

Bispecific Antibodies on Hematological Malignancies: A Novel Mechanism of Action That May Contribute to Therapeutic Efficacy

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ABSTRACT

Bispecific antibodies (BsAbs) act through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) leading to T-cell activation and serial lysis of tumor cells. The aim of the present study is to explore the mechanism of action (MOA) and the *in vitro* effect of BsAbs on hematological samples with the PharmaFlow platform. For this purpose, whole bone marrow (BM) and peripheral blood (PB) from 44 samples from 3 different hematological diseases (34 AML, 3 ALL and 7 CLL) and two AML cell lines were tested with CD3-CD123 (for AML patients and cell lines) or CD3-CD19 (for ALL and CLL patients) BsAbs in the PharmaFlow platform, an innovative proprietary method that uses flow cytometry (FCM) to efficiently count the number of tumor cells killed by activated T-cells. We analyzed the populations of leukemic cells, activated T-cells, and residual normal cells. Additional key parameters were also used to explore the MOA after BsAb exposure at different time incubations (24h-144h), such as the effective E:T ratio (the number of T-cells that kill a number of leukemic cells), real basal E:T ratio, tumor antigen expression, T-cell expansion, and expression of immune checkpoint proteins on target and effector cells before and after cell culture. For some experiments, fluorescence-activated cell sorting (FACS) was performed to evaluate T-cell cytotoxicity after BsAb exposure. Most of the samples demonstrated T-cell activation and effective lysis of tumor cells after BsAb exposure independent of TAA expression and in a dose-response manner. Once sorted, these T-cells could kill tumor cells in the absence of BsAb, as well as tumor cells that did not express the TAA target. Interestingly, these activated T-cells selectively killed tumor cells with low cytotoxicity in residual normal cells from the same patients. Moreover, differential T-cell cytotoxicity was observed between samples. We observed samples with leukemic resistance or no T-cell activity (especially in CLL with CD3-CD19), as well as others with higher T-cell cytotoxicity and minimal number of activated T-cells (especially in AML with CD3-CD123). The integration of all the predictive parameters (E:T ratios, Tumor-Specific Antigen (TSA) expression, etc.) allowed us to generate an *in vitro* response model and select samples with higher T-cell cytotoxicity after the BsAb exposure.

