Ruxolitinib, the Janus kinase inhibitor (JAK), is regarded as effective and relatively safe drug with overall response rate ranging from 45% to 100% in both acute and chronic graft-versus-host disease (GvHD) in the pediatric population. However, 28–55% of children with GvHD do not respond or do not tolerate ruxolitinib. This results in a lower median survival for these patients. Treatment-related toxicities including cytopenia, liver toxicity, and infections often requires ruxolitinib dose reductions or the drug withdrawal which may limit treatment efficacy. Most patients who discontinue ruxolitinib experience a return to near baseline symptoms. Thrombocytopenic patients have median survival of less than 1 year after discontinuation of the therapy. The factors underlying intolerance and resistance to the drug are not fully understood. However, variable pharmacokinetics (PK) and pharmacodynamic (PD) profiles observed in children may impact the drug effectiveness.

Therefore, the aim of the project is to analyze particular determinants of resistance and intolerance of ruxolitinib in children and, thus, answering the following scientific questions:

1. Which of the investigated factors may influence pharmacokinetics of ruxolitinib and its two main metabolites (M18 and M27) in children:
   A) selected single nucleotide polymorphism (SNP) type mutations in CYP3A4 and CYP2C9 isoenzymes, drug transporters (ABCB1, ABCG2) and in components of JAK/STAT cellular signaling pathway (JAK2, MPL, CALR) encoding genes;
   B) drug-drug interactions with inducers, inhibitors and substrates of CYP3A4, CYP2C9, ABCB1 and ABCG2?
2. Which pharmacodynamic parameters can be used for functional assessment of response to the treatment with ruxolitinib?
3. How do the changes of selected cytokines (IL-8, 1B, IL-6, IL-10, TNF, IL-12p70, IL-12, IL-18, IL-17, IL-22, TNF-α, TGF-B, IFN-γ) and phosphorylated STATs (STAT1, STAT3 and STAT5) factors profile at the mRNA and protein level in the peripheral blood lymphocytes may be associated with response to the treatment with ruxolitinib?
4. Is it possible to develop a pharmacokinetic-pharmacodynamic model (PK/PD) allowing to predict the exposure–response relationship for toxicity and efficacy of ruxolitinib in children?

Pediatric patients (n=50) treated with ruxolitinib will be recruited from the Department of Pediatric Oncology, Hematology and Transplantology of Poznan University of Medical Sciences. Plasma concentrations of the drug, M18 and M27 will be analyzed by the validated LC-MS/MS method, while selected interleukins and expression of phosphorylated STAT1, STAT3 and STAT5 will be determined by flow cytometer. Molecular study will include analysis of SNP polymorphisms and gene expression of metabolizing enzymes (CYP3A4 and CYP2C9), of drug transporters (ABCB1 and ABCG2) and of JAK/STAT signaling pathway (JAK2, MPL, CALR).

Our project will enable the identification of determinants of the pharmacokinetics and pharmacodynamics of ruxolitinib and will help predict the response to treatment and identify the risk of treatment failure or intolerance, especially in younger children, who are characterized by high variability between patients and low correlation of drug concentrations with the pharmacological effect. An innovative aspect of the project is determination of PK profile not only for ruxolitinib but also for its active metabolites M18 and M27. The proposed study assumes thorough pharmacogenetic analysis including determinants of inter-patient variability in PK and PD influencing a response to the drug. As a result of the project, a population pharmacokinetic-pharmacodynamic (PK/PD) model will be developed allowing to determine clinically relevant factors of the response to treatment with ruxolitinib. As there is currently no published popPK/PD model for ruxolitinib in the pediatric population with MPNs and GvHD, our project aims to fill the existing gap in population-based PK/PD modeling of ruxolitinib and provide data that can be used to optimize ruxolitinib treatment of children with MPN and GvHD in various clinical scenarios.