Göteborg, 2022

Novel immunotherapies for metastatic uveal melanoma
-from bench to clinical biomarkers

Akademisk avhandling

Som för avläggande av medicine doctorsexamen vid Sahlgrenska akademin Göteborgs universitet kommer att offentligen försvaras i hörsal, K Isaksson, Medicinaregatan 16, 15th December 2022, klockan 13:00
av Vasu R Sah

Fakultetopponent: Associate Professor Andreas Lundqvist, Department of Oncology-Pathology, Karolinska Institutet, Sverige

Avhandlingen baseras på följande delarbeten

I. **Sah VR**, Karlsson J, Jespersen H, Lindberg MF, Nilsson LM, Ny L, Nilsson JA. Epigenetic therapy to enhance therapeutic effects of PD-1 inhibition in therapy-resistant melanoma. Melanoma research. vol. 32,4 (2022): 241-248.

II. **Sah VR**, Jespersen H, Karlsson J, Nilsson L, Bergkvist M, Johansson I, Carneiro A, Helgadottir H, Levin M, Ullenhag G, Ståhlberg A, Bagge RO, Nilsson JA, Ny L. Novel biomarkers identified in patients with metastatic uveal melanoma treated with combined epigenetic therapy and checkpoint immunotherapy. Submitted

III. **Sah VR***, Karlsson J*, Bucher V, Iqbal M, Saxena A, Johansson M, Bagge RO, Ny L, Nilsson L, Nilsson JA. Phenotypes and spatial localization of T cells in uveal melanoma metastases. Manuscript

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER

GÖTEBORGS UNIVERSITET

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Novel immunotherapies for metastatic uveal melanoma
-from bench to clinical biomarkers

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Abstract
Immunotherapy has revolutionized cancer treatment, with durable long-term effects in a proportion of patients. However, in around half of these patients, there are little to no effects. Patients with metastatic uveal melanoma (mUM), a rare form of melanoma originating from the eye has lack of effective treatments. The aim of this thesis is to discover novel immunotherapies and biomarkers for treatment of metastatic uveal melanoma (mUM).
In **paper 1**, we show HDAC inhibitor increases and BET inhibitor decreases levels of HLA (human leukocyte antigen) and PDL1 (programmed death ligand -1) on uveal melanoma cell lines. The combination of Entinostat and PD-1 (programmed death -1) inhibition resulted in enhanced effects of PD-1 inhibition both with in-vitro and in-vivo studies, whereas BET inhitor JQ1 did not. Using PDL1 knockout tumor cells, combined with Entinostat, helped in gaining mechanistic understanding. This translational work from paper I, provided the foundation of a phase II clinical trial PEMDAC (NCT02697630), in metastatic uveal melanoma. In **paper 2**, we perform clinical biomarker discovery for a two year follow up of patients treated in PEMDAC trial. We observe all patients w.r.t progression free survival and overall survival, assessing the efficacy and survival long term. This led us to a comprehensive analysis of patient samples from the pre-treatment stage, followed longitudinally to the end of study. We discovered tumor and chemokine signatures as novel biomarkers predicting clinical responses. Moreover, the discovered chemokine axis, essential for T-cells migration, induce tertiary lymphoid structures (TLS)-like entities at the metastasis sites, correlating to clinical benefit. In **paper 3,** we develop patient xenografts (PDX) models of mUM, and further investigated these tumors using an ex-vivo screening platform. The PDX tumors were used to grow 3-D spheroids in-vitro, co-cultured together with their autologous and allogenic tumor infiltrating lymphocytes (TILs). Using a NOG-IL2 transgenic mice, matched tumor and TILs were assessed with subcutaneous and liver-met mUM PDX models. The ex-vivo screen and patient biopsies were evaluated further, with T cell receptor (TCR) and single cell sequencing, in identifying T- reactive clones with anti-tumor immunity. Furthermore, using a highly multiplex technique, patient biopsies were interrogated for tumor immune spatial inter-play, leading to identification of similar tumor-reactive T cell subsets, building a cross-functional discovery platform for mUM.

Keywords: Metastatic uveal melanoma, Patient-derived xenografts, immunotherapy, biomarker discovery, tumor infiltrating lymphocytes, PD-1 inhibition, histone deacetylase inhibition, Multiplex imaging, Single cell sequencing.

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