



<b>Title</b>	Evaluation of oncogenic cysteinyl leukotriene receptor 2 as a therapeutic target for uveal melanoma
<b>Authors(s)</b>	Slater, Kayleigh, Hoo, Pei Sian, Buckley, A. M., Piulats, J. M., Villanueva, A., Portela, A., Kennedy, Breandán
<b>Publication date</b>	2018-09
<b>Publication information</b>	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." Springer, September 2018. <a href="https://doi.org/10.1007/s10555-018-9751-z">https://doi.org/10.1007/s10555-018-9751-z</a> .
<b>Publisher</b>	Springer
<b>Item record/more information</b>	<a href="http://hdl.handle.net/10197/9533">http://hdl.handle.net/10197/9533</a>
<b>Publisher's statement</b>	The final publication is available at Springer via <a href="http://dx.doi.org/10.1007/s10555-018-9751-z">http://dx.doi.org/10.1007/s10555-018-9751-z</a> .
<b>Publisher's version (DOI)</b>	<a href="https://doi.org/10.1007/s10555-018-9751-z">10.1007/s10555-018-9751-z</a>

Downloaded 2026-05-02 00:29:32

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

1 **TITLE**

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

4 **AUTHORS**

5 Slater K<sup>1,2</sup>, Hoo PS<sup>1,3</sup>, Buckley AM<sup>3</sup>, Piulats JM<sup>4</sup>, Villanueva A<sup>5,6</sup>, Portela A<sup>5</sup>, Kennedy BN<sup>1\*</sup>

6

7 **AFFILIATIONS**

8 **1** UCD School of Biomolecular & Biomedical Science, UCD Conway Institute, University College Dublin, Dublin,  
9 Ireland

10 **2** Genomics Medicine Ireland Limited, Dublin, Ireland

11 **3** Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Trinity College Dublin,  
12 Ireland

13 **4** Department of Medical Oncology, Catalan Institute of Oncology – IDIBELL, Barcelona, Spain

14 **5** Xenopat S.L., Barcelona, Spain

15 **6** Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology (ICO), Bellvitge  
16 Institute for Biomedical Research (IDIBELL), Barcelona, Spain

17 \* Email: [brendan.kennedy@ucd.ie](mailto:brendan.kennedy@ucd.ie) Tel: +3531 7166740

18

19 **AUTHOR ORCID ID**

20 **KS** 0000000155668399

21 **PSH** 0000000219942589

22 **AMB** 0000000250808580

23 **JMP** 0000000236061724

24 **AV** 0000000241646671

25 **AP** 0000000321842584

26 **BNK** 0000000179914689

27 **FUNDING**

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

31 **ACKNOWLEDGEMENTS**

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

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

35 **Abstract**

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

46 **Epidemiology and aetiology of uveal melanoma**

47 Uveal melanomas, which arise from the choroid (85-90% of cases), ciliary body (5-8% of cases) or iris (3-5% of  
48 cases), account for approximately 5.2% of all primary melanomas [1]. Although considered a rare disease, incidence  
49 ranges from < 2 per million to > 8 per million across Europe [2], uveal melanoma is the primary intraocular tumour  
50 found in adults. The overall incidence of uveal melanoma has remained relatively constant in comparison to other  
51 cancer types, but varies by race, sex and country [3]. Males have greater disease incidence than females and uveal  
52 melanoma is more common among Caucasians than non-Caucasians [4]. Interestingly, the National Cancer Registry  
53 Ireland reports an estimated 62 new cases of neoplasms of the eye and adnexa diagnosed in Ireland between 2015 -  
54 2017 [5], this compares to approximately 1,700 new cases per year in the United States [6] and 430 new cases per  
55 year in the United Kingdom [7], suggesting that Ireland has a higher incidence of the disease per capita (1.3 cases per  
56 100,000 per year in Ireland versus 0.52 cases per 100,000 per year in the U.S.).

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

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

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

71 **Prognosis of uveal melanoma**

72 ***Treatment of primary uveal melanoma***

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

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

#### 84 ***Treatment of metastatic uveal melanoma***

85 Despite advances in the treatment of the primary ocular tumour, the prognosis of patients that develop metastatic uveal  
86 melanoma remains poor and the effect of ocular therapy on metastasis and survival remains uncertain [19].  
87 Approximately 50% of patients develop metastatic disease, with the liver being the most common site (89% of  
88 metastatic patients), followed by the lung, bone and soft tissue [20]. The median overall survival from diagnosis of  
89 metastatic uveal melanoma ranges from less than 6 months to 13.4 months, with only 8% of patients surviving beyond  
90 2 years [20, 21].

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

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

#### 106 **Genetic alterations in uveal melanoma**

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

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

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

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

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

127 Uveal melanomas are predominantly characterised by mutations in *GNAQ* and *GNA11* (a paralog of *GNAQ*), both of  
128 which encode for G-protein alpha subunits and share 90% amino acid sequence homology [34]. Overall, 83% of uveal  
129 melanomas contain mutations in either *GNAQ* or *GNA11*, however, these mutations do not correlate with prognosis  
130 [35]. *GNAQ* mutations occur almost exclusively at codon 209 and result in glutamine to leucine (p.Gln209Leu), or  
131 proline (p.Gln209Pro) amino acid substitutions. In both cases, this mutation occurs within the GTPase domain and  
132 results in a constitutively active G-protein [36]. Similarly, mutations in *GNA11* are predominantly found at position  
133 Q209 and result in similar downstream consequences [35] In 2016, a recurrent hotspot mutation in *PLCB4*, a  
134 downstream target of GNAQ/GNA11 was identified in 2 of 28 samples assayed [37]. PLCβ4 is activated upon binding  
135 of a G-protein subunit, resulting in cleavage of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to produce diacylglycerol  
136 (DAG) and inositol triphosphate (IP3), and calcium release from the cell. The *PLCB4* hotspot mutation is also a gain-  
137 of-function mutation leading to constitutive activation of the MAPK/ERK pathway.