METHODS

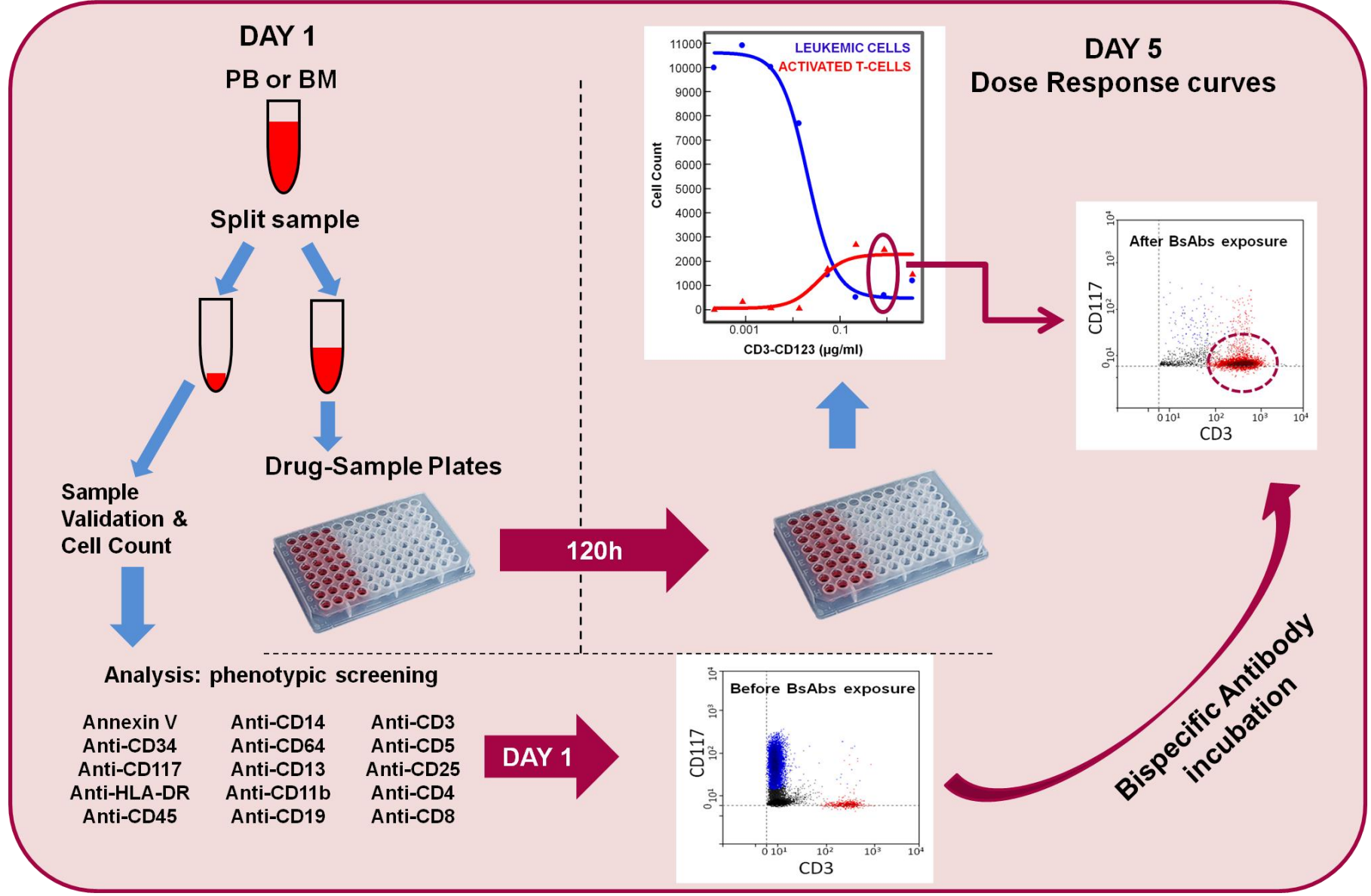


Figure 1. Screening set-up and Workflow

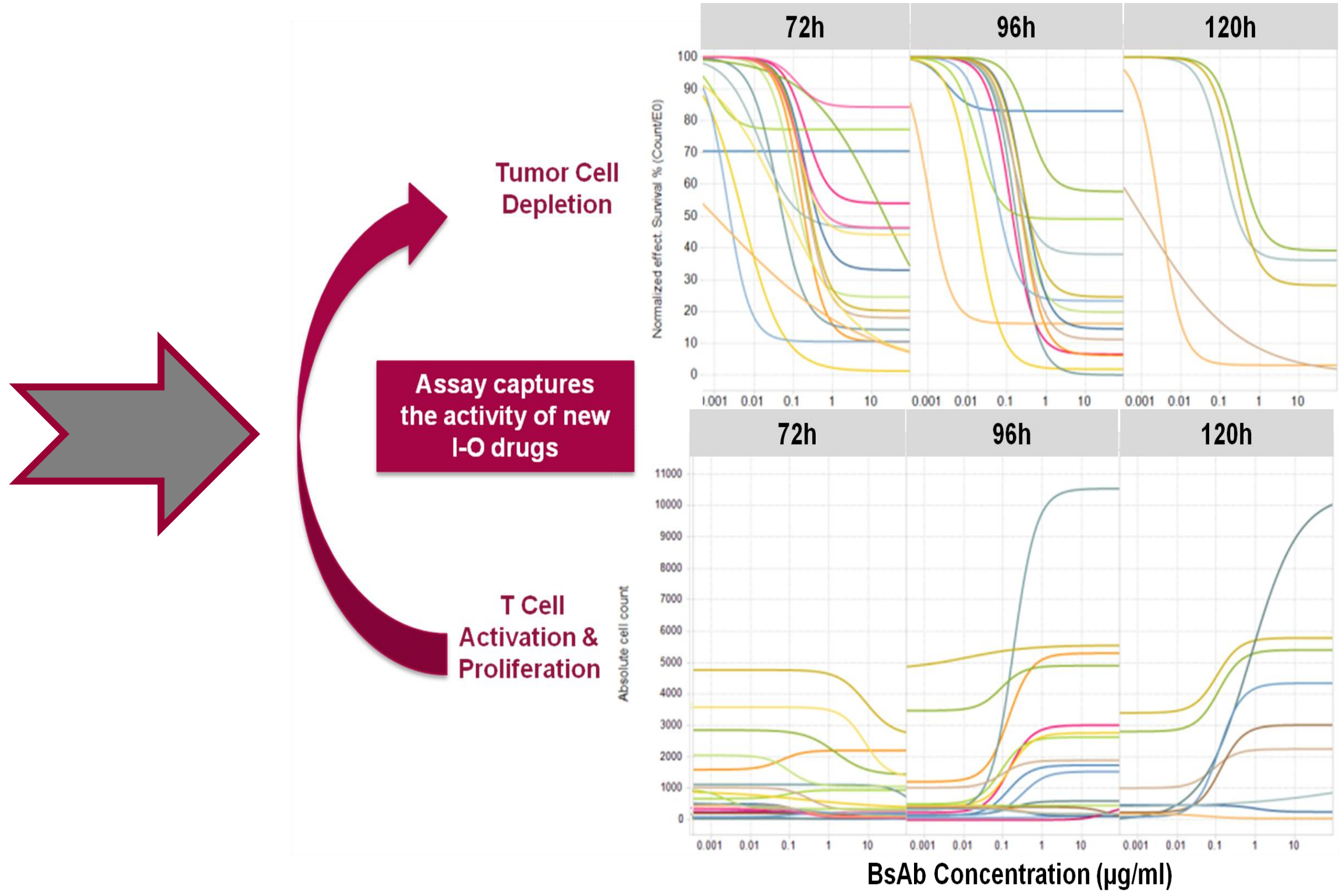


Figure 2. BsAb decrease leukemic cells and increase activated T-cells in a time and a concentration manner. Dose response curves to assess the CD3-CD123 bispecific antibody activity at different time points (72-96-120h) in AML samples. Upper panel displays leukemic cell depletion curves. The survival 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.

