**Genetic variants of the *Butyrophilin-like 2 (BTNL2)* gene in uveal melanoma**

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Significant progress has recently been made in understanding the molecular pathology of uveal melanoma (UM). It is well known that genetic alterations, such as monosomy 3, polysomy 8q and *BAP1* gene inactivating mutations, are associated with a poor prognosis in UM, whereas a gain in chromosome 6p is associated with a more favorable outcome. 1 The pathways by which these genetic aberrations influence the processes involved in tumor dissemination and ultimate colonisation, however, are not fully understood.

In this issue of *JAMA Ophthalmology*, Amaro et al. present an extensive analysis of the *BTNL2* gene in UM and its association with macrophage infiltrates in these tumors. 2 Chromosome 6p harbors the *BTNL2* gene, which is a member of the butyrophilin-like B7 family of immunoregulators. 3 *BTNL2* gene polymorphisms have been implicated in a number of diseases, such as sarcoidosis; rheumatoid arthritis; inflammatory bowel disease; type 1 diabetes and systemic lupus erythematosus. It has also been associated with prostate cancer. 4 The role of this gene has not yet been studied in UM. It is believed that the gene may be involved in immune surveillance as a negative T-cell regulator by decreasing T-cell proliferation and cytokine release, which would be pro-tumor progression. 3,5-6 Hence it would seem to be contradictory to the more indolent course associated with a chromosome 6p gain in UM.

Amaro et al. investigated the expression and missense variant frequencies of the *BTNL2* gene in UM samples from patients of treated in Italy and Germany, UM cell lines as well as in human macrophages (after in vitro polarization into M1 and M2 subsets) by real-time polymerase chain reaction and multiplex ligation-dependent probe amplification. They found that BTNL2 was expressed in UM specimens and UM cell lines at highly variable levels with no correlation with the amplification of chromosome 6p. However, not all of the examined UM samples were demonstrated in the Results, and hence it was unclear which cohorts were included and how the authors selected the ones to highlight. Interestingly, there was also no difference seen in cell lines derived from primary or metastatic UM. The authors also demonstrated that there was no correlation between the frequencies of missense variants with UM risk. Furthermore, no association was found between ethnic groups. The unexplained discrepancies noted in the allele single nucleotide variant frequencies may have been due to the different methodologies used by other investigators.

The *BTNL2* gene was also expressed in both M1 and M2 macrophages, but at significantly higher levels in the latter subtype. This finding would be consistent with the immunosuppressive and tumorigenic activity associated with the M2 subtype. Chronic inflammation is a hallmark of both primary and metastatic UM (*Coupland et al., unpublished*), and is thought to be a key mediator in all steps of tumorgenesis – from initiation, through to progression and metastasis. The main infiltrating inflammatory cells are macrophages, particularly the M2-polarized subtype of the tumor associated macrophages (TAMs). A high density of M2 macrophages has also been reported with monosomy 3. 7 It would have been of interest had the investigators confirmed that the macrophages were truly polarized into M1 and M2 in UM by immunohistochemistry (CD68; CD163), and that greater expression of the *BTNL2* gene was seen in CD163+ macrophages.

In conclusion, Amaro et al. presented a very interesting analysis of the variable expression of *BTNL2* gene in UM, which has not been previously reported. Furthermore, they demonstrate expression of this gene in M2 macrophages, which adds further novel information to the current literature examining the microenvironment of UM, advocating the role of inflammation in their development and progression.

**References**

1. Coupland SE, Lake SL, Zeschnigk M, Damato BE. [Molecular pathology of uveal melanoma.](http://www.ncbi.nlm.nih.gov/pubmed/23222563) *Eye* 2013;27:230-242.

2. Adriana Amaro, Federica Parodi, Konrad Diedrich, Giovanna Angelini, Cornelia Götz, Silvia Viaggi,et al. Analysis

of the expression and single nucleotide variant frequencies of the B7 immune regulatory family member

Butyrophilin-like 2 (BTNL2) gene in uveal melanoma. *JAMA Ophthal*. 2016; In press.

3. Nguyen T, Liu XK, Zhang Y, Dong C. BTNL2, a butyrophilin-like molecule that functions to inhibit T

343 cell activation. *J Immunol.* Jun 15 2006;176:7354-7360.

4. Fitzgerald LM, Kumar A, Boyle EA, et al. Germline missense variants in the BTNL2 gene are associated with prostate cancer susceptibility. *Cancer Epidemiol Biomarkers Prev.* 2013;22:1520-1528.

5. Orozco G, Eerligh P, Sanchez E, et al. Analysis of 360 a functional BTNL2 polymorphism in type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus. *Hum Immunol.* 2005;66:1235-1241.

6. Valentonyte R, Hampe J, Huse K, et al. Sarcoidosis is associated with a truncating splice site mutation in BTNL2. *Nat Genet.*  2005;37:357-364.

7. Bronkhorst IH, Jager MJ. Inflammation in uveal melanoma. *Eye* 2013;27:217-223.