A NOVEL NATIVE ENVIRONMENT METHOD FOR EVALUATING IMMUNE-ONCOLOGY COMPOUNDS BY FLOW CYTOMETRY

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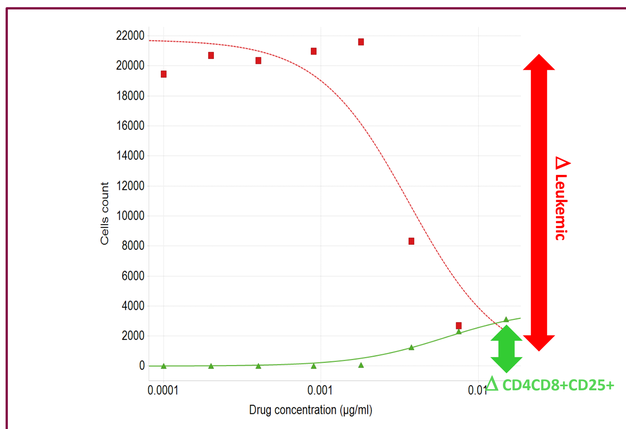
INTRODUCTION AND AIM

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) 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, 8-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.



	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

Figure 1: Illustrative example to calculate the effective E:T ratio in a fresh CLL sample after CD3-CD19 exposure in our Native Environment Assay.

RESULTS

BsAbs works in fresh samples even at very low and real effector-to-target ratios in both AML or CLL samples with both T-cell activation and lysis of tumor cells.

Sample	BsAb	Pathology	Source	Basal E:T	#CD5CD25- 0h	#CD5+CD25+ h**	EC_{50}
#1	CD3/CD123	AML	BM	1:104	122	161	0.0241
#1	CD3/CD123	AML	PB	1:16	988	689	0.0372
#2	CD3/CD123	AML	BM	1:2,2	6356	2026	0.0195
#2	CD3/CD123	AML	PB	1:1,2	9272	2597	0.0226
#3	CD3/CD123	AML	BM	1:12	1386	1415	0.0356
#3	CD3/CD123	AML	PB	1:5	2619	2398	0.0203
#4	CD3/CD123	AML	BM	1:29	805	1113	0.1126
#4	CD3/CD123	AML	PB	1:5	4390	5373	0.1108
#5	CD3/CD123	AML	BM	1:10	1254	1143	0.001
#1	CD3/CD19	CLL	PB	1:27	998	4085	0.0039
#2	CD3/CD19	CLL	PB	1:58	550	8916	0.0092
#3	CD3/CD19	CLL	PB	1:86	581	2478	0.0035
#4	CD3/CD19	CLL	PB	1:78	491	14080	0.0046
#5	CD3/CD19	CLL	PB	1:30	1101	7570	0.0107
#6	CD3/CD19	CLL	PB	1:85	168	1530	0.0087
#7	CD3/CD19	CLL	PB	1:516	30	5	0.001

Table 1: Basal effector (T-Cells) to target (blast or B-cells) ratios on AML or CLL samples. Absolute cell count were measured with the ExviTech® platform at the basal level (0h) or after the BsAb exposure (72h for AML and 120h for CLL). Low staring number of T-cells in the assay are enough to mediate kill of cancer cells.**

