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

Daniel Primo[1], David Martínez-Cuadrón[2], Pau Montesinos[2], Pilar Hernández[1], Julián Gorrochategui[1], María Luisa Vicente[1], Cristina Gómez[1], Joaquín Martínez[3], Joan Ballesteros[1]
[1]Vivia Biotech, Madrid; [2]Hematology, Hospital Universitari i Politècnic La Fe de Valencia, Valencia, Spain; [3]Hospital Universitario 12 de Octubre, Madrid, Spain

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 and *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 CD123xCD3 BsAb and 7 CLL and 3 B-ALL samples with Blinatumumab. When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform efficiently count 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. EC_{50} or E_{max} 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 (CD25+) 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 EC_{50} or E_{max}, even more marked between CLL samples. The integration of effective E:T ratios, EC_{50}, E_{max}, 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 all the BsAbs leave a significant proportion of live cells, even at the higher BsAb concentrations or with a remarkable expansion of activated T-cells that suggest the use of immunecheckpoint to unblock this immnunoresistant status.

METHODS

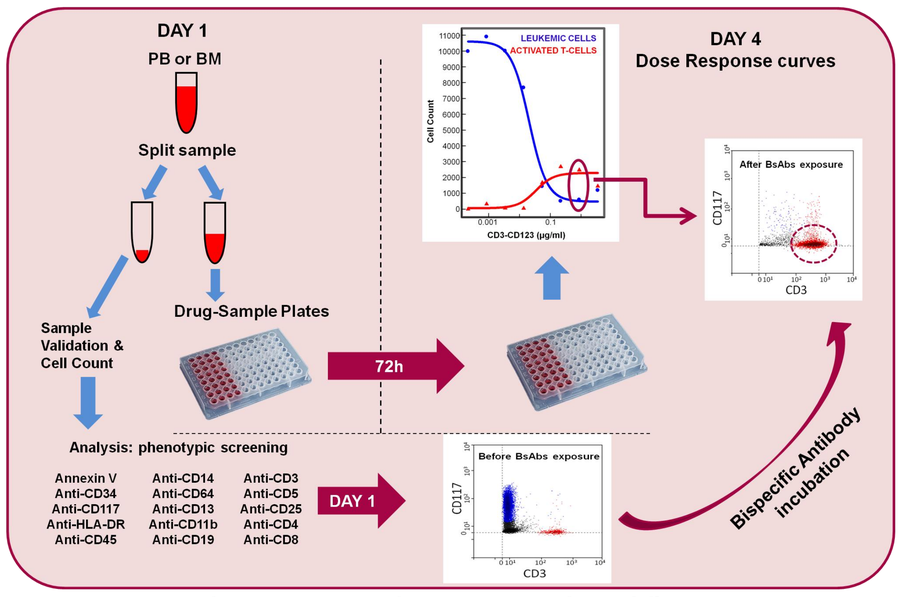
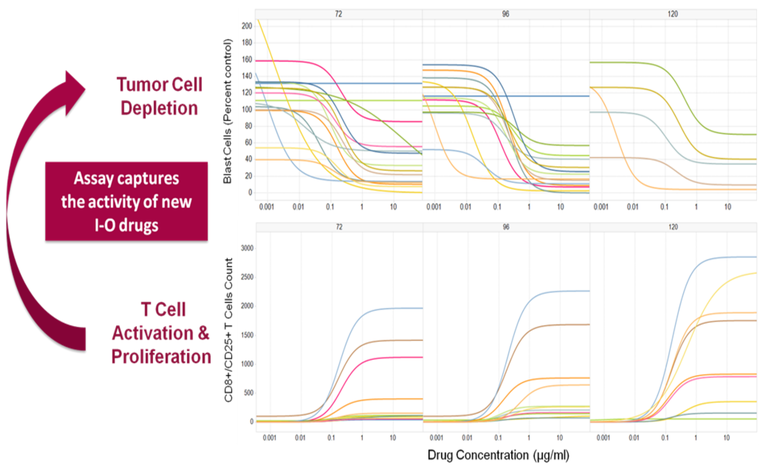


Figure 1. Screening set-up and Workflow

RESULTS

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



Dose response curves to assess the CD3-CD123 bispecific antibody activity at different time points (72-96-120h) in AML samples. Upper panel displays leukemic cell depletion curves. The survival index (y-axis) ranges from 100% to 0% displaying the leukemic cell depletion after exposure to dose response CD3-CD123 bispecific antibody concentrations (x-axis). Bottom panel shows the simultaneous T-cell activation and proliferation along different time incubations. Absolute cell count of activated T-cells (y-axis) after CD3-CD123 bispecific antibody dose response concentrations (x-axis) is displayed.

