Review

Do ERBB Receptors Serve as a Nexus for Oncogenic Signals in Uveal Melanoma?

Niusha Raeesian and David J. Riese *

Posted Date: 1 April 2026

doi: 10.20944/preprints202604.0001.v1

Keywords: uveal melanoma; targeted therapy; signal transduction; Galphaq; Galpha11; PI3K/AKT/mTOR; RAS-MAPK; GPCR-EGFR crosstalk; ERBB heterodimerization

 doi:10.20944/preprints202604.0001.v1

Review

Do ERBB Receptors Serve as a Nexus for Oncogenic Signals in Uveal Melanoma?

Niusha Raeesian [1,2] and David J. Riese [1,3,*]

[1] Department of Drug Discovery and Development, Harrison College of Pharmacy, Auburn University, Auburn, AL 36849 USA

[2] Program in Molecular Biotechnology, Department of Applied Biosciences and Process Engineering, Anhalt University of Applied Sciences, Köthen, Germany

[3] Cancer Biology and Immunology Research Program, O'Neal Comprehensive Cancer Center, Heersink College of Medicine, University of Alabama-Birmingham, Birmingham, AL 35233 USA

* Correspondence: djr0009@auburn.edu

Abstract

Uveal melanomas (UMs) metastasize at a high frequency, and metastatic UMs are associated with very poor clinical outcomes. Consequently, there is considerable interest in the genetics and biochemistry of UM and how these insights may lead to novel and effective approaches for treating UM. This work reviews evidence that elevated signaling by the CYSLTR2/Gaq/Ga11 pathway drives UM. This work focuses on the large number of signaling pathways that appear to be activated by the CYSLTR2/Gaq/Ga11 pathway in UM, including pathways not thought to be effectors of canonical CYSLTR2/Gaq/Ga11 signaling. This work reviews evidence suggesting that ERBB receptor tyrosine kinases may mediate CYSLTR2/Gaq/Ga11 signaling in UM, thereby accounting for the non-canonical CYSLTR2/Gaq/Ga11 signaling observed in UM. Thus, this work describes novel targets for UM therapy.

Keywords: uveal melanoma; targeted therapy; signal transduction; Galphaq; Galpha11; PI3K/AKT/mTOR; RAS-MAPK; GPCR-EGFR crosstalk; ERBB heterodimerization

1. Introduction to Uveal Melanoma

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, accounting for approximately 4-5% of all melanomas. Its worldwide incidence is reported to range from 4 to 7 cases per million individuals annually [1–13]. UM consists of any malignant lesion derived from melanocytes of the uveal tissues (choroid, ciliary body, and iris). UM most frequently arises in choroid tissues (90%) and less frequently in the ciliary body (6%) and iris (4%) [4,11,14–18].

UM typically presents in adulthood, with a peak incidence around the age of 60 [4,14,16]. The goals of UM treatment are to prevent metastatic spread and conserve the eye and vision [4,15,19]. As noted elsewhere in this review, the primary tumor treatment options are considered relatively effective. Nonetheless, the prognosis for UM patients is often poor, as UM frequently metastasizes to the liver, presumably before or during the treatment of the primary tumor. This challenge is exacerbated by the fact that effective targeted therapies for metastatic UM have yet to be discovered [4,6,11,13,17,18,20–31]. In fact, the 2025 NCCN guidelines indicate that the preferred therapy for metastatic UM is a clinical trial [4,32].

UM is more common in Caucasian populations, particularly at higher latitudes, and among individuals with light-colored skin and irises, suggesting that solar ultraviolet light exposure contributes to the pathogenesis of UM [2,6,7,17,33–39]. Thus, it is somewhat surprising that UM most frequently arises in choroid tissues, as these tissues are relatively protected from sunlight [11,40–42]. UM does not typically exhibit the same genetic changes as cutaneous skin melanoma (CM), which is also strongly associated with sunlight exposure [3,4,6,11,30,43–47]. Nonetheless, like CM, UM is

 doi:10.20944/preprints202604.0001.v1

more common among men than women [2,4,14,16]. Moreover, epidemiologic studies of CM predict that UM is more common among military service members and retirees than among civilians [48]. In this review, we propose that ERBB receptor signaling may act as a critical intermediate in CYSLTR2/G_{aq}/G_{a11}-driven oncogenic signaling in uveal melanoma.

2. Treatment of Uveal Melanoma

The typical treatment for primary UM includes radiotherapy (either plaque brachytherapy or external radiation therapy), photocoagulation, transpupillary thermotherapy, and various forms of tumor removal, such as transscleral resection, endoresection, and removal of the entire eye (enucleation). Thus, UM treatment is commonly associated with visual handicap or facial disfigurement [11,18,26,49]. The treatment of primary UM is typically effective, as relatively few patients experience recurrence at the primary tumor site. However, approximately 50% of UM patients eventually develop metastatic disease. Roughly 90% of metastatic melanomas target the liver, whereas the lung and bone are targeted to a lesser extent [3,4,9,11,13,21,26,27,30,50].

Metastatic UM appears to arise from hematogenous spread of micrometastases before or during treatment of the primary tumor; even enucleation or exenteration (removal of the eye and surrounding tissues) has little effect on the progression to metastatic disease [4,6,8,17–19,51–56]. Metastatic UM is typically fatal, and has a median overall survival of 4 to 15 months [3,4,8,9,11,18,24–27,30,56–61]. However, the median time to progression to metastatic UM is measured in years [18,20,21,29,62], as the mortality rate at 10-15 years after diagnosis of the primary tumor is approximately 40-50% [8,18,20,21,62]. The long latency of progression and mortality suggests that the latent micrometastases must acquire additional genetic changes (relative to the primary tumor) to become clinically relevant.

Circulating tumor DNA (ctDNA) is a biomarker of UM progression to metastatic disease [18,63–65]. Hence, strategies for targeting metastatic UM should be reserved for patients with ctDNA harboring alterations characteristic of UM with high metastatic potential [13,26,66,67], as the benefits of therapy are more likely to outweigh any adverse effects in these patients.

Unfortunately, as noted earlier, there is no effective standard treatment for metastatic UM [4,11,17,31,32]. Nonetheless, several advances in treatment paradigms have been made, particularly those aimed at reducing tumor cell immune evasion [4,13,17].

Immune checkpoint inhibitors (ICIs), including antibodies against PD-1 (e.g., pembrolizumab, nivolumab), PD-L1 (e.g., atezolizumab), and CTLA-4 (e.g., ipilimumab), are effective against CM and other tumors [4,68–86]. Single ICI agents exhibit limited therapeutic efficacy against metastatic UM. Combinations of ICIs have elicited surprisingly modest responses, presumably due to the relatively low mutational and neoantigen burden of these tumor cells, particularly those that harbor wild-type *MBD4* alleles [4,7,17,32,47,87–99]. Thus, future experimentation may identify those metastatic UM subtypes that exhibit more robust responses to combinations of ICIs.

Tumor-infiltrating lymphocyte (TIL) therapy has shown promising activity against metastatic UM [4,100]. However, this approach requires preparing TILs for each patient, which limits scalability and makes it relatively expensive [4,101]. Nonetheless, given that TILs appear to be more effective than ipilimumab against metastatic CM [101–103], further investigation of TIL therapy seems warranted, particularly if TIL therapy can overcome the limitation of ICI effectiveness in low-mutational burden tumors.

Uveal melanomas and CMs commonly overexpress gp100, a 100 kDa glycosylated transmembrane protein involved in melanosome maturation [104,105]. Moreover, gp100 is a target for anti-CM TILs and anti-CM vaccines [104,106–108]. Consequently, agents targeting gp100 have been extensively studied as potential anti-UM therapies.

Anti-GP100 agents include tebentafusp, an ImmTAC (immunomobilizing monoclonal T-cell receptor) targeting cancer. Tebentafusp consists of a modified T cell receptor (TCR) that recognizes a gp100 peptide in the context of a specific human leukocyte antigen (HLA-A*0201). This modified TCR is fused to a single-chain antibody against CD3, a molecule expressed by T lymphocytes that

 doi:10.20944/preprints202604.0001.v1

plays a crucial role in their activation. Thus, tebentafusp binds tumor cells that present the gp100 peptide in the context of HLA-A*0201; tebentafusp then recruits and activates T lymphocytes, ultimately resulting in the formation of a "lytic immune synapse" of the target tumor cell and the cytotoxic T lymphocyte [4,104,109].

Some of the earliest data emerging from the pivotal tebentafusp phase III clinical trial (NCT03070392 [110]) reported that HLA-A*0201 metastatic UM patients treated with tebentafusp exhibited 73% overall survival at 1 year, whereas control patients treated with the investigators' choice of therapy (pembrolizumab, dacarbazine, or ipilimumab) showed 59% overall survival at 1 year. Likewise, progression-free survival at 6 months was higher in the tebentafusp group (31%) than in the control group (19%). Adverse cytokine-mediated or skin-related events occurred infrequently (2%) and led to trial discontinuation [109]. Thus, in January 2022, tebentafusp was granted Breakthrough Therapy designation by the United States Food and Drug Administration [7,11,17,30,47,111–113].

Additional data from NCT03070392 indicated that the median overall survival of HLA-A*0201 metastatic UM patients was 21.6 months in the tebentafusp group and 16.9 months in the control group (0.68 hazard ratio [95% confidence interval of 0.54 – 0.87]). The three-year survival was 27% in the tebentafusp group and 18% in the control group. By approximately 64 months, all control patients had died, while approximately 15% of the patients in the tebentafusp group were alive [110,112]. This modest effect on survival highlights the need for additional innovation in metastatic UM therapy, particularly since only approximately 50% of Caucasians and fewer Hispanics and African-Americans are HLA-A*0201 positive [4,17,18,104,113–117].

Most CMs harbor mutations in genes (e.g., *BRAF*, *NF1*, *NRAS*) that encode proteins involved in the RAF/RAS/MAPK signaling pathway, leading to increased signaling through this pathway. These molecular insights have driven the use of BRAF and MEK inhibitors to treat *BRAF*-mutant CMs [70,78,83–86,118–122]. The most commonly mutated genes in UMs are *GNAQ*, *GNA11*, and *BAP1*. Unfortunately, these insights have not yet yielded strategies to prevent or treat metastatic UMs [6,7,11,17,18,30,32]. Thus, in the next section, we will discuss how molecular characterization of UMs has revealed potential future strategies for preventing or treating metastatic UMs.

3. CYSLTR2/Gαq/Gα$_{11}$ Signaling in UM

A. CYSTLR2, G$_{aq}$, and G$_{a11}$

CYSLTR2 encodes cysteinyl-leukotriene receptor 2, a G protein-coupled receptor that signals through heterotrimeric G proteins containing G$_{aq}$ or G$_{a11}$ subunits (Figure 1) [123–125]. The most common genetic alterations in UM involve mutations in the *GNAQ* or *GNA11* genes [4,6,11,13,18], resulting in constitutive canonical GPCR/G$_{aq}$/G$_{a11}$ signaling through loss of G$_{aq}$ or G$_{a11}$ GTPase activity [13]. Likewise, mutations in *CYSLTR2* that stimulate G$_{aq}$ or G$_{a11}$ signaling appear to drive UM [4,6,11,13,30,126,127].

B. PLCb4

Phospholipase C beta 4 (PLCb4) cleaves the membrane lipid phosphatidylinositol 4,5 bisphosphate (PIP2), producing inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). G$_{\alpha q}$ and G$_{\alpha 11}$ directly stimulate PLCβ4 [128]. This suggests that PLCb4 may mediate CYSLTR2/G$_{aq}$/G$_{a11}$ signaling in UM. Indeed, activating mutations in *PLCB4* are found in UM [3,4,6,11,129,130]. Activating mutations in *GNAQ*, *GNA11*, *CYSLTR2*, and *PLCB4* are mutually exclusive [4,13,30], suggesting that these genes encode components of a single signaling pathway that drives UM by activating RAS-MAPK and PKC signaling [11,131,132].

 doi:10.20944/preprints202604.0001.v1

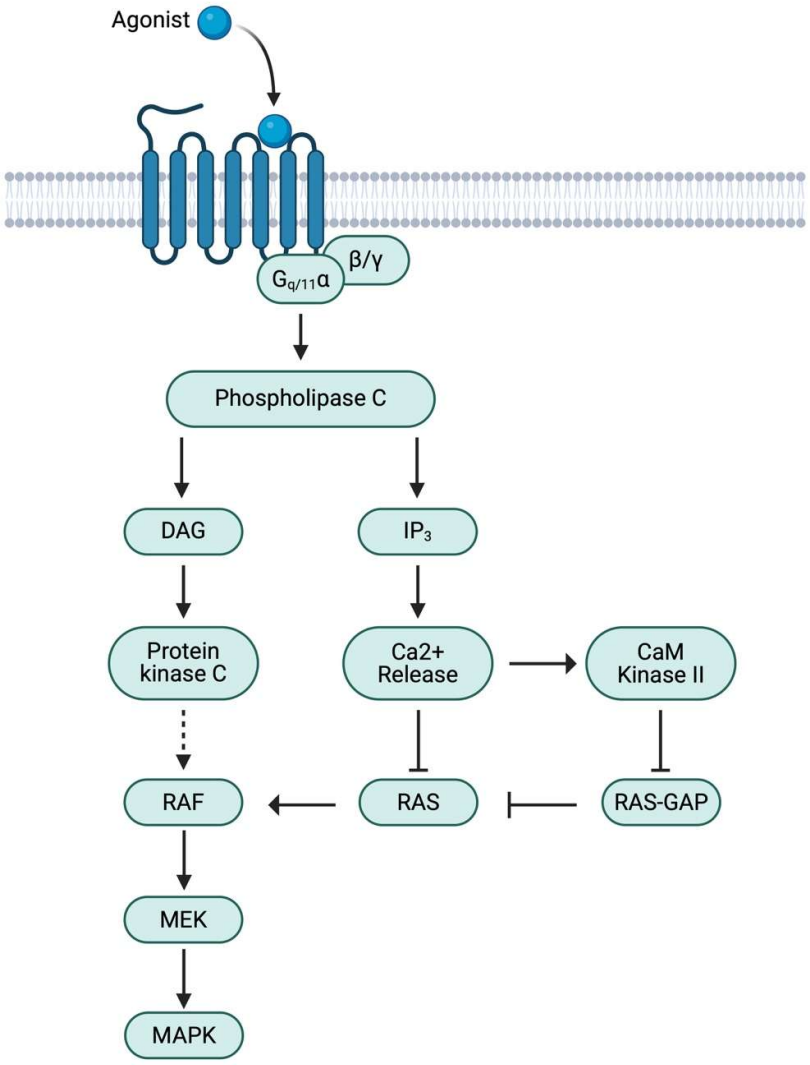

Figure 1. GPCR/Gaq/Ga11 Coupling to the RAS/MAPK Pathway.

