TURUN YLIOPISTO
UNIVERSITY OF TURKU

NOVEL IMMUNE AND GENETIC DRIVERS OF MELANOMA

Integrative and New Preclinical Models to Uncover the Impact of New Chromosomal and Transcriptomic Changes on Tumor Progression and Immunity

Wang Meng

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1886 | MEDICA – ODONTOLOGICA | TURKU 2025

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

NOVEL IMMUNE AND GENETIC DRIVERS OF MELANOMA

Integrative and New Preclinical Models to Uncover the Impact of New Chromosomal and Transcriptomic Changes on Tumor Progression and Immunity

Wang Meng

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1886 | MEDICA – ODONTOLOGICA | TURKU 2025

University of Turku

Faculty of Medicine
Institute of Biomedicine
Pathology
Turku Doctoral Programme of Molecular Medicine

Supervised by

Associate Professor Carlos Rogerio Figueiredo
Institute of Biomedicine
University of Turku
Turku, Finland

Reviewed by

Associate Professor Sanna Iivanainen
Cancer Center
Oulu University Hospital
Oulu, Finland

Assistant Professor Joe Yeong Poh Sheng
Duke-NUS Medical School &
Singapore General Hospital
Singapore

Opponent

Professor Roger Chammas
Comprehensive Center for Precision Oncology
University of São Paulo
São Paulo, Brazil

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin Originality Check service.

Cover Image: Medical Immuno-Oncology Research Group

ISBN 978-952-02-0203-3 (PRINT)
ISBN 978-952-02-0204-0 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2025

“I am among those who think that science has great beauty.”

— Maria Salomea Skłodowska-Curie

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Pathology
MENG WANG: NOVEL IMMUNE AND GENETIC DRIVERS OF MELANOMA
Doctoral Dissertation, 140 pp.
Turku Doctoral Programme of Molecular Medicine
May 2025

ABSTRACT

Genetic instabilities drive melanoma development, disrupting immune editing and leading to resistance against revolutionary therapies like immune checkpoint therapies (ICT), as seen in uveal (UM) and cutaneous (CM) subtypes. This thesis adopts a multidisciplinary approach integrating analytical tools in patient samples to reveal key drivers of uveal and cutaneous melanoma malignancy. It further reverses to the bench side to validate an important driver by creating a novel preclinical model that provides new foundations for future biological and therapeutic studies.

In CM, integration of multiple transcriptomic datasets from patients who had received ICT provided new insights into ICT resistance, often linked to cold tumors with deficient antigen presentation. Impaired antitumor immune responses were frequently associated with reduced β2-microglobulin (β2M) levels, which correlated with poor ICT responses in CM. Integrative transcriptomic analyses identified novel β2M-associated biomarkers, with *CD1D* playing a critical role in natural killer T (NKT) cell functions. Epigenetic profiling showed that methylation regulated β2M and *CD1D* expression, influencing ICT outcomes. Modulation of these pathways was suggested to enhance ICT efficacy in CM.

In UM, *BAP1* loss consistently drove aggressive tumor behavior across populations. Chromosomal analyses in Southeast Asian UM patients revealed distinct genetic features, including less frequent monosomy 3 and more frequent chromosome 1q gains, both linked to poor progression-free survival. These findings underscored the critical and consistent role of *BAP1* loss while highlighting the importance of region-specific molecular profiling. To investigate *BAP1*-dependent tumor-immune changes, CRISPR-engineered *BAP1*$^{-/-}$ mouse melanocyte models were developed, demonstrating lipid metabolic reprogramming and immunosuppressive traits characteristic of high-risk UM. This model offers a robust platform for testing novel immunotherapies targeting *BAP1*-driven pathways.

This research established a comprehensive preclinical framework to connect clinical observations with translational applications in melanoma. By elucidating key genetic and immune drivers, it provided a robust platform for developing and refining novel therapeutic strategies, particularly immunotherapies.

KEYWORDS: Melanoma, *BAP1* loss, tumor microenvironment, Immune suppression, CRISPR/Cas9 gene-editing, preclinical model

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen Laitos
Patologia
MENG WANG: MELANOOMAN UUDET IMMUUNIT JA GENEETTISET OHJEET
Väitöskirja, 140 s.
Molekyylilääketieteen tohtoriohjelma
Toukokuu 2025

TIIVISTELMÄ

Geneettiset epävakaudet edistävät melanooman kehittymistä, häiriten immuunieditointia ja aiheuttaen vastustuskykyä immuunijarrupistehoidoille (ICT), kuten havaitaan sekä uveal- (UM) että ihomelanoomatyypeissä (CM). Tämä väitöskirja yhdistää analyyttiset työkalut potilasnäytteisiin paljastaakseen UM:n ja CM:n maligniteetin keskeiset ajurit ja kehittää laboratorioympäristössä uuden prekliinisen mallin tulevia biologisia ja terapeuttisia tutkimuksia varten.

CM:ssä useiden ICT-hoitoa saaneiden potilaiden transkriptiomisten tietokokonaisuuksien integrointi tarjosi uusia näkemyksiä ICT-resistenssistä, joka liittyi usein kylmiin kasvaimiin, joilla on puutteellinen antigeenin esittely. Heikentyneet immuunivasteet kasvaimia vastaan yhdistettiin usein alentuneisiin β2-mikroglobuliinin (β2M) tasoihin, jotka korreloivat heikkojen ICT-hoitovasteiden kanssa CM:ssä. Integroivat transkriptiomiset analyysit tunnistivat uusia β2M:ään liittyviä biomarkkereita, joista CD1D:llä on keskeinen rooli luonnollisten tappajasolujen (NKT) toiminnassa. Epigeneettinen profilointi osoitti, että metylointi säätelee β2M:n ja CD1D:n ilmentymistä, mikä vaikuttaa ICT-hoidon lopputuloksiin. Näiden reittien moduloinnin ehdotettiin parantavan ICT:n tehokkuutta CM:ssä.

UM:ssä *BAP1*:n menetys osoittautui johdonmukaisesti aggressiivisen kasvainkäyttäytymisen ajuriksi eri populaatioissa. Kaakkois-Aasian UM-potilaiden kromosomianalyysit paljastivat erottuvia geneettisiä piirteitä, kuten harvinaisemman monosomia 3:n ja yleisemmän kromosomi 1q:n lisäykset, jotka molemmat yhdistettiin heikkoon etenemisvapaaseen eloonjäämiseen. Nämä havainnot korostivat *BAP1*:n keskeistä ja johdonmukaista roolia, samalla kun ne painottivat alueellisesti spesifin molekyyliprofiloinnin merkitystä. *BAP1*-riippuvaisten kasvain-immuniteettimuutosten tutkimiseksi kehitettiin CRISPR-tekniikalla tuotettu $BAP1^{-/-}$ hiiren melanosyyttimalli, joka osoitti lipidimetabolian uudelleenohjelmointia ja immuunisuppressiivisia piirteitä, jotka ovat tyypillisiä korkean riskin UM:lle. Tämä malli tarjoaa vankan alustan uusien *BAP1*-riippuvaisten reittien kohdistamiseen tähtäävien immunoterapioiden testaamiseen.

Tutkimus loi prekliinisen viitekehyksen, joka yhdistää kliiniset havainnot ja käänteentekevät sovellukset melanooman tutkimuksessa, tarjoten perustan uusien terapeuttisten strategioiden, erityisesti immunoterapioiden, kehittämiselle.
AVAINSANAT: Melanooma, *BAP1*-puutos, kasvaimen mikroympäristö, immuunisuppressio, CRISPR/Cas9-geenin muokkaus, prekliininen malli

Table of Contents

Abbreviations

3Es	Elimination, Equilibrium, and Escape
5-hmC	5-hydroxymethylcytosine
5-mC	5-methylcytosine
APC	Antigen-Presenting Cell
APM	Antigen-Processing Machinery
ARF6	ADP-Ribosylation Factor 6
BLI	Bioluminescence imaging
CAFs	Cancer-Associated Fibroblasts
CAMs	Cancer-Associated Macrophages
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A
CI	Cell Index
CIITA	Class II Major Histocompatibility Complex Transactivator
CT	Computed Tomography
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4
CTLs	Cytotoxic T Lymphocytes
DAB	Diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DC	Dendritic Cell
DEGs	Differentially Expressed Genes
DNMTs	DNA Methyltransferases
DSP	Digital Spatial Profiling
ECM	Extracellular Matrix
EMT	Epithelial-Mesenchymal Transition
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
GEMMs	Engineered Mouse Models
GPCRs	G-Protein-Coupled Receptors
GSEA	Gene Set Enrichment Analysis
GVAX	GM-CSF-Secreting Tumor Vaccine
H&E	Hematoxylin and Eosin
HATs	Histone Acetyltransferases

HC	Hierarchical Clustering
HDACs	Histone Deacetylases
HLA	Human Leukocyte Antigens
HLA-DM	Human Leukocyte Antigen-DM
ICB	Immune Checkpoint Blockade
IGF-1	Insulin-like Growth Factor 1
IGFR-1	Insulin-like Growth Factor Receptor 1
IHC	Immunohistochemistry
IL-10	Interleukin-10
IOP	Intraocular Pressure
KO	knockout
LOOC	Liverpool Ocular Oncology Centre
MAPK	Mitogen-Activated Protein Kinase
MDSCs	Myeloid-Derived Suppressor Cells
MHC	Major Histocompatibility Complex
MIF	Macrophage Migration Inhibitory Factor
mIHC	Multiplex Immunohistochemistry
MIORG	Medical Immuno-Oncology Research Group
mUM	Metastatic Uveal Melanoma
Neo-MoDC	Neoantigen-Loaded Monocyte-Derived Dendritic Cell
NKT	Natural Killer T Cells
OCT	Optical Coherence Tomography
OS	Overall Survival
PAP	Papanicolaou
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
PD-1	Programmed Cell Death-1
PDAC	Pancreatic Ductal Adenocarcinoma
PDK1	Phosphoinositide-Dependent Kinase 1
PDX	Patient-Derived Xenograft
PI3K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
PLCβ	Phospholipase C- β
RTK	Receptor Tyrosine Kinase
RTKs	Receptor Tyrosine Kinases
scRNA-seq	Single-cell RNA sequencing
SERI	Singapore Eye Research Institute
SGH	Singapore General Hospital
sgRNA	single-guide RNA
STAT3	Signal Transducer and Activator of Transcription 3

TAMs	Tumor-Associated Macrophages
TAP	Transporter Associated with Antigen Processing
TET	Ten-Eleven Translocation
TGF-β	Transforming Growth Factor Beta
TILs	Tumor-Infiltrating Lymphocytes
TME	Tumor Microenvironment
TOPP	Translational Ophthalmic Pathology Platform
Tregs	Regulatory T Cells
UM	Uveal Melanoma
β2M	beta-2-microglobulin

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I **Wang MM**, Koskela SA, Mehmood A, Langguth M, Maranou E, Figueiredo CR. Epigenetic control of *CD1D* expression as a mechanism of resistance to immune checkpoint therapy in poorly immunogenic melanomas. *Frontiers in Immunology*. 2023;14.

II ***Chen C, ***Wang MM**, Lim AST, Heng EYH, Tien SL, Yu SS, Tan G, Chan JY, Chan ASY. Genetic Landscape of Uveal Melanoma in Southeast Asia: High 1q Gains and Unique Patterns of Metastasis Risk. *Eye and Vis*. 2025 ***Equal contribution***

III **Wang MM**, Chen C, Lynn MN, Figueiredo CR, Tan WJ, Lim TS, Coupland SE, Chan ASY. Applying Single-Cell Technology in Uveal Melanomas: Current Trends and Perspectives for Improving Uveal Melanoma Metastasis Surveillance and Tumor Profiling. *Frontiers in Molecular Biosciences*. 2021;7(422).

IV **Wang MM**, Li YH, Ho CEH, Yu W, Coupland SE, Chan ASY, Figueiredo CR. New Pre-Clinical CRISPR-Based *BAP1* Knockout Tumor Model Recapitulates Tumorigenesis and Immune Evolution of Melanoma. *Manuscript*. 2025.

V **Wang MM**, Coupland SE, Tero A, Figueiredo CR. Resistance to immune checkpoint therapies by tumour-induced T cell desertification and exclusion: key mechanisms, prognostication, and new therapeutic opportunities. *British Journal of Cancer*. 2023

1 Introduction

Melanoma, a group of malignancies originating from melanocytes, poses significant clinical challenges due to its aggressive nature, varied genetic drivers, and immune suppression (Wagstaff et al. 2022). Among its subtypes, cutaneous melanoma (CM) (Balch et al. 2024) and uveal melanoma (UM) (Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, and Shields 2020) stand out for their distinct biological and clinical characteristics. CM, arising in the skin, is often marked by a high tumor mutational burden (TMB) and variable response to immune checkpoint therapies (ICT) (Sharma et al. 2021b). In contrast, UM, originating in the eye, exhibits low TMB, a highly immune-suppressive tumor microenvironment (TME), and a strong predilection for liver metastasis (Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, and Shields 2020). These differences underline the necessity for tailored therapeutic approaches and a deeper understanding of the molecular mechanisms underpinning tumor progression and immune evasion.

Immune suppression in CM is frequently associated with the absence of tumor-infiltrating lymphocytes (TILs), the presence of immune-suppressive cells such as myeloid-derived suppressor cells (MDSCs), and defects in antigen presentation mechanisms (Sharma et al. 2021a). One molecule of interest is *CD1D*, a non-classical major histocompatibility complex (MHC-I)-like molecule, which plays a key role in presenting lipid antigens to natural killer T (NKT) cells (Mori, Lepore, and De Libero 2016). Dysregulation of *CD1D*, potentially driven by epigenetic mechanisms such as β2-microglobulin (*B2M*) loss, can suppress antitumor immune responses and promote immune evasion (Kalaora et al. 2022). Understanding the interplay between *CD1D* expression and ICT resistance offers a promising avenue for overcoming therapeutic challenges in CM.

UM, the most common primary intraocular malignancy in adults, displays unique patterns of metastasis and resistance to treatments (Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, and Shields 2020). Chromosomal aberrations, such as losses of chromosomes 1p, 3, 6q, 8p, and 16q, and gains in 6p and 8q, also have a prognostic role in UM (Harbour 2012; Abdel-Rahman et al. 2011; Dogrusoz and Jager 2018; Gallenga et al. 2022; Harbour 2009).

Other chromosome alterations have not been well studied in multiple UM cohorts, so further chromosomal aberrations analysis can significantly advance our understanding of UM in different regions. Furthermore, BRCA1-associated protein 1 (*BAP1*) loss, a hallmark alteration in UM, is strongly associated with poor prognosis, aggressive tumor behavior, and immune suppression. *BAP1* encodes a nuclear deubiquitinase involved in chromatin remodeling, DNA damage repair, and cell cycle regulation (Masclef et al. 2021; Louie and Kurzrock 2020; Kwon, Lee, and Lee 2023). Despite its established role in promoting tumor progression and therapy resistance, the mechanisms by which *BAP1* loss influences immune modulation and tumorigenesis remain incompletely understood.

While preclinical models have been widely applied in melanoma research, existing systems often fall short of capturing the complex immune interactions and specific genetic contexts of interest. Xenograft models, which rely on immunodeficient mice, are inadequate for studying immunotherapies due to the absence of a functional immune system (Cao and Jager 2015; Némati et al. 2010; Kageyama et al. 2017; Nemati et al. 2014; Heegaard, Spang-Thomsen, and Prause 2003). Similarly, widely used syngeneic models often harbor confounding genetic alterations, complicating the delineation of specific molecular mechanisms (Rusciano, Lorenzoni, and Burger 1994; Fidler, Gersten, and Budmen 1976; Richards et al. 2020; De Waard‐Siebinga et al. 1995). To address these limitations, this thesis employed a CRISPR-based approach to develop an immunocompetent *BAP1* knockout model, providing a novel platform to investigate *BAP1*-driven mechanisms in melanoma progression and immune modulation.

By leveraging clinical and preclinical data, this thesis employed a reverse translational approach to connect clinical observations with experimental research. Integrating multi-omic analyses elucidated the molecular mechanisms driving tumor progression and immune suppression in melanoma, explicitly focusing on *CD1D* dysregulation in CM and 1q gains in UM. The development of a CRISPR-engineered *BAP1* knockout model further enabled in-depth exploration of the immune-related consequences of *BAP1* loss, addressing critical gaps in understanding its role in therapy resistance. This comprehensive strategy highlighted the importance of translating molecular discoveries into actionable therapeutic interventions, aiming to enhance treatment outcomes for melanoma patients.

The work presented in this thesis advances the understanding of melanoma biology, providing a foundation for precision medicine approaches. By unraveling the genetic and immunological complexities of melanoma subtypes, this research contributes to the broader field of cancer biology for novel therapeutic interventions that target immune resistance and tumor progression.

2 Review of the Literature

2.1 Hallmarks of Cancer

The concept of the Hallmarks of Cancer, introduced by Hanahan and Weinberg in their seminal 2000 paper (Hanahan and Weinberg 2000) and further expanded in 2011 (Hanahan and Weinberg 2011), has become a cornerstone in modern cancer biology. This framework distilled the complexity of malignant transformation into a set of core biological capabilities that are progressively acquired during the multistep development of human tumors. In their original formulation, six hallmarks were identified: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg 2000). Though mechanistically diverse, these features were conceptualized as universal principles underpinning the pathogenesis of most cancers.

In the updated framework, two emerging hallmarks, reprogramming energy metabolism and evading immune destruction, were added, alongside two enabling characteristics: genome instability and tumor-promoting inflammation (Hanahan and Weinberg 2011). Together, these ten dimensions reflect the evolving understanding of cancer not as a singular disease entity, but as a heterogeneous constellation of dynamic biological processes driven by genetic, epigenetic, and microenvironmental factors. Crucially, this model offers a unifying lens through which to analyze both the molecular underpinnings and the clinical behavior of cancers, shaping how researchers identify therapeutic targets, classify tumor subtypes, and interpret treatment responses.

The hallmarks model also supports the paradigm shift toward precision oncology (Singhal et al. 2025). As genomic and transcriptomic profiling become increasingly integrated into routine clinical care, these hallmark traits can often be traced to specific molecular alterations within individual tumors (Pleasance et al. 2022). This facilitates the development of tailored treatment strategies that align with the unique biological context of each patient's cancer (Tsimberidou et al. 2019). In this way, the hallmarks serve not only as an abstract theoretical construct but also as a practical framework that bridges basic science and translational medicine (Hanahan 2022).

Within this broader theoretical landscape, the present thesis narrows its focus to a specific and clinically significant cancer type: melanoma. While the hallmark capabilities provide a universal scaffold, their manifestation and interplay in melanoma follow distinct molecular trajectories that reflect the cancer's unique biological properties (Jain et al. 2023). Melanoma is characterized by a high mutational burden, aggressive metastatic potential, and notable immune responsiveness, traits that offer both challenges and opportunities for therapeutic innovation (Jardim et al. 2021). Accordingly, the following section delves into the molecular mechanisms of melanoma-specific tumor progression. It maps its biology in light of the hallmark traits while examining the particular pathways, mutations, and cellular interactions that drive its malignancy.

2.2 Immune Modulation, Resistance Mechanisms and Therapeutic Opportunities in Melanoma

The dynamic interplay between genetic instability and immune modulation is fundamental in driving cancer progression and shaping therapeutic resistance. Cancer evolution is intricately linked to the immune system's ability to recognize and eliminate transformed cells, a process conceptualized through the immunoediting model, which unfolds in three distinct phases: elimination, equilibrium, and escape (3Es), as first proposed by Lloyd Old and later expanded upon by his studies (Dunn, Old, and Schreiber 2004b). In the elimination phase, the innate and adaptive immune responses work together to detect and destroy nascent tumor cells. However, some cancer cells acquire mutations that enable them to persist, leading to the equilibrium phase, where immune pressure selects for variants with enhanced survival capacity. Eventually, in the escape phase, tumor cells develop mechanisms to evade immune detection and suppression, allowing unchecked proliferation and progression (Dunn, Old, and Schreiber 2004b).

The advent of ICT has revolutionized cancer treatment by harnessing the body’s immune system to target malignancies, particularly in highly immunogenic cancers such as metastatic CM (Ghemrawi et al. 2024). ICT agents, including programmed death-1 (PD-1) inhibitors (Ishida et al. 1992), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (Krummel and Allison 1995) inhibitors, and their combinations, have demonstrated remarkable efficacy in a subset of patients, leading to durable remissions and significantly improving survival rates (Boutros et al. 2016). T-cell-based cancer therapies emerged from a series of landmark discoveries that revealed how tumours co-opt physiological “brakes” on adaptive immunity. In the mid-1990s, James P. Allison and colleagues demonstrated that CTLA-4 functions as a negative regulator of T-cell activation; antibody-mediated blockade of CTLA-4 in murine models not only augmented effector responses but also precipitated

complete, immune-mediated tumour rejection (Krummel and Allison 1995; Leach, Krummel, and Allison 1996). Almost in parallel, Tasuku Honjo's group had cloned and described PD-1 as an inducible receptor on activated lymphocytes (Ishida et al. 1992). Subsequent work established PD-1 ligand interactions as a distinct inhibitory pathway that restrains peripheral T-cell activity and promotes tolerance, a finding that, once therapeutically neutralised, revitalised anti-tumour immunity even in the context of chronically antigen-exposed, exhausted T-cell populations. The translational arc from these fundamental insights to first-in-human antibodies (ipilimumab in 2011 for CTLA-4; nivolumab and pembrolizumab in 2014 for PD-1) culminated in durable responses across multiple malignancies and was recognised with the 2018 Nobel Prize in Physiology or Medicine, awarded jointly to Allison and Honjo. Against this historical backdrop, the modern immuno-oncology landscape is increasingly defined by strategic modulation of these and other checkpoints, adoptive T-cell transfer, and neoantigen-targeted vaccination approaches that collectively reposition the host immune system as a dynamic, druggable ecosystem. However, despite these successes, resistance to ICT remains a formidable clinical challenge. Both primary resistance, where tumors fail to respond to ICT from the outset, and acquired resistance, where initial responders eventually relapse, undermine the long-term effectiveness of these therapies (Queirolo et al. 2019; Sharma and Allison 2015a).

The clinical implications of ICT resistance are particularly pronounced in melanoma. A recent 10-year follow-up study published in the *New England Journal of Medicine* (Wolchok et al. 2025) reported that more than 50% of melanoma patients experience disease relapse within a decade of ICT initiation. This alarming statistic underscores the need for a deeper understanding of the mechanisms underpinning immune evasion. While CM has been extensively studied in the context of immune resistance, UM, a rarer and less immunogenic melanoma subtype, presents unique challenges, given its intrinsic resistance to ICT and the distinct tumor microenvironment shaped by intraocular immune privilege (Gelmi and Jager 2024).

This section delves into the key resistance mechanisms contributing to ICT failure, drawing insights from CM and UM. These mechanisms encompass various biological adaptations, including tumor-intrinsic genetic and epigenetic alterations, immune microenvironment modulation, and metabolic reprogramming. A comprehensive understanding of these resistance pathways is critical for informing novel therapeutic strategies to overcome immune suppression. By exploring recent advancements in biomarker identification, combination therapies, and next-generation immunotherapies, this discussion seeks to bridge gaps in ICT efficacy and pave the way for more personalized and durable treatment approaches in melanoma management.

2.2.1 Tumor-Induced Immunosuppressive Networks

The ability of tumors to evade immune surveillance and modulate the immune system to their advantage represents a significant barrier to effective cancer immunotherapy (Tang et al. 2021). Tumors employ various strategies to suppress immune activity, including the alteration of antigen presentation, the recruitment of immunosuppressive cell populations, and the secretion of inhibitory cytokines that create a tolerogenic microenvironment (Munn and Bronte 2016). This immunosuppressive network hinders the function of effector T cells and antigen-presenting cells, ultimately allowing tumors to persist and metastasize despite host immune responses (Saleh and Elkord 2020). Understanding these mechanisms is crucial for developing therapeutic strategies to counteract immune evasion and enhance the efficacy of ICT. The TME is vital in immune suppression (Arner and Rathmell 2023), shaped by intrinsic and extrinsic mechanisms that enable immune evasion. Low TMB has long been recognized as a hallmark of immune-resistant melanomas, particularly in UM (Budczies et al. 2024), which inherently possesses a low TMB compared to CM (Kang et al. 2020). However, tumors with adequate TMB often develop adaptive resistance mechanisms, including antigen presentation deficiencies, immune cell exclusion, and regulatory signaling pathways that suppress effective immune responses (Zhou et al. 2022). Tumor-induced immunosuppression can be broadly categorized into intrinsic factors associated with tumor cells and extrinsic factors involving the TME, contributing to immune evasion and resistance to immunotherapies.

2.2.1.1 Tumor-Intrinsic Factors Leading to Immune Evasion

Cancer progression is heavily influenced by the ability of tumor cells to evade immune surveillance through establishing immunosuppressive networks. These networks operate at multiple levels, including direct tumor-intrinsic alterations that impair immune recognition and tumor-extrinsic modifications of the TME that hinder immune cell infiltration and activation. Effective immune responses, particularly in ICT, require successful priming and expansion of tumor-specific T cells (Principe et al. 2020). However, many tumors employ various mechanisms to suppress these processes, leading to resistance to immunotherapy. The balance between immune activation and suppression is crucial, and tumor-driven immunosuppressive strategies significantly contribute to therapy resistance and tumor persistence (Ricci 2025). Understanding these mechanisms is essential for developing novel therapeutic approaches that enhance immune system recognition of tumors and improve patient outcomes.

Multiple intrinsic mechanisms have evolved in tumor cells to escape immune detection and destruction. A key strategy involves downregulating major

histocompatibility complex (MHC) molecules, particularly MHC class I, essential for antigen presentation to cytotoxic T lymphocytes (CTLs) (Sharma and Allison 2020). Loss of beta-2-microglobulin (β2M), a critical component of MHC class I, has been frequently observed in tumors, leading to impaired antigen presentation, rendering tumor cells invisible to CD8+ T cell-mediated immunity (Wen et al. 2023). Additionally, gene mutations involved in the antigen-processing machinery (APM), such as transporter associated with antigen processing (TAP) and proteasomal subunits, further limit antigen presentation (Dunn, Old, and Schreiber 2004a). These tumor-intrinsic alterations significantly reduce the effectiveness of ICT, particularly therapies targeting PD-1/PD-L1 and CTLA-4 (Xie et al. 2024).

Another intrinsic mechanism of immune evasion is the activation of the WNT/β-catenin signaling pathway. This pathway has been shown to suppress the infiltration of antigen-presenting cells (APCs), including dendritic cells (DCs), into the TME, thereby impairing T cell priming (Luke et al. 2019; Spranger, Bao, and Gajewski 2015a). Tumors with high WNT/β-catenin activity are characterized by poor immune cell infiltration and resistance to checkpoint inhibitors (Spranger, Bao, and Gajewski 2015a). Similarly, activation of the signal transducer and activator of transcription 3 (STAT3) pathway in tumor cells has been linked to immune evasion by promoting an immunosuppressive TME and inhibiting DC maturation (Zou et al. 2020).

Furthermore, tumor cells often produce immunosuppressive cytokines, such as transforming growth factor-beta (TGF-β) (Herber et al. 2010; Ohno et al. 2016; Chan et al. 2010; Bobadilla et al. 2013; Kobie et al. 2003; Ganesh and Massagué 2018) and interleukin-10 (IL-10), which impair immune activation and promote regulatory T cell (Treg) expansion (Herber et al. 2010; Ohno et al. 2016; Chan et al. 2010; Bobadilla et al. 2013; Kobie et al. 2003; Ganesh and Massagué 2018). TGF-β has promoted an exclusionary TME by inhibiting CD8+ T cell infiltration and fostering a tolerogenic environment (Herber et al. 2010; Ohno et al. 2016; Chan et al. 2010; Bobadilla et al. 2013; Kobie et al. 2003; Ganesh and Massagué 2018). IL-10, on the other hand, suppresses antigen presentation by downregulating MHC class II molecules and reducing the expression of co-stimulatory molecules on APCs (Herber et al. 2010; Ohno et al. 2016; Chan et al. 2010; Bobadilla et al. 2013; Kobie et al. 2003; Ganesh and Massagué 2018). These intrinsic tumor-driven mechanisms collectively contribute to the formation of immune-cold tumors, which exhibit minimal immune cell infiltration and poor responsiveness to immunotherapy (Wang et al. 2025).

2.2.1.2 Role of the Tumor Microenvironment in Hindering Immune Cell Infiltration

The TME is critical in suppressing immune responses and preventing effective T cell infiltration. Tumors that lack sufficient immune infiltration, often termed "cold tumors," are refractory to immune checkpoint blockade therapies (Chen and Mellman 2017a; Hanley and Thomas 2020). A key feature of these tumors is the presence of an immunosuppressive TME characterized by dysfunctional APCs, regulatory immune cells, and a dense extracellular matrix that acts as a physical and biochemical barrier to immune cell entry (Ramia et al. 2019).

Cancer-associated fibroblasts (CAFs) are a significant component of the TME that contribute to immune exclusion by producing extracellular matrix components such as collagen and fibronectin, which physically impede immune cell migration into the tumor core (Ford et al. 2020). Tumors that employ mechanisms to exclude T cells frequently hinder their infiltration by forming a fibrotic barrier. This process arises from the complex interactions between secreted factors produced by CAFs, which exhibit a myofibroblastic phenotype, and tumor-associated macrophages (TAMs) (Hanley and Thomas 2020). TAMs are another prominent component of the immunosuppressive TME. These cells often adopt an M2-like phenotype associated with the secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) and TGF-β, both suppressing effector T cell activity (Wynn and Barron 2010; Mariathasan et al. 2018). TAMs also express immune checkpoint ligands, including PD-L1, further inhibiting T cell responses (Petty et al. 2021). Additionally, TAMs contribute to metabolic immunosuppression by depleting essential nutrients required for T cell function, such as L-arginine, via arginase-1 activity, thereby impairing T cell proliferation and cytokine production (Martinenaite et al. 2025).

Beyond cellular components, soluble factors within the TME further restrict immune infiltration. Elevated levels of vascular endothelial growth factor (VEGF) not only promote tumor angiogenesis but also exert immunosuppressive effects by inhibiting the maturation of dendritic cells and increasing the presence of myeloid-derived suppressor cells (MDSCs), which block T cell activation (Bourhis et al. 2021; Yang, Yan, and Liu 2018). MDSCs interfere with T cell function by producing reactive oxygen species (ROS) and peroxynitrite, suppressing T cell receptor (TCR) signaling and promoting T cell exhaustion (Schouppe et al. 2013; Wang, Kuang, et al. 2021).

These tumor-intrinsic and microenvironmental factors collectively establish a formidable immunosuppressive network that prevents effective immune infiltration and function. Overcoming these barriers requires innovative therapeutic strategies, including the use of combination therapies that target immune checkpoints alongside modulators of the TME, such as TGF-β inhibitors, VEGF blockade, and metabolic reprogramming approaches (Liu, Liu, et al. 2021). By addressing both tumor-

intrinsic and extrinsic mechanisms of immune evasion, it may be possible to convert "cold" tumors into "hot" tumors, thereby improving responses to immune-based therapies and enhancing overall patient outcomes in cancer immunotherapy.

2.2.2 Biomarkers and Emerging Mechanisms of Immune Cell Exclusion in Melanoma Immunotherapy

A critical obstacle to effective immunotherapy is the inability of T cells to infiltrate the TME, resulting in so-called "desert" or "excluded" tumors. Identifying robust biomarkers that predict immune cell exclusion and resistance to ICT is paramount to refining patient stratification and optimizing therapeutic strategies (Taube et al. 2014; Tumeh, Harview, Yearley, Shintaku, Taylor, Robert, Chmielowski, Spasic, Henry, Ciobanu, et al. 2014). The scarcity of predictive biomarkers with high reproducibility across different melanoma cohorts remains a challenge, necessitating comprehensive molecular and genetic investigations. Recent advancements in multi-omics approaches have provided valuable insights into the complex interplay between tumor-intrinsic and immune-modulating factors that regulate immune cell exclusion (Kovács and Győrffy 2022; Coleman et al. 2023). This section explores the molecular and genetic underpinnings that shape immune cell absence and the distinct immunological challenges in CM and UM. The integration of findings from CM and UM underscores the complexity of immune resistance mechanisms in melanoma. While CM offers a model for studying antigen presentation and immune exclusion, UM provides insights into genetic drivers like *BAP1* that uniquely shape the TME (Figueiredo et al. 2020). By leveraging these insights, future therapeutic strategies can address both tumor-intrinsic and extrinsic factors, improving ICT efficacy across melanoma subtypes.

2.2.2.1 Immune Evasion in the TME and Biomarker Strategies for Immunotherapy Response

The absence of immune cells, especially T cells, in the tumor microenvironment can be attributed to a combination of tumor-intrinsic factors, stromal components, and immune-modulating molecules. Genetic alterations within tumor cells influence immune cell trafficking and function, thereby shaping the immunosuppressive landscape of melanoma. For example, in CM, antigen presentation is frequently impaired by the loss of β2M, a critical component of the MHC-I complex, which prevents cytotoxic T lymphocytes (CTLs) from recognizing and targeting tumor cells (Yang, Halima, and Chan 2023). The landmark Nature Communications study on β2M highlighted not only its essential role in MHC-I-mediated peptide antigen

presentation but also its association with HLA-related molecules like CD1d, which present glycoproteins and glycolipids to NKT cells (Borg et al. 2007).

The efficacy of ICT is significantly hindered by the emergence of resistance, affecting more than half of treated patients, including those who initially respond but later relapse (Wolchok et al. 2025). This highlights the critical need for predictive biomarkers that can accurately forecast both short- and long-term clinical outcomes. However, one of the significant obstacles in ICT biomarker development is the scarcity of predictive markers with robust and reproducible accuracy across different cancer cohorts. Ideally, biomarkers should be validated in their original study populations and demonstrate predictive reliability in independent patient groups. Addressing this limitation may require identifying distinct biomarker sets tailored to specific tumour types and implementing combination therapies to enhance treatment efficacy across diverse patient populations. ICT response biomarkers have historically been classified into tumour-intrinsic factors and phenotypic immunohistochemistry markers, such as PD-L1 expression and CD8+ tumour-infiltrating lymphocytes (TILs) (Taube et al. 2014; Tumeh, Harview, Yearley, Shintaku, Taylor, Robert, Chmielowski, Spasic, Henry, Ciobanu, et al. 2014). Despite their association with ICT outcomes, these biomarkers have limitations. The predictive capacity of PD-L1 is constrained by its biological complexity, site-specific expression variability, and technical inconsistencies in measurement, while CD8+ TILs, though linked to positive ICT responses, have not been universally validated for prognostic use across multiple cancer types (Li et al. 2021). Moreover, recent studies underscore the importance of T cell spatial distribution and activation status within the tumour microenvironment, suggesting that these parameters may be more relevant for predicting ICT efficacy than mere TIL presence (Ostroumov et al. 2018; Waldman, Fritz, and Lenardo 2020). For instance, high TIL levels in primary UM have been paradoxically associated with poor prognosis, potentially due to a regulatory rather than cytotoxic T cell phenotype (Triozzi et al. 2019). This phenomenon is particularly evident in immune-privileged organs such as the eye and liver, where local immunosuppressive mechanisms may inhibit T cell activation and lead to ICT resistance. Consequently, it is increasingly apparent that isolated biomarkers lack the predictive power necessary for guiding ICT response assessments, necessitating a more integrative and multi-dimensional approach.

Integrating multi-omics data with clinical datasets from large-scale trials facilitates the development of advanced machine learning-based biomarker models for ICT response prediction (Kovács and Győrffy 2022; Coleman et al. 2023). These computational approaches offer valuable insights into tumour-immune interactions, helping to uncover novel resistance mechanisms. For example, transcriptomic analyses have identified stabilin-1 (STAB1)-associated M2 macrophage signatures that predict ICT resistance in cold tumours, highlighting the role of macrophage-

induced T cell dysfunction rather than impaired T cell infiltration (Hollmén, Figueiredo, and Jalkanen 2020). Additionally, in ovarian cancer, an 18-gene panel, including TAP1, ICOS, and CD2, has been identified as a marker for immunologically "desert" tumours, complementing histopathological assessments of intratumoral T cell levels (Mlynska et al. 2020). Furthermore, a comprehensive 166-gene immunotherapy signature, which includes IFNγ-inducible genes such as IDO1 and JAK/STAT, along with checkpoint-related genes like LAG3, CTLA-4, and PD-L1, has been found to differentiate T cell-inflamed tumours from T cell-excluded ones, suggesting its potential use in pre-screening patients for ICT suitability (Trujillo et al. 2018). Notably, SOCS1, a key JAK/STAT signaling regulator, has been identified as an immune modulator that suppresses melanoma immunogenicity, making it a promising therapeutic target for enhancing ICT responses (Liau et al. 2018). Emerging biomarkers also include transcriptional signatures linked to dendritic cell-mediated antigen presentation. This may indicate effective MHC class II antigen priming and helper T cell activation, further refining ICT response predictions (Vander Lugt et al. 2014). Despite these advancements, clinical validation remains imperative. Ensuring these biomarkers exhibit consistent predictive performance across diverse patient cohorts while maintaining strong correlations with histopathological features and established immunogenicity-related genes will be critical for their successful translation into clinical practice. The continued development and validation of multi-omic and computational biomarker models hold the potential to revolutionize precision immunotherapy, enabling more personalized and effective treatment strategies for cancer patients.

2.2.2.2 Unique Immune Challenges in Intraocular Tumor Environments

UM presents a distinct model for studying immune suppression in tumors, particularly within the context of immunosuppressive TME. Unlike CM, which demonstrates high responsiveness to ICT, metastatic UM (mUM) remains refractory mainly due to both T cell desertification and exclusion mechanisms (Yarchoan, Hopkins, and Jaffee 2017) . This immune resistance persists despite the embryological similarities between uveal and cutaneous melanocytes, emphasizing the role of the TME in shaping immune evasion. Notably, mUM serves as a paradigm for investigating molecular barriers associated with ICT resistance in cold tumors, where immune infiltration is minimal and immunosuppressive factors predominate (Rossi et al. 2021).

BAP1 deficiency emerges as a pivotal factor in UM's immunosuppressive landscape. The loss or mutation of *BAP1* drives tumor progression while fostering immune escape by impairing antigen presentation and modulating the immune microenvironment (Figueiredo et al. 2020). One study demonstrated that *BAP1* loss

in UM is correlated with the upregulation of immunosuppressive genes, including HLA-DR, CD38, and CD74, forming a suppressive immune axis (Figueiredo et al. 2020). Utilizing advanced spatial transcriptomics (NanoString GeoMX) and high-dimensional single-cell mass cytometry (CyTOF), the study characterized the immune landscape of both primary and metastatic UM, revealing striking similarities in immunosuppressive gene expression between these tumor stages (Figueiredo et al. 2020). Notably, CD8+ T lymphocytes within UM acquire a regulatory phenotype, while monocytes differentiate into tumor-associated macrophages (TAMs), reinforcing a tolerogenic environment. Furthermore, UM metastases to the liver exhibit activation of the macrophage migration inhibitory factor (MIF)/CD74 axis, exacerbating immune evasion in conjunction with *BAP1* deficiency.

Beyond *BAP1*-driven immune suppression, additional molecular pathways contribute to UM's ICT resistance. β-catenin (CTNNB1) upregulation has been implicated in dendritic cell (DC) dysfunction, preventing effective T cell priming and promoting immune exclusion in mUM (Figueiredo et al. 2020) . These observations align with emerging evidence that TME-driven stromal barriers contribute to ICT resistance in UM with *BAP1* loss. The dissection of these molecular mechanisms offers a framework for novel combinatorial therapeutic strategies to overcome ICT resistance in mUM. Additionally, metabolic dysregulation has become a critical factor in UM immune evasion. Our research identified a *BAP1*-dependent metabolic shift associated with adipophilin loss, a lipid storage protein implicated in tumor progression and immune modulation (Fiorentzis et al. 2017; Matareed et al. 2023). Notably, reduced adipophilin expression correlated with poor survival outcomes in UM patients, particularly those with nuclear *BAP1* loss. This metabolic reprogramming, which alters the TME composition and immune responsiveness, underscores the importance of targeting metabolic pathways to enhance ICT efficacy. A multidisciplinary approach led to identifying potential therapeutic agents, including adrenergic, retinoid, and glucocorticoid receptor agonists and MEK and RAF inhibitors, that could reverse the metabolic gene signature in UM cells (Matareed et al. 2023). Notably, carvedilol, a beta-blocker with established clinical use, successfully restored adipophilin expression, highlighting the potential for drug repurposing in UM treatment. In agreement with these findings, other studies have demonstrated that beta-blockers, widely used for managing infantile hemangiomas, could also impair UM cell viability, further supporting their potential role in UM therapy (Farhoumand et al. 2022).

2.2.3 Innovative Strategies to Overcome Therapeutic Resistance

The persistence of therapeutic resistance in cancer immunotherapy remains a significant obstacle to achieving durable responses, particularly in cold tumours characterized by poor immunogenicity and immune exclusion. Despite the success of ICT in some cancers, a substantial proportion of patients fail to respond due to inherent or acquired resistance mechanisms. These multifaceted resistance mechanisms involve tumour-intrinsic factors, such as defective antigen presentation and immune evasion strategies, as well as tumour-extrinsic factors, including an immunosuppressive TME and systemic regulatory mechanisms. Recent research efforts have focused on overcoming these barriers through novel immunotherapeutic interventions that enhance antigen presentation, modulate the TME, and integrate combinatorial approaches to reinvigorate anti-tumour immunity (Garg et al. 2024; Siska et al. 2017; Espona-Fiedler et al. 2024). Among these strategies, dendritic cell (DC)-based vaccines and targeted immunosuppressive blockade have emerged as promising avenues to circumvent therapeutic resistance and restore immune surveillance in resistant tumours.

2.2.3.1 DC-based Vaccination and Novel Immunotherapeutic Strategies to Enhance Tumour Immunogenicity

Therapeutic strategies to overcome resistance in cold tumours have increasingly focused on optimizing antigen presentation and boosting DC function. DCs are essential for initiating anti-tumour immune responses through their role in antigen uptake, processing, and presentation to T cells. However, defective antigen presentation in cold tumours leads to a failure in T cell priming and contributes to therapeutic resistance. Therefore, efforts to improve DC-based vaccination strategies have concentrated on enhancing DC maturation, antigen loading, and antigen presentation capabilities (Galon and Bruni 2019; Harari et al. 2020).

Personalized neoantigen-loaded monocyte-derived dendritic cell (Neo-MoDC) vaccines have demonstrated encouraging clinical efficacy in overcoming therapeutic resistance. A notable study reported that a Neo-MoDC vaccine (NCT03185429, Phase I clinical trial) followed by ICT triggered robust T cell responses against tumour neoantigens, leading to complete tumour regression for over 25 months in patients with metastatic gastric cancer (Guo et al. 2022). Despite these promising outcomes, challenges such as high costs, scalability limitations, and potential immune-related adverse events remain obstacles to widespread clinical application. Further research is required to refine antigen selection methodologies and improve the efficiency of personalized DC vaccine production.

