Koundanya VV, 2015). Four distinct cellular types are recognized in intraocular melanoma: Spindle-A cells (spindle-shaped cells with slender nuclei and lacking visible nucleoli), Spindle-B cells (spindle-shaped cells with larger nuclei and distinct nucleoli), Epithelioid cells (larger polygonal cells with one or more prominent nucleoli) and Intermediate cells (similar to but smaller than epithelioid cell). Thus, intraocular melanomas can be classified into: spindle cell nevus; spindle cell malignant melanoma; mixed cell melanoma; and epithelioid cell melanoma. Taking into account this classification, spindle cell tumours carry the best prognosis and epithelioid cell tumours the worst. However, epithelioid cell melanomas are infrequent and mixed-cell type melanomas predominate (Rishi P, Koundanya VV, 2015; Bethesda e Institute., 2016). Regarding the location of the tumour, juxtapapillary placed and more anteriorly placed tumours are more likely to be of epithelioid cell and are also more likely to metastasize and progress. Diffuse growth pattern has been shown to be associated with a higher incidence of extraocular extensions and higher metastatic potential. Presence of mitotic figures is highly suggestive of malignancy and is well-known risk factor for metastases. Heavy pigmentation has been found to be associated with epithelioid cell type, larger sized tumours, necrosis and the presence of macrophages (which increases risk of malignancy). A choroidal melanoma shows a more prominent vasculature which is associated with epithelioid cell type and large size of tumour (Rishi P, Koundanya VV, 2015).

Molecular risk factors include: tyrosinase-mRNA (enzyme involved in the synthesis of melanin) which can be used for the indirect quantification of circulating tumour cells; vascular endothelial growth factor (VEGF) which is known to be overexpressed in melanoma cases. It originates from abnormal new vessels within the tumour and hypoxia induced by the irregular blood flow; hepatocyte growth factor, which have an important role in the growth of cells in the liver; and insulin-like growth factor-1, which binds to IGF-1 receptor,

increasing the cell proliferation, preventing apoptosis and playing an important role in integrin adhesion to the extracellular matrix and invasion of basement membranes. They are haematological markers that allow the detection of distant metastases (Rishi P, Koundanya VV, 2015).

Gene and chromosome alterations, referred above (1.2.2 Molecular pathology of Uveal Melanoma), are a great risk factor for the development of metastases. The major chromosome alterations have been described in chromosomes 3, 6, 8 and 11 and are significantly correlated with the clinical high-risk factors for metastases, such as tumour size at diagnosis and epithelioid cell histopathology (Rishi P, Koundanya VV, 2015).

Cytogenetic alterations have provided important insights into the pathobiology of UM, so several groups have studied the use of gene expression profiling (GEP) in the detection of up-regulation or down-regulation of select genes in a tissue sample (obtained by biopsy). The technique involves isolating RNA from a tissue sample following by its conversion to complementary DNA, whose targets are subsequently hybridised to gene chips, and microarray analysis is performed. Taking into account gene expression profile (15-gene expression panel), uveal melanomas can be divided into two distinct prognostic classes: class 1 tumours which generally have the clinical and pathological features known to be associated with decreased metastatic risk, such as the presence of spindle cells; class 2 tumours which generally have more aggressive clinical and pathological features, with a higher risk for metastases. This test is commercially available for routine use in clinical practice (Harbour, 2012; Correa, 2016).

3. PRIMARY TREATMENT OF UVEAL MELANOMA

The goal of treatment of localized uveal melanoma is to preserve the vision and prevent metastases. The therapy selected to treat uveal melanoma depends on several factors, including tumour size, lesion location, general health of the patient, and patient preference. The primary treatment of uveal melanoma includes: radiotherapy (brachytherapy and proton beam radiotherapy), phototherapy (photocoagulation, transpupillary thermal therapy, and photodynamic therapy) and surgery (Local resection, enucleation and exenteration) (Kapoor *et al.*, 2015; Blum *et al.*, 2016).

While small and medium sized UMs can be successfully treated with a variety of methods, there is no consensus about the optimum management for thick (≥7 mm) UMs. Although

enucleation has been the treatment of choice for large UMs, in certain situations, such as the presence of a tumour in only remaining eye, poor vision in the fellow eye, or whenever a patient insists on avoiding enucleation, conservative modalities can be considered to preserve the disease eye (Naseripour *et al.*, 2016).

Radiotherapy has largely replaced enucleation for tumours of suitable location and dimension (less than 10 mm in thickness and 18 mm in largest basal diameter). Regarding larger tumours, there is some risk of vision loss and radiation complications because of neovascular glaucoma. Nevertheless, radiotherapy is sometimes used in patients with large tumours and who show preference for eye-sparing treatments (Weis *et al.*, 2016).

