Pharmaceutical evaluation of bispecific antibodies: A novel method to quantify their in vitro activity in hematological samples

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ABSTRACT

Background and Aim: The PharmaFlow automated flow platform has achieved 85% clinical correlation with AML samples with its novel Native Deconvoluted assay. Recently, novel Bispecific Antibodies (BsAbs) or analogous constructs that simultaneously bind a surface target on tumor cells and an associated TCR chain have been used as immunotherapy leading to T-cell activation and overall decline of tumor cells that carry the target. The aim of the present study is to develop and in vitro assay that incorporates multiple variables to better quantify the activity of bispecific antibodies and capture the interplay variability.

Methods: For this purpose, different fresh whole bone marrow (BM) or Peripheral Blood (PB) samples were tested with their corresponding BsAbs at 4 different concentrations in different time points (24-144h) in AML samples. Upper panel displays leukemia cell (CD3-CD19+) TAA (EGFRvIII) expression of different samples and lower panel shows the BM or PB sample staining with the corresponding BsAbs.

Results: Most of the samples present both T-cell activation (CD25+) and an effective lysis of tumor cells after BsAbs exposure in a dose dependent manner, even starting with low basal E T ratios in 94%. For BM, basal quantification of CD25 by FCM directly does not reflect a correlation with the in vivo response. By contrast, differences in T-cell cytoxicity or leukemic immunoresistance were observed between samples in terms of Emax EC50. CD4+ T cells more prone to generate TAA lytic effector cells. Interestingly, many of the samples for all the BsAbs leave a significant proportion of live cells, even at the highest BsAbs concentrations or with a remarkable expansion of activated T cells that support the use of immunomodulatory ligands in this immunoresistant disease.

Conclusions: We have developed an automated flow cytometry assay for bispecific antibodies screening that keep intact both in vitro effects to target (E/T) ratios and Native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform selects different in vitro T-cytotoxicity effects across patients identifying best patient candidates for adoptive immunotherapy with BsAbs. The integration of effective E T ratios and phenotypic/functional response to bispecific antibodies can be validated measuring dose responses with FACS sorted activated T Cells. This method can be used as a pharmacological indicator of tumor cell killing and can be used to identify tumor immune checkpoint phenotypes that may predict drug resistance. The integration of these parameters quantifies the BsAbs activity selecting cases with higher possibility to BsAbs response.

RESULTS

BSAb decrease leukemic cells and increased activated T-cells in a time and a concentration manner

Figure 1. Screening set-up and Workflow

Figure 2. Dose response curves to assess the CD3+CD19+ lymphoma cell killing activity at different time points (72-144h) in AML samples. Upper panel displays leukemia cell (CD3-CD19+) TAA (EGFRvIII) expression of different samples and lower panel shows the BM or PB sample staining with the corresponding BsAbs.

Figure 3. Representative example of the Emax (E) ratio from 2 AML samples (right): This method clearly stratifies patients with high (left side) vs low T-cell killing activity (right side).

Figure 4. New method E/Ratio tỷ (E) and I/O Activity Bispecific Abs

Figure 5. Pharmacological evaluation of bispecific antibodies: A novel method to quantify their in vitro activity in hematological samples

CONCLUSION

We report a novel proprietary ex vivo automated flow cytometry assay that adoptive immunotherapy both bispecific effector to target (E/T) ratios and native environment using whole BM or PB.

The PharmaFlow platform selects different in vitro T-cytotoxicity effects across patients identifying best patient candidates for adoptive immunotherapy with BsAbs with the integration of effective E T ratios and pharmacological parameters (EC50, Emax). Quantitative pharmacology of BsAbs in patient samples.

Our findings are consistent with a model in which BsAbs can enrich highly cytotoxic clonal T-subsets with Tumor Specific Antigen in some patients.

This assay enable evaluate multiple combinations with immunomodulators (PD1, CTLA-4, TIM-3, LAG-3) or BsAbs candidates for hematological diseases.

Clinical trials should not exclude patient for low expression of TargetCD3.

The design of multiple 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).