Another DC-based vaccine approach, GVAX, has shown promise in augmenting immune responses in resistant tumours. GVAX consists of whole tumour cells

genetically engineered to secrete GM-CSF, a key cytokine in recruiting and activating DCs (Hurwitz et al. 1998; van Elsas, Hurwitz, and Allison 1999). Clinical trials have demonstrated that GVAX in combination with ICT enhances tumour immunogenicity and improves overall survival (OS) in pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (Hopkins et al. 2018). However, in a phase II trial assessing GVAX with ipilimumab versus chemotherapy in metastatic PDAC, GVAX failed to confer a survival benefit (Wu, Bever, et al. 2020). This highlights the need for patient stratification based on tumour genetic and immune profiles to identify responders. Adoptive DC transfer is another innovative strategy to improve T cell priming in cold tumours. Unlike conventional DC-based vaccines, this approach involves ex vivo expansion and activation of tumour antigen-loaded DCs, which are then reinfused into patients. CD1c+ and plasmacytoid DCs have demonstrated efficacy in generating tumour-specific cytotoxic T lymphocyte (CTL) responses in stage IV melanoma patients (Phase I and Phase II study) (Schreibelt et al. 2016; Tel et al. 2013). Additionally, mRNA-based DC maturation strategies, such as the TriMix-DC approach, have been developed to enhance the immunogenic potential of DC vaccines by encoding CD40L, CD70, and TLR-4 (Benteyn et al. 2014). The feasibility and immunogenicity of TriMix-DC vaccination have been confirmed in phase II trials, particularly in combination with anti-CTLA-4 therapy (Wilgenhof et al. 2016). These advancements in DC-based vaccination strategies underscore their potential to overcome therapeutic resistance by enhancing antigen presentation and reinvigorating T cell responses. Future research should focus on optimizing DC vaccine formulations, identifying predictive biomarkers of response, and integrating combination therapies to maximize clinical efficacy.

2.2.3.2 Targeting Immunosuppressive Mechanisms and Rational Combinations to Overcome T Cell Exclusion

In addition to enhancing antigen presentation, addressing immunosuppressive mechanisms within the TME is crucial for overcoming therapeutic resistance. Tumours employ various immunosuppressive pathways to evade immune detection, including the upregulation of inhibitory cytokines, metabolic reprogramming, and recruitment of regulatory immune cells that suppress anti-tumour responses.

One promising approach involves targeting macrophage migration inhibitory factor (MIF), a cytokine implicated in immune suppression and tumour immune evasion. Preclinical studies have demonstrated that MIF blockade using small-molecule inhibitors, such as 4-ipp, enhances the efficacy of anti-CTLA-4 therapy by reducing tumour hypoxia and reprogramming metabolic pathways in metastatic melanoma (de Azevedo et al. 2020). However, due to the pleiotropic nature of MIF,

further research is required to elucidate its specific immunosuppressive mechanisms and validate the clinical utility of MIF-targeting therapies.

Another key immunosuppressive target is Clever-1, a scavenger receptor that modulates immune suppression within the TME. The MATINS trial (Phase I study, NCT03733990) explored the therapeutic potential of Clever-1 inhibition in cancer patients and reported a shift towards immune activation upon blockade (Rannikko et al. 2023). However, the study population's limited sample size and heterogeneity preclude definitive conclusions regarding its clinical efficacy. Further research is needed to refine Clever-1-targeting strategies and identify predictive biomarkers of response.

A more direct approach to overcoming T cell exclusion involves bispecific TCR-based therapies, such as tebentafusp, which has advanced to a Phase III stage of clinical testing. Tebentafusp is a bispecific TCR that binds a specific peptide-HLA complex on tumour cells while simultaneously recruiting and activating polyclonal T cells. This approach has demonstrated remarkable efficacy in mUM, a cancer type characterized by extreme T cell desertification (Chen and Carvajal 2022; Carvajal et al. 2022; Nathan et al. 2021). The phase III IMCgp100-202 trial confirmed that tebentafusp significantly improves survival in HLA-A*02:01-positive mUM patients compared to standard therapies (Nathan et al. 2021). However, the disconnect between tebentafusp's survival benefit and conventional radiological response criteria suggests the need for novel evaluation metrics to assess its clinical efficacy.

Beyond these targeted immunotherapeutic strategies, metabolic reprogramming has emerged as a potential means of overcoming therapeutic resistance. Inhibiting PKC with compounds such as darovasertib (IDE-196, open-label Phase II, NCT03947385/NCT05907954) has disrupted UM cell proliferation, thereby restoring immune control (Shoushtari et al. 2021). The implications of PKC inhibition on lipid metabolism and immune function further highlight its potential as a therapeutic target in resistant tumours (Park et al. 2022; Mehta and Mehta 2017). Similarly, the MEK inhibitor selumetinib has shown promise in UM (Phase III trial, SUMIT, NCT01974752), although its clinical benefit must be balanced against associated toxicities (Khan et al. 2022). Genome-editing technologies also offer an innovative approach to overcoming T cell exclusion. A first-in-human clinical trial demonstrated the feasibility of CRISPR-based, non-viral precision genome editing to engineer neoantigen-specific TCRs (neo-TCRs, Phase I) in patients with treatment-resistant solid tumours (Foy et al. 2023). This strategy enhances tumour infiltration and cytotoxicity of engineered T cells, effectively bypassing immune exclusion. However, improvements in sample acquisition, neo-TCR isolation, and optimization of initial cell dosing are required to maximize the therapeutic potential of this approach.

Finally, rational combination therapies integrating immune checkpoint inhibitors with VEGF-targeting agents (Phase I and III trials) have demonstrated significant anti-tumour synergy for patients with renal cell carcinoma and head and neck squamous cell carcinoma (Chau and Bilusic 2020; Vathiotis, Johnson, and Argiris 2021). Similarly, targeting IL-10 and STAT3 pathways (pre-clinical study) has been proposed to counteract immune suppression in resistant tumours (Yu, Mao, et al. 2018; Zou et al. 2020). Novel strategies, such as TGF-β inhibition using bifunctional Y-trap antibodies (Phase I and II trials), have also shown efficacy in promoting tumour-infiltrating lymphocyte expansion and reversing immune tolerance (Ravi et al. 2018).

Despite the promise of these combinatorial strategies, challenges remain regarding their safety, efficacy, and patient selection. Many of these approaches are associated with severe toxicities and benefit only a subset of patients, emphasizing the need for predictive biomarkers and personalized treatment regimens. Future research should prioritize refining combination therapies, exploring resistance mechanisms, and integrating immunotherapeutic innovations to optimize clinical outcomes for patients with immune-resistant tumours.

2.3 Molecular Mechanisms of Tumor Progression in Melanoma

Melanoma represents a heterogeneous group of malignancies arising from melanocytes, the pigment-producing cells primarily found in the skin, eyes, and mucosal surfaces (Schadendorf et al. 2015). Among the various subtypes, CM (Balch et al. 2024) and UM (Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020) are the most clinically significant due to their prevalence, aggressive nature, and distinct molecular and clinical characteristics. Despite these commonalities, CM and UM exhibit distinct genetic and molecular characteristics. UM, the most prevalent primary intraocular malignancy in adults, is highly aggressive and demonstrates a striking propensity for hematogenous dissemination, with the liver serving as the predominant site of metastasis in approximately 90% of cases (Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, and Shields 2020; Kaliki and Shields 2017; Coupland et al. 2013). UM does not carry the common mutations seen in CM, including BRAF, NRAS, NF1, and TERT mutations. Additionally, UM lacks a significant presence of ultraviolet (UV)-induced cytosine-to-thymine (C>T) transitions at pyrimidine dimers and has a notably low mutation burden, averaging 0.5 mutations per megabase (Mb), whereas CM has an average of 17 mutations per Mb (Johansson et al. 2016). The mechanisms underlying this organotropism are complex and involve alterations in angiogenesis, immune evasion, and hepatic-specific microenvironmental adaptations. In contrast, CM, which is far more common than UM, primarily originates from melanocytes in the skin and follows a different metastatic trajectory. Although early-stage CM is often curable with surgical excision, its advanced and metastatic forms exhibit significant morbidity and mortality due to the spread of malignant cells to distant organs, including the lungs, liver, brain, and bones (Ahmed, Qadir, and Ghafoor 2020; Naik 2021). This section explores the genetic and epigenetic alterations that drive tumor progression in both UM and CM, emphasizing the molecular features contributing to aggressiveness, metastasis, and immune suppression.

2.3.1 Key Molecular Drivers in Melanoma

The molecular mechanisms underlying melanoma pathogenesis are driven by distinct oncogenic events that influence tumor initiation, progression, and metastatic potential. The divergent molecular landscapes of UM and CM contribute to their unique biological behaviors and differential responses to therapy. These key molecular drivers shape the heterogeneity of melanoma, affecting disease prognosis and the development of targeted treatment strategies.

UM is the most common intraocular malignancy in adults (Gelmi and Jager 2024; Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020). Over recent decades, substantial progress has been made in understanding the genetic underpinnings of UM, with mutations in genes such as *GNAQ, GNA11, BAP1, SF3B1*, and *EIF1AX* emerging as critical drivers of tumorigenesis and disease progression (Figure 1, right panel) (Gelmi and Jager 2024; Fuentes-Rodriguez et al. 2024; Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020; Harbour 2012). Chromosomal abnormalities, particularly loss of one copy of chromosome 3 (monosomy 3) and gains in chromosome 8q, correlate with a poorer prognosis and increased metastatic potential (Gelmi and Jager 2024; Fuentes-Rodriguez et al. 2024; Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020; Harbour 2012). Tumor size and gene expression profiling (GEP) have refined prognostic stratification, allowing clinicians to identify high-risk patients more accurately (Gelmi and Jager 2024; Ida et al. 2022; Afshar et al. 2019; Dogrusoz and Jager 2018; Harbour and Chen 2013; Harbour 2012, 2009). Despite advancements in local treatment strategies, UM continues to be a significant cause of mortality, with approximately 50% of patients developing fatal metastatic disease, typically involving the liver (Gelmi and Jager 2024; Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020). At the cellular and molecular level, the chromosomal mutations characteristic of UM are frequently linked to immune cell infiltration within the TME, particularly T cells and macrophages that often exhibit immunosuppressive phenotypes (Herwig and Grossniklaus 2011; Bronkhorst and Jager 2012; Tosi et al. 2021; Herwig et al. 2013). The unique predilection of UM to metastasize to the liver is driven by the interaction between tumor cells and liver-specific chemokines and adhesion molecules, underscoring the critical role of the TME in UM progression and metastatic dissemination (Fuentes-Rodriguez et al. 2024; Bakalian et al. 2008; Gallenga et al. 2022; Rossi et al. 2021; Grossniklaus et al. 2016). This suggests that the TME is vital in UM disease progression and metastasis (Fuentes-Rodriguez et al. 2024; Bakalian et al. 2008; Gallenga et al. 2022; Rossi et al. 2021; Grossniklaus et al. 2016). The TME of UM comprises a complex ecosystem of tumor cells, immune cells, and stromal components that interact to promote tumor growth and immune evasion. Dysregulated signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, further contribute to UM cell proliferation and survival (Krishna et al. 2020). Cancer-associated macrophages (CAMs) and tumor-infiltrating lymphocytes (TILs) also play crucial roles in establishing an immunosuppressive milieu that allows UM cells to escape immune destruction (Krishna et al. 2020; de Azevedo et al. 2020; Fröhlich et al. 2023; Hayata et al. 2013; Souri et al. 2021). The phenomenon of epithelial-mesenchymal transition (EMT) further compounds the

complexity of UM, as it enables tumor cells to acquire mesenchymal-like properties, thereby enhancing their resistance to therapies and facilitating metastatic spread (Lv et al. 2022). Despite identifying key genetic mutations and pathways involved in UM, our understanding of how these molecular alterations reshape the TME remains incomplete. This knowledge gap is particularly relevant given the significant role the TME plays in modulating immune responses and contributing to therapeutic resistance.

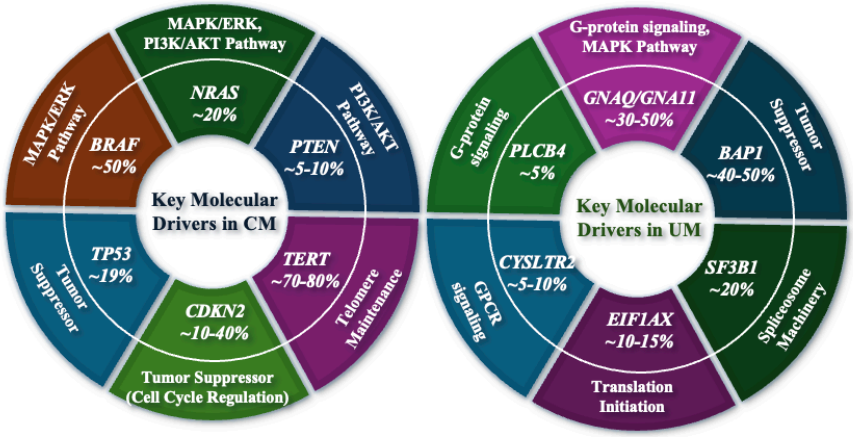

Figure 1. Key Molecular Driver Mutations Associated with CM (left panel) and UM (right panel). The left panel illustrates the principal driver mutations in CM, highlighting the activation of the MAPK/ERK and PI3K/AKT pathways. Major alterations include BRAF (~50%), NRAS (~20–25%), and NF1 (~10–15%), among others (e.g., TP53, TERT, PTEN), which are involved in tumor suppressor or cell cycle regulatory functions. The right panel shows the primary mutations in UM, emphasizing dysregulated G-protein signaling (e.g., GNAQ/GNA11, ~30–50%) and downstream pathways such as MAPK. Other key UM drivers include BAP1 (~40–50%), SF3B1 (~20%), EIF1AX (~10%), and PLCB4 or CYSLTR2 (less frequent), which together regulate transcription initiation, splicing, and cell growth. Approximate percentages indicate the prevalence of each mutation in CM or UM.

In contrast to UM, CM arises from skin melanocytes and is more genetically diverse, often characterized by a high tumor mutational burden (TMB) (Sharma et al. 2021b). Mutations in *BRAF*, *NRAS*, and *TERT* promoter regions are common in CM and play key roles in tumor initiation and progression (Figure 1, left panel) (Bai et al. 2017). The high mutational load in CM enables immune recognition and makes it more amenable to ICT (Ballotti, Cheli, and Bertolotto 2020), although resistance mechanisms, such as antigen presentation deficits and immune evasion, are increasingly recognized (Amodio et al. 2021). Epigenetic modifications also contribute to CM progression and therapeutic resistance(Jenkins, Barbie, and Flaherty 2018).

Both UM and CM share key features of TME-mediated progression. In UM, the liver-specific microenvironment facilitates metastatic spread through interactions between tumor cells and liver-resident macrophages, chemokines, and adhesion molecules (Fuentes-Rodriguez et al. 2024; Bakalian et al. 2008; Gallenga et al. 2022; Rossi et al. 2021; Grossniklaus et al. 2016). Similarly, in CM, the TME promotes EMT transition, angiogenesis, and immune suppression, which drive metastasis and resistance to therapy (Taki et al. 2021; Neophytou et al. 2021).

Additionally, the *BAP1* (BRCA1-associated protein 1) gene encodes a nuclear deubiquitinase with a critical tumor suppressor role in melanocytic tumors, including UM (Carbone et al. 2020) and specific subtypes of CM (Murali et al. 2013). Its functions span crucial cellular processes such as chromatin remodeling, DNA damage repair, cell cycle regulation, and apoptosis (Masclef et al. 2021; Louie and Kurzrock 2020; Kwon, Lee, and Lee 2023). Loss of *BAP1* expression is strongly associated with aggressive tumor behavior, immune evasion, and poor prognosis (Doria-Borrell and Pérez-García 2024; Zhang and Wu 2023; Carbone et al. 2020). In UM, *BAP1* loss occurs in approximately 47–50% of cases. It is a hallmark of high-risk Class 2 tumors, which exhibit a strong predilection for hepatic metastases and are resistant to conventional therapies (Chattopadhyay et al. 2016; Figueiredo et al. 2020; Ida et al. 2022; Wiesner, Obenauf, Murali, Fried, Griewank, Ulz, Windpassinger, Wackernagel, Loy, and Wolf 2011).

Similarly, in CM, *BAP1* loss is implicated in subtypes exhibiting heightened metastatic potential and immune suppression (Murali et al. 2013). The tumorigenic phenotype driven by *BAP1* loss results from its disruption of nuclear processes, leading to chromosomal instability, epigenetic dysregulation, and alterations in apoptotic pathways (Masclef et al. 2021; Louie and Kurzrock 2020; Kwon, Lee, and Lee 2023). Furthermore, *BAP1* loss reprograms the TME, promoting an immunosuppressive TME characterized by reduced infiltration of effector T cells and the expansion of TAMs with pro-tumorigenic profiles (Wiesner, Obenauf, Murali, Fried, Griewank, Ulz, Windpassinger, Wackernagel, Loy, Wolf, et al. 2011; Figueiredo et al. 2020). This immune-modulating effect contributes to the tumor's ability to evade immune surveillance and develop resistance to therapies, including immune checkpoint inhibitors (Figueiredo et al. 2020; Matareed et al. 2023; Strub, Ballotti, and Bertolotto 2020; Hossain et al. 2022). Beyond melanocytic tumors, *BAP1* loss is implicated in other malignancies, such as mesothelioma and renal cell carcinoma, underscoring its broad relevance in cancer biology and the necessity of studying its pathways to identify novel therapeutic strategies (Nasu et al. 2015; Peña-Llopis et al. 2012; Mochel et al. 2015; Leblay et al. 2017). Targeting *BAP1*-driven mechanisms represents a critical frontier in understanding melanoma progression, immune evasion, and resistance to therapy, with potential applications in developing precision medicine approaches for these challenging malignancies.

In summary, both UM and CM are driven by complex genetic and epigenetic alterations that shape their progression, aggressiveness, and therapeutic responses. While UM is characterized by chromosomal abnormalities like monosomy 3 and 8q gains and mutations in *GNAQ, GNA11*, and *BAP1* (Gelmi and Jager 2024; Fuentes-Rodriguez et al. 2024; Jager, Shields, Cebulla, Abdel-Rahman, Grossniklaus, Stern, Carvajal, Belfort, Jia, Shields, et al. 2020; Harbour 2012), CM exhibits a higher mutational burden with frequent mutations in *BRAF, NRAS*, and *TERT* (Bai et al. 2017). These genetic alterations drive tumorigenesis and significantly influence the TME, facilitating immune evasion, metastatic dissemination, and resistance to therapies. The role of *BAP1* loss stands out as a shared driver of aggressive phenotypes in both UM and CM, highlighting its importance in tumor progression and immune modulation. Despite advancements in our understanding of these molecular drivers, significant knowledge gaps remain, particularly in elucidating how these alterations interact with the TME to influence disease outcomes. Future research integrating multi-omic approaches and advanced technologies like single-cell sequencing will be critical in uncovering these interactions and developing innovative therapeutic strategies for both UM and CM.

2.3.2 Major Signaling Pathways in Melanoma Pathogenesis and Progression

Melanoma is characterized by genetic mutations that activate critical signaling pathways, driving oncogenic transformation, uncontrolled proliferation, and metastatic progression (Lawrence, Nguyen, et al. 2014). Among these, the MAPK pathway, also known as the RAS/RAF/MEK/ERK cascade, plays a pivotal role (Wellbrock and Hurlstone 2010). This pathway is primarily activated through receptor tyrosine kinases (RTKs) or integrin-mediated interactions with the extracellular matrix (ECM), leading to a cascade of phosphorylation events that culminate in ERK activation (Burotto et al. 2014). Once translocated to the nucleus, ERK modulates transcription factors that regulate cellular proliferation and differentiation (Burotto et al. 2014). Mutations in BRAF, notably the V600E variant, induce constitutive activation of this pathway, leading to uncontrolled cellular proliferation (Wellbrock and Hurlstone 2010). Similarly, NRAS mutations prevent GTP hydrolysis, maintaining the pathway in a hyperactive state (Mandalà, Merelli, and Massi 2014). Loss-of-function mutations in NF1 further contribute to the sustained activation of this oncogenic cascade, underscoring its central role in melanoma development (Gosman, Țăpoi, and Costache 2023). In addition to the MAPK pathway, the phosphoinositide 3-kinase (PI3K)/AKT pathway is a crucial driver of melanoma progression (Chamcheu et al. 2019). Activation of this pathway occurs upon insulin-like growth factor 1 (IGF-1) binding to its receptor (IGFR-1),

leading to increased production of phosphatidylinositol phosphate (PIP3) and subsequent recruitment of 3-phosphoinositide-dependent kinase 1 (PDK1) (Motwani and Eccles 2021). This activation facilitates phosphorylation of AKT, which in turn regulates proteins involved in cell survival, migration, and proliferation (Motwani and Eccles 2021). PTEN, a tumor suppressor that counteracts PI3K signaling, is frequently deleted or inactivated in melanoma, allowing unchecked AKT activation and contributing to tumor progression (Chawra et al. 2024). The Wnt/β-catenin signaling pathway is another key player in melanoma biology, governing processes such as proliferation, differentiation, and migration (Gajos-Michniewicz and Czyz 2020). Canonical Wnt signaling is initiated through interaction with frizzled (FZD) receptors and lipoprotein receptor-related proteins (LRP5/6), which leads to β-catenin accumulation and nuclear translocation (Komiya and Habas 2008). There, β-catenin interacts with transcription factors to regulate gene expression involved in melanoma progression (Komiya and Habas 2008). Aberrant activation of this pathway, often through overexpression of WNT5A, promotes metastasis by increasing melanoma cell motility (Gajos-Michniewicz and Czyz 2020). Other G-protein-coupled receptors (GPCRs) such as MC1R, EDNR, and CXCR contribute to melanoma progression by modulating various signaling cascades (Carlson et al. 2007). Similarly, transforming growth factor-beta (TGF-β) signaling promotes the EMT by upregulating N-cadherin and downregulating E-cadherin, enhancing melanoma cell invasiveness (Loh et al. 2019). Beyond these significant pathways, additional genetic alterations play critical roles in melanoma development. Cyclin-dependent kinase inhibitor 2A (CDKN2A) mutations disrupt cell cycle regulation, leading to uncontrolled proliferation (Ding et al. 2020). Loss of RB1 function and aberrations in CCND1 and CDK4/6 further promote cell cycle dysregulation (Kong et al. 2017). The interplay of these pathways underscores the complexity of melanoma pathogenesis and highlights potential therapeutic targets.

UM, on the other hand, is driven by a series of mutually exclusive genetic mutations that converge on a limited set of oncogenic signaling pathways, fundamentally shaping its pathogenesis and progression (Moore et al. 2016). One of the primary pathways implicated in UM involves the activation of GPCRs, particularly CysLT2R, which transmit oncogenic signals through *GNAQ* and *GNA11* mutations (Park et al. 2018). These alterations lead to the activation of key downstream effectors such as phospholipase C-β (PLCβ), protein kinase C (PKC), and ADP-ribosylation factor 6 (ARF6), ultimately influencing critical cellular pathways including the MAPK cascade, the PI3K/AKT survival pathway, and Rho GTPase signaling (Yoo et al. 2016). The MAPK pathway, in particular, is hyperactivated in UM, with oncogenic *GNAQ/GNA11* signaling initiating a cascade that stimulates RasGRP3, promoting RAS activation and sustained MAPK signaling, a hallmark of UM proliferation and survival (Van Raamsdonk et al. 2009). Similarly,

the PI3K/AKT pathway plays a crucial role in UM tumorigenesis, often activated through receptor tyrosine kinase (RTK) signaling and loss of PTEN, which enhances AKT/mTOR activity and promotes cell survival (Abdel-Rahman et al. 2006). Another significant signaling axis in UM is the TRIO/Rho/Rac GTPase pathway, which further drives UM progression and metastasis through cytoskeletal remodeling and transcriptional regulation via the Hippo-YAP/TAZ pathway (Park et al. 2018). Notably, ARF6 functions as a critical downstream effector of oncogenic *GNAQ* signaling, modulating multiple pathways, including PLCβ-PKC, MAPK, and β-catenin signaling, thereby contributing to the aggressive nature of UM (Yoo et al. 2016). Despite the extensive involvement of these pathways, the precise interplay between them and their role in UM progression remain areas of active investigation. The integration of secondary genetic alterations, such as *BAP1* loss and monosomy 3, further modifies the oncogenic landscape, leading to distinct prognostic subtypes of UM with varying molecular dependencies and potential therapeutic vulnerabilities (Robertson et al. 2017). Understanding the UM pathway at a mechanistic level is crucial for developing targeted therapies that can effectively disrupt these oncogenic networks and improve clinical outcomes.

2.3.3 Transcriptional and Epigenetic Regulation in Melanoma Progression

Transcriptional control plays a significant role in melanoma development, with key regulators such as *SOX10*, *MITF*, and Notch orchestrating critical aspects of melanocyte differentiation and tumor progression (Guo, Wang, and Li 2021). *SOX10*, a neural crest transcription factor, is essential for melanocyte lineage maintenance and melanoma cell survival (Goding 2000). Loss of *SOX10* disrupts tumor growth and migration, while its upregulation has been linked to resistance against targeted therapies such as BRAF inhibitors (Han et al. 2018). *SOX10* exerts its effects by activating *MITF*, a master regulator of melanocyte differentiation that governs genes involved in cell cycle progression, metabolism, and tumor survival (Hartman and Czyz 2015). The expression of *MITF* follows a "rheostat model," where low levels are associated with increased invasiveness, while moderate expression promotes proliferation (Carreira et al. 2006). Additionally, *MITF* downregulation has been implicated in resistance to BRAF-targeted therapies (Czarnecka et al. 2020). The Notch signalling pathway, another major regulator of melanoma pathogenesis, is highly active in melanoma cells (Pinnix et al. 2009). It promotes tumor growth through multiple mechanisms, including crosstalk with the MAPK pathway and β-catenin signaling (Balint et al. 2005). Increased Notch activity also enhances angiogenesis and contributes to therapy resistance (Bedogni et al. 2008). Similarly, Wnt/β-catenin signaling plays a dual role, promoting

melanoma progression in some contexts while acting as a tumor suppressor in others (Uka et al. 2020). This pathway's complex involvement in melanoma underscores further research's need to delineate its precise role in different genetic backgrounds (Webster and Weeraratna 2013; Gallagher et al. 2013).

Beyond genetic mutations and transcriptional modifications, epigenetics is increasingly recognized as a fundamental regulatory mechanism governing melanoma biology and its associated signaling pathways. Epigenetic modifications encompass heritable alterations in gene expression that occur without modifications to the DNA sequence (Gibney and Nolan 2010). These changes primarily arise through covalent modifications of histone tails or nucleosome complexes, thereby reshaping chromatin structure and influencing gene expression patterns (Goldberg, Allis, and Bernstein 2007). DNA methylation, histone modifications, non-coding RNAs, and, more recently, identified N6-methyladenosine (m6A) RNA methylation represent key epigenetic mechanisms in melanoma pathogenesis, with significant correlations to disease progression (Moran et al. 2018).

DNA methylation, one of the most extensively studied epigenetic modifications in oncology (Moran et al. 2018), involves the enzymatic addition of a methyl group to the cytosine residues of CpG dinucleotides, a process mediated by DNA methyltransferases (DNMTs). This modification is dynamically counterbalanced by the ten-eleven translocation (TET) enzyme family, which catalyzes the oxidation of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), ultimately promoting DNA demethylation (Tahiliani et al. 2009). Additionally, hypermethylation of tumor suppressor gene promoters, such as PTEN, P16INK4A, P14ARF, RASSF1A, and MGMT, has been widely documented in melanoma (Micevic, Theodosakis, and Bosenberg 2017). Such epigenetic silencing leads to functional deficiencies in these genes, thereby facilitating melanoma progression. Genome-wide promoter methylation analyses have further identified differentially methylated genes between melanoma and benign nevi, enriching key pathways governing differentiation, immune evasion, epithelial-to-mesenchymal transition, and metabolic reprogramming (Guo, Wang, and Li 2021).

Chromatin structure transitions between transcriptionally inactive heterochromatin and transcriptionally active euchromatin (Morrison and Thakur 2021). Histone modifications, primarily on histone N-terminal tails, regulate chromatin accessibility and influence fundamental biological processes, including gene transcription and DNA repair (Sadakierska-Chudy and Filip 2015; Bannister and Kouzarides 2011). Key histone modifications include acetylation, methylation, phosphorylation, and ubiquitination (Albini, Zakharova, and Ait-Si-Ali 2019). Histone acetylation is mediated by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs), modulating gene activation and repression, respectively (Bennett and Licht 2018). Similarly, histone methylation, occurring at

lysine or arginine residues, is regulated by methyltransferases and demethylases (Neganova et al. 2022). Systematic epigenomic profiling has revealed widespread loss of histone acetylation and H3K4 methylation at regulatory regions of cancer-associated genes in melanoma (Fiziev et al. 2017). Concurrently, dysregulated expression of HATs and HDACs has been implicated in tumorigenesis (Wu, Qiu, et al. 2020). Pharmacological restoration of histone acetylation through HDAC inhibitors has demonstrated substantial tumor-suppressive potential, with synergistic effects observed in combination with radiotherapy, MAPK pathway inhibitors, and immunotherapies via modulation of DNA repair, reactive oxygen species (ROS) generation, and PD-L1 expression (Guo, Wang, and Li 2021). Investigational HDAC inhibitors are currently being evaluated in early-phase clinical trials for refractory solid tumors, including melanoma (Rossi et al. 2024). Additionally, the HAT P300 has been identified as a key regulator of melanoma progression, with pharmacological inhibition demonstrating potent anti-tumor effects, particularly in MITF-high melanoma subtypes (Yan et al. 2013; Wang et al. 2018). Interestingly, P300 activation has been implicated in resistance to BRAF-targeted therapies, further supporting its potential as a therapeutic target (Zhang et al. 2021).

Histone methylation plays a complex role in melanoma progression, influenced by key enzyme families such as SETDB1, DOT1L, EZH2, and LSD1 (Sutopo, Kim, and Cho 2023). The histone methyltransferase SETDB1 catalyzes H3K9 methylation and is frequently amplified in melanoma, exhibiting oncogenic properties (Orouji et al. 2019). Metabolic reprogramming has been shown to elevate H3 trimethylation levels, enhancing metastatic capacity, which can be reversed through SETDB1 inhibition (Torrano et al. 2019; Orouji et al. 2019). In contrast, DOT1L, responsible for H3K79 methylation, is frequently mutated in melanoma, impairing nucleotide excision repair (NER) machinery recruitment and accelerating UV-induced melanoma formation (Zhu et al. 2018). EZH2, another histone methyltransferase, promotes melanoma progression by catalyzing H3K27 trimethylation, suppressing tumor suppressor genes (Simon and Lange 2008). EZH2 overexpression disrupts cilium function and PGC1α activity, driving Wnt/β-catenin and YAP signaling activation, thereby promoting metastasis (Luo et al. 2020; Zingg et al. 2018). Moreover, EZH2 is implicated in immunotherapy resistance, as it downregulates antigen presentation, interferon-γ signaling, and immune cell infiltration (Zingg et al. 2017). Targeting EZH2 may therefore enhance melanoma responsiveness to immunotherapies. Furthermore, H3K9 demethylases LSD1 and JMJD2C counteract oncogene-induced senescence, facilitating melanoma development (Yu, Schleich, et al. 2018). Interestingly, LSD1 depletion has increased tumor immunogenicity by promoting endogenous retroviral element (ERV) transcription and suppressing RNA-induced silencing complex activity, ultimately enhancing response to anti-PD-1 therapy (Sheng et al. 2018). These findings underscore the dual role of histone

methylation in melanoma, functioning as a tumor suppressor during initiation while fostering tumor progression at later stages.

2.4 Reverse Translational Research and Multi-Omic Approaches in Melanoma

After roughly half a century, when Baruj Benacerraf and George D. Snell first discovered the genes related to immune responses (Benacerraf and McDevitt 1972; Snell 1981), cancer immunology took unprecedented steps toward understanding the importance of antigen presentation and the connections between innate and adaptive immunity. The primordial works of tumor immunology opened the portals of a new frontier in cancer research, where we can harness the body's immune system to fight cancer. Immunotherapy has revolutionized the treatment of advanced metastatic solid tumors. ICTs that target immune regulators such as CTLA-4 and PD-1 have proven highly effective in some cancers, but not all tumors respond similarly. A particularly challenging subset of cancers is "cold" tumors, which evade immune detection and resist immunotherapy (Queirolo et al. 2019; Sharma and Allison 2015a). Yet, significant challenges persist: therapeutic responses vary widely among individuals, drug resistance often emerges following initial remission, and the molecular mechanisms governing tumor-immune interactions remain incompletely understood. In this evolving landscape, the concept of reverse translational research, where clinical insights feed directly back into laboratory investigations, has become increasingly influential (Sharma and Allison 2015a, 2015b; Sharma and Allison 2020).

At its core, reverse translational research strives to narrow the gap between bedside observations and bench-based experimentation by leveraging patient-derived data to refine mechanistic hypotheses, optimize experimental models, and identify clinically meaningful biomarkers (Sharma and Allison 2015a, 2015b; Sharma and Allison 2020; Ribas and Wolchok 2018; Tumeh, Harview, Yearley, Shintaku, Taylor, Robert, Chmielowski, Spasic, Henry, and Ciobanu 2014). The iterative feedback loop that characterizes reverse translational research entails not only the application of clinical findings to laboratory studies but also the return of those laboratory insights to the design of new clinical trials (Sheng et al. 2020). This dynamic synergy enables investigators to address real-world obstacles that arise in patient care, such as low response rates and immune-related adverse events, and to discover molecular pathways that can be selectively targeted or combined with existing therapies (Melero et al. 2015).

Within melanoma, this paradigm is further enriched by multi-omic technologies (McGranahan and Swanton 2015). These high-throughput approaches interrogate tumor and immune cells at multiple levels of regulation, from gene expression to protein function and metabolic pathways (Tirosh, Izar, Prakadan, Wadsworth, Treacy, Trombetta, Rotem, Rodman, Lian, and Murphy 2016; Jerby-Arnon et al. 2018). By integrating datasets from transcriptomic, epigenomic, proteomic, and metabolomic studies, researchers can obtain a more holistic view of the TME,

illuminating both the intricacies of immune cell infiltration and the heterogeneous nature of melanoma cells themselves (Li et al. 2016; Litchfield et al. 2020). Such multi-omic insights are increasingly being used to stratify patients based on molecular signatures, paving the way for personalized treatment regimens that improve upon the current standard of care (Luke et al. 2017b). Ultimately, the combined force of reverse translational research and multi-omic profiling holds the promise of delivering more precise, durable, and safe treatment strategies, marking a pivotal shift in how melanoma is studied and managed (Sharma and Allison 2020; Ribas and Wolchok 2018).

2.4.1 Foundations of Reverse Translational Research Approach: Bedside to Bench

Reverse translational research emerged from the recognition that patient-specific factors, including genetic mutations, epigenetic modifications, and variations in immune cell composition, can unmask critical vulnerabilities in melanoma that may not be apparent in standard bench-based models (Sharma and Allison 2015b; Champiat et al. 2017; Chang et al. 2015) Early clinical observations of differential responses to immune checkpoint blockade, including rare hyper progression and dramatically prolonged survival, underscored the limitations of conventional preclinical systems in capturing the full spectrum of tumor–immune interactions (Champiat et al. 2017; Wolchok et al. 2013). Consequently, clinical findings such as unusual response trajectories, patterns of primary and acquired resistance, and unexpected synergy between therapeutic agents have become pivotal launch points for laboratory investigations designed to decipher the underlying molecular and cellular mechanisms (Hugo et al. 2016; Sharma and Allison 2015b).

One prominent example is the striking tumor regressions observed in a subset of patients treated with anti-CTLA-4 antibodies, which catalyzed deeper mechanistic studies into how CTLA-4 blockade modulates T-cell priming, memory formation, and resistance pathways (Phan et al. 2003; Peggs et al. 2006). James Allison and Padmanee Sharma have prominently championed this reverse translational framework, emphasizing how systematic evaluation of clinical data can pinpoint new therapeutic targets and combinatorial strategies to be tested in refined preclinical systems (Sharma and Allison 2015a, 2015b; Sharma and Allison 2020). Leveraging insights from patients who develop resistance to anti-PD-1 therapies, researchers have constructed genetically engineered mouse models to explore signaling cascades, such as β-catenin activation, that foster T-cell exclusion or promote tumor survival (Spranger, Bao, and Gajewski 2015b; Gajewski, Schreiber, and Fu 2013). Likewise, clinical observations of metabolic reprogramming in immune-resistant tumors have fueled laboratory studies on the role of lactate dehydrogenase and

hypoxia-driven pathways in shaping tumor immunogenicity (Chang et al. 2015; Ho et al. 2015).

By continuously iterating between patient samples and bench-based experiments, reverse translational research ensures that potentially transformative clinical anecdotes, such as unexpected synergy between checkpoint inhibitors or rare cases of hyperprogression, do not remain isolated events but instead serve as catalysts for advancing melanoma biology (Champiat et al. 2017). This bidirectional process refines not only the design of experimental models but also the scope of subsequent clinical trials, as laboratory findings on resistance mechanisms or new immunomodulatory targets feed forward into next-generation therapeutic strategies (Chen and Mellman 2017b). In doing so, reverse translational research both accelerates and enriches the quest for more precise, durable, and broadly effective interventions against melanoma.

2.4.2 Multi-Omic Tools for Uncovering Novel Targets and Biomarker Validation

The success of reverse translational research in melanoma is increasingly linked to multi-omic techniques, which offer comprehensive insights into the molecular and cellular constituents of the TME (Lawrence, Stojanov, et al. 2014). Single-cell RNA sequencing (scRNA-seq) stands at the forefront of this revolution, enabling the dissection of heterogeneous tumor cell populations and the identification of subtly distinct, functionally significant subclones within individual tumors (Tirosh, Izar, Prakadan, Wadsworth, Treacy, Trombetta, Rotem, Rodman, Lian, and Murphy 2016; Durante et al. 2020). By capturing transcriptional states at single-cell resolution, scRNA-seq allows researchers to track tumor evolution and immune editing over time, revealing specialized cell types, such as exhausted T cells, tumor-associated macrophages, and immunoregulatory fibroblasts, that can drive immune evasion and therapeutic resistance (Jerby-Arnon et al. 2018).

Alongside scRNA-seq, bulk RNA-seq analysis remains a critical pillar of large-scale genomic studies. It enables the identification of global transcriptional signatures and broad stratification of patients based on expression profiles (Akbani et al. 2015). Bulk sequencing approaches are especially valuable for uncovering dominant molecular patterns that correlate with prognosis, relapse, or sensitivity to immune checkpoint blockade, thus guiding biomarker discovery and therapeutic development (Riaz et al. 2017).

Beyond transcriptomics, epigenetic profiling tools such as ATAC-seq uncover chromatin accessibility patterns associated with tumor aggressiveness, immune infiltration, and treatment outcomes (Llinàs-Arias et al. 2023). Proteomic and metabolomic approaches further reveal post-translational modifications and

metabolic dependencies that can be pharmacologically targeted, pinpointing pathways that mediate immune escape or metastatic dissemination (Chang et al. 2015). By integrating these multi-omic datasets, researchers construct predictive gene signatures correlating with clinical responses to checkpoint blockade or targeted therapies (Hugo et al. 2016).

Critically, hypothesized biomarkers emerging from multi-omic analyses, ranging from novel immune checkpoints to resistance-related enzymes and transcription factors, are validated in PDX, humanized mouse models, or genetically engineered systems, ensuring that findings are functionally relevant in preclinical settings (Gao et al. 2015). This closed-loop process epitomizes reverse translational research by grounding mechanistic hypotheses in patient data while simultaneously refining therapeutic targets to address real-world clinical challenges (Sharma and Allison 2020).

Ultimately, multi-omic tools expand the scope and depth of reverse translational research by clarifying the molecular interactions that govern tumor progression and immune responses (Chen and Mellman 2017b). As new high-throughput technologies evolve, incorporating spatial transcriptomics, high-dimensional cytometry, and advanced computational modelling, researchers can generate even more nuanced, multidimensional maps of tumor–immune crosstalk (Keren et al. 2019). This expanded toolkit will accelerate the identification of actionable targets and spur the development of next-generation immunotherapies designed to overcome the multifaceted obstacles of melanoma management (Ribas and Wolchok 2018). Through this iterative interplay between clinical observations and multi-omic discovery, the field stands poised to deliver personalized, effective, and enduring treatment strategies for melanoma patients (Luke et al. 2017b).

2.4.3 Insights from Single-Cell Profiling in Cutaneous and Uveal Melanoma

The field of oncology and pathology is undergoing transformative shifts with the integration of precision medicine as a clinical standard (McCart Reed et al. 2020). Single-cell profiling has emerged as a cornerstone of this shift, offering high-throughput molecular characterization of TME and cell subpopulations (Durante et al. 2020). Sc-RNAseq, in particular, provides unparalleled insights into cellular diversity and functional states within tumors. However, its limitation lies in the disruption of spatial organization, making it challenging to analyze cell-cell interactions within their native context. This limitation is addressed by digital spatial profiling (DSP), a powerful tool that preserves tissue architecture while enabling spatially resolved quantification of gene expression and protein abundance (Merritt et al. 2020). DSP utilizes highly multiplexed assays like the nCounter® barcoding

platform to visualize cell-to-cell interactions and generate context-specific data complementing scRNAseq findings (McCart Reed et al. 2020). While DSP is currently constrained by cost and technical demands, its integration with sc-RNAseq can enhance cluster analyses by spatially resolving molecularly defined cells. This approach is particularly valuable for TME studies, where the spatial dynamics of immune and stromal cells play crucial roles in tumor progression and immune evasion (Yan et al. 2022).

Despite these advancements, translating single-cell findings into clinical applications requires scalable and cost-effective methods. Multiplex immunohistochemistry (mIHC) addresses this need by allowing the simultaneous staining of multiple protein targets within a single tissue section. This technique bridges the gap between molecular discoveries and clinical implementation by translating gene expression profiles into clinically relevant protein markers, facilitating diagnostic and prognostic workflows (Tan et al. 2020; Yeong et al. 2020).

The combined application of scRNAseq, DSP, and mIHC provides a robust framework for comprehensive TME analysis, enabling researchers to correlate gene expression profiles with spatially resolved protein data (Zhu et al. 2024). This integrated approach offers profound insights into molecular pathways and cellular interactions driving tumor progression, metastasis, and therapeutic resistance (Lee et al. 2020). Such integration is particularly critical in UM, where improved prognostic tools and therapeutic strategies are urgently needed.

2.4.3.1 Single-Cell Transcriptomics and Genomics in Melanoma Progression

Single-cell transcriptomics and genomics have transformed melanoma research by offering unprecedented insights into tumor heterogeneity, evolutionary trajectories, and mechanisms of therapeutic resistance. Traditional bulk sequencing approaches average out the genomic and transcriptomic signals from mixed cell populations, masking the diversity and rare subpopulations that may drive disease progression. scRNA-seq and single-cell DNA sequencing (scDNA-seq) have emerged as powerful tools to deconvolute this complexity and provide a more detailed understanding of the molecular landscape of melanoma at the level of individual cells.

Melanoma exhibits significant intra- and inter-tumoral heterogeneity, influencing disease progression and treatment response (Ahmed and Haass 2018). ScRNA-seq has enabled the identification of distinct transcriptional states in melanoma cells, revealing that tumors consist of multiple coexisting cellular subpopulations with diverse functional roles (Joshi et al. 2014; Gerber et al. 2017; Tirosh, Izar, Prakadan, Wadsworth, Treacy, Trombetta, Rotem, Rodman, Lian, Murphy, et al. 2016). These studies have shown that melanoma cells exist along a

phenotypic spectrum ranging from proliferative to invasive states, with a subset of cells displaying an intermediate phenotype that can transition between these states. This plasticity underlies melanoma's ability to metastasize and evade therapy (Ho et al. 2018). Furthermore, scRNA-seq has been instrumental in distinguishing between benign nevi and malignant melanoma. The study from Kunz et al. (Kunz et al. 2018) used scRNA-seq to profile benign nevus cells and compare them with melanoma cells, identifying key molecular changes that drive early malignant transformation. By mapping the gene expression changes during tumorigenesis, this study provided new insights into the molecular determinants of melanoma initiation. In addition to defining tumor heterogeneity, scRNA-seq has facilitated the identification of melanoma subtypes with distinct molecular signatures. Gene expression profiling has revealed subtype-specific transcriptional programs correlating with patient prognosis and treatment response (Gerber et al. 2017).

