Research Repository UCD

Title	Evaluation of oncogenic cysteinyl leukotriene receptor 2 as a therapeutic target for uveal melanoma
Authors(s)	Slater, Kayleigh, Hoo, Pei Sian, Buckley, A. M., Piulats, J. M., Villanueva, A., Portela, A., Kennedy, Breandán
Publication date	2018-09
Publication information	Slater, Kayleigh, Pei Sian Hoo, A. M. Buckley, J. M. Piulats, A. Villanueva, A. Portela, and Breandán Kennedy. "Evaluation of Oncogenic Cysteinyl Leukotriene Receptor 2 as a Therapeutic Target for Uveal Melanoma" 37, no. 2–3 (September, 2018).
Publisher	Springer
Item record/more information	http://hdl.handle.net/10197/9533
Publisher's statement	The final publication is available at Springer via http://dx.doi.org/[10.1007/s10555-018-9751-z .
Publisher's version (DOI)	10.1007/s10555-018-9751-z

Downloaded 2023-04-07T10:52:29Z

The UCD community has made this article openly available. Please share how this access benefits you. Your story matters! (@ucd_oa)

© Some rights reserved. For more information

TITLE

EVALUATION OF ONCOGENIC CYSTEINYL LEUKOTRIENE RECEPTOR 2 AS A THERAPEUTIC TARGET FOR UVEAL MELANOMA

AUTHORS

Slater K^{1,2}, Hoo PS^{1,3}, Buckley AM³, Piulats JM⁴, Villanueva A^{5,6}, Portela A⁵, Kennedy BN^{1*}

AFFILIATIONS

1 UCD School of Biomolecular & Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, Ireland

2 Genomics Medicine Ireland Limited, Dublin, Ireland

3 Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Trinity College Dublin, Ireland

4 Department of Medical Oncology, Catalan Institute of Oncology – IDIBELL, Barcelona, Spain

5 Xenopat S.L., Barcelona, Spain

6 Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology (ICO), Bellvitge Institute for Biomedical Research (IDIBELL), Barcelona, Spain

* Email: brendan.kennedy@ucd.ie Tel: +3531 7166740

AUTHOR ORCID ID

KS 0000000155668399

PSH 0000000219942589

AMB 0000000250808580

JMP 0000000236061724

AV 0000000241646671

AP 0000000321842584

BNK 0000000179914689

FUNDING

Research related to some of the topics discussed in this review is funded by an Irish Research Council Employment Based Postgraduate Scholarship (EBP/2017/473). This project area has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 734907 (RISE/3D-NEONET project).

ACKNOWLEDGEMENTS

We wish to thank Noel Horgan, Jens Rauch and Sean Ennis for discussions and comments on the manuscript.

KEYWORDS: uveal melanoma, cysteinyl leukotriene receptor 2, cysteinyl leukotriene signalling, patient-derived xenograft models

Abstract

Uveal melanoma is a rare, but deadly, form of eye cancer that arises from melanocytes within the uveal tract. Although advances have emerged in treatment of the primary tumour, patients are still faced with vision loss, eye enucleation and lethal metastatic spread of the disease. Approximately 50% of uveal melanoma patients develop metastases, which occur most frequently to the liver. Metastatic patients encounter an extremely poor prognosis; as few as 8% survive beyond 2 years. Understanding of the genetic underpinnings of this fatal disease evolved in recent years with the identification of new oncogenic mutations that drive uveal melanoma pathogenesis. Despite this progress, the lack of successful therapies or a proven *standard-of-care* for uveal melanoma highlights the need for new targeted therapies. This review focuses on the recently identified *CYSLTR2* oncogenic mutation in uveal melanoma. Here, we evaluate the current status of uveal melanoma and investigate how to better understand the role of this *CYSLTR2* mutation in the disease and implications for patients harbouring this mutation.

Epidemiology and aetiology of uveal melanoma

Uveal melanomas, which arise from the choroid (85-90% of cases), ciliary body (5-8% of cases) or iris (3-5% of cases), account for approximately 5.2% of all primary melanomas [1]. Although considered a rare disease, incidence ranges from < 2 per million to > 8 per million across Europe [2], uveal melanoma is the primary intraocular tumour found in adults. The overall incidence of uveal melanoma has remained relatively constant in comparison to other cancer types, but varies by race, sex and country [3]. Males have greater disease incidence than females and uveal melanoma is more common among Caucasians than non-Caucasians [4]. Interestingly, the National Cancer Registry Ireland reports an estimated 62 new cases of neoplasms of the eye and adnexa diagnosed in Ireland between 2015 - 2017 [5], this compares to approximately 1,700 new cases per year in the United States [6] and 430 new cases per year in the United Kingdom [7], suggesting that Ireland has a higher incidence of the disease per capita (1.3 cases per 100,000 per year in Ireland versus 0.52 cases per 100,000 per year in the U.S.).

Uveal melanoma patients are often asymptomatic (30.2% of patients), with disease first diagnosed during routine ophthalmic examination [8]. Patients experience blurred vision, the presence of floaters and/or perceived flashes of light, visual loss and pain in the eye [8].

Risk factors associated with uveal melanoma include an inability to tan, the presence of light coloured eyes (blue or green), fair skin, ocular melanocytosis and the presence of germline mutations in *BAP1* (BRCA – associated protein-1), a tumour suppressor gene found on chromosome 3 [3]. The role of ultraviolet light remains unclear; many uveal melanomas show no evidence of the UV radiation mutational signature commonly found in cutaneous melanoma [9]. However, intermittent ultraviolet exposure through welding arcs and flames is reported as a significant risk factor for uveal melanoma [10].

Importantly, uveal melanoma is clinically and molecularly distinct from cutaneous melanoma, the most common melanoma subtype [11]. Therefore, recent advances in targeted therapies for the treatment of cutaneous melanoma have failed to alter the clinical outcomes of uveal melanoma patients [12]. Disease- and most importantly, mutation-specific therapies for uveal melanoma are critical and likely to provide the most promising therapeutic strategies for uveal melanoma patients.

Prognosis of uveal melanoma

Treatment of primary uveal melanoma

Treatment of the primary disease is surgical, (*e.g.* resection or enucleation) to remove the tumour from the eye, or, more conservative radiation or laser therapy, which aim to preserve the affected eye [13]. Enucleation involves complete removal of the eye and orbital recurrence of the cancer after primary enucleation is rare [14]. Enucleation is common in cases of large (> 8 mm), locally advanced tumours in which vision cannot be retained [4]. However, globe-conserving therapies have become increasingly popular after the 2006 Collaborative Ocular Melanoma Study confirmed no differences in survival between patients treated with iodine-125 brachytherapy and enucleation [15].

Brachytherapy for uveal melanoma involves placement of radioactive implants, most commonly emitting iodine-125 (¹²⁵I) or ruthenium-106 (¹⁰⁶R), directly on to the eye for several days [15, 16]. This allows for a concentrated dose of radiotherapy to be delivered directly to the tumour. Laser therapies, such as photodynamic therapy (PDT) and transpupillary thermotherapy (TTT) are also available, however, they are associated with a risk of local tumour recurrence [17, 18].