Effective E:T ratios on the AML samples

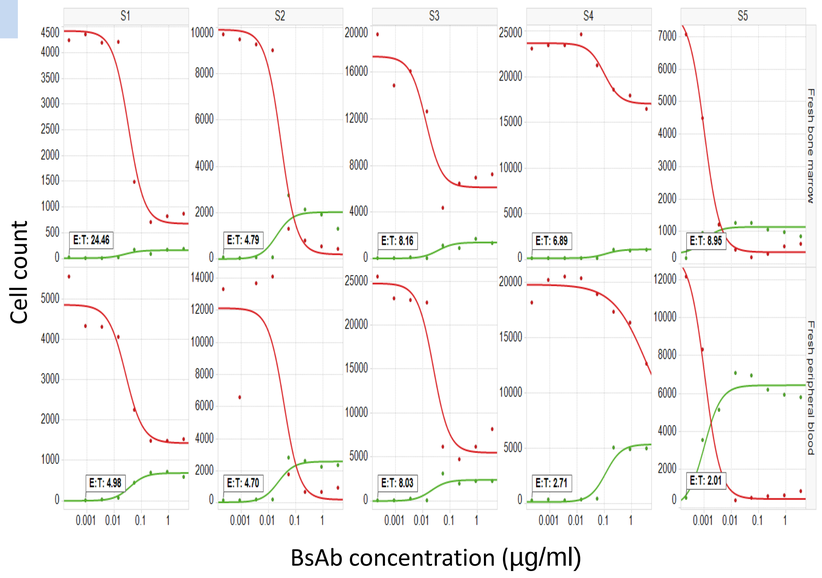


Figure 2: Effective E:T rations on the AML samples (N=5) after the CD3-CD123 exposure (72h). Upper panel correspond to the BM and bottom panel to the PB compartment. Red Line correspond to the Leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+).
BM T-cells are better killers in 3/5 samples (S1/S4/S5) consistent with BM immunosuppressed TILs.

Effective E:T ratios on the CLL samples

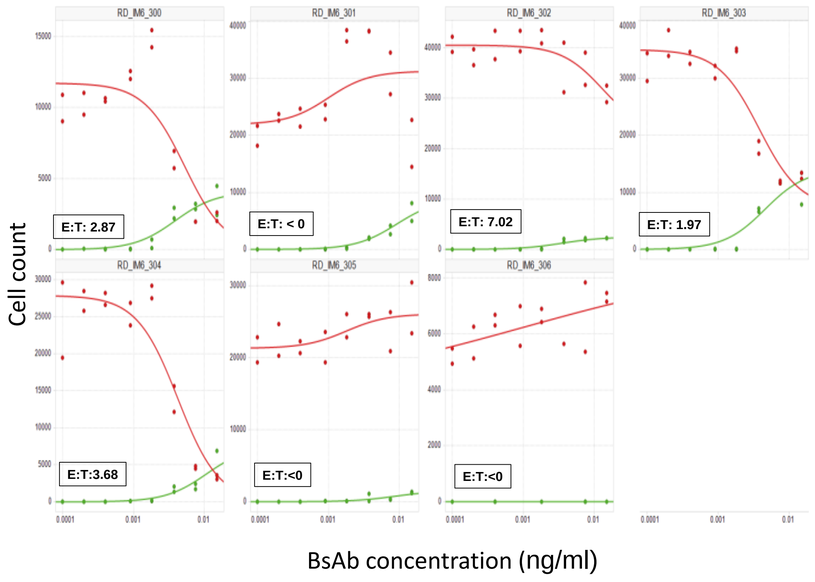


Figure 2: Effective E:T rations on the CLL samples (N=7) after the CD3-CD19 exposure (120h). Red Line correspond to the Leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+).
Effective E:T ratios, EC_{50} and E_{max} reflect both T-cell efficacy and B-cell immunoresistance identifying patients likely to receive blinatumumab therapy. Red Line correspond to the Leukemic cells and green line to the activated (CD25+) T-cells (CD4+ or CD8+).

