266 STING ACTIVATION IMPROVES T CELL ENGAGING IMMUNOTHERAPY OF ACUTE MYELOID LEUKEMIA

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Background The treatment landscape for Acute myeloid leukemia (AML) patients has changed dramatically in recent years, however the majority of patients will eventually relapse. Allogeneic stem cell transplantation has proven the power of T-cells in eradicating residual leukemic cells, but alternative strategies based on T-cell recruiting bispecifics have failed to replicate these responses. Resistance is mediated by the immunosuppressive tumor-microenvironment and secretion of immune dampening metabolites by AML cells. We hypothesized that combining the CD33 BiTE construct AMG 330 with a cGAS-STING agonist has the potential to reverse immunosuppressive mechanisms and augment anti-leukemic activity.

Methods *In vitro* co-culture assays of human T-cells and AML cell lines were performed to study the effect of AMG 330 in combination with the STING-agonist cGAMP. Cytotoxicity, degranulation, and cytokine secretion were assessed by flow-cytometry. Transcriptomic analysis and systematic CRISPRCas9 knockout-studies were conducted.

Results We observed markedly increased target cell lysis upon simultaneous addition of cGAMP and AMG 330. Notably the cGAMP-dependent enhancement of AML cell lysis increased over time and was most pronounced at low effector-to-target ratio. Moreover, cGAMP improved AMG 330 mediated killing of primary AML cells. We noted increased T-cell degranulation, as well as increased T-cell intrinsic levels of Granzyme B and IFNy in the presence of cGAMP. In addition, we observed a strong increase in inflammatory cytokine (IFNy, TNFa, IL-4) secretion.

RNA-sequencing of AMG 330 and cGAMP stimulated target (HL-60) and effector cells revealed type-I-IFN signatures in both cell types, while HL-60 cells also displayed a signature of IFNy signaling. We observed a distinct activated T-cell phenotype with markedly increased TNFa, IFNy and GZMB expression induced by the co-treatment.

Knockout-studies revealed that the enhanced phenotype was fully dependent on target cell intrinsic STING/IRF3 signaling and functional IFNy/TNFa signaling. Increased T-cell mediated IFNy production upon combinatory treatment was also dependent on functional target cell STING and INFy/TNFa signaling, implying a crosstalk between effector and target cells. Most notably, the effector cytokines IFNy and TNFa in turn boosted the expression of ISGs, as well as the secretion of type-I-interferons (IFNa, IFNl) by HL-60 cells in the presence of cGAMP.

Conclusions We propose a novel mechanism by which AMG 330-activated T-cells prime and sensitize AML target cells in a forward feedback loop towards STING activation, leading to increased type-I-IFN production. This leads to pronounced expression of effector cytokines and an overall cytotoxic T-cell phenotype, contributing to the beneficial effect of cGAMP in enhancing BiTE construct-mediated lysis.

Ethics Approval Peripheral blood or bone marrow samples were collected from healthy donors and patients with acute myeloid leukemia at initial diagnosis, relapse, or complete remission after written informed consent was received in accordance with the Declaration of Helsinki and approval was granted by the Institutional Review Board of the Ludwig-Maximilian-Universität (Munich, Germany, reference number: 216-08).

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