Taken from [125].

C. RAS-MAPK

The RAS-MAPK (mitogen-activated protein kinase) signaling pathway is a major regulator of cellular proliferation. This pathway is commonly activated in UM by CYSLTR2/Gαq/Gα11/PLCβ signaling (Figure 1) [11,13,125,131,133,134]; one mechanism may involve the phosphorylation of Thr133 in the RAS guanine nucleotide exchange factor RasGRP3, leading to GTP loading onto all three RAS isoforms. PKC may be responsible for this phosphorylation; however, the cited work did not demonstrate that PKC directly phosphorylates RasGRP3 [13,134]. Another mechanism may involve IP3-stimulated Ca^{2+} release, which activates CaM kinase II. CaM kinase II phosphorylates and inhibits the activity of the RAS GTPase-activating protein (RAS-GAP), resulting in decreased GTP hydrolysis by RAS and increased RAS signaling activity [124]. *MAPKAPK5*, which encodes the MAPK-activated protein kinase 5, is mutated in approximately 2% of UM cases. These mutations support the hypothesis that the RAS-MAPK pathway contributes to UM. However, frameshift mutations are most common among the predominant *MAPKAPK5* alterations in UM, suggesting that MAPKAPK5 may negatively regulate signaling by the RAS-MAPK pathway in UM [11,66].

 doi:10.20944/preprints202604.0001.v1

D. PKC

Protein kinase C (PKC) is a serine-threonine kinase, and DAG and Ca^{2+} activate many PKC isoforms. Thus, PKC signaling is elevated in UM, presumably due to elevated CYSLTR2/G_{aq}/G_{a11}/PLCb4 signaling [30]. PKC inhibitors have exhibited only modest effects in UM [30,135–137], suggesting that additional signaling pathways are activated in these tumors. Indeed, the combination of a PKC inhibitor and a MEK inhibitor exhibited greater effects than either drug alone in a preclinical model system [30,138]. Moreover, these data suggest that the MEK pathway is stimulated independently of PKC.

E. PI3K/AKT/mTOR

Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase that phosphorylates the membrane phospholipid phosphatidylinositol (4,5) bisphosphate (PIP2), yielding phosphatidylinositol (3,4,5) trisphosphate (PIP3). PIP3 recruits Protein Kinase B (PKB/AKT) to the membrane, enabling AKT phosphorylation (at Thr308 and Ser473) and signaling activity. PIP3 also stimulates signaling by PDK1 (phosphoinositide-dependent kinase-1), which phosphorylates AKT at Thr308. Ser473 of AKT is phosphorylated by a multiprotein complex (mTORC2) that features mTOR (mammalian target of rapamycin). The canonical PI3K/AKT/mTOR signaling pathway ultimately regulates the biochemical activity of GSK-3b (glycogen synthase kinase 3-beta), FOXOs (forkhead box O proteins), TSC1/2 (tuberous sclerosis complex 1/2 proteins), S6K (ribosomal S6 kinase), and other effectors [11,139].

Elevated signaling by the PI3K/AKT/mTOR pathway is a characteristic of UMs that exhibit elevated CYSLTR2/G_{aq}/G_{a11} signaling [11,30,140]. For example, the *GNAQ*-mutant Mel270 and 92-1 uveal melanoma cell lines exhibit elevated PI3K/AKT/mTOR signaling, as evidenced by phosphorylation of AKT and GSK3. Likewise, the PI3K inhibitor LY294002 inhibits the proliferation of the Mel270 and 92-1 cell lines. However, LY294002 does not inhibit ERK1/2 phosphorylation in these cell lines, suggesting that the PI3K/AKT/mTOR and MAPK signaling pathways independently drive UM proliferation. Indeed, combinations of PI3K and MEK inhibitors are more effective in inhibiting the proliferation of UM cell lines than either class of inhibitor alone [18,133,141]. However, the combination of the MEK inhibitor trametinib and the AKT inhibitor GSK2141795 (GSK795) did not significantly improve the survival of metastatic UM patients [18,142–144]. Additional signaling events may contribute to UM proliferation. The discrepancy may also be due to tumor heterogeneity and adaptive resistance mechanisms *in vivo*.

F. Hippo/YAP

The Yes-Associated Protein (YAP) is a component of transcription factor complexes that include the TAZ (Transcriptional co-Activator with PDZ-binding motif) protein and TEAD transcription factors. These complexes regulate cellular homeostasis in part by preventing abnormal cell proliferation. YAP/TAZ/TEAD complexes are regulated by the Hippo pathway, also known as the Salvador/Warts/Hippo (SWH) pathway. This pathway was initially discovered in *D. Melanogaster* and named after one of its major components, the Hippo protein kinase. However, this pathway exhibits substantial evolutionary conservation through mammals. Additional components downstream of Hippo include the mammalian STE20-like protein kinases (MST1/2) and the large tumor suppressor kinases 1/2 (LATS1/2) [145,146].

The Hippo/YAP pathway can be regulated by a variety of intracellular and extracellular signals, including GPCR- and receptor tyrosine kinase-mediated signaling. Hippo signaling initiates the MST/LATS kinase cascade, resulting in the phosphorylation of YAP/TAZ. Phosphorylated YAP/TAZ is retained in the cytoplasm and is degraded. Conversely, low levels of Hippo signaling cause reduced YAP/TAZ phosphorylation. Unphosphorylated YAP/TAZ translocates to the nucleus, driving cell proliferation [145,146]. The overexpression of YAP/TAZ is associated with poor clinical outcomes in breast, colorectal, and liver cancers [146].

Activating mutations in *GNAQ* or *GNA11* are associated with the dephosphorylation of YAP and TAZ in UM cells. Verteporfin, a drug that disrupts YAP/TEAD interactions, inhibits the proliferation of *GNAQ*/*GNA11*-mutant UM cells [30,147]. Focal adhesion kinase (FAK) stimulates

 doi:10.20944/preprints202604.0001.v1

YAP dephosphorylation via canonical and non-canonical pathways. Likewise, genetic ablation or pharmacological inhibition of FAK reduces proliferation in a *GNAQ*-mutant UM cell line [30,148]. Moreover, due to compensatory signaling, simultaneous inhibition of the RAS-MAPK and FAK/Hippo/YAP pathways may yield more favorable clinical responses than inhibiting either pathway individually [30,149].

G. JAK/STAT

Signal Transducers and Activators of Transcription (STAT proteins) are a family of transcription factors that regulate gene expression. Like many transcription factors, they reside in the cytoplasm and nucleus. The translocation of STAT proteins into the nucleus is required for them to function as transcription factors. STAT dimerization and translocation to the nucleus are commonly induced by STAT phosphorylation by members of the Janus kinase (JAK) family of non-receptor tyrosine kinases. Cytokine receptors and receptor tyrosine kinases are typically upstream stimuli of the JAK-STAT signaling pathway. Some STAT proteins stimulate the transcription of genes that encode proteins that inhibit apoptosis or stimulate cell proliferation, including Bcl-xL, cyclin D1, and c-myc. Likewise, elevated activity of a JAK/STAT pathway drives numerous cancers [150–153], and increased signaling by STAT3 [6,11,18,27,46] and STAT5 [11] is observed in UM. Because GPCR signaling is not typically directly coupled to JAK/STAT signaling, GPCR-ERBB receptor crosstalk may be responsible for the elevated JAK-STAT signaling observed in UM.

4. Could GPCR-ERBB Receptor Crosstalk Contribute to UM Tumorigenesis?

A. Overview of GPCR-ERBB Receptor Crosstalk

Epidermal growth factor receptor (EGFR/ERBB1) is a receptor tyrosine kinase (RTK) that is closely related to three other ERBB receptor tyrosine kinases (ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4). Crosstalk between GPCRs and ERBB receptors has been observed in numerous contexts and appears to occur via multiple mechanisms [128,154–159]. (a) Some GPCRs stimulate the activity of the Src non-receptor tyrosine kinase. Src directly phosphorylates EGFR tyrosine residues, thereby coupling EGFR to downstream signaling effectors [128,154–163]. (b) Some GPCRs complex with EGFR and stimulate EGFR tyrosine phosphorylation, presumably by stabilizing EGFR dimerization [154,155,164–166]. (c) A well-explored mechanism of GPCR-ERBB receptor crosstalk (Figure 2) appears to involve Src stimulation of the expression or activity of a matrix metalloproteinase (MMP) or an ADAM (a disintegrin and metalloproteinase). The metalloproteinase cleaves the transmembrane precursor form of an epidermal growth factor (EGF) family peptide hormone, releasing the mature hormone into the extracellular milieu. This enables ligand binding to ERBB receptors, and ligand-induced ERBB receptor dimerization and signaling [128,154–159,163,167–175]. (d) Stimulation of ERBB ligand maturation by MMP/ADAM activity by GPCRs may also be mediated by reactive oxygen species [170,172]. Direct evidence of CYSLTR2 and EGFR crosstalk has been observed in primary human bronchial fibroblasts, as the CYSLTR2 agonist leukotriene C4 (LTC_4) potentiates the mitogenic effect of EGF in these cells [176]. However, the first-generation EGFR tyrosine kinase inhibitor gefitinib failed to yield a significant benefit in a small number (n = 6) of unselected patients with metastatic choroidal melanoma [13,177]. Of course, this negative result may be due to the small sample size and a lack of biomarker-based patient selection. Moreover, EGFR inhibition alone may not adequately target ERBB family receptor signaling.

 doi:10.20944/preprints202604.0001.v1

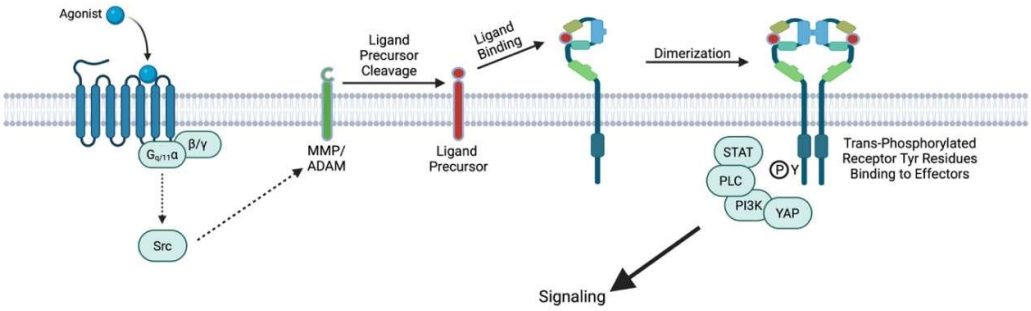

Figure 2. GPCR-EGFR Crosstalk.

Taken from [178].

B. Mechanism of Ligand-Dependent ERBB Receptor Signaling

Ligands for ERBB receptors include amphiregulin, betacellulin, EGF, epigen, epiregulin, heparin-binding EGF-like growth factor, neuregulin isoforms, and transforming growth factor alpha. As noted earlier, ERBB ligands are initially expressed as transmembrane precursor proteins that must be cleaved to enable the soluble form of the ligand to bind the extracellular region of the cognate receptor. Ligand binding stabilizes the ERBB receptor extracellular domains in a conformation that is competent for symmetrical dimerization of the ERBB receptor extracellular domain. Because a small fraction of EGFR that is present on the cell surface exists in the conformation that is competent for dimerization, overexpression of EGFR (and other ERBB RTKs) can cause ligand-independent symmetrical dimerization of the receptor extracellular domain. In both ligand-dependent and -independent signaling, this symmetrical dimerization of the ERBB extracellular domains is accompanied by asymmetrical dimerization of the ERBB intracellular domains. Consequently, within an ERBB receptor dimer, the kinase domain of one ERBB monomer phosphorylates tyrosine residues of the other ERBB monomer. Therefore, mechanistically, ERBB receptors do not undergo autophosphorylation; instead, transphosphorylation occurs within the dimer. ERBB receptors contain multiple tyrosine phosphorylation sites, each of which can bind distinct effector proteins via their SH2 or PTB domains. Thus, ERBB receptor phosphorylation stimulates numerous signaling pathways and biological responses, and differences in the sites of tyrosine phosphorylation for a given ERBB family receptor enable signaling specificity. [179–185].

C. Specificity of Ligand-Induced ERBB Receptor Signaling

This signaling specificity appears to be regulated by the juxtaposition of ERBB monomers within the dimer; the specificity of ERBB dimer conformations is likely to underlie many of the functional differences among the various ligands for a particular ERBB receptor [186–195]. Specificity also arises through heterodimerization by ERBB family receptors. This heterodimerization enables ligand-dependent signaling by ERBB2, which does not bind any EGF family member; heterodimerization also facilitates signaling by ERBB3, which possesses little tyrosine kinase activity. Hence, ligand-binding to EGFR, ERBB3, or ERBB4 may also elicit signaling by the other three ERBB receptors. Heterodimerization greatly diversifies ERBB receptor signaling, as the four ERBB receptors do not activate identical downstream signaling events. For example, EGFR and ERBB2 phosphorylation sites couple to the RAS/MAPK pathway via GRB2 and SOS [181,183,184,194]. Phosphorylated ERBB3 and ERBB4 each possess at least one PI3K binding site, whereas phosphorylated ERBB4 also possesses binding sites for YAP, STAT5a, STAT1, and PLCg [179,181,196–201].

One consequence of this specificity is that receptor homodimers and heterodimers may exhibit distinct signaling properties and may stimulate distinct biological effects. For example, ERBB4 homodimers commonly function as tumor suppressors by stimulating apoptosis or growth arrest.

 doi:10.20944/preprints202604.0001.v1

In contrast, ERBB4-EGFR or ERBB4-ERBB2 heterodimers function as oncoproteins by stimulating cell proliferation and suppressing apoptosis [179,200,202–204]. The mechanism of this specificity is unclear, although context-specific sites of ERBB receptor tyrosine phosphorylation confer other forms of ERBB receptor signaling specificity [179,186–195,205–208].

D. Non-Canonical Mechanisms of ERBB4 Signaling

ERBB4 exhibits signaling mechanisms distinct from those of canonical RTK signaling. Following ligand binding to ERBB4, the transmembrane metalloprotease tumor necrosis factor alpha (TNFa) converting enzyme (TACE) cleaves the ERBB4 extracellular domain near the transmembrane domain. This releases the 120 kDa extracellular domain into the extracellular milieu, where it can function as a ligand sink [179,209–218]. Then, the gamma-secretase complex cleaves the ERBB4 cytoplasmic domain near the transmembrane domain. This releases the 80 kDa cytoplasmic region of ERBB4 (4ICD) from the plasma membrane [179,211–214,216,219–223].