While scRNA-seq provides insights into transcriptional heterogeneity, scDNA-seq has been employed to characterize the genetic landscape of melanoma at single-cell resolution. Whole-genome amplification (WGA) and next-generation sequencing (NGS) approaches have allowed researchers to trace clonal evolution and identify mutational signatures associated with therapy resistance (Gawad, Koh, and Quake 2014; Yu et al. 2014). One major application of scDNA-seq in melanoma research is the analysis of copy number variations (CNVs), which play a critical role in tumor progression and drug resistance. Whole-genome sequencing of single melanoma cells has revealed that resistant subclones often harbor unique CNVs that distinguish them from treatment-sensitive populations (Knouse, Wu, and Hendricks 2017; Lawson et al. 2018; Tan et al. 2019). These findings suggest that the selective pressure of therapy drives the expansion of resistant clones, necessitating adaptive treatment strategies to overcome resistance.

Furthermore, single-cell genomic analyses have helped elucidate the mutational landscape of metastatic melanoma. Studies have identified recurrent mutations in driver genes such as BRAF, NRAS, and PTEN and novel mutations in epigenetic regulators and transcription factors (Luke et al. 2017a; Ho et al. 2018). By reconstructing the evolutionary trajectories of melanoma subclones, these studies have provided valuable insights into the dynamics of tumor progression and metastasis.

2.4.3.2 Dissecting the Tumor Microenvironment Using Single-Cell Technologies

The TME plays a crucial role in melanoma progression, immune evasion, and response to therapy. It comprises various cell types, including tumor cells, immune cells, fibroblasts, and endothelial cells, which interact in a dynamic and context-

dependent manner. Recent advancements in single-cell profiling technologies, including scRNA-seq and spatial transcriptomics, have provided unprecedented insights into the heterogeneity of the TME (An et al. 2024; Yuan et al. 2021). These techniques have enabled researchers to dissect the cellular and molecular composition of the TME at an unparalleled resolution, revealing distinct immune cell states, fibroblast subtypes, and endothelial cell populations that influence tumor progression and therapeutic response. Through single-cell analyses, novel immune resistance mechanisms have been uncovered, such as the emergence of dysfunctional or exhausted T cell populations, altered antigen presentation machinery, and interactions between tumor cells and stromal components that drive immune escape.

2.4.3.3 Single-Cell Profiling in Cutaneous Melanoma

Given that CM is significantly more prevalent than UM, single-cell technologies have been more extensively applied to the study of CM (Joshi et al. 2014; Gerber et al. 2017; Tirosh, Izar, Prakadan, Wadsworth, Treacy, Trombetta, Rotem, Rodman, Lian, Murphy, et al. 2016). This has allowed researchers to successfully identify and characterize malignant clones, subclones, and circulating tumor cell (CTC) transcriptomic profiles in CM, offering more profound insights into tumor heterogeneity and progression (Joshi et al. 2014; Gerber et al. 2017; Tirosh, Izar, Prakadan, Wadsworth, Treacy, Trombetta, Rotem, Rodman, Lian, Murphy, et al. 2016). Among the various single-cell methodologies, scRNA-seq remains the predominant approach for profiling melanoma at the molecular level. A notable scRNA-seq study compared benign nevus cells with malignant melanoma cells to elucidate the key molecular mechanisms underpinning early melanoma transformation (Kunz et al. 2018). Additionally, integrating scRNA-seq with pseudotime analysis in short-term melanoma cultures at varying stages of malignancy has facilitated the identification of dynamic gene expression changes associated with disease progression. These findings suggest that cutaneous melanoma development follows a distinct molecular trajectory, which may be leveraged to identify novel therapeutic targets to halt disease advancement (Loeffler-Wirth et al. 2018). Beyond tumor evolution, scRNA-seq has been instrumental in classifying melanoma subtypes based on unique gene expression signatures. These transcriptomic insights have refined the stratification of melanoma cases, potentially improving diagnostic accuracy and personalized treatment strategies for CM patients (Gerber et al. 2017).

The introduction of BRAF inhibitors and immunotherapy has significantly improved the treatment landscape for metastatic melanoma. However, intratumor heterogeneity presents a significant challenge, influencing resistance to these targeted therapies. Investigations into tumor heterogeneity using genome-wide

transcriptomics, single-cell phenotyping, and functional proteomics have provided valuable insights into the transition from drug-sensitive to drug-resistant melanoma cells. Such studies have identified key signaling pathways implicated in therapy resistance, which may inform the development of next-generation therapeutic interventions (Luke et al. 2017a). Moreover, single-cell transcriptomic profiling has unveiled several molecular pathways contributing to cellular adaptations in response to BRAF inhibitors (Luke et al. 2017a). Platforms such as Fluidigm C1 and 10× Genomics have been utilized to detect rare subpopulations of drug-resistant melanoma cells, with scRNA-seq combined with clustering algorithms aiding in characterizing resistance-associated signatures (Ho et al. 2018).

In the context of metastatic melanoma, single-cell isolation techniques have also been applied to the study of CTCs, leading to the identification of antibodies capable of enriching melanoma CTC populations (Ulmer et al. 2004; Karakousis, Yang, and Xu 2013). Initially developed for CM, these methodologies hold significant translational potential for UM research. Notably, studies have demonstrated a correlation between elevated melanoma CTC counts and poorer clinical outcomes, including reduced survival rates, increased tumor burden, and heightened proliferative activity in metastatic lesions (Ulmer et al. 2004; Karakousis, Yang, and Xu 2013; De Souza, Robertson, and Robertson 2017). These findings underscore the importance of CTC analysis in improving prognostic assessments and developing more effective therapeutic strategies for melanoma patients.

2.4.3.4 Single-Cell Profiling in Uveal Melanoma

Single-cell approaches for studying UM are still in their early stage, with only a limited number of studies available. Nevertheless, recent efforts have begun to explore single-cell transcriptomics to gain deeper insight into UM biology. In one of the first single-cell studies on UM, Durante et al. analyzed 59,915 tumor and non-neoplastic single cells isolated from eight primary and three metastatic UM samples using scRNA-seq on the 10× Genomics platform (Durante et al. 2020). Their findings demonstrated that gene expression patterns clustered similarly to the Class 1 and Class 2 GEP classifications used for UM prognostication. More importantly, they identified that among the 12 genes included in the DecisionDx-UM™ test, EIF1B, HTR2B, ECM1, CDH1, and ROBO1 were predominantly expressed in tumor cells, while SATB1 was primarily expressed in T cells. The remaining six genes were expressed in both tumor and immune cell populations (Durante et al. 2020).

CNV analysis at the single-cell level provided novel insights, challenging the previous notion that canonical CNVs occur as early events in UM evolution (Arozarena and Wellbrock 2019). Instead, their study indicated that UM cells

accumulate genetic alterations throughout tumor progression. While Class 1 UMs exhibited loss of 1p, 3, and 8p, Class 2 tumors were characterized by the gain of 6p and 6q. Additionally, five tumors displayed an initial gain of 8q, followed by the acquisition of 8p. Their findings also confirmed that signature driver mutations are associated with distinct CNV subclones, reinforcing the role of genomic evolution in UM pathogenesis. A key immunological finding of this study was that tumor-infiltrating immune cells predominantly expressed the checkpoint marker LAG3 rather than PD1 or CTLA4. This suggests that LAG3 could serve as a novel therapeutic target for immune checkpoint blockade in high-risk UM patients, who generally respond poorly to PD1 and CTLA4 inhibitors (Jindal 2018). This study exemplifies the power of single-cell technologies in refining our understanding of UM biology and validating insights obtained from bulk transcriptomic approaches (Karlson et al. 2020).

The detection and characterization of CTCs in UM have been extensively studied, with various melanoma-associated markers such as chondroitin sulfate proteoglycan (Ulmer et al. 2008; Suesskind et al. 2011; Eide et al. 2015), melanoma cell adhesion molecule (CD146), and high-molecular-weight melanoma-associated antigen (MHW-MAA) being utilized for identification (Tura et al. 2014). The standardized CellSearch® system isolates UM CTCs by immunomagnetic enrichment of CD146-positive cells followed by staining with MHW-MAA, while excluding leukocytes (CD45) and endothelial cells (CD34(Angi et al. 2013; Anand et al. 2019; Bidard et al. 2014; Bande et al. 2015; Terai et al. 2015).

2.5 Emerging Preclinical Models in Melanoma: Bridging Reverse Translational Insights and CRISPR Innovations

Building on the foundational concepts of reverse translational research introduced in Section 2.3, there is an increasing imperative to transform patient-derived discoveries into robust laboratory models that more accurately capture the complexity of melanoma. Traditional preclinical platforms, including various *in vivo* and *in vitro* systems, have provided important insights into tumor growth, metastatic spread, and therapeutic vulnerability (Stei, Loeffler, Holz, et al. 2016; van den Bosch et al. 2023). However, as multi-omic and single-cell analyses continue to reveal melanoma's profound genetic and immunological heterogeneity, a key question arises: How can these clinical insights be harnessed to construct models embody the molecular and microenvironmental diversity seen in patients? Addressing this question, a growing body of work employs CRISPR-based genome-editing strategies to recreate patient-specific mutations, thus narrowing the gap between bedside observations and mechanistic investigations (Laurent et al. 2024).

Among the genetic perturbations identified through reverse translational approaches, *BAP1* has become an especially significant focus in uveal melanoma, where its loss is frequently associated with disease progression and poorer clinical outcomes (Masoomian, Shields, and Shields 2018). The crucial role of *BAP1* has led to an emphasis on developing accurate laboratory systems capable of capturing its functional disruption. CRISPR-mediated manipulation of *BAP1* and other clinically relevant genes offers an avenue for refining established preclinical models, allowing researchers to test targeted therapies in settings that more faithfully reflect the biology of human tumors (Artegiani et al. 2019; Waters et al. 2024). This section examines the traditional *in vivo* and *in vitro* models that form the basis of melanoma research, illustrating how reverse translational discoveries, particularly those enabled by CRISPR-driven techniques, are guiding the evolution of improved preclinical frameworks for both UM and CM.

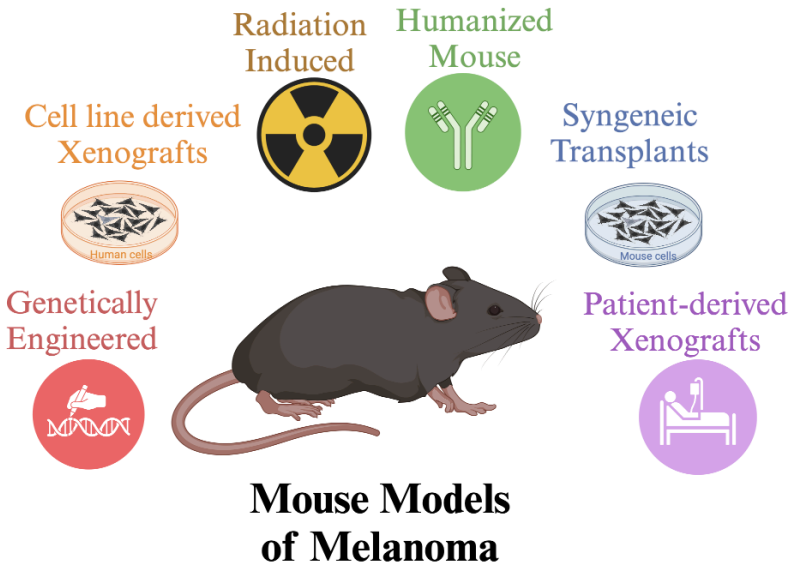

Figure 2. **Different mouse models used in melanoma research represent a distinct approach for studying tumor development, progression, and treatment responses**. Radiation-induced models (yellow) involve the exposure of mice to radiation to induce genetic mutations that mimic melanoma formation. Humanized mouse models (green) incorporate human immune cells or tissues into immunocompromised mice, facilitating studying human tumor-immune interactions. Syngeneic transplant models (blue) involve transplanting melanoma cells from genetically identical mice, allowing for immune-competent tumor studies. Patient-derived xenografts (PDX) (purple) are generated by implanting human melanoma tissue into immunodeficient mice, preserving patient-specific tumor characteristics for personalized medicine research. Genetically engineered models (red) introduce specific melanoma-associated genetic mutations in mice, enabling investigations into molecular mechanisms and targeted therapies. Cell line-derived xenografts (orange) are created by transplanting established melanoma cell lines into immunodeficient mice, serving as a widely used platform for preclinical drug screening. Each model provides unique advantages and limitations in melanoma research, contributing to developing improved therapeutic strategies.

2.5.1 Preclinical Models in Uveal Melanoma Research

In UM, animal models are essential in advancing our understanding of the TME and evaluating potential therapies (Stei, Loeffler, Holz, et al. 2016; van den Bosch et al. 2023). One of the primary obstacles in UM research is the lack of an ideal animal model that fully replicates the human disease. While animal models, including xenograft models in immunocompromised mice, have been instrumental in advancing our understanding of UM, they are limited in their ability to simulate the tumor-immune interactions crucial for studying immunotherapies (Stei, Loeffler, Holz, et al. 2016; van den Bosch et al. 2023). The absence of a functional immune system in these models precludes an accurate representation of the immune response

to UM, which is critical for developing effective therapies. Xenograft models, which use immunocompromised mice, lack a functional immune system for studying tumor-immune interactions and immunotherapies. Existing models often fail to replicate the specific mutations and chromosomal aberrations in human disease (Stei, Loeffler, Holz, et al. 2016; van den Bosch et al. 2023). The zebrafish model of UM has emerged as a valuable tool for investigating the genetic underpinnings of the disease. Zebrafish are genetically tractable, and their transparent embryos allow for real-time tumor development and metastasis visualization (Stei, Loeffler, Holz, et al. 2016; van den Bosch et al. 2023). The transgenic or xenotransplantation zebrafish model provides a valuable system for exploring the effects of specific genetic mutations and evaluating therapies in a whole-organism context. However, this model does not fully replicate intraocular disease, which remains a significant limitation (van den Bosch et al. 2023). To better understand the molecular events that drive UM metastasis, intraocular implantation of UM tumor cells with relevant genetic mutations remains essential. Current models often simulate metastasis by introducing melanoma cells into the liver, spleen, or tail vein to mimic hematogenous spread (van den Bosch et al. 2023; Grossniklaus et al. 1996; Yang, Cao, and Grossniklaus 2015). However, it is well established that the TME at metastatic sites, particularly in the liver and lungs, differs from that of the primary tumor (Grossniklaus et al. 1996; Yang, Cao, and Grossniklaus 2015). Therefore, developing metastatic models that accurately reflect the chromosomal and gene mutation pathways involved in UM metastasis is crucial.

2.5.1.1 Inoculation Method for Uveal Melanoma Model Development

Developing robust murine models of UM relies on the precise inoculation of tumor cells into mice's uvea environment, which facilitates the study of tumor progression, metastasis, and therapeutic responses (Lehrmann et al. 2022). Several inoculation techniques have been established, each with distinct advantages and limitations in replicating human UM pathology (Irigoyen et al. 2022; Wan et al. 2021). While some UM cell lines can successfully grow subcutaneously, allowing for more straightforward tumor measurement and drug response assessment, others demonstrate limited subcutaneous growth but thrive in uvea tissues, mirroring the tumor's natural microenvironment (Ozaki et al. 2016). As a result, orthotopic models, which more accurately reflect the human disease by establishing tumors in the uveal tract, have gained preference in preclinical research.

Orthotopic models of UM are categorized based on the site of tumor cell introduction, with inoculations targeting the iris, ciliary body, or choroid (Burnier et al. 2019). One of the earliest and most well-established methods involves the injection of tumor cells into the anterior chamber of the eye, leading to iris tumor

formation capable of metastasizing to distant sites (Niederkorn 1984). In an alternative approach, tumor cells are introduced into the suprachoroidal space via a translimbal injection technique. This allows for establishing tumors within the choroid and ciliary body, sites most commonly harbor UM in human patients (Dithmar, Rusciano, and Grossniklaus 2000). This method minimizes the risk of extraocular extension compared to transconjunctival inoculations and promotes the development of distant metastases, making it a valuable model for studying tumor dissemination. Another orthotopic strategy, intravitreal injection, involves delivering cells into the vitreous humor, an environment conducive to tumor proliferation. Although UM does not typically originate in the vitreous, injected tumor cells in this location invade adjacent uveal structures, mimicking disease progression (Kilian et al. 2016).

Beyond uvea inoculation methods, some models bypass the eye altogether to study metastatic colonization and tumor burden in visceral organs directly. Intravenous injection of UM cells via the retro-orbital sinus or tail vein simulates the later stages of metastasis by allowing tumor cells to disseminate hematogenously and establish secondary tumors, primarily in the liver and lungs. Additionally, intrasplenic inoculation has been developed to consistently generate liver metastases, reflecting the predominant site of UM metastasis in patients (Barisione et al. 2015; Gangemi et al. 2014; Jin et al. 2018). Direct implantation of UM cells or tumor fragments into the liver further enhances the fidelity of metastatic models, facilitating the study of organ-specific tumor growth dynamics (Kageyama et al. 2017; Ozaki et al. 2016).

Regardless of the inoculation method, non-invasive imaging techniques can monitor tumor progression and metastatic dissemination in real time. Bioluminescence imaging (BLI) (Sadikot and Blackwell 2005) is a widely used method in which tumor cells are genetically modified to express luciferase, enabling dynamic tracking of tumor burden when mice receive a luciferin substrate. Optical imaging systems (Conway, Carragher, and Timpson 2014) provide longitudinal assessment of tumor growth, facilitating the evaluation of therapeutic responses and metastatic potential in preclinical studies. Additionally, ophthalmic imaging modalities, including slit lamp biomicroscopy (Martin 2018) and optical coherence tomography (OCT) (Schmitt 1999), allow for detailed visualization of intraocular tumor development, structural changes, and tumor infiltration into adjacent uvea tissues. High-resolution imaging techniques such as magnetic resonance imaging (MRI) and ultrasound (Solnik et al. 2022) further enhance the ability to monitor tumor growth and metastatic progression in deeper tissues, including the liver and lungs, which are common sites of uveal melanoma metastasis. Integrating these imaging approaches provides a comprehensive strategy for tracking tumor evolution,

validating preclinical models, and assessing the efficacy of novel therapeutic interventions.

2.5.1.2 Syngeneic Melanoma Models for Uveal Melanoma Simulation

Syngeneic melanoma mouse models, initially developed for cutaneous melanoma research, have been widely utilized in the study of UM due to their ability to replicate key aspects of the disease, particularly its metastatic behavior (Richards et al. 2020). These models involve implanting melanoma cells into genetically identical mice, allowing tumor progression to be studied in an immunocompetent system (Jones et al. 2019; Dong et al. 2019; Van Raamsdonk et al. 2010). While the melanoma cell lines employed in these models are typically derived from cutaneous rather than uveal origins, they still provide valuable insights into intraocular tumor growth and metastatic spread, especially given that many of these lines display a natural propensity to metastasize to the liver (Van Raamsdonk et al. 2010), the primary site of UM metastasis in human patients. This capacity to model the full metastatic cascade, including local invasion, intravasation, circulation, extravasation, and colonization of distant organs, makes syngeneic models essential for UM research (Stei, Loeffler, Kurts, et al. 2016). Moreover, these models enable the examination of tumor-immune interactions within a fully functional immune system, which is an advantage over immunodeficient xenograft models. The ability to genetically modify host mice further enhances their utility, facilitating the investigation of specific host factors contributing to melanoma progression (Lattier et al. 2013; Stei, Loeffler, Kurts, et al. 2016).

The primary limitation of syngeneic models in UM research is that available mouse melanoma cell lines originate from cutaneous tumors rather than the uveal tract. Consequently, these cell lines harbor genetic and molecular alterations that differ from those driving human UM, which may influence tumor behavior and therapeutic responses. This divergence presents challenges in accurately modeling the disease at a molecular level. However, recent efforts have sought to develop more genetically relevant syngeneic models. For example, immortalized mouse melanocytes engineered to express canonical UM driver mutations, such as *GNAQ* and *GNA11* alterations, have been shown to undergo oncogenic transformation, forming tumors and metastases *in vivo* (Van Raamsdonk et al. 2010). The HCmel12 cutaneous melanoma cell line has also been identified as carrying the $GNA11^{Q209L}$ mutation, a key driver of UM. However, further characterization is needed to assess its suitability as a UM model (Schrage et al. 2015).

2.5.1.3 Xenograft Mouse Models for Uveal Melanoma

Xenograft models represent a widely employed approach in preclinical UM research, offering the advantage of using human-derived UM cell lines or tumor tissues implanted into mice (Zeng et al. 2022; Sugase et al. 2020). These models are particularly valuable for studying tumor biology, molecular signaling, and response to therapy, as the implanted cells retain key genetic and molecular features of the original patient tumors (Sugase et al. 2020; Yin et al. 2023). Given their reproducibility and ability to generate metastatic lesions in target organs such as the liver and lungs, xenograft models have been instrumental in advancing our understanding of UM progression and treatment strategies (Yin et al. 2023; Némati et al. 2010). Many recent studies investigating novel therapeutic approaches for UM have relied on xenograft systems, demonstrating their relevance in preclinical drug evaluation (Yin et al. 2023; Sugase et al. 2020; Nemati et al. 2023).

A significant development in xenograft modeling has been the emergence of patient-derived xenograft (PDX) models, in which fresh tumor specimens from UM patients, typically from metastatic lesions, are implanted into immunocompromised mice (Nemati et al. 2023). One particularly promising strategy involves the surgical implantation of metastatic UM tissues directly into the liver of NOD SCID gamma mice, achieving a high engraftment rate of approximately 83% (Kageyama et al. 2017). Notably, these models retain histological, genetic, and proteomic similarities to the original tumors, making them powerful tools for studying UM metastasis and potential therapeutic interventions. Furthermore, the ability to monitor tumor growth using computed tomography (CT) imaging enhances their utility for longitudinal studies (Solnik et al. 2022). PDX models hold considerable potential for personalized medicine, as they provide a platform for testing patient-specific drug responses, ultimately guiding tailored therapeutic strategies (Blanchard et al. 2024).

Despite their advantages, xenograft models face several challenges, primarily due to the requirement for immunocompromised mice to prevent rejection of the implanted human tumors (Lai et al. 2017; Chulpanova et al. 2020). Moreover, excluding functional immune components in traditional xenograft models presents a significant drawback, particularly in immunotherapy research. Given the increasing interest in immune-based treatments for UM, the lack of tumor-immune interactions in these models remains a critical limitation. While the relatively poor response of UM to immune checkpoint inhibitors such as PD-1 and CTLA-4 inhibitors (Algazi et al. 2016; Carvajal et al. 2017) partially mitigates this concern, other immune-modulatory pathways remain active research areas (Dougall et al. 2017; Yang et al. 2016). Ongoing efforts to develop humanized mouse models with functional human immune systems offer potential solutions. Still, they are currently hindered by challenges such as graft-versus-host disease and interspecies cytokine incompatibilities (Allen et al. 2019; Wege 2018).

The authentication of UM cell lines used in xenograft models is also critical. Some historically classified UM cell lines have been found to harbor mutations more characteristic of cutaneous melanoma, such as BRAF, and are now recognized as originating from cutaneous rather than uveal tumors (van der Kooij et al. 2019). Even when molecular characterization confirms UM origin, long-term in vitro passaging can alter gene expression profiles and chromosomal stability, leading to divergence from the parental tumor (Ben-David et al. 2018). Some older UM cell lines exhibit karyotypic differences from the original patient tumors, particularly concerning chromosome 3 status (Jager et al. 2016). Implanting fresh human tumor specimens directly into mice via PDX models represents one approach to mitigating these issues, as it preserves tumor heterogeneity and molecular characteristics more faithfully.

2.5.2 Preclinical Models in Cutaneous Melanoma Research

Unlike UM, the preclinical modeling of CM has advanced significantly, particularly in developing models that closely replicate the TME and immune system interactions (Viegas and Sarmento 2024). These models, including genetically engineered mouse models (GEMMs), syngeneic models, and PDX, have provided crucial insights into tumor progression, immune evasion, and therapeutic responses (Gercakova et al. 2024; Chulpanova et al. 2020). The integration of immunocompetent models has been especially valuable in evaluating immune-based therapies, making CM research more translationally relevant and facilitating the development of novel treatment strategies (Maulana et al. 2021).

2.5.2.1 Genetically Engineered Mouse and Allograft Models

One of the primary advantages of CM models is their ability to integrate functional immune components, enabling the study of immune checkpoint inhibitors, adoptive cell therapies, and cancer vaccines within an immunologically relevant context (Jaiswal, Liu, Pudakalakatti, Dutta, Jayaprakash, Bartkowiak, Ager, Wang, Reuben, Cooper, et al. 2020). Among these models, genetically engineered mouse models (GEMMs) have played a crucial role in elucidating the molecular pathways driving CM progression, particularly mutations in BRAF, NRAS, and PTEN, which are commonly observed in human CM (Neagu et al. 2016). GEMMs are specifically designed to reflect key melanoma-associated genetic alterations, such as mutations in BRAF, CDKN2A, CDK4, GNAQ, and NRAS, as well as environmental risk factors like UV radiation exposure (Neagu et al. 2016). The development of melanoma-specific GEMMs has been significantly advanced through the use of tissue-specific promoters, such as Tyr and MITF, which selectively target

melanocytes for transformation (Pérez-Guijarro et al. 2017). To enhance the physiological relevance of these models, UV radiation or chemical carcinogens such as DMBA are often incorporated, simulating natural disease progression (Wang et al. 2017). Some GEMMs employ the SV40 T-antigen under the control of the Tyr promoter to disrupt CDKN2A function, though CDKN2A loss alone is insufficient to induce robust melanoma formation (Oh et al. 2025). More sophisticated models have been developed by overexpressing HGF-cMET signaling, leading to constitutive activation of the RAS/RAF/MEK/ERK pathway, or by employing Tyr-Cre recombinase to drive oncogenic mutations in NRAS or BRAF while simultaneously deleting tumor suppressors such as Ink4a/Arf or Pten (Rebecca, Somasundaram, and Herlyn 2020). Despite their strengths, GEMMs often fail to fully recapitulate the high mutational burden and heterogeneity observed in human melanoma, limiting their ability to accurately model patient tumor evolution. However, advances in conditional gene expression systems, such as the Cre-recombinase/LoxP system, have provided more precise spatial and temporal control of oncogene activation, improving the physiological relevance of these models (Dankort et al. 2009).

Beyond murine models, alternative organisms such as canines and zebrafish have provided complementary approaches for studying melanoma. Canine melanoma, which frequently arises in the oral cavity, shares molecular similarities with human cutaneous melanoma and serves as a valuable spontaneous disease model for investigating immune responses to therapy (Hardwick 2021). Meanwhile, zebrafish models have been instrumental in high-throughput genetic screening and drug response studies in medulloblastoma (van Bree et al. 2024). Research using zebrafish has yielded critical insights into p53-BRAF interactions and the role of neural crest developmental pathways in melanoma formation (Dobre et al. 2021). Collectively, these diverse model systems contribute to a more comprehensive understanding of melanoma biology and therapeutic vulnerabilities, advancing the development of targeted treatment strategies.

2.5.2.2 Xenograft Models for Cutaneous Melanoma

In CM, xenograft models have been instrumental in elucidating tumor growth, metastatic potential, and therapeutic responses under *in vivo* conditions. Traditional xenograft approaches often involve subcutaneous implantation of human melanoma cell lines into immunocompromised mice, some exhibiting significant metastatic capacity after serial passaging (Yoshida 2020; Zeng et al. 2023). When cell lines have a lower propensity for metastasis, alternative methods, such as intracardiac or tail vein injections, are employed to force tumor cells to specific organs, including the brain and lungs (Morin et al. 2017). However, these techniques bypass key

metastatic processes such as intravasation and extravasation, limiting the model's ability to recapitulate the full metastatic cascade observed in patients.

PDX models have emerged as a valuable alternative to cell line-based xenografts by preserving the genetic and histological complexity of the original patient tumor (Yin et al. 2023; Patton et al. 2021). In PDX models for CM, fresh tumor specimens are directly engrafted into immunodeficient mice, enabling a more accurate representation of tumor behavior and therapeutic responses than conventional xenografts. While the requirement for immunocompromised hosts restricts the study of tumor–immune interactions, a critical consideration in the current era of immune checkpoint inhibitors, integrating PDX models into humanized mouse systems, has begun to address this shortcoming (Chiorazzi et al. 2023). By reconstituting aspects of the human immune system through adoptive transfer of tumor-infiltrating lymphocytes (TILs) or engraftment of CD34+ hematopoietic stem cells, these humanized PDX platforms allow for more nuanced exploration of immune responses to novel therapies, despite ongoing challenges such as graft-versus-host disease, cytokine incompatibilities, and suboptimal immune reconstitution.

Overall, xenograft and PDX models remain essential tools in CM research, as they retain crucial patient-specific tumor features. Their reliance on immunodeficient hosts, however, underscores the need for continued development of immune-humanized mice and advanced organoid systems, both of which offer avenues to simulate immunotherapeutic responses better. Ongoing refinements in genetic engineering, immune system reconstitution, and tumor modeling techniques promise to narrow the gap between laboratory findings and clinical outcomes, thereby enhancing the translational relevance of melanoma research.

2.5.2.3 Syngeneic and Humanized Mouse Models for Cutaneous Melanoma

Similarly, syngeneic models, which involve the transplantation of murine CM cell lines into immunocompetent mice, allow for the study of tumor-immune interactions and the evaluation of immune-modulatory agents in a setting where the host immune system remains functional. Additionally, syngeneic mouse models, which utilize tumor cells from the same genetic background as the host, are frequently employed to study immunotherapies (Jaiswal, Liu, Pudakalakatti, Dutta, Jayaprakash, Bartkowiak, Ager, Wang, Reuben, and Cooper 2020). Unlike xenograft models, syngeneic models possess a fully functional immune system, enabling researchers to evaluate the efficacy of immune checkpoint inhibitors like anti-PD-1 and anti-CTLA-4 antibodies in a physiologically relevant context (Sanmamed et al. 2016). Humanized mouse models, which involve the engraftment of human immune cells into immunodeficient mice, have also become valuable for studying human-specific immune responses to melanoma (Karnik et al. 2023; Yan et al. 2023). Furthermore,

zebrafish models are increasingly utilized to investigate melanoma metastasis due to their optical transparency and high genetic tractability, allowing for real-time tumor cell dissemination visualization (Lin, Jin, and Peng 2025). While these models have greatly enhanced our understanding of CM, replicating the complex interactions within the human TME remains challenging, particularly at metastatic sites. Continued refinement of these models is essential to bridge the gap between preclinical findings and clinical applications, ultimately leading to more effective treatments for metastatic melanoma.

2.5.3 CRISPR-Based Approaches and the Role of *BAP1* Loss

BAP1 loss plays a critical role in the tumorigenesis and immune modulation of both UM and CM. This genetic alteration profoundly reshapes the TME, driving immune suppression, metastatic progression, and resistance to therapy (Chattopadhyay et al. 2016; Figueiredo et al. 2020; Ida et al. 2022; Wiesner, Obenauf, Murali, Fried, Griewank, Ulz, Windpassinger, Wackernagel, Loy, and Wolf 2011). However, existing preclinical models for studying *BAP1*-loss melanocytic tumors face significant limitations. While useful for testing some targeted therapies, Xenograft models rely on immunodeficient mice and thus cannot investigate immune-mediated mechanisms central to *BAP1* loss. Furthermore, many cell lines used in these models harbor additional genetic alterations, complicating the isolation of *BAP1*-specific effects (Cao and Jager 2015; Némati et al. 2010; Kageyama et al. 2017; Nemati et al. 2014; Heegaard, Spang-Thomsen, and Prause 2003; Rusciano, Lorenzoni, and Burger 1994; Fidler, Gersten, and Budmen 1976; Richards et al. 2020; De Waard‐Siebinga et al. 1995). Recent studies have provided valuable insights into the molecular consequences of *BAP1* deletion. For instance, deletion of *BAP1* in B16-F10 melanoma cells by the CRISPR-Cas9 system has induced preferential downregulation of key genes, coinciding with increased ubiquitination of histone H2A at lysine 119. Transcriptomic analyses of *BAP1*-deficient cells further reveal dysregulation of multiple cellular functions, including extracellular matrix-receptor interactions and the MAPK signaling pathway, both of which may contribute to the enhanced metastatic and proliferative capacities observed in *BAP1*-deficient tumors (Luo et al. 2021). Additionally, studies on *BAP1* as a tumor suppressor in cholangiocarcinoma have demonstrated its role in chromatin accessibility and cellular plasticity. Using CRISPR/Cas9 to introduce *BAP1* loss-of-function mutations in normal human cholangiocyte organoids, researchers have identified *BAP1* as a key regulator of epithelial identity, controlling the expression of junctional and cytoskeletal components (Artegiani et al. 2019). The loss of *BAP1* in this context leads to increased cellular motility and a transition toward a more mesenchymal-like phenotype, emphasizing its role in tumor progression. Furthermore, engineering

human liver organoids with a combination of common cholangiocarcinoma mutations (TP53, PTEN, SMAD4, and NF1) has demonstrated that *BAP1* loss in this genetic background enhances the acquisition of malignant features upon xenotransplantation (Artegiani et al. 2019). These findings highlight the critical function of BAP1 in maintaining epithelial integrity through chromatin remodeling and suggest that its tumor-suppressive role extends beyond melanoma. The integration of organoid technology with CRISPR/Cas9-based gene editing provides a powerful experimental platform for dissecting the mechanistic underpinnings of *BAP1* function in cancer biology and underscores the necessity for more refined preclinical models that can accurately capture the immune and epigenetic landscape of *BAP1*-deficient melanomas.

Despite these advancements, a significant gap remains in the availability of suitable animal models that can accurately recapitulate the complex interactions between the tumor and its microenvironment. The lack of a robust and physiologically relevant in vivo system limits our ability to study the mechanisms governing metastasis, immune evasion, and therapeutic resistance in BAP1-deficient melanoma. Developing novel models that incorporate functional immune components and faithfully mimic the genetic and epigenetic landscape of human tumors is critical for advancing our understanding of melanoma progression and improving therapeutic interventions.

Addressing these gaps requires the development of immunocompetent preclinical models that accurately capture the molecular, cellular, and immune complexities of *BAP1*-loss melanocytic tumors. The goal is not merely to develop the best model for CM or UM but to create an immunocompetent preclinical system that faithfully represents *BAP1* loss and its multifaceted effects. Such models would enable researchers to investigate shared mechanisms of *BAP1* loss across melanoma subtypes and identify therapeutic strategies that mitigate its impact. Recent efforts to develop CRISPR/Cas9-based murine models of *BAP1* loss represent a promising step forward (van der Mijn et al. 2023). These models allow for precise genetic manipulation in an immune-competent context, facilitating the study of *BAP1*-specific mechanisms. Moreover, integrating scRNA-seq with these models provides a powerful approach for dissecting transcriptomic and microenvironmental alterations associated with *BAP1* loss, ultimately aiding in identifying novel therapeutic targets. By focusing on *BAP1* loss as a unifying factor, these efforts hold the potential to develop more effective therapies for both UM and CM, addressing the critical gaps in current preclinical research.

3 Aims

This thesis employs a reverse translational approach, leveraging clinical data to elucidate molecular mechanisms and advancing these findings through preclinical validation. Integrating multi-omic datasets from uveal and cutaneous melanoma, the different research projects investigate critical drivers of tumor progression and immune suppression, providing a comprehensive framework to comprehend melanoma biology across diverse contexts.

Structured in two interconnected stages, the thesis follows a cohesive research strategy: (1) Clinical Discovery, identifying novel clinical biomarkers and genomic features (*CD1D* and 1q gains, Aim 1) for future preclinical investigation; and (2) Preclinical Implementation, further exploring the clinically validated biomarker *BAP1* from extensive prior studies. This second stage addresses the lack of tools for studying *BAP1*'s immune-related implications by developing an unprecedented syngeneic, immunocompetent preclinical model (Aim 2). This model enables validation of *BAP1*'s role in tumor progression and immune modulation in vivo, culminating in its functional characterization and translational relevance (Aim 3). Together, these stages bridge clinical discoveries with preclinical advancements, establishing a robust framework for leveraging melanoma biomarkers in therapeutic contexts. Therefore, the specific aims were:

- Aim 1: Identifying Molecular Drivers of Tumor Progression and Immune Suppression in Melanoma Using Multi-Omic Integrative Analysis
- Aim 2: Developing a CRISPR-Based Model to Study *BAP1* Loss in Melanoma Tumorigenesis and Immune Modulation
- Aim 3: Unveiling the Mechanisms of *BAP1* Loss in Tumorigenesis and Immune Evasion: A Preclinical Perspective

Ultimately, this research established a comprehensive preclinical framework to bridge clinical observations with translational applications in melanoma. By integrating insights from genetic and immune drivers, including 1q gains, *CD1D* regulation, and *BAP1* loss, this work provided a platform for testing and refining novel therapeutic strategies, including immunotherapies.

4 Materials and Methods

4.1 Cell Line and Mice

Melan-a, a murine melanocyte cell line (SCC202) (Bennett, Cooper, and Hart 1987), was purchased from Merck Millipore. These cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin (Gibco) and incubated at 37° C in a 5% CO_2 humidified incubator.

C57BL/6 mice were used for *in vivo* validation. All mouse work was conducted in strict accordance with the guidelines and requirements set by the Committee for Animal Experimentation in Turku, Finland. These experiments adhere to the Finnish Act on Animal Experimentation (62/2006), which mandates compliance with the 3Rs principle (Replacement, Reduction, Refinement). The local Committee for Animal Experimentation has approved them under permit ESAVI/39072/2021, granted on 2.12.2021. Further validation of the mouse model will be carried out by our international collaborator at the Singapore Eye Research Institute (SERI) as part of a multi-center application and testing process. All animal work conducted at SERI has been approved by Singapore's Institutional Animal Care and Use Committee (IACUC) under Ref. 2022/SHS/1756. The Principal Investigator (PI) in charge ensures that animal suffering is minimized, employing appropriate sedation, analgesia, or anesthesia as needed. Ethical monitoring of tumor growth in both centers (Finland and Singapore) will include serial weekly assessments of behavior, weight, and pain. Animals with larger tumors or at risk of ocular perforation or severe intraocular pressure (IOP) elevation will be humanely euthanized earlier to prevent undue suffering. This method was developed by me, guided by the PI from SERI and MIORG, and mainly applied for Aim 2 and 3.

4.2 Patient Sample Collection and Clinical Examination

Formalin-fixed paraffin-embedded (FFPE) enucleated uveal melanoma (UM) samples were identified through a database search of UM biopsies conducted at the Singapore National Eye Centre (SNEC) between 2004 and 2018. All samples were collected prior to treatment and at the time of initial diagnosis. De-identified FFPE

sections were submitted to the Cytogenetics Laboratory at Singapore General Hospital for chromosomal aberration analysis using the OncoScan™ CNV Array (Agilent, Santa Clara, CA, USA).

A retrospective review of medical records was conducted to gather data on patient age at UM diagnosis, sex (male/female), tumor location, largest basal diameter and height, cell type, BAP1 immunohistochemistry staining, and pathological classification based on the TNM system (AJCC 8th edition) (Gershenwald and Scolyer 2018; Ogata et al. 2021). This method, developed by the PI (Dr. Anita Chan) from SERI, was mainly applied for Aim 1: the role of 1q gains in UM.

4.3 Primary Mouse Uveal Melanocyte Isolation

As my previous publication described (Wang, Chen, et al. 2021), uveal melanocytes will be dissociated and isolated from the uvea of 6-week-old C57BL/6 mice. Briefly, iris and ciliary body tissue were dissected from C57BL/6 mice uvea, and the tissue was cut into small pieces (<2mm) using scissors. Incubate the tissue with the digestion mix (100 ug/ml Liberase TL+150 ug/ml DNase I in RPMI 1640 medium) at 37^{o} C for 25-30 minutes while shaking gently. After that, place a 70um mesh strainer on a 50ml tube and rinse with PBS. Transfer the digested tissue into the filter. Using the rubber end of a syringe plunger, push the digested tissue through the strainer. Rinse the filter with the PBS and centrifuge the cell suspension at 300g for 10 min. The primary uvea cell suspension was cultured in the Melanocyte Growth Medium (PromoCell C-24010). For a positive selection of melanocytes, around 5 million primary uvea cell suspensions were incubated with mouse CD117 (c-Kit) MicroBeads (Milteny Biotec, 130-097-146) at 4^{o} C for 15 min. After washing twice, positively labeled melanocytes were eluted from the magnetic column by flushing with 1 mL of binding buffer. The enriched melanocytes were seeded into T25 flasks and cultured in the Melanocyte Growth Medium (PromoCell C-24010). Repeat the Magnetic-Activated Cell Sorting (MACS) twice for further melanocyte purification. This method was developed by me and mainly applied to Aim 2 of this study.

4.4 Cas9 sgRNA Plasmid and *BAP1* KO Clones Generation

To generate *BAP1* KO clones, the Cas9 sgRNA plasmid was constructed using the Guide-it CRISPR/Cas9 System (Green) (Cat. No. 632601) from Clontech Laboratories. The single-guide RNAs (sgRNAs) were designed using the CHOPCHOP platform (https://chopchop.cbu.uib.no) (Labun et al. 2019) to

target key regions of the BAP1 gene. Three sgRNAs were explicitly selected to target exon 1 and exon 14, critical for disrupting *BAP1* gene function.

These sgRNA sequences were carefully designed to maximize the likelihood of successful gene knockout by inducing frameshift mutations or indels within the targeted regions. Following sgRNA design, plasmid construction followed the manufacturer's protocol, which involved cloning the sgRNAs into a plasmid encoding the Cas9 endonuclease. The plasmids were transfected into melan-a mouse melanocyte cell lines using the Effectene Transfection Reagent (Qiagen), which ensures high-efficiency delivery of the CRISPR/Cas9 components. After 48 hours post-transfection, the cells were harvested and sorted by FACS. GFP-positive cells were isolated, indicating successful transfection and expression of the Cas9-sgRNA complex. Single GFP+ cells were sorted into 96-well plates and cultured in a conditioned medium to promote single-cell colony formation.

Several validation methods were employed to confirm successful *BAP1* knockout. Genomic DNA was extracted from the clones and subjected to Sanger sequencing to verify the presence of frameshift indels or larger deletions in the targeted regions of exon 1 and exon 14. Additionally, Western blot analysis confirmed the absence of BAP1 protein expression in selected clones, ensuring both gene and protein-level validation. Further, qRT-PCR was performed to assess the downregulation of *BAP1* gene expression. These *BAP1* KO clones were expanded and prepared for subsequent in vitro functional assays and in vivo studies to investigate the role of *BAP1* in tumorigenesis, immune evasion, and metastasis in uveal melanoma. This method was developed by me in collaboration with Allan Lee from SERI and mainly applied to Aim 2 of this study.

4.5 PCR and qPCR

To assess whether the CRISPR/Cas9 system successfully introduced mutations in the *BAP1* gene, a PCR assay was performed to amplify the targeted regions around exon 1 and exon 14. The PCR reaction was initiated with a pre-denaturation step at 95°C for 10 minutes. Thermal cycling consisted of 50 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes. Each PCR reaction was prepared in a total volume of 50 µl, containing PCR Master Mix (25 µl), forward and reverse primers (2 µl of 10 µM stock each), template DNA (5 µl), and nuclease-free water (18 µl). Primers were custom-designed and synthesized by Integrated DNA Technologies.

PCR products were separated on a 2% Tris-acetate-EDTA (TAE) gel and run at a constant voltage of 100V. Post-separation, the PCR amplicons were purified using a QIAquick Gel Extraction Kit (Qiagen) and sent for Sanger sequencing (Bio Basic

Asia) to confirm the presence of indels or larger deletions at the cut sites. DNA traces were analyzed using FinchTV and aligned with reference sequences using CLC Sequence Viewer.