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

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

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

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

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

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

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

### 175 **Cysteinyl leukotriene signalling**

176 The novel oncogenic mutations in *CYSLTR2* warrant further investigation of the associated signalling pathway in the  
177 pathogenesis and treatment of ocular melanoma. The cysteinyl leukotrienes (CysLTs), LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>, are a  
178 group of inflammatory, lipid, signalling molecules that mediate both acute and chronic inflammation. Indeed, cysteinyl  
179 leukotriene receptor antagonists are routinely prescribed in the treatment of asthma and allergic rhinitis [43, 44]. These  
180 eicosanoids are synthesized from arachidonic acid (AA) in the cell membrane upon cell activation. The 5-lipoxygenase  
181 enzyme (5-LOX) interacts with a 5-lipoxygenase activating protein (FLAP) which enhances the activity of 5-LOX to  
182 convert AA mobilised to the cytosol to the unstable leukotriene LTA<sub>4</sub> [45]. LTA<sub>4</sub> is further hydrolysed to LTB<sub>4</sub> or  
183 LTC<sub>4</sub> via LTC<sub>4</sub> synthase. Intracellularly synthesized LTC<sub>4</sub> is exported from the cell via multidrug resistance-associated  
184 proteins and rapidly metabolised to the remaining cysteinyl leukotrienes, LTD<sub>4</sub> or LTE<sub>4</sub> [46]. Synthesis of the CysLTs  
185 occurs predominantly in immune cells such as neutrophils, eosinophils, monocytes, macrophages and mast cells [47].

186 Thus, the CysLTs are a group of structurally similar but functionally different lipid mediators that exert their biological  
187 effects via binding to the GPCRs (G-protein-coupled receptors), CysLT<sub>1</sub> and CysLT<sub>2</sub><sup>(1)</sup> [48]. CysLT<sub>1</sub> and CysLT<sub>2</sub> are  
188 located on the plasma membrane [49, 50], however, both receptors possess the ability to localize to the nuclear  
189 membrane [51, 52]. LTC<sub>4</sub> and LTD<sub>4</sub> binds CysLT<sub>2</sub> with low, but equal affinity, LTD<sub>4</sub> and LTC<sub>4</sub> bind CysLT<sub>1</sub> with  
190 high and low affinity, respectively [49]. Neither receptor subtype exhibits substantial affinity for LTE<sub>4</sub> [48]. However,  
191 additional CysLT receptors, GPR17 and GPR99 have been reported. GPR17 is a G protein-coupled orphan receptor  
192 with homology to both the P2Y and CysLT receptors. GRP17 is reported as a ligand-independent negative regulator  
193 of CysLT<sub>1</sub> [53]. GPR99, also described as cysteinyl leukotriene receptor E (CysLTE) or CysLT<sub>3</sub> is proposed as a  
194 potential LTE<sub>4</sub> selective cysteinyl leukotriene receptor [46].

### 195 **Cysteinyl leukotriene signalling in cancer**

196 Cysteinyl leukotriene signalling is implicated in inflammation, bronchoconstriction, increased vascular permeability,  
197 mucus production and white blood cell recruitment [54-56]. A recent review evaluated links between CysLT receptors  
198 and many hallmarks of cancer including angiogenesis, sustained proliferative signalling, migration and invasion [57].  
199 Interestingly, overexpression of CysLT<sub>1</sub> presents in colorectal cancer, prostate cancer, renal cell carcinoma,  
200 transitional cell carcinoma and testicular cancer [58-61]. Tsai *et al.* conducted a large, population-based study to  
201 investigate the effect of leukotriene receptor antagonists on the risk of cancer development in newly diagnosed  
202 asthmatic patients. Leukotriene receptor antagonists decreased the risk of 14 different cancers analysed in a dose  
203 dependent manner, suggesting that CysLT receptor antagonism provides a cancer-protective effect [62].

204 Moore *et al.* identified the recurrent hotspot mutation in *CYSLTR2* as a driver oncogene [41]. The oncogenic properties  
205 of the CysLT<sub>2</sub> were later supported by Möller *et al.* who identified the same Leu129Gln hotspot mutation in blue nevi  
206 [63]. Interestingly, in other cancer types CysLT<sub>2</sub> exerts anti-cancer properties. CRC patients with high nuclear CysLT<sub>1</sub>  
207 expression have a poor prognosis, while patients with high nuclear CysLT<sub>2</sub> expression have a better overall prognosis,  
208 suggesting that CysLT<sub>2</sub> is protective in CRC [64]. Magnusson *et al.* reported a similar phenomenon in breast cancer  
209 patients, whereby patients with large tumours exhibiting high CysLT<sub>1</sub> and low CysLT<sub>2</sub> expression levels had a  
210 significantly reduced survival [65]. Indeed, it is suggested that regulation of CysLT<sub>2</sub>, leading to increased expression  
211 of the receptor, may have anti-tumour properties in CRC [66, 67].