The cytoplasmic region of ERBB4 contains a Bcl2 homology 3 (BH3) domain, which can bind pro- or anti-apoptotic proteins of the BAX/BAK or BCL-2 families. Homotypic 4ICD signaling triggers apoptosis by stimulating BAK oligomerization within the outer mitochondrial membrane (OMM). This may occur via 4ICD binding to BAK or through binding and sequestration of BCL-XL or other anti-apoptotic proteins [179,224,225]. BAK oligomerization triggers OMM permeability, resulting in increased cytochrome C efflux and caspase activation [179,217,223,226–228]. Signaling by ERBB4-EGFR or ERBB4-ERBB2 heterodimers suppresses 4ICD-induced apoptosis by an unknown mechanism.

The cytoplasmic region of ERBB4 also contains a nuclear localization signal [179,212,214,220,229,230] and two LXXLL motifs, which mediate interactions with nuclear (steroid) hormone receptors [179,231,232]. Homotypic 4ICD signaling regulates estrogen receptor alpha signaling and increases the sensitivity of breast cancer cells to tamoxifen [179,217,233–237]. The 4ICD apparently regulates the function of other transcriptional regulatory proteins, including YAP1 [179,199,217,223,234,235,238–240], STAT5a [199,217,229,235], ETO2 [217,221,234,235], TAB2-NCoR [216,217,234,235], and AP-2 [234,235,241].

E. Does Elevated ERBB4 Signaling Create Targets for Therapeutic Intervention in UM?

Given the diversity of ERBB receptor signaling, including non-canonical ERBB4 signaling, we postulate that crosstalk between the CYSLTR2/Gαq/Gα11 and ERBB receptors could be responsible for many of the changes in downstream signaling events implicated in UM, including changes in signaling by pro- and anti-apoptotic proteins [242–244], steroid hormone receptors [245–247], YAP [30,147–149], and STATs [6,11,18,27,46]. Nonetheless, we postulate that the CYSLTR2/Gαq/Gα11 pathway stimulates the RAS-MAPK pathway (and perhaps the PI3K/AKT/mTOR pathway, due to crosstalk with the RAS-MAPK pathway) independent of ERBB receptors. Therefore, we postulate that ERBB receptor signaling cooperates with the RAS-MAPK pathway (and perhaps the PI3K/AKT/mTOR) to drive UM cell proliferation, and that targeting ERBB receptors in combination with the RAS-MAPK pathway is likely to yield greater therapeutic effects than targeting either pathway alone.

This proposed mechanism resembles that for human skin cutaneous melanomas with wild-type *BRAF* alleles ("*BRAF*-WT melanomas"). In such tumors, *ERBB4* transcription or an *ERBB4* gain-of-function mutant allele appears to segregate with a gain-of-function *RAS* allele or a loss-of-function *NF1* allele. Thus, ERBB4 signaling appears to cooperate with the RAS-MAPK pathway to drive *BRAF*-WT melanomas [248,249]. Our lab is working to identify actionable targets within the ERBB4 signaling pathway in *ERBB4*-dependent *BRAF*-WT melanoma cell lines. For example, our preliminary observation that *ERBB2* is both sufficient and necessary for the proliferation of three *ERBB4*-dependent, *BRAF*-WT melanoma cell lines (IPC-298, MEL-JUSO, and MeWo) suggests that targeting signaling by ERBB4-ERBB2 heterodimers may be effective in some BRAF-WT melanomas [250].

Finally, four *ERBB4*-dependent BRAF-WT melanoma cell lines (IPC-298, MEL-JUSO, MeWo, and SK-MEL-2) harbor apparent driver mutations in *GNA11* or *PLCB4* [251]. These findings suggest

 doi:10.20944/preprints202604.0001.v1

that these cell lines display elevated expression of mature (soluble) ERBB receptor ligands, leading to increased signaling by ERBB4 heterodimers. Moreover, because *GNA11* or *PLCB4* driver mutations are found in many UMs, UMs may also depend on ERBB receptors, and insights into targeting *ERBB4*-dependent *BRAF*-WT melanomas may apply to UMs. Appropriate cell culture models of UM exist for such experiments [252–254].

5. Conclusions

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, annually accounting for 4-7 cases per million individuals worldwide. The typical treatment for primary UM includes radiotherapy, photocoagulation, thermotherapy, and surgery. These primary treatment options are, in some respects, quite effective, as the median time to progression to metastatic UM is measured in years. However, metastatic UM is relatively lethal, as the mortality rate at 10-15 years after diagnosis of the primary tumor is approximately 40-50%. This mortality is in part due to the absence of an effective standard treatment for metastatic melanoma.

Thus, there has been much interest in the molecular characterization of UM. As noted elsewhere, mutations in the CYSTLR2/G_{aq}/G_{a11}/PLCb4 GPCR signaling pathway appear to drive a significant percentage of UM. Hence, canonical effectors of this pathway have been implicated in UM, including PKC, the RAS/MAPK pathway, the PI3K/AKT/mTOR pathway, the Hippo/YAP pathway, and the JAK/STAT pathway. However, therapies targeting this extensive set of pathways are unlikely to be practical in UM treatment, particularly for preventing progression to metastatic UM (mUM).

We propose that ERBB receptor signaling lies upstream of many of these pathways and that targeting ERBB receptor signaling may be effective against UM by simultaneously blocking these pathways. This hypothesis is supported by the fact that the G_{aq}/G_{a11} signaling pathway can stimulate ERBB receptor signaling. Several mechanisms have been proposed for this crosstalk. However, one of the most common mechanisms involves Src-dependent stimulation of MMPs/ADAMs, which cleave the precursor, transmembrane forms of EGF family peptide hormones. The release of soluble, mature forms of EGF family hormones enables them to bind to ERBB receptors and stimulate receptor signaling. Receptor signaling may feature both ERBB receptor homodimerization and heterodimerization. Signaling by receptor heterodimers may be particularly important, as they enable more complex downstream signaling than receptor homodimers. Experimental data support the proposed G_{aq}/G_{a11}-ERBB crosstalk in UM. In human bronchial fibroblasts, the CYSLTR2 agonist LTC4 potentiates the mitogenic effect of EGF. Four *ERBB4*-dependent BRAF-WT melanoma cell lines possess apparent driver mutations in *GNA11* or *PLCB4*, suggesting that the elevated ERBB4 signaling in these *BRAF*-WT melanoma cell lines is due to increased maturation of soluble EGF family hormones. Human UM cell lines that possess driver mutations in *GNA11* or *GNAQ* could be used to test the hypothesis that these cell lines are ERBB-dependent.

Author Contributions: Conceptualization: D.J.R.; Writing – Original Draft Preparation: N.R. and D.J.R.; Writing – Review and Editing: N.R. and D.J.R.; Supervision – D.J.R. Funding Acquisition – D.J.R.

Funding: This work has been supported by the United States National Institutes of Health (R15CA280767).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: D.J.R. has been a consultant for Bristol-Myers Squibb, Eli Lilly and Company, and ImClone Systems. D.J.R. is no longer affiliated with these organizations. Thus, this work does not represent a financial conflict of interest.

 doi:10.20944/preprints202604.0001.v1

References

1. McLaughlin, C.C.; Wu, X.C.; Jemal, A.; Martin, H.J.; Roche, L.M.; Chen, V.W. Incidence of noncutaneous melanomas in the U.S. *Cancer* **2005**, *103*, 1000–1007, doi:10.1002/cncr.20866.
2. Singh, A.D.; Turell, M.E.; Topham, A.K. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology* **2011**, *118*, 1881–1885, doi:10.1016/j.ophtha.2011.01.040.
3. Kaliki, S.; Shields, C.L. Uveal melanoma: relatively rare but deadly cancer. *Eye (Lond)* **2017**, *31*, 241–257, doi:10.1038/eye.2016.275.
4. Damato, B.E.; Dukes, J.; Goodall, H.; Carvajal, R.D. Tebentafusp: T Cell Redirection for the Treatment of Metastatic Uveal Melanoma. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11070971.
5. Piperno-Neumann, S.; Piulats, J.M.; Goebeler, M.; Galloway, I.; Lugowska, I.; Becker, J.C.; Vihinen, P.; Van Calster, J.; Hadjistilianou, T.; Proenca, R.; et al. Uveal Melanoma: A European Network to Face the Many Challenges of a Rare Cancer. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11060817.
6. Sorrentino, F.S.; Culiersi, C.; Florido, A.; De Nadai, K.; Adamo, G.G.; Nasini, F.; Vivarelli, C.; Mura, M.; Parmeggiani, F. Genetic Features of Uveal Melanoma. *Genes (Basel)* **2024**, *15*, doi:10.3390/genes15111356.
7. Synoradzki, K.J.; Paduszynska, N.; Solnik, M.; Toro, M.D.; Bilmin, K.; Bylina, E.; Rutkowski, P.; Yousef, Y.A.; Bucolo, C.; Zweifel, S.A.; et al. From Molecular Biology to Novel Immunotherapies and Nanomedicine in Uveal Melanoma. *Curr Oncol* **2024**, *31*, 778–800, doi:10.3390/curroncol31020058.
8. Harbour, J.W. A prognostic test to predict the risk of metastasis in uveal melanoma based on a 15-gene expression profile. *Methods Mol Biol* **2014**, *1102*, 427–440, doi:10.1007/978-1-62703-727-3_22.
9. Onken, M.D.; Worley, L.A.; Ehlers, J.P.; Harbour, J.W. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res* **2004**, *64*, 7205–7209, doi:10.1158/0008-5472.CAN-04-1750.
10. Egan, K.M.; Seddon, J.M.; Glynn, R.J.; Gragoudas, E.S.; Albert, D.M. Epidemiologic aspects of uveal melanoma. *Surv Ophthalmol* **1988**, *32*, 239–251, doi:10.1016/0039-6257(88)90173-7.
11. Fuentes-Rodriguez, A.; Mitchell, A.; Guerin, S.L.; Landreville, S. Recent Advances in Molecular and Genetic Research on Uveal Melanoma. *Cells* **2024**, *13*, doi:10.3390/cells13121023.
12. Singh, S.R.K.; Malapati, S.J.; Kumar, R.; Willner, C.; Wang, D. NCDB Analysis of Melanoma 2004-2015: Epidemiology and Outcomes by Subtype, Sociodemographic Factors Impacting Clinical Presentation, and Real-World Survival Benefit of Immunotherapy Approval. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13061455.
13. Carvajal, R.D.; Sacco, J.J.; Jager, M.J.; Eschelman, D.J.; Olofsson Bagge, R.; Harbour, J.W.; Chieng, N.D.; Patel, S.P.; Joshua, A.M.; Piperno-Neumann, S. Advances in the clinical management of uveal melanoma. *Nat Rev Clin Oncol* **2023**, *20*, 99–115, doi:10.1038/s41571-022-00714-1.
14. Damato, B.E.; Coupland, S.E. Differences in uveal melanomas between men and women from the British Isles. *Eye (Lond)* **2012**, *26*, 292–299, doi:10.1038/eye.2011.272.
15. Damato, B. Progress in the management of patients with uveal melanoma. The 2012 Ashton Lecture. *Eye (Lond)* **2012**, *26*, 1157–1172, doi:10.1038/eye.2012.126.
16. Krantz, B.A.; Dave, N.; Komatsubara, K.M.; Marr, B.P.; Carvajal, R.D. Uveal melanoma: epidemiology, etiology, and treatment of primary disease. *Clin Ophthalmol* **2017**, *11*, 279–289, doi:10.2147/OPTH.S89591.
17. Gelmi, M.C.; Jager, M.J. Uveal melanoma: Current evidence on prognosis, treatment and potential developments. *Asia Pac J Ophthalmol (Phila)* **2024**, *13*, 100060, doi:10.1016/j.apjo.2024.100060.
18. Kulbay, M.; Marcotte, E.; Remtulla, R.; Lau, T.H.A.; Paez-Escamilla, M.; Wu, K.Y.; Burnier, M.N., Jr. Uveal Melanoma: Comprehensive Review of Its Pathophysiology, Diagnosis, Treatment, and Future Perspectives. *Biomedicines* **2024**, *12*, doi:10.3390/biomedicines12081758.
19. Damato, B. Ocular treatment of choroidal melanoma in relation to the prevention of metastatic death - A personal view. *Prog Retin Eye Res* **2018**, *66*, 187–199, doi:10.1016/j.preteyeres.2018.03.004.
20. Jensen, O.A. Malignant melanomas of the human uvea: 25-year follow-up of cases in Denmark, 1943--1952. *Acta Ophthalmol (Copenh)* **1982**, *60*, 161–182, doi:10.1111/j.1755-3768.1982.tb08371.x.
21. Kujala, E.; Makitie, T.; Kivela, T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci* **2003**, *44*, 4651–4659, doi:10.1167/iovs.03-0538.