To quantify the expression levels of *BAP1* in the modified clones, qPCR was employed to specifically target exon 1 and exon 14 regions of the *BAP1* gene. The analysis used the Roche LightCycler 480 (LC480) qPCR system. RNA was first extracted and reverse-transcribed to cDNA using the iScript RT reagent Kit (Bio-Rad). For qPCR, the reaction mixture contained 10 µl of SYBR Green Master Mix (Roche), 1 µl each of forward and reverse primers (10 µM), 2 µl of cDNA, and 6 µl of nuclease-free water in a final volume of 20 µl per well. The thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. Gene expression levels were normalized against *ACTB* as the internal control. Relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method, and results were presented as fold changes in *BAP1* expression compared to wild-type control cells. Each experiment was performed in triplicate, and the data were analyzed using LightCycler 480 Software (Roche). Primers were custom-designed and synthesized by Integrated DNA Technologies. This method was developed by me and mainly applied for Aim 2 of this study.

4.6 Western Blot

For protein extraction, cells were lysed in RIPA buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich) to ensure complete lysis and protein stabilization. Cell lysates were incubated on ice for 30 minutes and then centrifuged at 14,000g for 15 minutes at 4°C. The supernatants containing total proteins were collected and quantified using the Bio-Rad Protein Assay Kit (Bio-Rad) according to the manufacturer's instructions. Equal amounts of protein (25µg) were resolved on a 10% SDS-PAGE gel, depending on the size of the protein of interest, and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). The membranes were blocked with 5% non-fat milk and BSA in Tris-buffered saline with 0.1% Tween 20 (TBS-T) for 1 hour at room temperature to prevent nonspecific binding.

Primary antibodies against target proteins, such as BAP1 Rabbit mAb #13271 (Cell Signaling Technology, USA) and β-actin (loading control), were diluted (1:1000) in 5% BSA in TBS-T and incubated with the membranes overnight at 4°C. Following incubation, the membranes were washed three times with TBS-T and subsequently incubated with horseradish peroxidase (HRP)- conjugated secondary antibodies (Thermo Fisher Scientific) for one hour at room temperature. Protein bands were detected using the enhanced chemiluminescence (ECL) Western blot

detection reagent (GE Healthcare) and, visualized with the Fusion FX Imager, analyzed through the Fusion FX7 Edge 18.05 software. This method was developed by me and mainly applied to Aim 2 of this study.

4.7 Sanger Sequencing

PCR products were purified using the previously published method (Tan et al. 2021). The amplified PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's protocol. The purified DNA samples were eluted in 20 µl of nuclease-free water (Sigma-Aldrich) and prepared for Sanger sequencing.

Post-sequencing, the quality of the DNA traces was assessed using FinchTV software (Geospiza), ensuring high-fidelity sequencing reads. Multiple sequence alignments were conducted using CLC Sequence Viewer (Qiagen) to compare the sequenced data with reference sequences. A sequence homology search was performed using the NCBI blast algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm sequence identity and analyze potential sequence variations. This method was developed by me and mainly applied to Aim 2 of this study.

4.8 Cytology and IHC

For cytological analysis and immunohistochemistry (IHC), cell pellets or suspensions were pre-fixed in PreservCyt fixative as previously outlined (Wang, Tan, et al. 2021) and submitted to the Department of Anatomical Pathology and Cytology at Singapore General Hospital (SGH). Cell samples were centrifuged to prepare cytospin slides using a Thermo Scientific Shandon Cytospin (Shandon Scientific Ltd, Cheshire, UK) (Sng et al. 2007). These slides were subjected to Diff-Quik (DQ, Baxter, McGraw Park, IL) and Papanicolaou (PAP) staining following standard diagnostic procedures (Li Yan Khor 2017) and previously established IHC protocols (Chan et al. 2017; Anita SY Chan; Sunny Shen; Audrey Looi Lee Geok; Seah Lay Leng; 2014). For IHC, single staining was performed using antibodies against gp100 (Abcam, #ab137078), employing the Bond Polymer Refine Detection Kit (Leica Biosystems) with diaminobenzidine (DAB) as the chromogen. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E) to assess histopathological features. Cytospin slides and tissue sections were visualized under an Olympus BX42 microscope at 60x magnification (field diameter: 0.55mm). This method was developed by SGH and mainly applied to Aim 2 of this study.

The Human Protein Atlas database offers IHC protein staining data for 15,287 genes across 20 cancer types (Uhlen et al. 2017). In cutaneous melanoma tissues, protein expression levels are assessed in amelanotic areas using 3,3'-

diaminobenzidine (DAB) staining, with scale bars of 50 and 20 μm. The expression levels of β2M, CD1d, and CD1b in the cell membrane and cytosol were quantified via an automated machine learning method, which evaluates marker intensity, color, density, and object size and circularity, as previously described (Matareed et al. 2023). Bar plots illustrate the frequency (%) of positive expression relative to the total area, presented as Means ± Standard Error of Mean (SEM) from five independently quantified tissue areas in melanoma samples. This method was developed by my supervisor and mainly applied to Aim 1 of this study: the potential epigenetic mechanisms regulating *CD1D* in melanoma.

For clinical FFPE samples, BAP1 IHC was conducted using the antibody clone sc-28383 from Santa Cruz Biotechnology (Dallas, TX, USA). Automated immunostaining techniques were validated in the Clinical Pathology Laboratory of the Singapore General Hospital. Lung adenocarcinoma was used as an external positive control, and positive normal endothelial cells served as internal controls. This method was developed by SGH and mainly applied to Aim 1: the role of 1q gains in UM.

4.9 RNA and DNA Extraction

Cell suspensions were first centrifuged at 500g for 3 minutes to pellet the cells, followed by washing three times with 1 mL of sterile PBS to remove any residual media or contaminants. After the final wash, the supernatant was discarded, and the cells were lysed using a TRIzol reagent to facilitate RNA isolation. The lysate was thoroughly mixed by pipetting up and down, ensuring complete disruption of cell membranes. The Direct-zol RNA Kit (Zymo Research) was used according to the manufacturer's protocol for RNA extraction. This kit allows for the direct purification of RNA from the TRIzol lysate, simplifying the extraction process. An essential step in this protocol was the removal of genomic DNA contamination, which was achieved by an on-column DNase I treatment. This step was critical to ensure the purity of the RNA for downstream applications, such as reverse transcription and qPCR. After the RNA was purified, it was eluted in 20 μL of RNase-free water and immediately stored at -80°C to prevent degradation. In parallel, genomic DNA was extracted from separate aliquots of the same cell suspensions using the DNeasy Blood & Tissue Kit (Qiagen), following the standard protocol for cultured cells. After the extraction, the genomic DNA was re-suspended in 50 μL of RNase-free water and stored at -20°C. RNA and DNA yields were quantified using a NanoDrop spectrophotometer, and their quality was assessed by measuring the A260/A280 ratios. This method was developed by me and mainly applied to Aim 1-3.

4.10 Cell Proliferation Assay

Cell proliferation was assessed in real-time using the xCELLigence Real-Time Cell Analysis (RTCA) system (ACEA Biosciences) (Roshan Moniri et al. 2015), which measures electrical impedance across microelectrodes embedded in the wells of an E-Plate 96. The impedance data generated by the system are directly proportional to the number of cells, their viability, and their morphological status, allowing continuous monitoring of cell proliferation dynamics. In this study, *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ melanocyte clones were evaluated for differences in proliferation.

The E-Plate 96 was prepared by adding 50 µL of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) to each well. The plate was then placed into the RTCA station, and baseline impedance readings were recorded to establish background cell index (CI) values. This was followed by incubating the plate at 37°C in a humidified atmosphere containing 5% CO_2 for 30 minutes to ensure equilibration. Following the baseline measurement, *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ melanocyte clones were harvested and resuspended in a complete medium. Cells were seeded into the E-Plate at a concentration of 5×10^3 cells per well in 100 µL of medium, and an additional 50 µL of medium was added to bring the final volume to 150 µL per well. Each clone was plated in triplicate to ensure reproducibility, and wells containing only medium (without cells) were included as negative controls for background impedance correction. After seeding, the plate was placed into the xCELLigence system for continuous monitoring. Impedance measurements, reflected as the CI, were recorded at 15-minute intervals for the first 6 hours to capture the initial cell adhesion phase, followed by measurements every 30 minutes for the remaining duration of the experiment. This continuous, label-free method allowed for detailed monitoring of cell proliferation over time. The cell index data were analyzed using the RTCA software, which provided a comprehensive view of the proliferation kinetics of *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ melanocyte clones. All experiments were conducted in three independent replicates to confirm the consistency and reproducibility of the data. This method was developed by me and mainly applied to Aim 2-3.

4.11 Wound Healing Assay

1×10^6 cells/well of *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ mouse melanocyte clones were seeded in a 6-well plate and cultured for 24 hours. A sterile 1000 µL pipette tip created a scratch (wound) on the cell monolayer. After the wound was made, the cells were cultured in fresh medium at 37°C with 5% CO2. Wound width measurements were taken at 0, 6, 24, 30, 48, 54, 72, and 78 hours using a Nikon ECLIPSE TS100 inverted microscope at 4× magnification. Six fields per well were imaged and the percentage of wound closure was calculated using the ImageJ MRI Wound Healing Tool, and

the experiment was repeated independently three times. This method was developed by me and mainly applied to Aim 2-3.

4.12 Cell Invasion Assay

For the Matrigel Invasion Assay, Transwell inserts (8 μm pore size; Corning) were coated with 50 μL of Matrigel (BD Biosciences) diluted 1:3 in cold serum-free medium and allowed to solidify at 37°C for 1 hour. *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ mouse melanocyte clones (2 × 10^5 cells/well) were seeded into the upper chamber of the Transwell in a serum-free medium. The lower chamber was filled with a medium containing 10% FBS as a chemoattractant. The cells were incubated at 37°C with 5% CO_2 for 24 hours. After incubation, non-invading cells on the upper surface of the membrane were carefully removed using a cotton swab. Invaded cells on the lower surface of the membrane were fixed with 4% paraformaldehyde for 20 minutes. After fixation, the cells were permeabilized with 0.1% Triton X-100 for 5 minutes, followed by washing with phosphate-buffered saline (PBS). The cells were stained with DAPI (4',6-diamidino-2-phenylindole) solution (1 μg/mL in PBS) for 10 minutes in the dark at room temperature to visualize the nuclei. The number of invaded cells was counted under a fluorescence microscope (Nikon Eclipse or Olympus BX51) at 10× magnification. Pictures were taken from at least five randomly selected fields per well to quantify the number of invaded cells. The assay was performed in triplicate, and the results were analyzed by counting the number of DAPI-positive cells (nuclei) using ImageJ software (Suarez-Arnedo et al. 2020) to assess the invasion capacity. Data were presented as the mean number of invaded cells per field. This method was developed by me and mainly applied to Aim 2-3.

4.13 Adherent Colony Formation Assay

1000 cells/well of *BAP1*$^{+/+}$ and *BAP1*$^{-/-}$ mouse melanocyte clones were seeded into 6-well plates in 3 mL of complete growth medium and cultured at 37°C in a humidified incubator with 5% CO2. The cells should grow undisturbed for 10-14 days until visible colonies are formed. The medium was changed every 3-4 days to ensure optimal growth conditions. Once the colonies reached a suitable size (defined as a cluster of at least 50 cells per colony), the growth medium was gently removed, and the cells were washed twice with PBS to remove any residual medium. The colonies were then fixed with 4% paraformaldehyde for 15 minutes at room temperature. After fixation, the colonies were stained with 0.5% crystal violet solution (in 20% methanol) for 30 minutes to ensure thorough staining of all colonies. The excess stain was gently washed away with distilled water, and the plates were left to dry at room temperature. After drying, colonies were imaged using

a Nikon Eclipse microscope or scanned for quantification. The colonies were quantified using The NIS-Elements Advanced Software (V4.20.23, Nikon, Melville, NY, USA), applying an automated machine learning quantification method based on marker intensity, color, density, and object size and circularity, as described previously (Wang et al. 2023). Only colonies containing at least 50 cells were included in the analysis. Three independent experiments were performed, and the results were expressed as the mean colony count per condition. This method was developed by me and mainly applied to Aim 2-3.

4.14 Soft Agar Colony Formation Assay

The soft agar colony formation assay was performed to assess the anchorage-independent growth capabilities of the $BAP1^{+/+}$ and $BAP1^{-/-}$ melanocyte clones. A base layer (1ml) of 1% agarose in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin was first prepared and poured into 6-well plates. The agar was allowed to solidify at room temperature for approximately 30 minutes. Once the base layer had solidified, a second layer containing 0.5% agarose and 10K cells suspended in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin was poured over the base layer. This layer was also allowed to solidify at room temperature. After solidification, 1 mL of complete RPMI-1640 medium was added to each well to keep the agar hydrated throughout the experiment. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 4-6 weeks, during which time the medium was replenished every 3-4 days to maintain proper nutrient levels. After the incubation period, colonies were stained with 0.005% crystal violet. The number of colonies formed in each well was imaged using a Nikon Eclipse microscope or scanned for quantification. The colonies were quantified using The NIS-Elements Advanced Software (V4.20.23, Nikon, Melville, NY, USA), applying an automated machine learning quantification method described previously (Wang et al. 2023). Each experiment was performed in triplicate to ensure reproducibility and colony counts were compared between $BAP1^{+/+}$ and $BAP1^{-/-}$ clones. This method was developed by me and mainly applied to Aim 2-3.

4.15 Animal Experiments

We will utilize a syngeneic murine UM model to study the TME and transcriptomic differences between Class 1 low-risk and Class 2 high-risk primary UM and Class 2 primary UM and Class 2 metastatic tumors. The model was generated through the subcutaneous (Figueiredo et al. 2015), intraocular injection of murine melanocytic

cell lines, specifically Melan-a cells with *BAP1*$^{+/+}$ or Class 1 UM and Melan-a cells with *BAP1*$^{-/-}$ for Class 2 UM in C57BL/6 mice. These injections were performed using established anterior chamber and ciliary body injection methods, enabling the formation of primary UM tumors that mimic human Class 1 and Class 2 tumors (Grossniklaus et al. 1996; Yang, Cao, and Grossniklaus 2015).

Once the tumors were induced, weekly monitoring was conducted to assess tumor progression. Tumor size will be measured via slit lamp clinical examination and anterior optical coherence tomography (OCT), with serial *in vivo* images taken for consistent tracking. Tumor volume (TV) will be calculated using the following formula: TV = Mean largest height × Mean basal diameter, and the tumor growth rate was determined as TV/time. In addition to tumor size monitoring, general health assessments were performed, including behavioral observations, weight tracking, and pain monitoring to ensure animal welfare. If tumors reach a size that poses a risk of ocular perforation or cause severe intraocular pressure (IOP) elevation, mice will be sacrificed early to adhere to ethical guidelines. Tissue samples were collected for scRNAseq and outsourced to commercial platforms. These analyses enable the profiling of immune cell populations and the identification of differentially expressed genes (DEGs) within the TME and tumor tissues. Additionally, the TME was analyzed for immune cell infiltration, focusing on T cells and macrophages. This method was developed by me, guided by PIs from MIORG and SERI, and mainly applied to Aim 3.

4.16 Single-cell RNAseq and Data Analysis

scRNAseq was performed using the 10x Genomics Chromium platform, explicitly utilizing the 10x Genomics Flex assay for fixed cells. Following the manufacturer's guidelines, uvea and cutaneous tumor tissues were first dissected from mice and fixed according to the Flex 10x Genomics protocol, allowing for sample preservation before processing. After fixation and disassociation, the cells were stored at -80°C until further processing. Upon thawing, cell viability was assessed to ensure adequate preservation of cell integrity, and the cells were subsequently washed and counted. Approximately 10,000 cells per sample were used for GEM (gel bead-in-emulsion) generation, leveraging the 10x Chromium Flex 3' Library & Gel Bead Kit. Following GEM formation, reverse transcription was performed inside individual GEMs to capture mRNA transcripts and synthesize cDNA. This was followed by cDNA amplification, library construction, and sequencing using an Illumina NovaSeq 6000 platform, targeting a depth of at least 10,000 reads per cell.

Raw sequencing data were processed using the Cell Ranger (version 6.0, 10x Genomics) pipeline, which aligned reads to the mouse reference genome (GRCm38) and generated a gene-barcode matrix for each sample. Quality control and filtering

steps were performed using Seurat (version 5.0) (Freytag et al. 2018) in R. Cells with high mitochondrial content (>15%) and low unique molecular identifiers (UMIs) were excluded to ensure data quality. Data normalization, scaling, and clustering were conducted within the Seurat framework. Significant principal components were identified through PCA, and cell clustering was performed using the first 50 principal components at a resolution of 0.3. Dimensionality reduction was visualized using UMAP plots, facilitating the exploration of distinct cellular subpopulations within the tumor microenvironment. Cell type annotation was guided by known marker genes, with differential expression analysis performed using the "FindMarkers" function. Genes with an adjusted p-value < 0.05 and a log2 fold change >1 were considered significant. Further pathway analysis was conducted using clusterProfiler to identify key biological processes enriched within specific cell clusters. Immune cell deconvolution was performed using both SingleR and CIBERSORTx (Huang et al. 2021). At the same time, cell-cell communication was explored with the CellChat package to investigate interactions between different cell types within the tumor microenvironment. Visualization tools such as UMAP, heatmaps, and violin plots were employed to depict gene expression patterns, and the results were cross-validated with published datasets to ensure the robustness of the findings. To address batch effects in single-cell RNA sequencing data, I utilized the R package Harmony (Korsunsky et al. 2019), specifically designed to handle batch variation by integrating datasets from different experimental conditions while maintaining the biological variation of interest. This method, including the scripts in R, was developed by me after multiple bioinformatics training in Finland and Singapore and mainly applied to Aim 3.

4.17 Bulk RNA-seq Data Sets and Analysis

RNA quality and concentration were assessed using a Nanodrop 2000 spectrophotometer (Thermo Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies), with only samples showing RNA integrity number (RIN) values above 8.0 being used for further analysis. Library preparation was performed using the NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs), and sequencing was conducted on the Illumina NovaSeq 6000 platform to achieve a read depth of at least 30 million paired-end reads per sample. Before alignment, the raw sequencing data were subjected to quality control using FastQC (version 0.11.9) to check for read quality. Reads were aligned to the mouse reference genome (GRCm38) using the STAR aligner (version 2.7.9a). The aligned reads were then quantified at the gene level using featureCounts (version 2.0.1) from the Subread package. Reads were aligned to the mouse reference genome (GRCm38) using the STAR aligner (version 2.7.9a). Data visualization, including heatmaps, volcano

plots, and principal component analysis (PCA), was performed using ggplot2 and pheatmap in RStudio. This method was developed by me in collaboration with bioinformaticians at Duke-NUS medical school and mainly applied to Aim 2.

For the data analysis focusing on the *CD1D* study (Aim 1), mRNA expression and survival data from The Cancer Genome Atlas SKCM GDC dataset were obtained via the Xena Functional Genomics Explorer (Cline et al. 2013). Methylation data were retrieved from the Genomic Data Commons (GDC) legacy archive, focusing on the following methylation sites: B2M (cg18696027), SPI1 (cg06147863), and CD1D (cg13844591). Data were extracted in tab-separated values (TSV) format. RNA-seq datasets from immune checkpoint therapy (ICT) patients were collected from the anti-PD1 Riaz cohort (Riaz et al. 2017) (GSE91061, SRP094781), anti-PD1 and anti-PD1/anti-CTLA4 Gide cohort (Gide et al. 2019) (PRJEB23709), anti-PD1 Hugo cohort (Hugo et al. 2016)(GSE78220), and anti-PD1 Liu cohort (Liu et al. 2019) (phs000452.v3.p1), available through Gene Expression Omnibus (GEO), Sequence Read Archive (SRA), European Nucleotide Archive (ENA), and database of Genotypes and Phenotypes (dbGaP). Survival analysis was conducted using the survival and survminer R packages, with a quartile range of 0.25–0.75 to identify the minimum p-value. Kaplan-Meier plots were generated using the log-rank test to visualize overall survival. This method was developed by me in collaboration with bioinformaticians at MIORG.

4.18 Determination of Chromosomal Aberrations

Genome-wide copy number variations (CNVs) and copy number-neutral loss of heterozygosity (CN-LOH) were analyzed using the OncoScan™ CNV Array (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's protocol. CEL files generated during chip scanning were imported into Chromosome Analysis Suite (ChAS) version 4.0 (Applied Biosystems, Carlsbad, CA, USA) and processed using the FFPE Analysis NA33 workflow. Annotation was performed based on the hg19 genome version. This method was developed by the bioinformaticians from SGH and mainly applied for Aim 1: the role of 1q gains in UM.

4.19 Statistical Analysis

All statistical analyses will be conducted to evaluate experimental data rigorously. Initial data normality will be assessed using the Shapiro-Wilk normality test to guide the selection of appropriate statistical tests. Non-normally distributed data will be employed using non-parametric tests such as the Mann-Whitney U and Spearman's correlation tests. Pearson's correlation test will be applied for normally distributed data to assess correlations between variables. A p-value threshold of less than 0.05

will be considered statistically significant for all analyses. Differences in gene expression between experimental groups will be analyzed using a two-tailed, unpaired t-test. Multivariate regression models, implemented in R Studio, will be used to identify factors independently associated with tumor growth and the composition of the TME while controlling for potential confounding variables. These models will allow us to assess the influence of multiple factors on tumor progression and immune cell infiltration. Survival analysis will use Kaplan-Meier survival plots generated with GraphPad Prism 9.0 and R Studio packages “survival” and “survminer.” The log-rank test will be used to evaluate statistical significance between different survival curves, with a p-value threshold of less than 0.05 indicative of a significant difference in survival outcomes. All results will be presented as mean ± standard error of mean (SEM) or median with interquartile range (IQR), depending on the data distribution, and graphical representations will be generated to clarify the findings. This method was developed by me, guided by my supervisor from MIORG.

5 Results

5.1 *CD1D* as a Molecular Driver of Immune Checkpoint Therapy Resistance in Melanoma

CD1D has emerged as a critical regulator of ICT resistance in melanoma, primarily through its interplay with β2M and the surrounding tumor microenvironment. Recent analyses of multi-omic datasets have revealed that β2M expression is closely linked to the antigen-presenting functions of *CD1D*, suggesting that disruptions in this pathway contribute significantly to tumor immune evasion (Wang et al. 2023). Notably, epigenetic modifications appear to govern *CD1D* expression levels, thereby shaping the capacity of melanoma cells to engage immune effector populations.
These insights lay the groundwork for Aim 1 of this thesis, which focuses on uncovering additional biomarkers and molecular pathways that drive melanoma progression and immune suppression. By leveraging comprehensive genomic and transcriptomic datasets, the study underscores the importance of integrating large-scale bioinformatics approaches to bridge clinical observations with preclinical findings. This multi-omic perspective ultimately provides a more nuanced understanding of how melanomas circumvent immune surveillance, offering new avenues for therapeutic intervention. Although direct citations of this work are still emerging, several recent studies have explored epigenetic and immune-evasion pathways, referencing concepts akin to the *CD1D* (Eakins et al. 2024; Mukherjee et al. 2024; Zhang et al. 2023). By contextualizing this work within the rapidly evolving research on TME modulation, it becomes evident that this study contributes to an expanding body of knowledge seeking to bridge gaps in ICT efficacy.

5.1.1 *CD1D* as a Key Mediator of Immune Response and ICT Resistance in Melanoma

CD1D plays a crucial role in antigen presentation, particularly in activating NKT cells, contributing to antitumor immunity (Hara et al. 2021). Our study revealed that *CD1D* expression was significantly impacted by β2M levels, forming a distinct molecular cluster associated with immune response regulation (**I: Figure 1A**). While β2M loss has been extensively linked to MHC-I downregulation and ICT resistance,

our findings indicated that β2M deficiency also alters *CD1D*-associated antigen presentation, potentially disrupting NKT cell activation. Correlation analysis across melanoma patient cohorts demonstrates that *CD1D* clustering patterns shift in patients with low β2M expression, suggesting a functional dependency between these molecules (**I: Figure 1B & 1C**). Structural analysis further confirms direct interactions between CD1d and β2M, indicating that *CD1D* stability is likely affected by β2M depletion (**I: Figure 1D**). These findings highlight the critical role of *CD1D* in immune surveillance and suggest that its dysregulation contributes to ICT resistance.

5.1.2 Epigenetic Modulation of *CD1D* and Its Impact on Immune Checkpoint Therapy Outcomes

Given the strong association between *CD1D* expression and ICT response, we conducted an in-depth investigation into the underlying regulatory mechanisms that influence *CD1D* transcription in melanoma. Our comprehensive analysis of publicly available melanoma patient datasets revealed that *CD1D* downregulation is primarily driven by epigenetic modifications rather than direct promoter methylation (**I: Figure 2C, upper panel**). Notably, we identified a significant correlation between *CD1D* expression levels and the methylation status of SPI1, a key transcription factor responsible for regulating *CD1D* transcription (**I: Figure 2C, upper panel**). This regulatory relationship suggests that *SPI1* acts as an upstream modulator of *CD1D* expression, and any disruption in *SPI1* transcription may have cascading effects on *CD1D*-mediated antigen presentation. Further investigation demonstrated that increased methylation of the *SPI1* promoter leads to its transcriptional repression, effectively silencing *SPI1* expression. This suppression, in turn, results in downstream repression of *CD1D*, reinforcing a mechanism by which melanoma cells may evade immune detection. Importantly, this epigenetic silencing of *SPI1* and subsequent *CD1D* downregulation were strongly correlated with poor survival outcomes in patients undergoing ICT (**I: Figure 2D, right panel**). The observed association between *SPI1* methylation and diminished *CD1D* expression underscores a broader regulatory mechanism that extends beyond genetic alterations, highlighting the role of tumor-induced epigenetic remodeling in shaping immune evasion strategies. These findings suggest that epigenetic modifications targeting *SPI1* serve as a critical checkpoint in the regulation of *CD1D* expression, ultimately influencing immune responses and resistance to ICT. The suppression of *CD1D*-mediated antigen presentation due to *SPI1* methylation could impair NKT cell activation, further compromising the ability of the immune system to mount an effective response against melanoma. Understanding these epigenetic interactions may open new therapeutic avenues, where targeting *SPI1* demethylation or restoring

CD1D expression could enhance ICT efficacy and improve patient survival outcomes.

5.1.3 *CD1D* Downregulation Impairs NKT Cell Activation and Limits ICT Efficacy

CD1D is a non-polymorphic MHC class I-like molecule that is crucial for presenting glycolipid antigens, such as α-galactosylceramide, to a specialized subset of T lymphocytes known as NKT cells, which serve as a bridge between innate and adaptive immunity (Lee and Webb 2023). This process of glycolipid antigen presentation is central to early tumor surveillance and immune activation, particularly in melanoma. In our expanded analysis of patient samples, we observed that *CD1D* expression is significantly enriched in both tumor cells and dendritic cells in individuals responding to ICT, in sharp contrast to its marked downregulation in non-responders (**I: Figure 2E**). Further emphasizing the potential functional consequences of this dysregulation, immunohistochemical data from the Human Protein Atlas indicate that *CD1D* localizes predominantly to the cytoplasm of melanoma cells rather than their cell membrane, suggesting that crucial antigen presentation mechanisms may be compromised (**I: Figure 2F, 2G**). Consistent with human clinical findings, transcriptomic profiles of murine melanoma models also reveal substantial decreases in *CD1D* expression in anti-PD1-resistant tumors, highlighting the relevance of this pathway in therapeutic resistance (**I: Figure 3A, right panel**). Intriguingly, patients exhibiting a robust intratumor NKT cell signature demonstrated superior ICT responses, whereas non-responders showed a reduction in NKT cell activation (**I: Figure 3B, left panel**). Notably, only the activated NKT cell phenotype remained enriched in responders, underscoring the critical role *CD1D* plays in sustaining effective NKT cell-mediated responses (**I: Figure 3B, center and right panel**). Taken together, these findings suggest that when *CD1D* is epigenetically repressed, particularly via *SPI1* downregulation, NKT cell activation is blunted, thereby limiting the efficacy of ICT in melanoma. Such insights highlight a potential therapeutic avenue wherein restoration of *CD1D* expression, possibly through reversing epigenetic silencing, may bolster NKT cell function and ultimately improve clinical outcomes in melanoma.

5.2 Novel Insights into Chromosomal Aberrations and *BAP1* Loss in a Southeast Asian Uveal Melanoma Cohort

In the second segment of Aim 1 of the thesis, this work explores the unique genetic landscape of UM within a Southeast Asian cohort, highlighting the high prevalence of 1q gains and the loss of the tumor suppressor gene *BAP1*. These chromosomal alterations stand out as critical contributors to metastatic risk and overall survival, underscoring their significance in the disease course. By contrasting these findings with data reported in Western populations, this investigation reveals distinctive patterns of chromosomal imbalance that could be closely tied to regional genetic backgrounds and environmental factors. In particular, *BAP1* loss appears to represent a pivotal molecular event, aligning with heightened metastatic potential and worse clinical outcomes. Taken together, these novel insights enrich our understanding of UM pathogenesis in Southeast Asia, laying a foundation for refined risk stratification and the development of targeted therapeutic strategies tailored to the genetic context of this population.

5.2.1 High 1q Gains and Unique Patterns of Metastasis Risk in SEA UM cohort

Analysis of FFPE tumor samples from 20 UM patients treated at a tertiary care center in Singapore identified significant differences in the genetic profiles of the SEA cohort compared to Western cohorts, such as the TCGA-UVM dataset. Successful SNP array results were obtained from 14 samples, while six samples yielded insufficient DNA (**II: Table 2**). A total of 78 gains, 48 losses, and 2 CN-LOH events were identified in 11 samples, with three samples displaying a normal cytogenetic profile (**II: Table 2**). No significant correlation was found between FFPE block age and SNP array success, as samples older than ten years retained a 70% success rate. Failed cases exhibited higher necrosis and melanophage content, potentially compromising DNA quality.

Cytogenetic alterations in the Asian cohort showed distinct patterns compared to the TCGA-UVM cohort. Chromosome 6q losses were nearly twice as frequent in the SEA cohort (50%) than in TCGA-UM (28%), while chromosome 6p gains, associated with longer PFS in TCGA-UM, did not demonstrate statistical significance in SEA patients (**II: Figure 2A**). Chromosome 9 alterations, including 9p losses and 9q gains, were more prevalent in the SEA cohort and significantly correlated with extended PFS, a finding not observed in the TCGA-UM cohort (**II: Figure 2C**). Monosomy 3 was less frequent in the SEA cohort (14%) than TCGA-UVM (53%), though its presence remained a strong predictor of shorter PFS in both groups (**II: Figure 2**). Gains in chromosome 1q were associated with shorter PFS in

the SEA cohort (p=0.0289), a trend absent in TCGA-UM (**II: Figure 2C**). Chromosome 8 aberrations displayed similar frequencies across both cohorts, with 8p alterations significantly linked to shorter PFS in both groups. However, 8q gains correlated with reduced survival in TCGA-UVM did not reach significance in the SEA cohort. These findings suggest that 1q gains are a distinct and critical driver of tumor aggressiveness and metastasis in the SEA population, independent of the lower frequency of monosomy 3 in this cohort (14% in the SEA cohort vs. 53% in TCGA-UVM). These observations highlight a unique pathway of tumor progression in Southeast Asian UM, which differs from the predominantly monosomy 3-driven progression observed in Western populations.

5.2.2 The Prognostic Significance of *BAP1* Loss and Its Role in UM Progression

Despite the distinct chromosomal alterations observed between the SEA and Western cohorts, *BAP1* loss consistently emerged as a robust indicator of poor prognosis. In the SEA cohort, immunohistochemical analysis demonstrated that 20% of cases exhibited *BAP1* loss (**II: Table 2**). In line with findings from Western populations, the absence of BAP1 in SEA samples was strongly correlated with reduced survival (**II: Figure 2B**).

These observations were further supported by a pilot study conducted in Singapore, which employed SNP arrays and a customized single-cell workflow. Single-cell imaging revealed both *BAP1*-negative and *BAP1*-positive cells, alongside activated CD163 macrophages in a patient sample that displayed high-risk histological features, including epithelioid morphology and increased tumor dimensions (large basal diameter and height). Notably, the same sample exhibited copy-neutral loss of heterozygosity on 3p21.31p21.2, gain of 8q, and losses of 1p and 6q, all recognized as high-risk cytogenetic events. Consistent with these genomic findings, immunohistochemical staining confirmed the loss of *BAP1* expression (**III: Figure 1**).

This consistency underscores the universal role of *BAP1* loss in driving poor outcomes in uveal melanoma, regardless of regional or population-specific genetic backgrounds. However, it is noteworthy that not all metastatic cases in the SEA cohort exhibited BAP1 loss, suggesting that other genetic alterations, such as 1q gains, may also contribute to metastatic potential in this population. While the genetic landscape of UM in the SEA cohort diverges significantly from that of Western populations, particularly in the frequency and impact of chromosomal aberrations such as 1q gains and 9q gains, **the role of *BAP1* loss remains consistent as a robust predictor of poor outcomes**. This work provides compelling evidence of these distinctions, emphasizing the importance of integrating population-specific

data to refine prognostic tools while reaffirming the universal significance of *BAP1* loss in UM biology. Additionally, recent findings have validated the critical role of *BAP1* loss in driving metabolic alterations in UM, specifically affecting lipid metabolism, which contributes to its metastatic risk and immune suppression (Figueiredo et al. 2020; Matareed et al. 2023). These metabolic changes, characterized by dysregulated lipid storage and lipolysis, underscore the impact of *BAP1* loss on tumor progression and highlight its role in resistance to current immunotherapies. Identifying adipophilin as a marker of these metabolic shifts (Matareed et al. 2023) further strengthens the case for investigating *BAP1* loss in preclinical models.

These findings align with **Aim 1** of this thesis, contributing to identifying novel biomarkers and advancing translational research into UM progression and therapeutic strategies. This thesis builds on these insights, utilizing preclinical models to explore how *BAP1* loss drives lipolysis and metabolic reprogramming, ultimately providing a platform for testing targeted interventions to mitigate metastatic risk and overcome immunotherapy resistance.

5.3 Mechanisms of *BAP1* Loss in Tumorigenesis and Immune Evasion in Melanoma

This section further examines how the loss of the tumor suppressor gene *BAP1* contributes to tumorigenesis and enables immune evasion in melanoma. Recognizing a critical gap in available research tools, a novel syngeneic, immunocompetent preclinical model was developed to rigorously assess the immunological impact of *BAP1* under physiological conditions. Building on *BAP1*'s status as a clinically validated biomarker, this model provides a robust *in vivo* platform for investigating the mechanistic underpinnings of tumor progression and immune modulation. These efforts directly address **Aim 2** of this thesis by creating an appropriate model system to study *BAP1*'s immune-related functions and subsequently fulfill **Aim 3** through the functional characterization of *BAP1* and its translational potential in melanoma therapy. Through this integrated approach, a more comprehensive understanding of *BAP1*'s role in melanoma biology is achieved, revealing additional avenues for therapeutic intervention.

5.3.1 *BAP1* Depletion Drives Epithelioid Transformation and Alters Growth Dynamics in Melanoma

The establishment of CRISPR-engineered *BAP1* KO melanocyte clones forms a critical aspect of this study, providing the foundational tools for investigating UM's molecular and cellular dynamics. Using the immortalized mouse melanocyte cell line (Melan-a) (Bennett, Cooper, and Hart 1987), I developed a highly optimized CRISPR/Cas9 gene-editing system to effectively disrupt the *BAP1* gene, a tumor suppressor implicated in UM progression and therapeutic resistance. In this study, the tumorigenic potential and morphological characteristics of *BAP1* KO clones established in **Aim 2** were first systematically investigated *in vitro*, providing insight into how the loss of *BAP1* impacts cell behavior. CRISPR-engineered *BAP1* KO melanocyte clones resulted in profound morphological alterations, suggesting a key role for *BAP1* in maintaining normal cellular architecture. Compared to wild-type (WT) melanocytes, *BAP1* KO cells exhibited a more epithelioid morphology, with irregular cellular boundaries and increased single-cell size, indicating a shift toward a more aggressive phenotype (**IV: Figure 2A**). Histological analyses confirmed that these changes were consistent across multiple KO clones, reinforcing the importance of *BAP1* in regulating cell structure and adhesion. Notably, despite these morphological shifts, all *BAP1* KO clones retained positive gp100 staining, indicating that they remained within the melanocytic lineage (**IV: Figure 2A**).

Further investigation into the functional consequences of *BAP1* loss revealed significant variability in proliferation and anchorage-independent growth. Colony formation assays demonstrated that while several *BAP1* KO clones exhibited

enhanced proliferation, others showed reduced growth capacity (**IV: Figure 2B**). Anchorage-independent growth, a hallmark of tumorigenicity, also varied among the clones. For example, KO#3 formed a more significant number of colonies in soft agar assays compared to WT, suggesting increased tumorigenic potential. However, other KO clones, such as KO#4, showed a marked reduction in colony formation (**IV: Figure 2C**). These findings underscore the complexity of *BAP1* loss in driving tumor progression, indicating that while *BAP1* deficiency promotes aggressive cellular features, additional factors may influence the extent of tumorigenicity across different cellular contexts. The variability observed among *BAP1* KO clones suggests that the impact of *BAP1* loss is not uniform but dependent on other molecular and microenvironmental interactions that shape tumor development.

5.3.2 *BAP1* Loss Promotes Tumor Progression by Enhancing Migration, Invasion, and Metastatic Potential

In addition to altering cellular morphology and proliferation, *BAP1* loss significantly enhanced the migratory and invasive capabilities of melanoma cells. Wound-healing assays demonstrated that *BAP1* KO clones exhibited a markedly increased rate of wound closure compared to WT cells, suggesting a heightened migratory potential (**IV: Figure 3A**). This increased motility was further validated using transwell invasion assays, which revealed that clones with a complete loss of *BAP1* (KO#2, KO#3, KO#4) demonstrated significantly higher invasion through a porous membrane compared to WT cells. Interestingly, KO#1, which retained partial *BAP1* expression, did not exhibit a significant increase in invasion, suggesting that even partial *BAP1* activity may be sufficient to restrain invasive behaviors (**IV: Figure 3B**). These findings suggest a potential dose-dependent role for *BAP1* in regulating metastatic properties, where its complete loss significantly exacerbates tumor cell migration and invasion.

To investigate whether the increased invasive capacity observed *in vitro* translated into more aggressive tumor growth *in vivo*, *BAP1* KO clones were introduced into immunocompetent C57BL/6 mice through intraocular and subcutaneous injections. Tumor progression was assessed using slit-lamp imaging and optical coherence tomography (OCT), which revealed that *BAP1* KO clones exhibited rapid tumor growth and extensive invasion of the anterior chamber, leading to structural disruption of the iris (**IV: Figure 4A-C**). Intraocular pressure (IOP) measurements consistently showed significantly elevated levels in eyes injected with KO#1-3 clones, correlating with the degree of tumor expansion (**IV: Figure 4D**). In subcutaneous tumor models, *BAP1* KO clones also demonstrated significantly increased tumor volume compared to WT, with KO#2 exhibiting the most aggressive growth trajectory (**IV: Figure 4E-F**). Importantly, KO#4, which showed reduced

proliferation in vitro, failed to establish robust tumor growth *in vivo*, further reinforcing the idea that *BAP1* loss alone is not the sole determinant of tumorigenicity but rather interacts with other intrinsic and extrinsic factors to modulate tumor progression. The combination of elevated IOP, significant tumor growth in both intraocular and systemic environments, and structural disruption caused by the aggressive *BAP1* KO clones, particularly KO#2, reinforces the critical role of *BAP1* in tumor development and progression.

5.3.3 Transcriptional Reprogramming Induced by *BAP1* KO: Impact on Tumor-Intrinsic and Immune-Regulatory Pathways

The widespread phenotypic changes observed following *BAP1* loss were accompanied by extensive transcriptional alterations that reshaped gene expression networks. Bulk RNA sequencing of WT and *BAP1* KO clones revealed distinct clustering patterns, as demonstrated by principal component analysis (PCA) and hierarchical clustering, where fully silenced *BAP1* KO clones (KO#2, KO#3, KO#4) formed transcriptionally distinct groups, while KO#1, which retained partial *BAP1* expression, clustered closer to WT cells (**IV: Figure 5A**). Differentially expressed gene (DEG) analysis revealed that clones with complete *BAP1* loss exhibited widespread transcriptional reprogramming, highlighted shared and unique transcriptional changes across the KO clones (**IV: Figure 5B-C**). While common pathways were affected across all *BAP1*-loss clones, clone-specific expression patterns were evident, indicating that each clone may adapt uniquely to *BAP1* loss. The unsupervised hierarchical clustering of Pearson's correlation (**IV: Figure 5D**) supported this observation, showing increased clustering diversity in KO clones (C1-C6), which points to broader transcriptional reorganization. This increased heterogeneity likely contributes to these clones' varied tumorigenic and immune-evasive capacities. These transcriptional shifts are critical in enhancing the tumorigenic potential and promoting immune evasion in *BAP1*-loss UM. The distinct separation in gene expression profiles, especially in fully silenced *BAP1* clones, underscores the complex reprogramming of cellular pathways that drive aggressive tumor behavior. This highlights the need to further explore the transcriptional and functional consequences of *BAP1* loss to better understand its role in UM progression and immune suppression within the TME.

Gene Set Enrichment Analysis (GSEA) further delineated functional pathway changes induced by *BAP1* loss. KO#2 was particularly enriched in pathways associated with cell proliferation, immune suppression, and cytokine signaling, suggesting a strong link between *BAP1* deficiency and tumor immune escape (**IV: Figure 5E**). KO#3, while also enriched in proliferative pathways, showed less

involvement in immune regulatory functions, while KO#4 was predominantly associated with pathways involved in cellular differentiation and growth regulation. These findings indicate that while *BAP1* loss drives core tumorigenic processes, its impact on immune and metabolic functions may vary across tumor clones, highlighting the need for further investigation into the context-dependent roles of *BAP1* in tumor progression.

The volcano plot in Figure 1 further illustrates the scale of transcriptional dysregulation in *BAP1* KO clones, particularly KO#2, which exhibited the most significant changes in gene expression. Upregulated genes in KO#2 include *Mrgprb1*, *Ephb1*, and *Tgfbr3*, which are involved in immune modulation, proliferation, and tumor progression. Downregulated genes such as *Gpr37* and *Parp12* also implicate changes in pathways related to cellular stress responses and DNA repair mechanisms. Notably, genes associated with immune evasion, including *MIF*, *TIM3*, *IL-17*, and *S100A10L*, were dysregulated in KO#2, highlighting the role of *BAP1* in regulating immune-related pathways in UM. These findings are further supported by previous studies identifying a correlation between BAP1 loss and immune suppression in UM. The dysregulation of genes such as *DOCK10*, *PARP1*, *Galectin 3*, and macrophage inhibitory factor (*MIF*) suggests a complex interaction between *BAP1* loss and immune escape mechanisms. *PARP1* binding protein was notably upregulated, implicating its role in tumor progression and resistance to apoptosis. Moreover, the *DOCK10* gene, associated with epithelial-mesenchymal transition (EMT), was significantly dysregulated in KO#2, providing further evidence of its involvement in metastasis and tumor invasiveness.

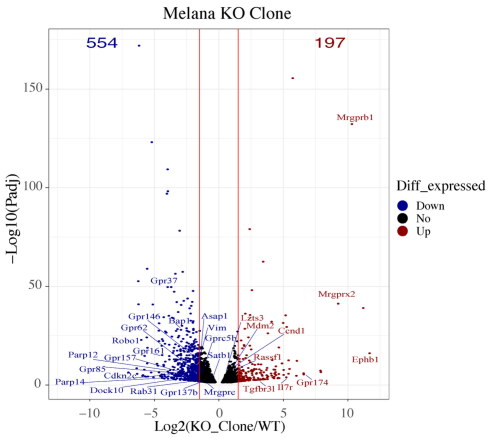

Figure 3. Volcano plot of differential gene expression in *BAP1* KO#2 clone compared to WT. The x-axis represents the log2 fold change (KO vs. WT), while the y-axis shows the negative log10 of the adjusted p-value, indicating the significance of the differential expression. Genes upregulated in the *BAP1* KO clone are marked in red (right side), while downregulated genes are in blue (left side).