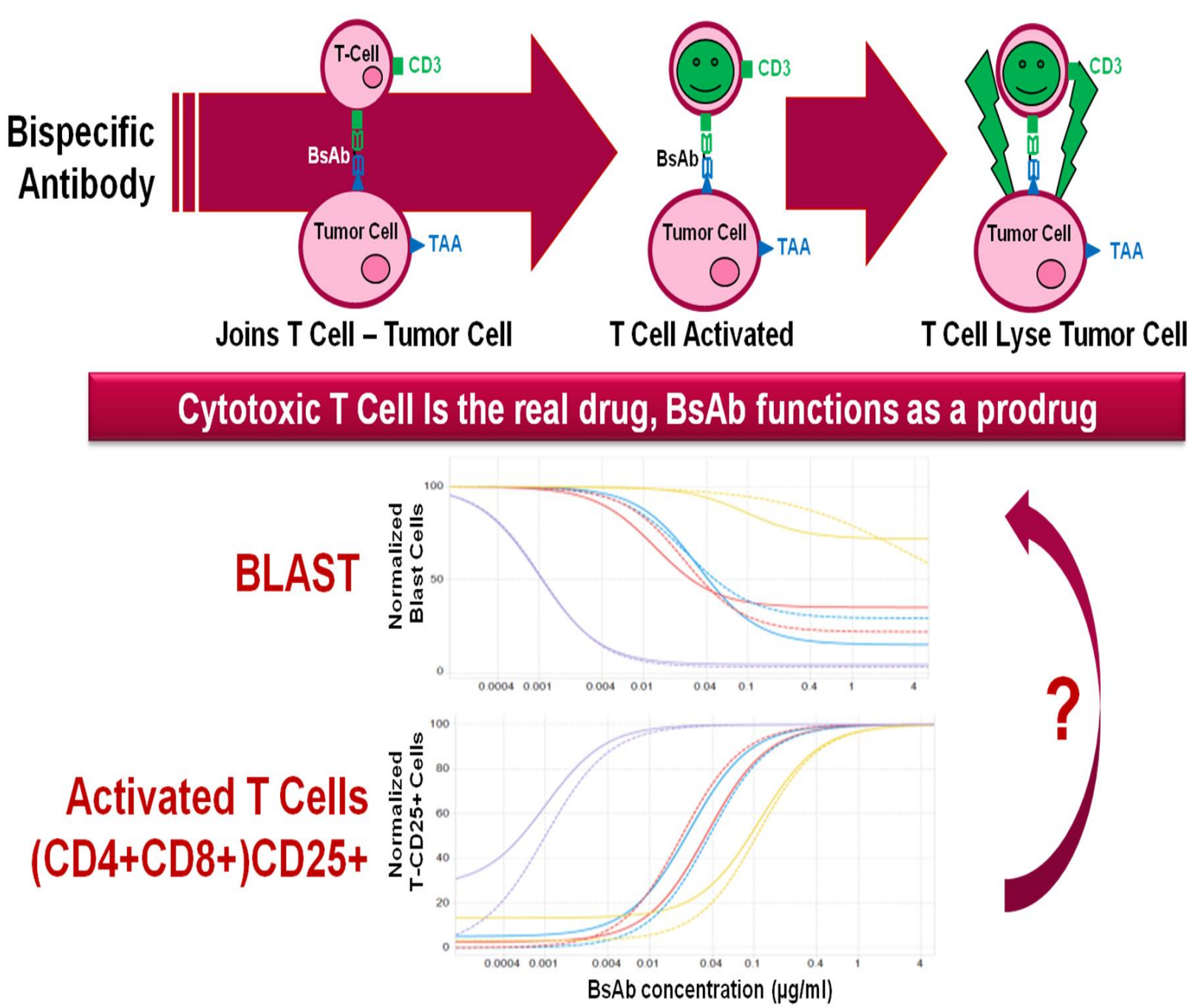
Quantitative Pharmacology for Bispecific Antibodies Activity In Patient Samples

- EC50 tumor depletion (same T Cell proliferation)**
 - When very low, predicts patient may respond at low doses
 - When very high, predicts resistant patient
- Effective E:T Ratio equivalent standard EC50**
 - Can be validated measuring dose responses with FACS sorted activated T Cells
 - High Effective E:T Ratios predicts sensitive patients
- Emax**
 - Emax near 100% required for a sensitive patient
- Kinetics of response**