 doi:10.20944/preprints202604.0001.v1

22. Carvajal, R.D.; Schwartz, G.K.; Tezel, T.; Marr, B.; Francis, J.H.; Nathan, P.D. Metastatic disease from uveal melanoma: treatment options and future prospects. *Br J Ophthalmol* **2017**, *101*, 38–44, doi:10.1136/bjophthalmol-2016-309034.
23. Aronow, M.E.; Topham, A.K.; Singh, A.D. Uveal Melanoma: 5-Year Update on Incidence, Treatment, and Survival (SEER 1973-2013). *Ocul Oncol Pathol* **2018**, *4*, 145–151, doi:10.1159/000480640.
24. Khoja, L.; Atenafu, E.G.; Suciu, S.; Leyvraz, S.; Sato, T.; Marshall, E.; Keilholz, U.; Zimmer, L.; Patel, S.P.; Piperno-Neumann, S.; et al. Meta-analysis in metastatic uveal melanoma to determine progression free and overall survival benchmarks: an international rare cancers initiative (IRCI) ocular melanoma study. *Ann Oncol* **2019**, *30*, 1370–1380, doi:10.1093/annonc/mdz176.
25. Rantala, E.S.; Hernberg, M.; Kivela, T.T. Overall survival after treatment for metastatic uveal melanoma: a systematic review and meta-analysis. *Melanoma Res* **2019**, *29*, 561–568, doi:10.1097/CMR.0000000000000575.
26. Jager, M.J.; Shields, C.L.; Cebulla, C.M.; Abdel-Rahman, M.H.; Grossniklaus, H.E.; Stern, M.H.; Carvajal, R.D.; Belfort, R.N.; Jia, R.; Shields, J.A.; et al. Uveal melanoma. *Nat Rev Dis Primers* **2020**, *6*, 24, doi:10.1038/s41572-020-0158-0.
27. Bustamante, P.; Piquet, L.; Landreville, S.; Burnier, J.V. Uveal melanoma pathobiology: Metastasis to the liver. *Semin Cancer Biol* **2021**, *71*, 65–85, doi:10.1016/j.semcancer.2020.05.003.
28. Roelofsen, C.D.M.; Wierenga, A.P.A.; van Duinen, S.; Verdijk, R.M.; Bleeker, J.; Marinkovic, M.; Luyten, G.P.M.; Jager, M.J. Five Decades of Enucleations for Uveal Melanoma in One Center: More Tumors with High Risk Factors, No Improvement in Survival over Time. *Ocul Oncol Pathol* **2021**, *7*, 133–141, doi:10.1159/000509918.
29. Stalhammar, G. Comprehensive causes of death in uveal melanoma: mortality in 1530 consecutively diagnosed patients followed until death. *JNCI Cancer Spectr* **2023**, *7*, doi:10.1093/jncics/pkad097.
30. Liu, X.L.; Run-Hua, Z.; Pan, J.X.; Li, Z.J.; Yu, L.; Li, Y.L. Emerging therapeutic strategies for metastatic uveal melanoma: Targeting driver mutations. *Pigment Cell Melanoma Res* **2024**, *37*, 411–425, doi:10.1111/pcmr.13161.
31. van Gils, W.; Lodder, E.M.; Mensink, H.W.; Kilic, E.; Naus, N.C.; Bruggenwirth, H.T.; van Ijcken, W.; Paridaens, D.; Luyten, G.P.; de Klein, A. Gene expression profiling in uveal melanoma: two regions on 3p related to prognosis. *Invest Ophthalmol Vis Sci* **2008**, *49*, 4254–4262, doi:10.1167/iovs.08-2033.
32. NCCN Guidelines: Melanoma: Uveal, Version 1.2025. Available online: https://www.nccn.org/professionals/physician_gls/pdf/uveal.pdf (accessed on July 23, 2025).
33. Weis, E.; Shah, C.P.; Lajous, M.; Shields, J.A.; Shields, C.L. The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch Ophthalmol* **2006**, *124*, 54–60, doi:10.1001/archopht.124.1.54.
34. Virgili, G.; Gatta, G.; Ciccolallo, L.; Capocaccia, R.; Biggeri, A.; Crocetti, E.; Lutz, J.M.; Paci, E.; Group, E.W. Incidence of uveal melanoma in Europe. *Ophthalmology* **2007**, *114*, 2309–2315, doi:10.1016/j.ophtha.2007.01.032.
35. Shields, C.L.; Kaliki, S.; Cohen, M.N.; Shields, P.W.; Furuta, M.; Shields, J.A. Prognosis of uveal melanoma based on race in 8100 patients: The 2015 Doyne Lecture. *Eye (Lond)* **2015**, *29*, 1027–1035, doi:10.1038/eye.2015.51.
36. Houtzagers, L.E.; Wierenga, A.P.A.; Ruys, A.A.M.; Luyten, G.P.M.; Jager, M.J. Iris Colour and the Risk of Developing Uveal Melanoma. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21197172.
37. Wu, M.; Yavuzyigitoglu, S.; Brosens, E.; Ramdas, W.D.; Kilic, E.; Rotterdam Ocular Melanoma Study, G. Worldwide Incidence of Ocular Melanoma and Correlation With Pigmentation-Related Risk Factors. *Invest Ophthalmol Vis Sci* **2023**, *64*, 45, doi:10.1167/iovs.64.13.45.
38. Schmidt-Pokrzywniak, A.; Jockel, K.H.; Bornfeld, N.; Sauerwein, W.; Stang, A. Positive interaction between light iris color and ultraviolet radiation in relation to the risk of uveal melanoma: a case-control study. *Ophthalmology* **2009**, *116*, 340–348, doi:10.1016/j.ophtha.2008.09.040.
39. Holly, E.A.; Aston, D.A.; Char, D.H.; Kristiansen, J.J.; Ahn, D.K. Uveal melanoma in relation to ultraviolet light exposure and host factors. *Cancer Res* **1990**, *50*, 5773–5777.

 doi:10.20944/preprints202604.0001.v1

40. Lawrence, M.S.; Stojanov, P.; Polak, P.; Kryukov, G.V.; Cibulskis, K.; Sivachenko, A.; Carter, S.L.; Stewart, C.; Mermel, C.H.; Roberts, S.A.; et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **2013**, *499*, 214–218, doi:10.1038/nature12213.
41. Royer-Bertrand, B.; Torsello, M.; Rimoldi, D.; El Zaoui, I.; Cisarova, K.; Pescini-Gobert, R.; Raynaud, F.; Zografos, L.; Schalenbourg, A.; Speiser, D.; et al. Comprehensive Genetic Landscape of Uveal Melanoma by Whole-Genome Sequencing. *Am J Hum Genet* **2016**, *99*, 1190–1198, doi:10.1016/j.ajhg.2016.09.008.
42. Chalmers, Z.R.; Connelly, C.F.; Fabrizio, D.; Gay, L.; Ali, S.M.; Ennis, R.; Schrock, A.; Campbell, B.; Shlien, A.; Chmielecki, J.; et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* **2017**, *9*, 34, doi:10.1186/s13073-017-0424-2.
43. Van Raamsdonk, C.D.; Bezrookove, V.; Green, G.; Bauer, J.; Gaugler, L.; O'Brien, J.M.; Simpson, E.M.; Barsh, G.S.; Bastian, B.C. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* **2009**, *457*, 599–602, doi:10.1038/nature07586.
44. Van Raamsdonk, C.D.; Griewank, K.G.; Crosby, M.B.; Garrido, M.C.; Vemula, S.; Wiesner, T.; Obenauf, A.C.; Wackernagel, W.; Green, G.; Bouvier, N.; et al. Mutations in GNA11 in uveal melanoma. *N Engl J Med* **2010**, *363*, 2191–2199, doi:10.1056/NEJMoa1000584.
45. Harbour, J.W. The genetics of uveal melanoma: an emerging framework for targeted therapy. *Pigment Cell Melanoma Res* **2012**, *25*, 171–181, doi:10.1111/j.1755-148X.2012.00979.x.
46. van der Kooij, M.K.; Speetjens, F.M.; van der Burg, S.H.; Kapiteijn, E. Uveal Versus Cutaneous Melanoma; Same Origin, Very Distinct Tumor Types. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11060845.
47. Wessely, A.; Koch, E.A.T.; Vera, J.; Berking, C.; Heppt, M.V. Identifying biomarkers and novel therapeutic targets in uveal melanoma. *J Dtsch Dermatol Ges* **2024**, *22*, 29–32, doi:10.1111/ddg.15225.
48. Riemenschneider, K.; Liu, J.; Powers, J.G. Skin cancer in the military: A systematic review of melanoma and nonmelanoma skin cancer incidence, prevention, and screening among active duty and veteran personnel. *J Am Acad Dermatol* **2018**, *78*, 1185–1192, doi:10.1016/j.jaad.2017.11.062.
49. Reichstein, D.A.; Brock, A.L. Radiation therapy for uveal melanoma: a review of treatment methods available in 2021. *Curr Opin Ophthalmol* **2021**, *32*, 183–190, doi:10.1097/ICU.0000000000000761.
50. Collaborative Ocular Melanoma Study, G. Assessment of metastatic disease status at death in 435 patients with large choroidal melanoma in the Collaborative Ocular Melanoma Study (COMS): COMS report no. 15. *Arch Ophthalmol* **2001**, *119*, 670–676, doi:10.1001/archopht.119.5.670.
51. Baum, S.H.; Westekemper, H.; Bechrakis, N.E.; Mohr, C. Conjunctival and uveal melanoma: Survival and risk factors following orbital exenteration. *Eur J Ophthalmol* **2022**, *32*, 612–619, doi:10.1177/1120672121995131.
52. Heng, J.S.; Perzia, B.M.; Sinard, J.H.; Pointdujour-Lim, R. Local recurrence of uveal melanoma and concomitant brain metastases associated with an activating telomerase promoter mutation seven years after secondary enucleation. *Am J Ophthalmol Case Rep* **2022**, *27*, 101607, doi:10.1016/j.ajoc.2022.101607.
53. Negretti, G.S.; Gurudas, S.; Gallo, B.; Damato, B.; Arora, A.K.; Sivaprasad, S.; Sagoo, M.S. Survival analysis following enucleation for uveal melanoma. *Eye (Lond)* **2022**, *36*, 1669–1674, doi:10.1038/s41433-021-01710-y.
54. Marshall, E.; Romaniuk, C.; Ghaneh, P.; Wong, H.; McKay, M.; Chopra, M.; Coupland, S.E.; Damato, B.E. MRI in the detection of hepatic metastases from high-risk uveal melanoma: a prospective study in 188 patients. *Br J Ophthalmol* **2013**, *97*, 159–163, doi:10.1136/bjophthalmol-2012-302323.
55. Lorenzo, D.; Piulats, J.M.; Ochoa, M.; Arias, L.; Gutierrez, C.; Catala, J.; Cobos, E.; Garcia-Bru, P.; Dias, B.; Padron-Perez, N.; et al. Clinical predictors of survival in metastatic uveal melanoma. *Jpn J Ophthalmol* **2019**, *63*, 197–209, doi:10.1007/s10384-019-00656-9.
56. Onken, M.D.; Worley, L.A.; Tuscan, M.D.; Harbour, J.W. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn* **2010**, *12*, 461–468, doi:10.2353/jmoldx.2010.090220.
57. Tschentscher, F.; Husing, J.; Holter, T.; Kruse, E.; Dresen, I.G.; Jockel, K.H.; Anastassiou, G.; Schilling, H.; Bornfeld, N.; Horsthemke, B.; et al. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* **2003**, *63*, 2578–2584.

 doi:10.20944/preprints202604.0001.v1

58. Gamel, J.W.; McLean, I.W.; McCurdy, J.B. Biologic distinctions between cure and time to death in 2892 patients with intraocular melanoma. *Cancer* **1993**, *71*, 2299–2305, doi:10.1002/1097-0142(19930401)71:7<2299::aid-cncr2820710721>3.0.co;2-g.
59. Kath, R.; Hayungs, J.; Bornfeld, N.; Sauerwein, W.; Hoffken, K.; Seeber, S. Prognosis and treatment of disseminated uveal melanoma. *Cancer* **1993**, *72*, 2219–2223, doi:10.1002/1097-0142(19931001)72:7<2219::aid-cncr2820720725>3.0.co;2-j.
60. Luyten, G.P.; Mooy, C.M.; Eijkenboom, W.M.; Stijnen, T.; Hellemons, L.P.; Luider, T.M.; de Jong, P.T. No demonstrated effect of pre-enucleation irradiation on survival of patients with uveal melanoma. *Am J Ophthalmol* **1995**, *119*, 786–791, doi:10.1016/s0002-9394(14)72786-2.
61. Lorigan, J.G.; Wallace, S.; Mavligit, G.M. The prevalence and location of metastases from ocular melanoma: imaging study in 110 patients. *AJR Am J Roentgenol* **1991**, *157*, 1279–1281, doi:10.2214/ajr.157.6.1950883.
62. Stalhammar, G.; Herrspiegel, C. Long-term relative survival in uveal melanoma: a systematic review and meta-analysis. *Commun Med (Lond)* **2022**, *2*, 18, doi:10.1038/s43856-022-00082-y.
63. Bidard, F.C.; Madic, J.; Mariani, P.; Piperno-Neumann, S.; Rampanou, A.; Servois, V.; Cassoux, N.; Desjardins, L.; Milder, M.; Vaucher, I.; et al. Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. *Int J Cancer* **2014**, *134*, 1207–1213, doi:10.1002/ijc.28436.
64. Cabel, L.; Riva, F.; Servois, V.; Livartowski, A.; Daniel, C.; Rampanou, A.; Lantz, O.; Romano, E.; Milder, M.; Buecher, B.; et al. Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study. *Ann Oncol* **2017**, *28*, 1996–2001, doi:10.1093/annonc/mdx212.
65. Park, J.J.; Diefenbach, R.J.; Byrne, N.; Long, G.V.; Scolyer, R.A.; Gray, E.S.; Carlino, M.S.; Rizos, H. Circulating Tumor DNA Reflects Uveal Melanoma Responses to Protein Kinase C Inhibition. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13071740.
66. Robertson, A.G.; Shih, J.; Yau, C.; Gibb, E.A.; Oba, J.; Mungall, K.L.; Hess, J.M.; Uzunangelov, V.; Walter, V.; Danilova, L.; et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma. *Cancer Cell* **2017**, *32*, 204–220 e215, doi:10.1016/j.ccell.2017.07.003.
67. Harbour, J.W.; Onken, M.D.; Roberson, E.D.; Duan, S.; Cao, L.; Worley, L.A.; Council, M.L.; Matatall, K.A.; Helms, C.; Bowcock, A.M. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* **2010**, *330*, 1410–1413, doi:10.1126/science.1194472.
68. Darvin, P.; Toor, S.M.; Sasidharan Nair, V.; Elkord, E. Immune checkpoint inhibitors: recent progress and potential biomarkers. *Exp Mol Med* **2018**, *50*, 1–11, doi:10.1038/s12276-018-0191-1.
69. Yun, S.; Vincelette, N.D.; Green, M.R.; Wahner Hendrickson, A.E.; Abraham, I. Targeting immune checkpoints in unresectable metastatic cutaneous melanoma: a systematic review and meta-analysis of anti-CTLA-4 and anti-PD-1 agents trials. *Cancer Med* **2016**, *5*, 1481–1491, doi:10.1002/cam4.732.
70. Champion, P.J.; Bluestein, J.R.; Quinn, A.E.; Bell, S.D.; Kiley, J.H.; Wakefield, M.R.; Fang, Y. A Consolidated Review of Contemporary Targeted and Immunotherapeutic Options for Melanoma. *Biomedicines* **2025**, *13*, doi:10.3390/biomedicines13061388.
71. Timis, T.; Buruiana, S.; Dima, D.; Nistor, M.; Muresan, X.M.; Cenariu, D.; Tigu, A.B.; Tomuleasa, C. Advances in Cell and Immune Therapies for Melanoma. *Biomedicines* **2025**, *13*, doi:10.3390/biomedicines13010098.
72. Wang, X.; Ma, S.; Zhu, S.; Zhu, L.; Guo, W. Advances in Immunotherapy and Targeted Therapy of Malignant Melanoma. *Biomedicines* **2025**, *13*, doi:10.3390/biomedicines13010225.
73. Saleem, M.; Watson, A.E.; Anwaar, A.; Jasser, A.O.; Yusuf, N. Optimizing Immunotherapy: The Synergy of Immune Checkpoint Inhibitors with Artificial Intelligence in Melanoma Treatment. *Biomolecules* **2025**, *15*, doi:10.3390/biom15040589.
74. Triggiano, G.; Pezzicoli, G.; Tucci, M. Immunotherapy in Advanced Cutaneous Melanoma: From the Optimal Treatment Duration to the Impact on Survival in Case of Early Discontinuation Due to Immune-Related Adverse Events. *Biomolecules* **2025**, *15*, doi:10.3390/biom15050651.
75. Wang, T.; Ma, W.; Zou, Z.; Zhong, J.; Lin, X.; Liu, W.; Sun, W.; Hu, T.; Xu, Y.; Chen, Y. PD-1 blockade treatment in melanoma: Mechanism of response and tumor-intrinsic resistance. *Cancer Sci* **2025**, *116*, 329–337, doi:10.1111/cas.16398.

