Harnessing zebrafish xenograft models for ocular melanoma treatment discovery

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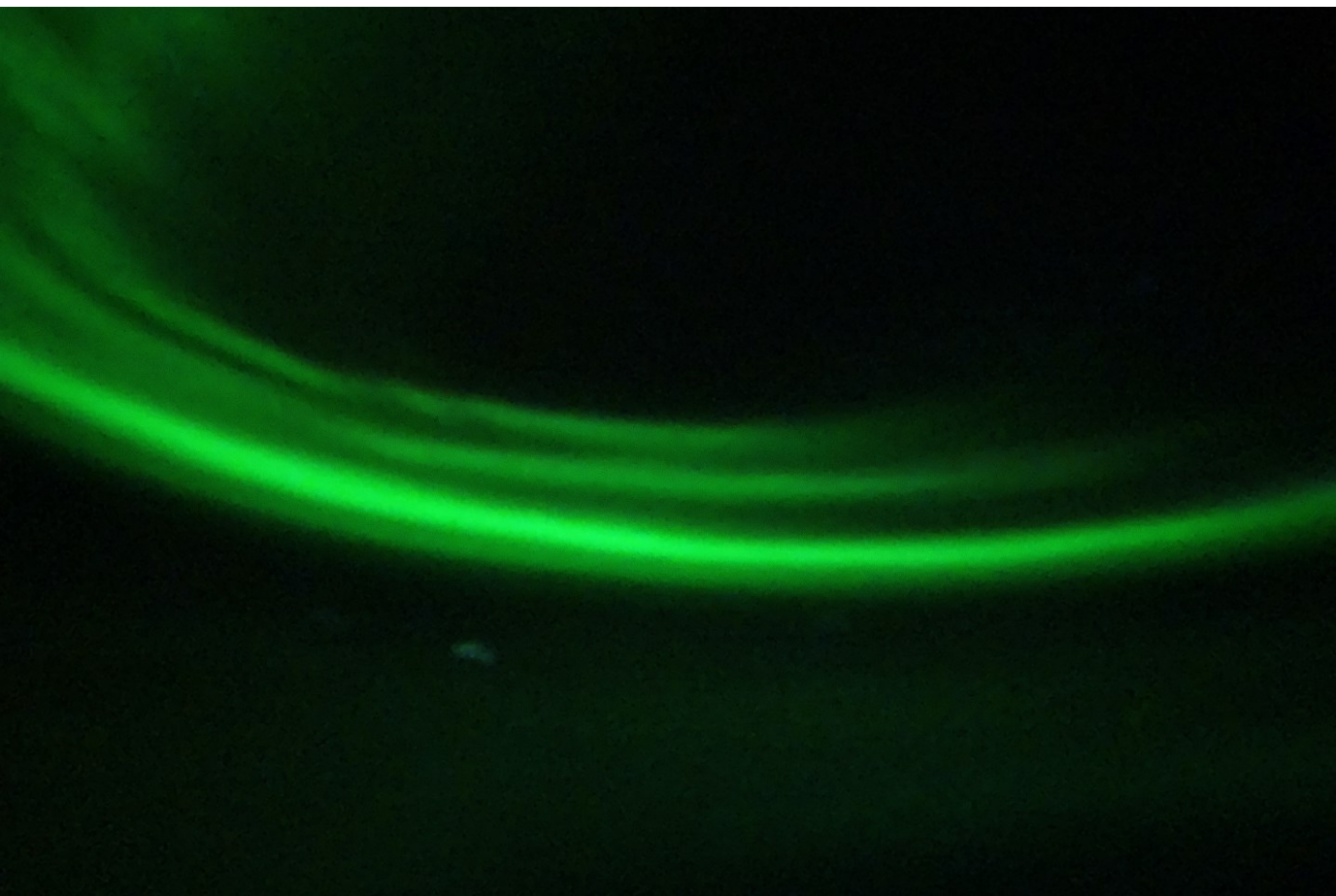

Chapter 1

General Introduction

1. Melanoma: Incidence and classifications

Melanoma can be categorized based on its origin: the skin, the eye, and the mucosal tissues, which include the ocular conjunctival tissues. Overall, melanomas as a group constitute the 17th most common form of cancer worldwide and the cutaneous type is the deadliest form of skin cancer, accounting for 80% of deaths [1]. According to GLOBOCAN, cutaneous melanoma mainly occurs in countries with predominantly fair-skinned individuals: in 2020, the highest incidence rates were observed in Australia and New Zealand, followed by Western Europe, Northern America, and Northern Europe [2]. During the past decade (2013-2023), the number of new invasive melanoma cases in the United States of America increased annually by 27 percent, and it is still rising [3]. Overall, melanoma is particularly prevalent among white males, with an incidence of 34.7 and 22.1 cases per 100,000 white men and women, respectively [4].

Cutaneous melanoma is the most common subtype of melanoma, accounting for more than 90% of all melanoma cases. Besides cutaneous melanoma, ocular melanoma (OM) is the second most frequent non-cutaneous malignant melanoma in adults [5]. OM can be divided into uveal melanoma (UM), conjunctival melanoma (CoM), and other sites of OM, accounting for 85%, 5%, and 10% of the OM cases respectively [6]. In Europe, the incidence of UM is estimated to be approximately 5 to 7 cases per million people per year, with CoM at 0.3 to 0.8 per million individuals per year [7]. Despite this low frequency, UM mortality is quite high, with up to 50% of cases dying from metastases. Once metastases have developed, median survival is around 6 to 8 months [8,9]. For CoM, 5-year survival is better, around 90% [10]. There is a great lack of treatment options for metastases and one needs to ascertain the pathogenesis of OM and find targeted therapeutic drugs to prevent and treat OM metastasis.

Melanoma arises from the malignant change of melanocytes, the cells responsible for synthesizing melanin, a photoprotective pigment [11]. These melanocytes differentiate from precursor cells (melanoblasts), originating from neural crest cells. Melanoblasts differentiate into mature melanocytes, subsequently producing melanin at anatomically specific sites, including the gastrointestinal tract, ocular structures, genitalia, paranasal sinuses, and meninges [12]. However, they can become malignant and risk factors for malignant transformation include sun exposure, immunosuppression, a positive melanoma family history, and certain congenital genetic mutations [13]. In cutaneous melanoma, UV radiation is considered a major contributor to melanoma development through its harmful effects on the skin and direct DNA damage [14]. In addition, somatic gene mutations play a vital role in prognosis. Since there is a strong correlation between patient prognosis and some specific gene mutations, such as those in BRAF and NRAS, many scientists focus on

gene mutations to find new treatments. Here, I will explore the distinguishing features of UM and CoM, including their genetic profiles, the process of cancer development, and strategies employed for their treatment.

1.1. Uveal Melanoma

UM is a rare type of OM that arises in the choroid, ciliary body, or iris of the eye [15]. UM differs from cutaneous melanoma in its characteristics such as anatomical spread, causative mutations, and therapeutic response. The liver, lungs, bones, and skin are the most frequent sites for UM metastasis [16]. Half of the UM patients exhibit poor prognosis, due to metastatic progression. In addition to prognostic clinical factors such as tumor location, a large diameter, and extra-ocular extension, male gender and older age are important risk factors for metastasis [17,18]. About 50% of patients will develop metastases despite effective treatment of the intra-ocular tumor by radiotherapy or enucleation [19]. As for the genomic landscape, UM shows specific chromosomal aberrations and gene mutations, both of which are strongly associated with clinical outcomes [20]. The details can be seen in **Table 1.**

In primary UM, common chromosome aberrations include losses of 1p, 3, 6q, 8p, and 16q and gains in 6p and 8q [21]. The chromosomal evolution in aggressive UM starts with a Guanine nucleotide-binding protein subunit alpha-q (GNAq/GNA11) mutation, succeeded by loss of one copy of chromosome 3 and/or a BRCA-Associated Protein 1 (BAP1) mutation, and gain of chromosome 8q [22]. In general, mutations or alterations in chromosome copy numbers of UM can influence specific innate immune responses, which are associated with a poor prognosis [23]. For instance, the initial influx of macrophages is associated with the gain of chromosome 8q, while BAP1 mutation is correlated with additional T cell infiltration in the tumor microenvironment [24,25]. The presence of local infiltrating T cells and macrophages in UM is associated with a poor prognosis, not a good prognosis.

Additional genetic profile studies are in progress. Using whole exome sequencing of UM, a recurrent gain of function in the phospholipase C beta 4 (PLCB4) gene was discovered which encodes a protein that is a downstream target of GNAq/GNA11 [26]. This mutation in the Y-domain of the highly conserved catalytic core of PLCB4 is mutually exclusive with the GNAq and GNA11 mutations [27]. Mutations in the telomerase reverse transcriptase (TERT) promoter are infrequent [28]. More frequent are mutations in the eukaryotic translation initiation factor 1A (EIF1AX) gene [29]. Recently, another gene, SF3B1 (splicing factor 3 subunit B1) was also reported to be mutated in 10% to 21% of cases of UM [30].

Table 1. *Chromosomal Aberrations and Gene Mutations in UM*

Genomic Profile		Description	References
Chromosomal Aberrations	Monosomy 3	Robust predictor of metastasis and poor prognosis Increased metastatic potential	[31,32]
	Gain of chromosome 8q	Increased macrophage infiltration in patient samples Activation of c-Myc gene	[33,34]
		Increasing metastatic potential poor prognosis	[35,36]
	Gain of chromosome 6p	Positive predictor for improved prognosis	[37]
Gene Mutations	GNAq and GNA11	Occurs as an early event Regulates MAPK pathway Sensitiveness to MEK inhibition	[38-40]
	BAP1	Mutated in aggressive tumors and in 80% of metastasized melanoma Results in hyper ubiquitination of H2A Increased expression of macrophage-attracting cytokines	[41-44]
	EIF1AX	Associated with low-risk tumors Exclusive with BAP1 mutation and inversely correlated with metastasis	[45,46]
	SF3B1	Driver gene associated tumors with intermediate risk of metastasis	[47-49]

Abbreviations: GNAq: Guanine nucleotide-binding protein subunit alpha-q; GNA11: Guanine nucleotide-binding protein submit alpha-11; MAPK: Mitogen-activated protein kinase; BAP1: BRCA-Associated Protein 1; BRCA: Breast Cancer gene 1; EIF1AX: Eukaryotic translation initiation factor 1A, X-linked; SF3B1: Splicing factor 3b subunit 1.

1.2. Conjunctival Melanoma

CoM (conjunctival melanoma) is another type of OM and arises from melanocytes located amongst the basal cells of the conjunctival epithelium. CoM are biologically different from their uveal counterparts and are more similar to cutaneous and mucous membrane melanomas [50]. The incidence of CoM in Europe and the US is around 0.2-0.7 cases per million annually in Caucasians, especially the elderly [51]. It usually presents with pigmented lesions that are most commonly located on the bulbar conjunctiva. CoM spreads directly toward the orbit or through lymphatic and hematic vessels [52]. Distant metastases are frequently found in the liver, lungs, and brain. The 10-year mortality rate for CoM has been reported as approximately 30% [53]. Treatment strategies for CoM patients generally include local excision, brachytherapy, and added cryotherapy. Local recurrence is still observed in 26-61% of cases after treatment [54,55].

At the genetic level, CoM is primarily characterized by B-Raf proto-oncogene, serine/threonine kinase (BRAF), NRAS proto-oncogene, GTPase (NRAS), Neurofibromin 1 (NF1) mutations, and mutations in the loss of phosphatase and tensin homolog (PTEN) gene [56,57]. The most common chromosomal aberrations and gene mutations in CoM are summarized in **Table 2**. Other genetic alterations associated with CoM are found in the cyclin-dependent kinase inhibitor 1A (CDKN1A) and Runt-related transcription factor 2 (RUNX2) gene located on 6p21.2, which are commonly overexpressed in primary CoM [58]. TERT promoter mutations are observed in 32-40% of CoM, leading to increased expression which is associated with cellular immortality [59,60]. Reverse transcriptase inhibitors like azidothymidine (AZT) and telomerase inhibitors such as imetelstat (GRN163L) are possible candidates for targeted therapies against CoM with TERT promoter mutations [61,62]. In recurring CoM, Heat shock protein 90 (HSP90) expression is found to be higher than in conjunctival nevi [63]. MutL homolog 1 (MLH1) involved in DNA repair and tissue inhibitor of metalloprotease type 2 (TIMP2) encoding for matrix metalloproteinase crucial for tissue homeostasis are amplified in metastatic CoM [64]. A deletion in the DNA repair gene, O-6-methylguanine-DNA methyltransferase (MGMT) has been observed in various cancer types including CoM and cutaneous melanoma [65].

