Venetoclax, a BH3-mimetic, is a small molecule inhibitor targeting BCL-2. Since its approval, the combination therapy of hypomethylating agents and Venetoclax (HMA+Ven) has emerged as a standard of care for acute myeloid leukemia (AML) patients unfit for intensive chemotherapy. Nonetheless, clinical challenges persist as patients develop upfront resistance or relapse in the mechanisms inexplicable by current knowledge. Previous studies have identified genetic (TP53 and RTK/RAS pathway mutations) and phenotypic (monocytic differentiation) factors as associated with resistance. In this context, we hypothesized that an integrative assessment of genotypic and phenotypic components in large cohort could elucidate the patterns of treatment evasion. To determine the predictors of treatment response, we analyzed the clinical characteristics of 208 newly diagnosed, secondary, and relapse/refractory AML patients treated with Decitabine and Venetoclax (Dec+Ven). While the French-American-British (FAB) classification was not associated with treatment response, mutations in KRAS and TP53 were significantly associated with nonresponse. To identify the mutational and immunophenotypic characteristics of the cells are resistant toward treatment, we further analyzed 60 samples sequentially collected from 33 newly diagnosed and secondary AML patients from the clinical cohort using a single-cell DNA-antibody sequencing (DAb-seq). At relapse, stem-like cells were largely eradicated and replaced by more differentiated cells, and we identified three patterns of phenotypic shifts: monocytic shift, erythroid shift, and mixed monocytic/erythroid shift. Notably, one relapse patient underwent a phenotypic transition, beginning as monocyte-like phenotype (diagnosed as FAB-M5) but ending as erythroid-like phenotype. The phenotypic shifts were not always associated with the expansion of new mutations, and the patterns of genotypic shifts remained nebulous. However, among the five baseline-relapse pairs, we found that one case had an erythroid shift accompanied by the expansion of NRAS mutation, and another had a monocytic shift accompanied by the expansion of KRAS mutation. To substantiate the observations on the phenotypic level, we used single-cell RNA sequencing on four patients with relapse or refractory periods to study the underlying mechanisms. We confirmed not only that monocytic or erythroid cells were indeed predominant at the time of relapse or resistance, but also that they had increased MCL1 or BCL2L1 expression in each compartment, respectively. In conclusion, phenotypic shifts from stem-like cells to more differentiated cells were frequently observed in AML resistance toward Dec+Ven therapy, a finding that is consistent with known dependency of these cells on non-BCL-2 anti-apoptotic proteins. Hence, close monitoring of these phenotypic alterations throughout the course of treatment may potentially facilitate the precise identification of patients predisposed to relapse or develop resistance.

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