Keratoconus (KTCN) is an eye disease in which the cornea gradually becomes thinner, which leads to a disruption of its normal curvature and the formation of a characteristic conical shape. It can cause vision impairment and, in advanced stages, even vision loss. The prevalence of KTCN in the general population is estimated to range from 1/2000 to 1/375 and varies among patients of different ethnic groups. It is one of the main indications for corneal transplantation in developed countries.

Abnormalities associated with KTCN can be observed in all 5 layers of the human cornea, from the outermost (1) epithelium, with deposition of iron particles, breaks in (2) the Bowman layer, through altered collagen fiber distribution within (3) the stroma and ruptures in (4) the Descemet's membrane, to the innermost (5) endothelium, which may show elongation and cell damage. Despite the recognized morphological changes in the cornea, the alterations at the molecular level in cells specific for individual corneal layers in KTCN have not yet been explained. Due to the complex structure of the cornea, in which different cell types are involved and under homeostatic conditions create a unique environment enabling normal vision, the KTCN cone structure should be studied at the single-cell level.

In our study, we hypothesize that specific cell subtypes, and in particular, their altered dynamics, cell cycle, and their mutual interactions, in individual corneal layers, may contribute to the formation of the characteristic for KTCN conical shape. As a part of the project, for the first time, the single cell RNA sequencing (scRNA-seq) will be performed on the consecutive cryosections of the same corneas used previously for the spatial transcriptomics (ST), and integration of data from both approaches will be conducted. **We assume that the combination of scRNA-seq and ST data, in what we call a 'bridged' assessment, will allow us to localize transcriptionally characterized single cells in the context of their native tissue and fill in the missing mechanistic aspects in the etiology of KTCN.** The project aims to compare the KTCN and control corneas regarding the: **1)** characteristic cell subtypes and cellular composition using scRNA-seq; **2)** the changes in the dynamics, cell cycle and mutual interactions of individual cell types/subtypes; and **3)** determining the contribution of cell subtypes to the mechanism of KTCN cone formation using an experimental model of human corneal cryosection.

The study will be conducted on human corneas collected from 5 patients with KTCN who underwent penetrating keratoplasty, and 5 corneas from a tissue bank, originating from deceased donors, constituting the control group. Subsequently, for each O.C.T.-embedded cornea from the KTCN and control group, the cryosections will be obtained, which will be used to isolate single cells using the experimental protocol we are developing. Samples prepared in this way will then be used to prepare NGS libraries, which will then be used for scRNA-seq. The obtained transcriptomic data for single cells will be subjected to bioinformatic analysis, in terms of characterization of cone-specific cell subtypes, cellular composition, changes in dynamics, cell cycle, and intercellular interactions in KTCN. In addition, an integrative analysis will be performed, which will include transcriptomic, genomic, and other epigenomic data from the same biological material from our previous and current KTCN-related projects. The obtained results will be verified using immunofluorescence staining of selected marker genes characteristic of newly identified cell subtypes in KTCN in an extended set of corneas of an additional 5 corneas from KTCN and 5 control corneas.

The study will enable the use of multiple consecutive cryosections of the cornea for a multi-omic approach, which is considered to be most suitable for multifactorial diseases such as KTCN. The maximum exploitation of material collected from particular patients for many different analyses allows for obtaining a comprehensive picture, allowing for drawing more detailed conclusions from the generated data, leading to a deeper understanding of the etiology of the disease. The generated for the first time the integrated **bridged scRNAseq-ST assessment** results will be publicly available in the repository and will allow for reprocessing in global KTCN research.