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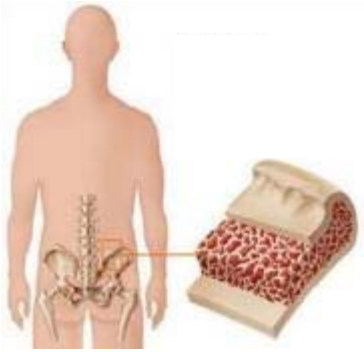
Sep 2016

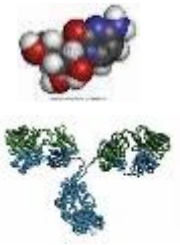
A New Mechanism Of Action For Bispecific Antibodies Activating Tumor- Specific Antigen T Cells

Drug Profiling
& Biomarker Discovery
to Personalize Treatments

Pioneers in Automated Flow Cytometry
Precision Medicine in Hematological Malignancies

Patient samples
Blood & bone marrow





Drugs
Active ingredients

ExviTech
(Ex vivo - Technology)
Automated Flow Cytometry

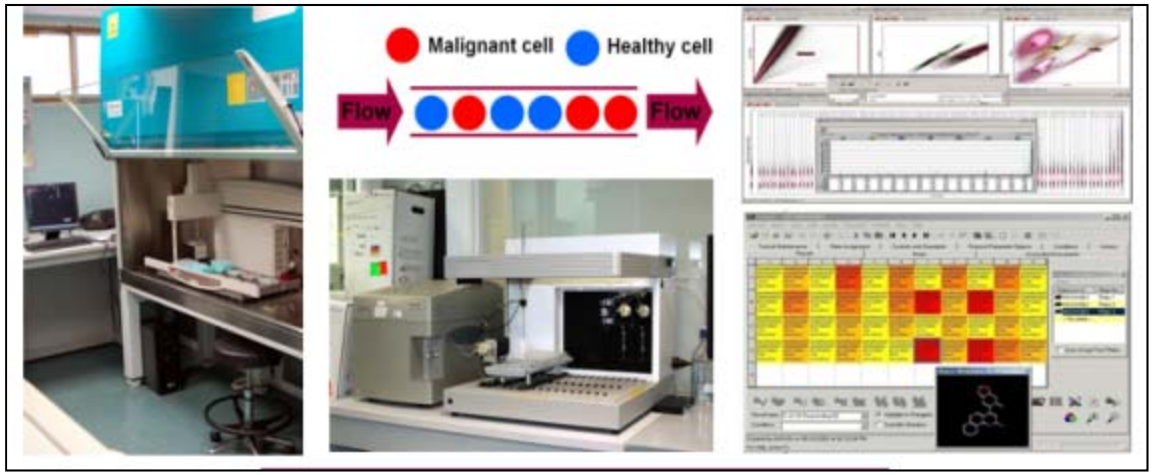
Precision Medicine Test Hospitals & Doctors

Pharma Service Evaluate drug candidates

Key Innovations Achieve Sound Pharmacology

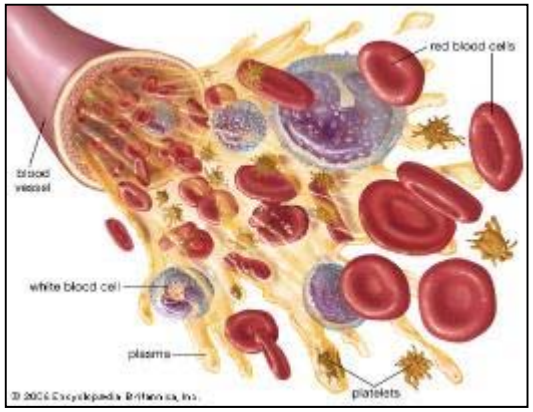
- **Vivia has overcome shortcomings of >30 years measuring drug sensitivity in patient samples:**

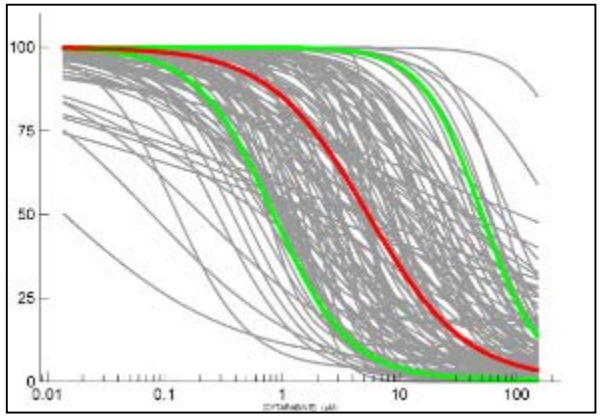
1. **Automation** capable of measuring up to 2,000 points per sample
2. Evaluating drug effects **selectively** in the **cancer cell subpopulation**
3. Measuring **exact % live tumor cells**, not apoptosis
4. **Whole sample** maintaining **Native Environment**
5. **PKPD Population Models** analysis



Automation & live single cancer cells

Whole Sample + **PKPD Population Models**





Vivia delivers high content translational data that correlates with clinical outcome

Native Environment Assay is Clinically Predictive
82% Clinical Correlation with 1st Line AML Treatment N=155

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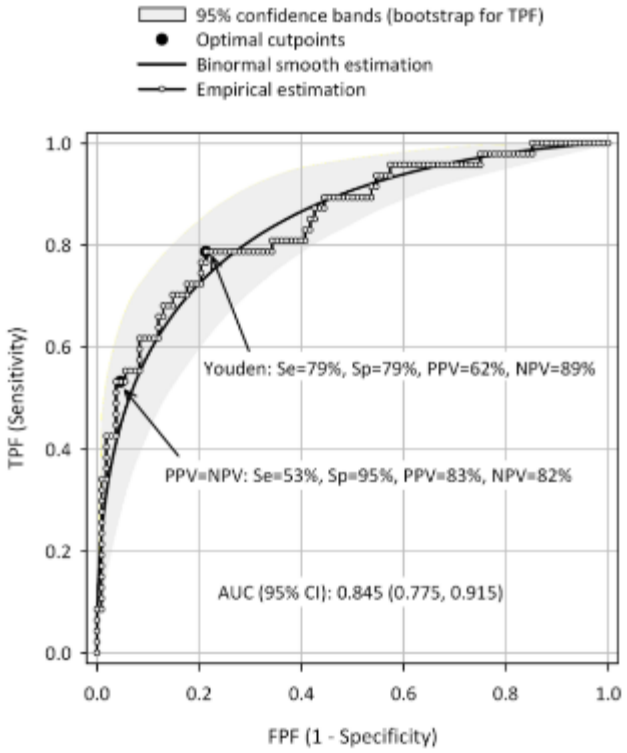
ROC Curve



Clinical Correlation

Exvivo response	Clinical outcome: RESISTANT	Clinical outcome: SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	25 *16.1%*	5 *3.2%*	*83.33*	30 *19.4%*
SENSITIVE	22 *14.2%*	103 *66.5%*	Negative predictive value % *82.40*	125 *80.6%*
	Sensitivity % *53.19*	Specificity % *95.37*	Prediction rate % *82.58*	
Subtotal	47 *30.3%*	108 *69.7%*		N 155 *100.0%*

When we say "sensitive" we'll be right 83%

When we say "resistant" we'll be right 82%

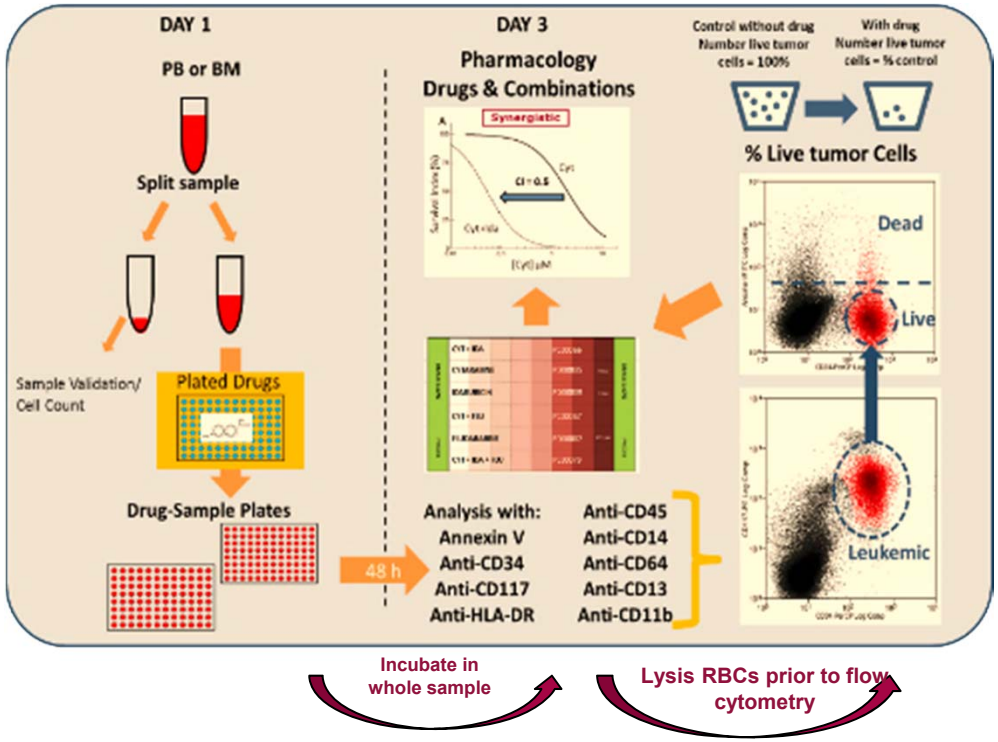
83% Correlation significantly higher than 70% 1st line response rate

VIVIA PM TEST AML
Suitable for 1st line treatment
Improves Induction Response Rates

Operation as a Centralized Diagnostic Laboratory

- Patients **bone marrow or peripheral blood** sample received within **24 hours** from extraction, and up to 72h with max efficiency

- **ExviTech Platform** automated analysis with proprietary software



The **whole sample** is incubated at different times and 8 different concentrations of each single drug and combination

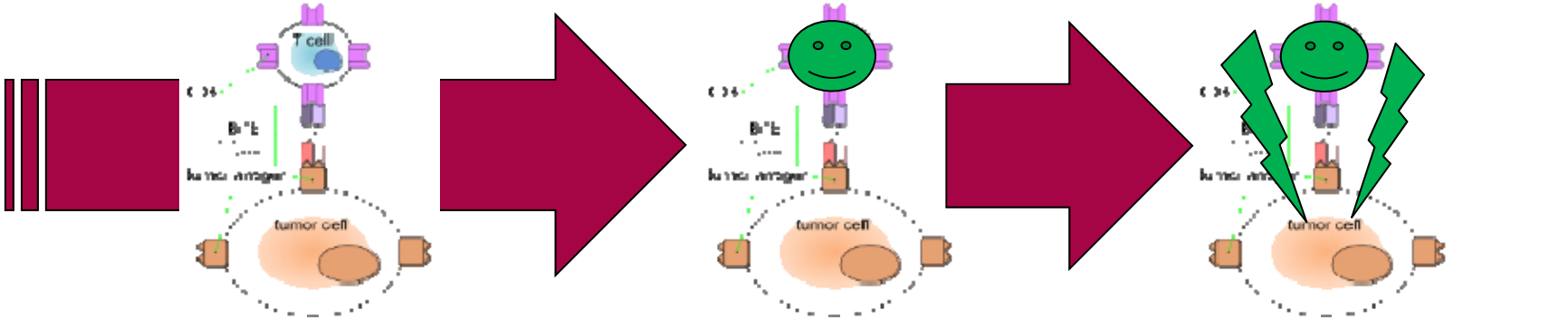
- **Exact number of cells per well**, to compare number of live tumor & T cells with/without drug