3.1 Surgery

Local resection of the tumour can be performed either a transretinal (endoresection) or transscleral (exoresection) approach (Blum *et al.*, 2016). Local resection is used to remove select (typically medium sized or large) uveal melanoma with retention of the eye (Simpson *et al.*, 2014). Endoresection is more suitable for posterior uveal melanomas (Pereira *et al.*, 2013). The complications involving these surgeries include vitreous haemorrhage and retinal detachment. Furthermore, there is some concern regarding local recurrence (risk factor for metastases) and iatrogenic tumour spread. However, these can be minimized by adjuvant radiotherapy (Blum *et al.*, 2016). Some centres irradiate the uveal melanoma before endoresection or place a radioactive plaque over the tumours base after transscleral resection (Simpson *et al.*, 2014).

Enucleation involves surgical removal of the eye. Although, historically, it was the treatment of choice, nowadays it has been shift towards vision-sparing treatments due to recent advances in radiotherapy (Blum *et al.*, 2016; Weis *et al.*, 2016). Nevertheless, patients with lesions exceeding 10 mm in thickness and/or 18 mm in diameter are still indicated for the treatment with enucleation because of the complications connected to delivering high doses of radiation to the eye, including vision loss and loss of the eye (Weis *et al.*, 2016). Furthermore, enucleation can also be used in patients with little chance to save vision, which is usually the case for large, advanced UM, tumours located near the optic disc, tumours presenting with extensive bleeding or retinal detachment, or vitreous haemorrhage. In terms of survival, several studies have showed no differences in mortality rates comparing surgical treatments and conservative treatments (Pereira *et al.*, 2013).

3.2 Brachytherapy

Brachytherapy involves suturing a radioactive plaque, temporarily, to the episclera to deliver a fixed dose directly to the tumour. Operative localization of the plaque placement is guided by translumination, ophthalmoscopic observation or ultrasonography. The surgery is performed either under local or general anaesthesia. Extraocular muscles and conjunctiva are reattached in order to not disturb brachytherapy (Blum *et al.*, 2016; Simpson *et al.*, 2014). The plaque remains in place for 2-5 days, depending on the type and activity of the radioactive source, and it is then removed under similar operative conditions. The most common radioisotopes used in the plaques are iodine-125 (^{125}I) and ruthenium-106 (^{106}Ru). ^{106}Ru is generally preferred in Europe, whereas ^{125}I is commonly prescribed in United States (Kapoor *et al.*, 2015; Blum *et al.*, 2016). Other radioisotopes include cobalt-60 (^{60}Co) and palladium-103 (^{103}Pd). The choice of isotope is often based on tumour depth since they have different penetration rates (Kapoor *et al.*, 2015). ^{125}I plaques emit gamma radiation which has deeper penetration but increased toxicity to surrounding healthy tissues. ^{106}Ru plaques emit beta radiation, having less deep penetration compared to ^{125}I plaques, but lower toxicity to surrounding healthy tissues. Thus, due to their lower penetration, ^{106}Ru plaques are unsuitable for thick tumours, and their use is generally restricted to tumours less than 6 mm in apical thickness (Blum *et al.*, 2016; Simpson *et al.*, 2014). According to Blum *et al.* (2016), regular ophthalmologic examinations should be performed following brachytherapy to assesses radiation-induced damage, including retinopathy, papillopathy, exudative retinal detachment and cataract which can develop 2 to 5 years following initial treatment.

Correct plaque placement is essential for good clinical outcomes. The target volume should include the tumours base and safety margin. According to the American Brachytherapy society, ^{106}Ru plaques achieve larger physical safety margins than ^{103}Pd and ^{125}I plaques (Simpson *et al.*, 2014). The use of eccentric plaque positioning has been shown to provide good local control for tumours within 5 mm of the optic disc or fovea. Slotted plaques incorporate the optic nerve into the plaque, allowing the entire tumour and a 2 mm-free margin to be encompassed by the plaque (Pereira *et al.*, 2013). Plaque orifices should exceed the largest tumour diameter as to create a tumour-free margin of safety to prevent geographic miss (Simpson *et al.*, 2014).

There is a lack of internationally accepted dosimetry standards for each radionuclide. According to American Brachytherapy Society dose prescriptions for uveal melanoma typically range from 70 to 100 Gy to the tumour apex and dose rates should not be less than

the COMS historical standard of 0.60 Gy/h for ^{125}I plaques. Dose modifications may be appropriate taking into account different tumour sizes, implant durations, threshold doses to critical normal ocular structures, and the use of alternate radionuclide sources (Simpson *et al.*, 2014).