Treatment of metastatic uveal melanoma

Despite advances in the treatment of the primary ocular tumour, the prognosis of patients that develop metastatic uveal melanoma remains poor and the effect of ocular therapy on metastasis and survival remains uncertain [19]. Approximately 50% of patients develop metastatic disease, with the liver being the most common site (89% of metastatic patients), followed by the lung, bone and soft tissue [20]. The median overall survival from diagnosis of metastatic uveal melanoma ranges from less than 6 months to 13.4 months, with only 8% of patients surviving beyond 2 years [20, 21].

Unfortunately, the prognosis for metastatic patients is bleak. There remains no proven *standard-of-care* available for metastatic uveal melanoma patients [13]. Dacarbazine, a chemotherapeutic used in cutaneous melanoma, has limited therapeutic benefit in uveal melanoma [22]. Fundamental molecular differences in the two melanomas are the obvious reason. Uveal melanomas generally lack the *BRAF* mutations common to cutaneous melanoma and which is an established target for treating disseminated cutaneous disease [23]. Given that >80% of uveal melanomas possess mutations that drive constitutive activation of the MAPK/ERK pathway, drugs targeting this pathway are of major interest [24]. Selumetinib, a small molecule inhibitor of MEK1/2, resulted in improved progression-free survival versus chemotherapy in a phase II clinical trial of uveal melanoma patients [22]. However, no improvement in overall patient survival was reported [22]. Similarly, in a phase III double-blind study, a combination of selumetinib plus dacarbazine did not significantly improve progression free survival in metastatic uveal melanoma patients versus dacarbazine alone [25].

In summary, there is an overwhelming unmet clinical need to develop targeted therapies to improve the prognosis of uveal melanoma patients. Given that the majority of the driver mutations identified to date in uveal melanoma lead to constitutive activation of the MAPK/ERK pathway via aberrant Gαq signalling, the associated G proteins and G protein-coupled receptors represent enticing therapeutic targets for the prevention and/or treatment of the disease.

Genetic alterations in uveal melanoma

Notably, the primary mutations linked with development and progression of uveal melanoma are entirely distinct from those in cutaneous melanoma. Cutaneous melanoma has one of the highest mutational loads amongst cancer types, while uveal melanoma has a low mutational burden [26]. Roberson *et al.* reported a median somatic mutation density of 1.1 per Mb in uveal melanoma, which was markedly lower than in cutaneous melanoma, other melanoma subtypes or other solid tumours [9]. The lack of *bona fide* mutations in uveal melanoma has meant that the scope for targeted therapies is quite limited, with no successful targeted therapies to date.

Uveal melanoma can be subdivided into molecular classes, Class 1 or 2, based on a 15-gene assay developed by Onken *et al.* [27, 28]. In terms of 5-year risk of developing metastases, patients with Class 2 tumours harbour a 72% risk, whereas Class 1 tumours harbour a 21% risk [29].

Several chromosomal abnormalities associated with uveal melanoma can inform a patient's prognosis and their likelihood of metastasis [30]. 8q and 6p gains are frequently observed in uveal melanoma [31], as are losses in 1p, 6q and chromosome 3 [32]. Loss of 1p and chromosome 3, and gain of 8q are associated with worse patient prognosis and often found in Class 2 tumours, whereas gain of 6p is associated with a better patient outcome and commonly associated with Class 1 tumours [27, 33]. In particular, monosomy 3 is an extremely important prognostic test and is frequently associated with metastasis and Class 2 tumours [33].

Additional analysis of 80 uveal melanomas from TCGA (The Cancer Genome Atlas <https://cancergenome.nih.gov/>) identified four distinct and clinically relevant disease subtypes; two associated with monosomy 3 and poor patient prognosis and two associated with disomy 3 and a more positive patient prognosis [9]. Disomy 3 uveal melanomas

were further divided into transcription-based clusters 1 and 2, while monosomy 3 uveal melanoma were further divided into transcription-based clusters 3 and 4 [9].

Uveal melanomas are predominantly characterised by mutations in *GNAQ* and *GNA11* (a paralog of *GNAQ*), both of which encode for G-protein alpha subunits and share 90% amino acid sequence homology [34]. Overall, 83% of uveal melanomas contain mutations in either *GNAQ* or *GNA11*, however, these mutations do not correlate with prognosis [35]. *GNAQ* mutations occur almost exclusively at codon 209 and result in glutamine to leucine (p.Gln209Leu), or proline (p.Gln209Pro) amino acid substitutions. In both cases, this mutation occurs within the GTPase domain and results in a constitutively active G-protein [36]. Similarly, mutations in *GNA11* are predominantly found at position Q209 and result in similar downstream consequences [35]. In 2016, a recurrent hotspot mutation in *PLCB4*, a downstream target of *GNAQ*/*GNA11* was identified in 2 of 28 samples assayed [37]. *PLCβ4* is activated upon binding of a G-protein subunit, resulting in cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce diacylglycerol (DAG) and inositol triphosphate (IP₃), and calcium release from the cell. The *PLCB4* hotspot mutation is also a gain-of-function mutation leading to constitutive activation of the MAPK/ERK pathway.

Recurrent mutations in splicing factor *SF3B1* occur at codon 625 in approximately 18.6% of tumours and are associated with low-grade uveal melanomas with good prognosis [38]. Similarly, mutations in *EIF1AX* are associated with better patient outcomes [39]. *SF3B1* and *EIF1AX* mutations appear to occur most frequently in uveal melanomas with disomy 3, which rarely metastasize and are often grouped into the Class 1 category [39].

BAP1 (BRCA associated protein-1) mutations are found in approximately 84% of metastasizing uveal melanoma tumours [40]. *BAP1* maps to chromosome 3p21.1 and is implicated as a tumour suppressor gene [26]. Both somatic and germline mutations in *BAP1* occur in uveal melanoma patients [40]. *SF3B1* and *BAP1* mutations are almost mutually exclusive, as also suggested for *BAP1* and *EIF1AX* [41], suggesting that they represent alternative pathways in tumour progression [38].

Recently, Moore *et al.* analysed DNA data from 136 uveal melanoma patients and identified seven significantly mutated codons in six genes [41]. Amongst those identified were *GNAQ*, *GNA11*, *PLCB4*, *SF3B1*, and *EIF1AX*; all previously linked to uveal melanoma. Interestingly, they also identified a c.386T>A mutation in cysteinyl leukotriene receptor 2 (*CYSLTR2*) which encodes a p.Leu129Gln substitution not previously described in the literature [41].