- Highest operational efficiency; 5.000 cells/s & 1.000 wells/day, **trillions of single cell data /day**

Cell Counting immune-phenotypic cell populations: mechanism unbiased & enables immune-therapy assessment (grow T-Cells vs tumor depletion)

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

Bispecific Antibody

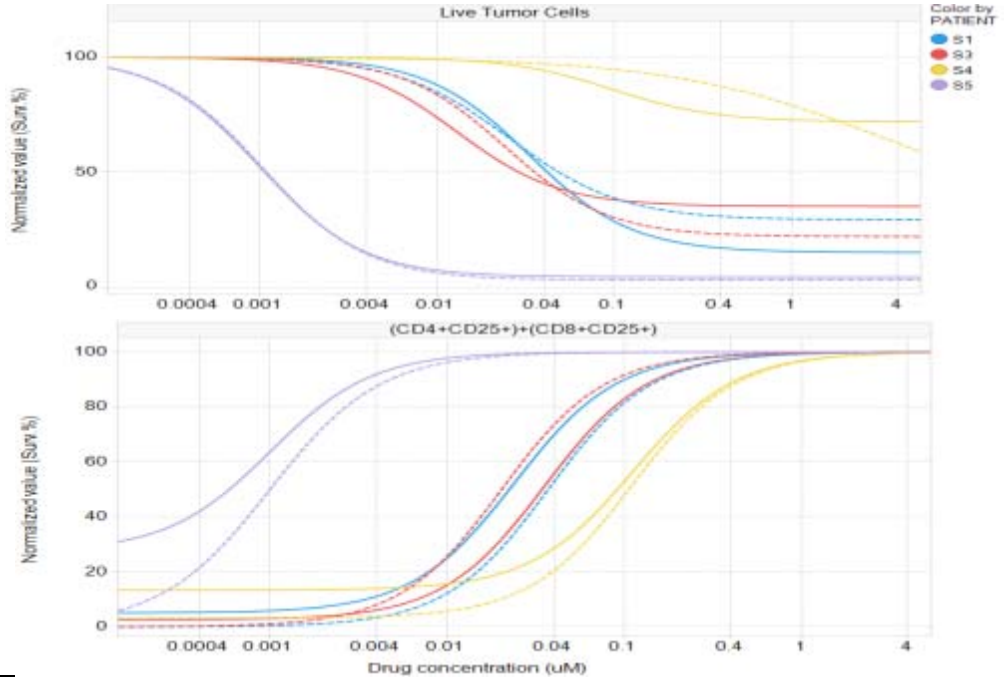


Joins T Cell – Tumor Cell | **T Cell Activated** | **T Cell Lyse Tumor Cell**

Cytotoxic T Cell Is the real drug, bispecific Ab functions as a prodrug

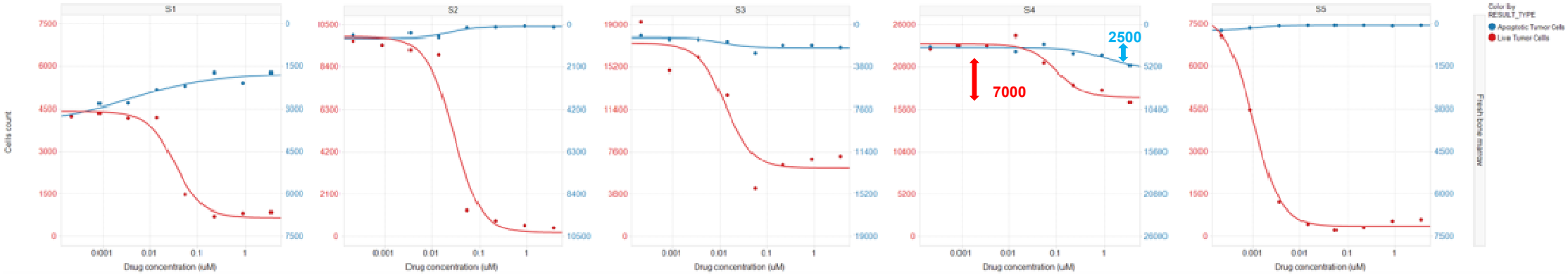
BLAST

Activated T Cells (CD4+CD8+)CD25+



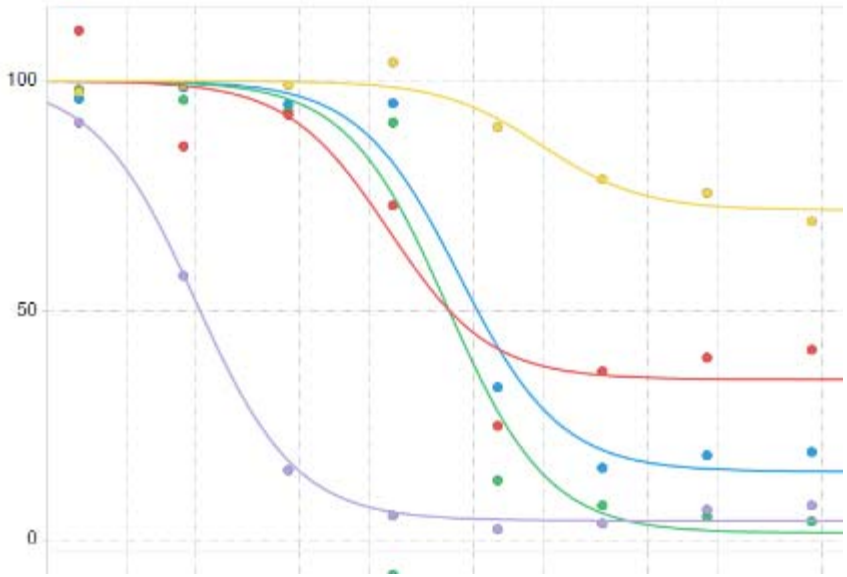
?

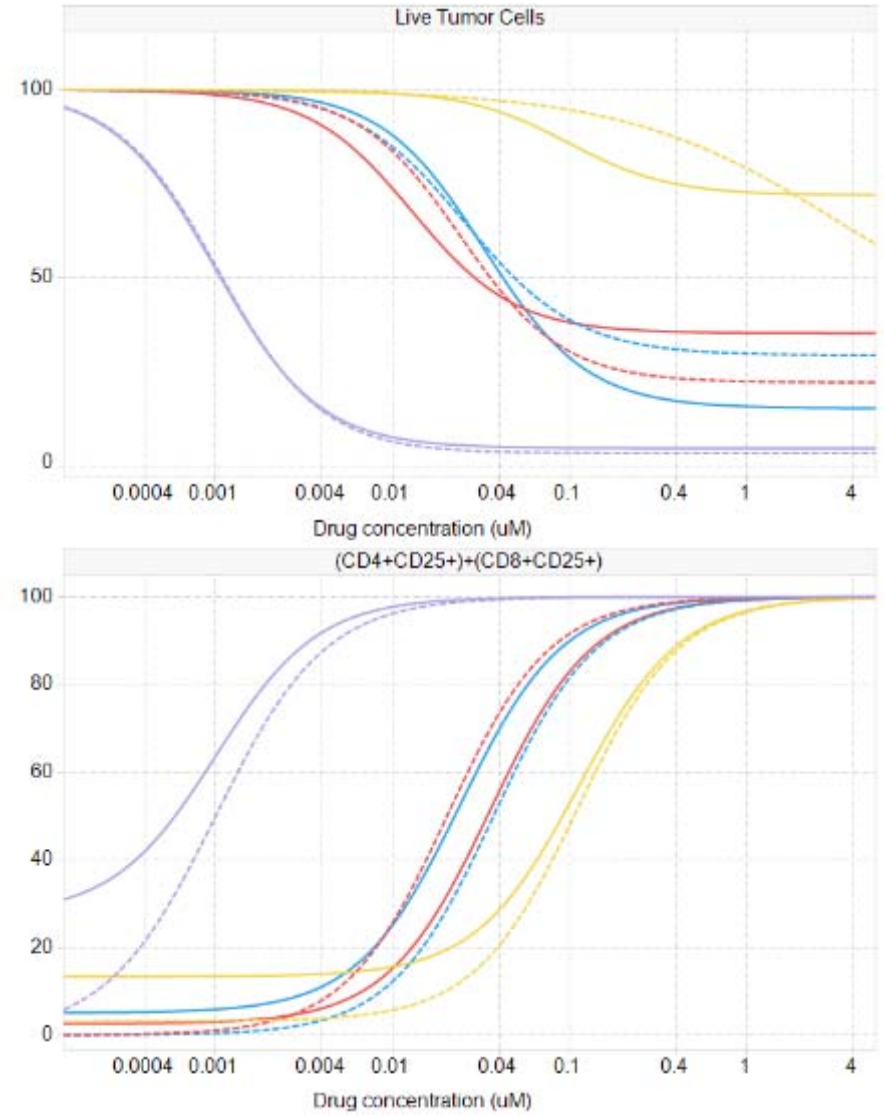
Mechanism of action for T-cells after BITE exposure. AML (N=5)



BITEs mediate their action through direct lysis (S1, S2, S3, S5) or activating pro-apoptotic components (S4).