The most common radiation side effects include optic neuropathy, maculopathy, cataract, and neovascular glaucoma, numerous studies of which have shown these to be dose dependent (Pereira *et al.*, 2013)**.**

3.3 Charged-particle Radiation Therapy

Charged-particle radiation therapy may be delivered using protons, carbon ions or helium ions and allows for more focused radiation treatment, collimated charged-particle beams peaking at the desired tissue depth and stopping thereafter. This has dosimetric advantage since it allows for the delivery of large radiation doses for adequate tumour treatment without sparing of surrounding structures. Nevertheless, the five most commonly reported adverse effects associated with charged-particle radiation therapy were glaucoma, radiation retinopathy, cataract formation, optic neuropathy and enucleation due to complications. Furthermore, charged-particle radiation therapy often also administers radiation to the anterior segment in order to reach posterior UM tumours. Thus, a strong correlation has been shown between the percentage of lens and anterior camera involvement and the development of neovascular glaucoma. However, it is possible to reduce radiation complications through certain charged-particle radiation therapy techniques, such as the use of notched beams, adjunctive TTT or phototherapy, and the treatment through a closed eyelid. These techniques are still under investigation. According to UM registries, patients with larger tumours and tumours near the optic disk and fovea are preferentially referred to be treated with charged-particle radiation therapy (Pereira *et al.*, 2013).

Proton beam therapy (PBT) is a type of charged particle therapy which uses proton beams. Firstly, the patient is submitted to a surgical placement of tantalum marker rings, which are placed at the tumour border on the sclera and served as radiographic markers of the tumour edge for treatment planning and daily image guidance. This is not necessary for iris melanomas since they are visible externally. Following surgery, the position of tantalum markers is confirmed by an X-ray image (Mishra e Daftari, 2016; Damato *et al.*, 2013). A 3D computer model of the eye is generated. The optimal gaze is established in order to minimize the dose to critical structures (optic nerve, macula, lens and cornea). The

appropriate depth of beam penetration necessary to encompass the target volume is determined, dose-volume-histograms are produced for each structure and dose distribution is assessed. The relationship of the rings to the beam and collimator are projected to ensure proper planned tumour coverage (Mishra e Daftari, 2016). In choroidal melanomas, proton beam radiotherapy is administered with safety margins of 2.0-2.5 mm. Nevertheless, these can be reduced if there is risk of optic nerve and fovea damage. On the other side, as ciliary body melanomas tend to develop diffuse spread circumferentially, safety margins of up to 4 mm should be used in such cases. With iris melanomas safety margins of 3 mm or one clock hour are advised. When iris tumour shows diffuse growth or seeding, radiotherapy should be delivered to the entire anterior segment (Damato *et al.*, 2013). The radiation is delivered for 4-5 days and the dose is generally 56-60 Gy in four daily fractions up to 70 Gy in five daily fractions (Mishra e Daftari, 2016). After proton beam is complete patients should be followed closely either for local recurrence as distant disease. This follow-up includes clinical examination, ocular ultrasound and liver function tests (Mishra e Daftari, 2016).

Several reports on proton beam therapy describe a 5-year local control of 95% or greater for uveal melanomas, with a 5-year overall survival of approximately 80% (small tumours ranging 95-98%; medium 80-86% and large 60%). Regarding enucleation rates, at 5 years is approximately 10% and at 15 years is approximately 15% (Mishra e Daftari, 2016).

According to Damato *et al.* (2013), proton beam therapy has the widest inclusion criteria and can be administered as primary treatment or salvage therapy for local tumour recurrence after plaque radiotherapy, phototherapy or surgical resection.

3.4 Stereotactic Radiosurgery

Stereotactic radiosurgery (SRS) uses emission of high-dose gamma radiation concentrated over a small volume. Radiation energy is delivered to a well-defined area with little exposure to surrounding tissue. This ability to direct radiation to a precise target is advantageous when treating tumours near the macula and optic disc (Sikuade *et al.*, 2015). SRS works by damaging the DNA of the target cells, which then lose the ability to reproduce, causing tumours to shrink. Furthermore, the high dose of radiation delivered causes blood vessels to close off over time following treatment, robbing the tumour of its blood supply. This technique is not a surgical procedure, requiring just local anaesthesia. Thus, is suitable for patients who are contraindicated to general anaesthesia.

Sikuade *et al.*, 2015 have studied their experience of the use of SRS and proton beam therapy to treat posterior uveal melanoma over a 10 year period. They verified that both treatments achieved excellent local control rates, concluding that these techniques are effective treatments for large choroidal melanomas or tumours unsuitable for plaque radiotherapy.

3.5 Photocoagulation

Photocoagulation uses a focused laser beam to destroy vessels that supply blood to tumour cells. Nevertheless, this technique present high rates of complications and has been substituted by a more recent variation using infrared radiation called Transpupillary Thermal therapy (TTT) (Pereira *et al.*, 2013).