Activated T-cells selectively kill tumor cells

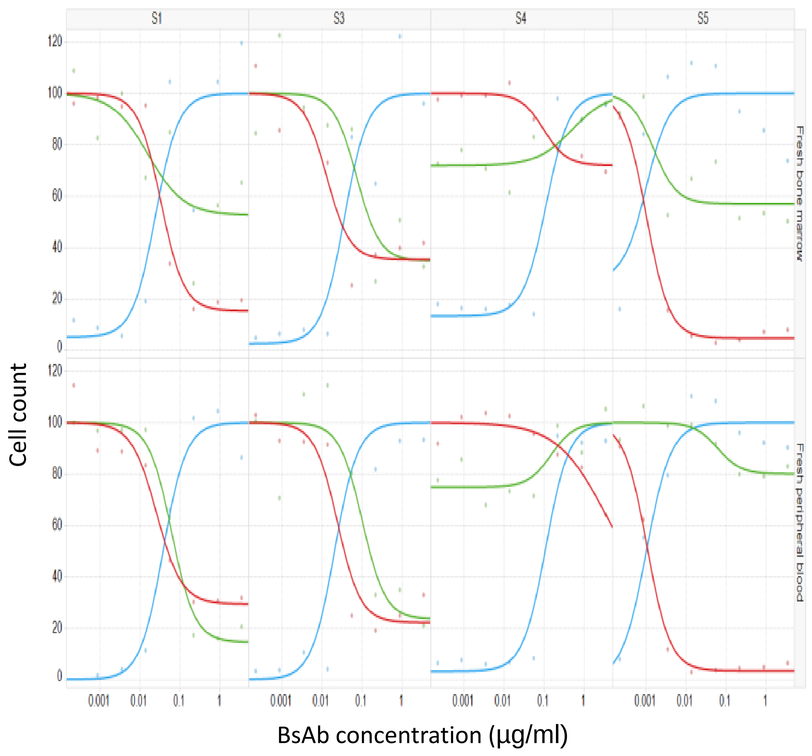


Figure 4: Simultaneous representation of the DR curves for the activated T-cells (blue line), leukemic cells (red line) and the residual B-cells (green line) in 4 AML samples after the BsAb (CD3-CD123) exposure in the BM (upper panel) or the PB (bottom panel) compartment. Results reflect how B-cells how activated T-cells kill more selectively tumor cells in comparison to CD19+ B-cells in BM or PB

PD-1 increase T-cell activity

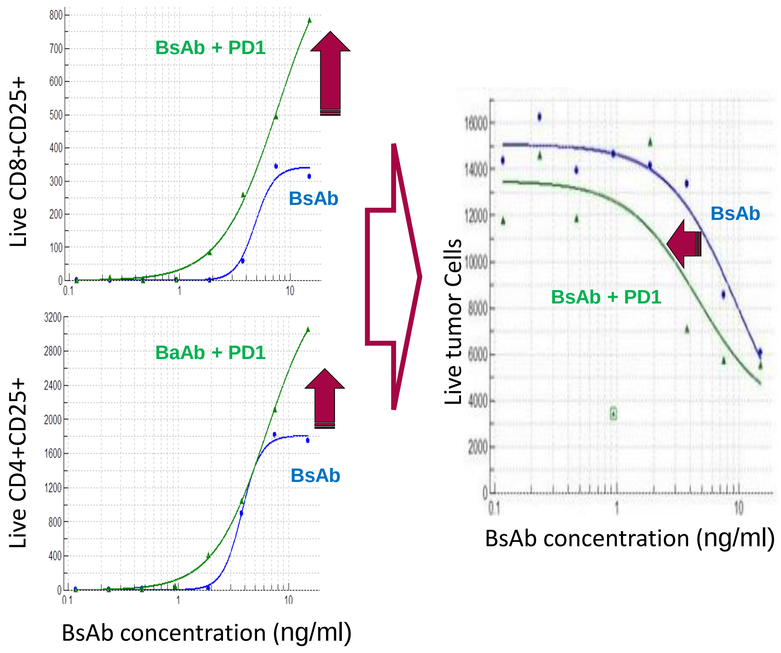


Figure 5: Absolute cell counts in a CLL sample for activated T-cells (left panels) and Live tumor cells (right panel) incubated with CD3-CD19 in presence (green lines) or absence (blue lines) of the immunomodulatory inhibitor checkpoint PD1.
Results show increased numbers of both activated T-cells (CD4 and CD8) in presence of PD1, enhancing the overall tumor killing.

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.**