CD3xCD123 AML Samples Dose Response Curves

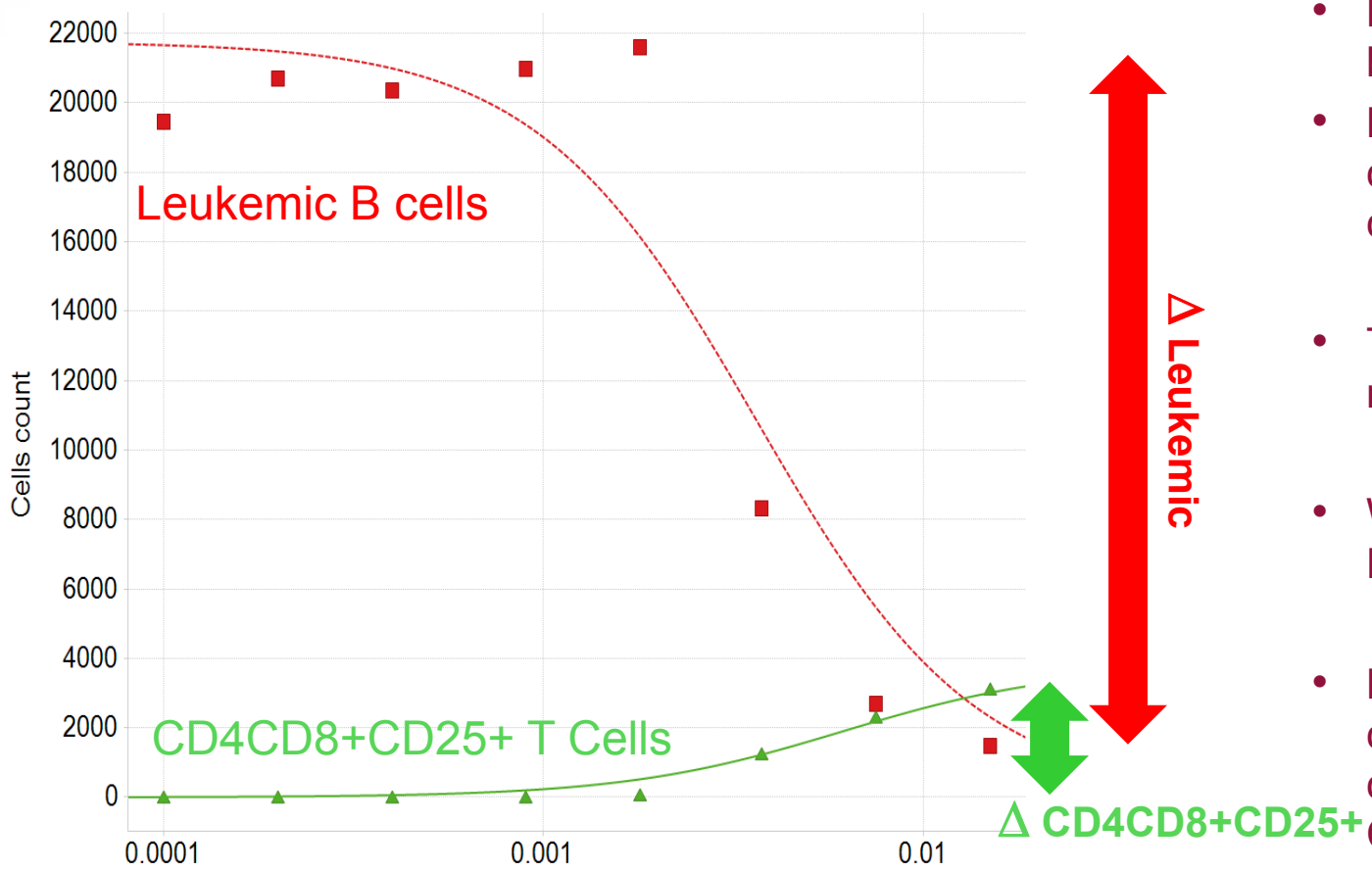




Different samples
Show different EC50s & Emax
For Tumor cell depletion

EC50s Tumor depletion are the same for T Cell activation

New Measurement of Activity for Bispecific Antibodies Effective E:T Ratios for Blinatumumab on CLL



	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

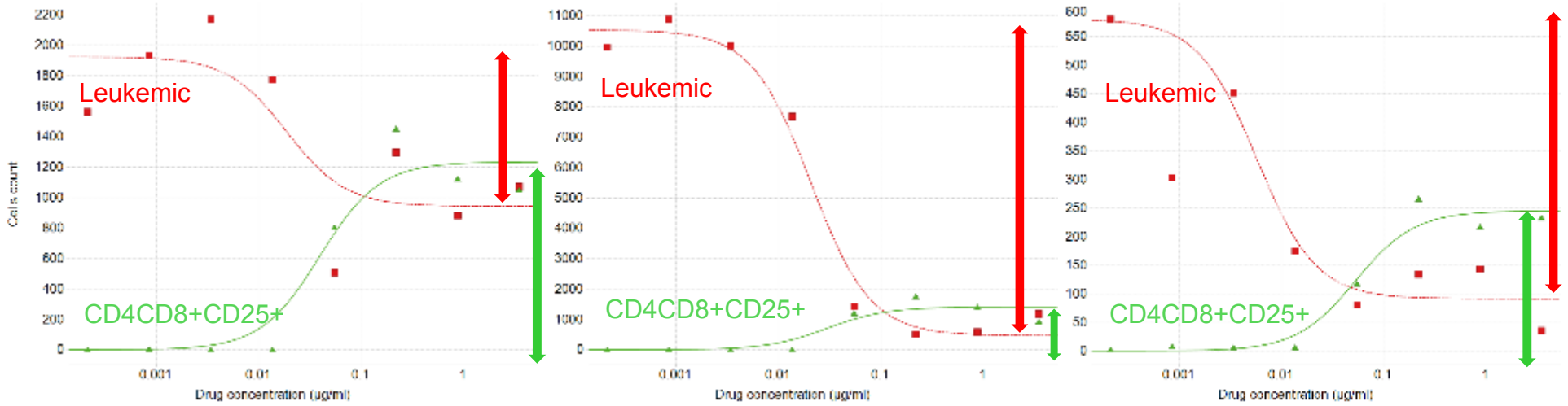
  Δ CD4CD8+CD25+ : Δ Leukemic
- Measures how many cancer cells are killed by each cytotoxic T Cell, i.e. the T Cell cancer-killing activity
- Effective E:T Ratios are different than Basal E:T ratios and may represent a better measurement of bispecific antibody activity

New Measurement of Activity for Bispecific Antibodies Effective E:T Ratios for CD3xCD123 on AML

Sample 1
Effective E:T Ratio = 1:0.8

Sample 2
Effective E:T Ratio = 1:7

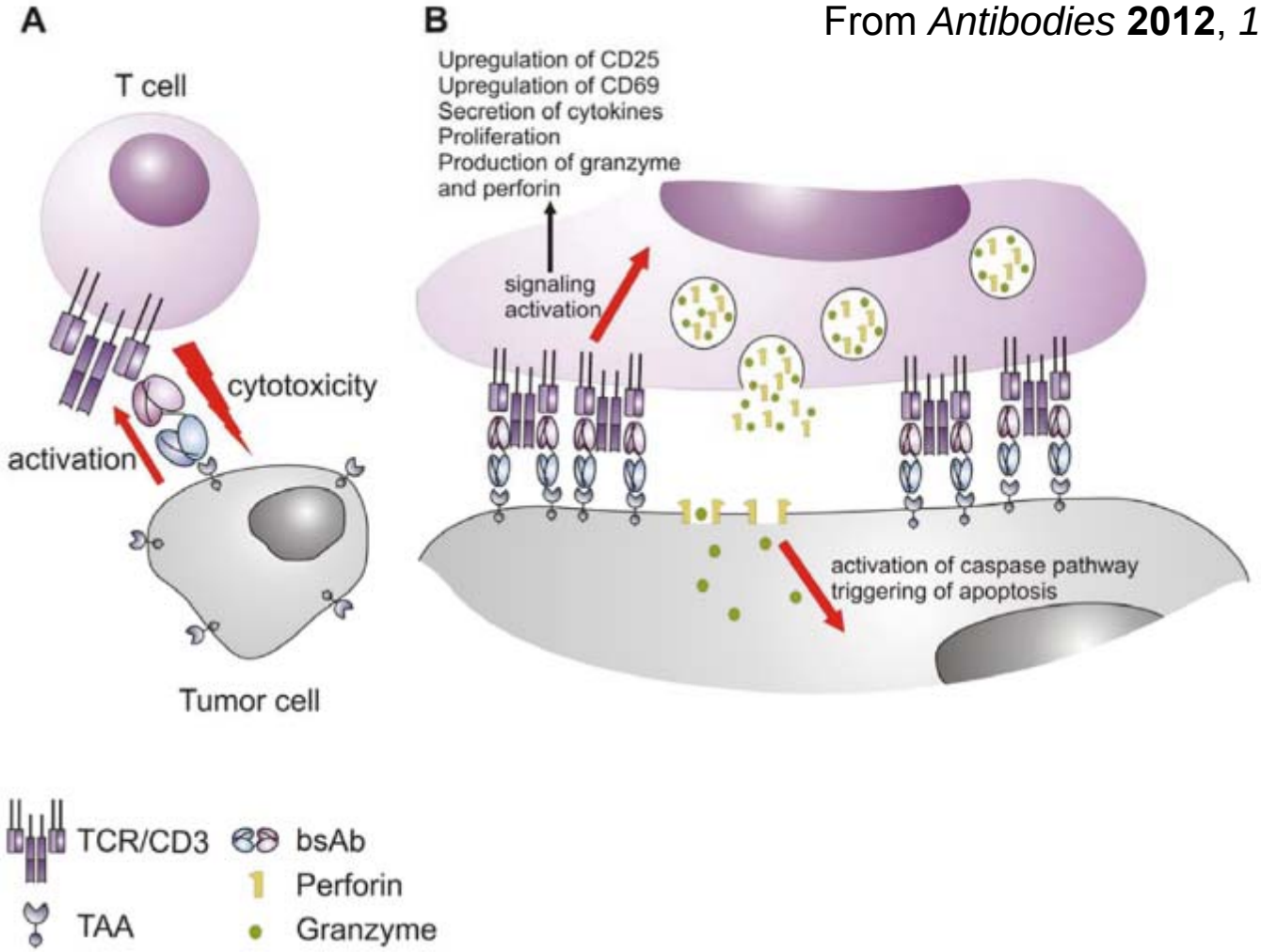
Sample 3
Effective E:T Ratio = 1:2



- Different samples have different Effective E:T Ratios (**Δ CD4CD8+CD25+ : Δ Leukemic**)
- Measures how many cancer cells are killed by each cytotoxic T cell (0.8, 7, and 2 above)
- Effective E:T Ratios are different than Basal E:T ratios and may represent a better measurement of clinical efficacy
- Effective E:T Ratios require only 2 points in triplicate and can be measured at each hospital flow cytometry facility and qualifies as PD marker for clinical trials

MOA: BsAbs Promote Direct Tumor Lysis by Proximity

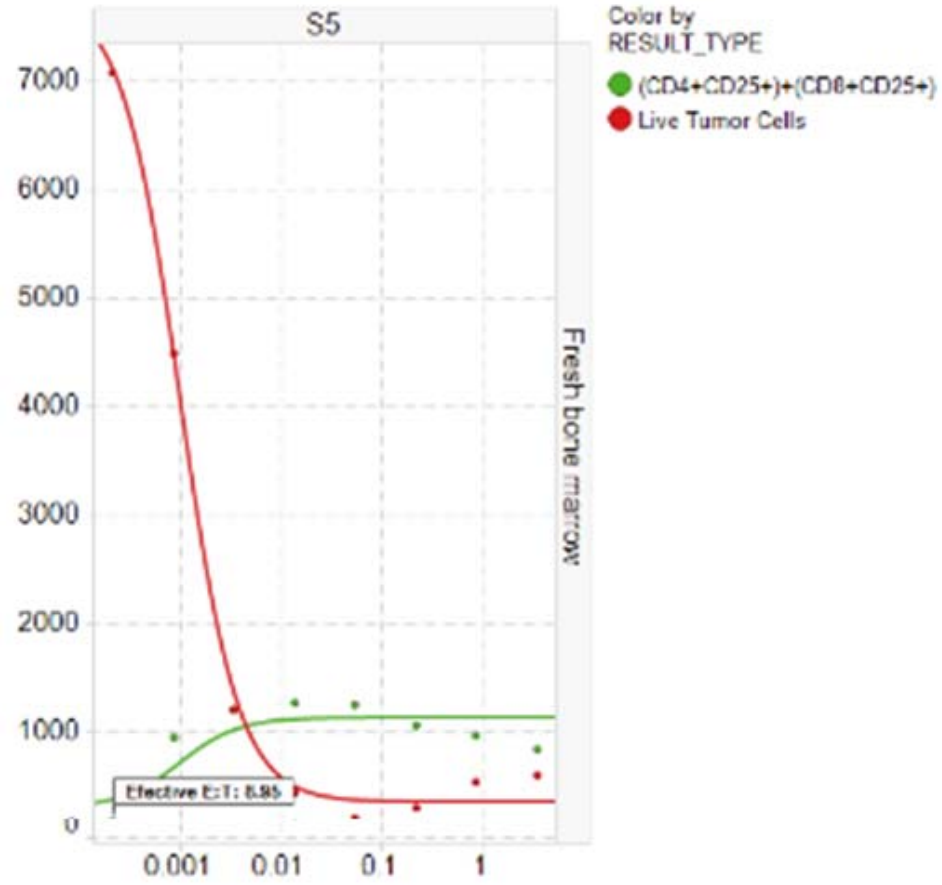
From *Antibodies* **2012**, *1*(2), 172-198