 doi:10.20944/preprints202604.0001.v1

76. Lens, M.; Schachter, J. Immune Checkpoint Inhibitors in the Treatment of Advanced Melanoma in Older Patients: An Overview of Published Data. *Cancers (Basel)* **2025**, *17*, doi:10.3390/cancers17111835.
77. Muto, Y.; Fujimura, T.; Asano, Y. Recent Advances in Immunotherapy for Melanoma: Perspectives on the Development of Novel Treatments: A Mini Review. *Cancers (Basel)* **2025**, *17*, doi:10.3390/cancers17132265.
78. Latoni, D.I.; Kim, S.H.; Tsao, H. Systemic Therapies for Metastatic Melanoma. *Dermatol Clin* **2025**, *43*, 483–494, doi:10.1016/j.det.2025.03.004.
79. Pham, J.P.; Staeger, R.; Joshua, A.M.; Liu, J.; da Silva, I.P.; Dummer, R.; Goldinger, S.M. An updated review of immune checkpoint inhibitors in cutaneous oncology: Beyond melanoma. *Eur J Cancer* **2025**, *214*, 115121, doi:10.1016/j.ejca.2024.115121.
80. Maul, L.V.; Ramelyte, E.; Dummer, R.; Mangana, J. Management of metastatic melanoma with combinations including PD-1 inhibitors. *Expert Opin Biol Ther* **2025**, *25*, 1–12, doi:10.1080/14712598.2025.2485315.
81. McCombe, A.; van Akkooi, A.C.J. An evaluation of nivolumab and ipilimumab for the treatment of resectable stage III melanoma. *Expert Rev Anticancer Ther* **2025**, 1–7, doi:10.1080/14737140.2025.2522944.
82. Patel, S.P.; Sheth, R.A.; Davis, C.; Medina, T. Combination Immunotherapy With Nivolumab Plus Ipilimumab in Melanoma of Unknown Primary. *J Clin Oncol* **2025**, *43*, 907–911, doi:10.1200/JCO-24-01802.
83. Tasdogan, A.; Sullivan, R.J.; Katalinic, A.; Lebbe, C.; Whitaker, D.; Puig, S.; van de Poll-Franse, L.V.; Massi, D.; Schadendorf, D. Cutaneous melanoma. *Nat Rev Dis Primers* **2025**, *11*, 23, doi:10.1038/s41572-025-00603-8.
84. Pathak, S.; Puckett, Y. Cutaneous Malignant Melanoma: Guideline-Based Management and Interprofessional Collaboration. In *StatPearls*; Treasure Island (FL), 2025.
85. Shah, A.; Decoste, R.; Vanderbeck, K.; Sharma, A.; Roy, S.F.; Naert, K.; Osmond, A. Molecular-Guided Therapy for Melanoma in Canada: Overview of Current Practices and Recommendations. *J Cutan Med Surg* **2025**, *29*, 290–297, doi:10.1177/12034754241303057.
86. Swetter, S.M.; Johnson, D.; Albertini, M.R.; Barker, C.A.; Bateni, S.; Baumgartner, J.; Bhatia, S.; Bichakjian, C.; Boland, G.; Chandra, S.; et al. NCCN Guidelines(R) Insights: Melanoma: Cutaneous, Version 2.2024. *J Natl Compr Canc Netw* **2024**, *22*, 290–298, doi:10.6004/jnccn.2024.0036.
87. Wessely, A.; Steeb, T.; Erdmann, M.; Heinzerling, L.; Vera, J.; Schlaak, M.; Berking, C.; Heppt, M.V. The Role of Immune Checkpoint Blockade in Uveal Melanoma. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21030879.
88. Wierenga, A.P.A.; Cao, J.; Luyten, G.P.M.; Jager, M.J. Immune Checkpoint Inhibitors in Uveal and Conjunctival Melanoma. *Int Ophthalmol Clin* **2019**, *59*, 53–63, doi:10.1097/IIO.0000000000000263.
89. van der Kooij, M.K.; Joosse, A.; Speetjens, F.M.; Hospers, G.A.; Bisschop, C.; de Groot, J.W.; Koornstra, R.; Blank, C.U.; Kapiteijn, E. Anti-PD1 treatment in metastatic uveal melanoma in the Netherlands. *Acta Oncol* **2017**, *56*, 101–103, doi:10.1080/0284186X.2016.1260773.
90. Johansson, P.A.; Stark, A.; Palmer, J.M.; Bigby, K.; Brooks, K.; Rolfe, O.; Pritchard, A.L.; Whitehead, K.; Warrier, S.; Glasson, W.; et al. Correction to: Prolonged stable disease in a uveal melanoma patient with germline MBD4 nonsense mutation treated with pembrolizumab and ipilimumab. *Immunogenetics* **2019**, *71*, 511, doi:10.1007/s00251-019-01120-1.
91. Johansson, P.A.; Stark, A.; Palmer, J.M.; Bigby, K.; Brooks, K.; Rolfe, O.; Pritchard, A.L.; Whitehead, K.; Warrier, S.; Glasson, W.; et al. Prolonged stable disease in a uveal melanoma patient with germline MBD4 nonsense mutation treated with pembrolizumab and ipilimumab. *Immunogenetics* **2019**, *71*, 433–436, doi:10.1007/s00251-019-01108-x.
92. Rodrigues, M.; Mobuchon, L.; Houy, A.; Fievet, A.; Gardrat, S.; Barnhill, R.L.; Popova, T.; Servois, V.; Rampanou, A.; Mouton, A.; et al. Outlier response to anti-PD1 in uveal melanoma reveals germline MBD4 mutations in hypermutated tumors. *Nat Commun* **2018**, *9*, 1866, doi:10.1038/s41467-018-04322-5.
93. Kastelan, S.; Antunica, A.G.; Oreskovic, L.B.; Pelcic, G.; Kasun, E.; Hat, K. Immunotherapy for Uveal Melanoma - Current Knowledge and Perspectives. *Curr Med Chem* **2020**, *27*, 1350–1366, doi:10.2174/0929867326666190704141444.
94. Alexandrov, L.B.; Kim, J.; Haradhvala, N.J.; Huang, M.N.; Tian Ng, A.W.; Wu, Y.; Boot, A.; Covington, K.R.; Gordenin, D.A.; Bergstrom, E.N.; et al. The repertoire of mutational signatures in human cancer. *Nature* **2020**, *578*, 94–101, doi:10.1038/s41586-020-1943-3.

 doi:10.20944/preprints202604.0001.v1

95. Johansson, P.A.; Brooks, K.; Newell, F.; Palmer, J.M.; Wilmott, J.S.; Pritchard, A.L.; Broit, N.; Wood, S.; Carlino, M.S.; Leonard, C.; et al. Whole genome landscapes of uveal melanoma show an ultraviolet radiation signature in iris tumours. *Nat Commun* **2020**, *11*, 2408, doi:10.1038/s41467-020-16276-8.
96. Yarchoan, M.; Hopkins, A.; Jaffee, E.M. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med* **2017**, *377*, 2500–2501, doi:10.1056/NEJMc1713444.
97. Johnson, D.B.; Bao, R.; Ancell, K.K.; Daniels, A.B.; Wallace, D.; Sosman, J.A.; Luke, J.J. Response to Anti-PD-1 in Uveal Melanoma Without High-Volume Liver Metastasis. *J Natl Compr Canc Netw* **2019**, *17*, 114–117, doi:10.6004/jnccn.2018.7070.
98. Algazi, A.P.; Tsai, K.K.; Shoushtari, A.N.; Munhoz, R.R.; Eroglu, Z.; Piulats, J.M.; Ott, P.A.; Johnson, D.B.; Hwang, J.; Daud, A.I.; et al. Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-L1 antibodies. *Cancer* **2016**, *122*, 3344–3353, doi:10.1002/cncr.30258.
99. Helgadottir, H.; Hoiom, V. The genetics of uveal melanoma: current insights. *Appl Clin Genet* **2016**, *9*, 147–155, doi:10.2147/TACG.S69210.
100. Chandran, S.S.; Somerville, R.P.T.; Yang, J.C.; Sherry, R.M.; Klebanoff, C.A.; Goff, S.L.; Wunderlich, J.R.; Danforth, D.N.; Zlott, D.; Paria, B.C.; et al. Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study. *Lancet Oncol* **2017**, *18*, 792–802, doi:10.1016/S1470-2045(17)30251-6.
101. Retel, V.P.; Steuten, L.M.G.; Geukes Foppen, M.H.; Mewes, J.C.; Lindenberg, M.A.; Haanen, J.; van Harten, W.H. Early cost-effectiveness of tumor infiltrating lymphocytes (TIL) for second line treatment in advanced melanoma: a model-based economic evaluation. *BMC Cancer* **2018**, *18*, 895, doi:10.1186/s12885-018-4788-5.
102. Rosenberg, S.A.; Yang, J.C.; Sherry, R.M.; Kammula, U.S.; Hughes, M.S.; Phan, G.Q.; Citrin, D.E.; Restifo, N.P.; Robbins, P.F.; Wunderlich, J.R.; et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res* **2011**, *17*, 4550–4557, doi:10.1158/1078-0432.CCR-11-0116.
103. Andersen, R.; Donia, M.; Ellebaek, E.; Borch, T.H.; Kongsted, P.; Iversen, T.Z.; Holmich, L.R.; Hendel, H.W.; Met, O.; Andersen, M.H.; et al. Long-Lasting Complete Responses in Patients with Metastatic Melanoma after Adoptive Cell Therapy with Tumor-Infiltrating Lymphocytes and an Attenuated IL2 Regimen. *Clin Cancer Res* **2016**, *22*, 3734–3745, doi:10.1158/1078-0432.CCR-15-1879.
104. Martinez-Perez, D.; Vinal, D.; Solares, I.; Espinosa, E.; Feliu, J. Gp-100 as a Novel Therapeutic Target in Uveal Melanoma. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13235968.
105. Wagner, S.N.; Wagner, C.; Schultewolter, T.; Goos, M. Analysis of Pmel17/gp100 expression in primary human tissue specimens: implications for melanoma immuno- and gene-therapy. *Cancer Immunol Immunother* **1997**, *44*, 239–247, doi:10.1007/s002620050379.
106. Kawakami, Y.; Eliyahu, S.; Jennings, C.; Sakaguchi, K.; Kang, X.; Southwood, S.; Robbins, P.F.; Sette, A.; Appella, E.; Rosenberg, S.A. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. *J Immunol* **1995**, *154*, 3961–3968.
107. Bakker, A.B.; Schreurs, M.W.; Tafazzul, G.; de Boer, A.J.; Kawakami, Y.; Adema, G.J.; Figdor, C.G. Identification of a novel peptide derived from the melanocyte-specific gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line. *Int J Cancer* **1995**, *62*, 97–102, doi:10.1002/ijc.2910620118.
108. Schwartzentruber, D.J.; Lawson, D.H.; Richards, J.M.; Conry, R.M.; Miller, D.M.; Treisman, J.; Gailani, F.; Riley, L.; Conlon, K.; Pockaj, B.; et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med* **2011**, *364*, 2119–2127, doi:10.1056/NEJMoa1012863.
109. Nathan, P.; Hassel, J.C.; Rutkowski, P.; Baurain, J.F.; Butler, M.O.; Schlaak, M.; Sullivan, R.J.; Ochsenreither, S.; Dummer, R.; Kirkwood, J.M.; et al. Overall Survival Benefit with Tebentafusp in Metastatic Uveal Melanoma. *N Engl J Med* **2021**, *385*, 1196–1206, doi:10.1056/NEJMoa2103485.
110. Safety and Efficacy of IMCgp100 Versus Investigator Choice in Uveal Melanoma. Available online: https://clinicaltrials.gov/study/NCT03070392 (accessed on August 10, 2025).
111. FDA approves tebentafusp-tebn for unresectable or metastatic uveal melanoma. Available online: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-tebentafusp-tebn-unresectable-or-metastatic-uveal-melanoma (accessed on August 11, 2025).