This activating, recurrent hotspot mutation in *CYSLTR2* was identified in 4 of 136 uveal melanoma patient samples analysed from different cohorts [41]. Interestingly, this mutation was found only in patients lacking *GNAQ*, *GNA11* or *PLCB4* mutations, all of which are established driver mutations in uveal melanoma. The presence of mutually exclusive somatic mutations in *GNAQ*, *GNA11*, *CYSLTR2* and *PLCB4* was further confirmed in a comprehensive analysis of patient samples in the Rare Tumor Project of The Cancer Genome Atlas (TCGA) by Robertson *et al* [9]. As mutually exclusive mutations often operate in the same pathway, this data suggests that the newly identified *CYSLTR2* mutation is associated with the same pathway as previously identified driver mutations, highlighting the importance of this *CYSLTR2*/*Gaq*/11/*PLCB4* pathway and of *Gaq* signalling in uveal melanoma oncogenesis.

Mutations in *GNAQ* and *GNA11* are not predictive of prognosis or the likelihood of metastases. However, patients lacking *GNAQ* or *GNA11* mutations have worse disease-free and overall survival than those with these mutations. This suggests that patients harbouring alternative mutations such as *CYSLTR2* or *PLCB4* may have a worse prognosis than those carrying *GNAQ*/*GNA11* mutations [35].

Activating mutations in *GNAQ* or *GNA11* are found in >80% of all uveal melanomas, irrespective of tumour class, and are also frequent in blue nevi, melanocytic nevi found in the dermal layer of the skin. Mutations in either *CYSLTR2* or *PLCB4* likely account for an additional 8-10% of activating mutations. Robertson *et al.* reported that neither *CYSLTR2* nor *PLCB4* mutations preferentially localized to a specific subset of uveal melanoma, consistent with mutations in these genes functioning like *GNAQ* and *GNA11* mutations to drive tumorigenesis without initiating metastasis [9]. One theory suggests that the mutation associated with the *CYSLTR2*/*Gaq*/11/*PLCB4* pathway occur early in tumour progression and are important initiating events but are not sufficient for malignant transformation. In contrast, genomic *BAP1* pathway mutations occur later in the progression of uveal melanoma and likely correspond with tumour metastasis [40]. Thus, simultaneous targeting of both *Gaq* coupled receptor signalling and *BAP1*

signalling pathway mutations might have synergistic therapeutic effects in the treatment of uveal melanoma. Targeting of the *BAP1* pathway has proven effective in different cancer types. Indeed, olaparib, an oral PARP inhibitor, has anti-tumour activity in metastatic breast cancer patients with germline *BRCA* mutations [42].

Cysteinyl leukotriene signalling

The novel oncogenic mutations in *CYSLTR2* warrant further investigation of the associated signalling pathway in the pathogenesis and treatment of ocular melanoma. The cysteinyl leukotrienes (CysLTs), LTC₄, LTD₄ and LTE₄, are a group of inflammatory, lipid, signalling molecules that mediate both acute and chronic inflammation. Indeed, cysteinyl leukotriene receptor antagonists are routinely prescribed in the treatment of asthma and allergic rhinitis [43, 44]. These eicosanoids are synthesized from arachidonic acid (AA) in the cell membrane upon cell activation. The 5-lipoxygenase enzyme (5-LOX) interacts with a 5-lipoxygenase activating protein (FLAP) which enhances the activity of 5-LOX to convert AA mobilised to the cytosol to the unstable leukotriene LTA₄ [45]. LTA₄ is further hydrolysed to LTB₄ or LTC₄ via LTC₄ synthase. Intracellularly synthesized LTC₄ is exported from the cell via multidrug resistance-associated proteins and rapidly metabolised to the remaining cysteinyl leukotrienes, LTD₄ or LTE₄ [46]. Synthesis of the CysLTs occurs predominantly in immune cells such as neutrophils, eosinophils, monocytes, macrophages and mast cells [47].

Thus, the CysLTs are a group of structurally similar but functionally different lipid mediators that exert their biological effects via binding to the GPCRs (G-protein-coupled receptors), CysLT₁ and CysLT₂⁽¹⁾ [48]. CysLT₁ and CysLT₂ are located on the plasma membrane [49, 50], however, both receptors possess the ability to localize to the nuclear membrane [51, 52]. LTC₄ and LTD₄ binds CysLT₂ with low, but equal affinity, LTD₄ and LTC₄ bind CysLT₁ with high and low affinity, respectively [49]. Neither receptor subtype exhibits substantial affinity for LTE₄ [48]. However, additional CysLT receptors, GPR17 and GPR99 have been reported. GPR17 is a G protein-coupled orphan receptor with homology to both the P2Y and CysLT receptors. GPR17 is reported as a ligand-independent negative regulator of CysLT₁ [53]. GPR99, also described as cysteinyl leukotriene receptor E (CysLTE) or CysLT₃ is proposed as a potential LTE₄ selective cysteinyl leukotriene receptor [46].

Cysteinyl leukotriene signalling in cancer

Cysteinyl leukotriene signalling is implicated in inflammation, bronchoconstriction, increased vascular permeability, mucus production and white blood cell recruitment [54-56]. A recent review evaluated links between CysLT receptors and many hallmarks of cancer including angiogenesis, sustained proliferative signalling, migration and invasion [57]. Interestingly, overexpression of CysLT₁ presents in colorectal cancer, prostate cancer, renal cell carcinoma, transitional cell carcinoma and testicular cancer [58-61]. Tsai *et al.* conducted a large, population-based study to investigate the effect of leukotriene receptor antagonists on the risk of cancer development in newly diagnosed asthmatic patients. Leukotriene receptor antagonists decreased the risk of 14 different cancers analysed in a dose dependent manner, suggesting that CysLT receptor antagonism provides a cancer-protective effect [62].

Moore *et al.* identified the recurrent hotspot mutation in *CYSLTR2* as a driver oncogene [41]. The oncogenic properties of the CysLT₂ were later supported by Möller *et al.* who identified the same Leu129Gln hotspot mutation in blue nevi [63]. Interestingly, in other cancer types CysLT₂ exerts anti-cancer properties. CRC patients with high nuclear CysLT₁ expression have a poor prognosis, while patients with high nuclear CysLT₂ expression have a better overall prognosis, suggesting that CysLT₂ is protective in CRC [64]. Magnusson *et al.* reported a similar phenomenon in breast cancer patients, whereby patients with large tumours exhibiting high CysLT₁ and low CysLT₂ expression levels had a significantly reduced survival [65]. Indeed, it is suggested that regulation of CysLT₂, leading to increased expression of the receptor, may have anti-tumour properties in CRC [66, 67].

Two *CYSLTR2* mutations, p.Arg136His and p.Arg136Cys, were identified in colorectal cancer [41]. However, with exception to the Leu129Gln hotspot mutation in uveal melanoma and blue nevi, *CYSLTR2* is not significantly mutated in any other cancer types, nor have other hotspot mutations been identified, suggesting this is a unique driver mutation in uveal melanoma and blue nevi. However, *CYSLTR2* is overexpressed in certain acute myeloid leukaemia subtypes [68].

