intratumoral TLR3 expression and T-cell infiltration is currently analyzed.

**Results** Global deficiency of murine Tlr3, or TRIF, induced significantly increased digestive tumor formation, associated with increased morbidity indicating a tumor suppressive role. Coherently, TLR3 expression is highly significantly decreased in human colorectal cancer compared to normal mucosa, significantly correlated with poor survival. TLR3 deficient cell lines showed reduced migration and slightly declined proliferation suggesting an oncogenic role on the cell-autonomous level. Nevertheless, gene expression analysis revealed that the dsRNA induced expression of T-cell attracting cytokines CXCL10 and CXCL11 in colon cancer cell lines is exclusively dependent on TLR3. These cytokines were shown to favor a TH1-type antitumoral response.

**Conclusions** TLR3 favors tumor suppression in vivo, presumably resulting from non-cell-autonomous factors such as the production of CXCL10 and 11 and resulting T-cell infiltration. This may outweigh the putative cell-autonomous oncogenic functions of TLR3 deficiency.


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**Materials and Methods** The bifunctional SIRPα-CD123 antibody was generated by fusing an extracellular domain of the SIRPα receptor, which functions as the CD47 blocking domain, to the CD123 antibody. The biological activity of the SIRPα-CD123 antibody was examined using AML-derived MOLM-13 cells, primary AML patient material and patient-derived xenografted (PDX) AML cells with NOD.Cg-Prkdcscid IL2rgtm1WjlSj/SzJ (NSG) mice.

**Results** The SIRPα fusion improved the binding of the bifunctional SIRPα-CD123 antibody to AML cells compared to a conventional CD123 antibody. The SIRPα-CD123 antibody enhanced the elimination of the AML-derived MOLM-13 cells by antibody-dependent cellular cytotoxicity via NK cells. Additionally, the cytocytotoxicity was confirmed using primary patient-derived AML cells. Furthermore, an improved cytocytotoxicity towards leukemia initiating AML PDX cells was observed with the SIRPα-CD123 antibody using luciferase bioluminescence in vivo imaging. With regards to the inhibition of CD47 signaling, we were able to show a blockade of CD47 on CD123+CD47+ cells by the SIRPα-CD123 antibody. Correspondingly, a significant increase in phagocytosis of primary patient-derived AML cells mediated by monocyte-derived macrophages was observed in both allogenic and autologous setting. We were also able to show a preferential binding to MOLM-13 in the presence of a 20-fold excess of red blood cells indicating a potential low on-target off-leukemia toxicity.

**Conclusions** The bifunctional SIRPα-CD123 fusion antibodies target the CD123+CD47+ cells and stimulate their phagocytosis by blocking the inhibitory CD47 signal. The dual mode of action of the SIRPα-CD123 has the potential to deplete the AML LSCs through NK cell cyctotoxicity and macrophage-mediated phagocytosis while restricting the CD47 related off-target on-leukemia toxicity.

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**Background** Despite advances in the development of novel strategies against acute myeloid leukemia (AML), treatment options are limited and most patients relapse. Relapse occurs due to the persistence of chemotherapy-resistant leukemic stem cells (LSCs), which re-initiate the outgrowth of the disease, highlighting the need of targeting LSCs to improve patient survival. LSCs are characterized by the expression of the interleukin-3 receptor α, also known as CD123. CD123 is expressed on AML blasts and LSCs, and shows a moderate expression on normal hematopoietic stem cells, claiming CD123 as a suitable target antigen. CD47 is a ubiquitously expressed immune checkpoint upregulated on LSCs where it acts as an immune escape mechanism. CD47 transmits a ‘don’t eat me’ signal upon its interaction with the signal regulatory protein alpha (SIRPα) receptor on macrophages, thus inhibiting phagocytosis. In order to efficiently eliminate LSCs, we have designed a bifunctional antibody that specifically targets CD123 and simultaneously blocks CD47. Importantly, our strategy restricts the benefits of the CD47 blockade to CD123+AML cells. Thus, we hypothesize a lower risk for on-target off-leukemia toxicity.

**Materials and Methods** The bifunctional SIRPα-CD123 antibody gets CD123 and simultaneously blocks CD47. Importantly, our strategy restricts the benefits of the CD47 blockade to CD123+AML cells. Thus, we hypothesize a lower risk for on-target off-leukemia toxicity.