Bispecific T-cell engager antibodies may contribute to reactivate pre-existing Tumor specific T-Cells

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INTRODUCTION

Cancer immunotherapy aim to harness the power and durability of immune responses. Bispecific T-Cell engager antibodies (BsAbs) redirect T-cells to kill tumor cells by proximity, independent of the intrinsic antigen-specific TCR recognition. However, we have previously demonstrated that following incubation of Acute Myeloid Leukemia (AML) samples with a CD3xCD123 BsAb, isolating BsAb-activated T Cells by fluorescence-activated cell sorting (FACS), removing BsAb by wash steps, and adding new autologous blasts, these BsAb-activated T Cells in the absence of the BsAb was able to kill blasts. We hypothesized that the recognition and killing by these isolated activated T cells may be through classical TCR-HLA antigen-specific recognition. Interestingly, clinically a subset of patients develops a long-term response after BsAb therapy that could point an expansion of tumor-specific cytotoxic T-lymphocytes against AML.

OBJECTIVE

We aim to identify patients with a TCR-dependent blast-T cell interaction after in vitro incubation with a CD3xCD123 BsAb on Bone Marrow (BM) from AML patients to potentially select patients with a more durable response after BsAb therapy.

MATERIAL & METHODS

Whole BM were tested in the PharmaFlow platform, an innovative proprietary method that uses flow cytometry (FCM) to efficiently count the number of tumor cells killed by activated T-cells with a CD3xCD123 BsAb (Creative Biolabs) at 120h at different doses. If the killing capacity of the BsAb could be measured by the PharmaFlow platform, then the activated T-cells were isolated, washed several times to remove BsAb, and co-incubated with new autologous isolated blast cells from a cryopreserved vial (Figure 3). For bloking experiments, tumor cells were stained with PKH67 and co-incubated at 0.5:1 ratio at 37ºC for two different time points (4h & 24h). The strategy to define and block the TCR interaction was analyze the T-cells expressing PKH67 in the doublet zone by FSC-A/FSC-H. The Doublets positive cells (CD5+PKH67+) consist of activated T-cells bound to autologous PKH-blast cells. For blocking experiments, HLA A,B,C (blast Cells), αβTCR (T-cells) or both were incubated 1h at 37ºC before the co-culture (Figure 6).

RESULTS

We Hypothesize A New MOA BsAb
BsAb activate Tumor-Associated Antigen (TAA) T Cells

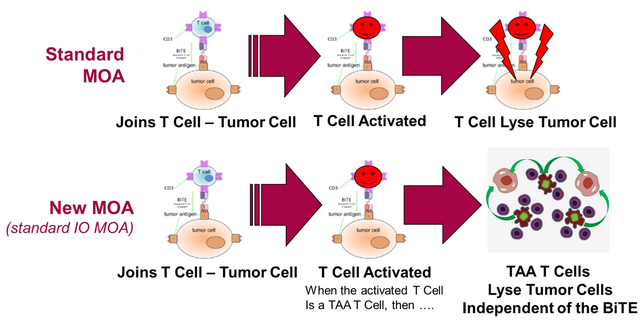


Figure 1. Mechanism of action of BsAb. In the standard MoA (higher panel), BsAb bring T cells in proximity to tumor cells, which activate T cells that kill tumor cells independent of any Tumor-Associated Surface Antigen (TAA) specific recognition. However, in bone marrow of AML patients, some of the T cells would be TAA T cells, which could also be activated by the BsAb. We hypothesize a new MoA (lower panel), where the BsAb activates tumor specific TAA T cells. The difference between these MoA is that these TAA T cells shown be capable of continued serial killing of tumor cells without the BsAb.

Tumor Cell Killing by BsAb-Activated T Cells Isolated, in the Absence of BsAb

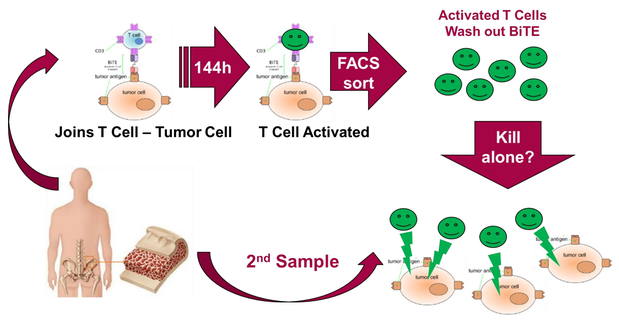


Figure 2. To test the hypothesis of the new MoA (Fig. 1), we designed the experiment illustrated above: First, we generated activated T-lymphocytes by incubation with an AML BM sample with a CD3xCD123 BsAb. Second, isolation of the actvated T cells and washing to remove the BsAb. Third, evaluation of its killing capacity on a second vial from the same sample.

