**Effect of split intravenous dosing of oncolytic adenovirus TILT-123 on normal tissue versus tumor macrophages and virus bioavailability in patients with advanced solid tumors**

E. Jirovec (1), D.C.A. Quixabeira (1, 2), K.J. Jalkanen (3), J.H.A. Clubb (1,2), T. Kudling (1), S. A. Pakola (1), V. Arias (1), T. Alanko (4), R. Korpisaari (4), M. Jaakkola (3), J. Kononen (4), J. Sormunen (4), T. Pellinen (5), L. Haybout (1,2), C. Kistler (2), S. Sorsa (1,2), R. Havunen (1,2), J.M. Santos (1,2), V. Cervera-Carrascon (1,2), A. Hemminki (1,2,3).

1) Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland

2) TILT Biotherapeutics Ltd, Helsinki, Finland

3) Comprehensive Cancer Center, Helsinki University Hospital, Helsinki, Finland

4) Docrates Cancer Center, Helsinki, Finland

5) Digital Microscopy and Molecular Pathology Unit, Institute for Molecular Medicine Finland, Helsinki, Finland

**Background**

Intratumoral administration of oncolytic viruses is the most commonly used delivery method but limits the broader clinical applicability of this therapy. Igrelimogene litadenorepvec (Ad5/3-E2F-d24-hTNF-IRES-hIL2; TILT-123), is a chimeric oncolytic adenovirus suitable for intravenous delivery due to its capsid modification, enabling partial evasion of neutralizing antibodies and potentially enhancing its clinical utility.

**Methods**

TILT-123 was evaluated as a fully intravenous regimen in six patients with advanced solid tumors in a cohort of TUNIMO (NCT04695327). Patients received $1x10^{12}$ viral particles of TILT-123 twice daily on days 1, 3, 8, 10, 22, 43, and 64. Splitting the dose aimed to increase TILT-123 bioavailability by modulating macrophage mediated clearance. Analyses of pre- and post-treatment blood, serum and tumors included multiplex immunofluorescence, virus immunohistochemistry (IHC), qPCR, and serum proteomics.

**Results**

Virus quantification results suggest increased TILT-123 concentration and persistence in blood over time. Successful TILT-123 transduction of post-treatment tumors was evidenced by IHC staining for viral proteins and qPCR analysis. Preliminary analysis of metastatic liver biopsies showed accumulation of cytotoxic lymphocytes and macrophages in tumor nests, while macrophages in normal tissues decreased. Analysis of intratumoral macrophages indicated an increase with the split dose regimen compared to biopsies from patients treated with a single-dose regimen.

**Conclusions**

A fully intravenous split-dose regimen of TILT-123 resulted in increased viral presence over time, tumor transduction and induced immunological changes in patients with advanced solid tumors. Macrophages in tumors increased but decreased in normal tissues suggesting the utility of the split dose regimen in attenuating anti-adenoviral normal tissue sinks. These findings support the feasibility of split-dose systemic administration of TILT-123 in future clinical trials.