This raises an interesting question about the role played by the different cysteinyl leukotriene receptors in various cancer subtypes. Increased expression of endogenous CysLT₂ has a protective effect linked to negative regulation of

CysLT₁ [69, 70]. Lack of CysLT₂ receptors may facilitate the formation of CysLT₁ homodimers, leading to heightened LTD₄ signalling which may promote a pro-tumorigenic phenotype [48]. Constitutive activation of CysLT₂ in uveal melanoma acts as an oncogene, suggesting opposing effects to those documented in colorectal and breast cancer. It will be interesting to determine if the oncogenic *CYSLTR2* mutation influences CysLT₁ signalling, expression or localization.

Cysteinyl leukotriene receptor 2 as a uveal melanoma oncogene

The CysLT₂ mutation associated with uveal melanoma and more recently, blue nevi, occurs at Leu129, which is situated in transmembrane helix 3, a functional hub of the receptor. This mutation leads to constitutive activation of the receptor and endogenous signalling, leaving it unresponsive to leukotriene stimulation *in vitro* [41]. Moore *et al.* characterised the oncogenic potential of this mutation by stably expressing the mutant Leu129Gln CysLT₂ in melanoma cells [41]. Mutant Leu129Gln, but not wild-type CysLT₂, conferred TPA(12-O-Tetradecanoylphorbol-13-acetate)-independent growth *in vitro* [41]. In agreement, siRNA mediated knockdown of exogenous *CYSLTR2* reduced the growth of melanoma cells grown in the presence or absence of TPA but had no effect on those expressing the wild-type receptor [41]. This exciting preliminary *in vitro* data suggests that inhibition of CysLT₂ in patients harbouring this oncogenic mutation may have therapeutic potential in the treatment of uveal melanoma.

Melanoma cells applied by Moore *et al.* are a melanocyte, non-tumorigenic cell line derived from the embryonic skin of 18-day-old C57BL mice and require phorbol-esters such as TPA for growth [71]. While melanoma cells are commonly used in melanoma research [35, 36], it will be important to also investigate the effects of this oncogenic mutation in human derived uveal melanoma cells. When mutant Leu129Gln was stably expressed in Mel290 cells, a human uveal melanoma cell line lacking *GNAQ* or *GNA11* mutations, the expression of melanocyte-lineage specific genes was significantly upregulated by RT-qPCR analysis compared to empty vector and wild-type control [41]. It will be interesting to examine whether expression of the oncogenic Leu129Gln mutation alters the cellular phenotype or additional hallmarks of cancer in uveal melanoma *in vitro* and *in vivo*. The effect of knockdown, or indeed knockout of the receptor in uveal melanoma cells also remains to be established. Similar experiments could also be conducted and validated using the Mel285 uveal melanoma cancer cell line, which is also reported as wild-type for both *GNAQ* and *GNA11* [72].

To strengthen the CysLT₂/Gaq/11/PLCβ4 pathway hypothesis, steps should be taken to examine the downstream signalling effects associated with the constitutively active Leu129Gln *CYSLTR2* mutation. Given that the best understood signalling pathway in uveal melanoma is the MAPK/ERK pathway, which is known to be activated by *GNAQ* and *GNA11* mutations [34], it is likely the *CYSLTR2* mutation upregulates this pathway. *GNAQ* and *GNA11* mutated uveal melanoma cell lines cause increased expression of phosphorylated MEK and phosphorylated ERK, which can be abolished via knockdown of the respective gene [36, 73]. In *GNAQ* and *GNA11* mutated cell lines, MAPK pathway activation occurs as a result of PKC activation [73]. As such, levels of p-MARCKS, a substrate of PKC, are detectable in uveal melanoma cells harbouring these mutations and can also be suppressed following knockdown [73]. Similar results would be expected from cell lines expressing the *CYSLTR2* mutation.

Given the well documented role of CysLT receptors in angiogenesis and inflammation, additional IHC and expression analysis could examine the vascular and inflammatory status of the Leu129Gln expressing cells. Cysteinyl leukotriene receptor antagonists can promote anti-angiogenic activity via a VEGF-independent pathway [74, 75]. It will be interesting to examine the levels of VEGF and other associated angiogenic markers in the oncogene background. Given the cross-regulation that occurs between the CysLT receptor subtypes, investigation into the effect of the Leu129Gln mutation on the expression of CysLT₁ is warranted.

Moore *et al.* also reported tumorigenic properties of the Leu129Gln *CYSLTR2* mutation *in vivo*. Leu129Gln expressing cells engrafted subcutaneously into immunocompromised mice significantly accelerated tumour formation versus the empty vector control [41]. These findings demand further investigation using additional model organisms and more advanced preclinical tumour models to evaluate the role of cysteinyl leukotriene signalling in uveal melanoma *in vivo* and to determine the relevance of *CYSLTR2* mutations to the patient disease.

Patient derived xenograft (PDX) models of uveal melanoma

Patient-derived xenograft (PDX) models have become a powerful tool in cancer research. PDX models are generated when cancerous cells or tissue taken directly from a patient's tumour are implanted into an immunocompromised mouse. Accumulating evidence suggests that PDX models have major advantages over the traditional cell line derived xenograft models as they show less divergence from the original patient tumour and more closely resemble the patient sample in terms of histology, gene expression, therapeutic response and metastatic behaviour [76-78].

Heterotopic uveal melanoma PDX models were previously generated, with a 28% engraftment success rate [79, 80]. Tumours taken from primary ocular tumours or metastases were implanted into the interscapular fat pad of SCID (severe combined immunodeficiency) female mice [80]. While useful for pharmacological studies, subcutaneous PDX models come with limitations. Firstly, they present a low engraftment rate and a slow tumour growth. Moreover, as expected, the vast majority of human solid tumours that grow subcutaneously in mice do not metastasize.

Orthotopic PDX (PDOX) or orthoxenografts are generated when the tumour is implanted into the organ of its origin. PDOX models better recall molecular features, histology, metastasis and drug response patterns, making them more suitable for translational research [81]. Recently, PDOX models using uveal melanoma liver metastases were developed with 10 of 12 hepatic metastasis specimens successfully xenografted into immunocompromised mice [82]. Similarly, orthotopic transplantation of uveal melanoma tumours directly into the eye will be extremely important to truly model the correct tumour environment. Exciting preliminary data shows the successful development of orthotopic uveal melanoma xenografts implanted directly into the eye (*Figures 1 & 2*). To our knowledge, this is the first report of successful orthotopic transplantation into the eye. These PDOX models will undoubtedly prove invaluable tools in the field of uveal melanoma research and for the identification of therapeutic strategies.

Figure 1 SCID mouse with orthotopically engrafted uveal melanoma tumour (T). This PDOX model was generated from human tumour tissue obtained from enucleation. A small tumour fragment was mechanically disaggregated, mixed with Matrigel and injected into eye.

Figure 2 Histology of PDOX model of uveal melanoma showing evidence of tumour growth in the ciliary body. Tumour cells are spindle shaped, heavily pigmented in some areas with uniform nuclei and are arranged in a spiral pattern.