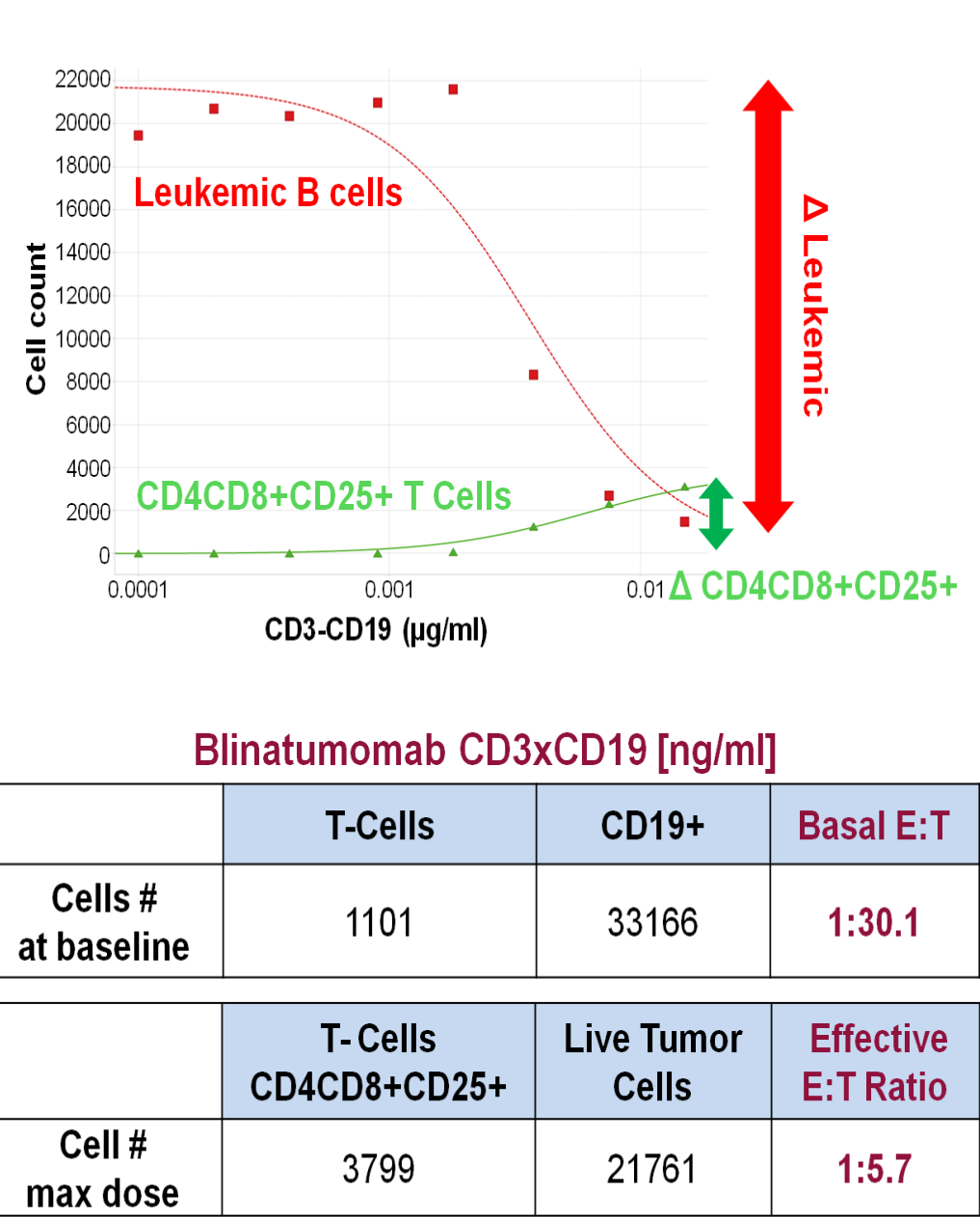
The integration of all these parameters quantifies the BsAb activity selecting cases with higher possibility of BsAb response.

