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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Dual-Targeting CD33/CD123 NANOBODY ® T Cell Engager Targeting Leukemia Blasts and Stem/Progenitor Cells in Relapsed AML

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Acute myeloid leukemia (AML) is the most common adult hematologic malignancy. Relapse is common and salvage treatment with cytotoxic chemotherapy is rarely curative. Immunotherapy targeting surface antigens expressed in leukemia cells has emerged as an effective approach for AML treatment for patients who are older/unfit for or relapsed from/refractory to current regimens (Isidori A, et al. Frontiers Oncol. 2021). CD123 and CD33, two clinically validated targets in AML, are jointly expressed on blasts and leukemic stem cells (LSC) in >95% of AML patients. However, their expression is heterogenous between subclones and between patients which may impact the efficacy of single-targeting agents in certain patient populations. We recently reported a novel dual-targeting CD33/CD123 NANOBODY® T-cell engager (CD33/CD123-TCE) and demonstrated the anti-leukemia activity of CD33/CD123-TCE in AML with good safety profile *in vivo* (Dullaers M, et. al, JITC. 2022). Here we showed that CD33/CD123-TCE effectively targets AML blasts and LSC in relapsed AML.

The cell surface expression profiling of samples from 55 patients with relapsed/refractory AML showed that all samples were positive for CD123 and CD33. The frequency of subpopulations in all samples co-expressing CD33 and CD123 is the most prominent, followed by single expression of CD123, and CD33. CD33-CD123- subset represented a minority.

To investigate the anti-leukemia effect of CD33/CD123 TCE, we treated 18 primary samples for 7 days and assessed apoptosis by flow cytometry. Treatment of CD33/CD123 TCE at 2.5nM (low dose) and 25nM (high dose) induced more than 50% specific apoptosis in majority of bulk AML, with no significant difference between low and high dose of CD33/123 TCE.

Analysis of cytotoxicity towards CD34+ AML-LSC revealed that CD33/CD123 TCE at low and high doses resulted in similar specific apoptosis of CD34+ cells, half of samples reached higher than 50% of apoptosis. Colony-forming assay in 2 primary samples further confirmed that CD33/CD123 TCE effectively inhibited the clonogenicity of primary AML cells expressing CD123 and CD33.

While the average activity of CD33/CD123 TCE was comparable to single-targeting CD33-TCE and CD123-TCE reagents, in a subset of patient samples (11% of all samples), the dual-targeting CD33/CD123-TCE induced higher apoptosis in bulk and CD34+ AML cells compared to single-targeting controls, supporting utility of dual targeting.

Concomitantly, CD3+ T cell expansion was observed in all dual- and single treatment groups, with a trend toward stronger amplification by CD33/CD123-TCE.

Next, we examined AML and T cell phenotypes using mass cytometry (CyTOF) to characterize the impact of the TCE treatments. UMAP plus FlowSOM analysis revealed heterogenous subsets of LSC within samples and across patients. LSC subsets defined by CD34+ are known to co-express various surface markers associated with LSC-drug resistance and disease relapse including CD135 (FLT3), CD117 (c-kit), CLL-1, PD-L1 and TIM-3. These subsets were found highly sensitive to CD33/CD123-TCE, with subset CD34+CD38+CD117+PD-L1+CD123+ and CD34+CD38- showing greater sensitivity to CD33/CD123-TCE. Despite diverse expression of leukemic markers, CD33/CD123-TCE depleted CD34+ AML-LSC in all 3 samples tested by CyTOF, whereas single-targeting CD123-TCE and CD33-TCE were only effective in 2/3 samples.

Analysis of T cell subsets revealed that dual- or single- targeting TCE expanded CD4+ helper and CD8+ cytotoxic subsets in all samples compared to control. CD4+ and CD8+ cells co-expressing CD69, TIM-3 or PD-1 were expanded by TCE in all three samples. Expansion of central and effector memory T cells defined by CCR7+CD45RA- and CCR7-CD45RA- in CD4+

and CD8+ subsets were seen in all treatment groups in 2 samples, whereas the highest expansion of CCR7-CD45RA-CD4+ effector memory subsets by CD33/CD123-TCE was seen in 1 sample.

Taken together, the CD33/CD123 dual-targeting NANOBODY® TCE exhibits potent anti-AML activity and offers a broad patient coverage. Immunophenotypic profiling confirmed the effectiveness of CD33/CD123-TCE in eliminating various blast- and LSC-subsets, activating CD4+ and CD8+ T cells or expanding central/effector memory CD4+ and CD8+ T cells. These dual-targeting immune engagers may constitute an effective treatment option for patients who are older/unfit for or relapsed from/refractory to current regimens.