Table 2. *Chromosomal Aberrations and gene mutations in CoM*

Genetic Profile		Descriptions	References
Chromosomal Aberration	Loss of 1p, 3q, 6q, 8p, 9p, 10, 11q, 12q, 13, 15p and 16q, and gain of 1q, 3p, 6p, 7, 8q, 11q, 12p, 14p and 17q	Most frequent alterations in CoM; most not correlated with metastasis and invasion 10q deletion with BRAF mutation is correlated with shorter survival, lymphatic invasion, and major tumor thickness Several onco-suppressor genes such as PTEN are located in 10q	[50,66,67]
Gene Mutations	BRAF	Found in 50% of both primary and metastatic CoM Most mutations are V600E, followed by V600K, V600D and V600R Associated with reduced metastases free-survival Observed in young males and associated with pigmentation	[68-70]
	NRAS	Involved in regulating cell division. Found in 20% of CoM cells NRAS mutations in CoM are mutually exclusive with BRAF mutations	[69,71,72]
	NF1	Found in 30% of CoM Co-occurs with NRAS and BRAF mutations Common in cutaneous melanomas with UV exposure	[73-75]
	KIT	Found in 2-7% of CoM Mutually exclusive with BRAF and NRAS Associated with older-age Partly sensitive to pharmacological inhibition	[76-78]
	PTEN	Mutually exclusive with NF1 mutations	[79,80]

	An AKT/mTOR pathway inhibitor Co-suppressor abrogated by nuclear-cytoplasmic transport in neoplastic conditions Low expression in CoM

Abbreviations: AKT: Protein Kinase B; mTOR: Mammalian target of rapamycin; KIT: receptor tyrosine-protein kinase.

2. Ocular Melanoma development

In the clinic, UM can be divided into American Joint Committee on Cancer (AJCC) stages I to IV [81]. Choroidal and ciliary body tumors are classified on the basis of thickness and size, as well as extraocular extension. In Stage IV, the cancer has metastasized beyond the original tumor site to more distant areas of the body. The development of CoM is also classified according to the AJCC, with stages [82].

UM's distant spread involves diverse cell types and multi-step processes. Tumor cells require oxygen and nutrients to survive and proliferate, therefore need to reside in close proximity to blood vessels to access the blood circulation [33,83]. UM also needs to alter metabolic processes to meet their increased demands for energy and building blocks for rapid growth [84]. More importantly, UM demands immune privilege in the eyes and evades immune detection and suppression to achieve tumor metastasis [85]. The following overview will briefly explain tumor development from these three aspects of oncology: angiogenesis, metabolism, and immune response.

2.1. Angiogenesis

One of the most critical steps in metastasis formation in cancer is angiogenesis. As primary tumors expand, new blood vessels are required to transfer nutrients for melanoma proliferation. Besides, migration largely occurs through these vessels [86]. Generally, the newly formed microvessel network covers or embeds melanoma clones, triggering interactions between the tumors and vascular endothelial cells. This prompts the degradation of the basement membrane and outward endothelial cell extension forming a cable-like shape. Finally, cancer cells invade the vasculature and metastasize to other sites, such as the lung, liver, and cerebrum [87,88]. Angiogenesis initiates the uncontrolled proliferation of malignant tumor cells, which in turn, dramatically increases the consumption of oxygen and nutrients and eventually leads to cell starvation and hypoxia [89,90].

Tumor angiogenesis is stimulated by a variety of growth factors such as vascular endothelial-derived growth factor (VEGF), basic fibroblast growth factors (bFGF), platelet-derived growth factor (PDGF), and transforming growth factors α and β (TGF-α and β) [91]. These growth factors are involved in both autocrine and paracrine

regulation of melanoma progression. Malignant melanoma cells express VEGFR-2, VEGFR-1, and co-receptor neuropilin-1/2, which are not commonly expressed on most of the cancer cells [92]. Therefore, VEGF may be able to induce similar intracellular signaling responses in both endothelial and melanoma cells [93,94]. Placental growth factor (PIGF) can independently bind to VEGFR-1 and neuropilin-1/2 to induce intracellular signaling. FGF or PDGF form heterodimeric complexes with VEGF and interact with VEGFR-2 [95]. Other receptors expressed on both melanoma and endothelial cells include urokinase plasminogen activator and its receptor (uPA/uPAR, respectively) [96], chemokine receptors CXCR-1/2, and FGFR-1, which could induce similar signaling responses between melanoma and endothelial cells. [97]. Increased levels of VEGF have been found in eyes containing a UM [98,99].

At the same time, bFGF regulates endothelial cell proliferation and angiogenesis by both autocrine and paracrine mechanisms. Since bFGF is devoid of the classic signal peptide for secretion, tumor cells release this factor by exocytosis from the endoplasmic reticulum [94,100] Significant amounts of bFGF were found to be associated with the extracellular matrix as well as with the basement membrane of the newly-formed blood vessels in human cutaneous melanomas [101,102]. Digestion of extracellular matrix by matrix metalloproteinases of melanoma or endothelial origin promotes the release of matrix-bound bFGF, which, in turn, stimulates endothelial cell proliferation and vascular tube formation in melanomas [103]. Based on different mechanisms of these factors, serials of anti-angiogenic drugs have been developed and are under various stages of clinical trial.

2.2. Metabolism

It is now widely accepted that metabolism is a critical driver of cancer malignancy, with cancer cell proliferation commonly requiring an upregulation or “metabolic switch” towards a more glycolytic pathway to fuel the rapid energy requirements and for cancer cell growth, commonly known as the “Warburg Effect” [104]. In mammalian cells, the end product of glucose metabolism can be either lactate or, upon full oxidation of glucose via respiration in the mitochondria, CO_2. In tumors, the rate of glucose uptake dramatically increases and high levels of lactate are produced, even in the presence of oxygen and fully-functioning mitochondria [105]. Due to this process, tumor cells exhibit higher glucose uptake, more lactate production, and faster ATP generation compared to normal cells [106].

According to Warburg’s calculation, glucose uptake in tumor cells was about 47–70% higher compared to 2-18% in normal tissues, and tumor cells converted 66% of glucose uptake to lactate [107]. Warburg also observed that blood lactate concentration was higher in blood vessels leaving tumor tissues than the lactate

concentration in blood vessels entering tumors [108]. The higher glucose uptake in cancer cells is facilitated by increased expression of glucose transporters, such as glucose transporter 1 (GLUT1), on the cell membrane [109]. In addition, the rate of glucose metabolism through anaerobic glycolysis (lactate production) is 10-100 times faster than such production of complete aerobic respiration of glucose in the mitochondria [110]. Tumor microenvironments have limited availability of glucose and stromal cells and the immune compartment competes for nutrients. Despite the lower ATP yield, cells with a higher ATP production rate may gain a selective advantage when competing for shared and limited energy resources [111]. Besides, it has been reported that the cellular environment can induce massive ATP demand by altering the demand for ATP-dependent membrane pumps, followed by a rapid increase in aerobic glycolysis, while oxidative phosphorylation remains constant [112].

In addition to the metabolic benefits for the tumor, the Warburg Effect may present additional advantages for cell growth in a multicellular environment, such as acidification of the microenvironment and other metabolic crosstalk. For example, a recent study showed that tumor-derived lactate is a contributor to alternatively activated (M2) macrophage polarization [113]. Furthermore, the unique metabolic characteristics of cancer cells also present altered lipid metabolism, amino acid metabolism, and mitochondrial dysfunction. Yu *et al.* found that ocular melanoma histone lactylation drives oncogenesis by facilitating YTH N6-methyladenosine RNA-binding protein 2 (YTHDF2) expression [114]. Over the recent years, some therapies in UM have been through modulation of the Warburg effect. For instance, microRNA-216a-5p (miR-216a-5p) inhibits Hexokinase-2 (HK2) expression by directly targeting its 3′-UTR in uveal melanoma cells. miR-216a-5p dampens glycolysis by reducing HK activity, glucose uptake, lactate production, and increasing oxygen consumption rate resulting in suppressing UM growth [115].

2.3. Immune response

The tumor microenvironment (TME) contains stromal cells, endothelial cells, cancer-associated fibroblasts, and a repertoire of immune cells that play important roles in tumorigenesis [116]. In general, tumor-associated immune cells can be divided into two types: tumor-antagonizing and tumor-promoting immune cells. Tumor-antagonizing immune cells mainly consist of effector T cells (including CD8+ cytotoxic T cells and effector CD4+ T cells), natural killer (NK) cells, dendritic cells (DCs), M1-polarized macrophages, and N1-polarized neutrophils. On the other side, tumor-promoting immune cells mainly consist of regulatory T cells (Tregs), M2-polarized macrophages, and myeloid-derived suppressor cells (MDSCs) [117,118]. All these types of cells play various roles in the different stages of tumor progression.

Among immune cells, tumor-associated macrophages (TAMs) play an integral role in extracellular matrix degradation, tumor cell migration, and angiogenesis. They are recruited by chemokines released by the cancer cells or the TME, such as C-C motif ligand 2 (CCL2) [119]. Generally, macrophages are differentiated into two opposing phenotypes: classically activated macrophages (M1), and alternatively activated macrophages (M2). M1 phenotypes can be induced by Toll-like receptor stimulation in the presence of interferon-gamma and express proinflammatory cytokines, such as interleukin 12 (IL-12) [120]. M2 phenotypes are induced by interleukins 4 and 13 (IL-4 and IL-13), produced by CD4+ T helper 2 cells, which are associated with the production of arginase I (Arg1) and the anti-inflammatory interleukin 10 (IL-10) [121]. In addition, within the evolving characteristics of the internal environment, such as local anoxia, and levels of lactic acid, both M1 and M2 polarized immune cells can repolarize. In fact, some studies have shown that TAMs not only have the characteristics of M2 but also share some M1 signatures [122].

TAMs in malignant cancers can often promote tumor growth and metastasis, via the secretion of chemokines and cytokines such as interleukin 6, 8, and 10 (IL-6, IL-8, and IL-10), and transforming growth factor-β (TGF-β) [123,124]. Conversely, tumor cells can restrain macrophage activity to achieve immune escape. Various molecular mechanisms are involved in this immunosuppression. For instance, the programmed cell death protein 1/Ligand 1 (PD-1/L1) signaling pathway promotes the possibility of tumor immune escape because it can inhibit the normal function of anti-tumor macrophages [125]. The cluster of differentiation 47 (CD47) interacts with signal regulatory protein alpha (SIRPα) on macrophages leading to tyrosine phosphatase activation and preventing myosin accumulation at the phagocytic synapse. The SIRPα/CD47 pathway is referred to as the "do-not-eat-me" signal. In that sense, tumor cells with CD47 expression can be recognized as self-cells with normal physiological functions. Major histocompatibility complex (MHC) class I component β2-microglobulin plays a role evasion of the adaptive immune response [126]. However, while this is true for cutaneous melanoma and CoM, it is not for UM, where an increased HLA expression is related to the development of metastases: several studies performed in UM, the high human leukocyte antigen (HLA) expression is associated with loss of one chromosome 3/loss of BAP1 expression, and is associated with the presence of infiltrating lymphocytes and macrophages [127]. It has been suggested that the increased HLA expression inhibits NK cells from killing UM cells during their transport from the eye to the liver [127-129]. The interactions between TAMs and tumor cells have become a research hotspot in tumor immunotherapy.

In fact, the TME is a complex network of interactions between various cell types and molecules that play a crucial role in cancer development and progression. These cells are not unaided but are interdependent. For instance, the metabolite of tumors could

cause the differentiation and polarization of TAMs. Meanwhile, TAMs have been shown to be important drivers for cancer neovascularization. The angiogenesis further facilitates tumor access to nutrients and metastasis. According to the paper "Hallmarks of Cancer: New Dimensions", cancer is a complex mixed phenotypes and genotypes [130]. The ten core capabilities of cancer are each, by their definition as a hallmark, conceptually distinguishable, but aspects of their regulation are partially interconnected in many cancers. Understanding these connections between cells in TME will benefit exploring cancer development and clinical therapeutic research.

3. Therapeutics

When detected and treated early in their development, primary UM is usually curable via surgery, radiotherapy, or a combination of them. Patients with early-stage UM treated with enucleation or proton beam therapy have a 90% 5-year survival rate [131]. However, therapy options are limited after metastasis and, unfortunately, metastatic melanoma is typically detected at a relatively advanced state. Additional treatment options include targeted therapy and immunotherapy. Chemotherapy is the most commonly used treatment option for cancer, but has not been found to be useful for UM [132]. However, the administration of traditional chemotherapeutic agents at high doses always induces significant non-selective toxicity, immunosuppression, and acquired drug resistance.

Regarding targeted therapy, clinical trials with selumetinib, an MEK inhibitor, reported a higher progression-free survival among UM patients. However, no meaningful increase in overall survival was observed in comparison to the general chemotherapeutic temozolomide. For UM patients with BAP1 mutations, preclinical studies highlighted that treatment with a histone deacetylase (HDAC), such as valproic acid, inhibitor could be beneficial [133]. Because BAP1 mutations are associated with loss of melanocytic differentiation, treatment with HDAC inhibitors has been postulated to inhibit the growth of UM *in vivo* by inducing morphological differentiation [134]. The era of molecular targeted therapy was expanded following the discovery of BRAF mutations in several cancers, including cutaneous and conjunctival melanoma. This discovery led to the initial evaluation of BRAF inhibitors with initial trials showing 50% response rates as a single agent in patients with metastatic cutaneous melanoma [135,136].

