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

Confidential Document
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

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

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.

- **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.

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>T-Cells</th>
<th>CD19+</th>
<th>Basal E:T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells # at baseline</td>
<td>1101</td>
<td>33166</td>
<td>1:30.1</td>
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<table>
<thead>
<tr>
<th></th>
<th>T-Cells</th>
<th>Live Tumor Cells</th>
<th>Effective E:T Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell # max dose</td>
<td>3799</td>
<td>21761</td>
<td>1:5.7</td>
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</table>
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 ($\Delta$ CD4CD8+CD25+ : $\Delta$ 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

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

Joins T Cell – Tumor Cell

T Cell Activated

144h

FACS sort

Activated T Cells Without BiTE

2nd Sample
Measuring Dose Responses of Sorted Activated T Cells Without Bispecific Antibody

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.

<table>
<thead>
<tr>
<th></th>
<th>IC50 (T-Cells#)</th>
<th>E0 (Survival %)</th>
<th>Emax (% Survival)</th>
<th>AUC(0-1000)</th>
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</thead>
<tbody>
<tr>
<td>CD8+CD25+</td>
<td>67</td>
<td>84.8</td>
<td>13.7</td>
<td>29392.1</td>
</tr>
<tr>
<td>CD4+CD25+</td>
<td>336</td>
<td>96.5</td>
<td>0.0</td>
<td>44769.0</td>
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<tr>
<td>(CD4+CD25+) &amp; (CD8+CD25+) [1:1]</td>
<td>164</td>
<td>138.4</td>
<td>0.0</td>
<td>44499.9</td>
</tr>
</tbody>
</table>

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

**Cell Line**
- 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

**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

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

T cells travel throughout body Searching for tumor cells

Skin tumor Melanoma

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

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

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

- 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

- 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
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.
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