 doi:10.20944/preprints202604.0001.v1

112. Hassel, J.C.; Piperno-Neumann, S.; Rutkowski, P.; Baurain, J.F.; Schlaak, M.; Butler, M.O.; Sullivan, R.J.; Dummer, R.; Kirkwood, J.M.; Orloff, M.; et al. Three-Year Overall Survival with Tebentafusp in Metastatic Uveal Melanoma. *N Engl J Med* **2023**, *389*, 2256–2266, doi:10.1056/NEJMoa2304753.
113. Piulats, J.M.; Watkins, C.; Costa-Garcia, M.; Del Carpio, L.; Piperno-Neumann, S.; Rutkowski, P.; Hassel, J.C.; Espinosa, E.; de la Cruz-Merino, L.; Ochsenreither, S.; et al. Overall survival from tebentafusp versus nivolumab plus ipilimumab in first-line metastatic uveal melanoma: a propensity score-weighted analysis. *Ann Oncol* **2024**, *35*, 317–326, doi:10.1016/j.annonc.2023.11.013.
114. Marincola, F.M.; Venzon, D.; White, D.; Rubin, J.T.; Lotze, M.T.; Simonis, T.B.; Balkissoon, J.; Rosenberg, S.A.; Parkinson, D.R. HLA association with response and toxicity in melanoma patients treated with interleukin 2-based immunotherapy. *Cancer Res* **1992**, *52*, 6561–6566.
115. Metzelaar-Blok, J.A.; Hurks, H.M.; Naipal, A.; De Lange, P.; Keunen, J.E.; Claas, F.H.; Doxiadis, II; Jager, M.J. Normal HLA class I, II, and MICA gene distribution in uveal melanoma. *Mol Vis* **2005**, *11*, 1166–1172.
116. van Essen, T.H.; Bronkhorst, I.H.; Maat, W.; Verduyn, W.; Roelen, D.L.; Luyten, G.P.; Jager, M.J. A comparison of HLA genotype with inflammation in uveal melanoma. *Invest Ophthalmol Vis Sci* **2012**, *53*, 2640–2646, doi:10.1167/iovs.11-8901.
117. Ellis, J.M.; Henson, V.; Slack, R.; Ng, J.; Hartzman, R.J.; Katovich Hurley, C. Frequencies of HLA-A2 alleles in five U.S. population groups. Predominance Of A*02011 and identification of HLA-A*0231. *Hum Immunol* **2000**, *61*, 334–340, doi:10.1016/s0198-8859(99)00155-x.
118. Kendra, K.L.; Contreras, C.M.; Verschraegen, C.F.; Wu, R.C.; Hasanov, M. Management of Cutaneous Melanoma, With a Special Focus on Desmoplastic Melanoma. *Am Soc Clin Oncol Educ Book* **2025**, *45*, e472752, doi:10.1200/EDBK-25-472752.
119. Martin-Liberal, J.; Marquez-Rodas, I.; Cerezuela-Fuentes, P.; Soria, A.; Garicano, F.; Medina, J.; Garcia Galindo, R.; Oramas, J.; Luis Manzano, J.; Delgado, M.; et al. Challenges and perspectives in the management of BRAF-mutated metastatic melanoma: Systemic treatment sequencing and brain metastases. *Cancer Treat Rev* **2025**, *133*, 102886, doi:10.1016/j.ctrv.2025.102886.
120. Connor, C.; Carr, Q.L.; Sweazy, A.; McMasters, K.; Hao, H. Clinical Approaches for the Management of Skin Cancer: A Review of Current Progress in Diagnosis, Treatment, and Prognosis for Patients with Melanoma. *Cancers (Basel)* **2025**, *17*, doi:10.3390/cancers17040707.
121. Paik, A.; Purawarga Matada, G.S.; Ghara, A.; Pal, R.; Pavani, G.; Kamurthy, H. BRAF inhibitors in Melanoma: Structural Insights, Therapeutic Resistance, and Biological Evaluation of Quinazoline Derivatives. *Eur J Med Chem* **2025**, *296*, 117866, doi:10.1016/j.ejmech.2025.117866.
122. Javaid, A.; Peres, T.; Pozas, J.; Thomas, J.; Larkin, J. Current and emerging treatment options for BRAFV600-mutant melanoma. *Expert Rev Anticancer Ther* **2025**, *25*, 55–69, doi:10.1080/14737140.2025.2451722.
123. Kamato, D.; Thach, L.; Bernard, R.; Chan, V.; Zheng, W.; Kaur, H.; Brimble, M.; Osman, N.; Little, P.J. Structure, Function, Pharmacology, and Therapeutic Potential of the G Protein, Galpha/q,11. *Front Cardiovasc Med* **2015**, *2*, 14, doi:10.3389/fcvm.2015.00014.
124. Naor, Z.; Benard, O.; Seger, R. Activation of MAPK cascades by G-protein-coupled receptors: the case of gonadotropin-releasing hormone receptor. *Trends Endocrinol Metab* **2000**, *11*, 91–99, doi:10.1016/s1043-2760(99)00232-5.
125. Riese, D.J., 2nd. GPCR/Galphaq/Galpha11 Coupling to the RAS/MAPK Pathway. Available online: https://BioRender.com/avu6c8z (accessed on December 30, 2025).
126. Nell, R.J.; Menger, N.V.; Versluis, M.; Luyten, G.P.M.; Verdijk, R.M.; Madigan, M.C.; Jager, M.J.; van der Velden, P.A. Involvement of mutant and wild-type CYSLTR2 in the development and progression of uveal nevi and melanoma. *BMC Cancer* **2021**, *21*, 164, doi:10.1186/s12885-021-07865-x.
127. Moore, A.R.; Ceraudo, E.; Sher, J.J.; Guan, Y.; Shoushtari, A.N.; Chang, M.T.; Zhang, J.Q.; Walczak, E.G.; Kazmi, M.A.; Taylor, B.S.; et al. Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Nat Genet* **2016**, *48*, 675–680, doi:10.1038/ng.3549.
128. New, D.C.; Wong, Y.H. Molecular mechanisms mediating the G protein-coupled receptor regulation of cell cycle progression. *J Mol Signal* **2007**, *2*, 2, doi:10.1186/1750-2187-2-2.
129. Bol, K.F.; Donia, M.; Heegaard, S.; Kiilgaard, J.F.; Svane, I.M. Genetic Biomarkers in Melanoma of the Ocular Region: What the Medical Oncologist Should Know. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21155231.

 doi:10.20944/preprints202604.0001.v1

130. Johansson, P.; Aoude, L.G.; Wadt, K.; Glasson, W.J.; Warrier, S.K.; Hewitt, A.W.; Kiilgaard, J.F.; Heegaard, S.; Isaacs, T.; Franchina, M.; et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. *Oncotarget* **2016**, *7*, 4624–4631, doi:10.18632/oncotarget.6614.
131. Ma, J.; Weng, L.; Bastian, B.C.; Chen, X. Functional characterization of uveal melanoma oncogenes. *Oncogene* **2021**, *40*, 806–820, doi:10.1038/s41388-020-01569-5.
132. Phan, H.T.N.; Kim, N.H.; Wei, W.; Tall, G.G.; Smrcka, A.V. Uveal melanoma-associated mutations in PLCbeta4 are constitutively activating and promote melanocyte proliferation and tumorigenesis. *Sci Signal* **2021**, *14*, eabj4243, doi:10.1126/scisignal.abj4243.
133. Ambrosini, G.; Musi, E.; Ho, A.L.; de Stanchina, E.; Schwartz, G.K. Inhibition of mutant GNAQ signaling in uveal melanoma induces AMPK-dependent autophagic cell death. *Mol Cancer Ther* **2013**, *12*, 768–776, doi:10.1158/1535-7163.MCT-12-1020.
134. Chen, X.; Wu, Q.; Depeille, P.; Chen, P.; Thornton, S.; Kalirai, H.; Coupland, S.E.; Roose, J.P.; Bastian, B.C. RasGRP3 Mediates MAPK Pathway Activation in GNAQ Mutant Uveal Melanoma. *Cancer Cell* **2017**, *31*, 685–696 e686, doi:10.1016/j.ccell.2017.04.002.
135. Piperno-Neumann, S.; Larkin, J.; Carvajal, R.D.; Luke, J.J.; Schwartz, G.K.; Hodi, F.S.; Sablin, M.P.; Shoushtari, A.N.; Szpakowski, S.; Chowdhury, N.R.; et al. Genomic Profiling of Metastatic Uveal Melanoma and Clinical Results of a Phase I Study of the Protein Kinase C Inhibitor AEB071. *Mol Cancer Ther* **2020**, *19*, 1031–1039, doi:10.1158/1535-7163.MCT-19-0098.
136. Shoushtari, A.N.; Khan, S.; Komatsubara, K.; Feun, L.; Acquavella, N.; Singh-Kandah, S.; Negri, T.; Nesson, A.; Abbate, K.; Cremers, S.; et al. A Phase Ib Study of Sotrastaurin, a PKC Inhibitor, and Alpelisib, a PI3Kalpha Inhibitor, in Patients with Metastatic Uveal Melanoma. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13215504.
137. Piperno-Neumann, S.; Carlino, M.S.; Boni, V.; Loirat, D.; Speetjens, F.M.; Park, J.J.; Calvo, E.; Carvajal, R.D.; Nyakas, M.; Gonzalez-Maffe, J.; et al. A phase I trial of LXS196, a protein kinase C (PKC) inhibitor, for metastatic uveal melanoma. *Br J Cancer* **2023**, *128*, 1040–1051, doi:10.1038/s41416-022-02133-6.
138. Chen, X.; Wu, Q.; Tan, L.; Porter, D.; Jager, M.J.; Emery, C.; Bastian, B.C. Combined PKC and MEK inhibition in uveal melanoma with GNAQ and GNA11 mutations. *Oncogene* **2014**, *33*, 4724–4734, doi:10.1038/onc.2013.418.
139. Prabhu, K.S.; Kuttikrishnan, S.; Mariyam, Z.; Habeeba, U.; Panicker, A.J.; Masoodi, T.; Junejo, K.; Uddin, S. PI3 K/AKT/mTOR pathway and its role in breast cancer stem cells. *Naunyn Schmiedebergs Arch Pharmacol* **2025**, *398*, 16779–16795, doi:10.1007/s00210-025-04297-3.
140. Luo, J.; Li, H.; Xiu, J.; Zeng, J.; Feng, Z.; Zhao, H.; Li, Y.; Wei, W. Elevated ZNF704 expression is associated with poor prognosis of uveal melanoma and promotes cancer cell growth by regulating AKT/mTOR signaling. *Biomark Res* **2023**, *11*, 38, doi:10.1186/s40364-023-00471-y.
141. Babchia, N.; Calipel, A.; Mouriaux, F.; Faussat, A.M.; Mascarelli, F. The PI3K/Akt and mTOR/P70S6K signaling pathways in human uveal melanoma cells: interaction with B-Raf/ERK. *Invest Ophthalmol Vis Sci* **2010**, *51*, 421–429, doi:10.1167/iovs.09-3974.
142. Smit, K.N.; Jager, M.J.; de Klein, A.; Kiliç, E. Uveal melanoma: Towards a molecular understanding. *Prog Retin Eye Res* **2020**, *75*, 100800, doi:10.1016/j.preteyeres.2019.100800.
143. Shoushtari, A.N.; Kudchadkar, R.R.; Panageas, K.S.; Murthy, R.K.; Jung, M.; Shah, R.; O'Donnell, B.; Khawaja, T.T.; Shames, Y.; Prempeh-Keteku, N.A.; et al. A randomized phase 2 study of trametinib with or without GSK2141795 in patients with advanced uveal melanoma. *J Clin Oncol* **2016**, *34*, 9511, doi:10.1200/JCO.2016.34.15_suppl.9511.
144. Trametinib With or Without GSK2141795 in Treating Patients With Metastatic Uveal Melanoma. Available online: https://clinicaltrials.gov/study/NCT01979523?tab=results (accessed on December 26, 2025).
145. Zhang, L.; Zhang, F.; Liang, H.; Qin, X.; Mo, Y.; Chen, S.; Xie, J.; Hou, X.; Deng, J.; Hao, E.; et al. Hippo/YAP signaling pathway in colorectal cancer: regulatory mechanisms and potential drug exploration. *Front Oncol* **2025**, *15*, 1545952, doi:10.3389/fonc.2025.1545952.
146. Zhang, J.; Wu, H.; Ren, X.; Chen, Z.; Ye, S.; Chen, S.; Fang, J.; Wu, Q.; Zhao, T. Hippo/YAP signaling's multifaceted crosstalk in cancer. *Front Cell Dev Biol* **2025**, *13*, 1595362, doi:10.3389/fcell.2025.1595362.

 doi:10.20944/preprints202604.0001.v1

147. Yu, F.X.; Luo, J.; Mo, J.S.; Liu, G.; Kim, Y.C.; Meng, Z.; Zhao, L.; Peyman, G.; Ouyang, H.; Jiang, W.; et al. Mutant Gq/11 promote uveal melanoma tumorigenesis by activating YAP. *Cancer Cell* **2014**, *25*, 822–830, doi:10.1016/j.ccr.2014.04.017.
148. Feng, X.; Arang, N.; Rigiracciolo, D.C.; Lee, J.S.; Yeerna, H.; Wang, Z.; Lubrano, S.; Kishore, A.; Pachter, J.A.; Konig, G.M.; et al. A Platform of Synthetic Lethal Gene Interaction Networks Reveals that the GNAQ Uveal Melanoma Oncogene Controls the Hippo Pathway through FAK. *Cancer Cell* **2019**, *35*, 457–472 e455, doi:10.1016/j.ccell.2019.01.009.
149. Li, H.; Li, Q.; Dang, K.; Ma, S.; Cotton, J.L.; Yang, S.; Zhu, L.J.; Deng, A.C.; Ip, Y.T.; Johnson, R.L.; et al. YAP/TAZ Activation Drives Uveal Melanoma Initiation and Progression. *Cell Rep* **2019**, *29*, 3200–3211 e3204, doi:10.1016/j.celrep.2019.03.021.
150. Djidjik, R.; Lamara Mahammed, L.; Berkani, L.M.; Allam, I.; Benmoussa, A.H.; Gharnaout, M.; Belaid, B. JAK/STAT in human diseases: a common axis in immunodeficiencies and hematological disorders. *Front Immunol* **2025**, *16*, 1669688, doi:10.3389/fimmu.2025.1669688.
151. Jia, J.; Zhou, X.; Chu, Q. Mechanisms and therapeutic prospect of the JAK-STAT signaling pathway in liver cancer. *Mol Cell Biochem* **2025**, *480*, 1–17, doi:10.1007/s11010-024-04983-5.
152. Parveen, S.; Fatma, M.; Mir, S.S.; Dermime, S.; Uddin, S. JAK-STAT Signaling in Autoimmunity and Cancer. *Immunotargets Ther* **2025**, *14*, 523–554, doi:10.2147/ITT.S485670.
153. Sohrabi, S.; Alipour, S.; Ghahramanipour, Z.; Masoumi, J.; Baradaran, B. STAT signaling pathways in immune cells and their associated mechanisms in cancer pathogenesis. *Bioimpacts* **2025**, *15*, 30030, doi:10.34172/bi.30030.
154. Vazquez-Cuevas, F.G.; Reyna-Jeldes, M.; Velazquez-Miranda, E.; Coddou, C. Transactivation of receptor tyrosine kinases by purinergic P2Y and adenosine receptors. *Purinergic Signal* **2023**, *19*, 613–621, doi:10.1007/s11302-022-09913-y.
155. Wang, W.; Qiao, Y.; Li, Z. New Insights into Modes of GPCR Activation. *Trends Pharmacol Sci* **2018**, *39*, 367–386, doi:10.1016/j.tips.2018.01.001.
156. Kose, M. GPCRs and EGFR - Cross-talk of membrane receptors in cancer. *Bioorg Med Chem Lett* **2017**, *27*, 3611–3620, doi:10.1016/j.bmcl.2017.07.002.
157. Kilpatrick, L.E.; Hill, S.J. Transactivation of G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs): Recent insights using luminescence and fluorescence technologies. *Curr Opin Endocr Metab Res* **2021**, *16*, 102–112, doi:10.1016/j.coemr.2020.10.003.
158. George, A.J.; Hannan, R.D.; Thomas, W.G. Unravelling the molecular complexity of GPCR-mediated EGFR transactivation using functional genomics approaches. *FEBS J* **2013**, *280*, 5258–5268, doi:10.1111/febs.12509.
159. Heppner, D.E.; van der Vliet, A. Redox-dependent regulation of epidermal growth factor receptor signaling. *Redox Biol* **2016**, *8*, 24–27, doi:10.1016/j.redox.2015.12.002.
160. Biscardi, J.S.; Maa, M.C.; Tice, D.A.; Cox, M.E.; Leu, T.H.; Parsons, S.J. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. *J Biol Chem* **1999**, *274*, 8335–8343, doi:10.1074/jbc.274.12.8335.
161. Etkovitz, N.; Tirosh, Y.; Chazan, R.; Jaldety, Y.; Daniel, L.; Rubinstein, S.; Breitbart, H. Bovine sperm acrosome reaction induced by G-protein-coupled receptor agonists is mediated by epidermal growth factor receptor transactivation. *Dev Biol* **2009**, *334*, 447–457, doi:10.1016/j.ydbio.2009.08.002.
162. Tice, D.A.; Biscardi, J.S.; Nickles, A.L.; Parsons, S.J. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc Natl Acad Sci U S A* **1999**, *96*, 1415–1420, doi:10.1073/pnas.96.4.1415.
163. Wang, Z. Transactivation of Epidermal Growth Factor Receptor by G Protein-Coupled Receptors: Recent Progress, Challenges and Future Research. *Int J Mol Sci* **2016**, *17*, doi:10.3390/ijms17010095.
164. Maudsley, S.; Pierce, K.L.; Zamah, A.M.; Miller, W.E.; Ahn, S.; Daaka, Y.; Lefkowitz, R.J.; Luttrell, L.M. The beta(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. *J Biol Chem* **2000**, *275*, 9572–9580, doi:10.1074/jbc.275.13.9572.

