CCC Research Grants

COMPREHENSIVE CANCER CENTER VIENNA

MEDICAL UNIVERSITY OF VIENNA

Vienna Healthcare Group
University Hospital Vienna

The project is funded by the proceeds of the Krebsforschungslauf and donations to the Initiative Krebsforschung. www.krebsforschungslauf.at

Functional precision medicine of T-cell prolymphocytic leukemia (T-PLL)

Tea Pemovska[1,2], Alexander Pichler[1] and Philipp Staber[1,2]

[1]Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna
[2]Comprehensive Cancer Center Vienna, Medical University of Vienna and Vienna General Hospital

T-PLL

- Rare and aggressive mature T-lymphoid malignancy
  - -Proliferation of post-thymic prolymphocytes
  - -2% of all adult mature lymphocytic leukemias
- Median age of diagnosis is 61
  - -Slight male preponderance
- Median overall survival ~20 months
  - -Poor responses to alkylating agents or poly-chemotherapy
- Only therapy effective in large proportion of patients
  - -Alemtuzumab (anti-CD52 antibody)
  - -Improved response rate to 75% in first-line
  - -All patients eventually relapse within 12 months
- Key molecular event in T-PLL pathogenesis
  - -Constitutive transcriptional activation of genes of the T-cell leukemia 1 (TCL1) family
  - -Result of genomic inversions/translocations chr. 14