Moreover, immunotherapy, especially immune checkpoint inhibitor treatment, is a current standard of care for melanoma. Immune checkpoint proteins, such as PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), are co-inhibitory protein receptors expressed on the cell surface of lymphocytes whose primary physiologic role is to maintain self-tolerance and limit inflammatory responses in normal tissues [137]. An analysis of Danish UM patients observed partial responses in 7% of patients

to anti-PD-1 and 21% to concurrent anti-PD-1 plus anti-CTLA-4 [138]. Tumor cells present clear metabolic adaptations and identifying deregulated glycolysis pathways could offer new therapeutic targets. Besides, the immune cells and other cells that infiltrate melanoma tumors have metabolic particularities that, upon interaction within the tumor microenvironment, would favor tumorigenesis [139,140]. While immunotherapy with immune checkpoint inhibitor treatment has yet been successful in UM, it is a promising option for CoM, as it is for cutaneous melanoma metastases [132,141,142]. Analysing both tumor cell metabolism and the metabolic outline of immune cells can offer innovative insights into new therapy targets and cancer therapeutical approaches.

4. Ginsenosides

Seeking effective anticancer drugs and elucidating their mechanisms is an important task for OM research. Due to the rare incidence of OM, the range of drugs currently being studied is limited. One group of such drugs is ginsenosides, the active extract of ginseng. Ginsenosides are able to kill melanoma cells with relatively weak toxicity to healthy cells, thus gaining attraction among the chemical and natural anti-melanoma drug candidates [143]. Given the similarities between CoM and cutaneous melanoma, I will review the ginsenoside effects against melanoma as a whole.

Ginseng, the root of *Panax ginseng* C.A. Meyer *(P. ginseng)*, has been widely used as a natural tonic in Asian countries, including China and Korea, for thousands of years [144]. Accumulating clinical and experimental studies demonstrated that ginseng has many pharmacological effects, such as antidiabetic, antiaging, antidepressant, anticancer, and immunity enhancement [145-148]. Ginseng contains numerous active compounds, including ginseng saponins, peptides, polysaccharides, mineral oils, and fatty acids. Among its various active ingredients, ginseng saponins (ginsenosides) are known as the main bioactive agents with pharmacological activities [149]. Until now, more than 100 ginsenosides have been isolated and determined. The basic structure of ginsenosides consists of a steroidal core, with various sugar moieties. According to differences in the chemical compositions and configurations, ginsenosides are classified into 3 types: protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanane-type ginsenosides [150]. The chemical structures and classifications of ginsenosides are shown in **Figure 1**. Based on existing published clinical and experimental studies, we summarized the potential mechanisms of anti-melanoma effects of ginsenosides in **Table 3**, including anti-proliferation, pro-apoptosis, anti-angiogenesis, anti-metastasis, mediate metabolism, and immune regulation.

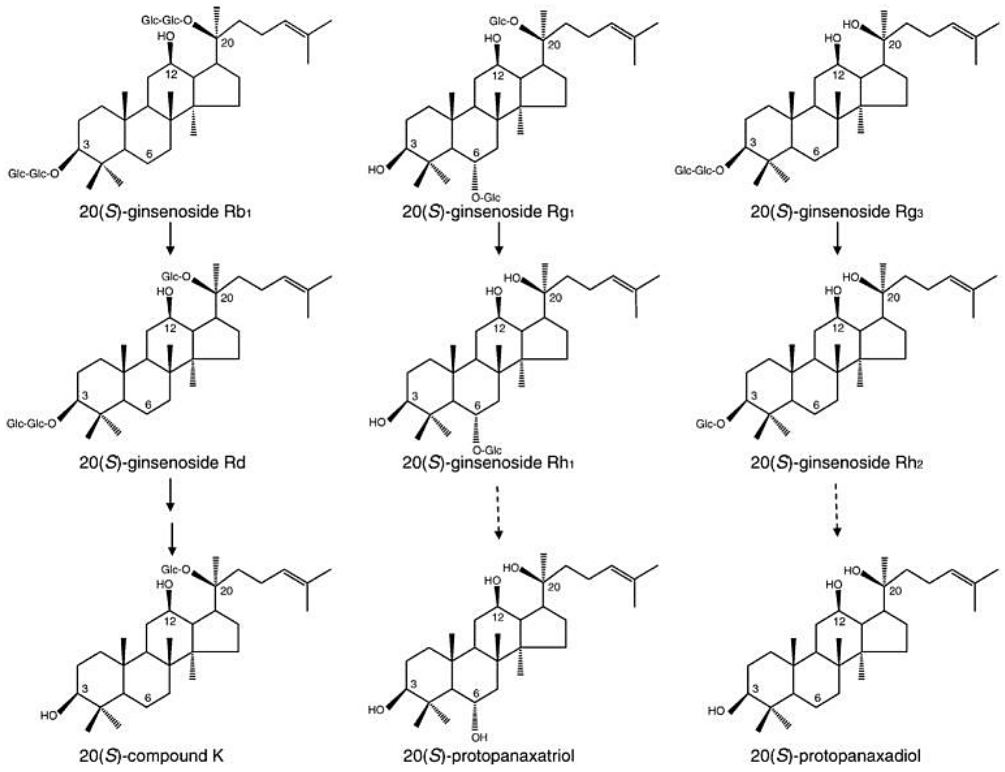

Figure 1. Structures and main metabolic pathways of 20(S)-ginsenoside Rb1, 20(S)-ginsenoside Rg1, and 20(S)- ginsenoside Rg3.

Table 3. *Functions and mechanisms of ginsenosides in melanoma*

Function	Ginsenoside	Mechanisms	Cell type	References
Anti-proliferation	Rg3	↓ DNA synthesis and induction of cell cycle arrest at S phase ↓ ERK and AKT pathways	B16	[151]
		↓ Expression of FUT4 ↓ Activation of EGFR/MAPK pathway	A375	[152]
		↑ Expression of HDAC3 ↑ Acetylation of P53	A375 and cutaneous melanoma patient tissue	[153]
		↓ Expression of NF-κB/p65	Melanoma xenograft in mice	[154]
	Rh2	↓ Tumor growth and improvement of survival time	B16-F10 melanoma mouse model	[155]
	PPD	↑ AMPK and subsequent ↓ mTOR phosphorylation ↑ c-Jun by inducing JNK phosphorylation	SK-MEL-28	[156]
Pro-apoptosis	Rk1	↓ Procaspase-8, procaspase-3, mutant p53 and Bcl-2 protein expression ↑ Fas, FasL, and Bax protein expression	SK-MEL-2	[157]
	Rh2	Depending on caspase-8 and caspase-3 pathway	A375-S2	[158]
	M1	↑ Expression of p27Kip1 ↓ Expression of c-Myc and cyclin D1	B16-BL6	[159]
	PPD	Induction of autophagy and apoptosis via inducing JNK phosphorylation	SK-MEL-28	[156]
		↑ Expression of p27Kip1 ↓ Expression of c-Myc and cyclin D1	B16-BL6	[160]

Anti-angiogenesis	Rg3	↓ VEGF dependent p38/ERK signaling ↓ Mobilization of EPCs	B16	[161]
Anti-metastasis	Rg3	↓ Lung metastasis when synergizing with ribonuclease inhibitor	B16-F10 melanoma mouse model	[162]
		↓ Cell colony formation, mobility and invasion abilities	B16F10	[163]
	Rb2	↓ Lung metastasis and tumor-associated angiogenesis	B16-BL6	[164]
Immune regulation	PPT	↑ Splenic NK cells to become cytotoxic to tumor cells	B16-BL6 melanoma mouse model	[165]
	Rg3	↑ Heat shock proteins and calreticulin expression; ↑ Proportion of CRT(+) CD11c(+) cells ↑ Uptake of dying tumor cells by DCs	B16F10 melanoma cells	[166]
	F1	Modulates Rho family GTPases resulting in dendrite retraction	B16F10	[167]
Associated anticancer effect with other chemotherapy drugs	Rh2	↑ Effects of SMI-4a on autophagy-inducing apoptosis by inhibiting AKT/mTOR signaling pathway.	A375 and G361	[168]
	Rh2	↑ Antitumor activity ↓ Genotoxic effect of cyclophosphamid	B16 melanoma mouse model	[169]
	F11	↓ Cisplatin-elevated blood urea nitrogen and creatinine levels Inversion of Bax/Bcl-2 ratio and the anti-oxidative and free radical levels induced by cisplatin ↑ Anti-tumor activity of cisplatin	Melanoma xenograft mouse models	[170]
	Rh2	↑ Anti-blastoma effect of the cytostatic drug	B16	[171]

	Rb1	melanogenesis synthesized by α-MSH	B16	[172]
	Rh4	↓ Cyclic AMP levels ↓ MITF and tyrosinase ↓ Melanin content and tyrosinase activity	B16	[173]
Anti-melanogenesis	23-O-methyl ginsenoside-Rg11	↓ Activity on α-MSH-stimulated melanogenesis through ↓ tyrosinase activity	B16	[174]
	PPT	↓ Melanin synthesis ↓ Genes encoding tyrosinase-related protein-1 and -2 through dephosphorylation of cAMP response element–binding protein	B16	[175]
	Rh23	↓ Potent melanogenesis and body pigmentation	B16 xenograft zebrafish models	[176]

Abbreviations: ERK: extracellular signal-regulated kinase; FUT4: fucosyltransferase IV; EGFR: Epidermal growth factor receptor; HDAC3: histone deacetylase 3; NF-κB: nuclear factor kappa B; AMPK: AMP-activated protein kinase; JNK: c-Jun N-terminal Kinase; Bcl-2: B-cell lymphoma 2; FasL: Fas ligand; Bax: Bcl-2 Associated X-protein; EPCs: Endothelial progenitor cells; DCs: dendritic cells; NK cells: natural killer cells; CRT: Calreticulin; mTOR: Mammalian target of rapamycin; α-MSH: α-melanocyte-stimulating-hormone; AMP: Adenosine monophosphate.

Among multiple types of ginsenosides, Rg3 is the most well-known in anti-tumor studies, as well as the most abundant in red ginseng extracts (40%). Due to the different spatial structures on C20 positions, there are two Rg3 enantiomers: 20(R)-Rg3 and 20(S)-Rg3, which exhibit different anti-tumoral characteristics. 20(S)-Rg3 has been reported to possess better anti-proliferative effects, whereas 20(R)-Rg3 has shown a better inhibition of cancer cell invasion and metastasis [177]. Furthermore, ginsenoside Rg3 may be an interesting CAM, as it has been shown to enhance the anti-tumor effects of conventional chemotherapeutic agents and to reduce drug-induced toxicity and chemotherapeutic resistance *in vitro* and *in vivo* [178]. Therefore, combination therapies using chemotherapeutic agents and ginsenoside may be an innovative and promising therapeutic strategy for the treatment of human cancer. However, the underlying mechanisms still need to be further elucidated.

Compound K (CK) is one of the main active metabolites of PPD-type ginsenosides. Interestingly, natural ginseng does not contain CK, which is usually produced by microbial or fungal enzymatic biotransformation of ginsenosides Rb1, Rb2, Rc, and Rd [179]. The fungal transformation was first discovered in the human intestinal system after the oral administration of ginseng. In this process, the oligosaccharides linked to the aglycone are gradually cleaved from the terminal sugars and further modified [180]. Several studies have indicated that CK exerts high anti-tumor roles with strong cytotoxic activity on tumor cells, such as in mouse highly metastatic melanoma (B16-BL6), human liver cancer (HepG2), and human highly metastatic lung cancer (95-D) cell lines [181]. *In vivo*, CK could also significantly inhibit lung metastasis induced by tumor inoculation of B16-BL6 melanoma cells in mice [182]. Similarly, CK could be transformed into PPD through a series of deglycosylation procedures by acid hydrolysis and intestinal bacterial actions. Compared to the parental components, PPD shows less polarity and easier absorbance in gastrointestinal tracts. Some studies revealed that oral administration of PPD exerted antineoplastic actions, which were more effective than its glycosides [183].

Ginsenosides with two molecules of glucose linked to C-3-OH have a lower inhibitory activity than those with one molecule: for example, Rh2 (one glucose at C-3) showed more potent pharmacological activities than Rg3 (two types of glucose at C-3) [184]. In concordance with this, PPD and CK showed much stronger anti-proliferation in some cancers than the original ginsenosides [185]. Structure-activity relationships indicate that glycosylation at C-3-OH on ginsenosides might be important for the inhibition of the chymotrypsin-like activity of the 20S proteasome which plays an important role in selective protein degradation and regulates cellular events in the anticancer process [181]. This may also explain the high efficiency of Rg3 in inhibiting tumor growth and inducing tumor apoptosis [186].

5. Glucosylceramidase GBA2

Mammalian β-glucosidases (GBA1, GBA2 and GBA3 β-glucosidases) hydrolyze β-glucosylceramide and play important functions in the metabolism of glycolipids and dietary glucosides, and also in signaling functions [187]. GBA1 is a lysosomal hydrolase whose deficiency causes Gaucher disease, the most prevalent inherited lysosomal storage disorder. Bile acid β-glucosidase, also known as GBA2, is a ubiquitous non-lysosomal glucosylceramidase. Subcellular fractionation analysis revealed that this enzyme is located at the plasma membrane, the cytosolic surface of the endoplasmic reticulum (ER) and/or Golgi. GBA3 is a cytosolic β-glucosidase, mostly present in the kidney, liver, spleen, intestine and lymphocytes of mammals, the function of which is still unclear [188].