Hypothesis: BsAb-efficacy is not dependent on the antigen specificity of bound T-cells - it essentially confers Tumor Associated Antigen-specificity to the entire contacted T-cell populous

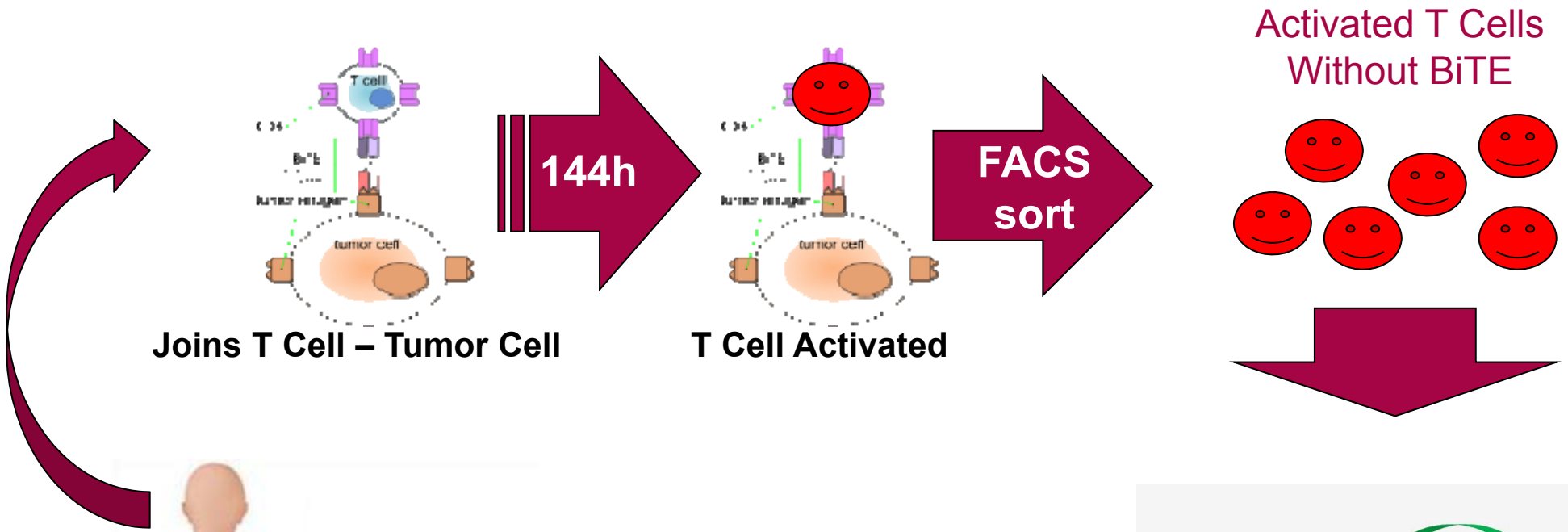
Are BiTEs the "missing link" in cancer therapy?, OncoImmunology, 4:6, 2015

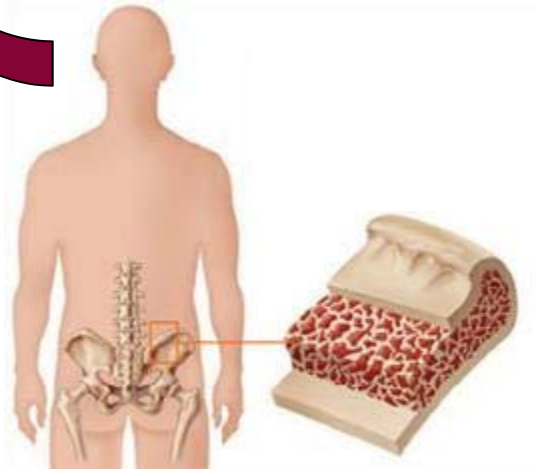
Samples with low % CD123+ in Tumor Cells show strong depletion blasts & high Effective E:T Ratio



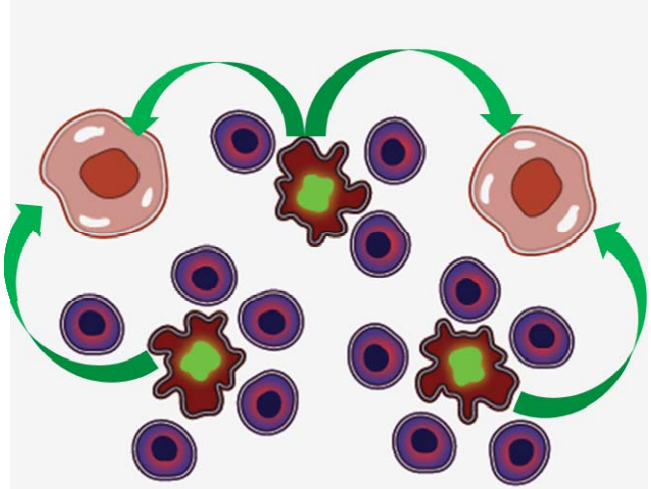
Activated cytotoxic T Cell kills blasts through a CD123 independent MOA

Activated Cytotoxic T Cell Kills Blasts Through a CD123 Independent MOA





2nd Sample



Measuring Dose Responses of Sorted Activated T Cells Without Bispecific Antibody

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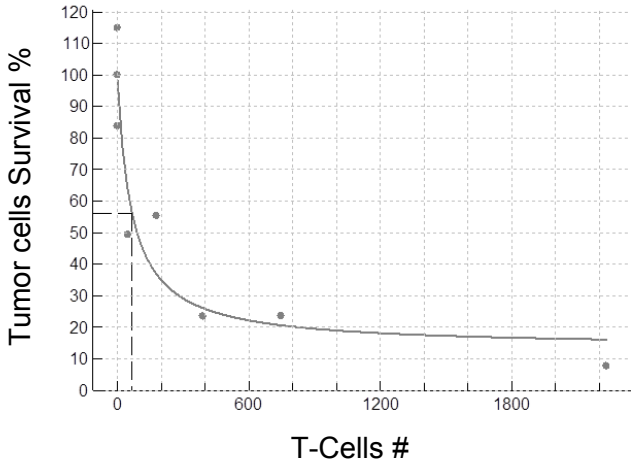
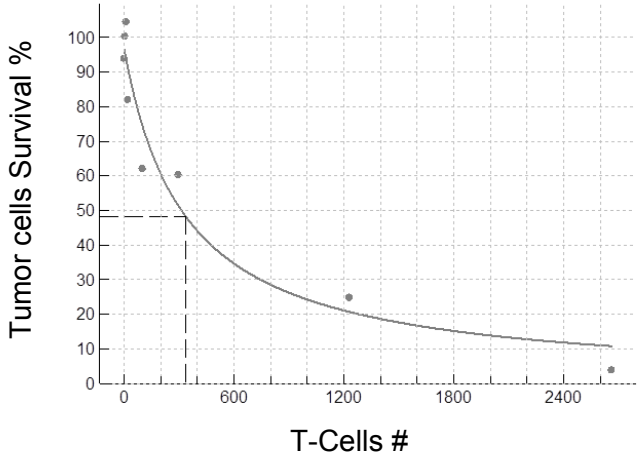
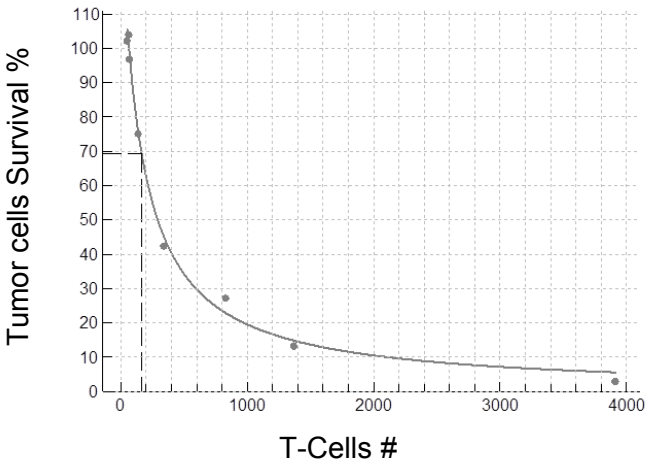
CD8+CD25+



CD4+CD25+



(CD8+CD25+) + (CD4+CD25+) 1:1



Tumor cells survival % estimated regarding plate no-drug control. Intercept dashed line correspond to IC50 value

	IC50 (T-Cells#)	E0 (Survival %)	Emax (% Survival)	AUC(0-1000)
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

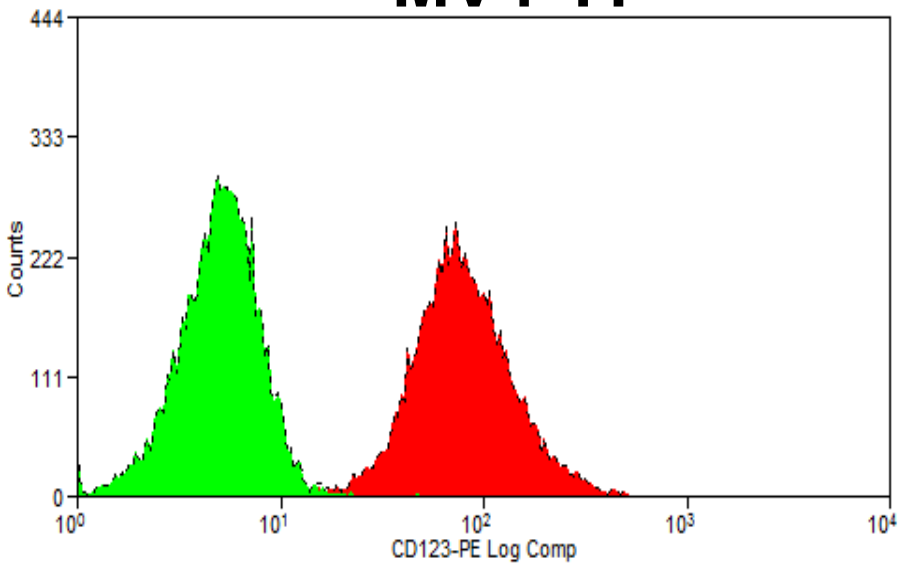
Both CD8 & CD4 activated T Cells kill tumor cells
CD8+ 5x more potent than CD4+
Effective E:T Ratios with CD4 & CD8 activated T Cells

Can FACS Sorted CD25+ T Cells Kill AML Cells Lines w/o CD123 Expression?