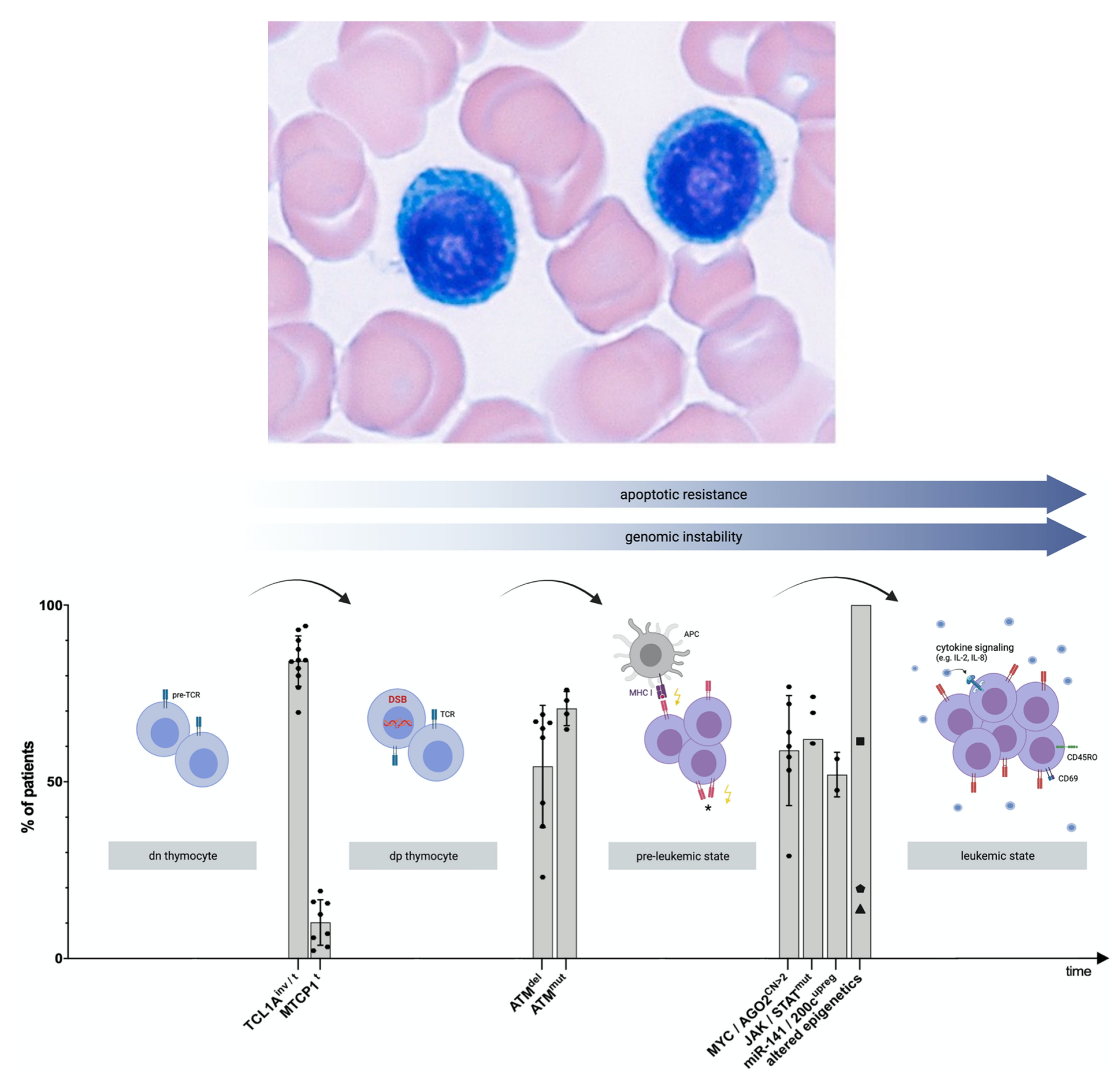

Figure 1. Peripheral blood smear and a model of clonal evolution of T-PLL cells. Schematic visualization explaining T-PLL's leukemogenesis, based on recent genomic profiling series and corresponding functional assessments. Adapted from Braun et al; Front. Oncol. 2021.

Open research questions in T-PLL

- Mechanisms of disease transformation and progression
- Clonal heterogeneity, complexity and dynamics
- Subclone-specific vulnerabilities and therapies

Project objectives and approach

- No treatments are specifically approved for T-PLL
  - -Highlighting the unmet medical need
  - -Deeper understanding of disease mechanisms
  - -Novel treatment strategies
- Hypothesis: Personalized treatments for T-PLL can be predicted by
  - -Dissecting the clonal variability within individual patients
  - -Learning general rules on clonal trajectories during disease progression and drug treatment

We specifically aim to (Fig.2):