These results provide critical insights into the immunosuppressive mechanisms driving *BAP1*-loss UM progression. The extensive transcriptional reprogramming observed in *BAP1* KO clones, particularly KO#2, underscores the central role of immune modulation in *BAP1*-associated tumorigenesis. The enrichment of immune suppression pathways, combined with the dysregulation of key immune-related genes, suggests that these pathways contribute significantly to the aggressiveness and metastatic potential of *BAP1*-loss tumors. The observed molecular heterogeneity across *BAP1* KO clones emphasizes the complexity of *BAP1*'s involvement in tumor biology. This diversity indicates that *BAP1* loss leads to various transcriptional responses, implicating multiple interconnected pathways in immune suppression and tumor progression. Therefore, a one-size-fits-all therapeutic strategy is unlikely to be effective. Instead, these findings highlight the necessity for personalized therapeutic approaches tailored to the specific molecular and immunological profiles of *BAP1*-loss tumors. By targeting the distinct immune suppression pathways and dysregulated gene networks in each clone, more effective therapeutic interventions can be developed, offering new hope for mitigating tumor growth and preventing metastasis in UM patients.

5.3.4 *BAP1* Loss Reshapes the Tumor Microenvironment and Recapitulates High-Risk Uveal Melanoma Features

Beyond its tumor-intrinsic effects, *BAP1* loss was found to significantly alter the composition and immune landscape of the TME, as revealed by scRNA-seq analyses. In subcutaneous models, TME profiling demonstrated that *BAP1* KO tumors exhibited increased T-cell infiltration, yet paradoxically maintained an immunosuppressive environment, potentially limiting effective antitumor immunity (**IV: Figure 6A & B**). A more diverse immune landscape was observed in the intraocular tumor model, where *BAP1*-loss tumors exhibited increased macrophage and neutrophil infiltration, alongside transcriptional signatures associated with immune activation and suppression (**IV: Figure 6C & D**).

Pathway enrichment analyses highlighted distinct transcriptional changes across multiple cell types within the TME. In the subcutaneous model, macrophages exhibited upregulated pathways associated with tumor-associated signaling, while downregulated pathways were enriched for immune-supportive functions, such as leukocyte chemotaxis and inflammatory responses, suggesting an immunosuppressive phenotype (**IV: Figure 8B**). Similarly, intraocular tumors demonstrated macrophage enrichment in pathways linked to the suppression of cytokine production and immune effector functions, further supporting a role for BAP1 loss in promoting immune evasion (**IV: Figure 8C**). Neutrophils in the intraocular tumor model displayed upregulation of immune-regulatory pathways

while downregulating biosynthetic and metabolic processes, indicating a shift toward a more suppressive phenotype within the TME (**IV: Figure 8D**). Additionally, fibroblasts and endothelial cells exhibited context-dependent transcriptional reprogramming, with intraocular tumors displaying an upregulation of pathways related to immune suppression and extracellular remodeling, while subcutaneous tumors exhibited a reduction in structural and signaling pathways (**IV: Figure 8E-H**).

To further validate the clinical relevance of these findings, the transcriptional profiles of *BAP1* KO tumors were compared to high-risk (class 2) human UM datasets. DEG analysis revealed a significant overlap in gene expression patterns between *BAP1* KO mouse models and high-risk human UM tumors, with 63% of upregulated genes and 237 downregulated genes shared between the two datasets (**IV: Figure 9D**). Functional enrichment analysis further confirmed that key pathways associated with immune suppression, metabolic reprogramming, and tumor progression were conserved between *BAP1* KO tumors and high-risk UM, underscoring the translational relevance of this model in studying the molecular and immunological mechanisms driving *BAP1*-related tumorigenesis (**IV: Figure 9E**). These alterations align with the hallmarks of class 2 tumors, including increased metabolic and signaling activity associated with tumor progression (Wu et al. 2024; Kim, Song, and Yim 2023; Matareed et al. 2023).

These findings establish that *BAP1* loss profoundly impacts tumor progression by driving morphological, proliferative, and invasive changes while simultaneously reprogramming the TME to promote immune evasion. The significant overlap between *BAP1* KO tumors and high-risk UM suggests that this model provides a valuable platform for investigating therapeutic strategies aimed at restoring anti-tumor immunity and targeting the molecular consequences of *BAP1* deficiency in melanoma.

6 Discussion

6.1 Epigenetic Regulation of *CD1D*: Implications for ICT and TME Modulation in Melanoma

The evolving landscape of ICT hinges fundamentally on the successful presentation of tumor antigens to T cells. Effective MHC-dependent antigen presentation initiates the cascade that generates a clonally expanded population of anti-tumor effector lymphocytes, setting the foundation for subsequent T-cell activation, exhaustion, and memory formation (**V: Figure 1**). These distinct stages underscore how clinical benefits from ICT are predicated on robust T-cell stimulation and the ability to avoid or reverse T-cell exhaustion (Sharma et al. 2021b). In line with emerging therapeutic strategies, efforts to bolster ICT efficacy frequently focus on modulating T-cell effector functions, targeting alternative checkpoints such as LAG-3, and harnessing co-stimulatory pathways (e.g., ICOS), as well as leveraging epigenetic modulators to promote long-lasting memory responses. However, many malignancies, particularly those deemed poorly immunogenic, resist ICT due to a failure to generate effective anti-tumor T cells (Dunn, Old, and Schreiber 2004a). This lack of immunogenicity cannot be attributed solely to low TMB because clinical observations indicate that the existence of tumor antigens and a functional antigen processing machinery are more decisive. Immunotherapy-responsive tumors often display high intratumoral T-cell infiltration (“hot” phenotype), in contrast to “cold” or “desert” tumors where T-cell populations are sparse, or “excluded” tumors in which T cells are trapped at the tumor periphery and prevented from efficient infiltration (**V: Figure 2**). These phenotypic distinctions further highlight the significance of intact antigen presentation pathways and underscore why defects in molecules such as β2M or CD1d can profoundly dampen immunosurveillance.

Within this context, our exploration of the β2M/CD1D axis as an epigenetically regulated immune resistance mechanism strongly aligns with the fundamental premise that effective T-cell priming relies on intact presentation machinery. While MHC class I molecules chiefly handle peptide antigen presentation, CD1d is critical for presenting glycolipid antigens to NKT cells, thereby complementing conventional T-cell responses. Yet β2M-deficient melanomas disrupt both these

channels, resulting in suboptimal T-cell and NKT cell activation. This dual pathway impairment in poorly immunogenic melanomas illuminates why concurrent epigenetic repression of *CD1D*, via mechanisms such as promoter methylation, can drastically impede tumor immune surveillance and ultimately diminish ICT responsiveness.

The epigenetic regulation of CD1D as a mechanism of immune resistance in melanoma aligns closely with Aim 1 of this thesis, which focuses on molecular drivers of tumor progression and immune suppression. This work emphasizes the β2M/CD1D axis, its role in antigen presentation, and its contribution to ICT resistance in poorly immunogenic melanomas (Wang et al. 2023). Among one of the first to identify and explore this topic, the significance of this research has already begun to resonate within the field, as evidenced by its thematic relevance in recent literature addressing immune modulation strategies and ICT optimization (Zhou et al. 2024). Although direct citations of this work are still emerging, several recent studies have explored epigenetic and immune-evasion pathways, referencing concepts akin to the CD1D (Eakins et al. 2024; Mukherjee et al. 2024; Zhang et al. 2023). By contextualizing this work within the rapidly evolving research on TME modulation, it becomes evident that this study contributes to an expanding body of knowledge seeking to bridge gaps in ICT efficacy.

The interactions between β2M and molecules such as CD1d, CD1b, and FCGRT have been shown to play a critical role in antigen presentation and immune regulation within APCs. These interactions are central to the effective priming of the immune response. In melanomas, deficiencies in β2M have profound consequences, particularly in reducing the efficiency of tumor antigen presentation via MHC-I, contributing to the failure of ICT in a subset of patients (Jenkins, Barbie, and Flaherty 2018). Interestingly, while β2M loss disrupts the conventional antigen presentation pathway, *CD1D* can still be transported to the cell surface independently. However, without β2M, *CD1D* undergoes abnormal glycosylation, leading to its rapid degradation and conversion into an immature glycoprotein state, thereby eliminating its functionality in glycolipid antigen presentation (Kim et al. 1999).

Antigen presentation constitutes a foundational step in initiating an effective anti-tumor response, particularly in the context of ICT. Beyond the MHC-mediated presentation of peptide antigens to T cells, non-peptide antigen presentation via β2M-associated molecules like *CD1D* plays a distinct role in activating NKT cells. *CD1D* presents glycolipid antigens, such as α-galactosylceramide (αGalCer), to NKT cells, initiating their activation and subsequent cytokine production, including IL-2 and IL-12. These cytokines act synergistically to restore exhausted CD8+ T cells, ultimately enhancing tumor clearance (Bendelac, Savage, and Teyton 2007; Kawano et al. 1997). The absence of β2M disrupts both MHC-I-dependent and

CD1D-mediated pathways, significantly reducing the activation of NKT cells and thereby contributing to an immunosuppressive TME. This dual disruption underscores the central role of β2M and *CD1D* in modulating tumor immunity and their critical importance in mediating the clinical benefits of ICT (Ardeniz et al. 2015).

Previous studies have reinforced the critical role of NKT cells in mediating anti-tumor immune responses, particularly in ICT-treated settings. In experimental models, NKT cell activation through *CD1D* stimulation by αGC in APCs has significantly improved outcomes in tumor-bearing mice. The cytokine response triggered by NKT cells, particularly the release of IL-2 and IL-12, restores CD8+ T cell functionality and promotes broader cytotoxic immune responses. Importantly, these interactions synergize with anti-PD1 therapy, enhancing tumor control and providing a mechanistic basis for integrating *CD1D*-mediated NKT activation with existing ICT protocols (Bae et al. 2018; Motohashi et al. 2009). Furthermore, NKT cells also facilitate the remodeling of the TME by increasing the levels of iNOS+CD206-M1 macrophages, further supporting the anti-tumor immune response (Paul et al. 2019).

Despite their critical role, NKT cells exhibit an exhausted phenotype in advanced cancer stages, characterized by impaired cytotoxicity and reduced activation potential. For example, studies in E0771 breast cancer and B16 melanoma models demonstrate that late-stage tumors are associated with a decline in NKT cell function, which correlates with immune escape and tumor progression (Liu, Li, et al. 2021). Interestingly, in clinical settings, increased NKT cell numbers have been observed in biopsies from melanoma patients responding to anti-PD1 therapies, suggesting that NKT cells may serve as a biomarker of ICT responsiveness and a potential therapeutic target (Kasanen et al. 2020; Krieg et al. 2018). A deeper understanding of the molecular mechanisms driving NKT cell activation, glycolipid antigen presentation, and their integration with anti-PD1 therapy is required to enhance ICT efficacy.

The differential expression of *CD1D* across cancers and its association with clinical outcomes have been observed in various contexts. Consistent with our findings, downregulation of *CD1D* has been reported in breast (Hix et al. 2011), cervical (Miura et al. 2010), and non-small cell lung cancers (65), where it is associated with poor prognosis. These studies suggest a broader role for *CD1D* in maintaining immune surveillance across tumor types. In melanoma, epigenetic mechanisms such as promoter methylation and histone modification play a significant role in silencing genes critical for immune regulation. For instance, the methylation of the *SPI1* promoter indirectly suppresses *CD1D* expression, reducing its availability for glycolipid antigen presentation. Although *SPI1* methylation has

been extensively studied in lymphoma, where it is downregulated and methylated in over 70% of patients, its role in melanoma remains underexplored (Melo et al. 2020).

These findings open avenues for exploring therapeutic interventions that target epigenetic mechanisms regulating *CD1D* expression. Epigenetic therapies, such as histone deacetylase (HDAC) and DNA methyltransferase inhibitors, have shown potential in restoring the expression of genes like β2M and *CD1D* (Woods et al. 2015). By reactivating these pathways, such therapies could reinvigorate NKT cell activity, enhance antigen presentation, and synergize with existing ICT approaches to improve patient outcomes (Luo et al. 2018; Tiper and Webb 2016). Moreover, combinatory therapeutic approaches integrating epigenetic modulators and immune checkpoint inhibitors may provide a more comprehensive strategy to overcome ICT resistance in poorly immunogenic melanomas.

As the opening chapter of this thesis **Aim 1**, the discovery of the β2M/*CD1D* axis as a central player in immune regulation highlights the critical importance of targeting epigenetic modulators to restore immune function. This work deepens our understanding of tumor immunology and the development of innovative combinatory therapies for metastatic melanoma by uncovering the molecular and epigenetic mechanisms driving immune resistance. It establishes a foundation for future translational research by bridging clinical observations with preclinical models.

6.2 Genetic and Phenotypic Factors Modulating Uveal Melanoma Outcomes in Southeast Asia

Based on the clinical study and the result from the paper included in this thesis, titled "*Genetic Landscape of Uveal Melanoma in Southeast Asia: High 1q Gains and Unique Patterns of Metastasis Risk*", our findings highlight significant differences in the genetic and phenotypic landscape of UM between SEA and Western populations. While monosomy 3 has been well-established as a key prognostic marker of poor outcomes(Gallenga et al. 2022; Coupland et al. 2013; Harbour 2009; Smit et al. 2020; Dogrusoz and Jager 2018; Gelmi and Jager 2024), it was the only chromosomal aberration in our study significantly associated with shorter PFS. Loss of *BAP1* nuclear expression further corroborated this association, matching data from the TCGA-UM cohort. However, the markedly lower frequency of monosomy 3 in our SEA cohort compared to Western studies (van Essen et al. 2016; Laurent et al. 2011) suggests that other mechanisms might play a greater role in driving metastatic risk in Asian UM.

Contrary to the widely reported association of chromosome 8q gains with poor prognosis(Dogrusoz and Jager 2018; Harbour 2012), our study found no significant link between 8q gains and reduced PFS. Instead, gains in chromosome 8p were uniquely associated with shorter PFS in the SEA cohort. This difference may stem from population-specific factors such as pigmentation and tumor biology. Patients in our cohort predominantly had brown irides and Fitzpatrick Skin Scale III–V, which could mitigate the adverse effects of 8q gains (Wierenga et al. 2022). These findings align with the hypothesis that chromosomal aberrations, including 8q gains, have a more pronounced impact in populations with lighter irides and Fitzpatrick Skin Scale I–II (Sen et al. 2022; Agrawal et al. 2024). Notably, the four patients in our study who developed metastases had Fitzpatrick Scale III skin tones, emphasizing the importance of skin tone over iris color as a potentially sensitive prognostic factor in SEA populations.

Our study underscores the significance of chromosome 1q gains in UM progression within the SEA population. We observed a notably higher frequency of 1q gains (20%) compared to the TCGA-UM cohort (6%), consistent with previous studies reporting frequencies of approximately 24% (Coupland et al. 2013). Chromosome 1q gains were strongly associated with shorter PFS, suggesting their critical role as a late-event driver of metastasis. Shain et al. (Shain et al. 2019) demonstrated that 1q gains are enriched in metastatic tumors, often following BAP1 loss. In SEA, delayed diagnosis likely contributes to the higher prevalence of 1q gains, as patients often present with advanced-stage disease.

Interestingly, the poor prognostic impact of 1q gains observed in our SEA cohort was not evident in the TCGA-UM cohort, indicating potential differences in genetic behavior between populations. Mechanistically, the formation of isochromosomes,

including 1q, may explain its prevalence. While genomic instability from monosomy 3 loss is a known driver of isochromosome formation, the low frequency of monosomy 3 in our SEA cohort suggests that 1q gains may occur independently of chromosome 3 loss. This finding highlights the importance of further research into the distinct pathways contributing to 1q gains in non-Western populations.

Chromosome 6p loss emerged as the most frequently observed aberration among metastatic cases in our cohort, present in 50% of these patients. This finding aligns with other studies linking 6p loss to increased metastatic risk (Harbour 2012). In our study, 6p loss occurred alongside other high-risk aberrations, such as 1q gains and 8q gains, even in the absence of monosomy 3. These observations suggest that, in SEA UM, non-monosomy 3 aberrations, particularly 6p loss, may drive aggressive tumor behavior. However, the limited sample size in our study restricts definitive conclusions. Larger cohort studies are needed to confirm the metastatic potential of 6p loss and its interplay with other chromosomal aberrations.

Our findings suggest that chromosome 9q gains may play a protective role in UM. Patients with 9q gains exhibited significantly longer PFS, although this observation requires further validation due to the limited cohort size. While chromosome 9q gains have not been widely studied as prognostic markers, a small case series of Vietnamese UM patients with 9q gains and no metastasis during a three-year follow-up supports this potential protective role. Interestingly, this trend was not observed in the TCGA-UM cohort, underscoring the need for additional research into population-specific genetic differences and their clinical significance.

6.3 *BAP1* Loss in Melanoma: Bridging Tumor Biology and Immune Modulation through CRISPR-Engineered Models

The *BAP1* gene has emerged as a critical tumor suppressor in melanocytic tumors, particularly in aggressive subtypes such as UM and CM. Its loss is associated with poor prognosis, tumor progression, and therapy resistance (Chattopadhyay et al. 2016; Figueiredo et al. 2020; Ida et al. 2022; Wiesner, Obenauf, Murali, Fried, Griewank, Ulz, Windpassinger, Wackernagel, Loy, and Wolf 2011). Despite its recognized role, preclinical models have failed to fully capture the molecular, transcriptomic, and TME complexities of melanocytic tumors characterized by *BAP1* loss (Cao and Jager 2015; Némati et al. 2010; Kageyama et al. 2017; Nemati et al. 2014; Heegaard, Spang-Thomsen, and Prause 2003; Rusciano, Lorenzoni, and Burger 1994; Fidler, Gersten, and Budmen 1976; Richards et al. 2020; De Waard‐Siebinga et al. 1995). Addressing these gaps, this study presents CRISPR-engineered *BAP1*$^{-/-}$ models that replicate the transcriptomic and phenotypic attributes of advanced melanoma tumors, enabling an investigation into the role of *BAP1* loss in tumor progression and immune modulation. These syngeneic models provide a robust framework to explore therapeutic strategies in an immune-competent setting, filling a significant gap in preclinical melanoma research. By targeting exon 1 and exon 14, this work aimed to disrupt *BAP1*'s ability to suppress tumor growth and modulate DNA damage repair mechanisms. The successful establishment of several *BAP1* KO clones provided a powerful platform to analyze how *BAP1* loss alters cell behavior and contributes to the aggressive nature of UM, which effectively recapitulates the molecular and genetic features observed in high-risk UM patients, particularly those with class 2 tumors.

The tumorigenic transformation observed in *BAP1*$^{-/-}$ cells, characterized by epithelial-like morphological changes, increased proliferation, and enhanced tumorigenic potential, is consistent with clinical findings linking *BAP1* loss to aggressive histopathological features, particularly the dominance of epithelioid cells in UM (Masoomian, Shields, and Shields 2018; Koopmans et al. 2014). The epithelial-like transformation observed in *BAP1* KO melanocytes closely mirrors the clinical presentation of aggressive histopathological features in *BAP1*-loss tumors, such as the dominance of epithelioid cell morphology in UM (Shah, Bourne, and Murali 2013). These findings reinforce the dual role of *BAP1* as both a tumor suppressor and a key regulator of cellular phenotype, which underpins the aggressive nature of *BAP1*-loss melanomas. Despite their aggressive tumorigenic behavior, *BAP1* KO cells retain melanocyte lineage markers, such as gp100. This duality underscores the potential of targeting lineage-specific antigens for therapeutic purposes. For example, gp100-based vaccines have demonstrated potential as immunotherapeutic agents in melanocytic tumors (Rezaei et al. 2021; Kos et al.

2019). However, their efficacy may be enhanced with strategies designed to counteract the immunosuppressive TME characteristic of *BAP1*-loss tumors, highlighting the need for combination therapies. In the randomised phase 2b KEYNOTE-942 trial, (Weber et al. 2024) it was shown that adjuvant administration of the personalised neo-antigen mRNA vaccine mRNA-4157 (V940) together with pembrolizumab significantly extended recurrence-free survival in patients with completely resected stage IIIB–I cutaneous melanoma while retaining a predominantly grade 1-2 safety profile (Terai and Sato 2024). Although BAP1 status was not a stratification factor, the finding that vaccine-primed, neo-antigen-specific T-cell immunity can deepen the efficacy of PD-1 blockade provides a mechanistic framework for overcoming the relative immunological quiescence that accompanies BAP1 deficiency. This framework is being tested prospectively in the phase 3 Adjuvant Trial in Ocular Melanoma (ATOM; NCT06246149), which is now randomising HLA-A*02:01–positive patients with high-risk uveal melanoma to receive six months of adjuvant tebentafusp, a bispecific T-cell-receptor fusion protein that redirects cytotoxic lymphocytes toward cells presenting the gp100 peptide, or observation. Tebentafusp already confers a survival benefit in metastatic uveal melanoma and directly exploits the lineage antigenicity retained by *BAP1*-KO cells in our models. The trial's incorporation of circulating-tumour-DNA monitoring further aligns with our observation that *BAP1*-loss tumours strive for immune escape through metabolic rewiring rather than rampant genomic instability, highlighting the utility of liquid biopsies to detect occult progression in this molecularly defined subgroup.

Our findings also reveal significant immunological and metabolic changes associated with *BAP1* loss, contributing to tumor progression and immune evasion (Han, Purwin, and Aplin 2021; Chang et al. 2024). Consistent with previous reports, our findings demonstrate that *BAP1* loss reshapes the TME, resulting in altered immune cell phenotypes, reduced cytotoxic T-cell infiltration, and increased M2-polarized macrophages (Kaler et al. 2022; Figueiredo et al. 2020; Zhang and Wu 2023). These changes align with *BAP1*-driven immunosuppressive mechanisms observed in various cancers, including UM, mesothelioma, and pancreatic ductal adenocarcinoma (Kaler et al. 2022; Figueiredo et al. 2020; Zhang and Wu 2023; Xu, Gao, Yang, Spils, Marti, Losmanová, Su, Wang, Zhou, Dorn, et al. 2024; Friedhoff, Schneider, Jurcic, Endris, Kirchner, Sun, Bolnavu, Pohl, Teroerde, Kippenberger, et al. 2023). The immunosuppressive TME presents significant challenges for ICIs, emphasizing the need for combinatorial therapeutic strategies to overcome these barriers (Xu, Gao, Yang, Spils, Marti, Losmanová, Su, Wang, Zhou, and Dorn 2024; Friedhoff, Schneider, Jurcic, Endris, Kirchner, Sun, Bolnavu, Pohl, Teroerde, and Kippenberger 2023).

Transcriptomic analyses further revealed enrichment of lipid metabolic pathways in *BAP1*$^{-/-}$ tumors, aligning with prior evidence that lipid metabolic reprogramming is a hallmark of *BAP1* loss (Matareed et al. 2023). Such reprogramming supports the energy demands and aggressive growth of *BAP1*-loss tumors while shaping the TME to promote immune tolerance. Lipid accumulation within the TME has been linked to impaired dendritic cell function, reduced T-cell activation, and a tolerogenic phenotype in tumor-associated macrophages (Qiao et al. 2023; Wang et al. 2022; Tiwary, Berzofsky, and Terabe 2019; Dallavalasa et al. 2021). These findings underscore the potential of therapeutic strategies targeting lipid metabolism to disrupt the interplay between metabolic reprogramming and immune suppression in *BAP1*-loss tumors (Zhang et al. 2024).

The heterogeneity displayed by *BAP1* KO clones, particularly regarding their tumorigenic and immunosuppressive characteristics, underscores the intricate role of *BAP1* in melanocytic tumors (Donati et al. 2022; Kumar et al. 2015; Donati and Kazakov 2024). Distinct phenotypic variations, such as the highly aggressive and immunosuppressive traits observed in KO#2, indicate that even in the absence of *BAP1* expression, intrinsic variability in tumor signaling pathways and adaptations within the TME can arise. This variability is likely driven by clonal differences in gene expression or epigenetic states that operate independently of *BAP1*. Similarly, human tumors with *BAP1* loss exhibit heterogeneity in both aggressiveness and immune modulation, though they often feature additional molecular alterations beyond the loss of *BAP1* (Donati et al. 2022; Kumar et al. 2015; Donati and Kazakov 2024). The phenotypic diversity among *BAP1* KO clones in our model thus provides a valuable opportunity to investigate the specific roles of *BAP1* loss and the mechanisms underlying this variability. Furthermore, the consistent enrichment of G-protein-coupled receptor signaling and transmembrane receptor activity observed in both *in vitro* and *in vivo* models emphasizes these pathways as intrinsic characteristics of *BAP1*-loss tumors, identifying them as promising targets for therapeutic intervention.

Comparative analysis between our *BAP1* KO models and human transcriptomic datasets validates the clinical relevance of our models for studying high-risk melanocytic tumors. The shared upregulation of lipid metabolic and signaling pathways highlights their role in tumor progression and immune modulation, consistent with findings in high-risk UM and CM (Matareed et al. 2023; Wang et al. 2023). Additionally, context-specific adaptations observed in BAP1-loss tumors, such as the prioritization of immune evasion in intraocular tumors versus increased proliferation in subcutaneous tumors, underscore the critical role of the TME in shaping tumor behavior. These findings emphasize the importance of integrating TME-targeted therapies with systemic approaches to address both intrinsic and extrinsic mechanisms of tumor resistance.

This study primarily examines whether *BAP1* loss replicates the tumorigenicity and microenvironmental characteristics of melanocytic tumors rather than investigating its role in metastatic dissemination to the liver, a defining feature of advanced UM (Kaliki and Shields 2017; Gallenga et al. 2022; Coupland et al. 2013; Harbour 2009; Smit et al. 2020; Dogrusoz and Jager 2018; Gelmi and Jager 2024),. Although metastatic modeling is outside the scope of this work, the syngeneic model presents a valuable foundation for future research into the metastatic processes of UM and CM driven exclusively by *BAP1* alterations. These findings establish a basis for subsequent studies utilizing the syngeneic framework of this model to develop targeted therapies aimed at counteracting *BAP1* loss. Furthermore, this model provides a critical platform for evaluating combinatorial immunotherapies designed to reverse the immunosuppressive environment linked to *BAP1* loss, intending to restore the efficacy of immune checkpoint therapies in resistant melanocytic tumors.

6.4 Limitations and Future Directions

This thesis is based on reverse translational research, leveraging clinical observations to elucidate molecular mechanisms of tumor progression and immune suppression and advancing these findings through preclinical validations. While significant progress has been achieved, the studies have inherent limitations that inform future research directions.

One limitation of the *CD1D* study (**Aim 1**) lies in the reliance on publicly available datasets and theoretical models to explore the functional role of β2M in antigen presentation and immune regulation. Although the findings highlight the potential epigenetic regulation of *CD1D* and its implications for phospholipid antigen presentation, the absence of in vivo validation limits the ability to confirm these mechanisms in ICT resistance. For instance, while β2M deficiencies are shown to impair MHC-I-mediated antigen presentation and lead to rapid *CD1D* degradation (Jenkins, Barbie, and Flaherty 2018; Kim et al. 1999), further studies employing knockout or knock-in models are required to validate these findings in a physiological setting. Additionally, the proposed mechanisms by which epigenetic modulation of *CD1D* contributes to immunoresistance remain underexplored. Future research should focus on functional studies that assess *CD1D* stability, trafficking, and antigen-presenting capabilities in β2M-deficient models.

In the UM genetic landscape of study (**Aim 1**), the SEA cohort analyzed in this thesis comprised a smaller sample size and predominantly early-stage tumors, reflecting the rarity of UM in the region. This discrepancy highlights the need for multi-regional studies integrating diverse cohorts to account for geographic and ethnic disparities in UM genetics. Additionally, the limited follow-up durations and the absence of advanced molecular profiling techniques, such as NGS, restricted the depth of analysis. While the study identified significant associations between chromosome 1q gains and metastatic risk, expanding the sample size and incorporating scRNA-seq will provide deeper insights into the functional consequences of these chromosomal alterations. Investigating alternative biomarkers, such as PRAME mRNA expression or IHC, may improve prognostic accuracy and refine risk stratification in SEA UM populations.

The preclinical implementation of the *BAP1*-loss study (**Aim 2-3**), which developed a CRISPR-based KO model, represents a significant advancement in understanding the role of *BAP1* in tumor progression and immune modulation. However, certain model aspects can be further optimized to enhance translational relevance. The use of immortalized melanocyte line Melan-a allowed for a controlled investigation of *BAP1* loss without the interference of additional driver mutations. However, Melan-a primarily reflects gene expression patterns associated with epigenetic characteristics of skin tissues, making it less suitable for studying intraocular melanocytic tumors (Jager, Shields, Cebulla, Abdel-Rahman,

Grossniklaus, Stern, Carvajal, Belfort, Jia, and Shields 2020; Kaliki and Shields 2017). A more representative model would involve immortalized intraocular melanocytes, but primary melanocytes were not utilized due to their limited growth potential and fragility (Zaidi, Fisher, and Rizos 2020). Moving forward, this model can serve as a foundation for developing improved systems that immortalize primary melanocytes from various tissues, enabling orthotopic studies of *BAP1*-loss melanocytic tumors.

Additionally, our model offers the first detailed insights into how *BAP1* loss impacts subcutaneous tumors' microenvironment while providing valuable data related to intraocular tumors. Although it does not utilize immortalized melanocytes with *BAP1* loss introduced directly into the eye, the model still replicates the transcriptomic characteristics of human UM tumors with *BAP1* loss. Expanding this methodology to include other melanocytic tumor types could provide deeper insights into *BAP1*'s role across melanocyte-derived malignancies. Addressing these limitations through longitudinal studies and developing more comprehensive modeling systems will be essential for advancing our understanding of *BAP1*-driven tumor biology.

The current *BAP1* KO model successfully replicated many aspects of human tumor biology but did not incorporate a metastatic component. Modeling liver metastasis, a hallmark of UM progression, represents an important area for future research. While differences between murine and human immune systems are a recognized challenge in preclinical studies, the *BAP1* KO model provided a robust platform for studying the TME and its role in immune suppression.

Future directions include the development of preclinical models using primary melanocytes to better mimic the native tumor environment. Enhancing metastatic modeling capabilities, including methods such as tail vein or splenic injections (Yang, Cao, and Grossniklaus 2015), will allow for more detailed studies of BAP1 loss in metastatic dissemination. Longitudinal studies to evaluate therapeutic responses, particularly combination therapies targeting metabolic pathways, immune suppression, and signaling aberrations, will refine translational applications and open new avenues for therapeutic development.

Addressing these limitations through future innovative experimental approaches, multi-regional collaborations during my postdoctoral research, and advanced molecular profiling will significantly advance the understanding of melanoma biology and immune suppression. This thesis has laid the groundwork for future studies to bridge clinical discoveries with translational applications, fostering the development of personalized therapies for uveal and cutaneous melanoma. These efforts will ultimately contribute to improved prognostic tools and therapeutic strategies, driving progress in the field of melanoma research.

7 Summary/Conclusions

This thesis comprehensively explores the molecular and immunological underpinnings of melanoma progression and immune suppression, employing a reverse translational approach to bridge clinical observations with preclinical insights. By integrating clinical datasets, multi-omic analyses, and novel preclinical models, this work has made significant strides in understanding the mechanisms driving tumor aggressiveness and therapy resistance in UM and cutaneous melanoma.

The first significant finding of this thesis focuses on the epigenetic regulation of *CD1D* as a mechanism of immune resistance in melanoma. Analysis of publicly available datasets revealed that the β2M/*CD1D* axis plays a vital role in antigen presentation and immune evasion. This work identifies *SPI1* promoter methylation as a key regulator of *CD1D* expression, providing a novel perspective on ICT resistance in poorly immunogenic melanomas. These findings suggest restoring *CD1D* functionality could enhance NKT cell-mediated immune responses, offering new therapeutic avenues for overcoming ICT resistance. This aspect of the thesis underscores the importance of epigenetic modulation in tumor immunity and sets the stage for future translational efforts targeting *CD1D* in melanoma.

The second major contribution of this thesis lies in elucidating the genetic landscape of UM in a SEA cohort. This study highlights significant differences in chromosomal aberrations compared to Western populations, with a notable emphasis on 1q gains as a critical driver of metastatic risk. The consistent association of *BAP1* loss with poor outcomes reinforces its universal role as a prognostic marker across populations. These findings illuminate the population-specific genetic features of UM while reaffirming the significance of *BAP1* loss and 1q gains as key contributors to tumor progression. The study's integration of clinical and genomic data provides a robust framework for refining prognostic tools and tailoring therapeutic strategies to diverse patient populations.

The third significant achievement of this thesis is developing a CRISPR-engineered, syngeneic, immunocompetent *BAP1*-loss model, addressing a critical gap in melanoma research. This model effectively replicates key features of *BAP1*-loss tumors, including **aggressive proliferation**, **immune evasion**, and **metabolic**

reprogramming, enabling the study of tumor progression and resistance mechanisms in a controlled setting. Validation through genomic, transcriptomic, and protein-level analyses confirmed the successful disruption of *BAP1*, with the resulting clones exhibiting hallmark phenotypes such as enhanced growth, epithelioid transformation, and enriched immune-suppressive pathways.

Transcriptomic profiling of the *BAP1*-loss model revealed significant overlaps with high-risk human melanoma datasets, particularly in **immune suppression** and **metabolic pathways**, underscoring the model's translational relevance. By capturing the complex interplay between tumor-intrinsic mechanisms and the tumor microenvironment, this model provides a robust platform for preclinical research, supporting the development of therapeutic strategies targeting *BAP1*-loss tumors, including combinatory approaches with immune and metabolic interventions.

This thesis also identifies critical limitations and avenues for future research. While the *CD1D* study highlighted epigenetic mechanisms of immune resistance, *in vivo* validation is needed to confirm these findings in a physiological context. The genetic analysis of UM in SEA populations provides valuable insights but requires larger cohorts and advanced molecular profiling to fully capture the complexity of these tumors. The CRISPR-engineered *BAP1*-loss model represents a significant advancement but would benefit from further refinement, including metastatic modeling and the use of primary melanocytes.

In conclusion, this thesis establishes a robust foundation for understanding the molecular and immunological drivers of melanoma progression and immune suppression. Bridging clinical discoveries with preclinical innovations advances melanoma research, providing critical insights for developing targeted therapies. Integrating epigenetic, genetic, and immunological perspectives offers a comprehensive framework for tackling melanoma therapy resistance and metastatic progression. These findings hold significant translational potential, paving the way for personalized therapeutic strategies that address the unique molecular landscapes of UM and CM. Through this work, we take an essential step toward improving outcomes for patients with high-risk melanomas, contributing to the broader goals of precision oncology.

Acknowledgments

This doctoral journey has been a profound collaboration between the Institute of Biomedicine, the University of Turku, and the Translational Ophthalmic Pathology-Immunology (TOP-IP) Platform at the Singapore Eye Research Institute. As the first project of its kind, I hope this work sets the foundation for many future partnerships, fostering a legacy of shared knowledge and scientific discovery internationally.

My deepest gratitude goes to my supervisor, Associate Professor Carlos Rogerio Figueiredo, whose guidance has shaped not only my scientific skills but also my outlook on research and life. Dear Carlos, thank you for introducing me to the world of immunotherapy, for guiding me through the intricacies of project planning and data analysis, and, above all, for teaching me to write with precision and purpose. Your mentorship has been an extraordinary blend of granting me the freedom to explore my ideas and offering a steady hand when I needed direction. You have provided the perfect balance of support and challenge, pushing me to grow as a scientist. Our conversations, ranging from the challenges of academia to the philosophies of life, have been a source of inspiration and strength. I will carry your words of encouragement and wisdom throughout my career.

I am equally grateful to Dr. Anita Chan, Head of the Department of Ocular Inflammation and Immunology at the Singapore Eye Research Institute. Dear Dr. Anita, this PhD journey might not have been possible without your trust and encouragement three years ago. Thank you for your unwavering support, for the generous resources you provided, and for the collaborations and publications that grew out of your vision. You gave me the space and trust to manage my responsibilities independently, allowing me to develop a unique and meaningful approach to my work. Your belief in me has meant the world.

I also extend my heartfelt thanks to my PhD Advisory Committee members, Professor Sarah Coupland and Professor Urpo Lamminmäki, for their invaluable feedback on my projects and publications. Your keen insights and attention to detail have refined my work and helped me elevate the quality of this dissertation.

To the organizations that funded this journey, including the Turku University Foundation, Cancer Foundation Finland, Finnish Melanoma Group, InFLAMES Research Flagship, Orion Research Foundation, Finnish Cultural Foundation,

VisionSave (Singapore), and SingHealth Fund (SHF) Research Grant, thank you for believing in the potential of this research and enabling me to pursue it to its fullest extent. Your support has been instrumental, not only financially but as an affirmation of the value of this work.

I am also blessed with family and friends who have been my pillars of strength. Dear Thiri Lynn, thank you for standing by my side through the highs and lows. You have cared for me during stressful times, when I was sick, and dealt with my allergies, lifting me when I was at my lowest. The adventures and travels you organized gave me joy and balance, allowing me to return to work with a renewed spirit. Dear Allan Lee, thank you for always being there to offer advice on my experiments, especially the complexities of CRISPR work and bioinformatic server setup. Your presence as a big brother in the lab has been invaluable, from guiding me through technical challenges to taking care of my precious cells when I was away. I cannot thank you enough for your reliability and friendship. To Augustine, my dear godfather and family, thank you for the endless laughter, for all the shared moments that brightened my days, and for your practical advice on reagents and equipment whenever I needed it. You have been a constant source of joy and wisdom, and I am so grateful to have you in my life. To Miika Kaunisto, I extend my sincere gratitude for your invaluable support in revising the Finnish version of my abstract, significantly enhancing its quality and clarity. Your friendship in Turku and assistance with unfamiliar matters have been a great source of help and comfort, and I am deeply appreciative. To Tommy, my dearest brother and best friend in so many aspects of life, thank you for taking care of me in daily life in Singapore, driving me around, and putting up with my pickiness in food. Your patience and help in looking after our parents whenever you can mean so much to me. I cherish everything you've done for me. To my beloved parents, thank you for educating me with love and patience, allowing me to study in advanced environments, and sending me to the best schools whenever possible. You shaped my character and, over time, came to understand and respect my choices in life. Your trust and unwavering support have been my foundation, and I am endlessly grateful for the sacrifices you made for me.

This thesis is not just a culmination of my work but a testament to the love, trust, and encouragement of everyone who stood by me. I am profoundly thankful to each of you for believing in me and helping me reach this stage. This achievement belongs as much to you as it does to me, and I am endlessly grateful. I love you all.

Date: 21 Feb 2025
Wang Meng (Mona)

References

Abdel-Rahman, M. H., B. N. Christopher, M. F. Faramawi, K. Said-Ahmed, C. Cole, A. McFaddin, A. Ray-Chaudhury, N. Heerema, and F. H. Davidorf. 2011. 'Frequency, molecular pathology and potential clinical significance of partial chromosome 3 aberrations in uveal melanoma', *Mod Pathol*, 24: 954-62.

Abdel-Rahman, M. H., Y. Yang, X. P. Zhou, E. L. Craig, F. H. Davidorf, and C. Eng. 2006. 'High frequency of submicroscopic hemizygous deletion is a major mechanism of loss of expression of PTEN in uveal melanoma', *J Clin Oncol*, 24: 288-95.

Afshar, A. R., B. E. Damato, J. M. Stewart, L. B. Zablotska, R. Roy, A. B. Olshen, N. M. Joseph, and B. C. Bastian. 2019. 'Next-Generation Sequencing of Uveal Melanoma for Detection of Genetic Alterations Predicting Metastasis', *Transl Vis Sci Technol*, 8: 18.

Agrawal, K. U., M. R. Barke, L. Chiang, R. Ni, R. S. Kim, Q. Zhang, and C. L. Shields. 2024. 'Correlation of fitzpatrick skin type and iris color with tumor size in 823 patients with uveal melanoma', *Asia Pac J Ophthalmol (Phila)*, 13: 100046.

Ahmed, Bilal, Muhammad Imran Qadir, and Saba Ghafoor. 2020. 'Malignant melanoma: skin cancer− diagnosis, prevention, and treatment', *Critical Reviews™ in Eukaryotic Gene Expression*, 30.

Ahmed, Farzana, and Nikolas K Haass. 2018. 'Microenvironment-driven dynamic heterogeneity and phenotypic plasticity as a mechanism of melanoma therapy resistance', *Frontiers in oncology*, 8: 173.

Akbani, Rehan, KadiráC Akdemir, B áArman Aksoy, Monique Albert, Adrian Ally, SamirkumaráB Amin, Harindra Arachchi, Arshi Arora, J áTodd Auman, and Brenda Ayala. 2015. 'Genomic classification of cutaneous melanoma', *Cell*, 161: 1681-96.

Albini, Sonia, Vlada Zakharova, and Slimane Ait-Si-Ali. 2019. 'Histone modifications.' in, *Epigenetics and regeneration* (Elsevier).

Algazi, Alain P, Katy K Tsai, Alexander N Shoushtari, Rodrigo R Munhoz, Zeynep Eroglu, Josep M Piulats, Patrick A Ott, Douglas B Johnson, Jimmy Hwang, and Adil I Daud. 2016. 'Clinical outcomes in metastatic uveal melanoma treated with PD‐1 and PD‐L1 antibodies', *Cancer*, 122: 3344-53.

Allen, T. M., M. A. Brehm, S. Bridges, S. Ferguson, P. Kumar, O. Mirochnitchenko, K. Palucka, R. Pelanda, B. Sanders-Beer, L. D. Shultz, L. Su, and M. PrabhuDas. 2019. 'Humanized immune system mouse models: progress, challenges and opportunities', *Nat Immunol*, 20: 770-74.

Amodio, Vito, Gianluca Mauri, Nicole M Reilly, Andrea Sartore-Bianchi, Salvatore Siena, Alberto Bardelli, and Giovanni Germano. 2021. 'Mechanisms of immune escape and resistance to checkpoint inhibitor therapies in mismatch repair deficient metastatic colorectal cancers', *Cancers*, 13: 2638.

An, Junsha, Yajie Lu, Yuxi Chen, Yuling Chen, Zhaokai Zhou, Jianping Chen, Cheng Peng, Ruizhen Huang, and Fu Peng. 2024. 'Spatial transcriptomics in breast cancer: providing insight into tumor heterogeneity and promoting individualized therapy', *Frontiers in Immunology*, 15: 1499301.

Anand, Kartik, Jason Roszik, Dan Gombos, Joshua Upshaw, Vanessa Sarli, Salyna Meas, Anthony Lucci, Carolyn Hall, and Sapna Patel. 2019. 'Pilot Study of Circulating Tumor Cells in Early-Stage and Metastatic Uveal Melanoma', *Cancers*, 11: 856.

Angi, Martina, Leila Khoja, Bertil Damato, Paul Lorigan, Caroline Dive, Sarah Coupland, and Helen Kalirai. 2013. 'Detection of circulating tumor cells in uveal melanoma using the CellSearch® system', *Investigative Ophthalmology & Visual Science*, 54: 4225-25.

Anita SY Chan; Sunny Shen; Audrey Looi Lee Geok; Seah Lay Leng;, Leonard Tan Hwan Cheong and Issam Al Jajeh. 2014. 'Orbital IgG4 Related Disease (IgG4RD): Incidence and Accompanying Histological Features Using the Latest Consensus Criteria', *Journal of Cytology & Histology*, 5: 286-90.

Ardeniz, Ö, S. Unger, H. Onay, S. Ammann, C. Keck, C. Cianga, B. Gerçeker, B. Martin, I. Fuchs, U. Salzer, A. İkincioğulları, D. Güloğlu, T. Dereli, R. Thimme, S. Ehl, K. Schwarz, A. Schmitt-Graeff, P. Cianga, P. Fisch, and K. Warnatz. 2015. 'β2-Microglobulin deficiency causes a complex immunodeficiency of the innate and adaptive immune system', *J Allergy Clin Immunol*, 136: 392-401.

