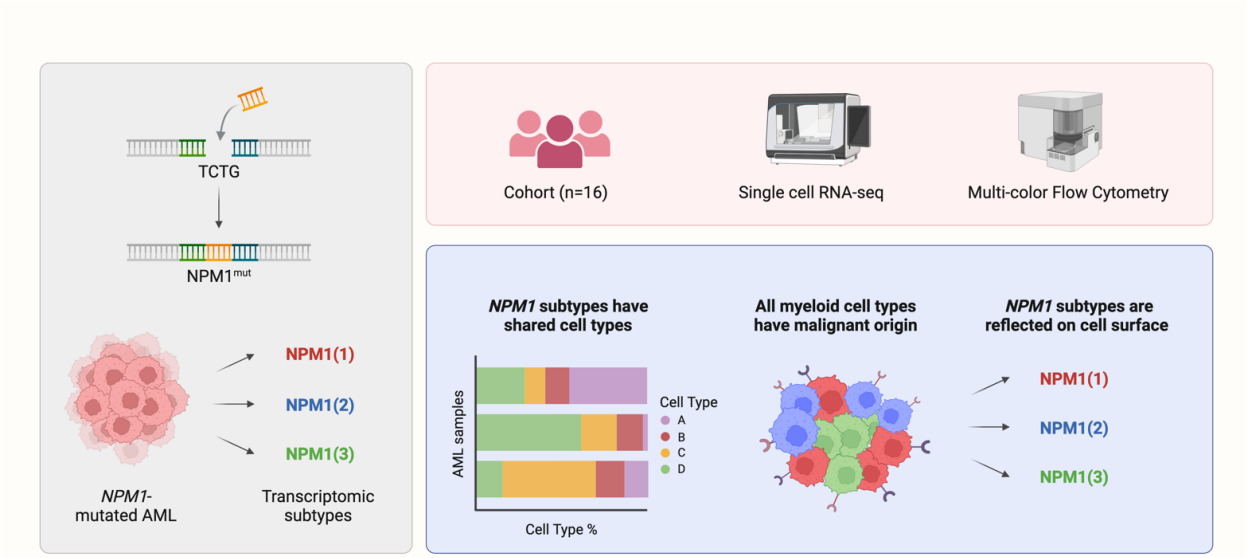
GRAPHICAL ABSTRACT



ABSTRACT

NPM1-mutated AML is one of the largest entities in international classification systems of myeloid neoplasms, which are based on integrating morphologic and clinical data with genomic data. Previous research, however, indicates that bulk transcriptomics-based subtyping may improve prognostication and therapy guidance. Here, we characterized the heterogeneity in *NPM1*-mutated AML by performing single-cell RNA-sequencing and spectral flow cytometry on 16 AML subjects belonging to three distinct transcriptional subtypes that we previously identified by bulk transcriptomics. Using single-cell expression profiling we generated a comprehensive atlas of *NPM1*-mutated AMLAML, collectively reconstituting complete myelopoiesis. The three *NPM1*-mutated subtypes showed consistent differences in proportions of myeloid cell clusters with distinct patterns in lineage commitment and maturational arrest. Nevertheless, malignant cells were detected across all myeloid cell clusters for all samples, indicating that *NPM1*-mutated

AMLAML is heavily skewed but not fully arrested in myelopoiesis. Same-sample multi-color spectral flow cytometry recapitulated these skewing patterns, indicating that the *NPM1*-mutated subtypes can be consistently identified across platforms. Moreover, our analyses highlighted differences in the abundance of rare hematopoietic stem and progenitor cells suggesting that skewing in two subtypes occurs early in myelopoiesis. To conclude, by harnessing single-cell RNA-sequencing and spectral flow cytometry, we provide a detailed description of three distinct and reproducible patterns in lineage skewing in *NPM1*-mutated AML that may have potential relevance for prognosis and treatment of patients with *NPM1*-mutated AML.