system by forming an immunosuppressive tumor microenvironment (TME). FoxP3+ Regulatory T cells (Tregs) and indoleamine-2,3-dioxygenase (IDO) are a part of the immunosuppressive TME in CM. In previous studies, IDO expression correlates with poor prognosis and greater Breslow's depth, but results concerning the role of FoxP3+ Tregs in CM have been controversial. Furthermore, the correlation between IDO and Tregs has not been substantially studied in CM, although IDO is known to be an important regulator of Tregs activity. To develop new therapeutic strategies, it is important to understand the role of immunosuppressive factors in CM.

Materials and Methods We investigated the associations of FoxP3+ Tregs, IDO+ tumor cells and IDO+ stromal immune cells with tumor stage, prognostic factors, and survival in CM. FoxP3 and IDO were immunohistochemically stained from 29 benign and 29 dysplastic nevi, 18 in situ -melanomas, 48 superficial and 62 deep melanomas and 67 lymph node metastases of CM. The number of FoxP3+ Tregs and IDO+ stromal immune cells was analysed quantitatively and the coverage and intensity of IDO+ tumor cells was evaluated semiquantitatively. Tumors were divided into IDO-negative and IDO-positive, containing less or more than 1% IDO+ melanoma cells of all tumor cells, respectively. P values equal to or less than 0.05 were considered statistically significant.

Results IDO+ stromal immune cells and FoxP3+ Tregs mainly accumulated in the areas with lymphocyte infiltration and thus resided mostly in the perilesional stroma. The number of FoxP3+ Tregs and IDO+ stromal immune cells were significantly higher in malignant melanomas compared with benign lesions. The increased expression of IDO in melanoma cells was associated with poor prognostic factors, such as recurrence, nodular growth pattern and increased mitotic count. Furthermore, the expression of IDO in melanoma cells was associated with reduced recurrence-free survival. We further showed that IDO-positive tumors contained significantly higher amounts of FoxP3+ Tregs and IDO+ stromal immune cells than IDO-negative tumors. However, the correlation between FoxP3+ Treg and IDO+ stromal immune cell counts was rather weak.

Conclusions Our results indicate that IDO expression is intimately involved in creating a TME conducive to tumor growth in CM. Thus, targeting IDO enzymatic pathway might be a worth of further studies in CM. Furthermore, we show that FoxP3+ Tregs appear to contribute to the immunosuppressive TME in CM, but their role may not be that critical to melanoma progression. The positive association of FoxP3+ Tregs with IDO+ melanoma cells, but not with IDO + stromal immune cells, indicates a complex interaction between IDO and Tregs in CM, which demands further studies. Support: Sigrid Juselius Foundation (S.P.-S.), Academy of Finland (S.P.-S.), The Paavo Koistinen Foundation (S.S.), Emil Aaltonen Foundation (S.S.) and North-Savo Cultural Foundation (S.S.).

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P02.09

HETEROMERIZATION OF UPA AND PAI-1 ENFORCES PRO-TUMORIGENIC NEUTROPHIL TRAFFICKING TO MALIGNANT TUMORS IN BREAST CANCER VIA VLDLR-DEPENDENT β 2 INTEGRIN CLUSTERING

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Background High tumor levels of urokinase-type plasminogen activator (uPA)-plasminogen activator inhibitor-1 (PAI-1) heteromers independently predict poor survival in early breast cancer. The pathogenetic role of this protein complex, however, remains largely obscure.

Material and Methods Neutrophil trafficking was analyzed in orthotopic (multi-channel flow cytometry) and heterotopic (ear; multi-channel *in vivo* microscopy) mouse models of 4T1 breast cancer, in a mouse peritonitis assay (multi-channel flow cytometry), as well as in the mouse cremaster muscle (multi-channel *in vivo* microscopy). Cytokine expression in tumors was determined by multiplex ELISA. Phenotypic and functional properties of primary mouse neutrophils, microvascular endothelial cells (cell line bEnd.3), macrophages (cell line RAW 264.7), and breast cancer cells (cell line 4T1) were characterized in different *in vitro* assays. uPA/PAI-1 expression and neutrophil infiltration in human breast cancer samples were assessed by RNA sequencing, immunohistochemistry, and ELISA.

Results and Discussion Here, we demonstrate that uPA-PAI-1 heteromerization multiplies the potential of the single proteins to attract pro-tumorigenic neutrophils. To this end, tumor-released uPA-PAI-1 activates peritumoral macrophages (VLDL receptor- and ERK/MAPK-pathway). This promotes neutrophil trafficking to cancerous lesions (enhanced β 2 integrin activation and clustering) and primes these immune cells towards a pro-tumorigenic phenotype (elevated neutrophil elastase expression), thus supporting tumor growth and metastasis. Blockade of uPA-PAI-1 heteromerization by a novel inhibitor effectively interfered with these events and prevented tumor progression.

