Endothelial cells line the blood vessels and decide on the proper functioning of the circulatory system. However, they also perform a different, very important, and less known function - they are an important element of the hematopoietic niche in the bone marrow. The hematopoietic niche is the place where hematopoietic stem cells (HSCs) reside. There are many indications that the niche determines HSC's ability to rebuild the hematopoietic system and protects the body against premature depletion of the HSC pool.

Despite very intensive research, it is still unclear which cells build a niche and how their activity is coordinated. HSC cells are very rare and difficult to recognize - their direct observation in the bone marrow has only recently become possible. Many previous studies have indicated that the niche for HSC cells is made up of arterioles and transient capillaries, built of atypical endothelium, the so-called type H (with some features of the venous and arterial endothelium), while the endothelium of the sinusoids is responsible for contact with activated cells leaving the marrow. Meanwhile, direct observations have shown that HSCs locate just at the sinusoids. How to explain such discrepancies? Based on our preliminary results, we believe that the sinusoid endothelium is not homogeneous, but contains groups of specialized, yet unrecognized, cells that directly contact HSCs, although the majority of sinusoid endothelial cells are responsible for the passage of activated cells from the bone marrow to the blood. The goal of our project is to get to know these hypothetical specialists.

In the research we will use a very modern methodology - most of the analyzes will be performed on single cells, so as to show the heterogeneity of the examined tissue. On single cells we will carry out analyzes of the activity of all genes (i.e. the so-called transcriptome profiling). The most important element will be selecting the right cells for analysis. We will do it thanks to the unique and recently developed transgenic mice in which HSC cells (and only them) shine in red. Another mouse strain has been designed such that the selected genes can be switched off or new genes can be specifically introduced into the HSC cells of a healthy mouse (an injection is made for this). The most interesting strains of mice will be created only during the project - we planned them so that the cells in which the HSCs directly contacted were shining in red (HSCs in turn will glow in green). Thanks to this we will be able to see and sort out those endothelial cells that actually contacted the HSCs. Then we will compare the transcriptome profiles of cells that interacted with HSC and the same seemingly neighboring cells that did not contact the HSCs. Bioinformatic analyzes will allow to indicate the molecular pathways responsible for the interaction of niche cells with stem cells. The actual significance of these pathways will then be checked in functional tests - either by removing selected cells or by turning off selected genes in them. This will allow us to: i) identify cells forming a hematopoietic niche; ii) indicate the molecular pathways that govern the function of the niche, iii) understand how the endothelial cells are plastic and able to learn new functions, iv) check how much the endothelium works by itself and how much it is regulated by hematopoietic and mesenchymal cells.

Thanks to the application of new research methods developed as part of the project, we have a chance to obtain information that has not been available so far, which is crucial for understanding the functioning of stem cells in the bone marrow. On this "which endothelium contacts HSCs" depends not only the unraveling basic biological mechanisms, but also the understanding of which drugs can affect HSC cells so that they can be successfully taken for transplantation, without activating other bone marrow cells. You can also suggest methods for preparing the patient for HSC transplantation, allowing to avoid irradiation damaging the bone marrow.