RESULTS

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



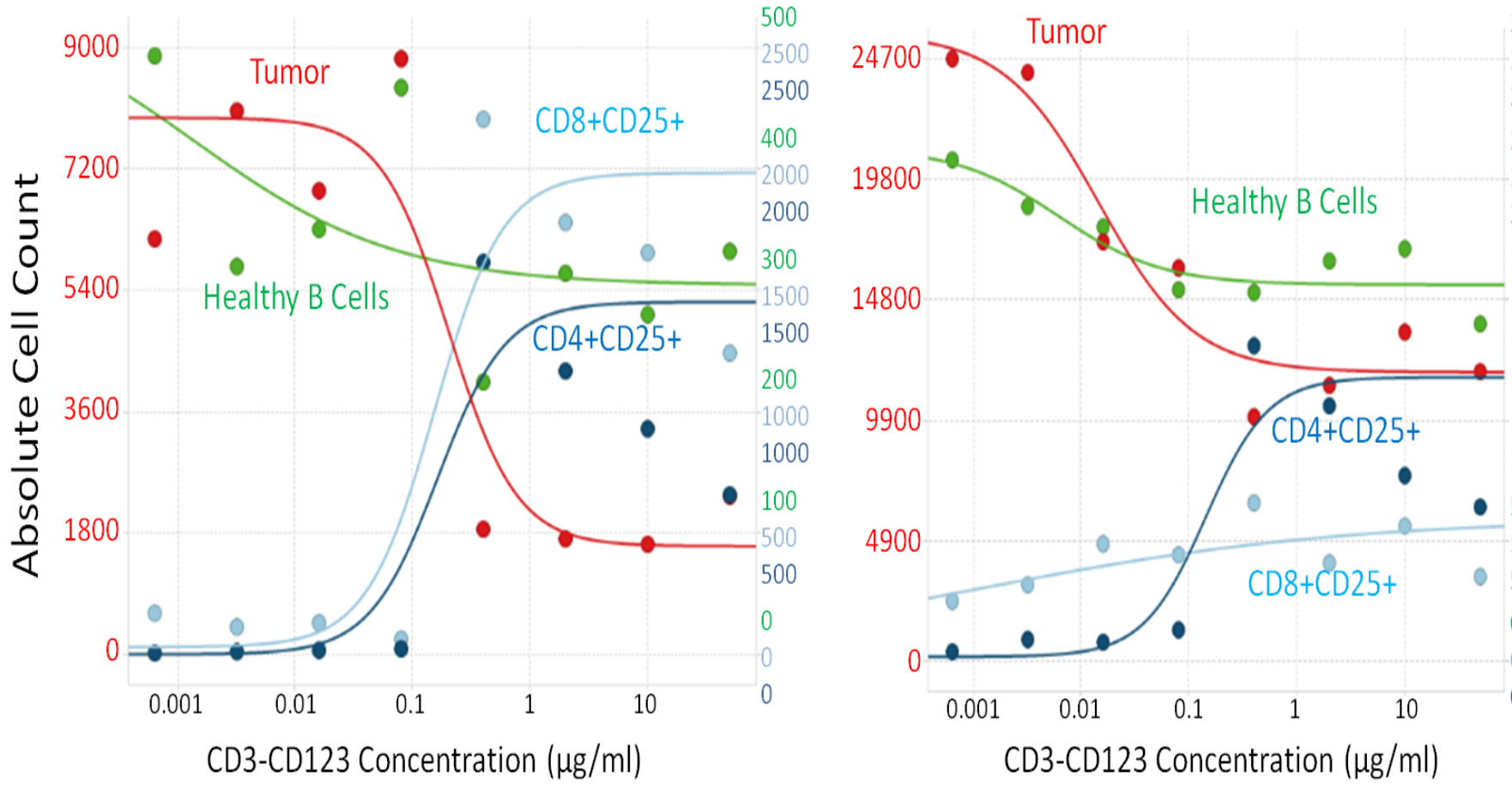
Activated T cells are the real drug: Effective E:T Ratios



	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

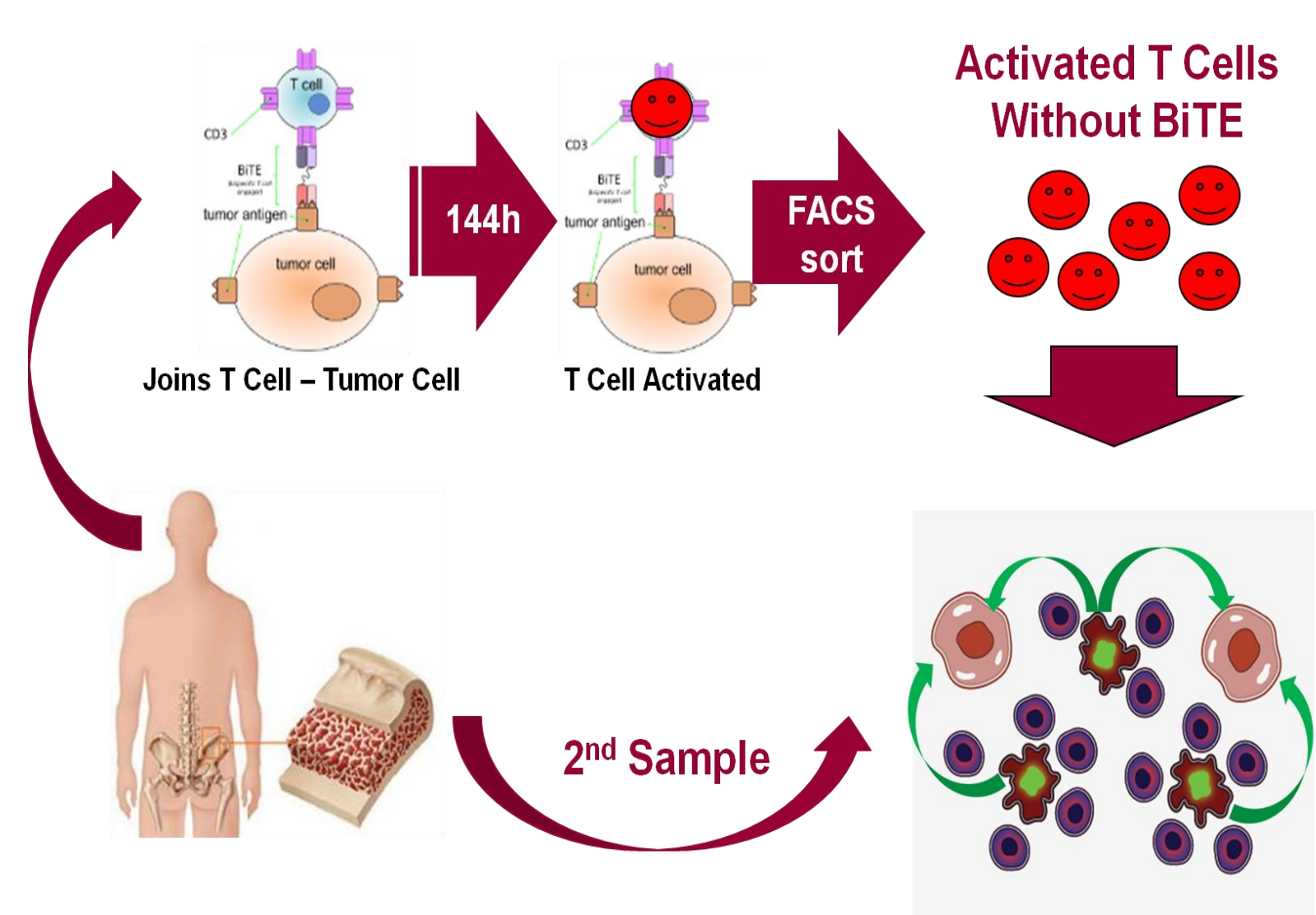
- Basal E:T ratios measure basal tumor vs total T cells
- Bispecific antibody induces cytotoxic CD4CD8+CD25+ T cells not present at basal
 - Δ CD4CD8+CD25+
- These cytotoxic T cells kill a number of leukemic cells
 - Δ Leukemic
- We define an Effective E:T Ratio as the ratio between
 - Δ CD4CD8+CD25+ : 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

