**Trainee Small Grant Scheme (TSGS 0419 02) Final Report**

**Title:** Transcriptome Profiling Reveals New Insights into the Immune Microenvironment and Upregulation of Novel Biomarkers in Metastatic Uveal Melanoma.

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**Background and aims:**

Metastatic uveal melanoma (mUM), typically involving the liver, is incurable. Our understanding of hepatic mUM and its tumour microenvironment (TME) needs significant advancement in order to identify potential therapeutic targets for this disease. We undertook transcriptome profiling of mUM and validated results using immunohistochemistry (IHC).

**Methods:**

Transcriptome profiling of 40 formalin-fixed paraffin-embedded mUM liver resections and 6 control liver specimens was undertaken. mUMs were assessed for morphology, nuclear BAP1 (nBAP1) expression, and their tumour microenvironments (TME) using an “immunoscore” (absent/altered/high) for tumour-infiltrating lymphocytes (TILs) and macrophages (TAMs). Transcriptomes were compared between mUM and control liver; intersegmental and intratumoural analyses were also undertaken.

**Results:**

Most mUM were epithelioid cell-type (75%), amelanotic (55%), and nBAP1-ve (70%). They had intermediate (68%) or absent (15%) immunoscores for TILs and intermediate (53%) or high (45%) immunoscores for TAMs. M2-TAMs were dominant in the mUM-TME, with upregulated expression of ANXA1, CD74, CXCR4, MIF, STAT3, PLA2G6, and TGFB1. Compared to control liver, mUM showed significant (p < 0.01) upregulation of 10 genes: DUSP4, PRAME, CD44, IRF4/MUM1, BCL2, CD146/MCAM/MUC18, IGF1R, PNMA1, MFGE8/lactadherin, and LGALS3/Galectin-3. Protein expression of DUSP4, CD44, IRF4, BCL-2, CD146, and IGF1R was validated in all mUMs, whereas protein expression of PRAME was validated in 10% cases; LGALS3 stained TAMs, and MFGEF8 highlighted bile ducts only. Please see graphic display.

**Conclusions:**

Intersegmental mUMs show differing transcriptomes, whereas those within a single mUM were similar. Our results show that M2-TAMs dominate mUM-TME with upregulation of genes contributing to immunosuppression. mUM significantly overexpress genes with targetable signalling pathways, and yet these may differ between intersegmental lesions.

The study was presented as an Oral Poster Presentation at Regional ACF Research Evening and published in the journal *Cancers*. Please see attached paper.

Krishna Y, Acha-Sagredo A, Sabat-Pośpiech D, Kipling N, Clarke K, Figueiredo C.R, Kalirai H, Coupland S.E. Transcriptome Profiling Reveals New Insights into the Immune Microenvironment and Upregulation of Novel Biomarkers in Metastatic Uveal Melanoma. Cancers 2020; 12(10) 2832:1-22.