CONTROL

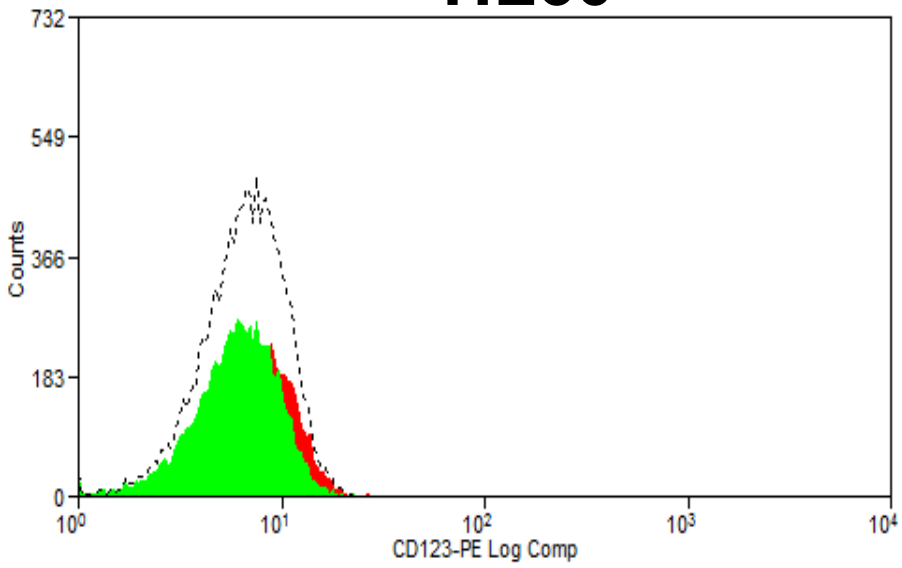
CD123

MV4-11



Positive

HL60



Negative

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

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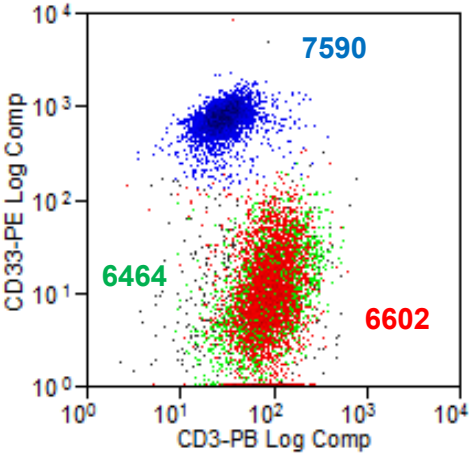
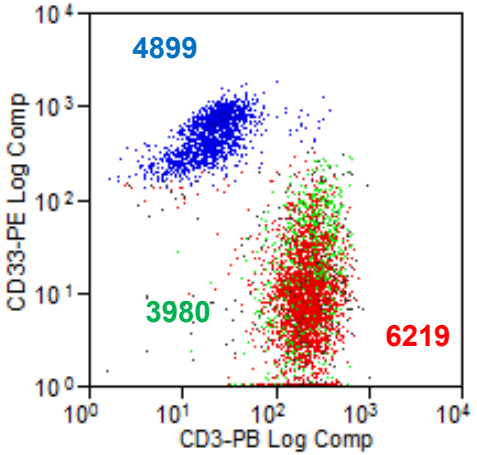
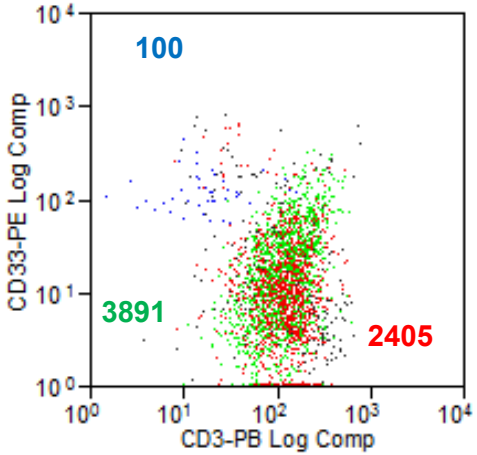
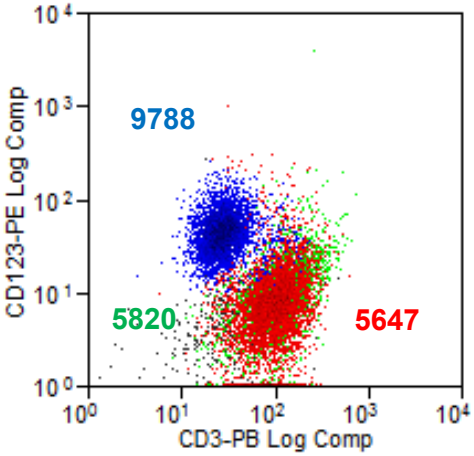
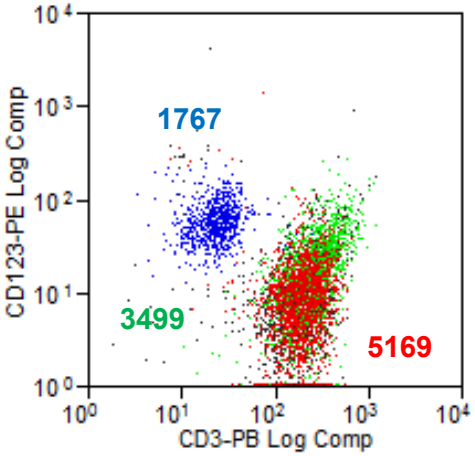
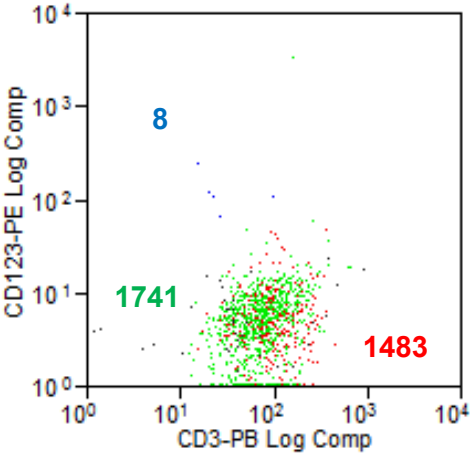
Cell Line
CD4
CD8

	Basal	4h	24h
HL60 No CD123			
MV4-11 Yes CD123			

FACS sorted activated cells kill in a CD123 independent MOA

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

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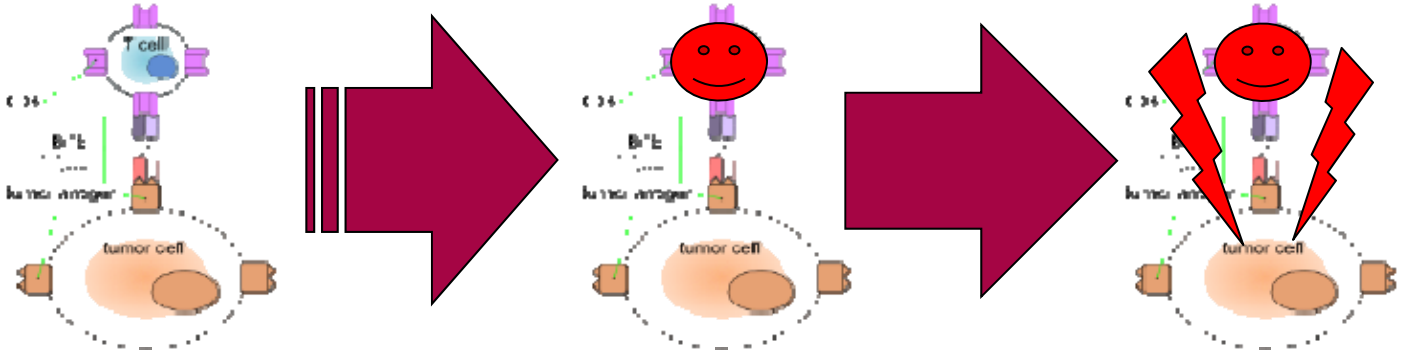
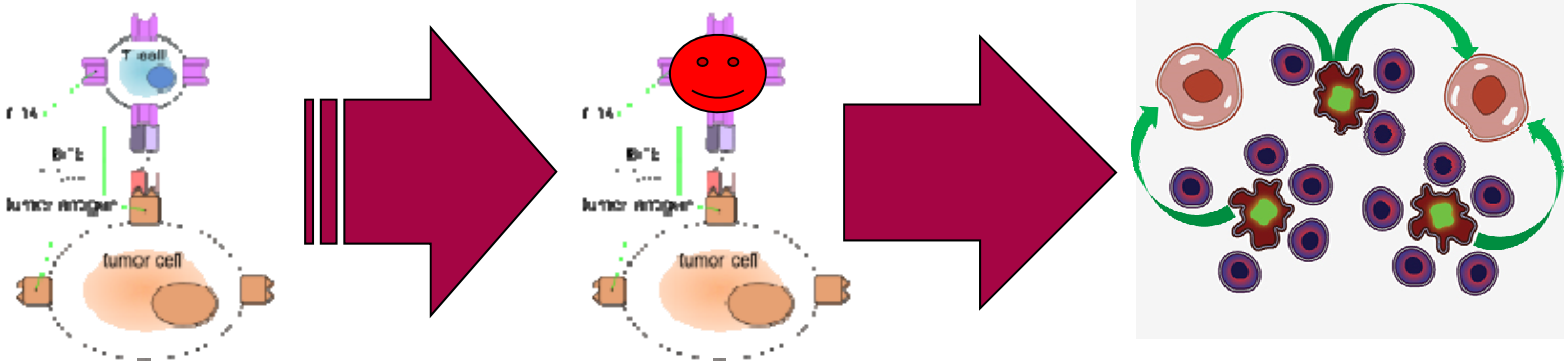
Standard MOA



Joins T Cell – Tumor Cell | **T Cell Activated** | **T Cell Lyse Tumor Cell**

New MOA



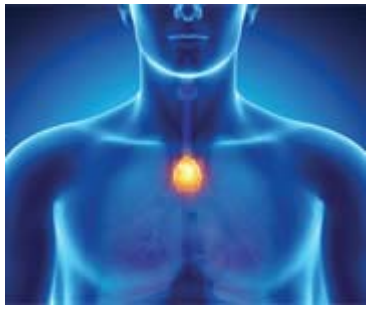
Joins T Cell – Tumor Cell | **T Cell Activated** | **Tumor-Specific-Antigen T Cell Lyse Tumor Cells (CD123 independent)**