Undoubtedly, large numbers of PDOX models are needed to accurately reflect the mutational diversity found in uveal melanoma and to reflect the different sites in which uveal melanomas are found (choroid, ciliary body and iris). It has been reported that tumours harbouring *GNA11* mutations grow significantly better than *GNAQ* mutated tumours and that metastatic tumours engrafted more successfully than those taken from the eye when implanted subcutaneously [79]. It will be of interest to see the effect of *CYSLTR2* and *PLCB4* mutations on PDX development. Given the rarity of these mutations in uveal melanoma patients it may take some time to generate PDX models with the desired mutations. However, the generation of PDOX models derived from patients harbouring the *CYSLTR2* mutation would allow for more in depth analysis of this mutation and its role in disease progression, metastasis and drug responsiveness. Once a successful PDOX model harbouring the *CYSLTR2* mutation is established, the tumour can be expanded to generate a tumour bearing colony of mice in molecular pathology and therapeutic efficacy can be analysed. Given the rarity of *CYSLTR2* mutations, this approach will offer a quicker and more comprehensive method of analysing the consequences of this mutation. It will be exciting to examine the effect of CysLT receptor antagonists in cell lines and *in vivo* models expressing the mutant *CYSLTR2*. However, given the constitutively active nature of the mutant receptor, it is likely that regular antagonists of the receptor will be ineffective.

Inverse agonists to target CysLTR₂

CysLT₁ antagonists, montelukast, zafirlukast and pranlukast are prescribed for the treatment of asthma and allergic rhinitis. BAY u9773 is a non-selective cysteinyl leukotriene receptor antagonist at both CysLT₁ and CysLT₂ receptors [83], while HAMI 3379 is described as a potent and selective CysLT₂ antagonist [84].

Aberrant expression and activity of GPCRs in cancer is well established and they have become a compelling therapeutic target in the disease. In order to effectively target the Leu129Gln mutation in *CYSLTR2* an inverse agonist that selectively targets this receptor will be required. Inverse agonists preferentially bind to and stabilize a constitutively active receptor, maintaining the receptor in an inactive state and thus have intrinsic negative activity [85]. This differs to a neutral antagonist which can block the actions of agonists and inverse agonists. Neutral antagonists exhibit equal preference for both the active and inactive state and have no intrinsic activity [85].

GPCRs represent one of the most common drug targets and yet there are few examples of anti-tumour agents that directly target these receptors [86]. Even fewer examples of inverse agonists as anti-cancer agents are available. However, ALX-065, a biparatopic nanobody that acts as inverse agonist, blocks spontaneous activation of the CXCR4 receptor and inhibits cell migration [87, 88], suggesting that inverse agonists may have the potential to act as successful chemotherapeutic agents.

Given that inverse agonists targeting CysLT₁ are currently in clinical use [89], it is certainly possible that an inverse agonist acting at CysLT₂ is available. Indeed, many compounds that were previously classified as antagonists, actually possess inverse agonist activity [90], suggesting that some anti-cancer GPCR antagonists may in fact mediate their effects through inverse agonism. BAY u9973 does not act as an inverse agonist at CysLT₁ [89], however, it exhibits weak potency at the human CysLT₁ and the exact activity of this drug at CysLT₂ remains to be studied. In addition, it will be important to test HAMI 3379 to determine if this selective antagonist possesses similar inverse agonist capabilities which could be used to target the constitutively active CysLT₂ receptor.

The relevance of a *CYSLTR2* mutation to the patient disease

The *CYSLTR2* mutation can be considered a rare mutation in a rare form of cancer. Moore *et al.* identified this mutation in 4 of 136 patients (~3% of study subjects) [41]. Three of the identified samples came from a cohort of 80 samples taken from the TCGA, while one additional sample came from a cohort of 22 samples from the University of Duisburg-Essen (UNI-UDE). In the United States, approximately 1,700 patients are diagnosed with this cancer each year [6], suggesting that a potential 51 newly diagnosed patients have CysLT₂ mutations.

The UNI-UDE sample came from the enucleated eye of a 77-year-old male treatment naïve for the disease. This tumour was positive for monosomy 3 and possessed a *BAP1* mutation. Sample V4 A9ED from TCGA was a stage IIIa tumour from a Caucasian male, diagnosed at 42 years old. Sample YZ A982 was a stage IIb tumour from a Caucasian female, diagnosed at 79 years old. Sample VD AA80 was a stage IIb tumour from a now deceased male of unknown ethnicity, diagnosed at 77 years old. Given the limited number of patient samples available it is difficult to extrapolate meaningful inferences from the data in terms of tumour and patient characteristics. In the future, with additional patient samples it will be possible to determine whether *CYSLTR2* mutations influence patient survival or the development of metastases.

Blue nevi are common melanocytic tumours that occur in the dermal layer of the skin [63]. Blue nevi generally lack *BRAF* and *NRAS* mutations commonly found in neoplasms of epithelial melanocytes [36]. Instead, blue nevi display a similar genetic profile to that found in uveal melanomas, and frequently possess recurrent activating mutations in *GNAQ* and *GNAI1* [36, 91]. *BAP1* mutations are reported in metastatic blue nevi, further strengthening the role of *BAP1* in metastatic potential and poor patient outcomes in certain cancer subtypes [92, 93]. Based on this knowledge and the additional findings of CysLT₂ and PLCβ4 mutations in uveal melanoma, Möller *et al.* sought to analyse a cohort of blue nevi lacking *GNAQ* or *GNAI1* mutations to determine if driver mutations in *CYSLTR2* and *PLCB4* are also present. 3% of tumours analysed harboured a mutation in *CYSLTR2*, which is identical to the frequency of the mutation reported in uveal melanoma [41, 63]. Moreover, the mutation in *CYSLTR2* was the same c.386T>A, L129Q, mutually exclusive, hotspot mutation identified in uveal melanoma samples by Moore *et al.* [63]. The three *CYSLTR2* mutations reported by Möller *et al.* were found in morphologically benign common blue nevi [63].

These findings highlight the strikingly similar genetic similarities between the two melanocytic tumour types affecting different organ systems, and that similar treatment strategies may be effective against both types of neoplasms.

Given the rare frequency of *CYSLTR2* mutations in uveal melanoma and blue nevi, it is important to continue to study large numbers of tumours to further understand the role of cysteinyl leukotriene receptor 2 in disease and to validate its utility as a therapeutic target. Similarly, the prognosis and survival of those patients identified with *CYSLTR2* mutations should be closely monitored. Furthermore, over-expression and CRISPR/Cas9 mediated knock-out or knock-in strategies targeting the cysteinyl leukotriene receptor 2 will help to further validate its role as a uveal melanoma oncogene and to test the therapeutic potential of targeting the receptor.