There is a frequently observed correlation between immune dysregulation and GBA dysfunction. For example, GBA2-deficient mice exhibit decreased serum levels of IL-6, TNFα, and reduced activation of signal transducer and activator of transcription 3 (STAT3) [189]. A study about Gaucher's disease reported the thymus exhibited impaired T-cell maturation, aberrant B-cell recruitment, enhanced antigen presentation, and impaired egress of mature thymocytes in mice in which the GBA gene is deleted in hematopoietic stem cells [190]. It is reported the macrophages generated from GBA mutation patients displayed an activated macrophage phenotype with increased activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome as a consequence of lysosomal storage and impaired autophagy. The results pointed to a fundamental role of GBA in immune regulation [191].

Interestingly, a transcriptomic study in multiple melanoma cell lines revealed a decreased level of GBA2 expression as compared to normal melanocytic cells [192]. Sorli *et al*., found that the inducible expression of GBA2 in human A375 melanoma cells led to enhanced GlcCer breakdown and ceramide formation, and resulted in significant inhibition of melanoma cell proliferation *in vitro* and *in vivo* [193]. This may indicate a potential ability for GBA2 to modulate tumor cell life or death switch. β-glucosidases are also involved in the biotransformation of ginsenosides and therefore may modify their anti-tumor functions. It has been reported that the anti-tumor activity of ginsenosides is associated with the number of glycosides [194]. The ginsenosides with many glycosyl have a large molecular weight and large steric hindrance, causing poor cell permeability [195]. The various effects of different ginsenosides suggest their enzymatic degradation. Currently, Halima *et al*., found that GBA2 could influence the anti-inflammatory effects of ginsenosides by converting them to active compounds [196]. The role of GBA2 in anti-tumor activity is still unknown. The connection between the ginsenosides and GBA2 is a new approach to exploring the metabolic and pharmacological mechanisms of ginsenosides in their anti-tumor functions.

6. Zebrafish as a model for screening anti-tumor drugs

The zebrafish (*Danio rerio*) has become a valuable non-mammalian vertebrate model widely used to study cancer biology and anti-cancer drug discovery. Benefits of zebrafish include the relatively high fecundity, cost-effective maintenance, dynamic visualization, and easy manipulation of embryos. Compared to the mouse model, the zebrafish model requires much less material to assess drug efficacy. Furthermore, zebrafish embryos can take up various small molecular weight compounds directly from water. Therefore, zebrafish are suitable for high-throughput drug screening and toxicity testing [197,198].

Zebrafish possess numerous characteristics that make them an attractive model for human cancer research. For example, the adaptive immune system of zebrafish does not reach maturity until 4 weeks post-fertilization [199]. This makes it possible to engraft human or mouse cancer cells into zebrafish larvae and avoid implantation rejection. Zebrafish have comparable vertebrate anatomy and express orthologues for 70% of human proteins, and paralogues of 84% of all known disease-related genes [200]. There is a high conservation of oncogenes and tumor-suppressor genes between zebrafish and humans, and various oncogenic transgenic zebrafish lines have been developed [201]. The histology of zebrafish tumors has been shown to be highly similar to tumors found in humans [202]. These characteristics allow extrapolation of cancer research outcomes obtained in fish back to humans.

Recent progress in zebrafish xenotransplantation studies and drug screening has shown that the zebrafish is a promising model for evaluating tumor metastasis *in vivo*. Currently, over 40 genetically engineered tumor models and many zebrafish xenograft models, including patient-derived xenografts (PDXs) in embryos and adult zebrafish have been developed [203,204]. PDXs are cancer models established by engrafting and effectively propagating human tumor materials in animal hosts [205]. PDX model grows in an animal host microenvironment, which includes vasculature that provides *in vivo* delivery of nutrients and oxygen, and host stromal cells that interact and communicate with the tumor cells. Compared to cell line-derived xenograft models, PDXs closely recapitulate the heterogeneity of primary tumors and retain their gene expression and mutation patterns [206,207].

The application of zebrafish-PDXs (zf-PDXs) has already led to several valuable preclinical discoveries. For instance, in 2019 the first larval zf-PDX co-clinical trial was initiated and olaparib plus temozolomide treatment was tested in an adult zf-PDXs xenograft model of rhabdomyosarcoma, a therapy that was later transferred to a clinical trial without additional prerequisite models [208]. A present ongoing phase II clinical trial of leflunomide combined with vemurafenib is the first to arise from an initial screen in zebrafish with cutaneous melanoma [209]. Many small molecules, that were observed to have tumor-killing activity in zebrafish, have been made into clinical trials [210]. In all, zf-PDXs are promising pre-clinical models in personalized medicine, for which they may be used to predict patient-specific drug responses and guide patient therapies.

7. Aim and outline of this thesis

The aim of this thesis was to develop novel treatment strategies for different types of eye melanoma utilizing zebrafish models. We first establish orthotopic and ectopic xenograft models for uveal and conjunctival melanoma by engraftment of the

immortalized cells derived from these tumors into zebrafish embryos. Next, we expanded these models with spheroids and zebrafish patient-derived xenografts for pre-clinical, personalized screening of anti-UM drug responses. We demonstrated that these models can be harnessed to explore the *in vivo* interactions of the tumor cells with blood vessels and macrophages leading to angiogenic response. We finally apply the CoM model to clarify the inhibitory effects of ginsenosides and correlate their structure with potential antitumoral mechanisms.

Chapter 1 presents an overview of the current understanding of ocular melanoma biology and the background of primary drug candidates. We outline ongoing research on UM and CoM, spanning genetic profiles, cancer development, and potential therapies. Additionally, we present an introduction and summary of ginsenosides' anti-melanoma research status, one of the most promising drug candidates against this disease.

Chapter 2 describes the generation and quantification of orthotopic and ectopic ocular melanoma xenografts (CoM) by engraftment of fluorescently labeled stable cell lines. We visualize and quantify post-engraftment cell migration and proliferation, facilitating imaging via epifluorescence and confocal microscopy. These models allow the use of chemical or genetic inhibition strategies within only 8 days. This platform enables effective screening of stable cell lines and supports precision medicine approaches for patients in the future.

Chapter 3 shows the pro-angiogenetic role of macrophages that have been recruited towards engrafted CoM cells. We reveal that CoM cells secrete lactate, which induces a pro-tumoral macrophage polarization supporting angiogenetic response towards engrafted CoM cells. Chemical inhibition of lactate secretion or ablation of macrophages attenuates angiogenesis. From this, we conclude that highly glycolytic CoM tumors likely progress through TAM-mediated angiogenesis.

In **Chapter 4**, we establish a platform to isolate, preserve, and transiently recover viable tissues by the generation of spheroid cultures derived from primary UM. All assessed tumor-derived samples formed spheroids in culture and stained positively for melanocyte-specific markers. These spheroids are labeled with fluorescence and xenografted into zebrafish through intravenous injection, a model that mimics the molecular features of disseminating UM. Drug treatment with navitoclax and everolimus validated the zebrafish patient-derived model as a versatile pre-clinical tool for screening anti-UM drugs and as a pre-clinical platform to foretell personalized drug responses.

In **Chapter 5**, we study the antitumoral effects of ginsenosides Rg3, CK, and PPD in CoM, noting the inconsistency of effects between *in vivo* and *in vitro*. Glycosylated ginsenosides exhibit antitumoral effects in zebrafish but not *in vitro*. We find that induction of GBA2 expression and activity due to xenograft-induced inflammation plays a key role in ginsenoside degradation, leading to the antitumoral function of these molecules *in vivo*. We further demonstrate that the ginsenoside PPD induces CoM apoptosis independently of the glucocorticoid receptor (GR).

Finally, **Chapter 6** summarizes the research findings presented in this thesis, discussing them within the current scientific context.

References

1. International, W.C.R.F. Skin cancer statistics. **2020**.
2. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca-Cancer J Clin* **2021**, *71*, 209-249.
3. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics, 2023. *CA: A Cancer Journal for Clinicians* **2023**, *73*, 17-48.
4. Bolick, N.L.; Geller, A.C. Epidemiology of Melanoma. *Hematol Oncol Clin N* **2021**, *35*, 57-72.
5. Krantz, B.A.; Dave, N.; Komatsubara, K.M.; Marr, B.P.; Carvajal, R.D. Uveal melanoma: epidemiology, etiology, and treatment of primary disease. *Clin Ophthalmol* **2017**, *11*, 279-289.
6. Kuk, D.; Shoushtari, A.N.; Barker, C.A.; Panageas, K.S.; Munhoz, R.R.; Momtaz, P.; Ariyan, C.E.; Brady, M.S.; Coit, D.G.; Bogatch, K.; et al. Prognosis of Mucosal, Uveal, Acral, Nonacral Cutaneous, and Unknown Primary Melanoma From the Time of First Metastasis. *Oncologist* **2016**, *21*, 848-854.
7. Laver, N.V.; McLaughlin, M.E.; Duker, J.S. Ocular Melanoma. *Arch Pathol Lab Med* **2010**, *134*, 1778-1784.
8. Rantala, E.S.; Hernberg, M.M.; Piperno-Neumann, S.; Grossniklaus, H.E.; Kivela, T.T. Metastatic uveal melanoma: The final frontier. *Prog Retin Eye Res* **2022**, *90,* 101041.
9. Nikol'skaia, I.; Aleksandrova, S.; Lopatina, N.; Debov, S. Fractionation and purification of DNA methylases and specific endonucleases from cells of Escherichia coli SK. *Biokhimiia (Moscow, Russia)* **1977**, *42*, 598-608.
10. Brouwer, N.J.; Marinkovic, M.; van Duinen, S.G.; Bleeker, J.C.; Jager, M.J.; Luyten, G.P.M. Treatment of conjunctival melanoma in a Dutch referral centre. *Br J Ophthalmol* **2018**, *102*, 1277-1282.
11. Jovanovic, P.; Mihajlovic, M.; Djordjevic-Jocic, J.; Vlajkovic, S.; Cekic, S.; Stefanovic, V. Ocular melanoma: an overview of the current status. *Int J Clin Exp Patho* **2013**, *6*, 1230-1244.

12. Carr, S.; Smith, C.; Wernberg, J. Epidemiology and Risk Factors of Melanoma. *Surg Clin N Am* **2020**, *100*, 1-12.
13. Trucco, L.D.; Mundra, P.A.; Hogan, K.; Garcia-Martinez, P.; Viros, A.; Mandal, A.K.; Macagno, N.; Gaudy-Marqueste, C.; Allan, D.; Baenke, F. Ultraviolet radiation–induced DNA damage is prognostic for outcome in melanoma. *Nature medicine* **2019**, *25*, 221-224.
14. Davis, E.J.; Johnson, D.B.; Sosman, J.A.; Chandra, S. Melanoma: What do all the mutations mean? *Cancer* **2018**, *124*, 3490-3499.
15. Miyamoto, C.; Balazsi, M.; Bakalian, S.; Fernandes, B.F.; Burnier, M.N. Uveal melanoma: Ocular and systemic disease. *Saudi J Ophthalmol* **2012**, *26*, 145-149.
16. Woodman, S.E. Metastatic Uveal Melanoma Biology and Emerging Treatments. *Cancer J* **2012**, *18*, 148-152.
17. Dogrusoz, M.; Jager, M.J. Genetic prognostication in uveal melanoma. *Acta Ophthalmologica* **2018**, *96*, 331-347.
18. Rola, A.C.; Taktak, A.; Eleuteri, A.; Kalirai, H.; Heimann, H.; Hussain, R.; Bonnett, L.J.; Hill, C.J.; Traynor, M.; Jager, M.J.; et al. Multicenter External Validation of the Liverpool Uveal Melanoma Prognosticator Online: An OOG Collaborative Study. *Cancers* **2020**, *12,* 477.
19. Branisteanu, D.C.; Bogdanici, C.M.; Branisteanu, D.E.; Maranduca, M.A.; Zemba, M.; Balta, F.; Branisteanu, C.I.; Moraru, A.D. Uveal melanoma diagnosis and current treatment options (Review). *Exp Ther Med* **2021**, *22,* 1-8.
20. Robertson, A.G.; Shih, J.L.; Yau, C.; Gibb, E.A.; Oba, J.; Mungall, K.L.; Hess, J.M.; Uzunangelov, V.; Walter, V.; Danilova, L.; et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma (vol 32, pg 204, 2017). *Cancer Cell* **2018**, *33*, 151-151.
21. Coupland, S.E.; Lake, S.L.; Zeschnigk, M.; Damato, B.E. Molecular pathology of uveal melanoma. *Eye* **2013**, *27*, 230-242.
22. Bakhoum, M.E.; Esmaeli, B. Molecular Characteristics of Uveal Melanoma: Insights from the Cancer Genome Atlas (TCGA) Project. *Cancers* **2019**, *11* 1061.
23. Narasimhaiah, D.; Legrand, C.; Damotte, D.; Remark, R.; Munda, M.; De Potter, P.; Coulie, P.G.; Vikkula, M.; Godfraind, C. DNA alteration-based classification of uveal melanoma gives better prognostic stratification than immune infiltration, which has a neutral effect in high-risk group. *Cancer Med-Us* **2019**, *8*, 3036-3046.
24. Gezgin, G.; Dogrusoz, M.; van Essen, T.H.; Kroes, W.G.M.; Luyten, G.P.M.; van der Velden, P.A.; Walter, V.; Verdijk, R.M.; van Hall, T.; van der Burg, S.H.; et al. Genetic evolution of uveal melanoma guides the development of an inflammatory microenvironment. *Cancer Immunol Immun* **2017**, *66*, 903-912.
25. Kaler, C.J.; Dollar, J.J.; Cruz, A.M.; Kuznetsoff, J.N.; Sanchez, M.I.; Decatur, C.L.; Licht, J.D.; Smalley, K.S.M.; Correa, Z.M.; Kurtenbach, S.; et al. BAP1 Loss Promotes Suppressive Tumor Immune Microenvironment via

