INTRODUCTION AND AIM

The Vivia Biotech ExviTech® automated flow platform has achieved 83% clinical correlation with AML samples with its novel Native Environment assay. Recently, novel Bi-specific antibodies (BsAbs) or analogous constructions acting through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) have been used as immunotherapy leading to T-cell activation and serial lysis of tumor cells. However, appropriate assays to capture their mechanism of action or patient to patient activity have not been developed. We aimed to incorporate our flow cytometry validated assay to evaluate new immune-oncology compounds in hematological malignancies.

METHODS

For this purpose, different fresh whole Bone Marrow (BM) or Peripheral Blood (PB) samples were tested with their corresponding BsAbs at 8 different concentrations. In this sense, we tested 10 AML (5 paired BM and PB) and 7 CLL samples with the CD3-CD123 (AML) or CD3-CD19 (CLL) BsAbs with the ExviTech® platform, that efficiently count by flow cytometry (FCM) how many tumor cells are killed by every activated T-cells, here called effective E:T ratio (Figure 1). For each sample, B-colour FCM staining was performed to simultaneously analyze the leukemic population, activated CD4 and CD8 T-cells and the residual normal cells. EC50 or Emax was calculated to evaluate potency or efficacy.

RESULTS

• 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 bspecific antibody activity

CONCLUSION

- We report an automated flow cytometry assay for immune-oncology drugs keeping intact both basal effector to target (E:T) ratios and Native environment using whole BM or PB.
- The ExviTech® 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).
- Our findings are consistent with a model in which BsAb can enrich highly cytotoxic clonal T-subsets with Tumor-Specific Antigen.
- This assay enable evaluate multiple combinations with immunomodulators (PD1, CTLA-4, TIM-3, LAG-3) or BsAbs candidates for hematological diseases.