POSTER 28: Application of high-dimensional spectral cytometry to the evaluation of drug responses in AML patient samples

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Acute myeloid Ieukemia (AML) is characterized by subclonal proliferation of myeloid progenitor/precursor cells, or blasts, that fail to terminally differentiate. The genetic profile of AML cells and subclones may guide therapeutic choices, such as eligibility for targeted therapies (FLT3, IDH). The identification of new genetic or epigenetic liabilities in AML and the exploitation of these vulnerabilities remains important for the development of therapeutic strategies.

Pre-clinical models such as ex vivo cultures of primary AML cells are extensively used towards the evaluation of drug effectiveness, as well as to discover markers for drug response or resistance. Although conventional flow-cytometry empowers efficient investigation of drug sensitivity, such analysis is hampered by the complex behavior of the different sub-fractions of blasts, that can self-maintain and/or respond differently towards therapeutics. The limited combination of markers as limited sample availability remain important issues.

To address these issues, and track specific cell populations throughout drug testing experiments, we have developed a 36-color flow cytometry-based panel (CYTEK AURORA), allowing a deep immunophenotyping of the major human bone marrow and peripheral blood cell subsets. In addition to markers enabling the analysis of normal and malignant immature hematopoietic cells and the definition of cell subsets within the different myeloid lineages, we have included intracellular markers to follow the expression of key lineage-specifying transcription factors (PU. I, GATA I) and oncogenes (MYC). Full-spectral cytometry has been conducted on limited samples (104 cells) and has allowed the immunophenotyping of small subclones (< 5% of total).

We have evaluated the utility of this analysis by analyzing the behavior of 3 primary AML samples exposed ex vivo to an inhibitor raised against the Brahma related gene I (BRO I) protein. BRGl is a member of the SWI/SNF complexes belonging to the family of ATP-dependent chromatin-remodeling complexes. Deregulation of SWI/SNF subunits are found in over 20% in all human cancers including AML, frequently driving oncogenic programs. In hematopoietic cells, BRO 1 through its A TPase activity mobilizes nucleosomes along the chromatin and modulate chromatin accessibility, impacting gene expression and lineage determination. The functional consequences of its inhibition in AML patient samples is currently characterized.