3.6 Transpupillary Thermal Therapy

TTT uses an infrared laser to deliver low-energy phototherapy through a dilated pupil to the tumour surface. Direct cell destruction induces tumour necrosis. Since infrared radiation is only capable of penetrating the surface tumour layer these technique is suitable for treating small tumours (<3 mm in apical height and <16 mm in basal largest diameter) or marginal recurrences following proton therapy (Pereira *et al.*, 2013; Blum *et al.*, 2016; Weis *et al.*, 2016). Initially this technique was developed as an adjunct therapy to plaque brachytherapy, but interest in TTT as a primary treatment grew since it could provide improved visual outcomes compared to plaque brachytherapy. Nevertheless, longer follow-up of initial studies using this technique as primary treatment for small posterior tumours revealed high risk of local recurrence, demonstrating its ineffectiveness in this setting. Besides that, TTT has revealed some complications, including branch retinal artery and vein occlusions, epiretinal membranes, cystoid macular oedema, and optic disk swelling (Pereira *et al.*, 2013).

3.7 Photodynamic Therapy

Photodynamic therapy (PTD) consists of the excitation of an intravenously administered photosensitizer by a specific wavelength applied to the target region, having non-thermal tissue effects. Through cellular, vascular and immunogenic pathways, which are not completely understood, generated free radicals and highly reactive singlet oxygen species induce cell and tissue destruction. The relative contribution of each pathway is thought to be dependent upon the characteristics of the photosensitizer, the treatment tissue and treatment parameters, including time and dose. Verteporfin is a second-generation

photosensitizer whose effects are thought to be attributed to a combination of vascular occlusion direct cytotoxicity and activation of the immune system. According to Pereira *et al.* (2013) there is some evidence that PDT is more effective in lightly pigmented melanomas than more densely pigmented tumours. Despite the limited clinical experience with PDT, small series using PDT with verteporfin as primary therapy have shown it to be effective in achieving complete regression. Moreover, its use as second-line therapy demonstrated partial effect in one study. Nevertheless, histopathologic studies of three UM cases 1 week after treatment with PDT (with verteporfin) and bevacizumab showed viable melanoma cell with no necrosis. Thus, they concluded that PDT is not effective in the treatment of these UMs. They consider that further studies are needed to determine optimal case-selection criteria, treatment parameters, and the efficacy of PDT with verteporfin as a primary or adjuvant treatment for UM (Pereira *et al.*, 2013).

Table 1 - Review of all the available treatment options. Adapted from KAPOOR, A. et al. - Management of uveal tract melanoma: A comprehensive review. Journal of the Egyptian National Cancer Institute, 28(2), 65–72.

Treatment	Used for	Comments
Radiotherapy		
Brachytherapy ^{106}Ru	Small/medium/large uveal melanoma	_____
Brachytherapy ^{125}I	<20 mm in basal diameter	_____
Proton Beam Therapy	Medium to large uveal melanoma, which cannot be treated with brachytherapy or resection	_____
Stereotactic Radiosurgery	Juxtapapillary uveal melanoma; patients unsuitable for brachytherapy or surgery	Lower availability
Phototherapy		
Transpupillary Thermal Therapy	Local recurrence and of adjuvant therapy of uveal melanoma	Very occasionally used; when considering preservation of vision since it avoids radiotherapy complications; it is not recommended routinely as a sole primary treatment.
Photodynamic Therapy	Small melanomas	Avoids radiotherapy complications
Surgery		
Exoresection +/- plaque	Medium to large melanoma with a narrow basal diameter	Rarely performed; always performed with brachytherapy to reduce the risk of recurrence.
Endoresection +/- radiotherapy	Medium sized uveal melanoma	_____
Enucleation	Large uveal melanoma	_____

4. ADJUVANT THERAPY

Adjuvant therapy consists of radiotherapy or systemic therapy, such as immunotherapy, chemotherapy and target therapy. Nevertheless, there are few studies related to adjuvant therapy in UM and according to Pereira *et al.* (2013) more studies with promising systemic therapies and combination treatments are needed.

5. TREATMENT OF METASTATIC UVEAL MELANOMA

Approximately half of patients with primary uveal melanoma will develop metastases. Although there are effective therapies to eradicate and prevent local recurrence of primary uveal melanomas, there are, to-date, no effective treatment for metastatic uveal melanoma. Thus, the long-term prognosis of metastatic disease is very poor. The median survival time from the development of distant metastases is between 4 and 15 months, and the 1-year survival rate is 10% to 15%. Nevertheless, several treatments have been studied, including chemotherapy, immunotherapy and molecularly target therapy (Blum *et al.*, 2016).

5.1 Systemic Chemotherapy

Response rates of metastatic uveal melanoma (MUM) to systemic chemotherapy are poor and survival rates of MUM have remained almost unchanged in the past 40 years. When MUM is restricted to a limited anatomic region, locoregional treatment modalities, such as surgical resection, intraarterial chemotherapy, transarterial percutaneous chemoembolization, selective internal radiation therapy, and radiofrequency ablation can be used to control the disease (Buder *et al.*, 2013). A variety of cytotoxic agents have been investigated, including dacarbazine, treosulfan, temozolomide, fotemustine, cisplatin, and combination therapies such as bleomycin, vincristine, lomustine, and dacarbazine, gemcitabine/treosulfan and dacarbazine/treosulfan. Nevertheless, in clinical practice responses are rarely seen and overall survival is questionable. The survival remains between 2 and 7 months, with only 15% of patients alive at 1 year. Higher response rates and median survival have been observed with chemotherapy administration directly to the hepatic artery (Pereira *et al.*, 2013). A better understanding of melanoma molecular biology has been critical in developing new treatments. New chemotherapeutic agents tested in uveal melanoma include docosahexaenoic acid–paclitaxel (a covalent conjugate of paclitaxel and docosahexaenoic acid) and vincristine sulphate liposomes infusion (sphingomyelin/cholesterol liposome encapsulated formulation of vincristine that results in extended drug circulation