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

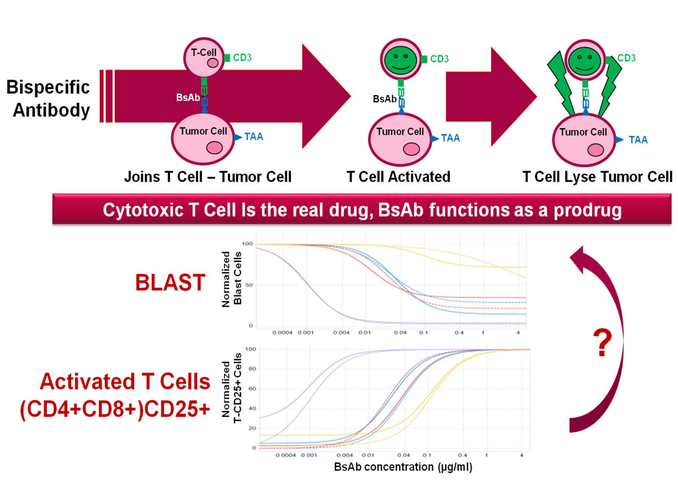
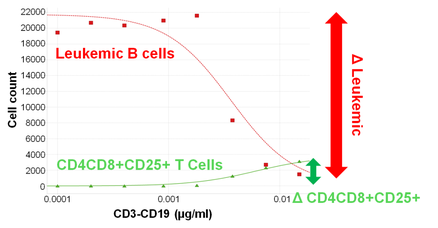


Figure 4. Activated T cells are the real drug: Effective E:T Ratios



Blinatumomab CD3xCD19 [ng/ml]

	T-Cells	CD19+	Basal E:T
Cells # at baseline	1101	33166	1:30.1

	T- Cells CD4CD8+CD25+	Live Tumor Cells	Effective E:T Ratio
Cell # max dose	3799	21761	1:5.7

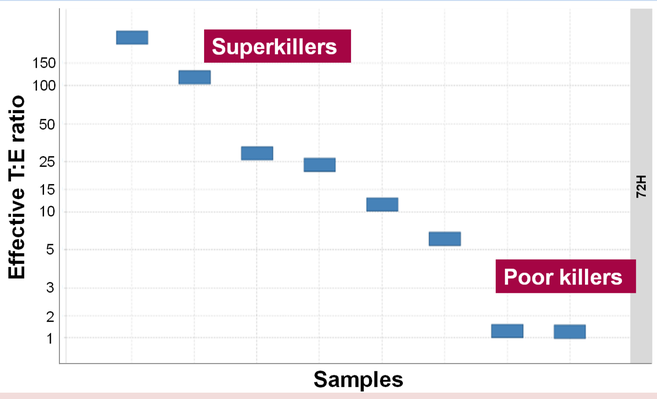
- Basal E:T ratios measure basal tumor vs total T cells
- Bispecific antibody induces cytotoxic CD4CD8+CD25+ T cells not present at basal
  - Δ CD4CD8+CD25+
- These cytotoxic T cells kill a number of leukemic cells
  - Δ Leukemic
- We define an Effective E:T Ratio as the ratio between
  Δ CD4CD8+CD25+ : Δ Leukemic
- Measures how many cancer cells are killed by each cytotoxic T Cell, i.e. the T Cell cancer-killing activity
- Effective E:T Ratios are different than Basal E:T ratios and may represent a better measurement of bispecific antibody activity

Quantitative Pharmacology for Bispecific Antibodies Activity In Patient Samples

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 cells suggest combination e.g. PD1
4. **Kinetics of response**

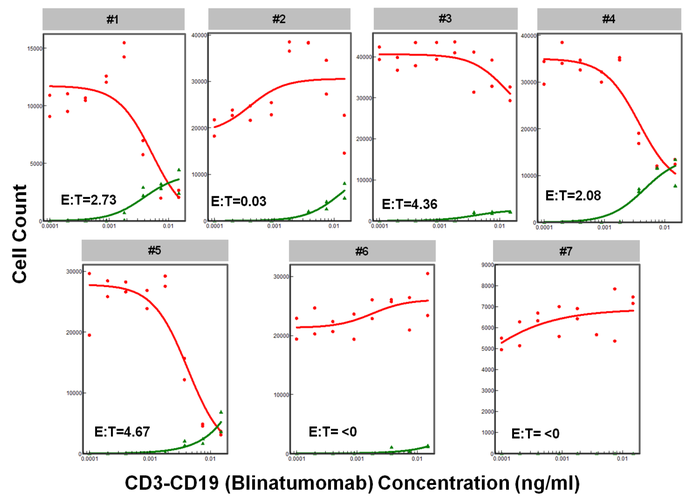
The integration of all these parameters quantifies the BsAb activity selecting cases with higher possibility to BsAb response.