Conclusion

There is an overwhelming unmet clinical need to develop new therapeutic strategies for the treatment of uveal melanoma. To date, no targeted therapy has proven successful in the treatment of this disease. The cysteinyl leukotrienes play an established role in inflammation and angiogenesis and have an established role in other cancer subtypes. Moreover, the cysteinyl leukotrienes have been successfully targeted in other diseases and antagonists have demonstrated anti-tumour properties *in vitro* and *in vivo*. The *CYSLTR2* hotspot mutation identified in uveal melanoma acts as an activating, oncogenic driver mutation and may have therapeutic potential in the subset of patients harbouring this mutation. Further *in vitro* and *in vivo* analysis is warranted to fully appreciate the implications of this mutation in terms of altered signalling, likelihood of metastasis and patient prognosis. Similarly, due to the low incidence of the disease, it is not feasible to conduct numerous clinical trials, especially those that are mutation specific. The development of orthotopic PDX models harbouring specific *CYSLTR2* mutations are likely the best way to model the patient disease and to determine the effectiveness of drug strategies targeting this mutation.

FOOTNOTES

⁽¹⁾ Correct nomenclature of the cysteinyl leukotriene receptors (CysLT₁ and CysLT₂) as per the IUPHAR/BPS Guide to PHARMACOLOGY [94].

AUTHOR CONTRIBUTIONS

KS was the primary author of the review. PSH and AMB contributed intellectual input. JMP, AV and AP were responsible for PDOX model development and drafted a section for the review. BNK contributed significant intellectual input, revised and edited the review. All authors reviewed the final manuscript.

CONFLICT OF INTEREST STATEMENT

KS is an employee of Genomics Medicine Ireland. AV is the chief scientific officer and co-founder of Xenopat S.L. AP is the chief executive officer and co-founder of Xenopat S.L.

The other authors declare no competing financial interests that could be construed as a potential conflict of interest.

399 BIBLIOGRAPHY

- 400 1. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous
401 melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on
402 Cancer and the American Cancer Society. *Cancer*. 1998;83(8):1664-78.
- 403 2. Virgili G, Gatta G, Ciccolallo L, Capocaccia R, Biggeri A, Crocetti E, et al. Incidence of uveal melanoma
404 in Europe. *Ophthalmology*. 2007;114(12):2309-15.
- 405 3. Krantz BA, Dave N, Komatsubara KM, Marr BP, Carvajal RD. Uveal melanoma: epidemiology, etiology,
406 and treatment of primary disease. *Clin Ophthalmol* 2017. p. 279-89.
- 407 4. Jovanovic P, Mihajlovic M, Djordjevic-Jocic J, Vlajkovic S, Cekic S, Stefanovic V. Ocular melanoma: an
408 overview of the current status. *Int J Clin Exp Pathol*. 2013;6(7):1230-44.
- 409 5. Registry NC. Cancer in Ireland 1994-2015 with estimates for 2015-2017: Annual Report of the National
410 Cancer Registry. NCR, Cork, Ireland 2017.
- 411 6. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival.
412 *Ophthalmology*. 2011;118(9):1881-5.
- 413 7. Keenan TD, Yeates D, Goldacre MJ. Uveal melanoma in England: trends over time and geographical
414 variation. *Br J Ophthalmol*. 2012;96(11):1415-9.
- 415 8. Damato EM, Damato BE. Detection and time to treatment of uveal melanoma in the United Kingdom: an
416 evaluation of 2,384 patients. *Ophthalmology*. 2012;119(8):1582-9.
- 417 9. Robertson AG, Shih J, Yau C, Gibb EA, Oba J, Mungall KL, et al. Integrative Analysis Identifies Four
418 Molecular and Clinical Subsets in Uveal Melanoma. *Cancer Cell*. 2017;32(2):204-20.e15.
- 419 10. Shah CP, Weis E, Lajous M, Shields JA, Shields CL. Intermittent and chronic ultraviolet light exposure and
420 uveal melanoma: a meta-analysis. *Ophthalmology*. 2005;112(9):1599-607.
- 421 11. Ali Z, Yousaf N, Larkin J. Melanoma epidemiology, biology and prognosis. *EJC Supplements*.
422 2013;11(2):81-91.
- 423 12. Pandiani C, Beranger GE, Leclerc J, Ballotti R, Bertolotto C. Focus on cutaneous and uveal melanoma
424 specificities. *Genes Dev*. 2017;31(8):724-43.
- 425 13. Yang J, Manson DK, Marr BP, Carvajal RD. Treatment of uveal melanoma: where are we now? *Ther Adv*
426 *Med Oncol*. 2018;10:1758834018757175.
- 427 14. Pham CM, Custer PL, Couch SM. Comparison of primary and secondary enucleation for uveal melanoma.
428 *Orbit*. 2017;36(6):422-27.
- 429 15. Diener-West M, Earle JD, Fine SL, Hawkins BS, Moy CS, Reynolds SM, et al. The COMS randomized
430 trial of iodine 125 brachytherapy for choroidal melanoma, III: initial mortality findings. COMS Report No. 18. *Arch*
431 *Ophthalmol*. 2001;119(7):969-82.
- 432 16. Naseripour M, Jaber R, Sedaghat A, Azma Z, Nojomi M, Falavarjani KG, et al. Ruthenium-106
433 brachytherapy for thick uveal melanoma: reappraisal of apex and base dose radiation and dose rate. *J Contemp*
434 *Brachytherapy* 2016. p. 66-73.
- 435 17. Zaldivar RA, Aaberg TM, Sternberg P, Jr., Waldron R, Grossniklaus HE. Clinicopathologic findings in
436 choroidal melanomas after failed transpupillary thermotherapy. *Am J Ophthalmol*. 2003;135(5):657-63.
- 437 18. Singh AD, Rundle PA, Berry-Brincat A, Parsons MA, Rennie IG. Extrascleral extension of choroidal
438 malignant melanoma following transpupillary thermotherapy. *Eye (Lond)*. England 2004. p. 91-3.
- 439 19. Damato B. Does ocular treatment of uveal melanoma influence survival? *Br J Cancer*. 2010;103(3):285-90.
- 440 20. Diener-West M, Reynolds SM, Agugliaro DJ, Caldwell R, Cumming K, Earle JD, et al. Development of
441 metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular
442 Melanoma Study Group Report No. 26. *Arch Ophthalmol*. 2005;123(12):1639-43.
- 443 21. Kuk D, Shoushtari AN, Barker CA, Panageas KS, Munhoz RR, Momtaz P, et al. Prognosis of Mucosal,
444 Uveal, Acral, Nonacral Cutaneous, and Unknown Primary Melanoma From the Time of First Metastasis.
445 *Oncologist*. 2016;21(7):848-54.
- 446 22. Carvajal RD, Sosman JA, Quevedo JF, Milhem MM, Joshua AM, Kudchadkar RR, et al. Effect of
447 selumetinib vs chemotherapy on progression-free survival in uveal melanoma: a randomized clinical trial. *Jama*.
448 2014;311(23):2397-405.
- 449 23. McArthur GA, Chapman PB, Robert C, Larkin J, Haanen JB, Dummer R, et al. Safety and efficacy of
450 vemurafenib in BRAF(V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): extended follow-up of a
451 phase 3, randomised, open-label study. *Lancet Oncol*. 2014;15(3):323-32.