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

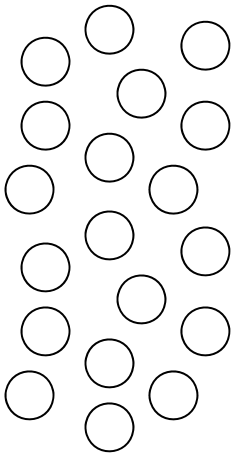
TILs in Solid Tumors:

TSA T Cells Immunosuppressed by Tumor Microenvironment

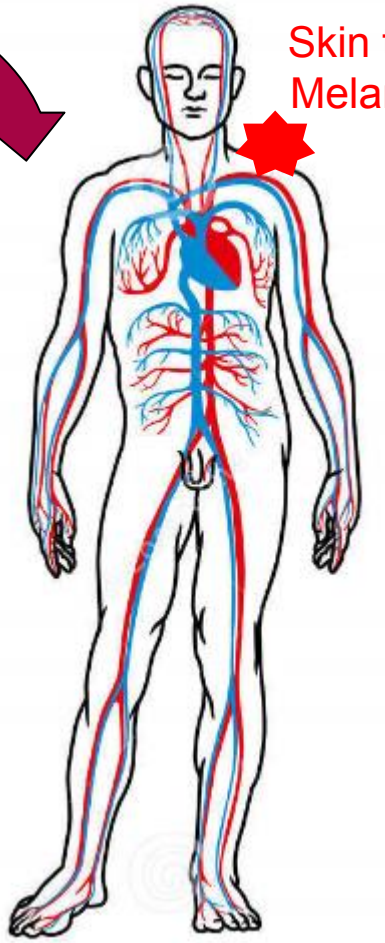
T cells travel throughout body
Searching for tumor cells



Thymus generates millions of T cells each recognize 1 foreign antigen

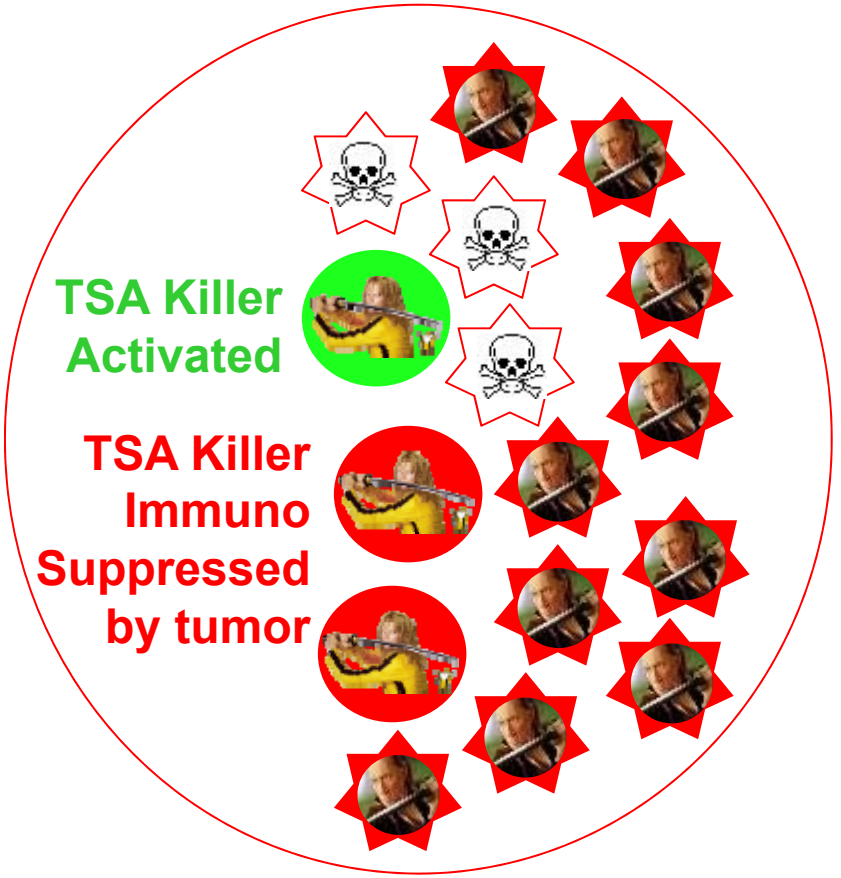


Few T cells recognize Selectively Tumor cells (e.g. neo-antigens) Tumor-Specific-Antigen (TSA) T Cells



Skin tumor
Melanoma

TUMOR



Tumor Infiltrated Lymphocytes (TILs)
Contain TSA T Cells but most are immunosuppressed by tumor microenvironment

How Can BM Samples With Low % T Cells Have TSA TILS?

- **Solid tumor Immuno-Oncology TILs**
  - TSA T Cells generated in Thymus travel to tumor site
  - Tumor microenvironment immunosuppresses TSA → TILs
  - Higher % TILs prognostic factor for immune therapy response
- **Hematological Malignancies → same in BM**
  - BM samples enriched in TILs
    - **Not appreciated before because is normal to have T cells in BM**
  - TSA T Cells present in 50-1.000 T-Cells/well
    - **vs 7.000-22.000 tumor cells in same well**
  - Higher % TILs → High Effective E:T Ratio → better patient response to bispecific therapy?

If Bone Marrow T Cells Are Enriched in TSA Immunosuppressed TILs
BM vs Peripheral Blood Activated T Cells should behave differently

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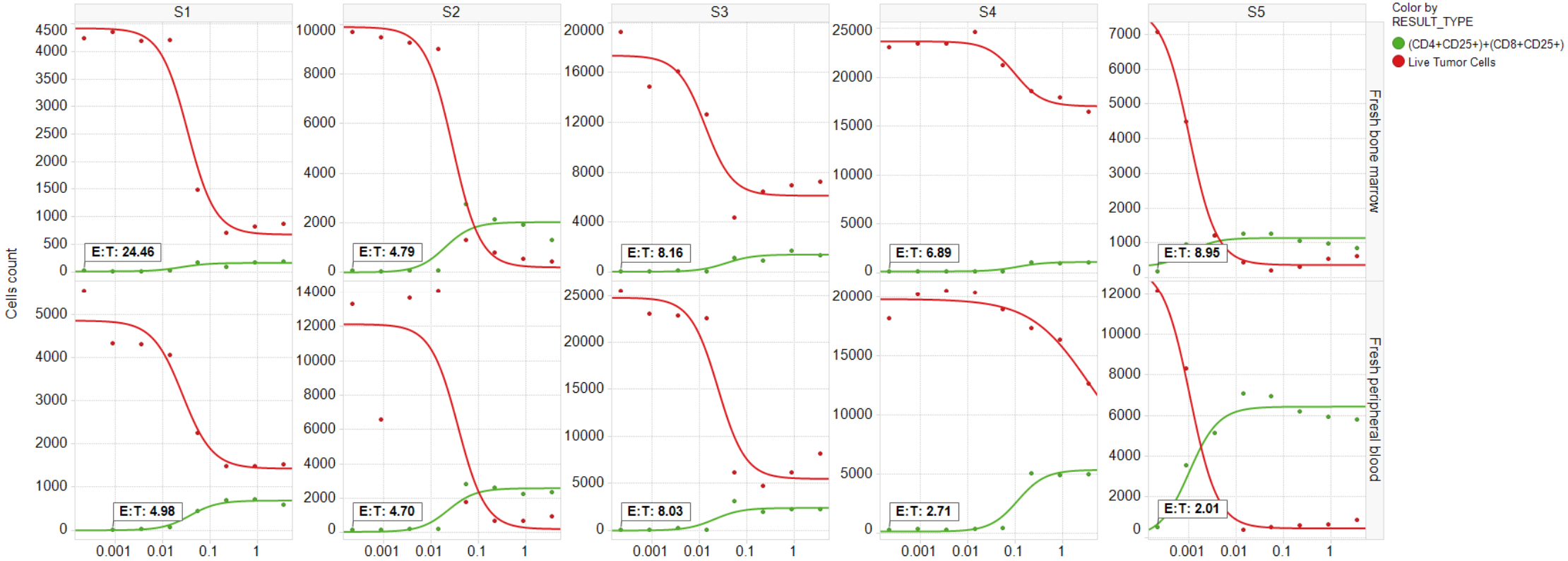
BM vs PB samples same AML patient incubated 72h with BsAb CD3xCD123