212 Two *CYSLTR2* mutations, p.Arg136His and p.Arg136Cys, were identified in colorectal cancer [41]. However, with  
213 exception to the Leu129Gln hotspot mutation in uveal melanoma and blue nevi, *CYSLTR2* is not significantly mutated  
214 in any other cancer types, nor have other hotspot mutations been identified, suggesting this is a unique driver mutation  
215 in uveal melanoma and blue nevi. However, *CYSLTR2* is overexpressed in certain acute myeloid leukaemia subtypes  
216 [68].

217 This raises an interesting question about the role played by the different cysteinyl leukotriene receptors in various  
218 cancer subtypes. Increased expression of endogenous CysLT<sub>2</sub> has a protective effect linked to negative regulation of

219 CysLT<sub>1</sub> [69, 70]. Lack of CysLT<sub>2</sub> receptors may facilitate the formation of CysLT<sub>1</sub> homodimers, leading to heightened  
220 LTD<sub>4</sub> signalling which may promote a pro-tumorigenic phenotype [48]. Constitutive activation of CysLT<sub>2</sub> in uveal  
221 melanoma acts as an oncogene, suggesting opposing effects to those documented in colorectal and breast cancer. It  
222 will be interesting to determine if the oncogenic *CYSLTR2* mutation influences CysLT<sub>1</sub> signalling, expression or  
223 localization.

#### 224 **Cysteinyl leukotriene receptor 2 as a uveal melanoma oncogene**

225 The CysLT<sub>2</sub> mutation associated with uveal melanoma and more recently, blue nevi, occurs at Leu129, which is  
226 situated in transmembrane helix 3, a functional hub of the receptor. This mutation leads to constitutive activation of  
227 the receptor and endogenous signalling, leaving it unresponsive to leukotriene stimulation *in vitro* [41]. Moore *et al.*  
228 characterised the oncogenic potential of this mutation by stably expressing the mutant Leu129Gln CysLT<sub>2</sub> in melan-  
229 a cells [41]. Mutant Leu129Gln, but not wild-type CysLT<sub>2</sub>, conferred TPA(12-O-Tetradecanoylphorbol-13-acetat)-  
230 independent growth *in vitro* [41]. In agreement, siRNA mediated knockdown of exogenous *CYSLTR2* reduced the  
231 growth of melan-a cells grown in the presence or absence of TPA but had no effect on those expressing the wild-type  
232 receptor [41]. This exciting preliminary *in vitro* data suggests that inhibition of CysLT<sub>2</sub> in patients harbouring this  
233 oncogenic mutation may have therapeutic potential in the treatment of uveal melanoma.

234 Melan-a cells applied by Moore *et al.* are a melanocyte, non-tumorigenic cell line derived from the embryonic skin of  
235 18-day-old C57BL mice and require phorbol-esters such as TPA for growth [71]. While melan-a cells are commonly  
236 used in melanoma research [35, 36], it will be important to also investigate the effects of this oncogenic mutation in  
237 human derived uveal melanoma cells. When mutant Leu129Gln was stably expressed in Mel290 cells, a human uveal  
238 melanoma cell line lacking *GNAQ* or *GNA11* mutations, the expression of melanocyte-lineage specific genes was  
239 significantly upregulated by RT-qPCR analysis compared to empty vector and wild-type control [41]. It will be  
240 interesting to examine whether expression of the oncogenic Leu129Gln mutation alters the cellular phenotype or  
241 additional hallmarks of cancer in uveal melanoma *in vitro* and *in vivo*. The effect of knockdown, or indeed knockout  
242 of the receptor in uveal melanoma cells also remains to be established. Similar experiments could also be conducted  
243 and validated using the Mel285 uveal melanoma cancer cell line, which is also reported as wild-type for both *GNAQ*  
244 and *GNA11* [72].

245 To strengthen the CysLT<sub>2</sub>/Gαq/11/PLCβ4 pathway hypothesis, steps should be taken to examine the downstream  
246 signalling effects associated with the constitutively active Leu129Gln *CYSLTR2* mutation. Given that the best  
247 understood signalling pathway in uveal melanoma is the MAPK/ERK pathway, which is known to be activated by  
248 *GNAQ* and *GNA11* mutations [34], it is likely the *CYSLTR2* mutation upregulates this pathway. *GNAQ* and *GNA11*  
249 mutated uveal melanoma cell lines cause increased expression of phosphorylated MEK and phosphorylated ERK,  
250 which can be abolished via knockdown of the respective gene [36, 73]. In *GNAQ* and *GNA11* mutated cell lines,  
251 MAPK pathway activation occurs as a result of PKC activation [73]. As such, levels of p-MARCKS, a substrate of  
252 PKC, are detectable in uveal melanoma cells harbouring these mutations and can also be suppressed following  
253 knockdown [73]. Similar results would be expected from cell lines expressing the *CYSLTR2* mutation.

