**Autotaxin as an emerging target in immunotherapy**

**Elisa Matas-Rico1, Maaike van Zon2, Irene van der Haar Avila3, Andrew Morris4, Jan Koster5, Inge Verbruggen3, Fernando Salgado-Polo1, Tomasz Ahrends3, Sander de Kivit3, ZOE Johnstone6, Stuart Farrow7, Anastassia Perrakis1, John Haenen2, Jannie Borst3, Ton Schumacher2, Joost van den Berg2, Wouter Mooonen1**

1Division of Biochemistry, 2Division of Immunology, 3Division of Tumour Biology and Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands. 4Division of Cardiovascular Medicine, Gill Heart Institute, University of Kentucky, Lexington, KY, USA. 5Amsterdam UMC, Department of Oncogenetics, Amsterdam, The Netherlands. 6Oncitura SA, Campus Biotech Innovation Park, Geneva, Switzerland

**7CRUK Therapeutic Discovery Laboratories, London, UK**

### Background

Autotaxin (ATX) or ENPP2, secreted by diverse cell types, produces lysophosphatidic acid (LPA) that activates G protein-coupled receptors (LPA1-6) to regulate multiple biological functions. ATX-LPA stimulates the motility of naïve T cells to promote their entry into lymph nodes via LPAR2, but how it affects the behavior of tumor-infiltrating lymphocytes (TILs) remains an open question in cancer immunotherapy.

### Aim

To determine how ATX regulates T-cell behavior and other cellular functions in the immunosuppressive tumor microenvironment, with focus on melanoma.

### Melanoma-conditioned medium is chemorepulsive for melanoma patient-derived TILs and circulating CD8+ T cells

- **A** Immunofluorescence showing ATX expression in cell lines and supernatants from MDAMB-453 and A375 melanoma cells. The basal level of ATX expression was higher in melanoma-conditioned medium. CCL10 was used as a positive control. Data are expressed as mean ± SEM (n=4), *p* <0.01 to *p* <0.001.

### LPA, and ATX (+LPC) inhibit the migration of melanoma TILs and circulating CD8+ T cells

- **A** LPA concentration gradient over time. LPA, ATX/LPC suppresses the basal migration of TILs. CCL10-induced TILs chemotaxis in LPA was added together with CCL10 to the bottom chamber. The basal migration rate of CD8+ T cells is inhibited by ATX/LPC. Data are expressed as mean ± SEM (n=4), *p* <0.01 to *p* <0.001.

### LPAR6 is the predominant LPA receptor in melanoma TILs and CD8+ T cells

- **A** Migration of melanoma TILs and CD8+ T cells to LPA with LPAR6 expression (relative to GAPDH) in melanoma TILs and CD8+ T cells.

### Melanoma-secreted ATX, rather than total LPA levels, determines TILs repulsion

- **A** Transcriptional profiling using RNA-seq technology showed differentially expressed genes in LPA-treated TILs (3 hrs). Ethanol-treated melanoma cultures (left, red up) are compared with whole-genome RNA-seq (left, blue down) as determined by whole-genome RNA-seq (left, blue down) expression (left, red up).

### Conclusion

Our work supports a model of the melanoma microenvironment whereby ATX, in complex with LPA, opposes cytotoxic T-cell infiltration via LPAR6, while stimulating melanoma cell dispersal and activating fibroblasts via LPAR1. Therefore, ATX inhibition may help improve the efficacy of targeted immunotherapy in melanoma and beyond.

### References

1. Ahrends, T. et al. (2017) CD4+ T cell help correlates with cytotoxic T cell effector program including cytokine receptor downregulation and increased tissue invasiveness. Immunity 46, 584-601