Background Novel therapies are needed for effective treatment of Acute Myeloid Leukemia (AML). Relapse is common and salvage treatment with cytotoxic chemotherapy is rarely curative. CD123 and CD33, two clinically validated targets in AML, are jointly expressed on blasts and leukemic stem cells in >95% of AML patients. However, their expression is heterogeneous between subclones and between patients which may impact the efficacy of single-targeting agents in some patient populations. We present here a dual targeting CD33/CD123 NANOBODY® T-cell engager (TCE) that was designed to decrease the risk of relapse from single antigen-negative clones and to increase coverage within and across patients.

Methods TCE-driven redirected T cell killing was evaluated in vitro against cells lines with different expression levels of both tumor antigens: MOLM-13, U-937 and KG1a. In vivo efficacy was addressed in a MOLM-13 disseminated CDX mouse model. PK, PD and safety were studied in an exploratory single-dose study in cynomolgous monkeys. Cytokine release was measured in an in vitro autologous PBMC setup.

Results The CD33/CD123 TCE killed AML tumor cells expressing one or both antigens in vitro. Compared to single-targeting control compounds, the CD33/CD123 TCE conferred equal or better ex vivo killing of AML blasts in most primary AML samples tested, suggesting a higher patient coverage. In a disseminated CDX mouse model of AML, the multispecific CD33/CD123 TCE cleared cancer cells in long bones as well as in soft tissues. As cytokine release syndrome is a well-documented adverse effect of TCE, the compound was tested in a cytokine release assay and shown to induce less cytokines compared to CD123 single-targeting control.

In an exploratory single-dose non-human primate study, the CD33/CD123 TCE revealed a favorable PK profile. Depletion of CD123 and CD33 expressing cells was observed at 0.04 µg/kg, without associated cytokine release syndrome nor signs of clinical toxicity.

Conclusions Taken together, the CD33/CD123 dual-targeting NANOBODY® TCE exhibits potent and safe anti-AML activity and promises a broad patient coverage.

Ethics Approval All in vivo experiments were approved by the Sanofi Ethical Committee and conducted in accordance with local and institutional Laws, Ethics and guidance in AAALAC accredited facilities.