24. Field MG, Harbour JW. GNAQ/11 Mutations in Uveal Melanoma: Is YAP the Key to Targeted Therapy? *Cancer cell*. 2014;25(6):714-15.
25. Carvajal RD, Piperno-Neumann S, Kapiteijn E, Chapman PB, Frank S, Joshua AM, et al. Selumetinib in Combination With Dacarbazine in Patients With Metastatic Uveal Melanoma: A Phase III, Multicenter, Randomized Trial (SUMIT). *Journal of Clinical Oncology*. 2018;36(12):1232-39.
26. Helgadottir H, Höiom V. The genetics of uveal melanoma: current insights. *Appl Clin Genet*. 2016. p. 147-55.
27. Onken MD, Worley LA, Tuscan MD, Harbour JW. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn*. 2010;12(4):461-8.
28. Harbour JW. A prognostic test to predict the risk of metastasis in uveal melanoma based on a 15-gene expression profile. *Methods Mol Biol*. 2014;1102:427-40.
29. Field MG, Harbour JW. Recent developments in prognostic and predictive testing in uveal melanoma. *Curr Opin Ophthalmol*. 2014;25(3):234-9.
30. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer*. 1998;83(2):354-9.
31. Kilic E, van Gils W, Lodder E, Beverloo HB, van Til ME, Mooy CM, et al. Clinical and cytogenetic analyses in uveal melanoma. *Invest Ophthalmol Vis Sci*. 2006;47(9):3703-7.
32. Staby KM, Gravdal K, Mork SJ, Heegaard S, Vintermyr OK, Krohn J. Prognostic impact of chromosomal aberrations and GNAQ, GNA11 and BAP1 mutations in uveal melanoma. *Acta Ophthalmol*. 2018;96(1):31-38.
33. Kaliki S, Shields CL, Shields JA. Uveal melanoma: Estimating prognosis. *Indian J Ophthalmol*. India2015. p. 93-102.
34. Shoushtari AN, Carvajal RD. GNAQ and GNA11 mutations in uveal melanoma. *Melanoma Res*. 2014;24(6):525-34.
35. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al. Mutations in GNA11 in Uveal Melanoma. *New England Journal of Medicine*. 2010;363(23):2191-99.
36. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*. 2009;457(7229):599-602.
37. Johansson P, Aoude LG, Wadt K, Glasson WJ, Warriar SK, Hewitt AW, et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. *Oncotarget*. 2016;7(4):4624-31.
38. Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet*. 2013;45(2):133-5.
39. Martin M, Masshofer L, Temming P, Rahmann S, Metz C, Bornfeld N, et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet*. 2013;45(8):933-6.
40. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*. 2010;330(6009):1410-3.
41. Moore AR, Ceraudo E, Sher JJ, Guan Y, Shoushtari AN, Chang MT, et al. Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Nat Genet*. 2016;48(6):675-80.
42. Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *New England Journal of Medicine*. 2017;377(6):523-33.
43. Bisgaard H. Pathophysiology of the cysteinyl leukotrienes and effects of leukotriene receptor antagonists in asthma. *Allergy*. 2001;56 Suppl 66:7-11.
44. D'Urzo AD, Chapman KR. Leukotriene-receptor antagonists. Role in asthma management. *Can Fam Physician*. 2000;46:872-9.
45. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. *Journal of Immunology* 2004. p. 1503-10.
46. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. *J Biol Chem*. 2013;288(16):10967-72.
47. Savari S, Vinnakota K, Zhang Y, A. S. Cysteinyl leukotrienes and their receptors: Bridging inflammation and colorectal cancer. *World Journal of Gastroenterology*2014. p. 968-77.
48. Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. *Clinical and Experimental Allergy*2012. p. 1313-20.
49. Lynch KR., O'Neill GP., Liu Q., Im DS., Sawyer N., Metters KM., et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature*1999. p. 789-93.

50. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem.* 2000;275(39):30531-6.
51. Jans DA, Xiao CY, Lam MH. Nuclear targeting signal recognition: a key control point in nuclear transport? *Bioessays.* 2000;22(6):532-44.
52. Servant MJ, Tenoever B, Lin R. Overlapping and distinct mechanisms regulating IRF-3 and IRF-7 function. *J Interferon Cytokine Res.* 2002;22(1):49-58.
53. Ciana P, et al. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *The EMBO Journal* 2006. p. 4615-27.
54. Lee KSea. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression. *The journal of allergy and clinical immunology*2004. p. 1093-99.
55. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis.* 1982;126(3):449-51.
56. Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, et al. Comparative airway and vascular activities of leukotrienes C-1 and D in vivo and in vitro. *Proc Natl Acad Sci U S A.* 1980;77(7):4354-8.
57. Burke L, Butler CT, Murphy A, Moran B, Gallagher WM, O'Sullivan J, et al. Evaluation of Cysteinyl Leukotriene Signaling as a Therapeutic Target for Colorectal Cancer. *Front Cell Dev Biol.* 2016;4.
58. Funao M, Matsuyama M, Naganuma T, Kawahito Y, Sano H, Nakatani T, et al. The cysteinylLT1 receptor in human renal cell carcinoma. *Mol Med Rep.* 2008;1(2):185-9.
59. Matsuyama M, Funao K, Hayama T, Tanaka T, Kawahito Y, Sano H, et al. Relationship between cysteinyl-leukotriene-1 receptor and human transitional cell carcinoma in bladder. *Urology.* 2009;73(4):916-21.
60. Matsuyama M, Funao K, Kawahito Y, Sano H, Chargui J, Touraine JL, et al. Expression of cysteinylLT1 receptor in human testicular cancer and growth reduction by its antagonist through apoptosis. *Mol Med Rep.* 2009;2(2):163-7.
61. Nielsen CK, Ohd JF, Wikstrom K, Massoumi R, Paruchuri S, Juhas M, et al. The leukotriene receptor CysLT1 and 5-lipoxygenase are upregulated in colon cancer. *Adv Exp Med Biol.* 2003;525:201-4.
62. Tsai MJ, Wu PH, Sheu CC, Hsu YL, Chang WA, Hung JY, et al. Cysteinyl Leukotriene Receptor Antagonists Decrease Cancer Risk in Asthma Patients. *Sci Rep.* 2016;6.
63. Moller I, Murali R, Muller H, Wiesner T, Jackett LA, Scholz SL, et al. Activating cysteinyl leukotriene receptor 2 (CYSLTR2) mutations in blue nevi. *Mod Pathol.* 2017;30(3):350-56.
64. Magnusson C, Mezhybovska M, Lorinc E, Fernebro E, Nilbert M, Sjolander A. Low expression of CysLT1R and high expression of CysLT2R mediate good prognosis in colorectal cancer. *Eur J Cancer.* 2010;46(4):826-35.
65. Magnusson C, Liu J, Ehrnstrom R, Manjer J, Jirstrom K, Andersson T, et al. Cysteinyl leukotriene receptor expression pattern affects migration of breast cancer cells and survival of breast cancer patients. *Int J Cancer.* 2011;129(1):9-22.
66. Magnusson C, Bengtsson AM, Liu M, Liu J, Ceder Y, Ehrnstrom R, et al. Regulation of cysteinyl leukotriene receptor 2 expression--a potential anti-tumor mechanism. *PLoS One.* 2011;6(12):e29060.
67. Mehdawi LM, Satapathy SR, Gustafsson A, Lundholm K, Alvarado-Kristensson M, Sjolander A. A potential anti-tumor effect of leukotriene C4 through the induction of 15-hydroxyprostaglandin dehydrogenase expression in colon cancer cells. *Oncotarget.* 2017;8(21):35033-47.
68. Maiga A, Lemieux S, Pabst C, Lavallee VP, Bouvier M, Sauvageau G, et al. Transcriptome analysis of G protein-coupled receptors in distinct genetic subgroups of acute myeloid leukemia: identification of potential disease-specific targets. *Blood Cancer J.* 2016;6(6):e431.
69. Jiang Y, Borrelli LA, Kanaoka Y, Bacskaï BJ, Boyce JA. CysLT(2) receptors interact with CysLT(1) receptors and down-modulate cysteinyl leukotriene-dependent mitogenic responses of mast cells. *Blood.* 2007;110(9):3263-70.
70. Kanaoka Y, Boyce JA. Cysteinyl Leukotrienes and Their Receptors; Emerging Concepts. *Allergy, Asthma & Immunology Research.* 2014;6(4):288-95.
71. Bennett DC, Cooper PJ, Hart IR. A line of non-tumorigenic mouse melanocytes, syngeneic with the B16 melanoma and requiring a tumour promoter for growth. *Int J Cancer.* 1987;39(3):414-8.
72. Griewank KG, Yu X, Khalili J, Sozen MM, Stempke-Hale K, Bernatchez C, et al. Genetic and molecular characterization of uveal melanoma cell lines. *Pigment Cell Melanoma Res.* 2012;25(2):182-7.
73. Chen X, Wu Q, Tan L, Porter D, Jager MJ, Emery C, et al. Combined PKC and MEK inhibition in uveal melanoma with GNAQ and GNA11 mutations. *Oncogene.* 2014;33(39):4724-34.
74. Reynolds AL. Phenotype based discovery of 2-[(E)-2-(QUINOLIN-2-YL)VINYL]PHENOL as a novel regulator of ocular angiogenesis. *The Journal of Biological Chemistry* 2016.