time and anticancer activity). Both treatments have showed limited responses in patients with uveal melanoma (Pereira *et al.*, 2013).

There are several clinical trials studying chemotherapy for the treatment of metastatic uveal melanoma or as adjuvant therapy of primary UM (See annex 1).

5.2 Systemic Immunotherapy

The eye is considered an immune-privileged organ which influences the immune response against UM cells and provides escape mechanisms for UM. The following factors play an important role in the immune-privilege of the eye: aqueous humour is rich in immunosuppressive proteins, such as transforming growth factor β (TGF-β), vasoactive intestinal peptide (VIP), α-melanocyte-stimulating hormone (α-MSH), and complement regulatory proteins (CRPs); the blood-eye barrier restricts inflammatory cell access to the eye; eye cells reduce major histocompatibility complex (MHC) class Ia expression to escape cytotoxic mediated lyses; and ocular cells express programmed death ligand-1(PD-L1) which inhibits T cell response. Thus, a deeper understanding of the interaction between immune and cancer cells is crucial to take the maxim benefit of immunotherapy (Oliva *et al.*, 2016).

UM cells impair the innate immunity through inhibition of the action of Natural killer cells by the production of macrophage inhibiting factor (MIF) and TGF-β and MHC class I upregulation. Furthermore, UM cells produce cytokines that lead to macrophage differentiation to M2 subtype, which promotes tumour growth instead of an effective immune response. To date, there are no treatments that target NK cells or macrophages. Regarding adaptive immune response, the mechanisms that interfere with immunity are the production of indoleamine 2,3-dioxygenase (IDO), overexpression of PD-L1, alteration of FasL expression, and resistance to perforin (Oliva *et al.*, 2016).

UM cells overexpress the PD-L1 receptor when exposed to interferon-γ (INF-γ), an immunostimulatory cytokine, with consequent suppression of T-cells by decreasing IL-2 production. UM cells come in contact with IFN-γ produced in the new organ when they metastasize, so UM cells do not express PD-L1 constitutively when they are in the immune-privileged microenvironment of the eye (Oliva *et al.*, 2016). Although anti-PD1 antibodies nivolumab and pembrolizumab have been approved by the US Food and Drug Administration (FDA) for the treatment metastatic cutaneous melanoma, there are no available results with anti-PD1 or anti-PD-L1 in metastatic UM (Blum *et al.*, 2016; Oliva *et al.*, 2016). There are

currently three clinical trial exploring this strategy: a phase II trial studying pembrolizumab in monotherapy (NCT02359851); two phase II trial studying the combinations of nivolumab and ipilimumab (NCT01585194 and NCT02626962) (Clinicaltrials.gov, [2016]).

An antibody against Cytotoxic T-lymphocyte associated protein-4 (CTLA-4), ipilimumab, has been approved to treat patients with metastatic uveal melanoma in the setting of immunotherapy. CTLA-4 is expressed by activated T-cells and transmits an inhibitory signal to T-cells, downregulating immune responses. Although experience with ipilimumab in metastatic UM is limited, it has demonstrated good overall survival and long-term survival in a proportion of patients (Oliva *et al.*, 2016).

5.3 Molecular Targeted Therapy

As a result of increased understanding of the oncogenic drivers in uveal melanoma several promising therapeutic targets have been identified. Target therapy is, actually, the most explored strategy for the treatment of cancer. It applies drugs that interfere with specific molecules involved in cell tumour progression and proliferation and, thus, block specific pathways to the growth and evolution of cancer cells (Pereira *et al.*, 2013).

Since MAPK pathway is activated in the majority of UMs, due to *GNAQ/GNA11* mutations, inhibitors of downstream effectors, such as MEK and protein kinase C (PKC), are currently under investigation. MEK inhibitors include trametinib and selumetinib. Both have shown clinical activity in clinical trials. A randomized phase II clinical trial showed that selumetinib-treated patients had better responses rates (14%) and improved progression-free survival (PFS) (15,9 weeks) compared to those who received temozolomide or dacarbazine chemotherapy (0% and 7 weeks, respectively). Nevertheless, other study of selumetinib plus dacarbazine vs dacarbazine alone did not showed a statistically significant improvement in PFS. A study of the combination of a MEK inhibitor with an Akt inhibitor demonstrated that MEK inhibition is sensitized by Akt inhibition (Blum *et al.*, 2016; Pereira *et al.*, 2013).