If Activated BM T Cells are TSA They Should Kill Selectively Tumor Cells and Not Healthy Cells

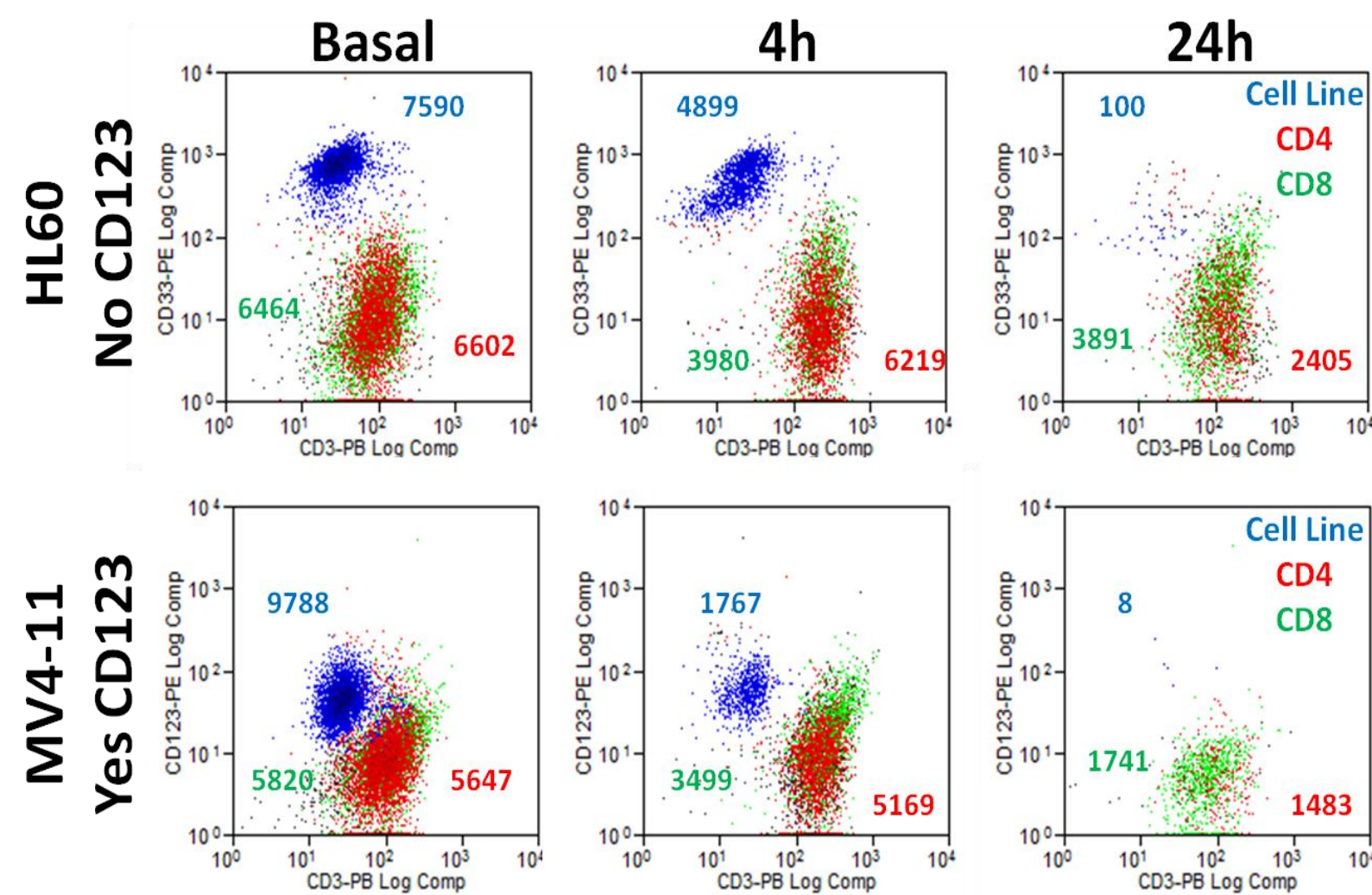


Activated proliferating T Cells kill tumor cells but not healthy B Cells within the same bone marrow sample