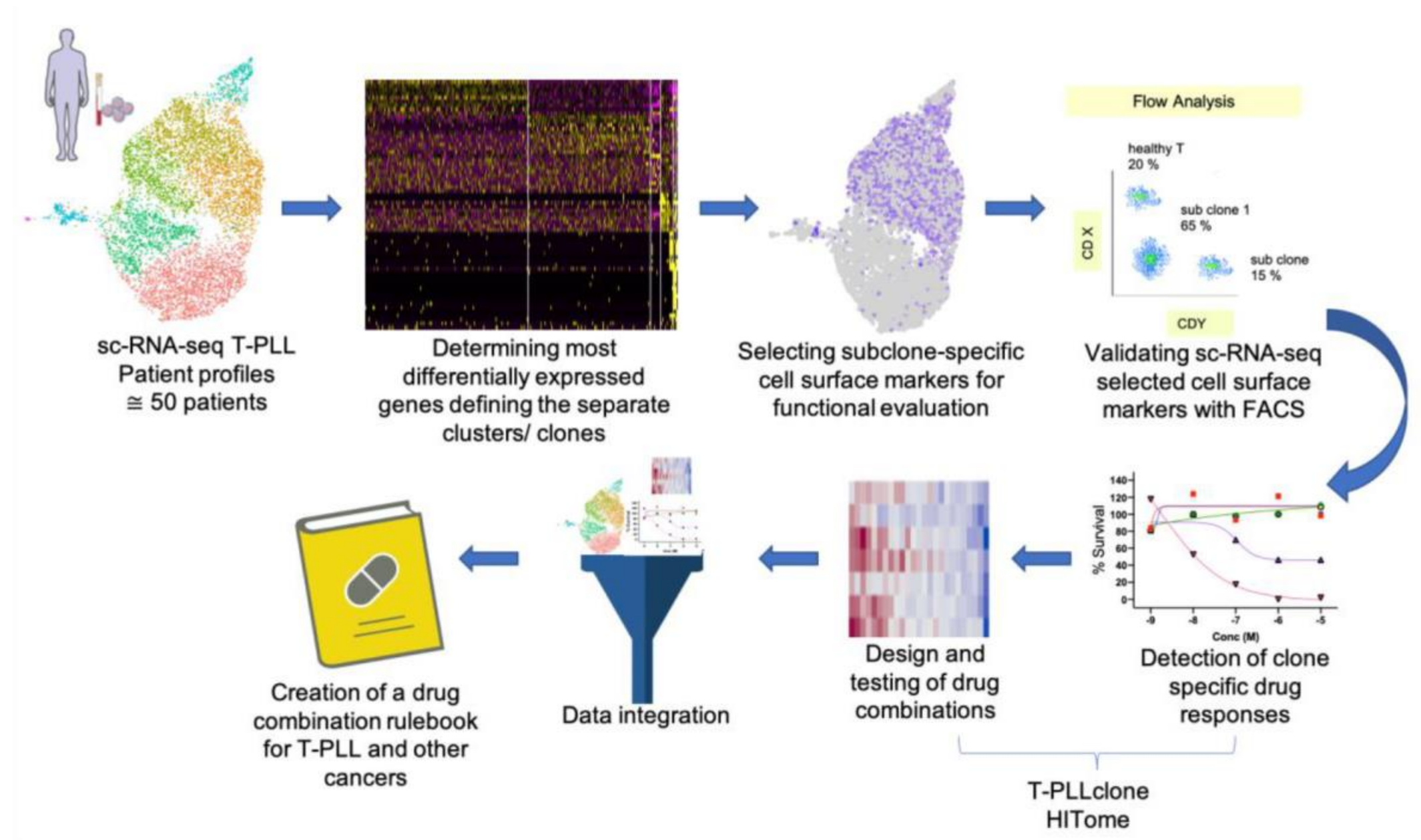

Figure 2. Project outline. We will generate in-depth genetic and epigenetic characterization on the single-cell level and measure the responsiveness of identified clones and subclones to clinical cancer drugs, thereby developing a novel rulebook of drug combinations for T-PLL and beyond.

1. Single-cell characterization of T-PLL

-Identify clonal composition and evolution during disease progression and therapy response by performing sc-RNA-seq on individual and sequential samples (Fig. 4)

-Identify gene signatures that correlate with conversion of T-PLL from inactive to an active disease state as well as with response to therapy

2. Functional characterization of T-PLL cells

-Identifying subclone-specific functional dependencies and drug resistance mechanisms by performing high-throughput FACS-based drug screening (Fig. 3)

-Establish T-PLL subclone-specific drug responses (HITome)

3. Develop a rulebook for effective drug combinations in T-PLL and beyond

-Integrate molecular and functional profiles to generate drug combination candidates

