MODE OF CYTOTOXIC ACTION OF T-CELL BISPECIFIC ANTIBODIES ON HEMATOLOGICAL MALIGNANCIES: A NOVEL IN VITRO APPROACH

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OBJECTIVES:
Bispecific antibodies (BsAbs) act through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) leading to T-cell activation and serial lysis of tumor cells. The aim of the present study is to explore the mechanism of action (MOA) and the in vitro efficacy of BsAbs on acute myeloid leukemia (AML) samples with the PharmaFlow platform.

METHODOLOGY:
Thirty-one whole fresh bone marrow (BM) samples and two AML cell line were tested with the CD3-CD123 BsAb in the PharmaFlow platform, an innovative proprietary method that uses flow cytometry (FCM) to efficiently count the number of tumor cells killed by each activated T-cells. We analyzed the populations of leukemic cells, activated T-cells, and residual normal cells. In addition, other key parameters were used to elicit the MOA after BsAb exposure at different time incubations (24h–144h), such as the effective E:T ratio and number of T-cells that kill a number of leukemic cells, real basal E:T ratio, tumor antigen expression, T-cell expansion, expression of immune checkpoint proteins on target and effector before and after cell culture. For some experiments, fluorescence-activated cell sorting (FACS) was performed to evaluate T-cell cytotoxicity after BsAb exposure.

RESULTS:
Most of the samples demonstrated T-cell activation and effective lysis of tumor cells after BsAb exposure independent of TAA expression and in a dose-response manner. Once sorted, these T-cells could kill tumor cells in the absence of BsAbs, as well as tumor cells that did not express the TAA target. Interestingly, these activated T-cells selectively killed tumor cells with low cytotoxicity in residual normal cells from the same patients. Moreover, differential T-cell cytotoxicity was observed between samples. We observed samples with leukemic persistence or no T-cell activity, as well as others with higher T-cell cytotoxicity and minimal number of activated T-cells. The integration of all the predictive parameters (E:T ratios, TSA expression, etc.) allowed us to generate an in vitro response model and select samples with higher T-cell cytotoxicity after the BsAb exposure.

CONCLUSIONS:
• Our findings are consistent with a model where, in addition to the standard MOA inducing tumor lysis by proximity, BsAbs can highly enrich cytotoxic clonal T-cell subsets with TSA and induce strong activation and proliferation of T-cells capable of killing tumor cells in an effective and selective manner.
• The PharmaFlow platform selects different in vitro T-cytotoxicity effects across patients identifying best patient candidates for adoptive antitumor immunotherapy with BsAbs with the integration of Effective E:T ratios and pharmacological parameters (EC50 & Emax): quantitative pharmacology of BsAbs in patient samples.
• New design of multi-specific antibodies from our new MOA are empowered by our screening of hundreds constructs ex vivo.
• CD8 opportunity may increase substantially the clinical outcomes (ISAs).

Figure 2. BsAb decrease leukemic cells and increase activated T-cells in a time and a concentration manner.

METHODS: Quantitative Pharmacology for Bispecific Antibodies Activity in Patient Samples

1. EC50 tumor depletion (same T Cell proliferation)
   - When very low, predicts patient may respond at low doses
   - When very high, predicts resistant patient
2. Effective E:T Ratio equivalent standard EC50
   - Can be validated measuring dose responses with FACS sorted activated T Cells
   - High Effective E:T Ratios predicts sensitive patients
3. Emax
   - Emax near 100% required for a sensitive patient
4. Kinetics of response
   - The combination of all these parameters quantifies the BsAb activity selection cases with higher possibility of BsAb response.

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ABSTRACT

Simple Version Immune-Tumor Response How Activated (CD25+) T Cells Lead to Tumor Depletion?

Activated Cytotoxic T Cells Kills Blasts through a CD123 Independent MOA

Can They Kill AML Cells Lines w/o CD123 Expression? YES

Measuring Dose Responses of Sorted Activated T Cells Without Bispecific Antibody

CONCLUSIONS

Standard MOA: BsAbs Promote Direct Tumor Lysis by Proximity
New MOA: BsAbs may activate Tumor-Specific Antigen T Cells

High Effective E:T Ratios (e.g. 2-5) samples may activate TSA T Cells
Low Effective E:T Ratios (e.g. 1-5) may kill only by low potency proximity