Activated Cytotoxic T Cell Kills Blasts Through a CD123 Independent MOA

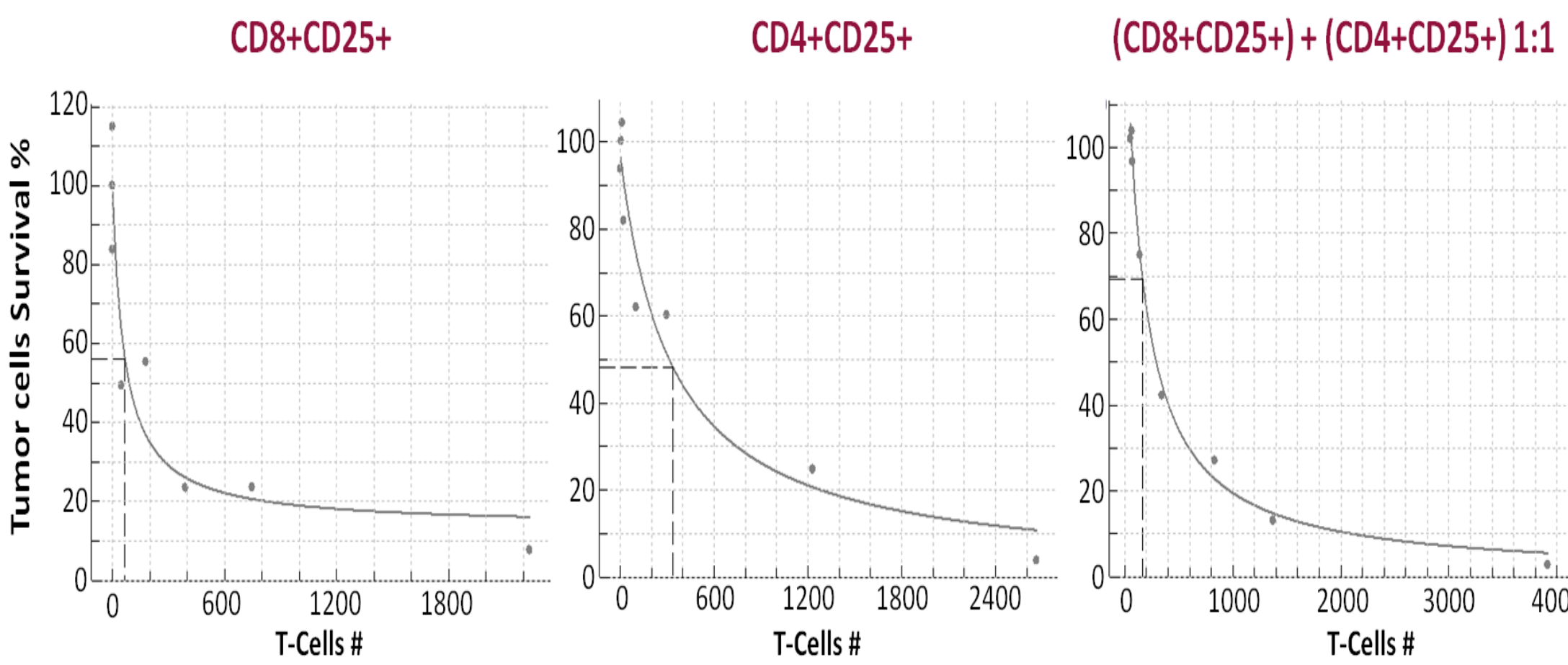


Can They Kill AML Cells Lines w/o CD123 Expression? YES



FACS sorted activated cells kill in a CD123 independent MOA

Measuring Dose Responses of Sorted Activated T Cells Without Bispecific Antibody

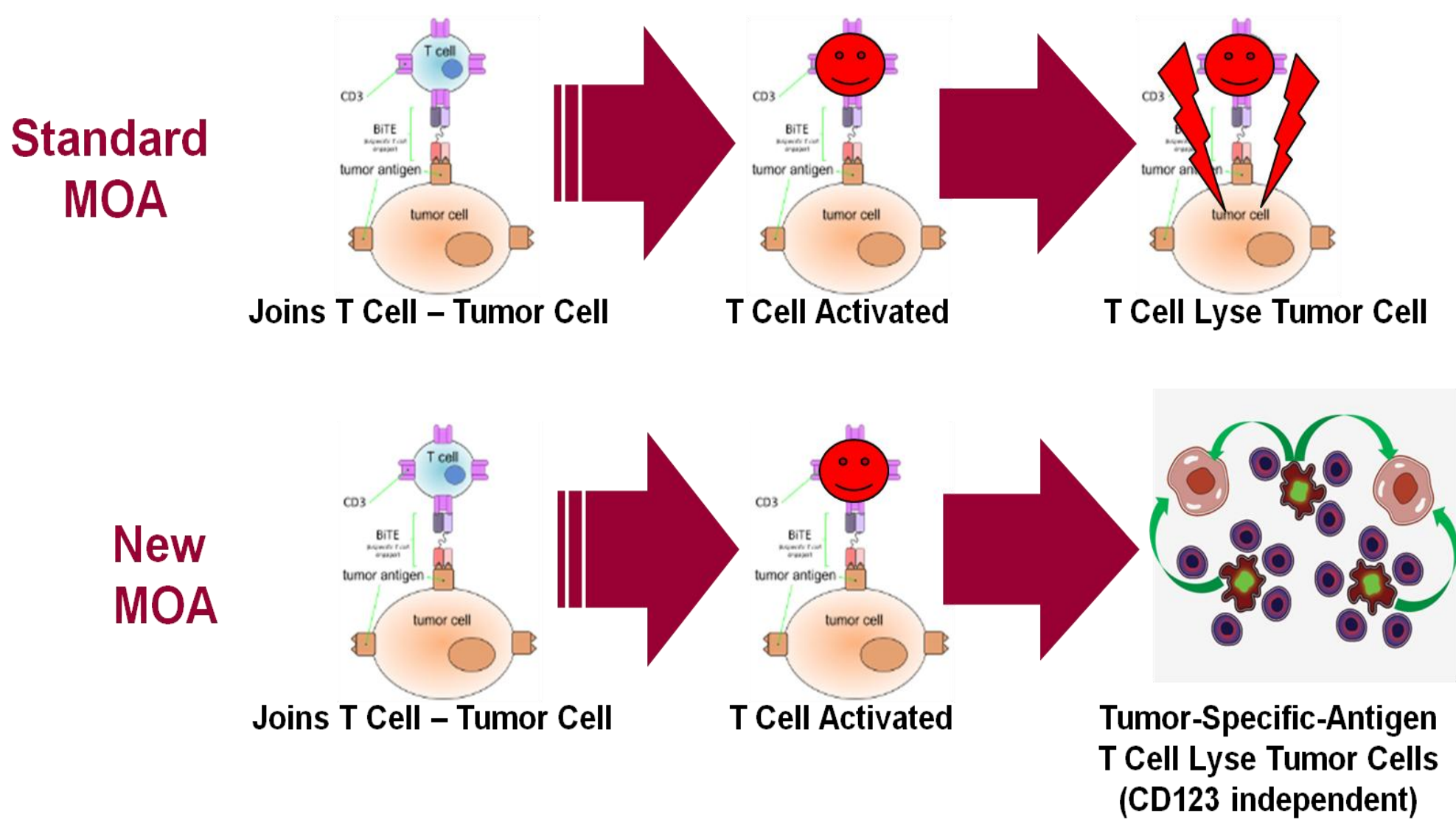


Percentage of tumor cells survival estimated relative to plate control with no-drug Intercept dashed line corresponds to EC50 value

	EC50 (T-Cells#)	E0 (% Survival)	Emax (% Survival)	AUC
CD8+CD25+	67	84.8	13.7	29392.1
CD4+CD25+	336	96.5	0.0	44769.0
(CD4+CD25+) & (CD8+CD25+) [1:1]	164	138.4	0.0	44499.9

Both CD8 & CD4 activated T Cells kill tumor cells CD8+ 5x more potent than CD4+ Effective E:T Ratios with CD4 & CD8 activated T Cells

Standard MOA: BsAbs Promote Direct Tumor Lysis by Proximity New MOA: BsAbs may activate Tumor-Specific-Antigen T Cells



High Effective E:T Ratios (e.g. 25) samples may activate TSA-T Cells Low Effective E:T Ratios (e.g. 1-5) may kill only by low potency proximity

CONCLUSIONS

- Our findings are consistent with a model where, in addition to the standard MOA inducing tumor cells lysis by proximity, BsAbs can highly enrich cytotoxic clonal T-cell subsets with TSA and induce strong activation and proliferation of T-cells, capable of killing tumor cells in an effective and selective manner.
- 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 (EC₅₀ & Emax): quantitative pharmacology of BsAbs in patient samples.
- 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).