Arner, Emily N, and Jeffrey C Rathmell. 2023. 'Metabolic programming and immune suppression in the tumor microenvironment', *Cancer Cell*, 41: 421-33.

Arozarena, I., and C. Wellbrock. 2019. 'Phenotype plasticity as enabler of melanoma progression and therapy resistance', *Nat Rev Cancer*, 19: 377-91.

Artegiani, Benedetta, Lisa van Voorthuijsen, Rik G. H. Lindeboom, Daniëlle Seinstra, Inha Heo, Pablo Tapia, Carmen López-Iglesias, Daniel Postrach, Talya Dayton, Rurika Oka, Huili Hu, Ruben van Boxtel, Johan H. van Es, Johan Offerhaus, Peter J. Peters, Jacco van Rheenen, Michiel Vermeulen, and Hans Clevers. 2019. 'Probing the Tumor Suppressor Function of BAP1 in CRISPR-Engineered Human Liver Organoids', *Cell Stem Cell*, 24: 927-43.e6.

Bae, E. A., H. Seo, B. S. Kim, J. Choi, I. Jeon, K. S. Shin, C. H. Koh, B. Song, I. K. Kim, B. S. Min, Y. D. Han, S. J. Shin, and C. Y. Kang. 2018. 'Activation of NKT Cells in an Anti-PD-1-Resistant Tumor Model Enhances Antitumor Immunity by Reinvigorating Exhausted CD8 T Cells', *Cancer Res*, 78: 5315-26.

Bai, Xue, Yan Kong, Zhihong Chi, Xinan Sheng, Chuanliang Cui, Xuan Wang, Lili Mao, Bixia Tang, Siming Li, and Bin Lian. 2017. 'MAPK pathway and TERT promoter gene mutation pattern and its prognostic value in melanoma

patients: a retrospective study of 2,793 cases', *Clinical cancer research*, 23: 6120-27.

Bakalian, Silvin, Jean-Claude Marshall, Patrick Logan, Dana Faingold, Shawn Maloney, Sebastian Di Cesare, Claudia Martins, Bruno F. Fernandes, and Miguel N. Burnier, Jr. 2008. 'Molecular Pathways Mediating Liver Metastasis in Patients with Uveal Melanoma', *Clinical cancer research*, 14: 951-56.

Balch, Charles, Alan N Houghton, Arthur J Sober, Seng-jaw Soong, Michael B Atkins, and John F Thompson. 2024. *Cutaneous melanoma* (Taylor & Francis).

Balint, Klara, Min Xiao, Chelsea C Pinnix, Akinobu Soma, Imre Veres, Istvan Juhasz, Eric J Brown, Anthony J Capobianco, Meenhard Herlyn, and Zhao-Jun Liu. 2005. 'Activation of Notch1 signaling is required for β-catenin–mediated human primary melanoma progression', *The Journal of clinical investigation*, 115: 3166-76.

Ballotti, Robert, Yann Cheli, and Corine Bertolotto. 2020. 'The complex relationship between MITF and the immune system: a Melanoma ImmunoTherapy (response) Factor?', *Molecular cancer*, 19: 1-12.

Bande, Manuel F, Maria Santiago, Laura Muinelo-Romay, Maria Jose Blanco, Purificacion Mera, Carmela Capeans, Maria Pardo, and Antonio Piñeiro. 2015. 'Detection of circulating melanoma cells in choroidal melanocytic lesions', *BMC research notes*, 8: 1-4.

Bannister, Andrew J, and Tony Kouzarides. 2011. 'Regulation of chromatin by histone modifications', *Cell research*, 21: 381-95.

Barisione, Gaia, Marina Fabbi, Alice Gino, Paola Queirolo, Laura Orgiano, Laura Spano, Virginia Picasso, Ulrich Pfeffer, Carlo Mosci, and Martine J Jager. 2015. 'Potential role of soluble c-Met as a new candidate biomarker of metastatic uveal melanoma', *JAMA ophthalmology*, 133: 1013-21.

Bedogni, Barbara, James A Warneke, Brian J Nickoloff, Amato J Giaccia, and Marianne Broome Powell. 2008. 'Notch1 is an effector of Akt and hypoxia in melanoma development', *The Journal of clinical investigation*, 118: 3660-70.

Ben-David, U., B. Siranosian, G. Ha, H. Tang, Y. Oren, K. Hinohara, C. A. Strathdee, J. Dempster, N. J. Lyons, R. Burns, A. Nag, G. Kugener, B. Cimini, P. Tsvetkov, Y. E. Maruvka, R. O'Rourke, A. Garrity, A. A. Tubelli, P. Bandopadhayay, A. Tsherniak, F. Vazquez, B. Wong, C. Birger, M. Ghandi, A. R. Thorner, J. A. Bittker, M. Meyerson, G. Getz, R. Beroukhim, and T. R. Golub. 2018. 'Genetic and transcriptional evolution alters cancer cell line drug response', *Nature*, 560: 325-30.

Benacerraf, Baruj, and Hugh O McDevitt. 1972. 'Histocompatibility-Linked Immune Response Genes: A new class of genes that controls the formation of specific immune responses has been identified', *Science*, 175: 273-79.

Bendelac, A., P. B. Savage, and L. Teyton. 2007. 'The biology of NKT cells', *Annu Rev Immunol*, 25: 297-336.

Bennett, Dorothy C, Philip J Cooper, and Ian R Hart. 1987. 'A line of non‐tumorigenic mouse melanocytes, syngeneic with the B16 melanoma and

requiring a tumour promoter for growth', *International journal of cancer*, 39: 414-18.

Bennett, Richard L, and Jonathan D Licht. 2018. 'Targeting epigenetics in cancer', *Annual review of pharmacology and toxicology*, 58: 187-207.

Benteyn, D., A. M. Van Nuffel, S. Wilgenhof, and A. Bonehill. 2014. 'Single-step antigen loading and maturation of dendritic cells through mRNA electroporation of a tumor-associated antigen and a TriMix of costimulatory molecules', *Methods Mol Biol*, 1139: 3-15.

Bidard, François‐Clément, Jordan Madic, Pascale Mariani, Sophie Piperno‐Neumann, Aurore Rampanou, Vincent Servois, Nathalie Cassoux, Laurence Desjardins, Maud Milder, and Isabelle Vaucher. 2014. 'Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma', *International journal of cancer*, 134: 1207-13.

Blanchard, Zannel, Elisabeth A Brown, Arevik Ghazaryan, and Alana L Welm. 2024. 'PDX models for functional precision oncology and discovery science', *Nature Reviews Cancer*: 1-14.

Bobadilla, K., E. Sada, M. E. Jaime, Y. González, L. Ramachandra, R. E. Rojas, S. Pedraza-Sánchez, C. Michalak, A. González-Noriega, and M. Torres. 2013. 'Human phagosome processing of Mycobacterium tuberculosis antigens is modulated by interferon-γ and interleukin-10', *Immunology*, 138: 34-46.

Borg, Natalie A., Kwok S. Wun, Lars Kjer-Nielsen, Matthew C. J. Wilce, Daniel G. Pellicci, Ruide Koh, Gurdyal S. Besra, Mandvi Bharadwaj, Dale I. Godfrey, James McCluskey, and Jamie Rossjohn. 2007. 'CD1d–lipid-antigen recognition by the semi-invariant NKT T-cell receptor', *Nature*, 448: 44-49.

Bourhis, M., J. Palle, I. Galy-Fauroux, and M. Terme. 2021. 'Direct and Indirect Modulation of T Cells by VEGF-A Counteracted by Anti-Angiogenic Treatment', *Front Immunol*, 12: 616837.

Boutros, C., A. Tarhini, E. Routier, O. Lambotte, F. L. Ladurie, F. Carbonnel, H. Izzeddine, A. Marabelle, S. Champiat, A. Berdelou, E. Lanoy, M. Texier, C. Libenciuc, A. M. Eggermont, J. C. Soria, C. Mateus, and C. Robert. 2016. 'Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination', *Nat Rev Clin Oncol*, 13: 473-86.

Bronkhorst, Inge H.G., and Martine J. Jager. 2012. 'Uveal Melanoma: The Inflammatory Microenvironment', *Journal of Innate Immunity*, 4: 454-62.

Budczies, Jan, Daniel Kazdal, Michael Menzel, Susanne Beck, Klaus Kluck, Christian Altbürger, Constantin Schwab, Michael Allgäuer, Aysel Ahadova, and Matthias Kloor. 2024. 'Tumour mutational burden: clinical utility, challenges and emerging improvements', *Nature Reviews Clinical Oncology*, 21: 725-42.

Burnier, Julia V, Christina Mastromonaco, Jade Marie Lasiste, and Miguel N Burnier. 2019. 'Animal Models in Uveal Melanoma', *Clinical Ophthalmic Oncology: Uveal Tumors*: 135-54.

Burotto, Mauricio, Victoria L Chiou, Jung‐Min Lee, and Elise C Kohn. 2014. 'The MAPK pathway across different malignancies: a new perspective', *Cancer*, 120: 3446-56.

Cao, Jinfeng, and Martine J Jager. 2015. 'Animal eye models for uveal melanoma', *Ocular oncology and pathology*, 1: 141-50.

Carbone, Michele, J William Harbour, James Brugarolas, Angela Bononi, Ian Pagano, Anwesha Dey, Thomas Krausz, Harvey I Pass, Haining Yang, and Giovanni Gaudino. 2020. 'Biological mechanisms and clinical significance of BAP1 mutations in human cancer', *Cancer discovery*, 10: 1103-20.

Carlson, J Andrew, Gerald P Linette, Andrew Aplin, Bernard Ng, and Andrzej Slominski. 2007. 'Melanocyte receptors: clinical implications and therapeutic relevance', *Dermatologic clinics*, 25: 541-57.

Carreira, Suzanne, Jane Goodall, Laurence Denat, Mercedes Rodriguez, Paolo Nuciforo, Keith S Hoek, Alessandro Testori, Lionel Larue, and Colin R Goding. 2006. 'Mitf regulation of Dia1 controls melanoma proliferation and invasiveness', *Genes & development*, 20: 3426-39.

Carvajal, R. D., M. O. Butler, A. N. Shoushtari, J. C. Hassel, A. Ikeguchi, L. Hernandez-Aya, P. Nathan, O. Hamid, J. M. Piulats, M. Rioth, D. B. Johnson, J. J. Luke, E. Espinosa, S. Leyvraz, L. Collins, H. M. Goodall, K. Ranade, C. Holland, S. E. Abdullah, J. J. Sacco, and T. Sato. 2022. 'Clinical and molecular response to tebentafusp in previously treated patients with metastatic uveal melanoma: a phase 2 trial', *Nat Med*, 28: 2364-73.

Carvajal, Richard D, Gary K Schwartz, Tongalp Tezel, Brian Marr, Jasmine H Francis, and Paul D Nathan. 2017. 'Metastatic disease from uveal melanoma: treatment options and future prospects', *British Journal of Ophthalmology*, 101: 38-44.

Chamcheu, Jean Christopher, Tithi Roy, Mohammad Burhan Uddin, Sergette Banang-Mbeumi, Roxane-Cherille N Chamcheu, Anthony L Walker, Yong-Yu Liu, and Shile Huang. 2019. 'Role and therapeutic targeting of the PI3K/Akt/mTOR signaling pathway in skin cancer: a review of current status and future trends on natural and synthetic agents therapy', *Cells*, 8: 803.

Champiat, Stéphane, Laurent Dercle, Samy Ammari, Christophe Massard, Antoine Hollebecque, Sophie Postel-Vinay, Nathalie Chaput, Alexander Eggermont, Aurélien Marabelle, and Jean-Charles Soria. 2017. 'Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1', *Clinical cancer research*, 23: 1920-28.

Chan, Anita S Y, Hardeep Mudhar, Sunny Yu Shen, Stephanie S Lang, Malee Fernando, Maryam Hazly Hilmy, Naomi Jayne Guppy, Ian Rennie, Lisa Dunkley, and Issam Al Jajeh. 2017. 'Serum IgG2 and tissue IgG2 plasma cell elevation in orbital IgG4-related disease (IgG4-RD): Potential use in IgG4-RD assessment', *British Journal of Ophthalmology*, 101: 1576-82.

Chan, L. L., B. K. Cheung, J. C. Li, and A. S. Lau. 2010. 'A role for STAT3 and cathepsin S in IL-10 down-regulation of IFN-gamma-induced MHC class II molecule on primary human blood macrophages', *J Leukoc Biol*, 88: 303-11.

Chang, Chih-Hao, Jing Qiu, David O'Sullivan, Michael D Buck, Takuro Noguchi, Jonathan D Curtis, Qiongyu Chen, Mariel Gindin, Matthew M Gubin, and Gerritje JW Van Der Windt. 2015. 'Metabolic competition in the tumor microenvironment is a driver of cancer progression', *Cell*, 162: 1229-41.

Chang, Hong, Mingxia Li, Linlin Zhang, Meng Li, Swee Hoe Ong, Zhiwei Zhang, Jie Zheng, Xiang Xu, Yu Zhang, Jing Wang, Xingjie Liu, Kairui Li, Yao Luo, Haiyun Wang, Zhichao Miao, Xi Chen, Jie Zha, and Yong Yu. 2024.

'Loss of histone deubiquitinase Bap1 triggers anti-tumor immunity', *Cellular Oncology*.

Chattopadhyay, C., D. W. Kim, D. S. Gombos, J. Oba, Y. Qin, M. D. Williams, B. Esmaeli, E. A. Grimm, J. A. Wargo, S. E. Woodman, and S. P. Patel. 2016. 'Uveal melanoma: From diagnosis to treatment and the science in between', *Cancer*, 122: 2299-312.

Chau, Vincent, and Marijo Bilusic. 2020. 'Pembrolizumab in combination with axitinib as first-line treatment for patients with renal cell carcinoma (RCC): evidence to date', *Cancer Management and Research*, 12: 7321.

Chawra, Himmat Singh, Mohit Agarwal, Anurag Mishra, Shailendra Singh Chandel, Ravindra Pal Singh, Gaurav Dubey, Neelima Kukreti, and Mithilesh Singh. 2024. 'MicroRNA-21's role in PTEN suppression and PI3K/AKT activation: Implications for cancer biology', *Pathology-Research and Practice*: 155091.

Chen, D. S., and I. Mellman. 2017a. 'Elements of cancer immunity and the cancer-immune set point', *Nature*, 541: 321-30.

Chen, Daniel S, and Ira Mellman. 2017b. 'Elements of cancer immunity and the cancer–immune set point', *Nature*, 541: 321-30.

Chen, L. N., and R. D. Carvajal. 2022. 'Tebentafusp for the treatment of HLA-A*02:01-positive adult patients with unresectable or metastatic uveal melanoma', *Expert Rev Anticancer Ther*, 22: 1017-27.

Chiorazzi, Michael, Jan Martinek, Bradley Krasnick, Yunjiang Zheng, Keenan J Robbins, Rihao Qu, Gabriel Kaufmann, Zachary Skidmore, Melani Juric, and Laura A Henze. 2023. 'Autologous humanized PDX modeling for immuno-oncology recapitulates features of the human tumor microenvironment', *Journal for ImmunoTherapy of Cancer*, 11.

Chulpanova, Daria S, Kristina V Kitaeva, Catrin S Rutland, Albert A Rizvanov, and Valeriya V Solovyeva. 2020. 'Mouse tumor models for advanced cancer immunotherapy', *International Journal of Molecular Sciences*, 21: 4118.

Cline, Melissa S, Brian Craft, Teresa Swatloski, Mary Goldman, Singer Ma, David Haussler, and Jingchun Zhu. 2013. 'Exploring TCGA pan-cancer data at the UCSC cancer genomics browser', *Scientific Reports*, 3: 2652.

Coleman, S., M. Xie, A. A. Tarhini, and A. C. Tan. 2023. 'Systematic evaluation of the predictive gene expression signatures of immune checkpoint inhibitors in metastatic melanoma', *Mol Carcinog*, 62: 77-89.

Conway, James RW, Neil O Carragher, and Paul Timpson. 2014. 'Developments in preclinical cancer imaging: innovating the discovery of therapeutics', *Nature Reviews Cancer*, 14: 314-28.

Coupland, S. E., S. L. Lake, M. Zeschnigk, and B. E. Damato. 2013. 'Molecular pathology of uveal melanoma', *Eye (Lond)*, 27: 230-42.

Czarnecka, Anna M, Ewa Bartnik, Michał Fiedorowicz, and Piotr Rutkowski. 2020. 'Targeted therapy in melanoma and mechanisms of resistance', *International Journal of Molecular Sciences*, 21: 4576.

Dallavalasa, S., N. M. Beeraka, C. G. Basavaraju, S. V. Tulimilli, S. P. Sadhu, K. Rajesh, G. Aliev, and S. V. Madhunapantula. 2021. 'The Role of Tumor Associated Macrophages (TAMs) in Cancer Progression, Chemoresistance, Angiogenesis and Metastasis - Current Status', *Curr Med Chem*, 28: 8203-36.

Dankort, D., D. P. Curley, R. A. Cartlidge, B. Nelson, A. N. Karnezis, W. E. Damsky, Jr., M. J. You, R. A. DePinho, M. McMahon, and M. Bosenberg. 2009. 'Braf(V600E) cooperates with Pten loss to induce metastatic melanoma', *Nat Genet*, 41: 544-52.

de Azevedo, R. A., E. Shoshan, S. Whang, G. Markel, A. R. Jaiswal, A. Liu, M. A. Curran, L. R. Travassos, and M. Bar-Eli. 2020. 'MIF inhibition as a strategy for overcoming resistance to immune checkpoint blockade therapy in melanoma', *Oncoimmunology*, 9: 1846915.

De Souza, L. M., B. M. Robertson, and G. P. Robertson. 2017. 'Future of circulating tumor cells in the melanoma clinical and research laboratory settings', *Cancer Lett*, 392: 60-70.

De Waard‐Siebinga, Itte, Derk‐Jan R Blom, Marieke Griffioen, Peter I Schrier, ED Hoogendoorn, Geoff Beverstock, Erik HJ Danen, and Marline J Jager. 1995. 'Establishment and characterization of an uveal‐melanoma cell line', *International journal of cancer*, 62: 155-61.

Ding, Lei, Jiaqi Cao, Wen Lin, Hongjian Chen, Xianhui Xiong, Hongshun Ao, Min Yu, Jie Lin, and Qinghua Cui. 2020. 'The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer', *International Journal of Molecular Sciences*, 21: 1960.

Dithmar, S, D Rusciano, and HE Grossniklaus. 2000. 'A new technique for implantation of tissue culture melanoma cells in a murine model of metastatic ocular melanoma', *Melanoma Research*, 10: 2-8.

Dobre, E. G., C. Constantin, M. Costache, and M. Neagu. 2021. 'Interrogating Epigenome toward Personalized Approach in Cutaneous Melanoma', *J Pers Med*, 11.

Dogrusoz, M., and M. J. Jager. 2018. 'Genetic prognostication in uveal melanoma', *Acta Ophthalmol*, 96: 331-47.

Donati, Michele, and Dmitry V. Kazakov. 2024. 'Beyond typical histology of BAP1-inactivated melanocytoma', *Pathology - Research and Practice*, 259: 155162.

Donati, Michele, Petr Martinek, Petr Steiner, Petr Grossmann, Tomas Vanecek, Liubov Kastnerova, Isabel Kolm, Martina Baneckova, Pietro Donati, and Irina Kletskaya. 2022. 'Novel insights into the BAP1-inactivated melanocytic tumor', *Modern pathology*, 35: 664-75.

Dong, Lei, Shuo You, Qing Zhang, Satoru Osuka, Narra S Devi, Stefan Kaluz, Jalisa H Ferguson, Hua Yang, Guoliang Chen, and Binghe Wang. 2019. 'Arylsulfonamide 64B inhibits hypoxia/HIF-induced expression of c-Met and CXCR4 and reduces primary tumor growth and metastasis of uveal melanoma', *Clinical cancer research*, 25: 2206-18.

Doria-Borrell, Paula, and Vicente Pérez-García. 2024. 'Understanding the intersection between placental development and cancer: Lessons from the tumor suppressor BAP1', *Communications Biology*, 7: 1053.

Dougall, W. C., S. Kurtulus, M. J. Smyth, and A. C. Anderson. 2017. 'TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy', *Immunol Rev*, 276: 112-20.

Dunn, G. P., L. J. Old, and R. D. Schreiber. 2004a. 'The immunobiology of cancer immunosurveillance and immunoediting', *Immunity*, 21: 137-48.

Dunn, Gavin P, Lloyd J Old, and Robert D Schreiber. 2004b. 'The three Es of cancer immunoediting', *Annu. Rev. Immunol.*, 22: 329-60.

Durante, M. A., D. A. Rodriguez, S. Kurtenbach, J. N. Kuznetsov, M. I. Sanchez, C. L. Decatur, H. Snyder, L. G. Feun, A. S. Livingstone, and J. W. Harbour. 2020. 'Single-cell analysis reveals new evolutionary complexity in uveal melanoma', *Nat Commun*, 11: 496.

Eakins, Jessica, Laura O'conner, Julie David, Mark Bates, Ola Ibrahim, Cara Martin, Hassan Rajab, Steven Gray, Feras Abu Saadeh, and Stavros Selemidis. 2024. 'EV270/# 1221 Ex vivo upregulation of CD1d expression to enhance the therapeutic benefit of iNKT cells of ovarian cancer', *International Journal of Gynecological Cancer*, 34: A224.

Eide, Nils, Ragnar S Faye, Hanne K Høifødt, Berit Sandstad, Geir Qvale, Rowan Faber, Peter Jebsen, Gunnar Kvalheim, and Øystein Fodstad. 2015. 'Immunomagnetic detection of micrometastatic cells in bone marrow of uveal melanoma patients: a paradox', *Acta ophthalmologica*, 93: 59-66.

Espona-Fiedler, Margarita, Cedric Patthey, Stina Lindblad, Irina Sarró, and Daniel Öhlund. 2024. 'Overcoming therapy resistance in pancreatic cancer: New insights and future directions', *Biochemical pharmacology*: 116492.

Farhoumand, L. S., M. Fiorentzis, M. M. Kraemer, A. Sak, M. Stuschke, T. Rassaf, U. Hendgen-Cotta, N. E. Bechrakis, and U. Berchner-Pfannschmidt. 2022. 'The Adrenergic Receptor Antagonist Carvedilol Elicits Anti-Tumor Responses in Uveal Melanoma 3D Tumor Spheroids and May Serve as Co-Adjuvant Therapy with Radiation', *Cancers (Basel)*, 14.

Fidler, Isaiah J, Douglas M Gersten, and Marilyn B Budmen. 1976. 'Characterization in vivo and in vitro of tumor cells selected for resistance to syngeneic lymphocyte-mediated cytotoxicity', *Cancer research*, 36: 3160-65.

Figueiredo, Carlos R, Helen Kalirai, Joseph J Sacco, Ricardo A Azevedo, Andrew Duckworth, Joseph R Slupsky, Judy M Coulson, and Sarah E Coupland. 2020. 'Loss of BAP1 expression is associated with an immunosuppressive microenvironment in uveal melanoma, with implications for immunotherapy development', *The Journal of Pathology*, 250: 420-39.

Figueiredo, Carlos R., Alisson L. Matsuo, Ricardo A. Azevedo, Mariana H. Massaoka, Natalia Girola, Luciano Polonelli, and Luiz R. Travassos. 2015. 'A novel microtubule de-stabilizing complementarity-determining region C36L1 peptide displays antitumor activity against melanoma in vitro and in vivo', *Scientific Reports*, 5: 14310.

Fiorentzis, M., H. Kalirai, P. Katopodis, and S. E. Coupland. 2017. 'Adipophilin expression in primary and metastatic uveal melanoma: a pilot study', *Graefes Arch Clin Exp Ophthalmol*, 255: 1049-51.

Fiziev, Petko, Kadir C Akdemir, John P Miller, Emily Z Keung, Neha S Samant, Sneha Sharma, Christopher A Natale, Christopher J Terranova, Mayinuer Maitituoheti, and Samirkumar B Amin. 2017. 'Systematic epigenomic analysis reveals chromatin states associated with melanoma progression', *Cell reports*, 19: 875-89.

Ford, K., C. J. Hanley, M. Mellone, C. Szyndralewiez, F. Heitz, P. Wiesel, O. Wood, M. Machado, M. A. Lopez, A. P. Ganesan, C. Wang, A. Chakravarthy, T. R. Fenton, E. V. King, P. Vijayanand, C. H. Ottensmeier, A. Al-Shamkhani,

N. Savelyeva, and G. J. Thomas. 2020. 'NOX4 Inhibition Potentiates Immunotherapy by Overcoming Cancer-Associated Fibroblast-Mediated CD8 T-cell Exclusion from Tumors', *Cancer Res*, 80: 1846-60.

Foy, S. P., K. Jacoby, D. A. Bota, T. Hunter, Z. Pan, E. Stawiski, Y. Ma, W. Lu, S. Peng, C. L. Wang, B. Yuen, O. Dalmas, K. Heeringa, B. Sennino, A. Conroy, M. T. Bethune, I. Mende, W. White, M. Kukreja, S. Gunturu, E. Humphrey, A. Hussaini, D. An, A. J. Litterman, B. B. Quach, A. H. C. Ng, Y. Lu, C. Smith, K. M. Campbell, D. Anaya, L. Skrdlant, E. Y. Huang, V. Mendoza, J. Mathur, L. Dengler, B. Purandare, R. Moot, M. C. Yi, R. Funke, A. Sibley, T. Stallings-Schmitt, D. Y. Oh, B. Chmielowski, M. Abedi, Y. Yuan, J. A. Sosman, S. M. Lee, A. J. Schoenfeld, D. Baltimore, J. R. Heath, A. Franzusoff, A. Ribas, A. V. Rao, and S. J. Mandl. 2023. 'Non-viral precision T cell receptor replacement for personalized cell therapy', *Nature*, 615: 687-96.

Freytag, Saskia, Luyi Tian, Ingrid Lönnstedt, Milica Ng, and Melanie Bahlo. 2018. 'Comparison of clustering tools in R for medium-sized 10x Genomics single-cell RNA-sequencing data', *F1000Research*, 7.

Friedhoff, Jana, Felix Schneider, Christina Jurcic, Volker Endris, Martina Kirchner, Angela Sun, Iulia Bolnavu, Laura Pohl, Miriam Teroerde, and Maximilian Kippenberger. 2023. 'BAP1 and PTEN mutations shape the immunological landscape of clear cell renal cell carcinoma and reveal the intertumoral heterogeneity of T cell suppression: A proof-of-concept study', *Cancer Immunology, Immunotherapy*, 72: 1603-18.

Friedhoff, Jana, Felix Schneider, Christina Jurcic, Volker Endris, Martina Kirchner, Angela Sun, Iulia Bolnavu, Laura Pohl, Miriam Teroerde, Maximilian Kippenberger, Constantin Schwab, Adam Kaczorowski, Stefanie Zschäbitz, Dirk Jäger, Markus Hohenfellner, Albrecht Stenzinger, Anette Duensing, and Stefan Duensing. 2023. 'BAP1 and PTEN mutations shape the immunological landscape of clear cell renal cell carcinoma and reveal the intertumoral heterogeneity of T cell suppression: a proof-of-concept study', *Cancer Immunology, Immunotherapy*, 72: 1603-18.

Fröhlich, L. M., H. Niessner, B. Sauer, S. Kämereit, E. Chatziioannou, S. Riel, T. Sinnberg, and B. Schittek. 2023. 'PARP Inhibitors Effectively Reduce MAPK Inhibitor Resistant Melanoma Cell Growth and Synergize with MAPK Inhibitors through a Synthetic Lethal Interaction In Vitro and In Vivo', *Cancer Res Commun*, 3: 1743-55.

Fuentes-Rodriguez, A., A. Mitchell, S. L. Guerin, and S. Landreville. 2024. 'Recent Advances in Molecular and Genetic Research on Uveal Melanoma', *Cells*, 13.

Gajewski, Thomas F, Hans Schreiber, and Yang-Xin Fu. 2013. 'Innate and adaptive immune cells in the tumor microenvironment', *Nature immunology*, 14: 1014-22.

Gajos-Michniewicz, Anna, and Malgorzata Czyz. 2020. 'WNT signaling in melanoma', *International Journal of Molecular Sciences*, 21: 4852.

Gallagher, Stuart J, Florian Rambow, Mayuko Kumasaka, Delphine Champeval, Alfonso Bellacosa, Véronique Delmas, and Lionel Larue. 2013. 'Beta-

catenin inhibits melanocyte migration but induces melanoma metastasis', *Oncogene*, 32: 2230-38.

Gallenga, C. E., E. Franco, G. G. Adamo, S. S. Violanti, P. Tassinari, M. Tognon, and P. Perri. 2022. 'Genetic Basis and Molecular Mechanisms of Uveal Melanoma Metastasis: A Focus on Prognosis', *Front Oncol*, 12: 828112.

Galon, Jérôme, and Daniela Bruni. 2019. 'Approaches to treat immune hot, altered and cold tumours with combination immunotherapies', *Nature reviews Drug discovery*, 18: 197-218.

Ganesh, K., and J. Massagué. 2018. 'TGF-β Inhibition and Immunotherapy: Checkmate', *Immunity*, 48: 626-28.

Gangemi, Rosaria, Adriana Amaro, Alice Gino, Gaia Barisione, Marina Fabbi, Ulrich Pfeffer, Antonella Brizzolara, Paola Queirolo, Sandra Salvi, and Simona Boccardo. 2014. 'ADAM 10 correlates with uveal melanoma metastasis and promotes in vitro invasion', *Pigment cell & melanoma research*, 27: 1138-48.

Gao, Hui, Joshua M Korn, Stéphane Ferretti, John E Monahan, Youzhen Wang, Mallika Singh, Chao Zhang, Christian Schnell, Guizhi Yang, and Yun Zhang. 2015. 'High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response', *Nature medicine*, 21: 1318-25.

Garg, Pankaj, Jyoti Malhotra, Prakash Kulkarni, David Horne, Ravi Salgia, and Sharad S Singhal. 2024. 'Emerging therapeutic strategies to overcome drug resistance in cancer cells', *Cancers*, 16: 2478.

Gawad, Charles, Winston Koh, and Stephen R Quake. 2014. 'Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics', *Proceedings of the National Academy of Sciences*, 111: 17947-52.

Gelmi, M. C., and M. J. Jager. 2024. 'Uveal melanoma: Current evidence on prognosis, treatment and potential developments', *Asia Pac J Ophthalmol (Phila)*, 13: 100060.

Gerber, Tobias, Edith Willscher, Henry Loeffler-Wirth, Lydia Hopp, Dirk Schadendorf, Manfred Schartl, Ulf Anderegg, Gray Camp, Barbara Treutlein, Hans Binder, and Manfred Kunz. 2017. 'Mapping heterogeneity in patient-derived melanoma cultures by single-cell RNA-seq', *Oncotarget*, 8: 846-62.

Gercakova, K., M. Poturnajova, S. Tyciakova, I. Rezuchova, and M. Matuskova. 2024. 'Mouse models for cancer research - current state and the perspective', *Neoplasma*, 71: 99-116.

Gershenwald, Jeffrey E, and Richard A Scolyer. 2018. 'Melanoma staging: American joint committee on cancer (AJCC) and beyond', *Annals of surgical oncology*, 25: 2105-10.

Ghemrawi, R., L. Abuamer, S. Kremesh, G. Hussien, R. Ahmed, W. Mousa, G. Khoder, and M. Khair. 2024. 'Revolutionizing Cancer Treatment: Recent Advances in Immunotherapy', *Biomedicines*, 12.

Gibney, ER, and CM Nolan. 2010. 'Epigenetics and gene expression', *Heredity*, 105: 4-13.

Gide, Tuba N, Camelia Quek, Alexander M Menzies, Annie T Tasker, Ping Shang, Jeff Holst, Jason Madore, Su Yin Lim, Rebecca Velickovic, and Matthew

Wongchenko. 2019. 'Distinct immune cell populations define response to anti-PD-1 monotherapy and anti-PD-1/anti-CTLA-4 combined therapy', *Cancer Cell*, 35: 238-55. e6.

Goding, Colin R. 2000. 'Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage', *Genes & development*, 14: 1712-28.

Goldberg, Aaron D, C David Allis, and Emily Bernstein. 2007. 'Epigenetics: a landscape takes shape', *Cell*, 128: 635-38.

Gosman, Laura Maria, Dana-Antonia Țăpoi, and Mariana Costache. 2023. 'Cutaneous melanoma: a review of multifactorial pathogenesis, immunohistochemistry, and emerging biomarkers for early detection and management', *International Journal of Molecular Sciences*, 24: 15881.

Grossniklaus, Hans E, Matthew W Wilson, Brent C Barron, and Michael J Lynn. 1996. 'Anterior vs posterior intraocular melanoma: metastatic differences in a murine model', *Archives of Ophthalmology*, 114: 1116-20.

Grossniklaus, Hans E., Qing Zhang, Shuo You, Conni McCarthy, Steffen Heegaard, and Sarah E. Coupland. 2016. 'Metastatic ocular melanoma to the liver exhibits infiltrative and nodular growth patterns', *Human Pathology*, 57: 165-75.

Guo, Weinan, Huina Wang, and Chunying Li. 2021. 'Signal pathways of melanoma and targeted therapy', *Signal transduction and targeted therapy*, 6: 424.

Guo, Z., Y. Yuan, C. Chen, J. Lin, Q. Ma, G. Liu, Y. Gao, Y. Huang, L. Chen, L. Z. Chen, Y. F. Huang, H. Wang, B. Li, Y. Chen, and X. Zhang. 2022. 'Durable complete response to neoantigen-loaded dendritic-cell vaccine following anti-PD-1 therapy in metastatic gastric cancer', *NPJ Precis Oncol*, 6: 34.

Han, Anna, Timothy J. Purwin, and Andrew E. Aplin. 2021. 'Roles of the BAP1 Tumor Suppressor in Cell Metabolism', *Cancer research*, 81: 2807-14.

Han, Shujun, Yibo Ren, Wangxiao He, Huadong Liu, Zhe Zhi, Xinliang Zhu, Tielin Yang, Yu Rong, Bohan Ma, and Timothy J Purwin. 2018. 'ERK-mediated phosphorylation regulates SOX10 sumoylation and targets expression in mutant BRAF melanoma', *Nature communications*, 9: 28.

Hanahan, Douglas. 2022. 'Hallmarks of cancer: new dimensions', *Cancer discovery*, 12: 31-46.

Hanahan, Douglas, and Robert A Weinberg. 2000. 'The hallmarks of cancer', *Cell*, 100: 57-70.

———. 2011. 'Hallmarks of cancer: the next generation', *Cell*, 144: 646-74.

Hanley, C. J., and G. J. Thomas. 2020. 'T-cell tumour exclusion and immunotherapy resistance: a role for CAF targeting', *Br J Cancer*, 123: 1353-55.

Hara, Ayaka, Ryo Koyama-Nasu, Mariko Takami, Takahide Toyoda, Takahiro Aoki, Fumie Ihara, Masayoshi Kobayashi, Seiichiro Hirono, Tomoo Matsutani, and Toshinori Nakayama. 2021. 'CD1d expression in glioblastoma is a promising target for NKT cell-based cancer immunotherapy', *Cancer Immunology, Immunotherapy*, 70: 1239-54.

Harari, Alexandre, Michele Graciotti, Michal Bassani-Sternberg, and Lana E Kandalaft. 2020. 'Antitumour dendritic cell vaccination in a priming and boosting approach', *Nature reviews Drug discovery*, 19: 635-52.

Harbour, J. W. 2009. 'Molecular prognostic testing and individualized patient care in uveal melanoma', *Am J Ophthalmol*, 148: 823-9 e1.

———. 2012. 'The genetics of uveal melanoma: an emerging framework for targeted therapy', *Pigment Cell Melanoma Res*, 25: 171-81.

Harbour, J. W., and R. Chen. 2013. 'The DecisionDx-UM Gene Expression Profile Test Provides Risk Stratification and Individualized Patient Care in Uveal Melanoma', *PLoS Curr*, 5.

Hardwick, L. 2021. 'A Comparative View on Molecular Alterations and Potential Therapeutic Strategies for Canine Oral Melanoma', *Vet Sci*, 8.

Hartman, Mariusz L, and Malgorzata Czyz. 2015. 'MITF in melanoma: mechanisms behind its expression and activity', *Cellular and Molecular Life Sciences*, 72: 1249-60.

Hayata, K., M. Iwahashi, T. Ojima, M. Katsuda, T. Iida, M. Nakamori, K. Ueda, M. Nakamura, M. Miyazawa, T. Tsuji, and H. Yamaue. 2013. 'Inhibition of IL-17A in tumor microenvironment augments cytotoxicity of tumor-infiltrating lymphocytes in tumor-bearing mice', *PloS one*, 8: e53131.

Heegaard, S, M Spang-Thomsen, and JU Prause. 2003. 'Establishment and characterization of human uveal malignant melanoma xenografts in nude mice', *Melanoma Research*, 13: 247-51.

Herber, D. L., W. Cao, Y. Nefedova, S. V. Novitskiy, S. Nagaraj, V. A. Tyurin, A. Corzo, H. I. Cho, E. Celis, B. Lennox, S. C. Knight, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, S. Antonia, M. Fishman, R. L. Ferris, V. E. Kagan, and D. I. Gabrilovich. 2010. 'Lipid accumulation and dendritic cell dysfunction in cancer', *Nat Med*, 16: 880-6.

Herwig, M. C., C. Bergstrom, J. R. Wells, T. Höller, and H. E. Grossniklaus. 2013. 'M2/M1 ratio of tumor associated macrophages and PPAR-gamma expression in uveal melanomas with class 1 and class 2 molecular profiles', *Exp Eye Res*, 107: 52-8.

Herwig, M. C., and H. E. Grossniklaus. 2011. 'Role of macrophages in uveal melanoma', *Expert Rev Ophthalmol*, 6: 405-07.

Hix, L. M., Y. H. Shi, R. R. Brutkiewicz, P. L. Stein, C. R. Wang, and M. Zhang. 2011. 'CD1d-expressing breast cancer cells modulate NKT cell-mediated antitumor immunity in a murine model of breast cancer metastasis', *PloS one*, 6: e20702.

Ho, Ping-Chih, Jessica Dauz Bihuniak, Andrew N Macintyre, Matthew Staron, Xiaojing Liu, Robert Amezquita, Yao-Chen Tsui, Guoliang Cui, Goran Micevic, and Jose C Perales. 2015. 'Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses', *Cell*, 162: 1217-28.

Ho, Y. J., N. Anaparthy, D. Molik, G. Mathew, T. Aicher, A. Patel, J. Hicks, and M. G. Hammell. 2018. 'Single-cell RNA-seq analysis identifies markers of resistance to targeted BRAF inhibitors in melanoma cell populations', *Genome Res*, 28: 1353-63.

Hollmén, Maija, Carlos R. Figueiredo, and Sirpa Jalkanen. 2020. 'New tools to prevent cancer growth and spread: a 'Clever' approach', *British Journal of Cancer*, 123: 501-09.

Hopkins, A. C., M. Yarchoan, J. N. Durham, E. C. Yusko, J. A. Rytlewski, H. S. Robins, D. A. Laheru, D. T. Le, E. R. Lutz, and E. M. Jaffee. 2018. 'T cell

receptor repertoire features associated with survival in immunotherapy-treated pancreatic ductal adenocarcinoma', *JCI Insight*, 3.

Hossain, Sultana Mehbuba, Gregory Gimenez, Peter A Stockwell, Peter Tsai, Cristin G Print, Janusz Rys, Bozena Cybulska-Stopa, Magda Ratajska, Agnieszka Harazin-Lechowska, and Suzan Almomani. 2022. 'Innate immune checkpoint inhibitor resistance is associated with melanoma sub-types exhibiting invasive and de-differentiated gene expression signatures', *Frontiers in Immunology*, 13: 955063.

Huang, Qianhui, Yu Liu, Yuheng Du, and Lana X Garmire. 2021. 'Evaluation of cell type annotation R packages on single-cell RNA-seq data', *Genomics, Proteomics and Bioinformatics*, 19: 267-81.

Hugo, Willy, Jesse M Zaretsky, LU Sun, Chunying Song, Blanca Homet Moreno, Siwen Hu-Lieskovan, Beata Berent-Maoz, Jia Pang, Bartosz Chmielowski, and Grace Cherry. 2016. 'Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma', *Cell*, 165: 35-44.

Hurwitz, A. A., T. F. Yu, D. R. Leach, and J. P. Allison. 1998. 'CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma', *Proc Natl Acad Sci U S A*, 95: 10067-71.

Ida, C. M., J. Pulido, P. T. Greipp, J. J. Garcia, T. W. Olsen, L. Dalvin, and D. R. Salomao. 2022. 'BAP1 Immunostain Status in Intraocular Biopsy Specimens for Uveal Melanoma Highly Correlates with Other Prognostic Markers', *Ocul Oncol Pathol*, 8: 22-29.

Irigoyen, Cristina, Asier Amenabar Alonso, Jorge Sanchez-Molina, María Rodríguez-Hidalgo, Araceli Lara-López, and Javier Ruiz-Ederra. 2022. 'Subretinal injection techniques for retinal disease: a review', *Journal of clinical medicine*, 11: 4717.

Ishida, Yasumasa, Yasutoshi Agata, Keiichi Shibahara, and Tasuku Honjo. 1992. 'Induced expression of PD‐1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death', *The EMBO journal*, 11: 3887-95.

Jager, M. J., J. A. Magner, B. R. Ksander, and S. R. Dubovy. 2016. 'Uveal Melanoma Cell Lines: Where do they come from? (An American Ophthalmological Society Thesis)', *Trans Am Ophthalmol Soc*, 114: T5.

Jager, Martine J, Carol L Shields, Colleen M Cebulla, Mohamed H Abdel-Rahman, Hans E Grossniklaus, Marc-Henri Stern, Richard D Carvajal, Rubens N Belfort, Renbing Jia, and Jerry A Shields. 2020. 'Uveal melanoma', *Nature reviews Disease primers*, 6: 24.

Jager, Martine J., Carol L. Shields, Colleen M. Cebulla, Mohamed H. Abdel-Rahman, Hans E. Grossniklaus, Marc-Henri Stern, Richard D. Carvajal, Rubens N. Belfort, Renbing Jia, Jerry A. Shields, and Bertil E. Damato. 2020. 'Uveal melanoma', *Nature reviews Disease primers*, 6: 24.

Jain, Paras, Maalavika Pillai, Atchuta Srinivas Duddu, Jason A Somarelli, Yogesh Goyal, and Mohit Kumar Jolly. 2023. "Dynamical hallmarks of cancer: Phenotypic switching in melanoma and epithelial-mesenchymal plasticity." In *Seminars in Cancer Biology*, 48-63. Elsevier.

Jaiswal, A. R., A. J. Liu, S. Pudakalakatti, P. Dutta, P. Jayaprakash, T. Bartkowiak, C. R. Ager, Z. Q. Wang, A. Reuben, Z. A. Cooper, C. Ivan, Z. Ju, F. Nwajei, J. Wang, M. A. Davies, R. E. Davis, J. A. Wargo, P. K. Bhattacharya, D. S. Hong, and M. A. Curran. 2020. 'Melanoma Evolves Complete Immunotherapy Resistance through the Acquisition of a Hypermetabolic Phenotype', *Cancer Immunol Res*, 8: 1365-80.

Jaiswal, Ashvin R, Arthur J Liu, Shivanand Pudakalakatti, Prasanta Dutta, Priyamvada Jayaprakash, Todd Bartkowiak, Casey R Ager, Zhi-Qiang Wang, Alexandre Reuben, and Zachary A Cooper. 2020. 'Melanoma evolves complete immunotherapy resistance through the acquisition of a hypermetabolic phenotype', *Cancer immunology research*, 8: 1365-80.

Jardim, Denis L, Aaron Goodman, Debora de Melo Gagliato, and Razelle Kurzrock. 2021. 'The challenges of tumor mutational burden as an immunotherapy biomarker', *Cancer Cell*, 39: 154-73.