Conclusions Here, we identified an already therapeutically targetable interplay between hemostasis and innate immunity that drives advanced stages of breast cancer as well as characterized the underlying mechanisms of this process. As a personalized immunotherapeutic strategy, blockade of uPA-PAI-1 heteromerization might be particularly beneficial for patients with highly aggressive uPA-PAI-1^{high} tumors. *This study was*

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P02.10 FOCUSCOPE: A SINGLE CELL, MULTI-OMICS SOLUTION TO SIMULTANEOUSLY ANALYZE TUMOR VARIANTS AND MICROENVIRONMENT

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Recent advances of high-throughput single cell sequencing technologies have greatly improved our understanding of the complex biological systems. Heterogeneous samples such as tumor tissues commonly harbor cancer cell-specific genetic variants and gene expression profiles, both of which have been shown to be related to the mechanisms of disease development, progression, and responses to treatment. Furthermore, stromal and immune cells within tumor microenvironment interact with cancer cells to play important roles in tumor responses to systematic therapy such as immunotherapy or cell therapy. However, most current high-throughput single cell sequencing methods detect only gene expression levels or epigenetics events such as chromatin conformation. The information on important genetic variants including mutation or fusion is not captured. To better understand the mechanisms of tumor responses to systematic therapy, it is essential to decipher the connection between genotype and gene expression patterns of both tumor cells and cells in the tumor microenvironment. We developed FocuSCOPE, a high-throughput multi-omics sequencing solution that can detect both genetic variants and transcriptome from same single cells. FocuSCOPE has been used to successfully perform single cell analysis of both gene expression profiles and point mutations, fusion genes, or intracellular viral sequences from thousands of cells simultaneously, delivering comprehensive insights of tumor and immune cells in tumor microenvironment at single cell resolution.

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P02.11 TREM1 AGONIST PY159 PROMOTES MYELOID CELL REPROGRAMMING AND UNLEASHES ANTI-TUMOR IMMUNITY

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Background Tumor-associated myeloid cells can impede productive anti-tumor immunity. One strategy for targeting immunosuppression is myeloid reprogramming, which drives immunosuppressive myeloid cells to acquire an immunostimulatory phenotype. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor expressed on neutrophils and subsets of monocytes and tissue macrophages. TREM1 associates with DAP12 adaptor and induces proinflammatory signaling, amplifies innate immune responses, and is implicated in the development of acute and chronic inflammatory diseases. TREM1 is also enriched in tumors, specifically on tumor-associated myeloid cells. To investigate the potential of TREM1 modulation as an anti-cancer therapeutic strategy, we developed PY159, an afucosylated humanized anti-TREM1 monoclonal antibody, and characterized it in the pre-clinical assays described below.

Materials and Methods An FcγR binding ELISA and a Jurkat TREM1/DAP12 NFAT-luciferase reporter cell line were used to assess PY159 binding to human FcγRs and TREM1 signaling, respectively. PY159 responses in human whole blood *in vitro* were evaluated by flow cytometry, transcriptional analysis of sorted leukocyte subsets, and measurement of secreted cytokines/chemokines by MSD. A Transwell system was used to evaluate PY159 effects on neutrophil chemotaxis. TREM1 expression in human tumors was validated by scRNAseq, immunohistochemistry, and flow cytometry. Anti-tumor efficacy of a surrogate anti-mouse TREM1 antibody, PY159m, was evaluated using syngeneic mouse tumor models, either as a single agent or in combination with anti-PD-1.

Results PY159 afucosylation increased its binding affinity for FcγR and its ability to activate TREM1/DAP12 signaling. In human blood assays, PY159 treatment did not induce depletion of TREM1-expressing cells. Rather, it upregulated monocyte activation markers, promoted neutrophil chemotaxis, and induced proinflammatory cytokines and chemokines, which was dependent on PY159 afucosylation. In human tumors, TREM1 was detected on tumor-associated neutrophils, tumor-associated macrophages, and monocytic myeloid-derived suppressive cells. PY159 induced proinflammatory cytokines and chemokines in dissociated human tumors *in vitro*, demonstrating that PY159 can reprogram tumor-associated myeloid cells. A surrogate anti-mouse TREM1 antibody, PY159m, exhibited anti-tumor efficacy in several syngeneic mouse tumor models, both as single-agent and in combination with anti-PD-1.

Conclusions These results show that PY159 is a TREM1 agonist that reprograms myeloid cells and unleashes anti-tumor immunity. PY159 safety and efficacy are currently being evaluated in first-in-human clinical trial (NCT04682431) involving patients resistant and refractory to standard of care therapies.

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