Upregulation of PROS1 in Class 2 Uveal Melanomas. *Cancers* **2022**, *14,* 3678.
26. van de Nes, J.A.P.; Koelsche, C.; Gessi, M.; Moller, I.; Sucker, A.; Scolyer, R.A.; Buckland, M.E.; Pietsch, T.; Murali, R.; Schadendorf, D.; et al. Activating CYSLTR2 and PLCB4 Mutations in Primary Leptomeningeal Melanocytic Tumors. *J Invest Dermatol* **2017**, *137*, 2033-2035.
27. Johansson, P.; Aoude, L.G.; Wadt, K.; Glasson, W.J.; Warrier, S.K.; Hewitt, A.W.; Kiilgaard, J.F.; Heegaard, S.; Isaacs, T.; Franchina, M.; et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. *Oncotarget* **2016**, *7*, 4624-4631.
28. van Poppelen, N.M.; van Ipenburg, J.A.; van den Bosch, Q.; Vaarwater, J.; Brands, T.; Eussen, B.; Magielsen, F.; Dubbink, H.J.; Paridaens, D.; Brosens, E.; et al. Molecular Genetics of Conjunctival Melanoma and Prognostic Value of TERT Promoter Mutation Analysis. *Int J Mol Sci* **2021**, *22*.
29. Martin, M.; Masshofer, L.; Temming, P.; Rahmann, S.; Metz, C.; Bornfeld, N.; van de Nes, J.; Klein-Hitpass, L.; Hinnebusch, A.G.; Horsthemke, B.; et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet* **2013**, *45*, 933-U296.
30. Furney, S.J.; Pedersen, M.; Gentien, D.; Dumont, A.G.; Rapinat, A.; Desjardins, L.; Turajlic, S.; Piperno-Neumann, S.; de la Grange, P.; Roman-Roman, S.; et al. SF3B1 mutations are associated with alternative splicing in uveal melanoma. *Cancer Discov* **2013**, *3*, 1122-1129.
31. Prescher, G.; Bornfeld, N.; Hirche, H.; Horsthemke, B.; Jockel, K.H.; Becher, R. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet* **1996**, *347*, 1222-1225.
32. Thomas, S.; Putter, C.; Weber, S.; Bornfeld, N.; Lohmann, D.R.; Zeschnigk, M. Prognostic significance of chromosome 3 alterations determined by microsatellite analysis in uveal melanoma: a long-term follow-up study. *Brit J Cancer* **2012**, *106*, 1171-1176.
33. Brouwer, N.J.; Gezgin, G.; Wierenga, A.P.A.; Bronkhorst, I.H.G.; Marinkovic, M.; Luyten, G.P.M.; Versluis, M.; Kroes, W.G.M.; van der Velden, P.A.; Verdijk, R.M.; et al. Tumour Angiogenesis in Uveal Melanoma Is Related to Genetic Evolution. *Cancers* **2019**, *11,* 979.
34. Singh, A.D.; Tubbs, R.; Biscotti, C.; Schoenfield, L.; Trizzoi, P. Chromosomal 3 and 8 Status Within Hepatic Metastasis of Uveal Melanoma. *Arch Pathol Lab Med* **2009**, *133*, 1223-1227.
35. Klatte, T.; Kroeger, N.; Rampersaud, E.N.; Birkhauser, F.D.; Logan, J.E.; Sonn, G.; Riss, J.; Rao, P.N.; Kabbinavar, F.F.; Belldegrun, A.S.; et al. Gain of chromosome 8q is associated with metastases and poor survival of patients with clear cell renal cell carcinoma. *Cancer* **2012**, *118*, 5777-5782.
36. Royds, J.A.; Sharrard, R.M.; Parsons, M.A.; Lawry, J.; Rees, R.; Cottam, D.; Wagner, B.; Rennie, I.G. C-Myc Oncogene Expression in Ocular Melanomas. *Graef Arch Clin Exp* **1992**, *230*, 366-371.
37. Drabarek, W.; Yavuzyigitoglu, S.; Obulkasim, A.; van Riet, J.; Smit, K.N.; van Poppelen, N.M.; Vaarwater, J.; Brands, T.; Eussen, B.; Verdijk, R.M. Multi-modality analysis improves survival prediction in enucleated uveal

melanoma patients. *Investigative ophthalmology & visual science* **2019**, *60*, 3595-3605.
38. Boru, G.; Cebulla, C.M.; Sample, K.M.; Massengill, J.B.; Davidorf, F.H.; Abdel-Rahman, M.H. Heterogeneity in Mitogen-Activated Protein Kinase (MAPK) Pathway Activation in Uveal Melanoma With Somatic GNAQ and GNA11 Mutations. *Invest Ophthalmol Vis Sci* **2019**, *60*, 2474-2480.
39. Harbour, J.W. The genetics of uveal melanoma: an emerging framework for targeted therapy. *Pigment Cell Melanoma Res* **2012**, *25*, 171-181.
40. Rapisuwon, S.; Qin, Y.; Roszik, J.; Carapeto, F.; Patel, S.; Carvajal, R.D. Systemic therapy for mucosal, acral, and uveal melanoma. *Cutaneous Melanoma* **2020**, 1301-1335.
41. Fukuda, T.; Tsuruga, T.; Kuroda, T.; Nishikawa, H.; Ohta, T. Functional link between BRCA1 and BAP1 through histone H2A, heterochromatin and DNA damage response. *Current cancer drug targets* **2016**, *16*, 101-109.
42. Gezgin, G.; Dogrusoz, M.; van Essen, T.H.; Kroes, W.G.M.; Luyten, G.P.M.; van der Velden, P.A.; Walter, V.; Verdijk, R.M.; van Hall, T.; van der Burg, S.H.; et al. Genetic evolution of uveal melanoma guides the development of an inflammatory microenvironment. *Cancer Immunol Immunother* **2017**, *66*, 903-912.
43. Harbour, J.W.; Onken, M.D.; Roberson, E.D.; Duan, S.; Cao, L.; Worley, L.A.; Council, M.L.; Matatall, K.A.; Helms, C.; Bowcock, A.M. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* **2010**, *330*, 1410-1413.
44. Koopmans, A.E.; Verdijk, R.M.; Brouwer, R.W.; van den Bosch, T.P.; van den Berg, M.M.; Vaarwater, J.; Kockx, C.E.; Paridaens, D.; Naus, N.C.; Nellist, M.; et al. Clinical significance of immunohistochemistry for detection of BAP1 mutations in uveal melanoma. *Mod Pathol* **2014**, *27*, 1321-1330.
45. Ewens, K.G.; Kanetsky, P.A.; Richards-Yutz, J.; Purrazzella, J.; Shields, C.L.; Ganguly, T.; Ganguly, A. Chromosome 3 status combined with BAP1 and EIF1AX mutation profiles are associated with metastasis in uveal melanoma. *Invest Ophthalmol Vis Sci* **2014**, *55*, 5160-5167.
46. Field, M.G.; Decatur, C.L.; Kurtenbach, S.; Gezgin, G.; Van Der Velden, P.A.; Jager, M.J.; Kozak, K.N.; Harbour, J.W. PRAME as an independent biomarker for metastasis in uveal melanoma. *Clinical cancer research* **2016**, *22*, 1234-1242.
47. Field, M.G.; Durante, M.A.; Decatur, C.L.; Tarlan, B.; Oelschlager, K.M.; Stone, J.F.; Kuznetsov, J.; Bowcock, A.M.; Kurtenbach, S.; Harbour, J.W. Epigenetic reprogramming and aberrant expression of PRAME are associated with increased metastatic risk in Class 1 and Class 2 uveal melanomas. *Oncotarget* **2016**, *7*, 59209-59219.
48. Helgadottir, H.; Hoiom, V. The genetics of uveal melanoma: current insights. *Appl Clin Genet* **2016**, *9*, 147-155.
49. Yavuzyigitoglu, S.; Koopmans, A.E.; Verdijk, R.M.; Vaarwater, J.; Eussen, B.; van Bodegom, A.; Paridaens, D.; Kilic, E.; de Klein, A.; Rotterdam Ocular Melanoma Study, G. Uveal Melanomas with SF3B1 Mutations: A Distinct

Subclass Associated with Late-Onset Metastases. *Ophthalmology* **2016**, *123*, 1118-1128.
50. Rossi, E.; Schinzari, G.; Maiorano, B.A.; Pagliara, M.M.; Di Stefani, A.; Bria, E.; Peris, K.; Blasi, M.A.; Tortora, G. Conjunctival Melanoma: Genetic and Epigenetic Insights of a Distinct Type of Melanoma. *Int J Mol Sci* **2019**, *20,* 5447.
51. Tuomaala, S.; Eskelin, S.; Tarkkanen, A.; Kivela, T. Population-based assessment of clinical characteristics predicting outcome of conjunctival melanoma in whites. *Invest Ophthalmol Vis Sci* **2002**, *43*, 3399-3408.
52. Missotten, G.S.; Gambrelle, J.; de Wolff-Rouendaal, D.; de Keizer, R. Epistaxis or epiphora as a sign for extension of a conjunctival melanoma. A series of six patients with nasolacrimal recurrence. *British journal of ophthalmology* **2010**, *94*, 1328-1331.
53. Missotten, G.S.; Keijser, S.; De Keizer, R.J.; De Wolff-Rouendaal, D. Conjunctival melanoma in the Netherlands: a nationwide study. *Invest Ophthalmol Vis Sci* **2005**, *46*, 75-82.
54. Blum, E.S.; Yang, J.; Komatsubara, K.M.; Carvajal, R.D. Clinical Management of Uveal and Conjunctival Melanoma. *Oncology (Williston Park)* **2016**, *30*, 29-32, 34-43, 48.
55. Jain, P.; Finger, P.T.; Fili, M.; Damato, B.; Coupland, S.E.; Heimann, H.; Kenawy, N.; N, J.B.; Marinkovic, M.; Van Duinen, S.G.; et al. Conjunctival melanoma treatment outcomes in 288 patients: a multicentre international data-sharing study. *Br J Ophthalmol* **2021**, *105*, 1358-1364.
56. Seregard, S. Conjunctival melanoma. *Survey of ophthalmology* **1998**, *42*, 321-350.
57. van Poppelen, N.M.; de Bruyn, D.P.; Bicer, T.; Verdijk, R.; Naus, N.; Mensink, H.; Paridaens, D.; de Klein, A.; Brosens, E.; Kiliç, E. Genetics of ocular melanoma: Insights into genetics, inheritance and testing. *Int J Mol Sci* **2020**, *22*, 336.
58. Lake, S.L.; Jmor, F.; Dopierala, J.; Taktak, A.F.; Coupland, S.E.; Damato, B.E. Multiplex ligation-dependent probe amplification of conjunctival melanoma reveals common BRAF V600E gene mutation and gene copy number changes. *Invest Ophthalmol Vis Sci* **2011**, *52*, 5598-5604.
59. Koopmans, A.E.; Ober, K.; Dubbink, H.J.; Paridaens, D.; Naus, N.C.; Belunek, S.; Krist, B.; Post, E.; Zwarthoff, E.C.; de Klein, A.; et al. Prevalence and implications of TERT promoter mutation in uveal and conjunctival melanoma and in benign and premalignant conjunctival melanocytic lesions. *Invest Ophthalmol Vis Sci* **2014**, *55*, 6024-6030.
60. Qin, Y.; Guo, H.; Tang, B.; Yang, S.M. The non-reverse transcriptase activity of the human telomerase reverse transcriptase promotes tumor progression (review). *Int J Oncol* **2014**, *45*, 525-531.
61. Humer, J.; Ferko, B.; Waltenberger, A.; Rapberger, R.; Pehamberger, H.; Muster, T. Azidothymidine inhibits melanoma cell growth in vitro and in vivo. *Melanoma Research* **2008**, *18*, 314-321.
62. Röth, A.; Harley, C.B.; Baerlocher, G.M. Imetelstat (GRN163L)-telomerase-based cancer therapy. *Small Molecules in Oncology* **2009**, 221-234.