C-kit is a transmembranar receptor with tyrosine kinase activity that acts in differentiation, proliferation and programmed cell death and has been shown to be overexpressed in uveal melanoma, being a potential oncogenic driver in this type of tumour. Imatinib is a c-kit inhibitor which demonstrated, in-vitro, to decrease the invasion capability of UM cells (Blum *et al.*, 2016; Pereira *et al.*, 2013). Nevertheless, according to Pereira *et al.* (2013) further studies are needed to address its effect in the treatment of primary uveal melanoma,

especially in high-risk patients. Sunitinib is a non-selective c-kit inhibitor that did not show improvement in PFS or overall survival in the SUAVE trial, a phase II clinical trial (Blum *et al.*, 2016).

C-met inhibitors have been also studied as a therapy to treat uveal melanoma. Inhibition of c-met was shown to prevent tumour growth in preclinical models of uveal melanoma. Cabozantinib is an example of a c-met inhibitor.

Loss of *BAP1* seems to be a difficult therapeutic challenge since it appears to represent a loss of a tumour suppressor, and direct therapies will require the re-initiation of function. Nevertheless, some treatment options, which have this mutation as target, have been studied. A study showed that *BAP1* loss results in an accumulation of mono-ubiquitin on histone H2A, altering the transcriptional profile within these cells. The use of Histone deacetylase inhibitors (HDACis), such as valproic acid, can reverse the accumulation of mono-ubiquitin (Woodman, 2012). Several clinical trials regarding the use of HDACis in uveal melanoma are ongoing (see annex 1).

Tumour angiogenesis is essential for tumour growth, progression and metastasis. VEGF is one of the major cytokines that influence tumour angiogenesis and can serve as a biomarker for metastatic UM since it appears to be significantly increased after metastatic development in UM patients. Bevacizumab is a monoclonal anti-VEGF antibody that showed to suppress in-vivo hepatic micrometastasis (Pereira *et al.*, 2013).

There are several clinical trials currently testing different types of molecular target therapies. (see annex 1)

6. LIVER-DIRECTED THERAPIES

The liver is the most common local of metastases of UM and the clinical course of patients with liver metastases is dependent on disease progression in the liver. The use of whole body PET-CT is useful for detection of liver and extra-hepatic metastases at the time of diagnosis of UM. Several approaches have been used to treat metastatic liver disease, including resection, immune-embolization, chemo-embolization, radio-embolization, isolated hepatic perfusion and percutaneous hepatic perfusion. However, there is no standard treatment for liver metastases (Pereira *et al.*, 2013).

6.1 Resection

Resection consists of the surgical removal of metastatic nodules and has been shown to improve the survival of patients with liver metastases of UM, either by single or multiple resections. Nevertheless, the presence of multiple liver metastases is a contraindication for major liver resection and, thus, only a limited number of patients are eligible for surgical treatment (Pereira *et al.*, 2013).

6.2 Chemoembolization

Chemoembolization combines artery embolization with the infusion of chemotherapeutic agents. This treatment has shown to improve the overall survival of patients with liver metastatic disease. Although, chemoembolization has been used for the treatment of liver metastases from uveal melanoma, there are no standard protocols and comparative trials demonstrating superior results of one particular chemotherapeutic agent over another. Several chemotherapeutic agents have been used for chemoembolization, including cisplatin alone and in combination with carboplatin, carboplatin in monotherapy, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCUN) and mitomycin C alone and in combination with cisplatin and doxorubicin (Eschelman, Gonsalves e Sato, 2013).

6.3 Immunoembolization

Immunoembolization consists of infusion of an immune-stimulating agent into the hepatic artery followed by embolization. Despite the abundance of immune cells, the liver tends to induce tolerance rather than immunity. Thus, the objective of immunoembolization is to destroy the tumour by embolization and, thus to control the tumour progression locally and to provide tumour antigens to the local immune system. It acts a local stimulation of the immune system which results in the development of a systemic immune response against tumour cells that may suppress the tumour growth (Eschelman *et al.*, 2013). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein secreted by the T-cells that stimulates immune cells, such as macrophages and dendritic cells and it has been used for immunoembolization of metastatic uveal melanoma, demonstrating to be well-tolerates and with acceptable toxicity profiles (Eschelman *et al.*, 2013; Pereira *et al.*, 2013).