254 Given the well documented role of CysLT receptors in angiogenesis and inflammation, additional IHC and expression  
255 analysis could examine the vascular and inflammatory status of the Leu129Gln expressing cells. Cysteinyl leukotriene  
256 receptor antagonists can promote anti-angiogenic activity via a VEGF-independent pathway [74, 75]. It will be  
257 interesting to examine the levels of VEGF and other associated angiogenic markers in the oncogene background.  
258 Given the cross-regulation that occurs between the CysLT receptor subtypes, investigation into the effect of the  
259 Leu129Gln mutation on the expression of CysLT<sub>1</sub> is warranted.

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

265

266 **Patient derived xenograft (PDX) models of uveal melanoma**

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

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

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

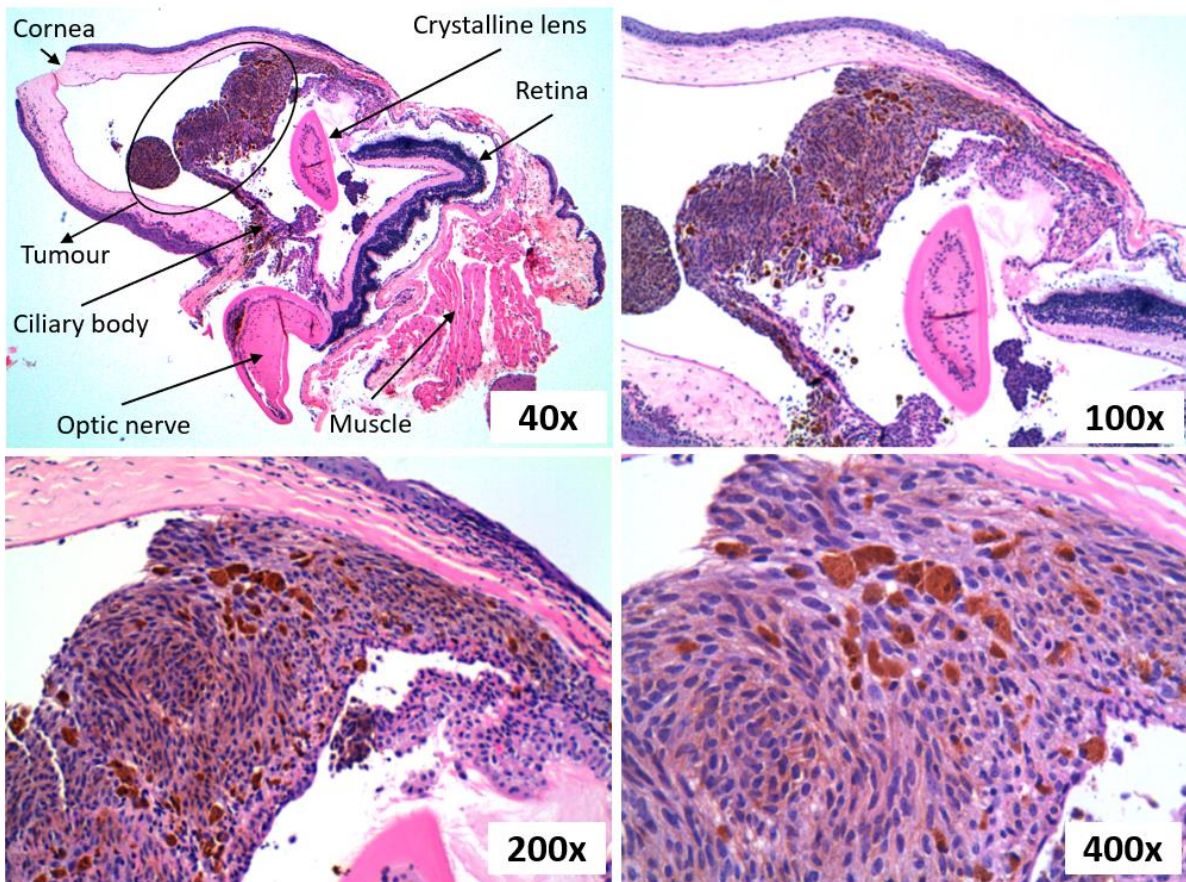
286



287

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

291



292

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

296 Undoubtedly, large numbers of PDOX models are needed to accurately reflect the mutational diversity found in uveal  
 297 melanoma and to reflect the different sites in which uveal melanomas are found (choroid, ciliary body and iris). It has  
 298 been reported that tumours harbouring *GNA11* mutations grow significantly better than *GNAQ* mutated tumours and  
 299 that metastatic tumours engrafted more successfully than those taken from the eye when implanted subcutaneously  
 300 [79]. It will be of interest to see the effect of *CYSLTR2* and *PLCB4* mutations on PDX development. Given the rarity  
 301 of these mutations in uveal melanoma patients it may take some time to generate PDX models with the desired  
 302 mutations. However, the generation of PDOX models derived from patients harbouring the *CYSLTR2* mutation would  
 303 allow for more in depth analysis of this mutation and its role in disease progression, metastasis and drug  
 304 responsiveness. Once a successful PDOX model harbouring the *CYSLTR2* mutation is established, the tumour can be  
 305 expanded to generate a tumour bearing colony of mice in molecular pathology and therapeutic efficacy can be  
 306 analysed. Given the rarity of *CYSLTR2* mutations, this approach will offer a quicker and more comprehensive method  
 307 of analysing the consequences of this mutation. It will be exciting to examine the effect of CysLT receptor antagonists  
 308 in cell lines and *in vivo* models expressing the mutant *CYSLTR2*. However, given the constitutively active nature of  
 309 the mutant receptor, it is likely that regular antagonists of the receptor will be ineffective.