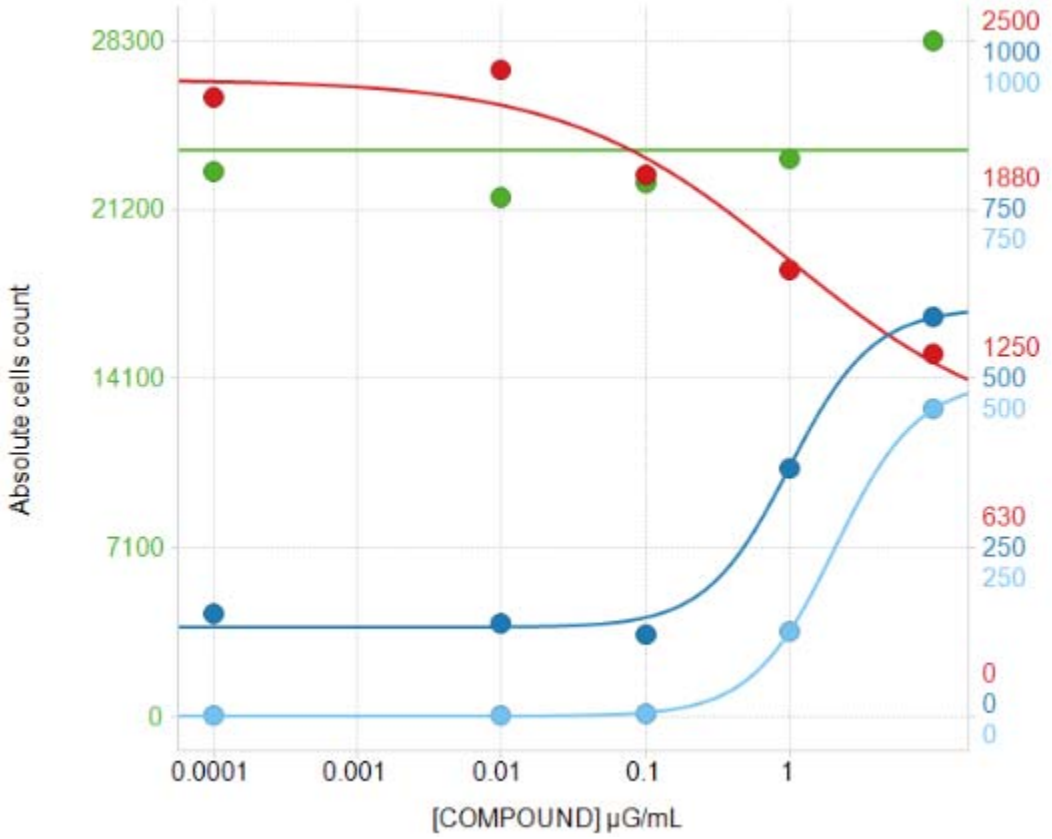


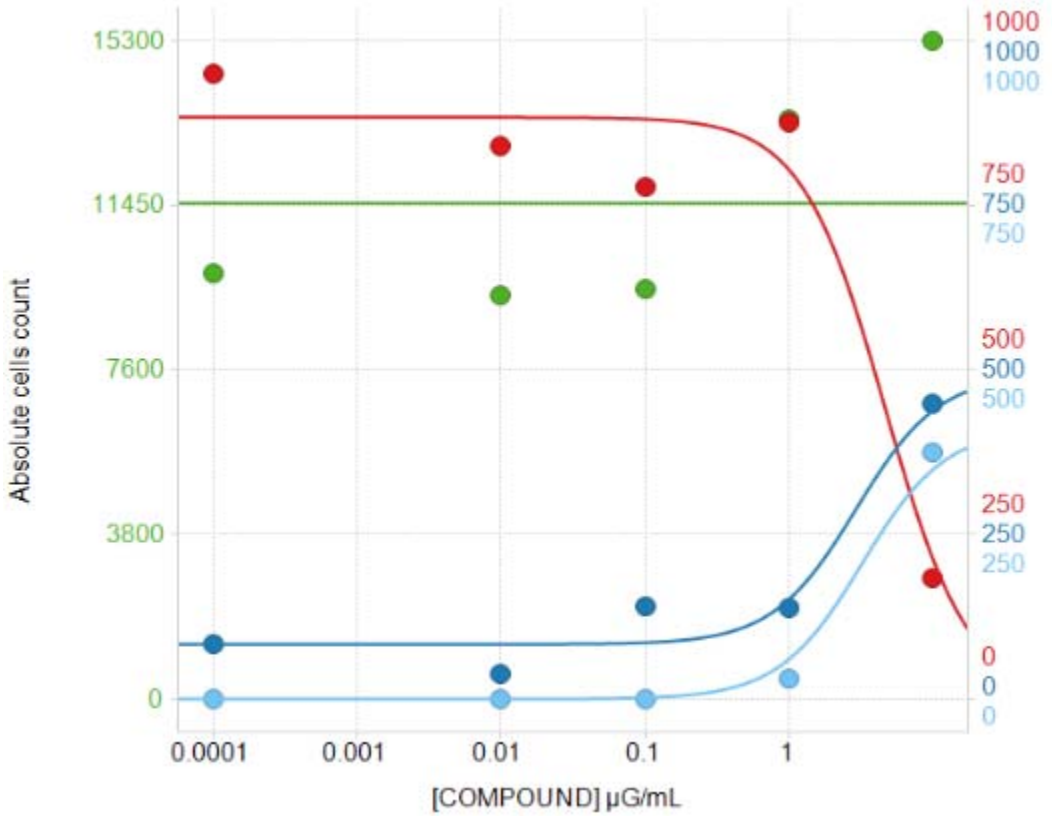
BM 25	BM 5	BM 8	BM 7	BM 9
PB 5	PB 5	PB 8	PB 3	PB 2

BM T cells are better killers in 3/5 samples
consistent with BM immunosuppressed TILs in 3/5 samples

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

2 Multiple Myeloma samples, T Cells induced by Bispecific Ab

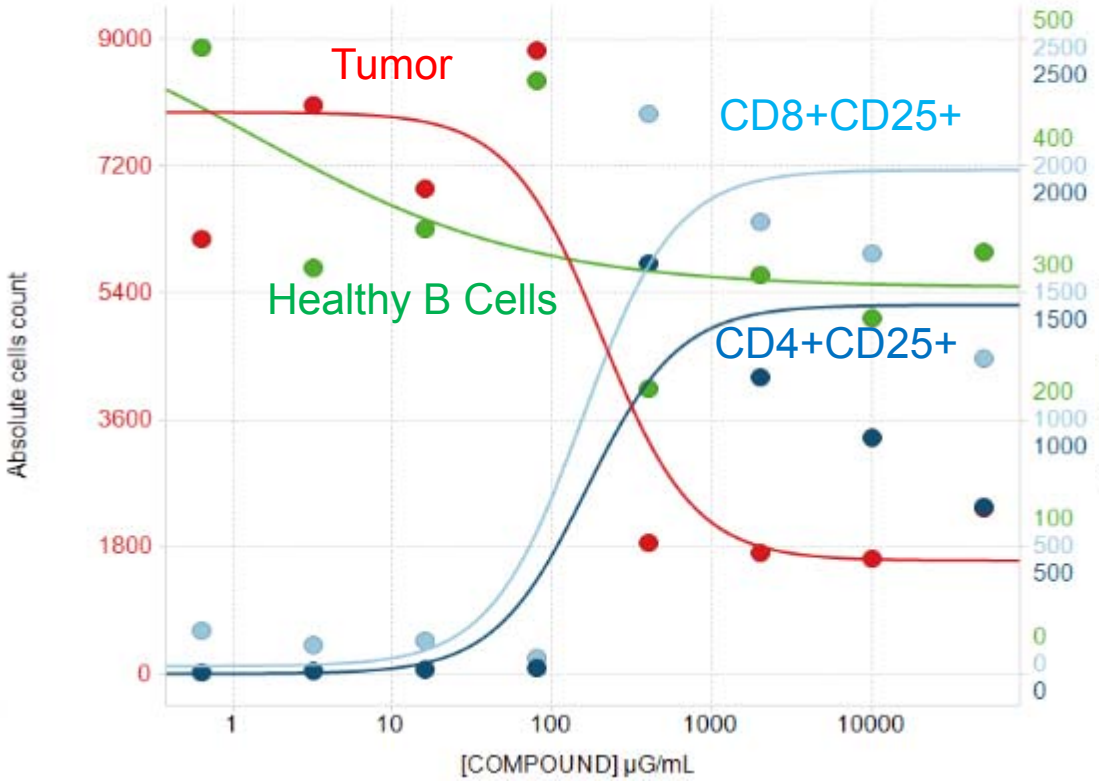


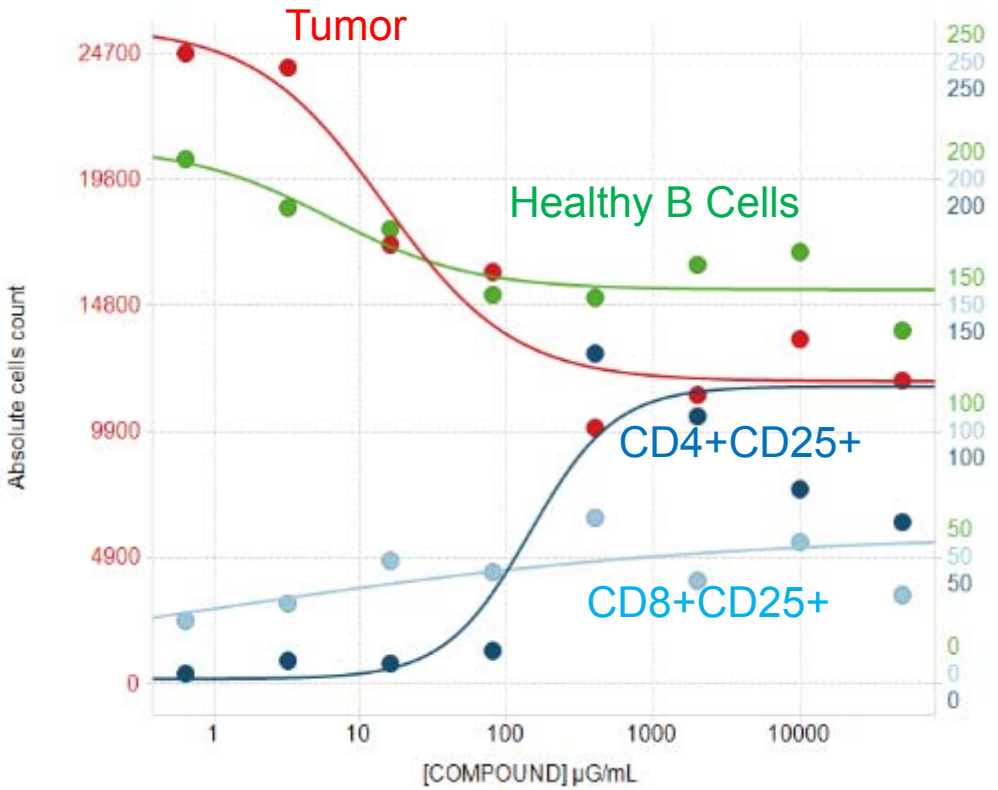


Activated proliferating T Cells kill tumor cells
but not healthy granulocytes within the same bone marrow sample

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

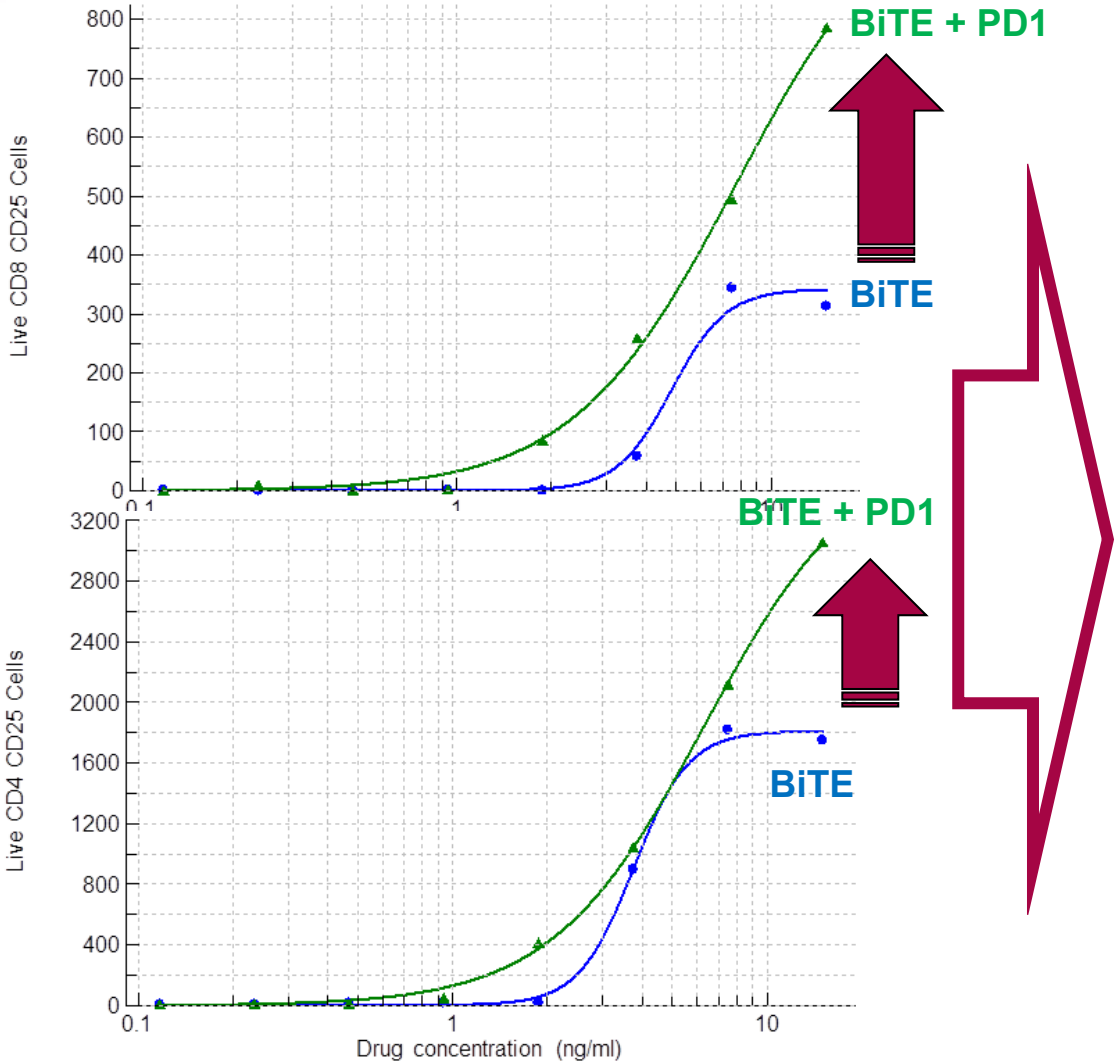
2 AML BM samples, T Cells induced by Bispecific Ab CD3xCD123