6.4 Radioembolization

Radioembolization using ytrrium-90 (^{90}Y) has been applied to treat patients with liver metastases from uveal melanoma. ^{90}Y is delivered to the liver through two types of microspheres: TheraSpheres (nonbiodegradable glass microspheres with a diameter of 20 to 30 μm) and SIR-Spheres (nonbiodegradable resin ^{90}Y microspheres with a diameter of 20 to 40 μm). Since SIR-Spheres presents lower activity (40 to 70 Bq per microsphere) compared to TheraSpheres (maximum 2500 Bq), more SIR-Spheres are necessary to deliver a similar dose. Nevertheless, the higher number of SIR-Spheres allows providing an embolic effect in addition to delivering radiation directly to the tumours. This is advantageous when tumours are hypervascular, numerous and dispersed throughout both lobes of liver (Eschelman *et al.*, 2013). According to clinicaltrials.gov database a clinical trial, studying the use of SIR-spheres® ^{90}Y for the treatment uveal melanoma metastasized to the liver, is currently recruiting participants (NCT01473004).

6.5 Isolated Hepatic Perfusion

Isolated Hepatic Perfusion (IHP) is a procedure where the liver is surgically isolated and perfused with a high concentration of the chemotherapeutic agent. This technique allows for locally perfuse the liver with a high dose of a chemotherapeutic agent, without the leakage of systemic circulation and, thus, avoiding undesirable systemic effects. The isolated hepatic perfusion of mephalan has been studied. Nevertheless, IHP requires a complex surgical procedure with considerable morbidity and mortality, which is largely related to veno-occlusive disease and hepatotoxicity (Ben-Shabat *et al.*, 2015; Pereira *et al.*, 2013).

6.6 Percutaneous Hepatic Perfusion

Percutaneous Hepatic Perfusion (PHP) with simultaneous chemofiltration has been developed as an alternative to IHP since decreases morbidity and mortality and can be repeated leading to a higher response rate and improved survival (by local control of the disease). PHP involves the placement of two catheters, one in the proper hepatic artery, to infuse the chemotherapeutic agent and the other in the inferior caval vein, to aspirate the chemosaturated blood returning through the hepatic veins and to avoid the leakage into the systemic circulation. Simultaneously, the blood returning from the hepatic veins is perfused through an extra-corporeal filtration system. After filtration, the blood is returned to the patient by a third catheter in the right internal jugular vein. Hemodynamic monitoring and

hemodynamic support is advised because of the hemodynamic instability resulting of this complex procedure (Leede, de *et al.*, 2016).

Current clinical trials on uveal melanoma management are presented in annex 1.

7. CRITICAL REVIEW

Uveal melanoma is a rare disease which can arise either in the anterior (iris) or posterior uveal tract (ciliary body and choroid). A variety of therapeutic options are available to treat primary uveal melanoma, such as surgery and radiotherapy. Several studies have shown that either conservative as radical (enucleation) treatments are not associated with a definitive survival benefit. This is one of the reasons that enucleation has been largely replaced by conservative modalities such as brachytherapy, proton beam radiation, stereotactic radiotherapy and tumour resection in recent years. These conservative treatments, in contrary to enucleation, allow for eye preservation. Among them, brachytherapy is the technique of choice. Enucleation is preferred for larger tumours and when there is a little chance to save vision.

Nonetheless, approximately half of patients with primary uveal melanoma will develop metastases, especially to the liver. Metastases are the main cause of mortality among patients with uveal melanoma and are a poor prognostic factor. More specific therapies have been used for the treatment of metastatic uveal melanoma, including systemic chemotherapy, immunotherapy, molecular targeted therapy and liver-directed therapies. Nevertheless, systemic chemotherapy has not improved the overall survival time in patients with UM. Regarding, molecular target therapy the results are not constant, with different clinical trials with the same drugs showing different conclusions. The diversity of pathways involved in the metastatic uveal melanoma make it difficult to find a specific effective drug for this highly lethal tumour. The main challenge of uveal melanoma management is to find an effective therapy for the high percentage of metastases observed in this pathology. Several clinical trials have been developed worldwide in order to resolve this problem. The better understanding of molecular biology of uveal melanoma has allowed developing new promising systemic therapies. However, further studies are needed to find an effective strategy to teat metastatic uveal melanoma. The combination of different therapies, which have different targets involved in UM, may be a good alternative for this lethal tumour.

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Annex I

Table 2 – Current open clinical trials on uveal melanoma with known status; Source: https://clinicaltrials.gov/ct2/results?term=Uveal+melanoma&recr=Open&pg=2