63. Schopf, F.H.; Biebl, M.M.; Buchner, J. The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol* **2017**, *18*, 345-360.
64. Kumari, A.; Bhawal, S.; Kapila, S.; Yadav, H.; Kapila, R. Health-promoting role of dietary bioactive compounds through epigenetic modulations: a novel prophylactic and therapeutic approach. *Critical Reviews in Food Science and Nutrition* **2022**, *62*, 619-639.
65. Christmann, M.; Verbeek, B.; Roos, W.P.; Kaina, B. O6-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* **2011**, *1816*, 179-190.
66. Griewank, K.G.; Westekemper, H.; Murali, R.; Mach, M.; Schilling, B.; Wiesner, T.; Schimming, T.; Livingstone, E.; Sucker, A.; Grabellus, F.; et al. Conjunctival melanomas harbor BRAF and NRAS mutations and copy number changes similar to cutaneous and mucosal melanomas. *Clin Cancer Res* **2013**, *19*, 3143-3152.
67. Tsao, H.; Mihm Jr, M.C.; Sheehan, C. PTEN expression in normal skin, acquired melanocytic nevi, and cutaneous melanoma. *Journal of the American Academy of Dermatology* **2003**, *49*, 865-872.
68. Larsen, A.C.; Dahl, C.; Dahmcke, C.M.; Lade-Keller, J.; Siersma, V.D.; Toft, P.B.; Coupland, S.E.; Prause, J.U.; Guldberg, P.; Heegaard, S. BRAF mutations in conjunctival melanoma: investigation of incidence, clinicopathological features, prognosis and paired premalignant lesions. *Acta Ophthalmol* **2016**, *94*, 463-470.
69. Larsen, A.C.; Dahmcke, C.M.; Dahl, C.; Siersma, V.D.; Toft, P.B.; Coupland, S.E.; Prause, J.U.; Guldberg, P.; Heegaard, S. A Retrospective Review of Conjunctival Melanoma Presentation, Treatment, and Outcome and an Investigation of Features Associated With BRAF Mutations. *JAMA Ophthalmol* **2015**, *133*, 1295-1303.
70. van den Hurk, K.; Niessen, H.E.; Veeck, J.; van den Oord, J.J.; van Steensel, M.A.; Zur Hausen, A.; van Engeland, M.; Winnepenninckx, V.J. Genetics and epigenetics of cutaneous malignant melanoma: a concert out of tune. *Biochim Biophys Acta* **2012**, *1826*, 89-102.
71. Zoroquiain, P.; Fernandes, B.F.; Gonzalez, S.; Novais, G.N.; Schalper, K.A.; Burnier, M.N., Jr. p16ink4a expression in benign and malignant melanocytic conjunctival lesions. *Int J Surg Pathol* **2012**, *20*, 240-245.
72. Jakob, J.A.; Bassett Jr, R.L.; Ng, C.S.; Curry, J.L.; Joseph, R.W.; Alvarado, G.C.; Rohlfs, M.L.; Richard, J.; Gershenwald, J.E.; Kim, K.B. NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer* **2012**, *118*, 4014-4023.
73. Rajkumar, S.; Berry, D.; Heney, K.A.; Strong, C.; Ramsay, L.; Lajoie, M.; Alkallas, R.; Nguyen, T.T.; Thomson, C.; Ahanfeshar-Adams, M.; et al. Melanomas with concurrent BRAF non-p.V600 and NF1 loss-of-function mutations are targetable by BRAF/MEK inhibitor combination therapy. *Cell Rep* **2022**, *39*, 110634.

74. Scholz, S.L.; Cosgarea, I.; Susskind, D.; Murali, R.; Moller, I.; Reis, H.; Leonardelli, S.; Schilling, B.; Schimming, T.; Hadaschik, E.; et al. NF1 mutations in conjunctival melanoma. *Br J Cancer* **2018**, *118*, 1243-1247.
75. Wiesner, T.; Kiuru, M.; Scott, S.N.; Arcila, M.; Halpern, A.C.; Hollmann, T.; Berger, M.F.; Busam, K.J. NF1 Mutations Are Common in Desmoplastic Melanoma. *Am J Surg Pathol* **2015**, *39*, 1357-1362.
76. Kaštelan, S.; Gverović Antunica, A.; Beketić Orešković, L.; Salopek Rabatić, J.; Kasun, B.; Bakija, I. Conjunctival melanoma-epidemiological trends and features. *Pathology & Oncology Research* **2018**, *24*, 787-796.
77. Gong, H.Z.; Zheng, H.Y.; Li, J. The clinical significance of KIT mutations in melanoma: a meta-analysis. *Melanoma Res* **2018**, *28*, 259-270.
78. Carvajal, R.D.; Antonescu, C.R.; Wolchok, J.D.; Chapman, P.B.; Roman, R.-A.; Teitcher, J.; Panageas, K.S.; Busam, K.J.; Chmielowski, B.; Lutzky, J. KIT as a therapeutic target in metastatic melanoma. *Jama* **2011**, *305*, 2327-2334.
79. Populoa, H.; Soares, P.; Rocha, A.S.; Silva, P.; Lopes, J.M. Evaluation of the mTOR pathway in ocular (uvea and conjunctiva) melanoma. *Melanoma Research* **2010**, *20*, 107-117.
80. Hopkins, B.D.; Hodakoski, C.; Barrows, D.; Mense, S.M.; Parsons, R.E. PTEN function: the long and the short of it. *Trends Biochem Sci* **2014**, *39*, 183-190.
81. Force, A.O.O.T. International Validation of the American Joint Committee on Cancer's 7th Edition Classification of Uveal Melanoma. *JAMA Ophthalmol* **2015**, *133*, 376-383.
82. Damato, B.; Coupland, S.E. Clinical mapping of conjunctival melanomas. *Br J Ophthalmol* **2008**, *92*, 1545-1549.
83. Lane, A.M.; Egan, K.M.; Yang, J.; Saornil, M.A.; Alroy, J.; Albert, D.; Gragoudas, E.S. An evaluation of tumour vascularity as a prognostic indicator in uveal melanoma. *Melanoma Research* **1997**, *7*, 237-242.
84. de Bruyn, D.P.; Bongaerts, M.; Bonte, R.; Vaarwater, J.; Meester-Smoor, M.A.; Verdijk, R.M.; Paridaens, D.; Naus, N.C.; de Klein, A.; Ruijter, G.J.G.; et al. Uveal Melanoma Patients Have a Distinct Metabolic Phenotype in Peripheral Blood. *Int J Mol Sci* **2023**, *24,* 5077.
85. Niederkorn, J.Y. Ocular immune privilege and ocular melanoma: parallel universes or immunological plagiarism? *Front Immunol* **2012**, *3*, 148.
86. Hashemi, G.; Dight, J.; Khosrotehrani, K.; Sormani, L. Melanoma Tumour Vascularization and Tissue-Resident Endothelial Progenitor Cells. *Cancers (Basel)* **2022**, *14,* 4216.
87. Patel, J.K.; Didolkar, M.S.; Pickren, J.W.; Moore, R.H. Metastatic pattern of malignant melanoma. A study of 216 autopsy cases. *Am J Surg* **1978**, *135*, 807-810.
88. Braeuer, R.R.; Watson, I.R.; Wu, C.J.; Mobley, A.K.; Kamiya, T.; Shoshan, E.; Bar-Eli, M. Why is melanoma so metastatic? *Pigment cell & melanoma research* **2014**, *27*, 19-36.

89. Ruocco, M.R.; Avagliano, A.; Granato, G.; Vigliar, E.; Masone, S.; Montagnani, S.; Arcucci, A. Metabolic flexibility in melanoma: A potential therapeutic target. *Semin Cancer Biol* **2019**, *59*, 187-207.
90. Viallard, C.; Larrivee, B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. *Angiogenesis* **2017**, *20*, 409-426.
91. Cho, W.C.; Jour, G.; Aung, P.P. Role of angiogenesis in melanoma progression: Update on key angiogenic mechanisms and other associated components. *Semin Cancer Biol* **2019**, *59*, 175-186.
92. Atzori, M.G.; Ceci, C.; Ruffini, F.; Trapani, M.; Barbaccia, M.L.; Tentori, L.; D'Atri, S.; Lacal, P.M.; Graziani, G. Role of VEGFR-1 in melanoma acquired resistance to the BRAF inhibitor vemurafenib. *J Cell Mol Med* **2020**, *24*, 465-475.
93. Rosenblatt, M.I.; Azar, D.T. Anti-angiogenic therapy: prospects for treatment of ocular tumors. In Proceedings of the Seminars in ophthalmology **2006**, *3,* 151-160.
94. Mahabeleshwar, G.H.; Byzova, T.V. Angiogenesis in melanoma. *Seminars in Oncology* **2007**, *34*, 555-565.
95. Ferrara, N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* **2004**, *25*, 581-611.
96. Berkenblit, A.; Matulonis, U.A.; Kroener, J.F.; Dezube, B.J.; Lam, G.N.; Cuasay, L.C.; Brunner, N.; Jones, T.R.; Silverman, M.H.; Gold, M.A. A6, a urokinase plasminogen activator (uPA)-derived peptide in patients with advanced gynecologic cancer: a phase I trial. *Gynecol Oncol* **2005**, *99*, 50-57.
97. Hearing, V.J.; Law, L.W.; Corti, A.; Appella, E.; Blasi, F. Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. *Cancer Research* **1988**, *48*, 1270-1278.
98. el Filali, M.; Ly, L.V.; Luyten, G.P.M.; Versluis, M.; Grossniklaus, H.E.; van der Velden, P.A.; Jager, M.J. Bevacizumab and intraocular tumors: an intriguing paradox. *Mol Vis* **2012**, *18*, 2454-2467.
99. Missotten, G.S.O.; Notting, I.C.; Schlingemann, R.O.; Zijlmans, H.J.; Lau, C.; Eilers, P.H.C.; Keunen, J.E.E.; Jager, M.J. Vascular endothelial growth factor A in eyes with uveal melanoma. *Arch Ophthalmol-Chic* **2006**, *124*, 1428-1434.
100. Zhang, X.P.; Li, N.L.; Guo, C.; Li, Y.D.; Luo, L.L.; Liu, Y.Q.; Duan, Y.Y.; Li, Z.D.; Xie, X.R.; Song, H.X.; et al. A vaccine targeting basic fibroblast growth factor elicits a protective immune response against murine melanoma. *Cancer Biol Ther* **2018**, *19*, 518-524.
101. Moccia, F.; Negri, S.; Shekha, M.; Faris, P.; Guerra, G. Endothelial Ca2+ Signaling, Angiogenesis and Vasculogenesis: Just What It Takes to Make a Blood Vessel. *Int J Mol Sci* **2019**, *20*.
102. Taverna, S.; Ghersi, G.; Ginestra, A.; Rigogliuso, S.; Pecorella, S.; Alaimo, G.; Saladino, F.; Dolo, V.; Dell'Era, P.; Pavan, A.; et al. Shedding of membrane vesicles mediates fibroblast growth factor-2 release from cells. *Journal of Biological Chemistry* **2003**, *278*, 51911-51919.
103. Plum, S.M.; Holaday, J.W.; Ruiz, A.; Madsen, J.W.; Fogler, W.E.; Fortier, A.H. Administration of a liposomal FGF-2 peptide vaccine leads to

abrogation of FGF-2-mediated angiogenesis and tumor development. *Vaccine* **2000**, *19*, 1294-1303.
104. Lu, J.R.; Tan, M.; Cai, Q.S. The Warburg effect in tumor progression: Mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett* **2015**, *356*, 156-164.
105. Feron, O. Pyruvate into lactate and back: From the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol* **2009**, *92*, 329-333.
106. Epstein, T.; Gatenby, R.A.; Brown, J.S. The Warburg effect as an adaptation of cancer cells to rapid fluctuations in energy demand. *Plos One* **2017**, *12,* e0185085.
107. Zhang, D.S.; Li, J.; Wang, F.Z.; Hu, J.; Wang, S.W.; Sun, Y.M. 2-Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy. *Cancer Lett* **2014**, *355*, 176-183.
108. Crowther, M.; Brown, N.J.; Bishop, E.T.; Lewis, C.E. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. *J Leukocyte Biol* **2001**, *70*, 478-490.
109. Nakajima, E.C.; Van Houten, B. Metabolic symbiosis in cancer: Refocusing the Warburg lens. *Mol Carcinogen* **2013**, *52*, 329-337.
110. Liberti, M.V.; Locasale, J.W. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci* **2016**, *41*, 211-218.
111. Pfeiffer, T.; Schuster, S.; Bonhoeffer, S. Cooperation and competition in the evolution of ATP-producing pathways. *Science* **2001**, *292*, 504-507.
112. Epstein, Y.; Roberts, W.O. The pathopysiology of heat stroke: an integrative view of the final common pathway. *Scand J Med Sci Spor* **2011**, *21*, 742-748.
113. Tariq, M.; Zhang, J.Q.; Liang, G.K.; Ding, L.; He, Q.J.; Yang, B. Macrophage Polarization: Anti-Cancer Strategies to Target Tumor-Associated Macrophage in Breast Cancer. *J Cell Biochem* **2017**, *118*, 2484-2501.
114. Yu, J.; Chai, P.; Xie, M.; Ge, S.; Ruan, J.; Fan, X.; Jia, R. Histone lactylation drives oncogenesis by facilitating m6A reader protein YTHDF2 expression in ocular melanoma. *Genome biology* **2021**, *22*, 1-21.
115. Liu, Y.; Huo, Y.; Wang, D.J.; Tai, Y.H.; Li, J.; Pang, D.B.; Zhang, Y.; Zhao, W.; Du, N.; Huang, Y.F. MiR-216a-5p/Hexokinase 2 axis regulates uveal melanoma growth through modulation of Warburg effect. *Biochemical and Biophysical Research Communications* **2018**, *501*, 885-892.
116. Arneth, B. Tumor Microenvironment. *Medicina-Lithuania* **2020**, *56,* 15.
117. Lei, X.; Lei, Y.; Li, J.K.; Du, W.X.; Li, R.G.; Yang, J.; Li, J.; Li, F.; Tan, H.B. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. *Cancer Lett* **2020**, *470*, 126-133.
118. Zhang, S.-W.; Wang, H.; Ding, X.-H.; Xiao, Y.-L.; Shao, Z.-M.; You, C.; Gu, Y.-J.; Jiang, Y.-Z. Bidirectional crosstalk between therapeutic cancer vaccines and the tumor microenvironment: Beyond tumor antigens. *Fundamental Research* **2022**.
119. Rao, Q.; Chen, Y.; Yeh, C.R.; Ding, J.; Li, L.; Chang, C.S.; Yeh, S.Y. Recruited mast cells in the tumor microenvironment enhance bladder cancer