Isolated BsAb-Activated T Cells Kill Cancer Cell Without BsAb They Behave As A Real Drug

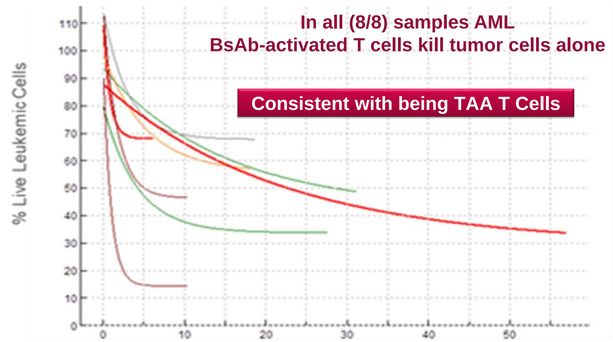
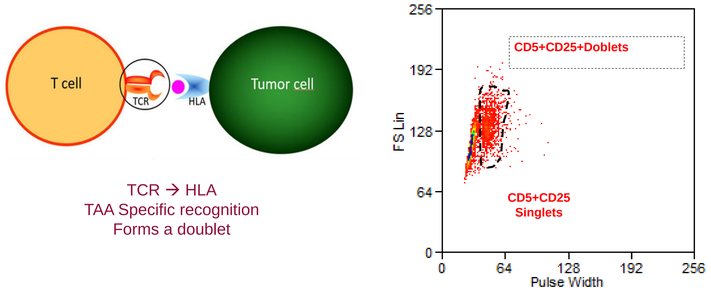


Figure 3. Evaluation of T-Cell killing ex vivo on 8 AML samples generating activated T-Cells by a CD3/CD123 BsAb and incubating with new autologous frozen AML blasts.

BsAb-Activated T Cells form Trogocytotic Singlets & Doublets
T Cell-Cancer Cell Like TAA T Cells



Singlets represent trogocytotic T Cells that have acquired pieces of the membrane form leukemic cells from earlier interactions, presumably doublets

TAA T Cells recognize cancer cells forming doblets by an immune synapse to proof that BsAb-activated T Cell doublets are TAA, if we add anti-TCR & anti-HLA antibody we should block recognition and doublet formation

Figure 4. Representation of a TCR-HLA recognition and an illustrative dot plot of doublets by flow cytometry

BsAb-Activated Trogocytotic T Cells Are Blocked by Anti TCR & HLA in 6/8 AML Samples, Supporting they are TSA T Cells

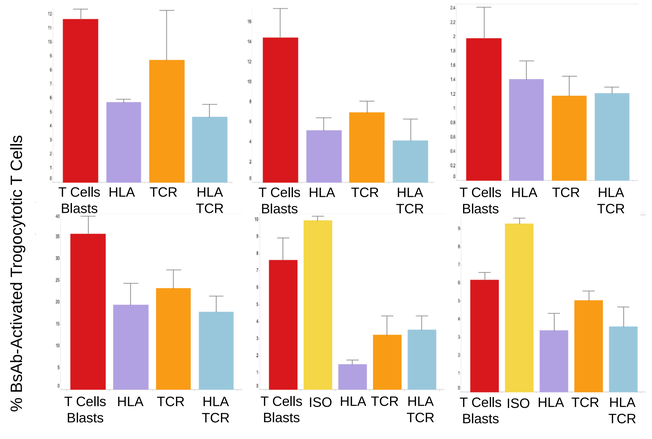


Figure 5. Bar diagrams representing the % of CD5+/PKH67+ in presence or absence of an isotype and blocking antibodies: HLA A,B,C (blast Cells), αβTCR (T-Cells).

CONCLUSIONS

- We speculate that proximate contact between T cells and blast cells through an BsAb could, in some cases, directly reactivate pre-existing Tumor-Associated Antigen T-cells from the BM to recognize and kill AML blasts by themselves.
- This secondary T-cell response require cross-presentation by classical APCs or probably the blasts directly, independently of the BsAb that can be blocked by Anti HLA or TCR antibodies.
- Reactivating the immune system could provide those patients with the benefits of long-term duration responses.
- Assays that could identify those patients may enable Precision Medicine tests for patient selection.

CONTACT INFORMATION

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