Clinical Trial	Identification number	Status	Observations
Diagnosis and Prognosis			
Assessing the Clinical Effectiveness of Serum Biomarkers in the Diagnosis of Metastatic Uveal Melanoma (UM)	NCT01438658	Recruiting	_____
BAP1 Testing in Instance Choroidal Nevi or Uveal Melanoma	NCT01925599	Recruiting	_____
Clinical and Histopathologic Characteristics of BAP1 Mutations	NCT01773655	Recruiting	_____
Cytogenetic Study of Ocular Melanoma	NCT00344799	Recruiting	_____
Optical Coherence Tomography of Retinal Abnormalities Associated With Choroidal Nevus, Choroidal Melanoma and Choroidal Melanoma Treated With Iodine-125 Brachytherapy	NCT00346372	Recruiting	_____
Definity for Ultrasound of Intraocular Tumors	NCT01930968	Not yet recruiting	_____
Surgery /Phototherapy			
Phase II Evaluating Endoresection of the Tumor Scar or Transpupillary Thermotherapy When Endoresection is Not Feasible After Proton Beam Therapy for the Treatment of Large Uveal Melanomas (Endoresection-Laser)	NCT02874040	Recruiting	_____
Radiotherapy			
Vascular Response to Brachytherapy Using Functional OCT	NCT01955941	Recruiting	I-125 plaque brachytherapy
Hypofractionated Stereotactic Linear Accelerator Radiotherapy of Uveal Melanoma	NCT00872391	Recruiting	Stereotactic LINAC radiotherapy
Study Assessing Two Models of Hypofractionated Protontherapy on Large Choroidal Melanomas (HYGROMEL)	NCT02602756	Recruiting	_____
Chemotherapy			
Randomized Phase III Study Comparing an Adjuvant Chemotherapy With Fotemustin to Intensive Surveillance in Patients With High Risk Uveal Melanoma (FOTEADJ)	NCT02843386	Recruiting	_____
Immunotherapy			
Phase II Study of Nivolumab in Combination With Ipilimumab for Uveal Melanoma	NCT01585194	Recruiting	Anti- PD-L1 Anti-CTLA-4
Trial of Nivolumab in Combination With Ipilimumab in Subjects With Previously Untreated Metastatic Uveal Melanoma (GEM1402)	NCT02626962	Recruiting	Anti- PD-L1 Anti-CTLA-4
Dendritic Cells Plus Autologous	NCT01983748	Recruiting	Vaccine

Tumor RNA in Uveal Melanoma			
Pembrolizumab in Treating Patients With Advanced Uveal Melanoma	NCT02359851	Recruiting	Anti- PD-L1
Glembatumumab Vedotin in Treating Patients With Metastatic or Locally Recurrent Uveal Melanoma	NCT02363283	Recruiting	Fully-human monoclonal antibody-drug conjugate that targets gpNMB
Immunotherapy Using Tumor Infiltrating Lymphocytes for Patients With Metastatic Ocular Melanoma	NCT01814046	Recruiting	Tumor Infiltrating Lymphocytes (TILs)
Study of CM-24 (MK-6018) Alone and In Combination With Pembrolizumab (MK-3475) in Participants With Selected Advanced or Recurrent Malignancies (MK-6018-001)	NCT02346955	Recruiting	CEACAM1 inhibitor Anti-PD-L1
Molecular targeted therapy			
A Phase I Study of LXS196 in Patients With Metastatic Uveal Melanoma	NCT02601378	Recruiting	PKC inhibitor
Intermittent Selumetinib for Uveal Melanoma	NCT02768766	Not yet recruiting	MEK Inhibitor
Adjuvant Sunitinib or Valproic Acid in High-Risk Patients With Uveal Melanoma	NCT02068586	Recruiting	MEK Inhibitor Histone deacetylase inhibitor
Trametinib With or Without GSK2141795 in Treating Patients With Metastatic Uveal Melanoma	NCT01979523	Recruiting	MEK Inhibitor AKT inhibitor
Crizotinib in High-Risk Uveal Melanoma Following Definitive Therapy	NCT02223819	Recruiting	ALK inhibitor ROS-1 inhibitor
Vorinostat in Treating Patients With Metastatic Melanoma of the Eye	NCT01587352	Recruiting	Histone deacetylase inhibitor
Trial of AEB071 in Combination With BYL719 in Patients With Melanoma	NCT02273219	Recruiting	PKC Inhibitor PI3Kα Inhibitor
Liver-directed therapies			
SIR-Spheres® 90Y Microspheres Treatment of Uveal Melanoma Metastasized to Liver	NCT01473004	Recruiting	Radioembolization
The Scandinavian Randomized Controlled Trial of Isolated Hepatic Perfusion for Uveal Melanoma Liver Metastases (SCANDIUM)	NCT01785316	Recruiting	_____
Combination therapies			
Epacadostat and Vaccine Therapy in Treating Patients With Stage III-IV Melanoma	NCT01961115	Recruiting	Histone deacetylase inhibitor Vaccine
Sorafenib and Radioembolization With Sir-Spheres® for the Treatment of Metastatic Ocular Melanoma	NCT01893099	Recruiting	Anti-angiogenic drug Radioembolization
Efficacy Study of Pembrolizumab With Entinostat to Treat Metastatic Melanoma of the Eye (PEMDAC)	NCT02697630	Not yet recruiting	Anti- PD-L1 Histone deacetylase inhibitor
Percutaneous Hepatic Perfusion vs Best Alternative Care in Patients With Hepatic-dominant Ocular Melanoma (FOCUS)	NCT02678572	Recruiting	Procedures: Melphalan; Transarterial chemoembolization Drugs: Dacarbazine;Ipilimumab; Pembrolizumab