ImmunoTools *special* Award 2021

Agnieszka Witalisz-Siepracka, PhD, PostDoc

Supervisor: Prof. Dr. Dagmar Stoiber-Sakaguchi

Division Pharmacology; Department for Pharmacology, Physiology and Microbiology, Karl Landsteiner University of Health Sciences, Dr.-Karl-Dorrek-Straße 30, 3500 Krems, Austria

Natural killer cells in myeloproliferative neoplasms

Myeloproliferative neoplasms (MPNs) – polycythaemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) – are a group of malignant bone marrow disorders characterised by unbalanced proliferation and differentiation of hematopoietic precursors resulting in abnormal numbers of blood cells. The Janus kinase (JAK) 2 V617F somatic mutation is the most common driver mutation detected in MPN patients. In ET and PMF more than 70% of the JAK2 non-mutated patients carry mutations in the Calreticulin gene. Both mutations lead to an aberrant activation of the JAK-Signal Transducer and Activator of Transcription (STAT) pathway, in particular the JAK2/STAT5 axis, which results in increased numbers of megakaryocytes, erythrocytes and thrombocytes as well as splenomegaly. Therefore, JAK inhibition is a relevant therapeutic strategy. Ruxolitinib, a JAK1/JAK2 inhibitor, has been approved for the treatment of intermediate and high risk PMF. Although Ruxolitinib treatment ameliorates the disease and increases the survival, it is not curative and patients require long-term treatment. In addition, it is associated with different side effects, such as recurrent infections due to its suppressive effects on the immune system. In particular, Ruxolitinib treatment leads to a severe impairment of natural killer (NK) cell survival and activity.

NK cells are innate lymphocytes providing the first line of defence against virally infected and transformed cells. We have previously shown that JAK1 is indispensable for NK cell survival, while loss of JAK2 does not affect NK cells. Therefore, it is

attractive to speculate that in contrast to currently used JAK1/2 inhibitor, JAK2-specific inhibitors will ameliorate the disease without suppressing the innate immune system. To date, two JAK2-specific inhibitors have reached the clinics, with Fedratinib being approved for PMF since 2020 and Pacritinib undergoing phase III clinical trials. Although both inhibitors show remarkable JAK2 selectivity over other JAKs, they both inhibit other off-target kinases such as FLT3 and BRD4. Therefore, it is of crucial importance to carefully assess their effects on the immune responses.

Using a mouse model of MPN, we are currently studying the effects of JAK2-specific inhibitors Fedratinib and Pacritinib on the disease progression, as well as on their effects on NK cell functionality. To further expand our findings and reduce animal experiments, we plan to test how MPN patients' NK cells react to the treatment with Fedratinib and Pacritinib *in vitro*. This approach requires an establishment of human primary NK cell cultures and functional assays in our laboratory. Therefore, we will isolate primary human NK cells from healthy donors and would use the ImmunoTools reagents to establish a thorough immunophenotyping flow cytometry panel (i.e. CD56, CD16, CD57, CD69, CD62L). Further, we want to perform functional analysis of effector molecule production using flow cytometry (IFNγ, TNFα, granzyme B and perforin) upon cytokine-induced activation (IL-2, IL-15 and IL-12). Establishing these assays in human primary NK cells would be a great methodological add-on in our laboratory. Moreover, this is a prerequisite to perform further experiments that would profoundly help in the assessment of immune suppressive side effects of currently developed JAK2-specifc inhibitors and might in the future influence the rational for the usage of JAK2-specific inhibitors in the clinics. We hope you consider our project relevant and are looking forward to your response.

ImmunoTools *special* AWARD for **Agnieszka Witalisz-Siepracka** includes 10 reagents

FITC - conjugated anti-human CD16, CD57,

PE - conjugated anti-human CD62L, IFN-g, TNFa,

APC - conjugated anti-human CD56, CD69,

recombinant human cytokines: rh IL-2, rh IL-15, rh IL-12

DETAILS more AWARDS