metastasis via modulation of ER beta/CCL2/CCR2 EMT/MMP9 signals. *Oncotarget* **2016**, *7*, 7842-7855.
120. Dander, E.; Fallati, A.; Gulic, T.; Pagni, F.; Gaspari, S.; Silvestri, D.; Cricri, G.; Bedini, G.; Portale, F.; Buracchi, C.; et al. Monocyte-macrophage polarization and recruitment pathways in the tumour microenvironment of B-cell acute lymphoblastic leukaemia. *Brit J Haematol* **2021**, *193*, 1157-1171.
121. Saeedifar, A.M.; Mosayebi, G.; Ghazavi, A.; Bushehri, R.H.; Ganji, A. Macrophage polarization by phytotherapy in the tumor microenvironment. *Phytother Res* **2021**, *35*, 3632-3648.
122. Buddingh, E.P.; Kuijjer, M.L.; Duim, R.A.J.; Burger, H.; Agelopoulos, K.; Myklebost, O.; Serra, M.; Mertens, F.; Hogendoorn, P.C.W.; Lankester, A.C.; et al. Tumor-Infiltrating Macrophages Are Associated with Metastasis Suppression in High-Grade Osteosarcoma: A Rationale for Treatment with Macrophage Activating Agents. *Clinical Cancer Research* **2011**, *17*, 2110-2119.
123. Luo, Y.P.; Zhou, H.; Krueger, J.; Kaplan, C.; Lee, S.H.; Dolman, C.; Markowitz, D.; Wu, W.Y.; Liu, C.; Reisfeld, R.A.; et al. Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J Clin Invest* **2006**, *116*, 2132-2141.
124. Zarif, J.C.; Taichman, R.S.; Pienta, K.J. TAM macrophages promote growth and metastasis within the cancer ecosystem. *Oncoimmunology* **2014**, *3,* e941374.
125. Moreno, B.H.; Ribas, A. Anti-programmed cell death protein-1/ligand-1 therapy in different cancers. *Brit J Cancer* **2015**, *112*, 1421-1427.
126. Gameiro, S.F.; Zhang, A.; Ghasemi, F.; Barrett, J.W.; Nichols, A.C.; Mymryk, J.S. Analysis of Class I Major Histocompatibility Complex Gene Transcription in Human Tumors Caused by Human Papillomavirus Infection. *Viruses-Basel* **2017**, *9,* 252.
127. Souri, Z.; Wierenga, A.P.A.; Mulder, A.; Jochemsen, A.G.; Jager, M.J. HLA Expression in Uveal Melanoma: An Indicator of Malignancy and a Modifiable Immunological Target. *Cancers* **2019**, *11,* 1132.
128. Jager, M.J.; Hurks, H.M.H.; Levitskaya, J.; Kiessling, R. HLA expression in uveal melanoma: There is no rule without some exception. *Hum Immunol* **2002**, *63*, 444-451.
129. Ma, D.; Luyten, G.P.; Luider, T.M.; Niederkorn, J.Y. Relationship between Natural-Killer-Cell Susceptibility and Metastasis of Human Uveal Melanoma-Cells in a Murine Model. *Investigative Ophthalmology & Visual Science* **1995**, *36*, 435-441.
130. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discovery* **2022**, *12*, 31-46.
131. Mishra, K.K.; Chiu-Tsao, S.T.; Orton, C.G. Particle therapy is ideal for the treatment of ocular melanomas. *Med Phys* **2016**, *43*, 631-634.
132. Carvajal, R.D.; Sacco, J.J.; Jager, M.J.; Eschelman, D.J.; Bagge, R.O.; Harbour, J.W.; Chieng, N.D.; Patel, S.P.; Joshua, A.M.; Piperno-Neumann, S. Advances in the clinical management of uveal melanoma. *Nat Rev Clin Oncol* **2023**, *20*, 99-115.

133. Wei, A.Z.; Maniar, A.B.; Carvajal, R.D. New targeted and epigenetic therapeutic strategies for the treatment of uveal melanoma. *Cancer Gene Ther* **2022**, *29*, 1819-1826.
134. Landreville, S.; Agapova, O.A.; Matatall, K.A.; Kneass, Z.T.; Onken, M.D.; Lee, R.S.; Bowcock, A.M.; Harbour, J.W. Histone Deacetylase Inhibitors Induce Growth Arrest and Differentiation in Uveal Melanoma. *Clinical Cancer Research* **2012**, *18*, 408-416.
135. Ackerman, A.; Klein, O.; McDermott, D.F.; Wang, W.; Ibrahim, N.; Lawrence, D.P.; Gunturi, A.; Flaherty, K.T.; Hodi, F.S.; Kefford, R.; et al. Outcomes of Patients With Metastatic Melanoma Treated With Immunotherapy Prior to or After BRAF Inhibitors. *Cancer* **2014**, *120*, 1695-1701.
136. Robinson, G.W.; Orr, B.A.; Gajjar, A. Complete clinical regression of a BRAF V600E-mutant pediatric glioblastoma multiforme after BRAF inhibitor therapy. *Bmc Cancer* **2014**, *14,* 1-5.
137. Seidel, J.A.; Otsuka, A.; Kabashima, K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, efficacy, and Limitations. *Front Oncol* **2018**, *8,* 86.
138. Alicea, G.M.; Rebecca, V.W. Emerging strategies to treat rare and intractable subtypes of melanoma. *Pigment Cell & Melanoma Research* **2021**, *34*, 44-58.
139. Neagu, M. Metabolic Traits in Cutaneous Melanoma. *Front Oncol* **2020**, *10,* 851.
140. Baghba, R.; Roshangar, L.; Jahanban-Esfahlan, R.; Seidi, K.; Ebrahimi-Kalan, A.; Jaymand, M.; Kolahian, S.; Javaheri, T.; Zare, P. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal* **2020**, *18,* 1-19.
141. Brouwer, N.J.; Verdijk, R.M.; Heegaard, S.; Marinkovic, M.; Esmaeli, B.; Jager, M.J. Conjunctival melanoma: New insights in tumour genetics and immunology, leading to new therapeutic options. *Prog Retin Eye Res* **2022**, *86,* 100971.
142. Esmaeli, B. Immune Checkpoint Inhibitor Therapy for Orbital and Ocular Adnexal Squamous Cell Carcinomas: International Society of Ocular Oncology President's Lecture, 2022. *Invest Ophthalmol Vis Sci* **2023**, *64*, 29.
143. Yue, P.Y.K.; Mak, N.K.; Cheng, Y.K.; Leung, K.W.; Ng, T.B.; Fan, D.T.P.; Yeung, H.W.; Wong, R.N.S. Pharmacogenomics and the Yin/Yang actions of ginseng: anti-tumor, angiomodulating and steroid-like activities of ginsenosides. *Chinese medicine* **2007**, *2*, 1-21.
144. Yun, T.K. Brief introduction of Panax ginseng CA Meyer. *J Korean Med Sci* **2001**, *16*, S3-S5.
145. El-Khayat, Z.; Hussein, J.; Ramzy, T.; Ashour, M. Antidiabetic antioxidant effect of Panax ginseng. *J Med Plants Res* **2011**, *5*, 4616-4620.
146. Sun, J.H.; Jiao, C.X.; Ma, Y.; Chen, J.G.; Wu, W.; Liu, S.Y. Anti-ageing effect of red ginseng revealed by urinary metabonomics using RRLC-Q-TOF-MS. *Phytochem Analysis* **2018**, *29*, 387-397.
147. Wang, G.-L.; He, Z.-M.; Zhu, H.-Y.; Gao, Y.-G.; Zhao, Y.; Yang, H.; Zhang, L.-X. Involvement of serotonergic, noradrenergic and dopaminergic systems

in the antidepressant-like effect of ginsenoside Rb1, a major active ingredient of Panax ginseng CA Meyer. *Journal of Ethnopharmacology* **2017**, *204*, 118-124.
148. Tao, R.Z.; Lu, K.Q.; Zong, G.F.; Xia, Y.W.; Han, H.K.; Zhao, Y.; Wei, Z.H.; Lu, Y. Ginseng polysaccharides: Potential antitumor agents. *J Ginseng Res* **2023**, *47*, 9-22.
149. Chen, X.J.; Zhang, X.J.; Shui, Y.M.; Wan, J.B.; Gao, J.L. Anticancer Activities of Protopanaxadiol- and Protopanaxatriol-Type Ginsenosides and Their Metabolites. *Evid-Based Compl Alt* **2016**, *2016*.
150. Nag, S.A.; Qin, J.J.; Wang, W.; Wang, M.H.; Wang, H.; Zhang, R.W. Ginsenosides as anticancer agents: in vitro and in vivo activities, structure-activity relationships, and molecular mechanisms of action. *Front Pharmacol* **2012**, *3,* 25.
151. Meng, L.B.; Ji, R.; Dong, X.M.; Xu, X.C.; Xin, Y.; Jiang, X. Antitumor activity of ginsenoside Rg3 in melanoma through downregulation of the ERK and Akt pathways. *International Journal of Oncology* **2019**, *54*, 2069-2079.
152. Shan, X.; Aziz, F.; Tian, L.L.; Wang, X.Q.; Yan, Q.; Liu, J.W. Ginsenoside Rg3-induced EGFR/MAPK pathway deactivation inhibits melanoma cell proliferation by decreasing FUT4/LeY expression. *International Journal of Oncology* **2015**, *46*, 1667-1676.
153. Shan, X.; Fu, Y.S.; Aziz, F.; Wang, X.Q.; Yan, Q.; Liu, J.W. Ginsenoside Rg3 Inhibits Melanoma Cell Proliferation through Down-Regulation of Histone Deacetylase 3 (HDAC3) and Increase of p53 Acetylation. *Plos One* **2014**, *9,* e115401.
154. Shan, X.; Tian, L.L.; Zhang, Y.M.; Wang, X.Q.; Yan, Q.; Liu, J.W. Ginsenoside Rg3 suppresses FUT4 expression through inhibiting NF-κB/p65 signaling pathway to promote melanoma cell death. *International journal of oncology* **2015**, *47*, 701-709.
155. Wang, M.; Yan, S.J.; Zhang, H.T.; Li, N.; Liu, T.; Zhang, Y.L.; Li, X.X.; Ma, Q.; Qiu, X.C.; Fan, Q.Y. Ginsenoside Rh2 enhances the antitumor immunological response of a melanoma mice model. *Oncology Letters* **2017**, *13*, 681-685.
156. Kang, S.; Kim, J.-E.; Song, N.R.; Jung, S.K.; Lee, M.H.; Park, J.S.; Yeom, M.-H.; Bode, A.M.; Dong, Z.; Lee, K.W. The ginsenoside 20-O-β-D-glucopyranosyl-20 (S)-protopanaxadiol induces autophagy and apoptosis in human melanoma via AMPK/JNK phosphorylation. *Plos One* **2014**, *9*, e104305.
157. Kim, J.S.; Joo, E.J.; Chun, J.; Ha, Y.W.; Lee, J.H.; Han, Y.; Kim, Y.S. Induction of apoptosis by ginsenoside Rk1 in SK-MEL-2-human melanoma. *Arch Pharm Res* **2012**, *35*, 717-722.
158. Fei, X.-F.; Wang, B.-X.; Tashiro, S.; Li, T.-J.; Ma, J.-S.; Ikejima, T. Apoptotic effects of ginsenoside Rh2 on human malignant melanoma A375-S2 cells. *Acta Pharmacologica Sinica* **2002**, *23*, 315-322.
159. Wakabayashi, C.; Murakami, K.; Hasegawa, H.; Murata, J.; Saiki, I. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the