 doi:10.20944/preprints202604.0001.v1

165. Salazar, N.; Munoz, D.; Kallifatidis, G.; Singh, R.K.; Jorda, M.; Lokeshwar, B.L. The chemokine receptor CXCR7 interacts with EGFR to promote breast cancer cell proliferation. *Mol Cancer* **2014**, *13*, 198, doi:10.1186/1476-4598-13-198.
166. Watt, H.L.; Kharmate, G.D.; Kumar, U. Somatostatin receptors 1 and 5 heterodimerize with epidermal growth factor receptor: agonist-dependent modulation of the downstream MAPK signalling pathway in breast cancer cells. *Cell Signal* **2009**, *21*, 428–439, doi:10.1016/j.cellsig.2008.11.012.
167. Filardo, E.J. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. *J Steroid Biochem Mol Biol* **2002**, *80*, 231–238, doi:10.1016/s0960-0760(01)00190-x.
168. Filardo, E.J.; Thomas, P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. *Endocrinology* **2012**, *153*, 2953–2962, doi:10.1210/en.2012-1061.
169. Fischer, O.M.; Hart, S.; Gschwind, A.; Ullrich, A. EGFR signal transactivation in cancer cells. *Biochem Soc Trans* **2003**, *31*, 1203–1208, doi:10.1042/bst0311203.
170. Mill, C.P.; Chester, J.A.; Riese, D.J., 2nd. EGFR may couple moderate alcohol consumption to increased breast cancer risk. *Breast Cancer (Dove Med Press)* **2009**, *1*, 31–38, doi:10.2147/bctt.s6254.
171. Higashiyama, S.; Nanba, D. ADAM-mediated ectodomain shedding of HB-EGF in receptor cross-talk. *Biochim Biophys Acta* **2005**, *1751*, 110–117, doi:10.1016/j.bbapap.2004.11.009.
172. Ohtsu, H.; Dempsey, P.J.; Eguchi, S. ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am J Physiol Cell Physiol* **2006**, *291*, C1–10, doi:10.1152/ajpcell.00620.2005.
173. Palanisamy, S.; Xue, C.; Ishiyama, S.; Naga Prasad, S.V.; Gabrielson, K. GPCR-ErbB transactivation pathways and clinical implications. *Cell Signal* **2021**, *86*, 110092, doi:10.1016/j.cellsig.2021.110092.
174. Graves, L.M.; Han, J.; Earp, H.S., 3rd. Transactivation of the EGF receptor: is the PDGF receptor an unexpected accomplice? *Mol Interv* **2002**, *2*, 208–212, doi:10.1124/mi.2.4.208.
175. Shah, B.H.; Catt, K.J. Matrix metalloproteinases in reproductive endocrinology. *Trends Endocrinol Metab* **2004**, *15*, 47–49, doi:10.1016/j.tem.2004.01.004.
176. Yoshisue, H.; Kirkham-Brown, J.; Healy, E.; Holgate, S.T.; Sampson, A.P.; Davies, D.E. Cysteinyl leukotrienes synergize with growth factors to induce proliferation of human bronchial fibroblasts. *J Allergy Clin Immunol* **2007**, *119*, 132–140, doi:10.1016/j.jaci.2006.08.028.
177. Patel, S.P.; Kim, K.B.; Papadopoulos, N.E.; Hwu, W.J.; Hwu, P.; Prieto, V.G.; Bar-Eli, M.; Zigler, M.; Dobroff, A.; Bronstein, Y.; et al. A phase II study of gefitinib in patients with metastatic melanoma. *Melanoma Res* **2011**, *21*, 357–363, doi:10.1097/CMR.0b013e3283471073.
178. Riese, D.J., 2nd. GPCR-EGFR Crosstalk. Available online: https://BioRender.com/p5kjegr (accessed on December 30, 2025).
179. Lucas, L.M.; Dwivedi, V.; Senfeld, J.I.; Cullum, R.L.; Mill, C.P.; Piazza, J.T.; Bryant, I.N.; Cook, L.J.; Miller, S.T.; Lott, J.H.t.; et al. The Yin and Yang of ERBB4: Tumor Suppressor and Oncoprotein. *Pharmacol Rev* **2022**, *74*, 18–47, doi:10.1124/pharmrev.121.000381.
180. Riese, D.J., 2nd; Stern, D.F. Specificity within the EGF family/ErbB receptor family signaling network. *Bioessays* **1998**, *20*, 41–48, doi:10.1002/(SICI)1521-1878(199801)20:1<41::AID-BIES7>3.0.CO;2-V.
181. Trombetta, D.; Fabrizio, F.P.; Di Maio, M.; Parente, P.; Melocchi, L.; Martini, M.; Sparaneo, A.; Latiano, T.P.; Graziano, P.; Rossi, G.; et al. NRG/ErbB signaling: on the trail of a molecular fingerprint in mucinous carcinoma. *Expert Opin Ther Targets* **2025**, *29*, 423–434, doi:10.1080/14728222.2025.2532390.
182. Al-Awadhi, S.S.A.; Patil, P.; Shetty, P.; Shetty, P.K.; Shetty, R.A.; Shetty, V.V. Potential role of epidermal growth factor receptors (EGFR) signaling in the pathogenesis and management of hepatocellular carcinoma. *Bioimpacts* **2025**, *15*, 30905, doi:10.34172/bi.30905.
183. Du, Y.; Karatekin, F.; Wang, W.K.; Hong, W.; Boopathy, G.T.K. Cracking the EGFR code: Cancer biology, resistance mechanisms, and future therapeutic frontiers. *Pharmacol Rev* **2025**, *77*, 100076, doi:10.1016/j.pharmr.2025.100076.
184. Tao, Z.; Shao, W.; Zhou, H.; Xia, S.; Zeng, J.; Ren, J.; Wang, X.; Zhu, H. Role of signaling pathways in lung cancer development and advances in targeted therapies (Review). *Oncol Lett* **2025**, *30*, 589, doi:10.3892/ol.2025.15335.

 doi:10.20944/preprints202604.0001.v1

185. Tito, C.; Masciarelli, S.; Colotti, G.; Fazi, F. EGF receptor in organ development, tissue homeostasis and regeneration. *J Biomed Sci* **2025**, *32*, 24, doi:10.1186/s12929-025-01119-9.
186. Riese, D.J., 2nd. Ligand-based receptor tyrosine kinase partial agonists: New paradigm for cancer drug discovery? *Expert Opin Drug Discov* **2011**, *6*, 185–193, doi:10.1517/17460441.2011.547468.
187. Burke, C.L.; Lemmon, M.A.; Coren, B.A.; Engelman, D.M.; Stern, D.F. Dimerization of the p185neu transmembrane domain is necessary but not sufficient for transformation. *Oncogene* **1997**, *14*, 687–696, doi:10.1038/sj.onc.1200873.
188. Burke, C.L.; Stern, D.F. Activation of Neu (ErbB-2) mediated by disulfide bond-induced dimerization reveals a receptor tyrosine kinase dimer interface. *Mol Cell Biol* **1998**, *18*, 5371–5379, doi:10.1128/mcb.18.9.5371.
189. Wilson, K.J.; Gilmore, J.L.; Foley, J.; Lemmon, M.A.; Riese, D.J., 2nd. Functional selectivity of EGF family peptide growth factors: implications for cancer. *Pharmacol Ther* **2009**, *122*, 1–8, doi:10.1016/j.pharmthera.2008.11.008.
190. Wilson, K.J.; Mill, C.; Lambert, S.; Buchman, J.; Wilson, T.R.; Hernandez-Gordillo, V.; Gallo, R.M.; Ades, L.M.; Settleman, J.; Riese, D.J., 2nd. EGFR ligands exhibit functional differences in models of paracrine and autocrine signaling. *Growth Factors* **2012**, *30*, 107–116, doi:10.3109/08977194.2011.649918.
191. Wilson, K.J.; Mill, C.P.; Cameron, E.M.; Hobbs, S.S.; Hammer, R.P.; Riese, D.J., 2nd. Inter-conversion of neuregulin2 full and partial agonists for ErbB4. *Biochem Biophys Res Commun* **2007**, *364*, 351–357, doi:10.1016/j.bbrc.2007.10.004.
192. Wilson, K.J.; Mill, C.P.; Gallo, R.M.; Cameron, E.M.; VanBrocklin, H.; Settleman, J.; Riese, D.J. The Q43L mutant of neuregulin 2beta is a pan-ErbB receptor antagonist. *Biochem J* **2012**, *443*, 133–144, doi:10.1042/BJ20110921.
193. Hu, C.; Leche, C.A., 2nd; Kiyatkin, A.; Yu, Z.; Stayrook, S.E.; Ferguson, K.M.; Lemmon, M.A. Glioblastoma mutations alter EGFR dimer structure to prevent ligand bias. *Nature* **2022**, *602*, 518–522, doi:10.1038/s41586-021-04393-3.
194. Gaba, S.; Sahu, M.; Chauhan, N.; Jain, U. Transforming growth factor alpha: Key insights into physiological role, cancer therapeutics, and biomarker potential (A review). *Int J Biol Macromol* **2025**, *310*, 143212, doi:10.1016/j.ijbiomac.2025.143212.
195. Freed, D.M.; Bessman, N.J.; Kiyatkin, A.; Salazar-Cavazos, E.; Byrne, P.O.; Moore, J.O.; Valley, C.C.; Ferguson, K.M.; Leahy, D.J.; Lidke, D.S.; et al. EGFR Ligands Differentially Stabilize Receptor Dimers to Specify Signaling Kinetics. *Cell* **2017**, *171*, 683–695 e618, doi:10.1016/j.cell.2017.09.017.
196. Desai, O.; Rathore, M.; Boutros, C.S.; Wright, M.; Bryson, E.; Curry, K.; Wang, R. HER3: Unmasking a twist in the tale of a previously unsuccessful therapeutic pursuit targeting a key cancer survival pathway. *Genes Dis* **2025**, *12*, 101354, doi:10.1016/j.gendis.2024.101354.
197. Ergun, Y. HER3 in breast cancer: molecular insights, clinical implications, and therapeutic horizons. *Expert Opin Ther Targets* **2025**, *29*, 481–489, doi:10.1080/14728222.2025.2540355.
198. Garrett, J.T.; Tendler, S.; Feroz, W.; Kilroy, M.K.; Yu, H. Emerging importance of HER3 in tumorigenesis and cancer therapy. *Nat Rev Clin Oncol* **2025**, *22*, 348–370, doi:10.1038/s41571-025-01008-y.
199. Citri, A.; Yarden, Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* **2006**, *7*, 505–516, doi:10.1038/nrm1962.
200. Yarden, Y.; Sliwkowski, M.X. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2001**, *2*, 127–137, doi:10.1038/35052073.
201. Arteaga, C.L.; Engelman, J.A. ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. *Cancer Cell* **2014**, *25*, 282–303, doi:10.1016/j.ccr.2014.02.025.
202. Pitfield, S.E.; Bryant, I.; Penington, D.J.; Park, G.; Riese, D.J., 2nd. Phosphorylation of ErbB4 on tyrosine 1056 is critical for ErbB4 coupling to inhibition of colony formation by human mammary cell lines. *Oncol Res* **2006**, *16*, 179–193, doi:10.3727/000000006783981134.
203. Mill, C.P.; Gettinger, K.L.; Riese, D.J., 2nd. Ligand stimulation of ErbB4 and a constitutively-active ErbB4 mutant result in different biological responses in human pancreatic tumor cell lines. *Exp Cell Res* **2011**, *317*, 392–404, doi:10.1016/j.yexcr.2010.11.007.