Jenkins, R. W., D. A. Barbie, and K. T. Flaherty. 2018. 'Mechanisms of resistance to immune checkpoint inhibitors', *Br J Cancer*, 118: 9-16.

Jerby-Arnon, Livnat, Parin Shah, Michael S Cuoco, Christopher Rodman, Mei-Ju Su, Johannes C Melms, Rachel Leeson, Abhay Kanodia, Shaolin Mei, and Jia-Ren Lin. 2018. 'A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade', *Cell*, 175: 984-97. e24.

Jin, Yanli, Ping Zhang, Yun Wang, Bei Jin, Jingfeng Zhou, Jing Zhang, and Jingxuan Pan. 2018. 'Neddylation blockade diminishes hepatic metastasis by dampening cancer stem-like cells and angiogenesis in uveal melanoma', *Clinical cancer research*, 24: 3741-54.

Jindal, V. 2018. 'Role of immune checkpoint inhibitors and novel immunotherapies in uveal melanoma', *Chin Clin Oncol*, 7: 8.

Johansson, P., L. G. Aoude, K. Wadt, W. J. Glasson, S. K. Warrier, A. W. Hewitt, J. F. Kiilgaard, S. Heegaard, T. Isaacs, M. Franchina, C. Ingvar, T. Vermeulen, K. J. Whitehead, C. W. Schmidt, J. M. Palmer, J. Symmons, A. M. Gerdes, G. Jönsson, and N. K. Hayward. 2016. 'Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4', *Oncotarget*, 7: 4624-31.

Jones, Nyasia M, Hua Yang, Qing Zhang, Vanessa M Morales-Tirado, and Hans E Grossniklaus. 2019. 'Natural killer cells and pigment epithelial-derived factor control the infiltrative and nodular growth of hepatic metastases in an Orthotopic murine model of ocular melanoma', *BMC cancer*, 19: 1-10.

Joshi, Powrnima, Barbara Jacobs, Adeeb Derakhshan, Lee R. Moore, Paul Elson, Pierre L. Triozzi, Ernest Borden, and Maciej Zborowski. 2014. 'Enrichment of circulating melanoma cells (CMCs) using negative selection from patients with metastatic melanoma', *Oncotarget*, 5: 2450-61.

Kageyama, Ken, Masahiro Ohara, Kengo Saito, Shinji Ozaki, Mizue Terai, Michael J Mastrangelo, Paolo Fortina, Andrew E Aplin, and Takami Sato. 2017. 'Establishment of an orthotopic patient-derived xenograft mouse model using uveal melanoma hepatic metastasis', *Journal of translational medicine*, 15: 1-19.

Kalaora, Shelly, Adi Nagler, Jennifer A. Wargo, and Yardena Samuels. 2022. 'Mechanisms of immune activation and regulation: lessons from melanoma', *Nature Reviews Cancer*, 22: 195-207.

Kaler, C. J., J. J. Dollar, A. M. Cruz, J. N. Kuznetsoff, M. I. Sanchez, C. L. Decatur, J. D. Licht, K. S. M. Smalley, Z. M. Correa, S. Kurtenbach, and J. W. Harbour. 2022. 'BAP1 Loss Promotes Suppressive Tumor Immune Microenvironment via Upregulation of PROS1 in Class 2 Uveal Melanomas', *Cancers (Basel)*, 14.

Kaliki, S, and Carol L Shields. 2017. 'Uveal melanoma: relatively rare but deadly cancer', *Eye*, 31: 241-57.

Kang, Kai, Fucun Xie, Jinzhu Mao, Yi Bai, and Xiang Wang. 2020. 'Significance of tumor mutation burden in immune infiltration and prognosis in cutaneous melanoma', *Frontiers in oncology*, 10: 573141.

Karakousis, G., R. Yang, and X. Xu. 2013. 'Circulating melanoma cells as a predictive biomarker', *J Invest Dermatol*, 133: 1460-2.

Karnik, Isha, Zhisheng Her, Shu Hui Neo, Wai Nam Liu, and Qingfeng Chen. 2023. 'Emerging preclinical applications of humanized mouse models in the discovery and validation of novel immunotherapeutics and their mechanisms of action for improved cancer treatment', *Pharmaceutics*, 15: 1600.

Kasanen, Henna, Micaela Hernberg, Siru Mäkelä, Oscar Brück, Susanna Juteau, Laura Kohtamäki, Mette Ilander, Satu Mustjoki, and Anna Kreutzman. 2020. 'Age-associated changes in the immune system may influence the response to anti-PD1 therapy in metastatic melanoma patients', *Cancer Immunology, Immunotherapy*, 69: 717-30.

Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, H. Koseki, and M. Taniguchi. 1997. 'CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides', *Science*, 278: 1626-9.

Keren, Leeat, Marc Bosse, Steve Thompson, Tyler Risom, Kausalia Vijayaragavan, Erin McCaffrey, Diana Marquez, Roshan Angoshtari, Noah F Greenwald, and Harris Fienberg. 2019. 'MIBI-TOF: A multiplexed imaging platform relates cellular phenotypes and tissue structure', *Science advances*, 5: eaax5851.

Khan, S., S. P. Patel, A. N. Shoushtari, G. Ambrosini, S. Cremers, S. Lee, L. Franks, S. Singh-Kandah, S. Hernandez, N. Sender, K. Vuolo, A. Nesson, P. Mundi, B. Izar, G. K. Schwartz, and R. D. Carvajal. 2022. 'Intermittent MEK inhibition for the treatment of metastatic uveal melanoma', *Front Oncol*, 12: 975643.

Kilian, Marta M, Karin U Loeffler, Christiane Pfarrer, Frank G Holz, Christian Kurts, and Martina C Herwig. 2016. 'Intravitreally injected HCmel12 melanoma cells serve as a mouse model of tumor biology of intraocular melanoma', *Current eye research*, 41: 121-28.

Kim, Do-Hee, Na-Young Song, and Hyungshin Yim. 2023. 'Targeting dysregulated lipid metabolism in the tumor microenvironment', *Archives of Pharmacal Research*, 46: 855-81.

Kim, H. S., J. Garcia, M. Exley, K. W. Johnson, S. P. Balk, and R. S. Blumberg. 1999. 'Biochemical characterization of CD1d expression in the absence of beta2-microglobulin', *J Biol Chem*, 274: 9289-95.

Knouse, K. A., J. Wu, and A. Hendricks. 2017. 'Detection of Copy Number Alterations Using Single Cell Sequencing', *J Vis Exp*.

Kobie, J. J., R. S. Wu, R. A. Kurt, S. Lou, M. K. Adelman, L. J. Whitesell, L. V. Ramanathapuram, C. L. Arteaga, and E. T. Akporiaye. 2003. 'Transforming growth factor beta inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines', *Cancer Res*, 63: 1860-4.

Komiya, Yuko, and Raymond Habas. 2008. 'Wnt signal transduction pathways', *Organogenesis*, 4: 68-75.

Kong, Yan, Xinan Sheng, Xiaowen Wu, Junya Yan, Meng Ma, Jiayi Yu, Lu Si, Zhihong Chi, Chuanliang Cui, and Jie Dai. 2017. 'Frequent genetic aberrations in the CDK4 pathway in acral melanoma indicate the potential for CDK4/6 inhibitors in targeted therapy', *Clinical cancer research*, 23: 6946-57.

Koopmans, Anna E, Robert M Verdijk, Rutger WW Brouwer, Thierry PP Van Den Bosch, Mike MP Van Den Berg, Jolanda Vaarwater, Christel EM Kockx, Dion Paridaens, Nicole C Naus, and Mark Nellist. 2014. 'Clinical significance of immunohistochemistry for detection of BAP1 mutations in uveal melanoma', *Modern pathology*, 27: 1321-30.

Korsunsky, Ilya, Nghia Millard, Jean Fan, Kamil Slowikowski, Fan Zhang, Kevin Wei, Yuriy Baglaenko, Michael Brenner, Po-ru Loh, and Soumya Raychaudhuri. 2019. 'Fast, sensitive and accurate integration of single-cell data with Harmony', *Nature methods*, 16: 1289-96.

Kos, Spela, Alessandra Lopes, Veronique Preat, Maja Cemazar, Ursa Lampreht Tratar, Bernard Ucakar, Kevin Vanvarenberg, Gregor Sersa, and Gaelle Vandermeulen. 2019. 'Intradermal DNA vaccination combined with dual CTLA-4 and PD-1 blockade provides robust tumor immunity in murine melanoma', *PloS one*, 14: e0217762.

Kovács, S. A., and B. Győrffy. 2022. 'Transcriptomic datasets of cancer patients treated with immune-checkpoint inhibitors: a systematic review', *J Transl Med*, 20: 249.

Krieg, C., M. Nowicka, S. Guglietta, S. Schindler, F. J. Hartmann, L. M. Weber, R. Dummer, M. D. Robinson, M. P. Levesque, and B. Becher. 2018. 'High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy', *Nat Med*, 24: 144-53.

Krishna, Yamini, Amelia Acha-Sagredo, Dorota Sabat-Pośpiech, Natalie Kipling, Kim Clarke, Carlos R. Figueiredo, Helen Kalirai, and Sarah E. Coupland. 2020. 'Transcriptome Profiling Reveals New Insights into the Immune Microenvironment and Upregulation of Novel Biomarkers in Metastatic Uveal Melanoma', *Cancers*, 12: 2832.

Krummel, Matthew F, and James P Allison. 1995. 'CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation', *The Journal of experimental medicine*, 182: 459-65.

Kumar, Raj, Michael Taylor, Benchun Miao, Zhenyu Ji, Jenny C. N. Njauw, Göran Jönsson, Dennie T. Frederick, and Hensin Tsao. 2015. 'BAP1 Has a Survival Role in Cutaneous Melanoma', *Journal of Investigative Dermatology*, 135: 1089-97.

Kunz, Manfred, Henry Löffler-Wirth, Michael Dannemann, Edith Willscher, Gero Doose, Janet Kelso, Tina Kottek, Birgit Nickel, Lydia Hopp, Jenny Landsberg, Steve Hoffmann, Thomas Tüting, Paola Zigrino, Cornelia Mauch, Jochen Utikal, Mirjana Ziemer, Hans-Joachim Schulze, Michael Hölzel, Alexander Roesch, Susanne Kneitz, Svenja Meierjohann, Anja Bosserhoff, Hans Binder, and Manfred Schartl. 2018. 'RNA-seq analysis identifies different transcriptomic types and developmental trajectories of primary melanomas', *Oncogene*, 37: 6136-51.

Kwon, Jongbum, Daye Lee, and Shin-Ai Lee. 2023. 'BAP1 as a guardian of genome stability: implications in human cancer', *Experimental & Molecular Medicine*, 55: 745-54.

Labun, Kornel, Tessa G Montague, Maximilian Krause, Yamila N Torres Cleuren, Håkon Tjeldnes, and Eivind Valen. 2019. 'CHOPCHOP v3: expanding the CRISPR web toolbox beyond genome editing', *Nucleic acids research*, 47: W171-W74.

Lai, Yunxin, Xinru Wei, Shouheng Lin, Le Qin, Lin Cheng, and Peng Li. 2017. 'Current status and perspectives of patient-derived xenograft models in cancer research', *Journal of hematology & oncology*, 10: 1-14.

Lattier, John M, Hua Yang, Susan Crawford, and Hans E Grossniklaus. 2013. 'Host pigment epithelium-derived factor (PEDF) prevents progression of liver metastasis in a mouse model of uveal melanoma', *Clinical & experimental metastasis*, 30: 969-76.

Laurent, Cécile, Fabien Valet, Nathalie Planque, Licia Silveri, Selma Maacha, Océane Anezo, Philippe Hupe, Corine Plancher, Cécile Reyes, and Benoit Albaud. 2011. 'High PTP4A3 phosphatase expression correlates with metastatic risk in uveal melanoma patients', *Cancer research*, 71: 666-74.

Laurent, Marine, Marine Geoffroy, Giulia Pavani, and Simon Guiraud. 2024. 'CRISPR-Based Gene Therapies: From Preclinical to Clinical Treatments', *Cells*, 13: 800.

Lawrence, Michael S, Petar Stojanov, Craig H Mermel, James T Robinson, Levi A Garraway, Todd R Golub, Matthew Meyerson, Stacey B Gabriel, Eric S Lander, and Gad Getz. 2014. 'Discovery and saturation analysis of cancer genes across 21 tumour types', *Nature*, 505: 495-501.

Lawrence, Sarah K, Dung Nguyen, Lauren Richards-Peterson, and Konstantine W Skordos. 2014. 'The metabolic drug-drug interaction profile of Dabrafenib: in vitro investigations and quantitative extrapolation of the P450-mediated DDI risk', *Drug Metabolism and Disposition*, 42: 1180-90.

Lawson, Devon A, Kai Kessenbrock, Ryan T Davis, Nicholas Pervolarakis, and Zena Werb. 2018. 'Tumour heterogeneity and metastasis at single-cell resolution', *Nature cell biology*, 20: 1349-60.

Leach, Dana R, Matthew F Krummel, and James P Allison. 1996. 'Enhancement of antitumor immunity by CTLA-4 blockade', *Science*, 271: 1734-36.

Leblay, Noémie, Frédéric Leprêtre, Nolwenn Le Stang, Amandine Gautier-Stein, Laurent Villeneuve, Sylvie Isaac, Denis Maillet, Françoise Galateau-Sallé, Céline Villenet, and Shéhérazade Sebda. 2017. 'BAP1 is altered by copy number loss, mutation, and/or loss of protein expression in more than 70%

of malignant peritoneal mesotheliomas', *Journal of Thoracic Oncology*, 12: 724-33.

Lee, Hye Won, Woosung Chung, Hae-Ock Lee, Da Eun Jeong, Areum Jo, Joung Eun Lim, Jeong Hee Hong, Do-Hyun Nam, Byong Chang Jeong, Se Hoon Park, Kyeung-Min Joo, and Woong-Yang Park. 2020. 'Single-cell RNA sequencing reveals the tumor microenvironment and facilitates strategic choices to circumvent treatment failure in a chemorefractory bladder cancer patient', *Genome Medicine*, 12: 47.

Lee, Michael S, and Tonya J Webb. 2023. 'Novel lipid antigens for NKT cells in cancer', *Frontiers in Immunology*, 14: 1173375.

Lehrmann, Daria, Nasrin Refaian, Michael Simon, Alexander C Rokohl, and Ludwig M Heindl. 2022. 'Preclinical models in ophthalmic oncology—A narrative review', *Annals of Eye Science*, 7: 14-14.

Li, Bo, Eric Severson, Jean-Christophe Pignon, Haoquan Zhao, Taiwen Li, Jesse Novak, Peng Jiang, Hui Shen, Jon C Aster, and Scott Rodig. 2016. 'Comprehensive analyses of tumor immunity: implications for cancer immunotherapy', *Genome biology*, 17: 1-16.

Li, Feng, Caichen Li, Xiuyu Cai, Zhanhong Xie, Liquan Zhou, Bo Cheng, Ran Zhong, Shan Xiong, Jianfu Li, and Zhuxing Chen. 2021. 'The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis', *EClinicalMedicine*, 41: 101134.

Li Yan Khor, Simin Zeng, Lee Hong Song, Issam Al Jajeh, Kiat Hon Lim, Angela Takano, Syed Salahuddin Ahmed, Jabed Iqbal, Wei Keat Wan, Rafay Azhar, Jacqueline Siok Gek Hwang, Sangeeta Mantoo. 2017. "Atypical' Pancreatic Endoscopic Ultrasound-Guided Fine Needle Aspiration Cytology: A Study of Interobserver Agreement', *Journal of the Pancreas*, 18(5): 409-14.

Liau, N. P. D., A. Laktyushin, I. S. Lucet, J. M. Murphy, S. Yao, E. Whitlock, K. Callaghan, N. A. Nicola, N. J. Kershaw, and J. J. Babon. 2018. 'The molecular basis of JAK/STAT inhibition by SOCS1', *Nat Commun*, 9: 1558.

Lin, Qizhuan, Libo Jin, and Renyi Peng. 2025. 'New Progress in Zebrafish Liver Tumor Models: Techniques and Applications in Hepatocellular Carcinoma Research', *International Journal of Molecular Sciences*, 26: 780.

Litchfield, Kevin, Stacey Stanislaw, Lavinia Spain, Lisa L Gallegos, Andrew Rowan, Desiree Schnidrig, Heidi Rosenbaum, Alexandre Harle, Lewis Au, and Samantha M Hill. 2020. 'Representative sequencing: unbiased sampling of solid tumor tissue', *Cell reports*, 31.

Liu, David, Bastian Schilling, Derek Liu, Antje Sucker, Elisabeth Livingstone, Livnat Jerby-Arnon, Lisa Zimmer, Ralf Gutzmer, Imke Satzger, and Carmen Loquai. 2019. 'Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma', *Nature medicine*, 25: 1916-27.

Liu, X., L. Li, F. Si, L. Huang, Y. Zhao, C. Zhang, D. F. Hoft, and G. Peng. 2021. 'NK and NKT cells have distinct properties and functions in cancer', *Oncogene*, 40: 4521-37.

Liu, Ze-Long, Jing-Hua Liu, Daniel Staiculescu, and Jiang Chen. 2021. 'Combination of molecularly targeted therapies and immune checkpoint

inhibitors in the new era of unresectable hepatocellular carcinoma treatment', *Therapeutic Advances in Medical Oncology*, 13: 17588359211018026.

Llinàs-Arias, Pere, Miquel Ensenyat-Mendez, Sandra Íñiguez-Muñoz, Javier IJ Orozco, Betsy Valdez, Matthew P Salomon, Chikako Matsuba, Maria Solivellas-Pieras, Andrés F Bedoya-López, and Borja Sesé. 2023. 'Chromatin insulation orchestrates matrix metalloproteinase gene cluster expression reprogramming in aggressive breast cancer tumors', *Molecular cancer*, 22: 190.

Loeffler-Wirth, H., H. Binder, E. Willscher, T. Gerber, and M. Kunz. 2018. 'Pseudotime Dynamics in Melanoma Single-Cell Transcriptomes Reveals Different Mechanisms of Tumor Progression', *Biology (Basel)*, 7.

Loh, Chin-Yap, Jian Yi Chai, Ting Fang Tang, Won Fen Wong, Gautam Sethi, Muthu Kumaraswamy Shanmugam, Pei Pei Chong, and Chung Yeng Looi. 2019. 'The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges', *Cells*, 8: 1118.

Louie, Bryan H, and Razelle Kurzrock. 2020. 'BAP1: Not just a BRCA1-associated protein', *Cancer treatment reviews*, 90: 102091.

Luke, J. J., R. Bao, R. F. Sweis, S. Spranger, and T. F. Gajewski. 2019. 'WNT/β-catenin Pathway Activation Correlates with Immune Exclusion across Human Cancers', *Clin Cancer Res*, 25: 3074-83.

Luke, J. J., K. T. Flaherty, A. Ribas, and G. V. Long. 2017a. 'Targeted agents and immunotherapies: optimizing outcomes in melanoma', *Nat Rev Clin Oncol*, 14: 463-82.

Luke, Jason J, Keith T Flaherty, Antoni Ribas, and Georgina V Long. 2017b. 'Targeted agents and immunotherapies: optimizing outcomes in melanoma', *Nature Reviews Clinical Oncology*, 14: 463-82.

Luo, C., E. Balsa, E. A. Perry, J. Liang, C. D. Tavares, F. Vazquez, H. R. Widlund, and P. Puigserver. 2020. 'H3K27me3-mediated PGC1α gene silencing promotes melanoma invasion through WNT5A and YAP', *J Clin Invest*, 130: 853-62.

Luo, N., M. J. Nixon, P. I. Gonzalez-Ericsson, V. Sanchez, S. R. Opalenik, H. Li, C. A. Zahnow, M. L. Nickels, F. Liu, M. N. Tantawy, M. E. Sanders, H. C. Manning, and J. M. Balko. 2018. 'DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer', *Nat Commun*, 9: 248.

Luo, Xin, Yuyan Xu, Yilei Li, Guiming Zhang, Sisi Huang, Xiaolian Liu, Zhonglu Ren, Shuwen Liu, and Le Yu. 2021. 'BAP1 deletion abrogates growth and metastasis of murine cutaneous melanoma', *Melanoma Research*, 31.

Lv, Y., L. He, M. Jin, W. Sun, G. Tan, and Z. Liu. 2022. 'EMT-Related Gene Signature Predicts the Prognosis in Uveal Melanoma Patients', *J Oncol*, 2022: 5436988.

Mandalà, Mario, Barbara Merelli, and Daniela Massi. 2014. 'Nras in melanoma: targeting the undruggable target', *Critical reviews in oncology/hematology*, 92: 107-22.

Mariathasan, S., S. J. Turley, D. Nickles, A. Castiglioni, K. Yuen, Y. Wang, E. E. Kadel, H. Koeppen, J. L. Astarita, R. Cubas, S. Jhunjhunwala, R. Banchereau, Y. Yang, Y. Guan, C. Chalouni, J. Ziai, Y. Şenbabaoğlu, S. Santoro, D. Sheinson, J. Hung, J. M. Giltnane, A. A. Pierce, K. Mesh, S. Lianoglou, J. Riegler, R. A. D. Carano, P. Eriksson, M. Höglund, L. Somarriba, D. L. Halligan, M. S. van der Heijden, Y. Loriot, J. E. Rosenberg, L. Fong, I. Mellman, D. S. Chen, M. Green, C. Derleth, G. D. Fine, P. S. Hegde, R. Bourgon, and T. Powles. 2018. 'TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells', *Nature*, 554: 544-48.

Martin, Raul. 2018. 'Cornea and anterior eye assessment with slit lamp biomicroscopy, specular microscopy, confocal microscopy, and ultrasound biomicroscopy', *Indian journal of ophthalmology*, 66: 195-201.

Martinenaite, Evelina, Inés Lecoq, Mia Aaboe-Jørgensen, Shamaila Munir Ahmad, Maria Perez-Penco, Hannah Jorinde Glöckner, Marion Chapellier, Lucía Lara de la Torre, Lars Rønn Olsen, Anne Mette Askehøj Rømer, Ayako Wakatsuki Pedersen, and Mads Hald Andersen. 2025. 'Arginase-1-specific T cells target and modulate tumor-associated macrophages', *Journal for ImmunoTherapy of Cancer*, 13: e009930.

Masclef, Louis, Oumaima Ahmed, Benjamin Estavoyer, Bruno Larrivée, Nathalie Labrecque, Anastasia Nijnik, and El Bachir Affar. 2021. 'Roles and mechanisms of BAP1 deubiquitinase in tumor suppression', *Cell Death & Differentiation*, 28: 606-25.

Masoomian, Babak, Jerry A Shields, and Carol L Shields. 2018. 'Overview of BAP1 cancer predisposition syndrome and the relationship to uveal melanoma', *Journal of current ophthalmology*, 30: 102-09.

Matareed, Maisoon, Eleftheria Maranou, Saara A Koskela, Arfa Mehmood, Helen Kalirai, Sarah E Coupland, and Carlos R Figueiredo. 2023. 'Novel prognostication biomarker adipophilin reveals a metabolic shift in uveal melanoma and new therapeutic opportunities', *The Journal of Pathology*, 260: 203-21.

Maulana, T. I., E. Kromidas, L. Wallstabe, M. Cipriano, M. Alb, C. Zaupa, M. Hudecek, B. Fogal, and P. Loskill. 2021. 'Immunocompetent cancer-on-chip models to assess immuno-oncology therapy', *Adv Drug Deliv Rev*, 173: 281-305.

McCart Reed, Amy E, James Bennett, Jamie R Kutasovic, Emarene Kalaw, Kaltin Ferguson, Joe Yeong, Peter T Simpson, and Sunil R Lakhani. 2020. 'Digital spatial profiling application in breast cancer: A user's perspective', *Virchows Archiv*, 477: 885-90.

McGranahan, Nicholas, and Charles Swanton. 2015. 'Biological and therapeutic impact of intratumor heterogeneity in cancer evolution', *Cancer Cell*, 27: 15-26.

Mehta, D., and K. D. Mehta. 2017. 'PKCβ: Expanding role in hepatic adaptation of cholesterol homeostasis to dietary fat/cholesterol', *Am J Physiol Gastrointest Liver Physiol*, 312: G266-g73.

Melero, Ignacio, David M Berman, M Angela Aznar, Alan J Korman, José Luis Pérez Gracia, and John Haanen. 2015. 'Evolving synergistic combinations

of targeted immunotherapies to combat cancer', *Nature Reviews Cancer*, 15: 457-72.

Melo, Ashanty M., Melissa J. Conroy, Emma K. Foley, Éilis Dockry, Eamon P. Breen, John V. Reynolds, Joanne Lysaght, and Derek G. Doherty. 2020. 'CD1d expression and invariant natural killer T-cell numbers are reduced in patients with upper gastrointestinal cancers and are further impaired by commonly used chemotherapies', *Cancer Immunology, Immunotherapy*, 69: 969-82.

Merritt, Christopher R, Giang T Ong, Sarah E Church, Kristi Barker, Patrick Danaher, Gary Geiss, Margaret Hoang, Jaemyeong Jung, Yan Liang, and Jill McKay-Fleisch. 2020. 'Multiplex digital spatial profiling of proteins and RNA in fixed tissue', *Nature biotechnology*, 38: 586-99.

Micevic, Goran, Nicholas Theodosakis, and Marcus Bosenberg. 2017. 'Aberrant DNA methylation in melanoma: biomarker and therapeutic opportunities', *Clinical epigenetics*, 9: 1-15.

Miura, S., K. Kawana, D. J. Schust, T. Fujii, T. Yokoyama, Y. Iwasawa, T. Nagamatsu, K. Adachi, A. Tomio, K. Tomio, S. Kojima, T. Yasugi, S. Kozuma, and Y. Taketani. 2010. 'CD1d, a sentinel molecule bridging innate and adaptive immunity, is downregulated by the human papillomavirus (HPV) E5 protein: a possible mechanism for immune evasion by HPV', *J Virol*, 84: 11614-23.

Mlynska, A., R. Vaišnorė, V. Rafanavičius, S. Jocys, J. Janeiko, M. Petrauskytė, S. Bijeikis, P. Cimmperman, B. Intaitė, K. Žilionytė, A. Barakauskienė, R. Meškauskas, E. Paberalė, and V. Pašukonienė. 2020. 'A gene signature for immune subtyping of desert, excluded, and inflamed ovarian tumors', *Am J Reprod Immunol*, 84: e13244.

Mochel, Mark C, Adriano Piris, Vania Nose, and Mai P Hoang. 2015. 'Loss of BAP1 expression in basal cell carcinomas in patients with germline BAP1 mutations', *American journal of clinical pathology*, 143: 901-04.

Moore, A. R., E. Ceraudo, J. J. Sher, Y. Guan, A. N. Shoushtari, M. T. Chang, J. Q. Zhang, E. G. Walczak, M. A. Kazmi, B. S. Taylor, T. Huber, P. Chi, T. P. Sakmar, and Y. Chen. 2016. 'Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma', *Nat Genet*, 48: 675-80.

Moran, Bruce, Romina Silva, Antoinette S Perry, and William M Gallagher. 2018. "Epigenetics of malignant melanoma." In *Seminars in cancer biology*, 80-88. Elsevier.

Mori, L., M. Lepore, and G. De Libero. 2016. 'The Immunology of CD1- and MR1-Restricted T Cells', *Annu Rev Immunol*, 34: 479-510.

Morin, Aurélie, Carmen Ruggiero, Estelle Robidel, Mabrouka Doghman-Bouguerra, Atze T Das, Rémy Castellano, Emmanuelle Josselin, Judith Favier, and Enzo Lalli. 2017. 'Establishment of a mouse xenograft model of metastatic adrenocortical carcinoma', *Oncotarget*, 8: 51050.

Morrison, Olivia, and Jitendra Thakur. 2021. 'Molecular complexes at euchromatin, heterochromatin and centromeric chromatin', *International Journal of Molecular Sciences*, 22: 6922.

Motohashi, S., K. Nagato, N. Kunii, H. Yamamoto, K. Yamasaki, K. Okita, H. Hanaoka, N. Shimizu, M. Suzuki, I. Yoshino, M. Taniguchi, T. Fujisawa,

and T. Nakayama. 2009. 'A phase I-II study of alpha-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer', *J Immunol*, 182: 2492-501.

Motwani, Jyoti, and Michael R Eccles. 2021. 'Genetic and genomic pathways of melanoma development, invasion and metastasis', *Genes*, 12: 1543.

Mukherjee, Oishi, Sambuddha Paul, Sumana Das, Sudeshna Rakshit, Geetha Shanmugam, Melvin George, and Koustav Sarkar. 2024. 'Doxorubicin induced epigenetic regulation of dendritic cell maturation in association with T cell activation facilitates tumor protective immune response in non-small cell lung cancer (NSCLC)', *Pathology-Research and Practice*, 253: 155004.

Munn, D. H., and V. Bronte. 2016. 'Immune suppressive mechanisms in the tumor microenvironment', *Curr Opin Immunol*, 39: 1-6.

Murali, Rajmohan, James S Wilmott, Valerie Jakrot, Hikmat A Al-Ahmadie, Thomas Wiesner, Stanley W McCarthy, John F Thompson, and Richard A Scolyer. 2013. 'BAP1 expression in cutaneous melanoma: a pilot study', *Pathology-Journal of the RCPA*, 45: 606-09.

Naik, Piyu Parth. 2021. 'Cutaneous malignant melanoma: a review of early diagnosis and management', *World journal of oncology*, 12: 7.

Nasu, Masaki, Mitsuru Emi, Sandra Pastorino, Mika Tanji, Amy Powers, Hugh Luk, Francine Baumann, Yu-an Zhang, Adi Gazdar, and Shreya Kanodia. 2015. 'High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma', *Journal of Thoracic Oncology*, 10: 565-76.

Nathan, P., J. C. Hassel, P. Rutkowski, J. F. Baurain, M. O. Butler, M. Schlaak, R. J. Sullivan, S. Ochsenreither, R. Dummer, J. M. Kirkwood, A. M. Joshua, J. J. Sacco, A. N. Shoushtari, M. Orloff, J. M. Piulats, M. Milhem, A. K. S. Salama, B. Curti, L. Demidov, L. Gastaud, C. Mauch, M. Yushak, R. D. Carvajal, O. Hamid, S. E. Abdullah, C. Holland, H. Goodall, and S. Piperno-Neumann. 2021. 'Overall Survival Benefit with Tebentafusp in Metastatic Uveal Melanoma', *N Engl J Med*, 385: 1196-206.

Neagu, Monica, Constantin Caruntu, Carolina Constantin, Daniel Boda, Sabina Zurac, Demetrios A Spandidos, and Aristidis M Tsatsakis. 2016. 'Chemically induced skin carcinogenesis: Updates in experimental models', *Oncology reports*, 35: 2516-28.

Neganova, Margarita E, Sergey G Klochkov, Yulia R Aleksandrova, and Gjumrakch Aliev. 2022. "Histone modifications in epigenetic regulation of cancer: Perspectives and achieved progress." In *Seminars in Cancer Biology*, 452-71. Elsevier.

Nemati, Fariba, Leanne de Koning, David Gentien, Franck Assayag, Emilie Henry, Khadija Ait Rais, Gaelle Pierron, Odette Mariani, Michèle Nijnikoff, and Gabriel Champenois. 2023. 'Patient Derived Xenografts (PDX) Models as an Avatar to Assess Personalized Therapy Options in Uveal Melanoma: A Feasibility Study', *Current Oncology*, 30: 9090-103.

Nemati, Fariba, Catherine de Montrion, Guillaume Lang, Laurence Kraus-Berthier, Guillaume Carita, Xavier Sastre-Garau, Aurélie Berniard, David Vallerand, Olivier Geneste, and Ludmilla de Plater. 2014. 'Targeting Bcl-2/Bcl-XL

induces antitumor activity in uveal melanoma patient-derived xenografts', *PloS one*, 9: e80836.

Némati, Fariba, Xavier Sastre-Garau, Cécile Laurent, Jérôme Couturier, Pascale Mariani, Laurence Desjardins, Sophie Piperno-Neumann, Olivier Lantz, Bernard Asselain, and Corine Plancher. 2010. 'Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors', *Clinical cancer research*, 16: 2352-62.

Neophytou, Christiana M, Myrofora Panagi, Triantafyllos Stylianopoulos, and Panagiotis Papageorgis. 2021. 'The role of tumor microenvironment in cancer metastasis: Molecular mechanisms and therapeutic opportunities', *Cancers*, 13: 2053.

Niederkorn, Jerry Y. 1984. 'Enucleation in consort with immunologic impairment promotes metastasis of intraocular melanomas in mice', *Investigative ophthalmology & visual science*, 25: 1080-86.

Ogata, Dai, Kenjiro Namikawa, Akira Takahashi, and Naoya Yamazaki. 2021. 'A review of the AJCC melanoma staging system in the TNM classification', *Japanese journal of clinical oncology*, 51: 671-74.

Oh, D., N. Hong, K. Eun, J. Lee, L. Cai, M. Kim, H. Choi, A. Jawad, J. Ham, M. G. Park, B. Kim, S. C. Lee, C. Moon, H. Kim, and S. H. Hyun. 2025. 'Generation of a genetically engineered porcine melanoma model featuring oncogenic control through conditional Cre recombination', *Sci Rep*, 15: 1616.

Ohno, Y., H. Kitamura, N. Takahashi, J. Ohtake, S. Kaneumi, K. Sumida, S. Homma, H. Kawamura, N. Minagawa, S. Shibasaki, and A. Taketomi. 2016. 'IL-6 down-regulates HLA class II expression and IL-12 production of human dendritic cells to impair activation of antigen-specific CD4(+) T cells', *Cancer Immunol Immunother*, 65: 193-204.

Orouji, E., A. Federico, L. Larribère, D. Novak, D. B. Lipka, Y. Assenov, S. Sachindra, L. Hüser, K. Granados, C. Gebhardt, C. Plass, V. Umansky, and J. Utikal. 2019. 'Histone methyltransferase SETDB1 contributes to melanoma tumorigenesis and serves as a new potential therapeutic target', *Int J Cancer*, 145: 3462-77.

Ostroumov, Dmitrij, Nora Fekete-Drimusz, Michael Saborowski, Florian Kühnel, and Norman Woller. 2018. 'CD4 and CD8 T lymphocyte interplay in controlling tumor growth', *Cellular and molecular life sciences*, 75: 689-713.

Ozaki, Shinji, Raja Vuyyuru, Ken Kageyama, Mizue Terai, Masahiro Ohara, Hanyin Cheng, Tim Manser, Michael J Mastrangelo, Andrew E Aplin, and Takami Sato. 2016. 'Establishment and characterization of orthotopic mouse models for human uveal melanoma hepatic colonization', *The American journal of pathology*, 186: 43-56.

Park, J. J., A. Stewart, M. Irvine, B. Pedersen, Z. Ming, M. S. Carlino, R. J. Diefenbach, and H. Rizos. 2022. 'Protein kinase inhibitor responses in uveal melanoma reflects a diminished dependency on PKC-MAPK signaling', *Cancer Gene Ther*, 29: 1384-93.

Park, John J., Russell J. Diefenbach, Anthony M. Joshua, Richard F. Kefford, Matteo S. Carlino, and Helen Rizos. 2018. 'Oncogenic signaling in uveal melanoma', *Pigment cell & melanoma research*, 31: 661-72.

Patton, E Elizabeth, Kristen L Mueller, David J Adams, Niroshana Anandasabapathy, Andrew E Aplin, Corine Bertolotto, Marcus Bosenberg, Craig J Ceol, Christin E Burd, and Ping Chi. 2021. 'Melanoma models for the next generation of therapies', *Cancer Cell*, 39: 610-31.

Paul, S., S. Chhatar, A. Mishra, and G. Lal. 2019. 'Natural killer T cell activation increases iNOS(+)CD206(-) M1 macrophage and controls the growth of solid tumor', *J Immunother Cancer*, 7: 208.

Peggs, Karl S, Sergio A Quezada, Alan J Korman, and James P Allison. 2006. 'Principles and use of anti-CTLA4 antibody in human cancer immunotherapy', *Current opinion in immunology*, 18: 206-13.

Peña-Llopis, Samuel, Silvia Vega-Rubín-de-Celis, Arnold Liao, Nan Leng, Andrea Pavía-Jiménez, Shanshan Wang, Toshinari Yamasaki, Leah Zhrebker, Sharanya Sivanand, and Patrick Spence. 2012. 'BAP1 loss defines a new class of renal cell carcinoma', *Nature genetics*, 44: 751-59.

Pérez-Guijarro, E., C. P. Day, G. Merlino, and M. R. Zaidi. 2017. 'Genetically engineered mouse models of melanoma', *Cancer*, 123: 2089-103.

Petty, A. J., R. Dai, R. Lapalombella, R. A. Baiocchi, D. M. Benson, Z. Li, X. Huang, and Y. Yang. 2021. 'Hedgehog-induced PD-L1 on tumor-associated macrophages is critical for suppression of tumor-infiltrating CD8+ T cell function', *JCI Insight*, 6.

Phan, Giao Q, James C Yang, Richard M Sherry, Patrick Hwu, Suzanne L Topalian, Douglas J Schwartzentruber, Nicholas P Restifo, Leah R Haworth, Claudia A Seipp, and Linda J Freezer. 2003. 'Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma', *Proceedings of the National Academy of Sciences*, 100: 8372-77.

Pinnix, Chelsea C, John T Lee, Zhao-Jun Liu, Ronan McDaid, Klara Balint, Levi J Beverly, Patricia A Brafford, Min Xiao, Benjamin Himes, and Susan E Zabierowski. 2009. 'Active Notch1 confers a transformed phenotype to primary human melanocytes', *Cancer research*, 69: 5312-20.

Pleasance, E, A Bohm, LM Williamson, JMT Nelson, Y Shen, M Bonakdar, E Titmuss, V Csizmok, K Wee, and S Hosseinzadeh. 2022. 'Whole-genome and transcriptome analysis enhances precision cancer treatment options', *Annals of Oncology*, 33: 939-49.

Principe, N., J. Kidman, S. Goh, C. M. Tilsed, S. A. Fisher, V. S. Fear, C. A. Forbes, R. M. Zemek, A. Chopra, M. Watson, I. M. Dick, L. Boon, R. A. Holt, R. A. Lake, A. K. Nowak, W. J. Lesterhuis, A. M. McDonnell, and J. Chee. 2020. 'Tumor Infiltrating Effector Memory Antigen-Specific CD8(+) T Cells Predict Response to Immune Checkpoint Therapy', *Front Immunol*, 11: 584423.

Qiao, Xuehan, Zhangmin Hu, Fen Xiong, Yufei Yang, Chen Peng, Deqiang Wang, and Xiaoqin Li. 2023. 'Lipid metabolism reprogramming in tumor-associated macrophages and implications for therapy', *Lipids in Health and Disease*, 22: 45.

Queirolo, Paola, Andrea Boutros, Enrica Tanda, Francesco Spagnolo, and Pietro Quaglino. 2019. "Immune-checkpoint inhibitors for the treatment of metastatic melanoma: a model of cancer immunotherapy." In *Seminars in cancer biology*, 290-97. Elsevier.

Ramia, E., A. M. Chiaravalli, F. Bou Nasser Eddine, A. Tedeschi, F. Sessa, R. S. Accolla, and G. Forlani. 2019. 'CIITA-related block of HLA class II expression, upregulation of HLA class I, and heterogeneous expression of immune checkpoints in hepatocarcinomas: implications for new therapeutic approaches', *Oncoimmunology*, 8: 1548243.

Rannikko, Jenna H, Loic Verlingue, Maria de Miguel, Annika Pasanen, Debbie Robbrecht, Tanja Skytta, Sanna Iivanainen, Shishir Shetty, Yuk Ting Ma, and Donna M Graham. 2023. 'Bexmarilimab-induced macrophage activation leads to treatment benefit in solid tumors: The phase I/II first-in-human MATINS trial', *Cell Reports Medicine*, 4.

Ravi, R., K. A. Noonan, V. Pham, R. Bedi, A. Zhavoronkov, I. V. Ozerov, E. Makarev, A. V Artemov, P. T. Wysocki, R. Mehra, S. Nimmagadda, L. Marchionni, D. Sidransky, I. M. Borrello, E. Izumchenko, and A. Bedi. 2018. 'Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFβ enhance the efficacy of cancer immunotherapy', *Nat Commun*, 9: 741.

Rebecca, Vito W., Rajasekharan Somasundaram, and Meenhard Herlyn. 2020. 'Pre-clinical modeling of cutaneous melanoma', *Nature communications*, 11: 2858.

Rezaei, Tayebeh, Elham Davoudian, Saeed Khalili, Mohammad Amini, Maryam Hejazi, Miguel de la Guardia, and Ahad Mokhtarzadeh. 2021. 'Strategies in DNA vaccine for melanoma cancer', *Pigment cell & melanoma research*, 34: 869-91.

Riaz, Nadeem, Jonathan J Havel, Vladimir Makarov, Alexis Desrichard, Walter J Urba, Jennifer S Sims, F Stephen Hodi, Salvador Martín-Algarra, Rajarsi Mandal, and William H Sharfman. 2017. 'Tumor and microenvironment evolution during immunotherapy with nivolumab', *Cell*, 171: 934-49. e16.

Ribas, Antoni, and Jedd D Wolchok. 2018. 'Cancer immunotherapy using checkpoint blockade', *Science*, 359: 1350-55.

Ricci, J. E. 2025. 'Tumor-induced metabolic immunosuppression: Mechanisms and therapeutic targets', *Cell Rep*, 44: 115206.

Richards, Jackson R, Jae Hyuk Yoo, Donghan Shin, and Shannon J Odelberg. 2020. 'Mouse models of uveal melanoma: Strengths, weaknesses, and future directions', *Pigment cell & melanoma research*, 33: 264-78.

Robertson, A. G., J. Shih, C. Yau, E. A. Gibb, J. Oba, K. L. Mungall, J. M. Hess, V. Uzunangelov, V. Walter, L. Danilova, T. M. Lichtenberg, M. Kucherlapati, P. K. Kimes, M. Tang, A. Penson, O. Babur, R. Akbani, C. A. Bristow, K. A. Hoadley, L. Iype, M. T. Chang, A. D. Cherniack, C. Benz, G. B. Mills, R. G. W. Verhaak, K. G. Griewank, I. Felau, J. C. Zenklusen, J. E. Gershenwald, L. Schoenfield, A. J. Lazar, M. H. Abdel-Rahman, S. Roman-Roman, M. H. Stern, C. M. Cebulla, M. D. Williams, M. J. Jager, S. E. Coupland, B. Esmaeli, C. Kandoth, and S. E. Woodman. 2017. 'Integrative

Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma', *Cancer Cell*, 32: 204-20.e15.

Roshan Moniri, Mani, Ada Young, Kelsey Reinheimer, Jarrett Rayat, Long-Jun Dai, and Garth L Warnock. 2015. 'Dynamic assessment of cell viability, proliferation and migration using real time cell analyzer system (RTCA)', *Cytotechnology*, 67: 379-86.

Rossi, A., F. Zacchi, A. Reni, M. Rota, S. Palmerio, J. Menis, A. Zivi, S. Milleri, and M. Milella. 2024. 'Progresses and Pitfalls of Epigenetics in Solid Tumors Clinical Trials', *Int J Mol Sci*, 25.

Rossi, Ernesto, Michela Croce, Francesco Reggiani, Giovanni Schinzari, Marianna Ambrosio, Rosaria Gangemi, Giampaolo Tortora, Ulrich Pfeffer, and Adriana Amaro. 2021. 'Uveal Melanoma Metastasis', *Cancers*, 13: 5684.

Rusciano, D, P Lorenzoni, and M Burger. 1994. 'Murine models of liver metastasis', *Invasion & metastasis*, 14: 349-61.

Sadakierska-Chudy, Anna, and Małgorzata Filip. 2015. 'A comprehensive view of the epigenetic landscape. Part II: Histone post-translational modification, nucleosome level, and chromatin regulation by ncRNAs', *Neurotoxicity research*, 27: 172-97.