75. Butler CT, Reynolds AL, Tosetto M, Dillon ET, Guiry PJ, Cagney G, et al. A Quinib Analogue and Cysteinyl Leukotriene Receptor Antagonist Inhibits Vascular Endothelial Growth Factor (VEGF)-independent Angiogenesis and Exerts an Additive Antiangiogenic Response with Bevacizumab. *J Biol Chem*. 2017;292(9):3552-67.
76. Loukopoulos P, Kanetaka K, Takamura M, Shibata T, Sakamoto M, Hirohashi S. Orthotopic transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying varying metastatic activity. *Pancreas*. 2004;29(3):193-203.
77. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat Med*. 2011;17(11):1514-20.
78. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. *Neuro Oncol*. 2012;14(5):574-83.
79. Carita G, Némati F, Decaudin D. Uveal Melanoma Patient-Derived Xenografts. *Ocular Oncology and Pathology*. 2015;1(3):161-69.
80. Nemati F, Sastre-Garau X, Laurent C, Couturier J, Mariani P, Desjardins L, et al. Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors. *Clin Cancer Res*. 2010;16(8):2352-62.
81. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts. *Nat Rev Cancer*. 2015;15(8):451-2.
82. Kageyama K, Ohara M, Saito K, Ozaki S, Terai M, Mastrangelo MJ, et al. Establishment of an orthotopic patient-derived xenograft mouse model using uveal melanoma hepatic metastasis. *Journal of Translational Medicine*. 2017;15:145.
83. Tudhope SR, Cuthbert NJ, Abram TS, Jennings MA, Maxey RJ, Thompson AM, et al. BAY u9773, a novel antagonist of cysteinyl-leukotrienes with activity against two receptor subtypes. *European Journal of Pharmacology*. 1994;264(3):317-23.
84. Wunder F, Tinel H, Kast R, Geerts A, Becker EM, Kolkhof P, et al. Pharmacological characterization of the first potent and selective antagonist at the cysteinyl leukotriene 2 (CysLT(2)) receptor. *Br J Pharmacol*. 2010;160(2):399-409.
85. Khilnani G, Khilnani AK. Inverse agonism and its therapeutic significance. *Indian Journal of Pharmacology*. 2011;43(5):492-501.
86. Lappano R, Maggiolini M. Pharmacotherapeutic Targeting of G Protein-Coupled Receptors in Oncology: Examples of Approved Therapies and Emerging Concepts. *Drugs*. 2017;77(9):951-65.
87. Ramsey DM, McAlpine SR. Halting metastasis through CXCR4 inhibition. *Bioorg Med Chem Lett*. 2013;23(1):20-5.
88. Innamorati G, Valenti MT, Giovino F, Carbonare LD, Parenti M, Bassi C. Molecular Approaches To Target GPCRs in Cancer Therapy. *Pharmaceuticals*. 2011;4(4):567-89.
89. Dupre DJ, Le Gouill C, Gingras D, Rola-Pleszczynski M, Stankova J. Inverse agonist activity of selected ligands of the cysteinyl-leukotriene receptor 1. *J Pharmacol Exp Ther*. 2004;309(1):102-8.
90. Bond RA, Ijzerman AP. Recent developments in constitutive receptor activity and inverse agonism, and their potential for GPCR drug discovery. *Trends Pharmacol Sci*. 2006;27(2):92-6.
91. Zembowicz A, Phadke PA. Blue nevi and variants: an update. *Arch Pathol Lab Med*. 2011;135(3):327-36.
92. Costa S, Byrne M, Pissaloux D, Haddad V, Paindavoine S, Thomas L, et al. Melanomas Associated With Blue Nevi or Mimicking Cellular Blue Nevi: Clinical, Pathologic, and Molecular Study of 11 Cases Displaying a High Frequency of GNA11 Mutations, BAP1 Expression Loss, and a Predilection for the Scalp. *Am J Surg Pathol*. 2016;40(3):368-77.
93. Perez-Alea M, Vivancos A, Caratu G, Matito J, Ferrer B, Hernandez-Losa J, et al. Genetic profile of GNAQ-mutated blue melanocytic neoplasms reveals mutations in genes linked to genomic instability and the PI3K pathway. *Oncotarget*. 2016;7(19):28086-95.
94. Rovati EG, Bäck M, Dahlén S-E, Drazen J, Evans JF, Shimizu T, et al. Leukotriene receptors: CysLT 2 receptor. <http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=270>: IUPHAR/BPS Guide to PHARMACOLOGY; 20/02/2018 [cited 2018 27/05].