-Validate drug combination candidates by HT-FACS based drug screens

FACS-based drug testing platform

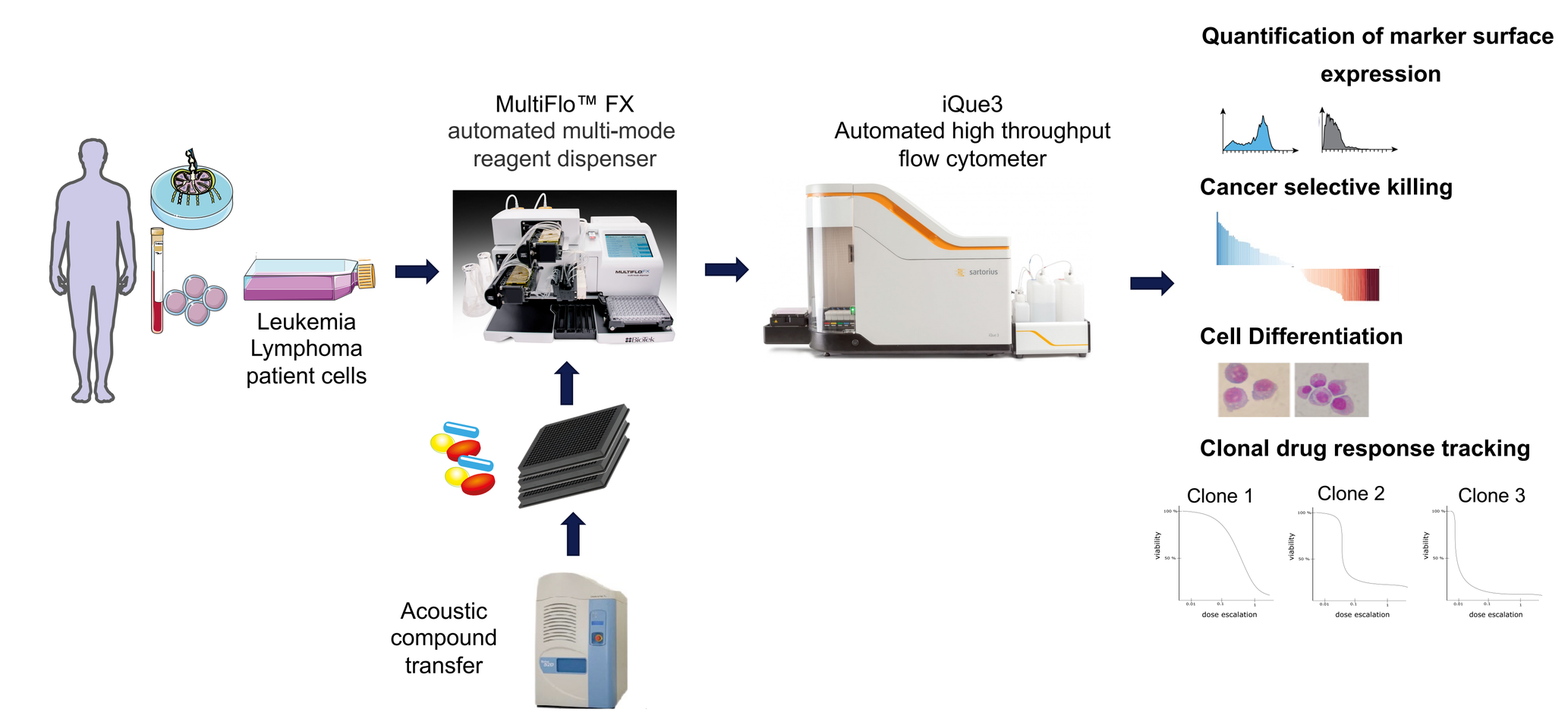

Figure 3. The platform can measure the response of (non-)adherent cells from primary liquid biopsies to chemical stimuli with single cell resolution in high throughput fashion for a variety of hematological disorders. We routinely test the sensitivity of 140 emerging and clinically relevant compounds and scored for leukemia selective responses.