ability to induce apoptosis in tumor cells. *Biochemical and Biophysical research communications* **1998**, *246*, 725-730.
160. Wang, W.; Rayburn, E.R.; Hang, J.; Zhao, Y.Q.; Wang, H.; Zhang, R.W. Anti-lung cancer effects of novel ginsenoside 25-OCH3-PPD. *Lung Cancer* **2009**, *65*, 306-311.
161. Kim, J.W.; Jung, S.Y.; Kwon, Y.H.; Lee, J.H.; Lee, Y.M.; Lee, B.Y.; Kwon, S.M. Ginsenoside Rg3 attenuates tumor angiogenesis via inhibiting bioactivities of endothelial progenitor cells. *Cancer Biol Ther* **2012**, *13*, 504-515.
162. Liu, J.; Chen, J.; Yu, L.; Tian, Y.; Cui, X.; Yan, Q.; Fu, L. Inhibitory effect of ginsenoside-Rg3 on lung metastasis of mouse melanoma transfected with ribonuclease inhibitor. *Zhonghua Zhong liu za zhi [Chinese Journal of Oncology]* **2004**, *26*, 722-725.
163. Lee, S.G.; Kang, Y.J.; Nam, J.O. Anti-Metastasis Effects of Ginsenoside Rg3 in B16F10 Cells. *J Microbiol Biotechn* **2015**, *25*, 1997-2006.
164. Sato, K.; Mochizuki, M.; Saiki, I.; Yoo, Y.C.; Samukawa, K.; Azuma, I. Inhibition of Tumor Angiogenesis and Metastasis by a Saponin of Panax-Ginseng, Ginsenoside-Rb2. *Biol Pharm Bull* **1994**, *17*, 635-639.
165. Hasegawa, H.; Suzuki, R.; Nagaoka, T.; Tezuka, Y.; Kadota, S.; Saiki, I. Prevention of growth and metastasis of murine melanoma through enhanced natural-killer cytotoxicity by fatty acid-conjugate of protopanaxatriol. *Biological and Pharmaceutical Bulletin* **2002**, *25*, 861-866.
166. Son, K.J.; Choi, K.R.; Lee, S.J.; Lee, H. Immunogenic Cell Death Induced by Ginsenoside Rg3: Significance in Dendritic Cell-based Anti-tumor Immunotherapy. *Immune Netw* **2016**, *16*, 75-84.
167. Kim, J.H.; Baek, E.J.; Lee, E.J.; Yeom, M.H.; Park, J.S.; Lee, K.W.; Kang, N.J. Ginsenoside F1 attenuates hyperpigmentation in B16F10 melanoma cells by inducing dendrite retraction and activating Rho signalling. *Experimental Dermatology* **2015**, *24*, 150-152.
168. Lv, D.L.; Chen, L.; Ding, W.; Zhang, W.; Wang, H.L.; Wang, S.; Liu, W.B. Ginsenoside G-Rh2 synergizes with SMI-4a in anti-melanoma activity through autophagic cell death. *Chinese Medicine* **2018**, *13,* 1-8.
169. Wang, Z.; Zheng, Q.; Liu, K.; Li, G.; Zheng, R. Ginsenoside Rh2 enhances antitumour activity and decreases genotoxic effect of cyclophosphamide. *Basic & clinical pharmacology & toxicology* **2006**, *98*, 411-415.
170. Wang, H.; Kong, L.; Zhang, J.; Yu, G.; Lv, G.; Zhang, F.; Chen, X.; Tian, J.; Fu, F. The pseudoginsenoside F11 ameliorates cisplatin-induced nephrotoxicity without compromising its anti-tumor activity in vivo. *Scientific reports* **2014**, *4*, 4986.
171. Razina, T.; Krylova, S.; Amosova, E.; Zueva, E.; Lopatina, K.; Popov, A.; Atopkina, L.; Kozlovskaia, E. Effect of ginsenoside Rh2 on the development of transferred tumors and chemotherapy efficiency. *Eksperimental'naia i Klinicheskaia Farmakologiia* **2010**, *73*, 27-30.
172. Wang, L.; Lu, A.-P.; Yu, Z.-L.; Wong, R.N.; Bian, Z.-X.; Kwok, H.-H.; Yue, P.Y.-K.; Zhou, L.-M.; Chen, H.; Xu, M. The melanogenesis-inhibitory effect

and the percutaneous formulation of ginsenoside Rb1. *AAPS PharmSciTech* **2014**, *15*, 1252-1262.
173. Jeong, Y.-M.; Oh, W.K.; Tran, T.L.; Kim, W.-K.; Sung, S.H.; Bae, K.; Lee, S.; Sung, J.-H. Aglycone of Rh4 inhibits melanin synthesis in B16 melanoma cells: possible involvement of the protein kinase A pathway. *Bioscience, biotechnology, and biochemistry* **2013**, *77*, 119-125.
174. Zhou, Q.L.; Yang, X.W. Four new ginsenosides from red ginseng with inhibitory activity on melanogenesis in melanoma cells. *Bioorg Med Chem Lett* **2015**, *25*, 3112-3116.
175. Lee, C.S.; Nam, G.B.; Park, J.S. Protopanaxatriol inhibits melanin synthesis through inactivation of the pCREB-MITF-tyrosinase signalling pathway in melanocytes. *Clin Exp Dermatol* **2019**, *44*, 295-299.
176. Lee, D.Y.; Kim, H.-G.; Lee, Y.-G.; Kim, J.H.; Lee, J.W.; Choi, B.-R.; Jang, I.-B.; Kim, G.-S.; Baek, N.-I. Isolation and quantification of ginsenoside Rh23, a new anti-melanogenic compound from the leaves of Panax ginseng. *Molecules* **2018**, *23*, 267.
177. Kim, Y.-J.; Choi, W.-I.; Jeon, B.-N.; Choi, K.-C.; Kim, K.; Kim, T.-J.; Ham, J.; Jang, H.J.; Kang, K.S.; Ko, H. Stereospecific effects of ginsenoside 20-Rg3 inhibits TGF-β1-induced epithelial–mesenchymal transition and suppresses lung cancer migration, invasion and anoikis resistance. *Toxicology* **2014**, *322*, 23-33.
178. Wang, Z.; Xie, C.; Huang, Y.; Lam, C.W.K.; Chow, M.S. Overcoming chemotherapy resistance with herbal medicines: past, present and future perspectives. *Phytochemistry Reviews* **2014**, *13*, 323-337.
179. Chu, L.L.; Hanh, N.T.Y.; Quyen, M.L.; Nguyen, Q.H.; Lien, T.T.P.; Do, K.V. Compound K Production: Achievements and Perspectives. *Life* **2023**, *13*, 1565.
180. Liu, J.; Wang, Y.; Yu, Z.; Lv, G.; Huang, X.; Lin, H.; Ma, C.; Lin, Z.; Qu, P. Functional mechanism of ginsenoside compound K on tumor growth and metastasis. *Integrative cancer therapies* **2022**, *21*, 15347354221101203.
181. Chen, X.-J.; Zhang, X.-J.; Shui, Y.-M.; Wan, J.-B.; Gao, J.-L. Anticancer activities of protopanaxadiol-and protopanaxatriol-type ginsenosides and their metabolites. *Evid-Based Compl Alt* **2016**, *2016*.
182. Taylor, P.; Noriega, R.; Farah, C.; Abad, M.-J.; Arsenak, M.; Apitz, R. Ajoene inhibits both primary tumor growth and metastasis of B16/BL6 melanoma cells in C57BL/6 mice. *Cancer Lett* **2006**, *239*, 298-304.
183. Shi, Z.-Y.; Zeng, J.-Z.; Wong, A.S.T. Chemical structures and pharmacological profiles of ginseng saponins. *Molecules* **2019**, *24*, 2443.
184. Chen, C.; Xia, J.; Ren, H.; Wang, A.; Zhu, Y.; Zhang, R.; Gan, Z.; Wang, J. Effect of the structure of ginsenosides on the in vivo fate of their liposomes. *Asian Journal of Pharmaceutical Sciences* **2022**, *17*, 219-229.
185. Lee, D.G.; Jang, S.-I.; Kim, Y.-R.; Yang, K.E.; Yoon, S.J.; Lee, Z.-W.; An, H.J.; Jang, I.-S.; Choi, J.-S.; Yoo, H.-S. Anti-proliferative effects of ginsenosides extracted from mountain ginseng on lung cancer. *Chinese journal of integrative medicine* **2016**, *22*, 344-352.

186. Shim, S.H.; Baek, K.-H.; Kim, Y.S. Inhibition of human 20S proteasome by ginsenosides from Panax ginseng. *Bulletin of the Korean Chemical Society* **2009**, *30*, 1385-1387.
187. Singh, G.; Verma, A.; Kumar, V. Catalytic properties, functional attributes and industrial applications of β-glucosidases. *3 Biotech* **2016**, *6*, 1-14.
188. Astudillo, L.; Therville, N.; Colacios, C.; Ségui, B.; Andrieu-Abadie, N.; Levade, T. Glucosylceramidases and malignancies in mammals. *Biochimie* **2016**, *125*, 267-280.
189. Aureli, M.; Samarani, M.; Murdica, V.; Mauri, L.; Loberto, N.; Bassi, R.; Prinetti, A.; Sonnino, S. Gangliosides and cell surface ganglioside glycohydrolases in the nervous system. *Glycobiology of the Nervous System* **2014**, 223-244.
190. Liu, J.; Halene, S.; Yang, M.; Iqbal, J.; Yang, R.; Mehal, W.Z.; Chuang, W.-L.; Jain, D.; Yuen, T.; Sun, L. Gaucher disease gene GBA functions in immune regulation. *Proceedings of the National Academy of Sciences* **2012**, *109*, 10018-10023.
191. Baden, P.; Yu, C.; Deleidi, M. Insights into GBA Parkinson's disease pathology and therapy with induced pluripotent stem cell model systems. *Neurobiol Dis* **2019**, *127*, 1-12.
192. Li, Y.; Chen, Y.; Jiang, L.; Zhang, J.; Tong, X.; Chen, D.; Le, W. Intestinal inflammation and Parkinson's disease. *Aging and disease* **2021**, *12*, 2052.
193. Sorli, S.C.; Colié, S.; Albinet, V.; Dubrac, A.; Touriol, C.; Guilbaud, N.; Bedia, C.; Fabrias, G.; Casas, J.; Ségui, B. The nonlysosomal β-glucosidase GBA2 promotes endoplasmic reticulum stress and impairs tumorigenicity of human melanoma cells. *The FASEB Journal* **2013**, *27*, 489-498.
194. Shibata, S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. *J Korean Med Sci* **2001**, *16*, S28-S37.
195. Wang, H.; Zheng, Y.; Sun, Q.; Zhang, Z.; Zhao, M.N.; Peng, C.; Shi, S.J. Ginsenosides emerging as both bifunctional drugs and nanocarriers for enhanced antitumor therapies. *J Nanobiotechnol* **2021**, *19,* 1-40.
196. Halima, M. Ginsenosides as selecti e glucocorticoid drugs: agonists, antagonists, and prodrugs.
197. Hason, M.; Bartůněk, P. Zebrafish models of cancer—new insights on modeling human cancer in a non-mammalian vertebrate. *Genes* **2019**, *10*, 935.
198. Lee, H.-C.; Lin, C.-Y.; Tsai, H.-J. Zebrafish, an in vivo platform to screen drugs and proteins for biomedical use. *Pharmaceuticals* **2021**, *14*, 500.
199. Zapata, A.; Diez, B.; Cejalvo, T.; Gutierrez-de Frias, C.; Cortés, A. Ontogeny of the immune system of fish. *Fish & shellfish immunology* **2006**, *20*, 126-136.
200. Osmani, N.; Goetz, J.G. Multiscale imaging of metastasis in zebrafish. *Trends in cancer* **2019**, *5*, 766-778.
201. Feitsma, H.; Cuppen, E. Zebrafish as a cancer model. *Molecular Cancer Research* **2008**, *6*, 685-694.

202. White, R.; Rose, K.; Zon, L. Zebrafish cancer: the state of the art and the path forward. *Nature Reviews Cancer* **2013**, *13*, 624-636.
203. Wu, J.-Q.; Zhai, J.; Li, C.-Y.; Tan, A.-M.; Wei, P.; Shen, L.-Z.; He, M.-F. Patient-derived xenograft in zebrafish embryos: a new platform for translational research in gastric cancer. *Journal of Experimental & Clinical Cancer Research* **2017**, *36*, 1-10.
204. Yin, J.; Zhao, G.; Kalirai, H.; Coupland, S.E.; Jochemsen, A.G.; Forn-Cuní, G.; Wierenga, A.P.; Jager, M.J.; Snaar-Jagalska, B.E.; Groenewoud, A. Zebrafish Patient-Derived Xenograft Model as a Preclinical Platform for Uveal Melanoma Drug Discovery. *Pharmaceuticals* **2023**, *16*, 598.
205. Costa, B.; Estrada, M.F.; Barroso, M.T.; Fior, R. Zebrafish Patient-Derived Avatars from Digestive Cancers for Anti-cancer Therapy Screening. *Current Protocols* **2022**, *2*, e415.
206. Invrea, F.; Rovito, R.; Torchiaro, E.; Petti, C.; Isella, C.; Medico, E. Patient-derived xenografts (PDXs) as model systems for human cancer. *Current opinion in biotechnology* **2020**, *63*, 151-156.
207. Linnekamp, J.F.; Hooff, S.R.v.; Prasetyanti, P.R.; Kandimalla, R.; Buikhuisen, J.Y.; Fessler, E.; Ramesh, P.; Lee, K.A.; Bochove, G.G.; de Jong, J.H. Consensus molecular subtypes of colorectal cancer are recapitulated in in vitro and in vivo models. *Cell Death & Differentiation* **2018**, *25*, 616-633.
208. Scott, C.; Becker, M.A.; Haluska, P.; Samimi, G. Patient-derived xenograft models to improve targeted therapy in epithelial ovarian cancer treatment. *Front Oncol* **2013**, *3*, 70425.
209. Yan, C.; Brunson, D.C.; Tang, Q.; Do, D.; Iftimia, N.A.; Moore, J.C.; Hayes, M.N.; Welker, A.M.; Garcia, E.G.; Dubash, T.D. Visualizing engrafted human cancer and therapy responses in immunodeficient zebrafish. *Cell* **2019**, *177*, 1903-1914. e1914.
210. Langenau, D.M. Cancer and zebrafish: mechanisms, techniques, and models. *Springer* **2016**, *916*.