310

311

## 312 **Inverse agonists to target CysLTR<sub>2</sub>**

313 CysLT<sub>1</sub> antagonists, montelukast, zafirlukast and pranlukast are prescribed for the treatment of asthma and allergic  
314 rhinitis. BAY u9773 is a non-selective cysteinyl leukotriene receptor antagonist at both CysLT<sub>1</sub> and CysLT<sub>2</sub> receptors  
315 [83], while HAMI 3379 is described as a potent and selective CysLT<sub>2</sub> antagonist [84].

316 Aberrant expression and activity of GPCRs in cancer is well established and they have become a compelling  
317 therapeutic target in the disease. In order to effectively target the Leu129Gln mutation in *CYSLTR2* an inverse agonist  
318 that selectively targets this receptor will be required. Inverse agonists preferentially bind to and stabilize a  
319 constitutively active receptor, maintaining the receptor in an inactive state and thus have intrinsic negative activity  
320 [85]. This differs to a neutral antagonist which can block the actions of agonists and inverse agonists. Neutral  
321 antagonists exhibit equal preference for both the active and inactive state and have no intrinsic activity [85].

322 GPCRs represent one of the most common drug targets and yet there are few examples of anti-tumour agents that  
323 directly target these receptors [86]. Even fewer examples of inverse agonists as anti-cancer agents are available.  
324 However, ALX-065, a biparatopic nanobody that acts as inverse agonist, blocks spontaneous activation of the CXC4  
325 receptor and inhibits cell migration [87, 88], suggesting that inverse agonists may have the potential to act as successful  
326 chemotherapeutic agents.

327 Given that inverse agonists targeting CysLT<sub>1</sub> are currently in clinical use [89], it is certainly possible that an inverse  
328 agonist acting at CysLT<sub>2</sub> is available. Indeed, many compounds that were previously classified as antagonists, actually  
329 possess inverse agonist activity [90], suggesting that some anti-cancer GPCR antagonists may in fact mediate their  
330 effects through inverse agonism. BAY u9973 does not act as an inverse agonist at CysLT<sub>1</sub> [89], however, it exhibits  
331 weak potency at the human CysLT<sub>1</sub> and the exact activity of this drug at CysLT<sub>2</sub> remains to be studied. In addition, it  
332 will be important to test HAMI 3379 to determine if this selective antagonist possesses similar inverse agonist  
333 capabilities which could be used to target the constitutively active CysLT<sub>2</sub> receptor.

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

335 The *CYSLTR2* mutation can be considered a rare mutation in a rare form of cancer. Moore *et al.* identified this mutation  
336 in 4 of 136 patients (~3% of study subjects) [41]. Three of the identified samples came from a cohort of 80 samples  
337 taken from the TCGA, while one additional sample came from a cohort of 22 samples from the University of Duisburg-  
338 Essen (UNI-UDE). In the United States, approximately 1,700 patients are diagnosed with this cancer each year [6],  
339 suggesting that a potential 51 newly diagnosed patients have CysLT<sub>2</sub> mutations.

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

348 Blue nevi are common melanocytic tumours that occur in the dermal layer of the skin [63]. Blue nevi generally lack  
349 *BRAF* and *NRAS* mutations commonly found in neoplasms of epithelial melanocytes [36]. Instead, blue nevi display  
350 a similar genetic profile to that found in uveal melanomas, and frequently possess recurrent activating mutations in  
351 *GNAQ* and *GNAI1* [36, 91]. *BAP1* mutations are reported in metastatic blue nevi, further strengthening the role of  
352 *BAP1* in metastatic potential and poor patient outcomes in certain cancer subtypes [92, 93]. Based on this knowledge  
353 and the additional findings of CysLT<sub>2</sub> and PLCβ4 mutations in uveal melanoma, Möller *et al.* sought to analyse a  
354 cohort of blue nevi lacking *GNAQ* or *GNAI1* mutations to determine if driver mutations in *CYSLTR2* and *PLCB4* are  
355 also present. 3% of tumours analysed harboured a mutation in *CYSLTR2*, which is identical to the frequency of the  
356 mutation reported in uveal melanoma [41, 63]. Moreover, the mutation in *CYSLTR2* was the same c.386T>A, L129Q,  
357 mutually exclusive, hotspot mutation identified in uveal melanoma samples by Moore *et al.* [63]. The three *CYSLTR2*  
358 mutations reported by Möller *et al.* were found in morphologically benign common blue nevi [63].

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

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

## 367 **Conclusion**

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

379

380

381

382

383

384

385

386

387

## 388 **FOOTNOTES**

389 <sup>(1)</sup> Correct nomenclature of the cysteinyl leukotriene receptors (CysLT<sub>1</sub> and CysLT<sub>2</sub>) as per the IUPHAR/BPS Guide  
390 to PHARMACOLOGY [94].

## 391 **AUTHOR CONTRIBUTIONS**

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

## 395 **CONFLICT OF INTEREST STATEMENT**

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

398 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, Jaberi 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.

