**Introduction**

Liquid biopsies are important samples for providing biomarkers in clinical studies in a non-invasive manner. They are particularly relevant for immuno-oncology trials, where regulation of circulating immune cells may reflect immune changes in tumours in response to immune targeting therapies. For example, RUX004, a potent and selective inhibitor of the Wnt pathway regulator porcupine is hypothesised to have immunomodulatory anti-cancer functions. Therefore as part of an RUX004 safety and tolerability study in cancer patients with solid tumours (NCT03447470), we aim develop methods to analyse immune response liquid biomarkers. This includes analysis of whole blood by flow cytometry to quantify a range of immune cell subsets and functional markers.

**Methods**

Flow cytometry analysis is carried out in house using 7 multi-colour panels, analysed on the ACEA Biosciences Novocyt 3000 flow cytometer (Fig 1). Antibodies included in these panels have been validated using healthy donor peripheral blood mononuclear cells (PBMCs), healthy donor whole blood and Biolegend VeriCells™.

**Results**

**Healthy donor whole blood**

Whole blood samples were collected from 3 healthy donors every 3-4 weeks to generate a longitudinal dataset.

Samples were stained with all 7 flow cytometry panels (Fig 1) to assess detectable immune cell populations in whole blood and variability over time in healthy donors.

All populations described in Fig 1 are detectable, with the exception of MDSCs, CD141+ mDCs and Tmoss.

The expression levels of functional T cell markers are also low.

**Conclusion**

We have established a protocol for the analysis of immune cell subsets by flow cytometry, suitable to assess potential therapy-induced changes in circulating immune cells as an exploratory end-point in patients with solid tumours enrolled in the RUX004 clinical study.

**References**

1. Li et al. (2019), 10.1038/s41598-018-38920-6
3. Wang et al. (2019), 10.1038/s41598-019-50628-3