



Supplementary Materials: High Cysteinyl Leukotriene Receptor 1 Expression Correlates with Poor Survival of Uveal Melanoma Patients and Cognate Antagonist Drugs Modulate the Growth, Cancer Secretome and Metabolism of Uveal Melanoma Cells

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**Figure S1.** Analysis of *CYSLTR1/CYSLTR2* expression and UM patient survival from The Cancer Genome Atlas (TCGA) adjusted by ESTIMATE stromal score. Survival curves in Figure 1 were adjusted by stromal infiltration. When the models are adjusted by stromal scores, there is no statistically significant relationship between high (red) *CYSLTR1* expression and disease-free survival (**A**) or overall survival (**B**) (p = 0.57 and p = 0.075, respectively). High expression of *CYSLTR2* (red) maintains a statistically significant relationship with disease-free survival (**C**) and overall survival (**D**) when the models are adjusted by stromal cell infiltration (p = 0.004 and p = 0.00027, respectively).



**Figure S2.** Patient TMA control tissue. Core-core correlations of manual versus digital pathology analysis. Clinical characteristics of patients included in the TMA. (**A**) Colorectal cancer tissue positive control for CysLT<sub>1</sub>. Colorectal cancer tissue positive control for CysLT<sub>2</sub>. Negative control (omission of antibody). (**B**) Correlation between manual score and the average H-score assigned by digital pathology analysis for CysLT<sub>1</sub> (R = 0.64) and CysLT<sub>2</sub> (R = 0.61). (**C**) Clinical characteristics of the patients included in the TMA analysed. (**D**) The relationship between prognostic clinical characteristics in UM and the expression of high CysLT<sub>1</sub> or CysLT<sub>2</sub>. Ciliary body involvement has a statistically significant relationship with high CysLT<sub>1</sub> expression (p = 0.041) as assessed by Pearson's Chi-Square test.



**Figure S3.** Cell line comparison data for the clonogenic assay. (A) Differences in the percentage survival fraction of Mel285 clones when treated for 24 versus 96 hours. Treatment with 20  $\mu$ M quininib (Q1) is significantly more effective at 96 hours. (B) Differences in the percentage survival

fraction of OMM2.5 clones when treated for 24 versus 96 h. Treatment with 20  $\mu$ M quininib (Q1) is significantly more effective at 96 h. (C) Differences in the percentage survival fraction of Mel285 versus OMM2.5 clones when treated for 24 h. 20  $\mu$ M quininib (Q1) is significantly more effective in Mel285 cells following 24-hour treatment. While 20  $\mu$ M 1,4–dihydroxy quininib (Q7) is significantly more effective in OMM2.5 cells following 24-hour treatment. (D) Differences in the percentage survival fraction of Mel285 versus OMM2.5 clones when treated for 96 hours. There were no statistically significant differences between cell lines following 96-hour treatment. Statistical analysis was performed using a paired t-test to compare within the same cell lines. An unpaired t-test was used to compare between different cell lines. Error bars are mean + S.E. \* p < 0.05; \*\* p < 0.01.



**Figure S4.** Factors unchanged by ELISA following 24 h treatment in Mel285 and OMM2.5 cells. Treatment with all CysLT targeting drugs tested (quininib (Q1), 1,4 –dihydroxy quininib (Q7), montelukast and HAMI 3379 (HAMI)) had no effect on the secretion of IFN-  $\gamma$  (**A**) or IL-4 (**B**) in Mel285 or OMM2.5 cells.



**Figure S5.** Generation of OMM2.5 cell line-derived orthotopic xenograft models of UM. Macroscopic appearance of an ocular tumour (**A**) 104 days after intraocular injection of 1 million OMM2.5 cells, or (**B**) 102 days after intraocular injection of disaggregated cells from ocular tumour (**A**). Macroscopic appearance and liver gross pathology of two representative mice injected with 1 million OMM2.5 cells in the liver, 98 days post-injection (**C**), 112 days post-injection(**D**), and of one representative mouse directly implanted with a OMM2.5 cell line-derived tumour fragment in the liver (**E**), 61 days post-implantation (**E**). H&E staining in representative sections of an ocular tumour from an OMM2.5 cell line-derived xenograft model. Original magnification ×50 (**F**), ×100 (**G**), ×200 (**H**).

Table S1. The association between CYSLTR1 and CYSLTR2 gene expression from TCGA and
prognosis were assessed by Cox proportional hazard regression models (A,B,C). To resolve a
violation of the proportional hazard assumption, the association between CYSLTR2 gene expression
and overall survival (D) was stratified by time from 0-20 months and beyond 20 months and analysed
by the likelihood ratio (LHR) test. The hazard ratios and corresponding confidence intervals for
CYSLTR1, CYSLTR2, sex, and age are reported.

(A) Disease-Free Survival CYSLTR1					
Variable	HR	95% CI	<i>p</i> -value		
Sex (male)	0.82	[0.44;1.20]	0.61		
Age	1.03	[1.01;1.05]	0.09		
CYSLTR1 (continuous)	1.05	[1.03;1.07]	0.01		
Likelihood ratio test	-	-	0.06		
(B) Overall Survival CYSLTR1					
Variable	HR	95% CI	<i>p</i> -value		
Sex (male)	1.47	[1.02;1.91]	0.39		
Age	1.05	[1.02;1.07]	0.02		
CYSLTR1 (continuous)	1.06	[1.04;1.08]	0.005		
Likelihood ratio test	-	-	0.004		
(C) Disease-Free Survival CYSLTR2					

Variable	HR	95% CI	<i>p</i> -value		
Sex (male)	0.73	[0.35;1.11]	0.41		
Age	1.03	[1.01;1.04]	0.12		
CYSLTR2 (continuous)	1.35	[1.25;1.45]	0.004		
Likelihood ratio test	-	-	0.01		
(D) Overall Survial CYSLTR2					
Variable	HR	95% CI	<i>p</i> -value		
Sex (male)	1.33	[0.89;1.77]	0.52		
Age	1.05	[1.03;1.07]	0.02		
CYSLTR2.adj:strata(tstart) tstart=0	1	[0.99;1.01]	0.47		
CYSLTR2.adj:strata(tstart) tstart=20	1.01	[1;1.02]	0.0002		
Likelihood ratio test	-	-	0.00003		



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