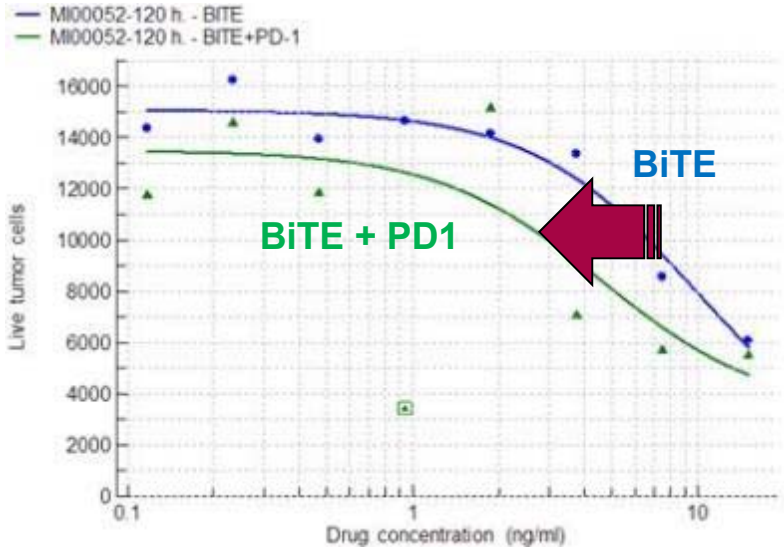




Activated proliferating T Cells kill tumor cells
but not healthy B Cells within the same bone marrow sample

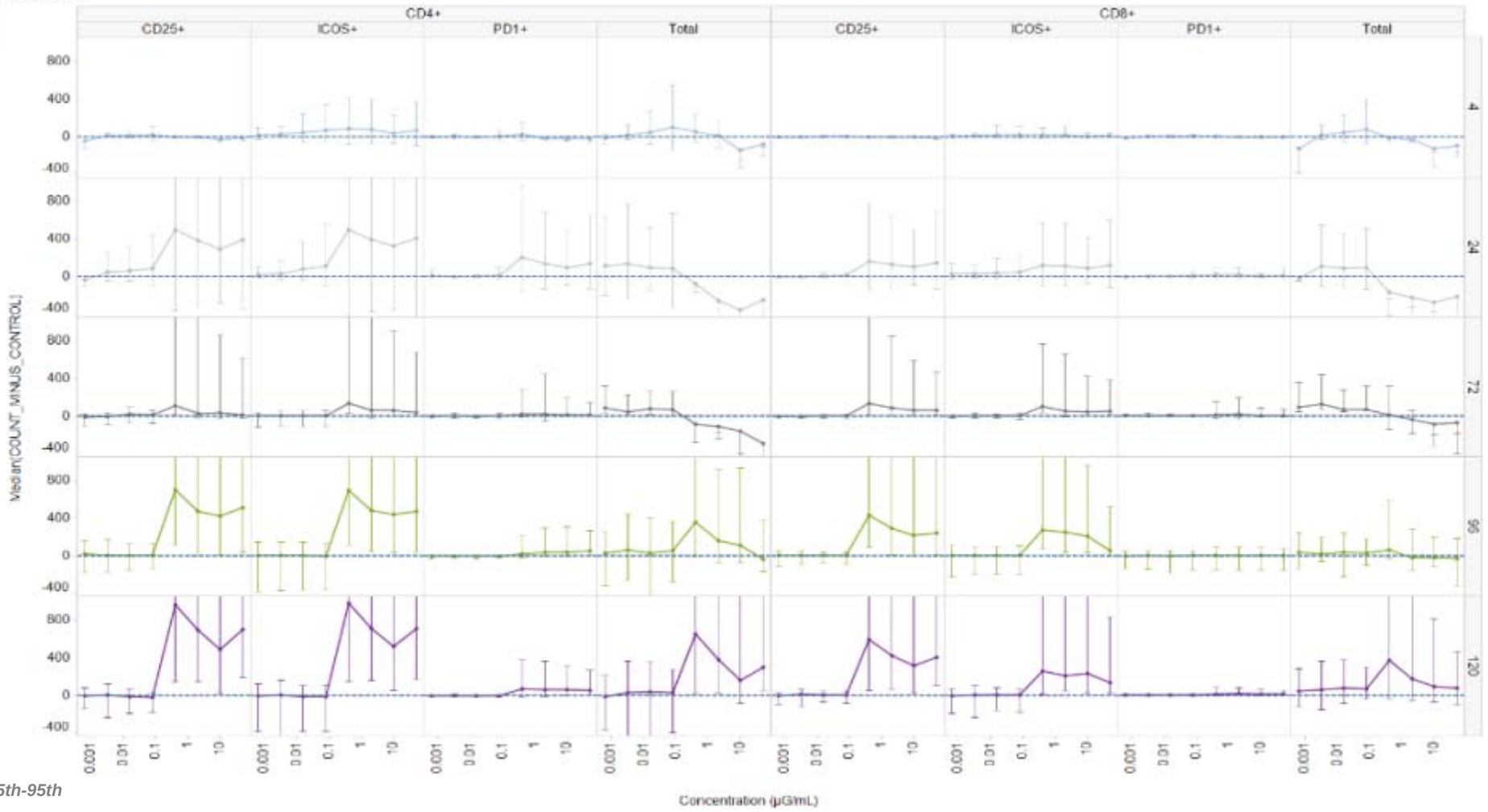
If Activated T Cells are TSA TILs
They Should Respond To PD1 (Nivolumab)





PD1 Increases activated CD4 & CD8 T Cells, enhancing tumor killing
Effect of PD1 more pronounced on more immunosuppressed samples with low Emax (CLL > ALL, AML, MM)

TSA T Cells Killers are ICOS+ (JP Allison, AACR 2016)
T Cell Immunophenotyping 5 AML Samples CD3xCD123



Error bars: Pct 5th-95th

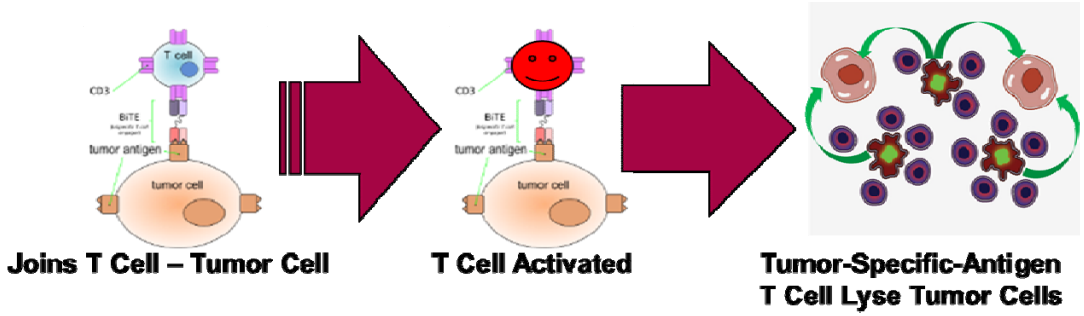
- **ICOS+ similar to CD25+ supporting these activated T cells are TSA**
- **PD1 expression weak at 5 days, higher at 6-7 days incubation**
- **Total T Cell counts decrease 48-72 h, non-effector memory T cells die**

New MOA Desirable for Bispecifics Discovery & Development

- **New MOA activating patient's TSA-T Cell is very desirable for bispecific candidates**
  - Higher efficacy T Cells (Effective E:T Ratios)
  - Higher safety, TSA T Cell kill selectively cancer cells
  - Occurs in addition to standard proximity MOA
- **Bispecific Development & Clinical**
  - Assay enables patient selection clinical trials (CDx)
  - Assay identifies combinations to maximize new MOA (PD1 etc…)
- **Bispecific Discovery**
  - Assay can be applied from hits to leads (we can screen 400 to 20.000 wells/sample)
  - Iterations of screens of 10 samples, discarding bad hits keeping best hits, so that best hits become validated in 50-100 samples

New MOA Suggest novel design of BsAbs
Alternative surface targets on T Cells & Tumor cells

New MOA



CD3xCDTumor

PD1xPDL1

CD3xCDTumor
PD1xPDL1

- **Bispecifics of Immune check point inhibitors & ligands also join T cells & tumor cells → same MOA**
  - Ideal for immuno-resistant tumor cells
- **Tetraspecifics may combine both designs**
  - E.g. CD3xCDTumor & PD1xPDL1
- **Vivia can screen 100s constructs/sample**
- **Enable partner with expertise BsAb designs**
- **Together High Throughput discovery of new wave of muti-specific Ab I-O candidates**

100s
1000s

Best I-O

Bispecific Ab Assay Cancer Indications

- **Established in Hematological Malignancies**
  - AML
  - MDS
  - MM
  - ALL
  - CLL
  - Available for Cryopreserved biobank samples of all above except CLL
- **Expanding to solid tumors**
  - Lung cancer pleural liquid
  - Ovarian cancer ascetic fluid

Conclusions

- Novel proprietary ex vivo assay for Bispecific Antibodies (BsAb) with novel measurement of activity “Effective E:T Ratios”
- Assay enable searching best of multiple combinations Bispecifics & SOC
- BsAbs generate CTLs that kill tumor independent of BsAb & target. These CTL may be Tumor-Specific-Antigen CTLs immunosuppressed in bone marrow, same as TILs in solid tumors. This should be very beneficial for patients and may be a criteria for bispecific optimization and patient selection
- Clinical trials should not exclude patient for low expression of targetxCD3
- New design of multi-specific antibodies from our new MOA empowered by our screening of 100’s constructs ex vivo
- Offered on a collaborative or service basis
- Novel Immuno-Oncology assay: Activated T Cells ex vivo by bispecific T cell engagers are tumor associated and thus better than CD3+CD28 non-selectively activated T Cells as representative of the immuno-oncology response. These bispecifics can be used as reagents for a novel I-O assay to explore new immunotherapies.

vivia BIOTECH

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