Sadikot, Ruxana T, and Timothy S Blackwell. 2005. 'Bioluminescence imaging', *Proceedings of the American Thoracic Society*, 2: 537-40.

Saleh, R., and E. Elkord. 2020. 'Acquired resistance to cancer immunotherapy: Role of tumor-mediated immunosuppression', *Semin Cancer Biol*, 65: 13-27.

Sanmamed, MF, C Chester, I Melero, and H Kohrt. 2016. 'Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies', *Annals of Oncology*, 27: 1190-98.

Schadendorf, Dirk, David E Fisher, Claus Garbe, Jeffrey E Gershenwald, Jean-Jacques Grob, Allan Halpern, Meenhard Herlyn, Michael A Marchetti, Grant McArthur, and Antoni Ribas. 2015. 'Melanoma', *Nature reviews Disease primers*, 1: 1-20.

Schmitt, Joseph M. 1999. 'Optical coherence tomography (OCT): a review', *IEEE Journal of selected topics in quantum electronics*, 5: 1205-15.

Schouppe, E., E. Van Overmeire, D. Laoui, J. Keirsse, and J. A. Van Ginderachter. 2013. 'Modulation of CD8(+) T-cell activation events by monocytic and granulocytic myeloid-derived suppressor cells', *Immunobiology*, 218: 1385-91.

Schrage, Ramona, Anna-Lena Schmitz, Evelyn Gaffal, Suvi Annala, Stefan Kehraus, Daniela Wenzel, Katrin M Büllesbach, Tobias Bald, Asuka Inoue, and Yuji Shinjo. 2015. 'The experimental power of FR900359 to study Gq-regulated biological processes', *Nature communications*, 6: 10156.

Schreibelt, G., K. F. Bol, H. Westdorp, F. Wimmers, E. H. Aarntzen, T. Duiveman-de Boer, M. W. van de Rakt, N. M. Scharenborg, A. J. de Boer, J. M. Pots, M. A. Olde Nordkamp, T. G. van Oorschot, J. Tel, G. Winkels, K. Petry, W. A. Blokx, M. M. van Rossum, M. E. Welzen, R. D. Mus, S. A. Croockewit, R. H. Koornstra, J. F. Jacobs, S. Kelderman, C. U. Blank, W. R. Gerritsen, C. J. Punt, C. G. Figdor, and I. J. de Vries. 2016. 'Effective Clinical

Responses in Metastatic Melanoma Patients after Vaccination with Primary Myeloid Dendritic Cells', *Clin Cancer Res*, 22: 2155-66.

Sen, M., K. R. Card, G. B. Caudill, N. R. Spitofsky, P. W. Dockery, A. R. Zaloga, J. S. Zeiger, C. F. DeYoung, S. J. Hamou, and C. L. Shields. 2022. 'Relationship between Fitzpatrick Skin Type and The Cancer Genome Atlas Classification with Melanoma-Related Metastasis and Death in 854 Patients at a Single Ocular Oncology Center', *Ophthalmic Genet*, 43: 742-55.

Shah, Akeesha A, T David Bourne, and Rajmohan Murali. 2013. 'BAP1 protein loss by immunohistochemistry: a potentially useful tool for prognostic prediction in patients with uveal melanoma', *Pathology*, 45: 651-56.

Shain, A. H., M. M. Bagger, R. Yu, D. Chang, S. Liu, S. Vemula, J. F. Weier, K. Wadt, S. Heegaard, B. C. Bastian, and J. F. Kiilgaard. 2019. 'The genetic evolution of metastatic uveal melanoma', *Nat Genet*, 51: 1123-30.

Sharma, P., and J. P. Allison. 2020. 'Dissecting the mechanisms of immune checkpoint therapy', *Nat Rev Immunol*, 20: 75-76.

Sharma, P., B. A. Siddiqui, S. Anandhan, S. S. Yadav, S. K. Subudhi, J. Gao, S. Goswami, and J. P. Allison. 2021a. 'The Next Decade of Immune Checkpoint Therapy', *Cancer Discov*, 11: 838-57.

Sharma, Padmanee, and James P Allison. 2015a. 'The future of immune checkpoint therapy', *Science*, 348: 56-61.

———. 2015b. 'Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential', *Cell*, 161: 205-14.

Sharma, Padmanee, Bilal A Siddiqui, Swetha Anandhan, Shalini S Yadav, Sumit K Subudhi, Jianjun Gao, Sangeeta Goswami, and James P Allison. 2021b. 'The next decade of immune checkpoint therapy', *Cancer discovery*, 11: 838-57.

Sheng, K. L., L. Kang, K. J. Pridham, L. E. Dunkenberger, Z. Sheng, and R. T. Varghese. 2020. 'An integrated approach to biomarker discovery reveals gene signatures highly predictive of cancer progression', *Sci Rep*, 10: 21246.

Sheng, W., M. W. LaFleur, T. H. Nguyen, S. Chen, A. Chakravarthy, J. R. Conway, Y. Li, H. Chen, H. Yang, P. H. Hsu, E. M. Van Allen, G. J. Freeman, D. D. De Carvalho, H. H. He, A. H. Sharpe, and Y. Shi. 2018. 'LSD1 Ablation Stimulates Anti-tumor Immunity and Enables Checkpoint Blockade', *Cell*, 174: 549-63.e19.

Shoushtari, A. N., S. Khan, K. Komatsubara, L. Feun, N. Acquavella, S. Singh-Kandah, T. Negri, A. Nesson, K. Abbate, S. Cremers, E. Musi, G. Ambrosini, S. Lee, G. K. Schwartz, and R. D. Carvajal. 2021. 'A Phase Ib Study of Sotrastaurin, a PKC Inhibitor, and Alpelisib, a PI3Kα Inhibitor, in Patients with Metastatic Uveal Melanoma', *Cancers (Basel)*, 13.

Simon, Jeffrey A., and Carol A. Lange. 2008. 'Roles of the EZH2 histone methyltransferase in cancer epigenetics', *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 647: 21-29.

Singhal, Akshat, Xiaoyu Zhao, Patrick Wall, Emily So, Guido Calderini, Alexander Partin, Natasha Koussa, Priyanka Vasanthakumari, Oleksandr Narykov, and Yitan Zhu. 2025. 'The Hallmarks of Predictive Oncology', *Cancer discovery*, 15: 271-85.

Siska, Peter J, Kathryn E Beckermann, W Kimryn Rathmell, and Scott M Haake. 2017. "Strategies to overcome therapeutic resistance in renal cell

carcinoma." In *Urologic Oncology: Seminars and Original Investigations*, 102-10. Elsevier.
Smit, K. N., M. J. Jager, A. de Klein, and E. Kiliç. 2020. 'Uveal melanoma: Towards a molecular understanding', *Prog Retin Eye Res*, 75: 100800.
Snell, George D. 1981. 'Studies in histocompatibility', *Science*, 213: 172-78.
Sng, K., M. E. Nga, S. Tan, and T. Walker. 2007. 'Analysis of Urine Cytology Tests in 120 Paired Cases', *Acta Cytologica*, 51: 782-87.
Solnik, Małgorzata, Natalia Paduszyńska, Anna M Czarnecka, Kamil J Synoradzki, Yacoub A Yousef, Tomasz Chorągiewicz, Robert Rejdak, Mario Damiano Toro, Sandrine Zweifel, and Katarzyna Dyndor. 2022. 'Imaging of uveal melanoma—Current standard and methods in development', *Cancers*, 14: 3147.
Souri, Z., A. P. A. Wierenga, W. G. M. Kroes, P. A. van der Velden, R. M. Verdijk, M. Eikmans, G. P. M. Luyten, and M. J. Jager. 2021. 'LAG3 and Its Ligands Show Increased Expression in High-Risk Uveal Melanoma', *Cancers (Basel)*, 13.
Spranger, S., R. Bao, and T. F. Gajewski. 2015a. 'Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity', *Nature*, 523: 231-5.
Spranger, Stefani, Riyue Bao, and Thomas F Gajewski. 2015b. 'Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity', *Nature*, 523: 231-35.
Stei, Marta M, Karin U Loeffler, Frank G Holz, and Martina C Herwig. 2016. 'Animal models of uveal melanoma: methods, applicability, and limitations', *BioMed research international*, 2016: 4521807.
Stei, Marta M, Karin U Loeffler, Christian Kurts, Tobias Hoeller, Christiane Pfarrer, Frank G Holz, and Martina C Herwig-Carl. 2016. 'Impact of macrophages on tumor growth characteristics in a murine ocular tumor model', *Experimental Eye Research*, 151: 9-18.
Strub, Thomas, Robert Ballotti, and Corine Bertolotto. 2020. 'The "ART" of epigenetics in melanoma: from histone "alterations, to resistance and therapies"', *Theranostics*, 10: 1777.
Suarez-Arnedo, Alejandra, Felipe Torres Figueroa, Camila Clavijo, Pablo Arbeláez, Juan C Cruz, and Carolina Muñoz-Camargo. 2020. 'An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays', *PloS one*, 15: e0232565.
Suesskind, Daniela, Anja Ulmer, Ursel Schiebel, Gerhard Fierlbeck, Bernhard Spitzer, Martin S Spitzer, Karl U Bartz‐Schmidt, and Salvatore Grisanti. 2011. 'Circulating melanoma cells in peripheral blood of patients with uveal melanoma before and after different therapies and association with prognostic parameters: a pilot study', *Acta ophthalmologica*, 89: 17-24.
Sugase, Takahito, Bao Q Lam, Meggie Danielson, Mizue Terai, Andrew E Aplin, J Silvio Gutkind, and Takami Sato. 2020. 'Development and optimization of orthotopic liver metastasis xenograft mouse models in uveal melanoma', *Journal of translational medicine*, 18: 1-14.
Sutopo, Natasha Christabella, Ji Hye Kim, and Jae Youl Cho. 2023. 'Role of histone methylation in skin cancers: Histone methylation–modifying enzymes as a new class of targets for skin cancer treatment', *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1878: 188865.

Tahiliani, Mamta, Kian Peng Koh, Yinghua Shen, William A Pastor, Hozefa Bandukwala, Yevgeny Brudno, Suneet Agarwal, Lakshminarayan M Iyer, David R Liu, and L Aravind. 2009. 'Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1', *Science*, 324: 930-35.

Taki, Mana, Kaoru Abiko, Masayo Ukita, Ryusuke Murakami, Koji Yamanoi, Ken Yamaguchi, Junzo Hamanishi, Tsukasa Baba, Noriomi Matsumura, and Masaki Mandai. 2021. 'Tumor immune microenvironment during epithelial–mesenchymal transition', *Clinical cancer research*, 27: 4669-79.

Tan, W. J., M. M. Wang, P. Ricciardi-Castagnoli, T. Tang, S. P. Chee, T. S. Lim, and A. S. Y. Chan. 2019. 'Single-cell MYD88 sequencing of isolated B cells from vitreous biopsies aids vitreoretinal lymphoma diagnosis', *Blood*, 134: 709-12.

Tan, Wei Chang Colin, Sanjna Nilesh Nerurkar, Hai Yun Cai, Harry Ho Man Ng, Duoduo Wu, Yu Ting Felicia Wee, Jeffrey Chun Tatt Lim, Joe Yeong, and Tony Kiat Hon Lim. 2020. 'Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy', *Cancer Communications*, 40: 135-53.

Tan, Wei Jian, Mona Meng Wang, Paola Ricciardi Castagnoli, Tiffany Tang, Anita Sook Yee Chan, and Tong Seng Lim. 2021. 'Single B-Cell Genomic Analyses Differentiate Vitreoretinal Lymphoma from Chronic Inflammation', *Ophthalmology*, 128: 1079-90.

Tang, T., X. Huang, G. Zhang, Z. Hong, X. Bai, and T. Liang. 2021. 'Advantages of targeting the tumor immune microenvironment over blocking immune checkpoint in cancer immunotherapy', *Signal Transduct Target Ther*, 6: 72.

Taube, J. M., A. Klein, J. R. Brahmer, H. Xu, X. Pan, J. H. Kim, L. Chen, D. M. Pardoll, S. L. Topalian, and R. A. Anders. 2014. 'Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy', *Clin Cancer Res*, 20: 5064-74.

Tel, J., E. H. Aarntzen, T. Baba, G. Schreibelt, B. M. Schulte, D. Benitez-Ribas, O. C. Boerman, S. Croockewit, W. J. Oyen, M. van Rossum, G. Winkels, P. G. Coulie, C. J. Punt, C. G. Figdor, and I. J. de Vries. 2013. 'Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients', *Cancer Res*, 73: 1063-75.

Terai, Mizue, Zhaomei Mu, David J Eschelman, Carin F Gonsalves, Ken Kageyama, Inna Chervoneva, Marlana Orloff, Ryan Weight, Michael J Mastrangelo, and Massimo Cristofanilli. 2015. 'Arterial blood, rather than venous blood, is a better source for circulating melanoma cells', *EBioMedicine*, 2: 1821-26.

Terai, Mizue, and Takami Sato. 2024. 'Individualised neoantigen cancer vaccine therapy', *The Lancet*, 403: 590-91.

Tiper, Irina V., and Tonya J. Webb. 2016. 'Histone deacetylase inhibitors enhance CD1d-dependent NKT cell responses to lymphoma', *Cancer Immunology, Immunotherapy*, 65: 1411-21.

Tirosh, Itay, Benjamin Izar, Sanjay M Prakadan, Marc H Wadsworth, Daniel Treacy, John J Trombetta, Asaf Rotem, Christopher Rodman, Christine Lian, and

George Murphy. 2016. 'Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq', *Science*, 352: 189-96.

Tirosh, Itay, Benjamin Izar, Sanjay M. Prakadan, Marc H. Wadsworth, 2nd, Daniel Treacy, John J. Trombetta, Asaf Rotem, Christopher Rodman, Christine Lian, George Murphy, Mohammad Fallahi-Sichani, Ken Dutton-Regester, Jia-Ren Lin, Ofir Cohen, Parin Shah, Diana Lu, Alex S. Genshaft, Travis K. Hughes, Carly G. K. Ziegler, Samuel W. Kazer, Aleth Gaillard, Kellie E. Kolb, Alexandra-Chloé Villani, Cory M. Johannessen, Aleksandr Y. Andreev, Eliezer M. Van Allen, Monica Bertagnolli, Peter K. Sorger, Ryan J. Sullivan, Keith T. Flaherty, Dennie T. Frederick, Judit Jané-Valbuena, Charles H. Yoon, Orit Rozenblatt-Rosen, Alex K. Shalek, Aviv Regev, and Levi A. Garraway. 2016. 'Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq', *Science (New York, N.Y.)*, 352: 189-96.

Tiwary, S., J. A. Berzofsky, and M. Terabe. 2019. 'Altered Lipid Tumor Environment and Its Potential Effects on NKT Cell Function in Tumor Immunity', *Front Immunol*, 10: 2187.

Torrano, Joachim, Abdullah Al Emran, Heinz Hammerlindl, and Helmut Schaider. 2019. 'Emerging roles of H3K9me3, SETDB1 and SETDB2 in therapy-induced cellular reprogramming', *Clinical epigenetics*, 11: 43.

Tosi, A., R. Cappellesso, A. P. Dei Tos, V. Rossi, C. Aliberti, J. Pigozzo, A. Fabozzi, M. Sbaraglia, S. Blandamura, P. Del Bianco, V. Chiarion-Sileni, and A. Rosato. 2021. 'The immune cell landscape of metastatic uveal melanoma correlates with overall survival', *J Exp Clin Cancer Res*, 40: 154.

Triozzi, Pierre L, Lynn Schoenfield, Thomas Plesec, Yogen Saunthararajah, Raymond R Tubbs, and Arun D Singh. 2019. 'Molecular profiling of primary uveal melanomas with tumor-infiltrating lymphocytes', *Oncoimmunology*, 8: e947169.

Trujillo, J. A., R. F. Sweis, R. Bao, and J. J. Luke. 2018. 'T Cell-Inflamed versus Non-T Cell-Inflamed Tumors: A Conceptual Framework for Cancer Immunotherapy Drug Development and Combination Therapy Selection', *Cancer Immunol Res*, 6: 990-1000.

Tsimberidou, Apostolia-Maria, David S Hong, Jennifer J Wheler, Gerald S Falchook, Filip Janku, Aung Naing, Siqing Fu, Sarina Piha-Paul, Carrie Cartwright, and Russell R Broaddus. 2019. 'Long-term overall survival and prognostic score predicting survival: the IMPACT study in precision medicine', *Journal of hematology & oncology*, 12: 1-12.

Tumeh, P. C., C. L. Harview, J. H. Yearley, I. P. Shintaku, E. J. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A. N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A. J. Gutierrez, T. R. Grogan, C. Mateus, G. Tomasic, J. A. Glaspy, R. O. Emerson, H. Robins, R. H. Pierce, D. A. Elashoff, C. Robert, and A. Ribas. 2014. 'PD-1 blockade induces responses by inhibiting adaptive immune resistance', *Nature*, 515: 568-71.

Tumeh, Paul C, Christina L Harview, Jennifer H Yearley, I Peter Shintaku, Emma JM Taylor, Lidia Robert, Bartosz Chmielowski, Marko Spasic, Gina Henry, and Voicu Ciobanu. 2014. 'PD-1 blockade induces responses by inhibiting adaptive immune resistance', *Nature*, 515: 568-71.

Tura, Aysegül, Julia Lüke, Hartmut Merz, Mihaela Reinsberg, Matthias Lüke, Martine J. Jager, and Salvatore Grisanti. 2014. 'Identification of Circulating Melanoma Cells in Uveal Melanoma Patients by Dual-Marker Immunoenrichment', *Investigative Ophthalmology & Visual Science*, 55: 4395-404.

Uhlen, Mathias, Cheng Zhang, Sunjae Lee, Evelina Sjöstedt, Linn Fagerberg, Gholamreza Bidkhori, Rui Benfeitas, Muhammad Arif, Zhengtao Liu, and Fredrik Edfors. 2017. 'A pathology atlas of the human cancer transcriptome', *Science*, 357: eaan2507.

Uka, Rexhep, Christian Britschgi, Anja Krättli, Claudia Matter, Daniela Mihic, Michal J Okoniewski, Marco Gualandi, Roger Stupp, Paolo Cinelli, and Reinhard Dummer. 2020. 'Temporal activation of WNT/β-catenin signaling is sufficient to inhibit SOX10 expression and block melanoma growth', *Oncogene*, 39: 4132-54.

Ulmer, A., O. Schmidt-Kittler, J. Fischer, U. Ellwanger, G. Rassner, G. Riethmuller, G. Fierlbeck, and C. A. Klein. 2004. 'Immunomagnetic enrichment, genomic characterization, and prognostic impact of circulating melanoma cells', *Clin Cancer Res*, 10: 531-7.

Ulmer, Anja, Julia Beutel, Daniela Süsskind, Ralf-Dieter Hilgers, Focke Ziemssen, Matthias Lüke, Martin Röcken, Martin Rohrbach, Gerhard Fierlbeck, and Karl-Ulrich Bartz-Schmidt. 2008. 'Visualization of circulating melanoma cells in peripheral blood of patients with primary uveal melanoma', *Clinical Cancer Research*, 14: 4469-74.

van Bree, N., A. S. Oppelt, S. Lindström, L. Zhou, L. Boutin, B. Coyle, F. J. Swartling, J. I. Johnsen, L. Bräutigam, and M. Wilhelm. 2024. 'Development of an orthotopic medulloblastoma zebrafish model for rapid drug testing', *Neuro Oncol*.

van den Bosch, Quincy CC, Annelies de Klein, Rob M Verdijk, Emine Killic, and Erwin Brosens. 2023. 'Uveal melanoma modeling in mice and zebrafish', *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*: 189055.

van der Kooij, M. K., F. M. Speetjens, S. H. van der Burg, and E. Kapiteijn. 2019. 'Uveal Versus Cutaneous Melanoma; Same Origin, Very Distinct Tumor Types', *Cancers (Basel)*, 11.

van der Mijn, Johannes C, Kristian B Laursen, Leiping Fu, Francesca Khani, Lukas E Dow, Dawid G Nowak, Qiuying Chen, Steven S Gross, David M Nanus, and Lorraine J Gudas. 2023. 'Novel genetically engineered mouse models for clear cell renal cell carcinoma', *Scientific Reports*, 13: 8246.

van Elsas, A., A. A. Hurwitz, and J. P. Allison. 1999. 'Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation', *J Exp Med*, 190: 355-66.

van Essen, T Huibertus, Sake I van Pelt, Inge HG Bronkhorst, Mieke Versluis, Fariba Némati, Cécile Laurent, Gregorius PM Luyten, Thorbald van Hall, Peter J van den Elsen, and Pieter A van der Velden. 2016. 'Upregulation of HLA

expression in primary uveal melanoma by infiltrating leukocytes', *PloS one*, 11: e0164292.

Van Raamsdonk, C. D., V. Bezrookove, G. Green, J. Bauer, L. Gaugler, J. M. O'Brien, E. M. Simpson, G. S. Barsh, and B. C. Bastian. 2009. 'Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi', *Nature*, 457: 599-602.

Van Raamsdonk, Catherine D, Klaus G Griewank, Michelle B Crosby, Maria C Garrido, Swapna Vemula, Thomas Wiesner, Anna C Obenauf, Werner Wackernagel, Gary Green, and Nancy Bouvier. 2010. 'Mutations in GNA11 in uveal melanoma', *New England Journal of Medicine*, 363: 2191-99.

Vander Lugt, B., A. A. Khan, J. A. Hackney, S. Agrawal, J. Lesch, M. Zhou, W. P. Lee, S. Park, M. Xu, J. DeVoss, C. J. Spooner, C. Chalouni, L. Delamarre, I. Mellman, and H. Singh. 2014. 'Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation', *Nat Immunol*, 15: 161-7.

Vathiotis, Ioannis A, Jennifer M Johnson, and Athanassios Argiris. 2021. 'Enhancing programmed cell death protein 1 axis inhibition in head and neck squamous cell carcinoma: Combination immunotherapy', *Cancer Treatment Reviews*, 97: 102192.

Viegas, J., and B. Sarmento. 2024. 'Bridging the gap between testing and clinics exploring alternative pre-clinical models in melanoma research', *Adv Drug Deliv Rev*, 208: 115295.

Wagstaff, William, Rimel N Mwamba, Karina Grullon, Mikhayla Armstrong, Piao Zhao, Bryce Hendren-Santiago, Kevin H Qin, Alexander J Li, Daniel A Hu, and Andrew Youssef. 2022. 'Melanoma: Molecular genetics, metastasis, targeted therapies, immunotherapies, and therapeutic resistance', *Genes & diseases*, 9: 1608-23.

Waldman, Alex D, Jill M Fritz, and Michael J Lenardo. 2020. 'A guide to cancer immunotherapy: from T cell basic science to clinical practice', *Nature Reviews Immunology*, 20: 651-68.

Wan, Chen-rei, Leroy Muya, Viral Kansara, and Thomas A Ciulla. 2021. 'Suprachoroidal delivery of small molecules, nanoparticles, gene and cell therapies for ocular diseases', *Pharmaceutics*, 13: 288.

Wang, D., Q. Ye, H. Gu, and Z. Chen. 2022. 'The role of lipid metabolism in tumor immune microenvironment and potential therapeutic strategies', *Front Oncol*, 12: 984560.

Wang, J., C. J. Perry, K. Meeth, D. Thakral, W. Damsky, G. Micevic, S. Kaech, K. Blenman, and M. Bosenberg. 2017. 'UV-induced somatic mutations elicit a functional T cell response in the YUMMER1.7 mouse melanoma model', *Pigment Cell Melanoma Res*, 30: 428-35.

Wang, L., Z. Kuang, D. Zhang, Y. Gao, M. Ying, and T. Wang. 2021. 'Reactive oxygen species in immune cells: A new antitumor target', *Biomed Pharmacother*, 133: 110978.

Wang, Mona Meng, Chuanfei Chen, Myoe Naing Lynn, Carlos R. Figueiredo, Wei Jian Tan, Tong Seng Lim, Sarah E. Coupland, and Anita Sook Yee Chan. 2021. 'Applying Single-Cell Technology in Uveal Melanomas: Current

Trends and Perspectives for Improving Uveal Melanoma Metastasis Surveillance and Tumor Profiling', *Frontiers in Molecular Biosciences*, 7.

Wang, Mona Meng, Saara A Koskela, Arfa Mehmood, Miriam Langguth, Eleftheria Maranou, and Carlos R Figueiredo. 2023. 'Epigenetic control of CD1D expression as a mechanism of resistance to immune checkpoint therapy in poorly immunogenic melanomas', *Frontiers in Immunology*, 14: 1152228.

Wang, Mona Meng, Wei Jian Tan, Tong Seng Lim, and Anita Sook Yee Chan. 2021. 'PreservCyt is an optimal fixative that permits cytologic and molecular analyses of vitreoretinal lymphoma biopsies', *Ocular Immunology and Inflammation*, 29: 430-39.

Wang, R., Y. He, V. Robinson, Z. Yang, P. Hessler, L. M. Lasko, X. Lu, A. Bhathena, A. Lai, T. Uziel, and L. T. Lam. 2018. 'Targeting Lineage-specific MITF Pathway in Human Melanoma Cell Lines by A-485, the Selective Small-molecule Inhibitor of p300/CBP', *Mol Cancer Ther*, 17: 2543-50.

Wang, Zehua, Chen Chen, Jiaoyu Ai, Yaping Gao, Lei Wang, Shurui Xia, Yongxu Jia, and Yanru Qin. 2025. 'The crosstalk between senescence, tumor, and immunity: molecular mechanism and therapeutic opportunities', *MedComm*, 6: e70048.

Waters, Andrew J, Timothy Brendler-Spaeth, Danielle Smith, Victoria Offord, Hong Kee Tan, Yajie Zhao, Sofia Obolenski, Maartje Nielsen, Remco van Doorn, and Jo-Ellen Murphy. 2024. 'Saturation genome editing of BAP1 functionally classifies somatic and germline variants', *Nature genetics*, 56: 1434-45.

Weber, Jeffrey S., Matteo S. Carlino, Adnan Khattak, Tarek Meniawy, George Ansstas, Matthew H. Taylor, Kevin B. Kim, Meredith McKean, Georgina V. Long, Ryan J. Sullivan, Mark Faries, Thuy T. Tran, C. Lance Cowey, Andrew Pecora, Montaser Shaheen, Jennifer Segar, Theresa Medina, Victoria Atkinson, Geoffrey T. Gibney, Jason J. Luke, Sajeve Thomas, Elizabeth I. Buchbinder, Jane A. Healy, Mo Huang, Manju Morrissey, Igor Feldman, Vasudha Sehgal, Celine Robert-Tissot, Peijie Hou, Lili Zhu, Michelle Brown, Praveen Aanur, Robert S. Meehan, and Tal Zaks. 2024. 'Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma (KEYNOTE-942): a randomised, phase 2b study', *The Lancet*, 403: 632-44.

Webster, Marie R, and Ashani T Weeraratna. 2013. 'A Wnt-er migration: the confusing role of β-catenin in melanoma metastasis', *Science signaling*, 6: pe11-pe11.

Wege, A. K. 2018. 'Humanized Mouse Models for the Preclinical Assessment of Cancer Immunotherapy', *BioDrugs*, 32: 245-66.

Wellbrock, Claudia, and Adam Hurlstone. 2010. 'BRAF as therapeutic target in melanoma', *Biochemical pharmacology*, 80: 561-67.

Wen, M., Y. Li, X. Qin, B. Qin, and Q. Wang. 2023. 'Insight into Cancer Immunity: MHCs, Immune Cells and Commensal Microbiota', *Cells*, 12.

Wierenga, A. P. A., N. J. Brouwer, M. C. Gelmi, R. M. Verdijk, M. H. Stern, Z. Bas, K. Malkani, S. G. van Duinen, A. Ganguly, W. G. M. Kroes, M. Marinkovic, G. P. M. Luyten, C. L. Shields, and M. J. Jager. 2022. 'Chromosome 3 and 8q Aberrations in Uveal Melanoma Show Greater Impact on Survival in

Patients with Light Iris versus Dark Iris Color', *Ophthalmology*, 129: 421-30.

Wiesner, T., A. C. Obenauf, R. Murali, I. Fried, K. G. Griewank, P. Ulz, C. Windpassinger, W. Wackernagel, S. Loy, I. Wolf, A. Viale, A. E. Lash, M. Pirun, N. D. Socci, A. Rütten, G. Palmedo, D. Abramson, K. Offit, A. Ott, J. C. Becker, L. Cerroni, H. Kutzner, B. C. Bastian, and M. R. Speicher. 2011. 'Germline mutations in BAP1 predispose to melanocytic tumors', *Nat Genet*, 43: 1018-21.

Wiesner, Thomas, Anna C Obenauf, Rajmohan Murali, Isabella Fried, Klaus G Griewank, Peter Ulz, Christian Windpassinger, Werner Wackernagel, Shea Loy, and Ingrid Wolf. 2011. 'Germline mutations in BAP1 predispose to melanocytic tumors', *Nature genetics*, 43: 1018-21.

Wilgenhof, S., J. Corthals, C. Heirman, N. van Baren, S. Lucas, P. Kvistborg, K. Thielemans, and B. Neyns. 2016. 'Phase II Study of Autologous Monocyte-Derived mRNA Electroporated Dendritic Cells (TriMixDC-MEL) Plus Ipilimumab in Patients With Pretreated Advanced Melanoma', *J Clin Oncol*, 34: 1330-8.

Wolchok, Jedd D, Vanna Chiarion-Sileni, Piotr Rutkowski, C Lance Cowey, Dirk Schadendorf, John Wagstaff, Paola Queirolo, Reinhard Dummer, Marcus O Butler, and Andrew G Hill. 2025. 'Final, 10-year outcomes with nivolumab plus ipilimumab in advanced melanoma', *New England Journal of Medicine*, 392: 11-22.

Wolchok, Jedd D, Harriet Kluger, Margaret K Callahan, Michael A Postow, Naiyer A Rizvi, Alexander M Lesokhin, Neil H Segal, Charlotte E Ariyan, Ruth-Ann Gordon, and Kathleen Reed. 2013. 'Nivolumab plus ipilimumab in advanced melanoma', *New England Journal of Medicine*, 369: 122-33.

Woods, D. M., A. L. Sodré, A. Villagra, A. Sarnaik, E. M. Sotomayor, and J. Weber. 2015. 'HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy with PD-1 Blockade', *Cancer Immunol Res*, 3: 1375-85.

Wu, A. A., K. M. Bever, W. J. Ho, E. J. Fertig, N. Niu, L. Zheng, R. M. Parkinson, J. N. Durham, B. Onners, A. K. Ferguson, C. Wilt, A. H. Ko, A. Wang-Gillam, D. A. Laheru, R. A. Anders, E. D. Thompson, E. A. Sugar, E. M. Jaffee, and D. T. Le. 2020. 'A Phase II Study of Allogeneic GM-CSF-Transfected Pancreatic Tumor Vaccine (GVAX) with Ipilimumab as Maintenance Treatment for Metastatic Pancreatic Cancer', *Clin Cancer Res*, 26: 5129-39.

Wu, Donglu, Ye Qiu, Yunshuang Jiao, Zhidong Qiu, and Da Liu. 2020. 'Small molecules targeting HATs, HDACs, and BRDs in cancer therapy', *Frontiers in oncology*, 10: 560487.

Wu, Yuting, Xi Pu, Xu Wang, and Min Xu. 2024. 'Reprogramming of lipid metabolism in the tumor microenvironment: a strategy for tumor immunotherapy', *Lipids in Health and Disease*, 23: 35.

Wynn, T. A., and L. Barron. 2010. 'Macrophages: master regulators of inflammation and fibrosis', *Semin Liver Dis*, 30: 245-57.

Xie, Qi, Xiaolin Liu, Rengyun Liu, Jingxuan Pan, and Jing Liang. 2024. 'Cellular mechanisms of combining innate immunity activation with PD-1/PD-L1 blockade in treatment of colorectal cancer', *Molecular cancer*, 23: 252.

Xu, Duo, Yanyun Gao, Haitang Yang, Marc Spils, Thomas M Marti, Tereza Losmanová, Min Su, Wenxiang Wang, Qinghua Zhou, and Patrick Dorn. 2024. 'BAP1 deficiency inflames the tumor immune microenvironment and is a candidate biomarker for immunotherapy response in malignant pleural mesothelioma', *JTO Clinical and Research Reports*, 5: 100672.

Xu, Duo, Yanyun Gao, Haitang Yang, Marc Spils, Thomas M. Marti, Tereza Losmanová, Min Su, Wenxiang Wang, Qinghua Zhou, Patrick Dorn, Yongqian Shu, and Ren-Wang Peng. 2024. 'BAP1 Deficiency Inflames the Tumor Immune Microenvironment and Is a Candidate Biomarker for Immunotherapy Response in Malignant Pleural Mesothelioma', *JTO Clinical and Research Reports*, 5: 100672.

Yan, Chi, Caroline A Nebhan, Nabil Saleh, Rebecca Shattuck-Brandt, Sheau-Chiann Chen, Gregory D Ayers, Vivian Weiss, Ann Richmond, and Anna E Vilgelm. 2023. 'Generation of orthotopic patient-derived xenografts in humanized mice for evaluation of emerging targeted therapies and immunotherapy combinations for melanoma', *Cancers*, 15: 3695.

Yan, G., M. S. Eller, C. Elm, C. A. Larocca, B. Ryu, I. P. Panova, B. M. Dancy, E. M. Bowers, D. Meyers, L. Lareau, P. A. Cole, S. D. Taverna, and R. M. Alani. 2013. 'Selective inhibition of p300 HAT blocks cell cycle progression, induces cellular senescence, and inhibits the DNA damage response in melanoma cells', *J Invest Dermatol*, 133: 2444-52.

Yan, Hailan, Jinghua Shi, Yi Dai, Xiaoyan Li, Yushi Wu, Jing Zhang, Zhiyue Gu, Chenyu Zhang, and Jinhua Leng. 2022. 'Technique integration of single-cell RNA sequencing with spatially resolved transcriptomics in the tumor microenvironment', *Cancer Cell International*, 22: 155.

Yang, H., C. M. Brackett, V. M. Morales-Tirado, Z. Li, Q. Zhang, M. W. Wilson, C. Benjamin, W. Harris, E. K. Waller, A. V. Gudkov, L. G. Burdelya, and H. E. Grossniklaus. 2016. 'The Toll-like receptor 5 agonist entolimod suppresses hepatic metastases in a murine model of ocular melanoma via an NK cell-dependent mechanism', *Oncotarget*, 7: 2936-50.

Yang, Hua, Jinfeng Cao, and Hans E Grossniklaus. 2015. 'Uveal melanoma metastasis models', *Ocular oncology and pathology*, 1: 151-60.

Yang, J., J. Yan, and B. Liu. 2018. 'Targeting VEGF/VEGFR to Modulate Antitumor Immunity', *Front Immunol*, 9: 978.

Yang, Kailin, Ahmed Halima, and Timothy A Chan. 2023. 'Antigen presentation in cancer—mechanisms and clinical implications for immunotherapy', *Nature Reviews Clinical Oncology*, 20: 604-23.

Yarchoan, Mark, Alexander Hopkins, and Elizabeth M Jaffee. 2017. 'Tumor mutational burden and response rate to PD-1 inhibition', *New England Journal of Medicine*, 377: 2500-01.

Yeong, Joe, Tira Tan, Zi Long Chow, Qing Cheng, Bernett Lee, Amanda Seet, Johnathan Xiande Lim, Jeffrey Chun Tatt Lim, Clara Chong Hui Ong, and Aye Aye Thike. 2020. 'Multiplex immunohistochemistry/immunofluorescence (mIHC/IF) for PD-L1 testing

in triple-negative breast cancer: a translational assay compared with conventional IHC', *Journal of Clinical Pathology*, 73: 557-62.

Yin, Jie, Gangyin Zhao, Helen Kalirai, Sarah E Coupland, Aart G Jochemsen, Gabriel Forn-Cuní, Annemijn PA Wierenga, Martine J Jager, B Ewa Snaar-Jagalska, and Arwin Groenewoud. 2023. 'Zebrafish patient-derived xenograft model as a preclinical platform for uveal melanoma drug discovery', *Pharmaceuticals*, 16: 598.

Yoo, J. H., D. S. Shi, A. H. Grossmann, L. K. Sorensen, Z. Tong, T. M. Mleynek, A. Rogers, W. Zhu, J. R. Richards, J. M. Winter, J. Zhu, C. Dunn, A. Bajji, M. Shenderovich, A. L. Mueller, S. E. Woodman, J. W. Harbour, K. R. Thomas, S. J. Odelberg, K. Ostanin, and D. Y. Li. 2016. 'ARF6 Is an Actionable Node that Orchestrates Oncogenic GNAQ Signaling in Uveal Melanoma', *Cancer cell*, 29: 889-904.

Yoshida, Go J. 2020. 'Applications of patient-derived tumor xenograft models and tumor organoids', *Journal of hematology & oncology*, 13: 4.

Yu, Chang, Jun Yu, Xiaotian Yao, William KK Wu, Youyong Lu, Senwei Tang, Xiangchun Li, Li Bao, Xiaoxing Li, and Yong Hou. 2014. 'Discovery of biclonal origin and a novel oncogene SLC12A5 in colon cancer by single-cell sequencing', *Cell research*, 24: 701-12.

Yu, G. T., L. Mao, L. Wu, W. W. Deng, L. L. Bu, J. F. Liu, L. Chen, L. L. Yang, H. Wu, W. F. Zhang, and Z. J. Sun. 2018. 'Inhibition of SRC family kinases facilitates anti-CTLA4 immunotherapy in head and neck squamous cell carcinoma', *Cell Mol Life Sci*, 75: 4223-34.

Yu, Y., K. Schleich, B. Yue, S. Ji, P. Lohneis, K. Kemper, M. R. Silvis, N. Qutob, E. van Rooijen, M. Werner-Klein, L. Li, D. Dhawan, S. Meierjohann, M. Reimann, A. Elkahloun, S. Treitschke, B. Dörken, C. Speck, F. A. Mallette, L. I. Zon, S. L. Holmen, D. S. Peeper, Y. Samuels, C. A. Schmitt, and S. Lee. 2018. 'Targeting the Senescence-Overriding Cooperative Activity of Structurally Unrelated H3K9 Demethylases in Melanoma', *Cancer Cell*, 33: 322-36.e8.

Yuan, Xiao, Jinxi Wang, Yixuan Huang, Dangang Shangguan, and Peng Zhang. 2021. 'Single-cell profiling to explore immunological heterogeneity of tumor microenvironment in breast cancer', *Frontiers in Immunology*, 12: 643692.

Zaidi, M. Raza, David E. Fisher, and Helen Rizos. 2020. 'Biology of Melanocytes and Primary Melanoma.' in Charles M. Balch, Michael B. Atkins, Claus Garbe, Jeffrey E. Gershenwald, Allan C. Halpern, John M. Kirkwood, Grant A. McArthur, John F. Thompson and Arthur J. Sober (eds.), *Cutaneous Melanoma* (Springer International Publishing: Cham).

Zeng, Mingtang, Chao Pi, Ke Li, Lin Sheng, Ying Zuo, Jiyuan Yuan, Yonggen Zou, Xiaomei Zhang, Wenmei Zhao, and Robert J Lee. 2022. 'Patient-derived xenograft: a more standard “Avatar” model in preclinical studies of gastric cancer', *Frontiers in oncology*, 12: 898563.

Zeng, Mingtang, Zijing Ruan, Jiaxi Tang, Maozhu Liu, Chengji Hu, Ping Fan, and Xinhua Dai. 2023. 'Generation, evolution, interfering factors, applications, and challenges of patient-derived xenograft models in immunodeficient mice', *Cancer Cell International*, 23: 120.

Zhang, Chao, and Shuai Wu. 2023. 'BAP1 mutations inhibit the NF-κB signaling pathway to induce an immunosuppressive microenvironment in uveal melanoma', *Molecular Medicine*, 29: 126.

Zhang, Feifei, Xiaofeng Tang, Song Fan, Xia Liu, Jun Sun, Cheng Ju, Yiping Liang, Renfeng Liu, Ruihao Zhou, Bo Yu, Changhua Zhang, Zhiping Zhang, Tiebang Kang, Guofu Huang, and Xiao-Bin Lv. 2021. 'Targeting the p300/NONO axis sensitizes melanoma cells to BRAF inhibitors', *Oncogene*, 40: 4137-50.

Zhang, Haixia, Shizhen Li, Dan Wang, Siyang Liu, Tengfei Xiao, Wangning Gu, Hongmin Yang, Hui Wang, Minghua Yang, and Pan Chen. 2024. 'Metabolic reprogramming and immune evasion: the interplay in the tumor microenvironment', *Biomarker Research*, 12: 96.

Zhang, Huimin, Sanwei Chen, Yuwei Zhang, Chenxi Tian, Jun Pan, Yu Wang, Shiyu Bai, Qielan Wu, Miya Su, and Di Xie. 2023. 'Antigen Priming Induces Functional Reprogramming in iNKT Cells via Metabolic and Epigenetic Regulation: An Insight into iNKT Cell-Based Antitumor Immunotherapy', *Cancer immunology research*, 11: 1598-610.

Zhou, Xiaoting, Yanghong Ni, Xiao Liang, Yi Lin, Biao An, Xiang He, and Xia Zhao. 2022. 'Mechanisms of tumor resistance to immune checkpoint blockade and combination strategies to overcome resistance', *Frontiers in Immunology*, 13: 915094.

Zhou, Xin, Ying Wang, Zhangqi Dou, Gloria Delfanti, Ourania Tsahouridis, Caroline Marnata Pellegry, Manuela Zingarelli, Gatphan Atassi, Mark G Woodcock, and Giulia Casorati. 2024. 'CAR-redirected natural killer T cells demonstrate superior antitumor activity to CAR-T cells through multimodal CD1d-dependent mechanisms', *Nature Cancer*: 1-15.

Zhu, B., S. Chen, H. Wang, C. Yin, C. Han, C. Peng, Z. Liu, L. Wan, X. Zhang, J. Zhang, C. G. Lian, P. Ma, Z. X. Xu, S. Prince, T. Wang, X. Gao, Y. Shi, D. Liu, M. Liu, W. Wei, Z. Wei, J. Pan, Y. Wang, Z. Xuan, J. Hess, N. K. Hayward, C. R. Goding, X. Chen, J. Zhou, and R. Cui. 2018. 'The protective role of DOT1L in UV-induced melanomagenesis', *Nat Commun*, 9: 259.

Zhu, Jie, Ke Zhang, Yuan Chen, Xinyu Ge, Junqing Wu, Peng Xu, and Jie Yao. 2024. 'Progress of single-cell RNA sequencing combined with spatial transcriptomics in tumour microenvironment and treatment of pancreatic cancer', *Journal of translational medicine*, 22: 563.

Zingg, D., J. Debbache, R. Peña-Hernández, A. T. Antunes, S. M. Schaefer, P. F. Cheng, D. Zimmerli, J. Haeusel, R. R. Calçada, E. Tuncer, Y. Zhang, R. Bossart, K. K. Wong, K. Basler, R. Dummer, R. Santoro, M. P. Levesque, and L. Sommer. 2018. 'EZH2-Mediated Primary Cilium Deconstruction Drives Metastatic Melanoma Formation', *Cancer Cell*, 34: 69-84.e14.

Zingg, Daniel, Natalia Arenas-Ramirez, Dilara Sahin, Rodney A. Rosalia, Ana T. Antunes, Jessica Haeusel, Lukas Sommer, and Onur Boyman. 2017. 'The Histone Methyltransferase Ezh2 Controls Mechanisms of Adaptive Resistance to Tumor Immunotherapy', *Cell reports*, 20: 854-67.

Zou, S., Q. Tong, B. Liu, W. Huang, Y. Tian, and X. Fu. 2020. 'Targeting STAT3 in Cancer Immunotherapy', *Mol Cancer*, 19: 145.

List of Figures, Tables and Appendices

Figures