Title
3D CLL model as a platform for AI-driven image analysis and integrative drug screening

Introduction
Chronic lymphocytic leukemia (CLL), the most common type of leukemia remains incurable. Despite recent application of targeted drugs, continuous treatment and emerging resistance are urgent socio-economic and clinical problems. This necessitates better understanding of CLL biology and drug-resistance, and further drug (combination) development. We have implemented a 3D CLL model which enables in vitro investigation of CLL spheroid architecture and patient-specific drug screening. Additionally, we set up an unbiased computational pipeline for the quantitative analysis of morphological changes in spheroids derived from patient material using live-cell imaging data. By integrating image processing and machine learning techniques, we seek to accurately assess in vitro drug responses and attempt to link this to clinical characteristics of patients to identify and predict therapeutic strategies.

Methods
We used a 3D CLL spheroid model as previously described by our group (Haselager, HemaSphere, 2023) to perform high-throughput drug screening. We combined live-cell imaging with end-point flow cytometry analysis to phenotype in vitro drug responses. Imaging data was analyzed by an AI model to extract and quantify spheroid features in an unbiased manner.

Results
Our 3D CLL model successfully generated spheroids from both treatment-naïve and refractory patient samples. High-throughput drug screening revealed varied responses across patients and drug types, also revealing drug-specific effects on spheroid morphology. Time-lapse imaging of spheroids in the presence of drugs showed dynamic changes in spheroid growth whereas other agents completely blocked spheroid growth or even formation. Using AI image analysis, we identified multiple parameters as a quantification of in vitro drug response, including dissociation, aggregation and externalization.

Conclusion
Here we characterize distinct drug-induced effects on spheroid architecture and cell-cell interactions. Linking this data to clinical patient data may ultimately lead to personalized treatment strategies.