A NOVEL METHOD TO CAPTURE THE PHARMACOLOGICAL ACTIVITY OF BISPECIFIC ANTIBODIES IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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ABSTRACT
A significant increase number of bispecific antibodies (BsAbs) leading to T-cell activation and serial lysis of tumor cells are currently in different clinical stages. However, no methods to stratify patients with remarkable antibody-mediated cytotoxicity are available to select patients with higher therapeutic potential in vivo for these constructions. The aim of the present study is to develop in vitro assay to better quantify the activity of BsAbs and capture the interpatient variability. Fresh whole Bone Marrow (BM) or Peripheral Blood (PB) were tested with their corresponding BsAbs at 8 different concentrations in different time points (24h-144h). We tested 31 AML BM samples with the CD123/CD3 BsAb and 7 CLL and 3 B-ALL samples with Blinatumumab. When appropriate, baseline quantification of TAA was performed by flow cytomtery (FCM). The PharmAFlow platform efficiently covered by FCM how many tumor cells are killed by every activated T-cells, here called effective E:T ratio. Eight-colour FCM staining was performed to simultaneously analyze the leukemic population, activated CD4 and CD8 T-cells and the residual normal cells. E50 or Emax was calculated to evaluate potency or efficacy. Kinetics of activity was measured repeating the dose response curves in 3 different days. Most of the samples present both T-cell activation (CD5+) and an effective lysis of tumor cells after BsAbs exposure in a time and dose dependent manner, even starting with low basal E:T ratios (<1:100). By contrast, differences in T-cell cytotoxicity or leukemic immunoresistance were observed between samples in terms of E50 or Emax even more marked between CLL samples. The integration of effective E:T ratios, E50 or Emax and kinetics allow us to generate an in vitro response model and select those samples with higher T-cell cytotoxicity after the different BsAbs exposure. Interestingly, many of the samples for the all BsAbs leave a significant proportion of live cells, even at the higher BsAbs concentrations or with a remarkable expansion of activated T-cells that suggest the use of immunocheckpoints to unlock this immunoresistant status.

RESULTS

1. EC50 tumor depletion (same T Cell proliferation)
   • Very similar across most samples
   • 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 predicted sensitive patients
3. Emax
   • Emax near 100% required for a sensitive patient
   • High % resistant tumor suggest combination e.g. PD1
4. Kinetics of response
   The integration of all these parameters quantifies the BsAb activity selecting cases with higher possibility to BsAb response.

CONCLUSIONS
• We report a novel proprietary ex vivo automated flow cytomtery assay for I-O drugs keeping intact both basal effector to target (E:T) ratios and native environment using whole BM or PB.
• The PharmAFlow platform selects different in vitro T-lytic activities across patients identifying best patient candidates for adoptive immunotherapy with BsAbs with the integration of effective E:T ratios and pharmacological parameters (E50 & Emax): quantitative pharmacology of BsAbs in patient samples.
• Our findings are consistent with a model in which BsAb 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 TargetsCD3.
• 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).

METHODS

Figure 1. Screening set-up and Workflow

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

Figure 3. Simple Version Immune-Tumor Response How Activated (CD25+) T Cells Lead to Tumor Depletion?

Figure 4. Activated T cells are the real drug: Effective E:T Ratios

Figure 5. New Method Effective E:T Ratios Captures I-O Activity Bispecific Abs

Figure 6. Effective E:T ratios on the CLL samples

Figure 7. Effective E:T ratios on the AML samples

Figure 8. Effective E:T ratios on the ALL samples

Figure 9. PD-1 Increases T-cell activity

Figure 10. E:T ratio for AML samples (N=4) after the CD3-CD123 exposure (72h). Upper panel correspond to the BM (blue lines) and lower panel to the PB (red lines). Percent to the corresponding TAA, cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+). BM T-cells are better killers in 35 samples #1, #4 and #5 consistent with BM immunosuppressed. Tumor Infiltrated Lymphocytes (TILs)

Figure 11. E:T ratio for AML samples (N=4) after the CD3-CD19 exposure (72h). Red line correspond to the Leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+). Samples #1 and #2 correspond to paired BM and PB patient sample, being BM T-cells (#1) better killers than PB (X).

Figure 12. Percentage of Emax near 100% required for a sensitive patient

Figure 13. High % resistant tumor suggest combination e.g. PD1

Figure 14. Kinetics of response

Figure 15. Values of E50 and Emax.