Transcriptional cell type identification by sc-RNA-seq

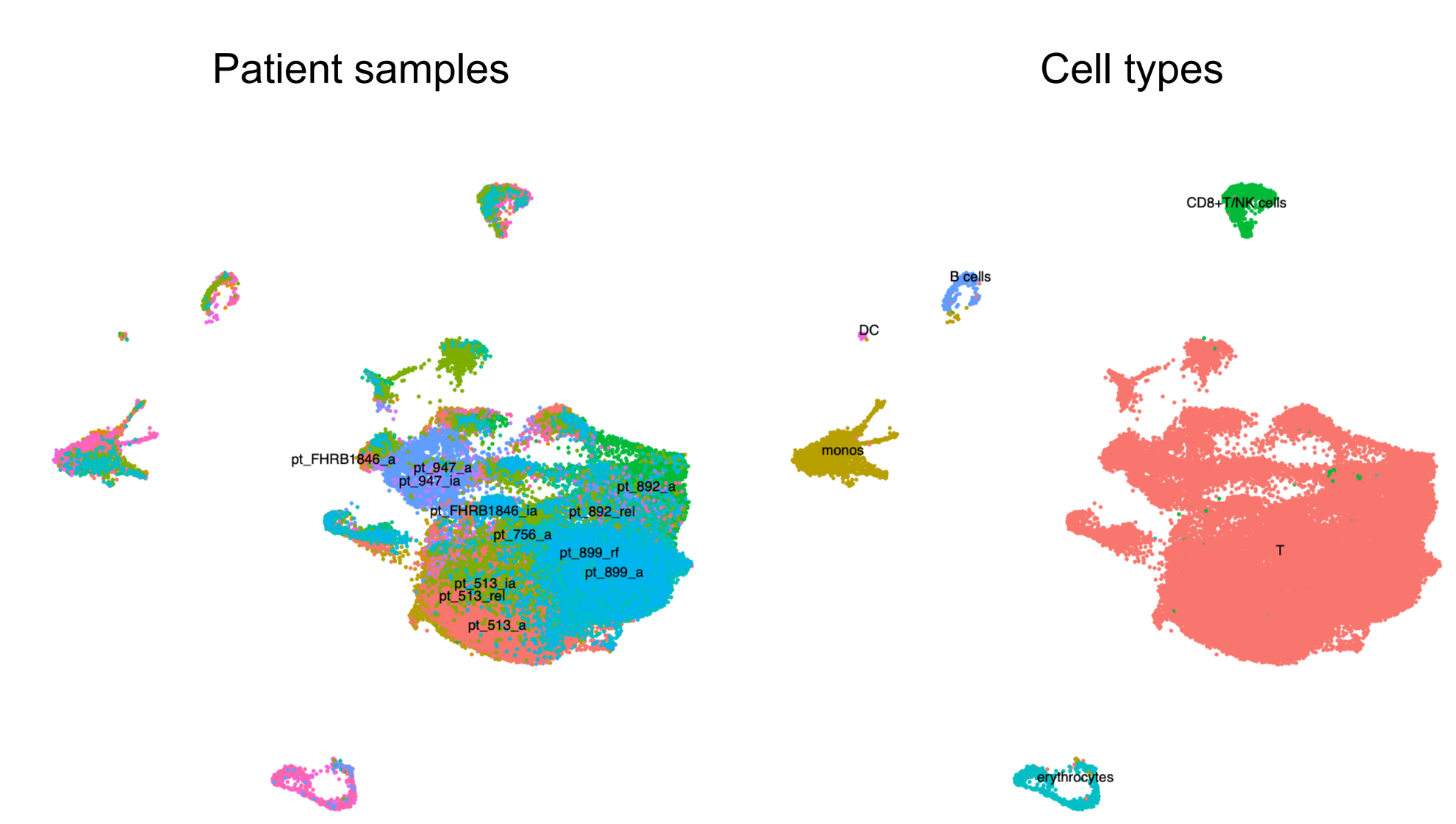

Figure 4. UMAP-plots after independent clustering of 26,000 single-cells from 12 patient derived peripheral blood samples colored either by patient (left) or cell type (right) using established marker genes.

Outlook

- Combination therapies are essential to overcome resistance mechanisms
- Improved approaches are needed to preselect sets of putative synergistic drugs
- This project could deliver an unprecedented understanding of how the subclone composition impacts drug sensitivity and resistance
- Our approach will illustrate novel functional insights into T-PLL disease biology

Contact: tea.pemovska@meduniwien.ac.at