 doi:10.20944/preprints202604.0001.v1

204. Mill, C.P.; Zordan, M.D.; Rothenberg, S.M.; Settleman, J.; Leary, J.F.; Riese, D.J., 2nd. ErbB2 Is Necessary for ErbB4 Ligands to Stimulate Oncogenic Activities in Models of Human Breast Cancer. *Genes Cancer* **2011**, *2*, 792–804, doi:10.1177/1947601911431080.
205. Shelly, M.; Pinkas-Kramarski, R.; Guarino, B.C.; Waterman, H.; Wang, L.M.; Lyass, L.; Alimandi, M.; Kuo, A.; Bacus, S.S.; Pierce, J.H.; et al. Epiregulin is a potent pan-ErbB ligand that preferentially activates heterodimeric receptor complexes. *J Biol Chem* **1998**, *273*, 10496–10505, doi:10.1074/jbc.273.17.10496.
206. Joslin, E.J.; Opresko, L.K.; Wells, A.; Wiley, H.S.; Lauffenburger, D.A. EGF-receptor-mediated mammary epithelial cell migration is driven by sustained ERK signaling from autocrine stimulation. *J Cell Sci* **2007**, *120*, 3688–3699, doi:10.1242/jcs.010488.
207. Gilmore, J.L.; Gonterman, R.M.; Menon, K.; Lorch, G.; Riese, D.J., 2nd; Robling, A.; Foley, J. Reconstitution of amphiregulin-epidermal growth factor receptor signaling in lung squamous cell carcinomas activates PTHrP gene expression and contributes to cancer-mediated diseases of the bone. *Mol Cancer Res* **2009**, *7*, 1714–1728, doi:10.1158/1541-7786.MCR-09-0131.
208. Gilmore, J.L.; Scott, J.A.; Bouizar, Z.; Robling, A.; Pitfield, S.E.; Riese, D.J., 2nd; Foley, J. Amphiregulin-EGFR signaling regulates PTHrP gene expression in breast cancer cells. *Breast Cancer Res Treat* **2008**, *110*, 493–505, doi:10.1007/s10549-007-9748-8.
209. Vecchi, M.; Carpenter, G. Constitutive proteolysis of the ErbB-4 receptor tyrosine kinase by a unique, sequential mechanism. *J Cell Biol* **1997**, *139*, 995–1003, doi:10.1083/jcb.139.4.995.
210. Rio, C.; Buxbaum, J.D.; Peschon, J.J.; Corfas, G. Tumor necrosis factor-alpha-converting enzyme is required for cleavage of erbB4/HER4. *J Biol Chem* **2000**, *275*, 10379–10387, doi:10.1074/jbc.275.14.10379.
211. Zhou, W.; Carpenter, G. Heregulin-dependent trafficking and cleavage of ErbB-4. *J Biol Chem* **2000**, *275*, 34737–34743, doi:10.1074/jbc.M003756200.
212. Ni, C.Y.; Murphy, M.P.; Golde, T.E.; Carpenter, G. gamma -Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. *Science* **2001**, *294*, 2179–2181, doi:10.1126/science.1065412.
213. Carpenter, G. ErbB-4: mechanism of action and biology. *Exp Cell Res* **2003**, *284*, 66–77, doi:10.1016/s0014-4827(02)00100-3.
214. Carpenter, G. Nuclear localization and possible functions of receptor tyrosine kinases. *Curr Opin Cell Biol* **2003**, *15*, 143–148, doi:10.1016/s0955-0674(03)00015-2.
215. Cheng, Q.C.; Tikhomirov, O.; Zhou, W.; Carpenter, G. Ectodomain cleavage of ErbB-4: characterization of the cleavage site and m80 fragment. *J Biol Chem* **2003**, *278*, 38421–38427, doi:10.1074/jbc.M302111200.
216. Schlessinger, J.; Lemmon, M.A. Nuclear signaling by receptor tyrosine kinases: the first robin of spring. *Cell* **2006**, *127*, 45–48, doi:10.1016/j.cell.2006.09.013.
217. Blobel, C.P.; Carpenter, G.; Freeman, M. The role of protease activity in ErbB biology. *Exp Cell Res* **2009**, *315*, 671–682, doi:10.1016/j.yexcr.2008.10.011.
218. Gilmore, J.L.; Riese, D.J., 2nd. secErbB4-26/549 antagonizes ligand-induced ErbB4 tyrosine phosphorylation. *Oncol Res* **2004**, *14*, 589–602, doi:10.3727/0965040042707907.
219. Ni, C.Y.; Yuan, H.; Carpenter, G. Role of the ErbB-4 carboxyl terminus in gamma-secretase cleavage. *J Biol Chem* **2003**, *278*, 4561–4565, doi:10.1074/jbc.M210504200.
220. Lee, H.J.; Jung, K.M.; Huang, Y.Z.; Bennett, L.B.; Lee, J.S.; Mei, L.; Kim, T.W. Presenilin-dependent gamma-secretase-like intramembrane cleavage of ErbB4. *J Biol Chem* **2002**, *277*, 6318–6323, doi:10.1074/jbc.M110371200.
221. Linggi, B.; Carpenter, G. ErbB-4 s80 intracellular domain abrogates ETO2-dependent transcriptional repression. *J Biol Chem* **2006**, *281*, 25373–25380, doi:10.1074/jbc.M603998200.
222. Linggi, B.; Cheng, Q.C.; Rao, A.R.; Carpenter, G. The ErbB-4 s80 intracellular domain is a constitutively active tyrosine kinase. *Oncogene* **2006**, *25*, 160–163, doi:10.1038/sj.onc.1209003.
223. Naresh, A.; Long, W.; Vidal, G.A.; Wimley, W.C.; Marrero, L.; Sartor, C.I.; Tovey, S.; Cooke, T.G.; Bartlett, J.M.; Jones, F.E. The ERBB4/HER4 intracellular domain 4ICD is a BH3-only protein promoting apoptosis of breast cancer cells. *Cancer Res* **2006**, *66*, 6412–6420, doi:10.1158/0008-5472.CAN-05-2368.
224. Kuwana, T.; Mackey, M.R.; Perkins, G.; Ellisman, M.H.; Latterich, M.; Schneiter, R.; Green, D.R.; Newmeyer, D.D. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell* **2002**, *111*, 331–342, doi:10.1016/s0092-8674(02)01036-x.

 doi:10.20944/preprints202604.0001.v1

225. Letai, A.; Bassik, M.C.; Walensky, L.D.; Sorcinelli, M.D.; Weiler, S.; Korsmeyer, S.J. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* **2002**, *2*, 183–192, doi:10.1016/s1535-6108(02)00127-7.
226. Miller, M.A.; Sullivan, R.J.; Lauffenburger, D.A. Molecular Pathways: Receptor Ectodomain Shedding in Treatment, Resistance, and Monitoring of Cancer. *Clin Cancer Res* **2017**, *23*, 623–629, doi:10.1158/1078-0432.CCR-16-0869.
227. Arienti, C.; Pignatta, S.; Tesei, A. Epidermal Growth Factor Receptor Family and its Role in Gastric Cancer. *Front Oncol* **2019**, *9*, 1308, doi:10.3389/fonc.2019.01308.
228. Segers, V.F.M.; Dugaucquier, L.; Feyen, E.; Shakeri, H.; De Keulenaer, G.W. The role of ErbB4 in cancer. *Cell Oncol (Dordr)* **2020**, *43*, 335–352, doi:10.1007/s13402-020-00499-4.
229. Williams, C.C.; Allison, J.G.; Vidal, G.A.; Burow, M.E.; Beckman, B.S.; Marrero, L.; Jones, F.E. The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone. *J Cell Biol* **2004**, *167*, 469–478, doi:10.1083/jcb.200403155.
230. Gallo, R.M.; Bryant, I.N.; Mill, C.P.; Kaverman, S.; Riese, D.J., 2nd. Multiple Functional Motifs Are Required for the Tumor Suppressor Activity of a Constitutively-Active ErbB4 Mutant. *J Cancer Res Ther Oncol* **2013**, *1*, 10.
231. Heery, D.M.; Kalkhoven, E.; Hoare, S.; Parker, M.G. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **1997**, *387*, 733–736, doi:10.1038/42750.
232. Edwards, D.P. The role of coactivators and corepressors in the biology and mechanism of action of steroid hormone receptors. *J Mammary Gland Biol Neoplasia* **2000**, *5*, 307–324, doi:10.1023/a:1009503029176.
233. Zhu, Y.; Sullivan, L.L.; Nair, S.S.; Williams, C.C.; Pandey, A.K.; Marrero, L.; Vadlamudi, R.K.; Jones, F.E. Coregulation of estrogen receptor by ERBB4/HER4 establishes a growth-promoting autocrine signal in breast tumor cells. *Cancer Res* **2006**, *66*, 7991–7998, doi:10.1158/0008-5472.CAN-05-4397.
234. Veikkolainen, V.; Vaparanta, K.; Halkilahti, K.; Iljin, K.; Sundvall, M.; Elenius, K. Function of ERBB4 is determined by alternative splicing. *Cell Cycle* **2011**, *10*, 2647–2657, doi:10.4161/cc.10.16.17194.
235. Wang, Z. ErbB Receptors and Cancer. *Methods Mol Biol* **2017**, *1652*, 3–35, doi:10.1007/978-1-4939-7219-7_1.
236. Wang, W.; Zhao, H.F.; Yao, T.F.; Gong, H. Advanced development of ErbB family-targeted therapies in osteosarcoma treatment. *Invest New Drugs* **2019**, *37*, 175–183, doi:10.1007/s10637-018-0684-8.
237. Naresh, A.; Thor, A.D.; Edgerton, S.M.; Torkko, K.C.; Kumar, R.; Jones, F.E. The HER4/4ICD estrogen receptor coactivator and BH3-only protein is an effector of tamoxifen-induced apoptosis. *Cancer Res* **2008**, *68*, 6387–6395, doi:10.1158/0008-5472.CAN-08-0538.
238. Komuro, A.; Nagai, M.; Navin, N.E.; Sudol, M. WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. *J Biol Chem* **2003**, *278*, 33334–33341, doi:10.1074/jbc.M305597200.
239. Omerovic, J.; Puggioni, E.M.; Napoletano, S.; Visco, V.; Fraioli, R.; Frati, L.; Gulino, A.; Alimandi, M. Ligand-regulated association of ErbB-4 to the transcriptional co-activator YAP65 controls transcription at the nuclear level. *Exp Cell Res* **2004**, *294*, 469–479, doi:10.1016/j.yexcr.2003.12.002.
240. Aqeilan, R.I.; Donati, V.; Palamarchuk, A.; Trapasso, F.; Kaou, M.; Pekarsky, Y.; Sudol, M.; Croce, C.M. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. *Cancer Res* **2005**, *65*, 6764–6772, doi:10.1158/0008-5472.CAN-05-1150.
241. Sundvall, M.; Veikkolainen, V.; Kurppa, K.; Salah, Z.; Tvorogov, D.; van Zoelen, E.J.; Aqeilan, R.; Elenius, K. Cell death or survival promoted by alternative isoforms of ErbB4. *Mol Biol Cell* **2010**, *21*, 4275–4286, doi:10.1091/mbc.E10-04-0332.
242. Mukherjee, N.; Dart, C.R.; Amato, C.M.; Honig-Frand, A.; Lambert, J.R.; Lambert, K.A.; Robinson, W.A.; Tobin, R.P.; McCarter, M.D.; Couts, K.L.; et al. Expression Differences in BCL2 Family Members between Uveal and Cutaneous Melanomas Account for Varying Sensitivity to BH3 Mimetics. *J Invest Dermatol* **2022**, *142*, 1912–1922 e1917, doi:10.1016/j.jid.2021.11.035.
243. Lubrano, S.; Cervantes-Villagrana, R.D.; Arang, N.; Officer, A.; Adame-Garcia, S.R.; Cuesta-Margolles, G.; Aplin, A.E.; Gutkind, J.S. Inhibition of anti-apoptotic BCL2 overcomes adaptive resistance to co-targeting of the protein kinase FAK and MEK in GNAQ-driven uveal melanoma. *J Biol Chem* **2025**, *301*, 110712, doi:10.1016/j.jbc.2025.110712.

 doi:10.20944/preprints202604.0001.v1

244. Nemati, F.; de Montrion, C.; Lang, G.; Kraus-Berthier, L.; Carita, G.; Sastre-Garau, X.; Berniard, A.; Vallerand, D.; Geneste, O.; de Plater, L.; et al. Targeting Bcl-2/Bcl-XL induces antitumor activity in uveal melanoma patient-derived xenografts. *PLoS One* **2014**, *9*, e80836, doi:10.1371/journal.pone.0080836.
245. Miller, M.; Schoenfield, L.; Abdel-Rahman, M.; Cebulla, C.M. Is Uveal Melanoma a Hormonally Sensitive Cancer? A Review of the Impact of Sex Hormones and Pregnancy on Uveal Melanoma. *Ocul Oncol Pathol* **2021**, *7*, 239–250, doi:10.1159/000514650.
246. Schoenfield, L.; Janse, S.; Kline, D.; Aronow, M.E.; Singh, A.D.; Craven, C.; Abdel-Rahman, M.; Cebulla, C.M. Estrogen Receptor Is Expressed in Uveal Melanoma: A Potential Target for Therapy. *Ocul Oncol Pathol* **2021**, *7*, 303–310, doi:10.1159/000512174.
247. Liu, K.; Zou, C.; Qin, B. The association between nuclear receptors and ocular diseases. *Oncotarget* **2017**, *8*, 27603–27615, doi:10.18632/oncotarget.15178.
248. Lucas, L.M.; Cullum, R.L.; Dwivedi, V.; Markham, J.A.; Woggerman, J.N.; Kelley, C.M.; Knerr, E.L.; Cook, L.J.; Lucas, H.C., 2nd; Waits, D.S.; et al. *ERBB4* drives the proliferation of *BRAF* WT melanoma cell lines. Available online: https://www.medrxiv.org/content/10.1101/2022.06.20.22276663v2 (accessed on February 3, 2024).
249. Lucas, L.M.; Cullum, R.L.; Markham, J.A.; Woggerman, J.N.; Dwivedi, V.; Mohamedelhassan, R.H.; Cook, L.J.; Kelley, C.M.; Knerr, E.L.; Kaufmann, D.P.; et al. *ERBB4* mutant alleles may drive *BRAF* WT melanomas. Available online: https://www.medrxiv.org/content/10.1101/2022.06.21.22276707v2 (accessed on February 3, 2024).
250. Dwivedi, V.; Lucas, L.M.; Cooke, R.; Davis, J.; Wilson, E.; Scott, M.; O'Daniel, K.; Dion, C.; Kerley, J.; Zelan, M.; et al. Human melanoma cell lines that possess wild-type BRAF alleles but are dependent on ERBB4 and ERBB2. Available online: https://doi.org/10.1101/2024.08.22.609260 (accessed on August 23, 2024).
251. Wilson, E.; Conway, A.; Riese 2nd, D.J. Cancer Cell Line Encyclopedia Data Suggest that Ligands for ERBB Family Receptors May Drive BRAF-WT Melanomas. Available online: https://doi.org/10.64898/2026.03.16.712185 (accessed on March 27, 2026).
252. MM28 - CRL-3295. Available online: https://www.atcc.org/products/crl-3295 (accessed on March 29, 2026).
253. MP41 - CRL-3297. Available online: https://www.atcc.org/products/crl-3297 (accessed on March 29, 2026).
254. MP46 - CRL-3298. Available online: https://www.atcc.org/products/crl-3298 (accessed on June 16, 2025).