Bispecific Antibodies on Hematological Malignancies: A Novel Mechanism of Action That May Contribute to Therapeutic Efficacy

ABSTRACT

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 in situ lysis of tumor cells. The aim of the present study is to explore the mechanism of action (MOA) and the in vitro effect of BsAbs on hematological samples with the PharmaFlow platform. For this purpose, whole bone marrow (BM) and peripheral blood (PB) from 44 samples from 3 different hematological diseases (34 AML, 3 ALL, and 7 CLL) and two AML cell lines were tested with CD3-CD123 for AML patients, and cell lines or CD3-CD19 (for ALL and CLL patients) BsAbs 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. We analyzed the populations of leukemic cells, activated T-cells, and residual normal cells. Additional key parameters were also used to explore the MOA after BsAbs exposure at different time incubations (24h-144h), such as the effective E:T ratio (the number of T-cells that kill a number of leukemic cells), real basal E:T ratio, tumor antigen expression, T-cell expansion, and expression of immune checkpoint proteins on target and effector cells before and after cell culture. For some experiments, fluorescence-activated cell sorting (FACS) was performed to evaluate T-cell cytotoxicity after BsAbs exposure. Most of the samples demonstrated T-cell activation and effective lysis of tumor cells after BsAbs exposure independent of TAA expression and MOA. We observed samples with leukemic resistance or no T-cell activity (especially in CLL with CD3-CD19), as well as others with higher T-cell cytotoxicity and minimal number of activated T-cells (especially in AML with CD3-CD123). The integration of all the predictive parameters (E:T ratios, Tumor-Specific Antigen (TSA) expression, etc.) allowed us to generate an in vitro response model and select samples with higher T-cell cytotoxicity after the BsAbs exposure.

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
3. Emax
   - Emax near 100% required for a sensitive patient
4. Kinetics of response

The integration of all these parameters quantifies the BsAbs activity selecting cases with higher possibility of BsAbs response.

RESULTS

Simple Version Immune-Tumor Response How Activated (CD3+) T Cells Lead to Tumor Depletion?

Activated T cells are the real drug: Effective E:T Ratios

CONCLUSIONS

- Our findings are consistent with a model where, in addition to the standard MOA inducing tumor cells 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 Tcytotoxicity 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.
- CDx opportunity may increase substantially the clinical outcomes (ISTs).