- 452 24. Field MG, Harbour JW. GNAQ/11 Mutations in Uveal Melanoma: Is YAP the Key to Targeted Therapy?  
453 *Cancer cell*. 2014;25(6):714-15.
- 454 25. Carvajal RD, Piperno-Neumann S, Kapiteijn E, Chapman PB, Frank S, Joshua AM, et al. Selumetinib in  
455 Combination With Dacarbazine in Patients With Metastatic Uveal Melanoma: A Phase III, Multicenter, Randomized  
456 Trial (SUMIT). *Journal of Clinical Oncology*. 2018;36(12):1232-39.
- 457 26. Helgadottir H, Höiom V. The genetics of uveal melanoma: current insights. *Appl Clin Genet*. 2016. p. 147-  
458 55.
- 459 27. Onken MD, Worley LA, Tuscan MD, Harbour JW. An accurate, clinically feasible multi-gene expression  
460 assay for predicting metastasis in uveal melanoma. *J Mol Diagn*. 2010;12(4):461-8.
- 461 28. Harbour JW. A prognostic test to predict the risk of metastasis in uveal melanoma based on a 15-gene  
462 expression profile. *Methods Mol Biol*. 2014;1102:427-40.
- 463 29. Field MG, Harbour JW. Recent developments in prognostic and predictive testing in uveal melanoma. *Curr*  
464 *Opin Ophthalmol*. 2014;25(3):234-9.
- 465 30. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic  
466 abnormalities with the outcome of patients with uveal melanoma. *Cancer*. 1998;83(2):354-9.
- 467 31. Kilic E, van Gils W, Lodder E, Beverloo HB, van Til ME, Mooy CM, et al. Clinical and cytogenetic  
468 analyses in uveal melanoma. *Invest Ophthalmol Vis Sci*. 2006;47(9):3703-7.
- 469 32. Staby KM, Gravdal K, Mork SJ, Heegaard S, Vintermyr OK, Krohn J. Prognostic impact of chromosomal  
470 aberrations and GNAQ, GNA11 and BAP1 mutations in uveal melanoma. *Acta Ophthalmol*. 2018;96(1):31-38.
- 471 33. Kaliki S, Shields CL, Shields JA. Uveal melanoma: Estimating prognosis. *Indian J Ophthalmol*.  
472 India2015. p. 93-102.
- 473 34. Shoushtari AN, Carvajal RD. GNAQ and GNA11 mutations in uveal melanoma. *Melanoma Res*.  
474 2014;24(6):525-34.
- 475 35. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al. Mutations in  
476 GNA11 in Uveal Melanoma. *New England Journal of Medicine*. 2010;363(23):2191-99.
- 477 36. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, et al. Frequent somatic  
478 mutations of GNAQ in uveal melanoma and blue naevi. *Nature*. 2009;457(7229):599-602.
- 479 37. Johansson P, Aoude LG, Wadt K, Glasson WJ, Warriar SK, Hewitt AW, et al. Deep sequencing of uveal  
480 melanoma identifies a recurrent mutation in PLCB4. *Oncotarget*. 2016;7(4):4624-31.
- 481 38. Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM. Recurrent mutations at  
482 codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet*. 2013;45(2):133-5.
- 483 39. Martin M, Masshofer L, Temming P, Rahmann S, Metz C, Bornfeld N, et al. Exome sequencing identifies  
484 recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet*. 2013;45(8):933-  
485 6.
- 486 40. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, et al. Frequent mutation of BAP1 in  
487 metastasizing uveal melanomas. *Science*. 2010;330(6009):1410-3.
- 488 41. Moore AR, Ceraudo E, Sher JJ, Guan Y, Shoushtari AN, Chang MT, et al. Recurrent activating mutations  
489 of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Nat Genet*. 2016;48(6):675-80.
- 490 42. Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer  
491 in Patients with a Germline BRCA Mutation. *New England Journal of Medicine*. 2017;377(6):523-33.
- 492 43. Bisgaard H. Pathophysiology of the cysteinyl leukotrienes and effects of leukotriene receptor antagonists in  
493 asthma. *Allergy*. 2001;56 Suppl 66:7-11.
- 494 44. D'Urzo AD, Chapman KR. Leukotriene-receptor antagonists. Role in asthma management. *Can Fam*  
495 *Physician*. 2000;46:872-9.
- 496 45. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in  
497 immune and inflammatory responses. *Journal of Immunology* 2004. p. 1503-10.
- 498 46. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl  
499 leukotriene receptor with a preference for leukotriene E4 ligand. *J Biol Chem*. 2013;288(16):10967-72.
- 500 47. Savari S, Vinnakota K, Zhang Y, A. S. Cysteinyl leukotrienes and their receptors: Bridging inflammation  
501 and colorectal cancer. *World Journal of Gastroenterology*2014. p. 968-77.
- 502 48. Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. *Clinical*  
503 *and Experimental Allergy*2012. p. 1313-20.
- 504 49. Lynch KR., O'Neill GP., Liu Q., Im DS., Sawyer N., Metters KM., et al. Characterization of the human  
505 cysteinyl leukotriene CysLT1 receptor. *Nature*1999. p. 789-93.

506 50. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human  
507 cysteinyl leukotriene 2 receptor. *J Biol Chem.* 2000;275(39):30531-6.

508 51. Jans DA, Xiao CY, Lam MH. Nuclear targeting signal recognition: a key control point in nuclear transport?  
509 *Bioessays.* 2000;22(6):532-44.

510 52. Servant MJ, Tenoever B, Lin R. Overlapping and distinct mechanisms regulating IRF-3 and IRF-7  
511 function. *J Interferon Cytokine Res.* 2002;22(1):49-58.