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



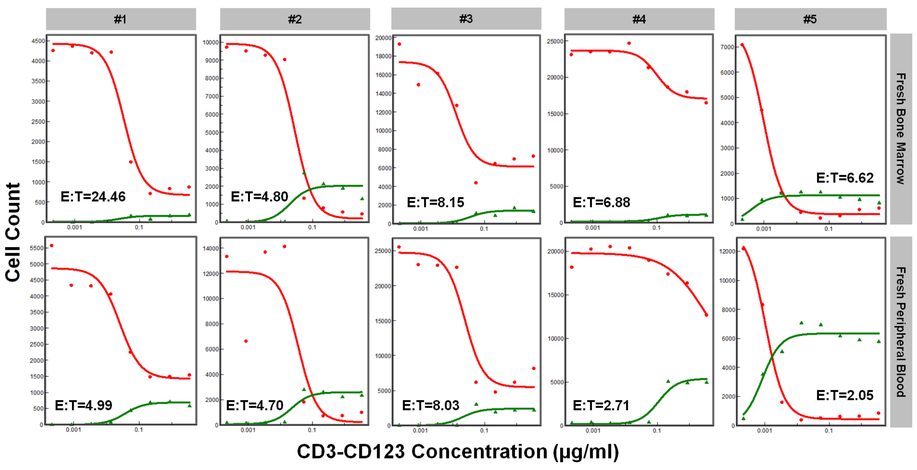
Representative example of the Effective T:E (y-axis) ratio from 8 AML samples (x-axis). This method clearly stratify patients with high (left side) vs low T-cell killing activity (right side).

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



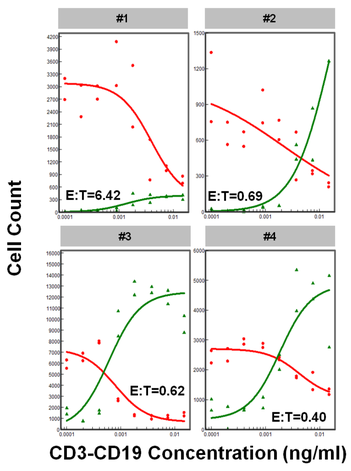
Effective E:T ratios on CLL samples (N=7) after the CD3-CD19 exposure (120h). Red Line correspond to the leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+). Effective E:T ratios, AUC values, EC_{50} and E_{max} reflect both T-cell efficacy and B-cell immunoresistance identifying patients likely to receive blinatumomab therapy.

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



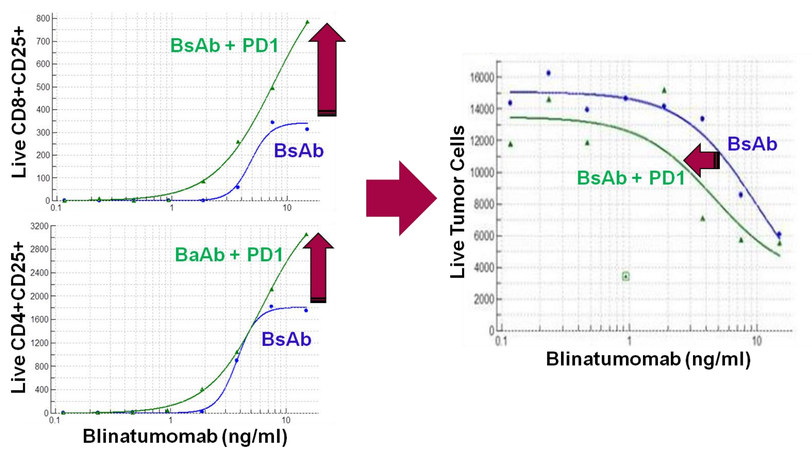
Effective E:T ratios on AML samples (N=5) after the CD3-CD123 exposure (72h). Upper panel correspond to the BM and bottom panel to the PB compartment. Red Line correspond to the Leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+). BM T-cells are better killers in 3/5 samples (#1, #4 and #5) consistent with BM immunosuppressed Tumor Infiltrated Lymphocytes (TILs).

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



Effective E:T ratios on ALL 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 (#2).

Figure 9. PD-1 increases T-cell activity



Absolute cell counts in a CLL sample for activated T-cells (left panels) and Live tumor cells (right panel) incubated with Blinatumomab in presence (green lines) or absence (blue lines) of the immunomodulatory inhibitor checkpoint PD1. Results show increased numbers of both activated T-cells (CD4 and CD8) in presence of PD1, enhancing the overall tumor killing.

CONCLUSIONS

- **We report a novel proprietary *ex vivo* automated flow cytometry 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-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 (EC_{50} & 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 TargetxCD3.**
- **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).**