512 53. Ciana P, et al. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-  
513 leukotrienes receptor. *The EMBO Journal* 2006. p. 4615-27.

514 54. Lee KSea. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular  
515 endothelial growth factor expression. *The journal of allergy and clinical immunology*2004. p. 1093-99.

516 55. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C4  
517 and D4, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis.* 1982;126(3):449-51.

518 56. Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, et al. Comparative airway and vascular  
519 activities of leukotrienes C-1 and D in vivo and in vitro. *Proc Natl Acad Sci U S A.* 1980;77(7):4354-8.

520 57. Burke L, Butler CT, Murphy A, Moran B, Gallagher WM, O'Sullivan J, et al. Evaluation of Cysteinyl  
521 Leukotriene Signaling as a Therapeutic Target for Colorectal Cancer. *Front Cell Dev Biol.* 2016;4.

522 58. Funao K, Matsuyama M, Naganuma T, Kawahito Y, Sano H, Nakatani T, et al. The cysteinylLT1 receptor  
523 in human renal cell carcinoma. *Mol Med Rep.* 2008;1(2):185-9.

524 59. Matsuyama M, Funao K, Hayama T, Tanaka T, Kawahito Y, Sano H, et al. Relationship between cysteinyl-  
525 leukotriene-1 receptor and human transitional cell carcinoma in bladder. *Urology.* 2009;73(4):916-21.

526 60. Matsuyama M, Funao K, Kawahito Y, Sano H, Chargui J, Touraine JL, et al. Expression of cysteinylLT1  
527 receptor in human testicular cancer and growth reduction by its antagonist through apoptosis. *Mol Med Rep.*  
528 2009;2(2):163-7.

529 61. Nielsen CK, Ohd JF, Wikstrom K, Massoumi R, Paruchuri S, Juhas M, et al. The leukotriene receptor  
530 CysLT1 and 5-lipoxygenase are upregulated in colon cancer. *Adv Exp Med Biol.* 2003;525:201-4.

531 62. Tsai MJ, Wu PH, Sheu CC, Hsu YL, Chang WA, Hung JY, et al. Cysteinyl Leukotriene Receptor  
532 Antagonists Decrease Cancer Risk in Asthma Patients. *Sci Rep.* 2016;6.

533 63. Moller I, Murali R, Muller H, Wiesner T, Jackett LA, Scholz SL, et al. Activating cysteinyl leukotriene  
534 receptor 2 (CYSLTR2) mutations in blue nevi. *Mod Pathol.* 2017;30(3):350-56.

535 64. Magnusson C, Mezhybovska M, Lorinc E, Fernebro E, Nilbert M, Sjolander A. Low expression of  
536 CysLT1R and high expression of CysLT2R mediate good prognosis in colorectal cancer. *Eur J Cancer.*  
537 2010;46(4):826-35.

538 65. Magnusson C, Liu J, Ehrnstrom R, Manjer J, Jirstrom K, Andersson T, et al. Cysteinyl leukotriene receptor  
539 expression pattern affects migration of breast cancer cells and survival of breast cancer patients. *Int J Cancer.*  
540 2011;129(1):9-22.

541 66. Magnusson C, Bengtsson AM, Liu M, Liu J, Ceder Y, Ehrnstrom R, et al. Regulation of cysteinyl  
542 leukotriene receptor 2 expression--a potential anti-tumor mechanism. *PLoS One.* 2011;6(12):e29060.

543 67. Mehdawi LM, Satapathy SR, Gustafsson A, Lundholm K, Alvarado-Kristensson M, Sjolander A. A  
544 potential anti-tumor effect of leukotriene C4 through the induction of 15-hydroxyprostaglandin dehydrogenase  
545 expression in colon cancer cells. *Oncotarget.* 2017;8(21):35033-47.

546 68. Maiga A, Lemieux S, Pabst C, Lavallee VP, Bouvier M, Sauvageau G, et al. Transcriptome analysis of G  
547 protein-coupled receptors in distinct genetic subgroups of acute myeloid leukemia: identification of potential  
548 disease-specific targets. *Blood Cancer J.* 2016;6(6):e431.

549 69. Jiang Y, Borrelli LA, Kanaoka Y, Bacskai BJ, Boyce JA. CysLT(2) receptors interact with CysLT(1)  
550 receptors and down-modulate cysteinyl leukotriene-dependent mitogenic responses of mast cells. *Blood.*  
551 2007;110(9):3263-70.

552 70. Kanaoka Y, Boyce JA. Cysteinyl Leukotrienes and Their Receptors; Emerging Concepts. *Allergy, Asthma  
553 & Immunology Research.* 2014;6(4):288-95.

554 71. Bennett DC, Cooper PJ, Hart IR. A line of non-tumorigenic mouse melanocytes, syngeneic with the B16  
555 melanoma and requiring a tumour promoter for growth. *Int J Cancer.* 1987;39(3):414-8.

556 72. Griewank KG, Yu X, Khalili J, Sozen MM, Stempke-Hale K, Bernatchez C, et al. Genetic and molecular  
557 characterization of uveal melanoma cell lines. *Pigment Cell Melanoma Res.* 2012;25(2):182-7.

558 73. Chen X, Wu Q, Tan L, Porter D, Jager MJ, Emery C, et al. Combined PKC and MEK inhibition in uveal  
559 melanoma with GNAQ and GNA11 mutations. *Oncogene.* 2014;33(39):4724-34.

560 74. Reynolds AL. Phenotype based discovery of 2-[(E)-2-(QUINOLIN-2-YL)VINYLL]PHENOL as a novel  
561 regulator of ocular angiogenesis. *The Journal of Biological Chemistry* 2016.

562 75. Butler CT, Reynolds AL, Tosetto M, Dillon ET, Guiry PJ, Cagney G, et al. A Quinib Analogue and  
563 Cysteinyl Leukotriene Receptor Antagonist Inhibits Vascular Endothelial Growth Factor (VEGF)-independent  
564 Angiogenesis and Exerts an Additive Antiangiogenic Response with Bevacizumab. *J Biol Chem.* 2017;292(9):3552-  
565 67.

566 76. Loukopoulos P, Kanetaka K, Takamura M, Shibata T, Sakamoto M, Hirohashi S. Orthotopic  
567 transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying  
568 varying metastatic activity. *Pancreas.* 2004;29(3):193-203.

569 77. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women  
570 with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat Med.*  
571 2011;17(11):1514-20.

572 78. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the  
573 molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. *Neuro Oncol.*  
574 2012;14(5):574-83.

575 79. Carita G, Némati F, Decaudin D. Uveal Melanoma Patient-Derived Xenografts. *Ocular Oncology and*  
576 *Pathology.* 2015;1(3):161-69.

577 80. Nemati F, Sastre-Garau X, Laurent C, Couturier J, Mariani P, Desjardins L, et al. Establishment and  
578 characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors.  
579 *Clin Cancer Res.* 2010;16(8):2352-62.

580 81. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous  
581 xenografts. *Nat Rev Cancer.* 2015;15(8):451-2.

582 82. Kageyama K, Ohara M, Saito K, Ozaki S, Terai M, Mastrangelo MJ, et al. Establishment of an orthotopic  
583 patient-derived xenograft mouse model using uveal melanoma hepatic metastasis. *Journal of Translational*  
584 *Medicine.* 2017;15:145.

585 83. Tudhope SR, Cuthbert NJ, Abram TS, Jennings MA, Maxey RJ, Thompson AM, et al. BAY u9773, a novel  
586 antagonist of cysteinyl-leukotrienes with activity against two receptor subtypes. *European Journal of*  
587 *Pharmacology.* 1994;264(3):317-23.

588 84. Wunder F, Tinel H, Kast R, Geerts A, Becker EM, Kolkhof P, et al. Pharmacological characterization of  
589 the first potent and selective antagonist at the cysteinyl leukotriene 2 (CysLT(2)) receptor. *Br J Pharmacol.*  
590 2010;160(2):399-409.

591 85. Khilnani G, Khilnani AK. Inverse agonism and its therapeutic significance. *Indian Journal of*  
592 *Pharmacology.* 2011;43(5):492-501.

593 86. Lappano R, Maggiolini M. Pharmacotherapeutic Targeting of G Protein-Coupled Receptors in Oncology:  
594 Examples of Approved Therapies and Emerging Concepts. *Drugs.* 2017;77(9):951-65.

595 87. Ramsey DM, McAlpine SR. Halting metastasis through CXCR4 inhibition. *Bioorg Med Chem Lett.*  
596 2013;23(1):20-5.

597 88. Innamorati G, Valenti MT, Giovinazzo F, Carbonare LD, Parenti M, Bassi C. Molecular Approaches To  
598 Target GPCRs in Cancer Therapy. *Pharmaceuticals.* 2011;4(4):567-89.

599 89. Dupre DJ, Le Gouill C, Gingras D, Rola-Pleszczynski M, Stankova J. Inverse agonist activity of selected  
600 ligands of the cysteinyl-leukotriene receptor 1. *J Pharmacol Exp Ther.* 2004;309(1):102-8.

601 90. Bond RA, Ijzerman AP. Recent developments in constitutive receptor activity and inverse agonism, and  
602 their potential for GPCR drug discovery. *Trends Pharmacol Sci.* 2006;27(2):92-6.

603 91. Zembowicz A, Phadke PA. Blue nevi and variants: an update. *Arch Pathol Lab Med.* 2011;135(3):327-36.

604 92. Costa S, Byrne M, Pissaloux D, Haddad V, Paindavoine S, Thomas L, et al. Melanomas Associated With  
605 Blue Nevi or Mimicking Cellular Blue Nevi: Clinical, Pathologic, and Molecular Study of 11 Cases Displaying a  
606 High Frequency of GNA11 Mutations, BAP1 Expression Loss, and a Predilection for the Scalp. *Am J Surg Pathol.*  
607 2016;40(3):368-77.

608 93. Perez-Alea M, Vivancos A, Caratu G, Matito J, Ferrer B, Hernandez-Losa J, et al. Genetic profile of  
609 GNAQ-mutated blue melanocytic neoplasms reveals mutations in genes linked to genomic instability and the PI3K  
610 pathway. *Oncotarget.* 2016;7(19):28086-95.

611 94. Rovati EG, Bäck M, Dahlén S-E, Drazen J, Evans JF, Shimizu T, et al. Leukotriene receptors: CysLT  
612 2 receptor. <http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=270>: IUPHAR/BPS  
613 Guide to PHARMACOLOGY; 20/02/2018 [cited 2018 27/05].

614