Improved T Cell Activation Bioassays for Development of Bispecific Antibodies and Engineered T Cell Immunotherapies

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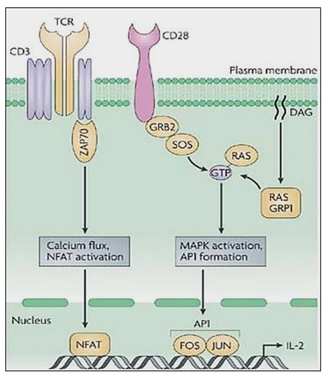
1. Introduction

Immunotherapy aims to boost a patient's own immune system to fight disease. In recent years, a variety of immunotherapy strategies aimed at inducing, strengthening or engineering T cell responses have emerged as promising approaches for the treatment of cancer and autoimmune disease.

Here we describe a platform of T cell activation bioassays for the development of CD3 bispecific antibodies and engineered T cell immunotherapies.

T Cell Activation Pathways

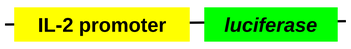


Lineberry-N et al. Nature Reviews Immunology 2007

Specifically, we developed two bioluminescent reporter-based bioassays to measure T cell activation via CD3 (NFAT-RE) or CD3 + CD28 (IL-2 promoter). These bioassays include the following:

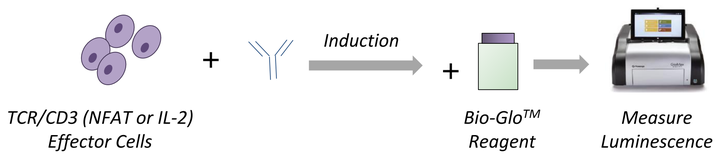
TCR/CD3 (NFAT) effector cells: Jurkat cells engineered with an NFAT-RE driving luciferase expression. Responds to CD3, but not CD28 stimulation.

TCR/CD3 (IL-2) effector cells: Jurkat cells engineered with an IL-2 promoter driving luciferase expression. Responds to CD3 and CD3+CD28 stimulation.



2. Both TCR/CD3 (NFAT) and TCR/CD3 (IL-2) Cells Respond to TCR/CD3 Stimulation

T Cell Activation Protocol

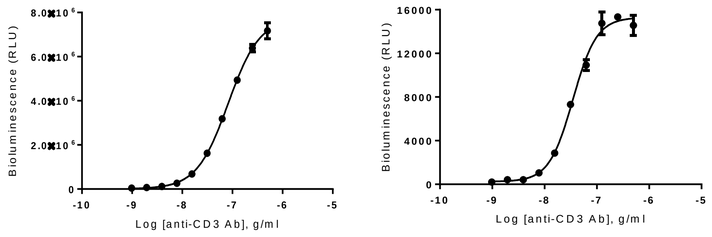


TCR/CD3 (NFAT)

EC50=82ng/ml
Fold Induction=165

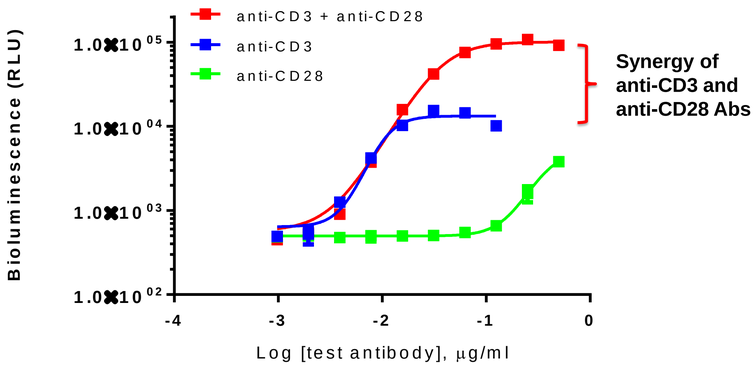
TCR/CD3 (IL-2)

EC50=35ng/ml
Fold Induction=76



TCR/CD3 (NFAT) (**Left**) and TCR/CD3 (IL-2) (**Right**) effector cells were stimulated with increasing concentrations of an anti-CD3 Ab.

3. T Cell CD3+CD28 Co-stimulation Measured using TCR/CD3 (IL-2) Effector Cells



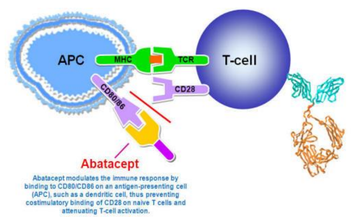
anti-CD3 + anti-CD28 Abs
- EC50=36ng/ml
- Fold Induction=230

anti-CD3 Ab:
- EC50=11ng/ml
- Fold Induction=31

anti-CD28 Ab:
- EC50=>368ng/ml
- Fold Induction=8

TCR/CD3 (IL-2) effector cells were stimulated with increasing concentrations of anti-CD28, anti-CD3 or a combination of anti-CD3+anti-CD28 Abs, as indicated.

4. Abatacept Modulates CD3+CD28 T Cell Activation using TCR/CD3 (IL-2) Cells



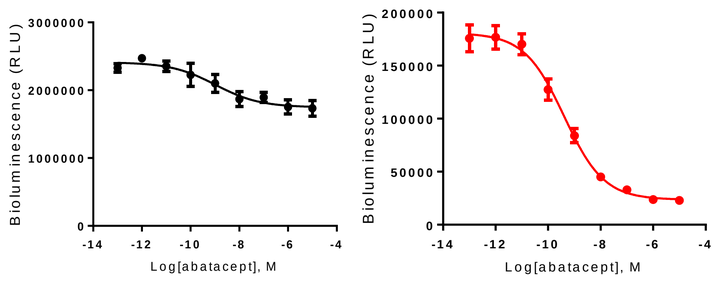
Experimental Design

(1) TCR/CD3 (NFAT or IL-2) effector cells are incubated with Raji (CD80/86$^+$) target cells

(2) T cell activation is induced via crosslinked anti-CD3 Ab and CD28 engagement by its ligand CD80/86 expressed on the Raji target cells

(3) Addition of a CTLA-4/IgG fusion protein (Abatacept) binds CD80/86 and inhibits CD28-mediated T cell activation.

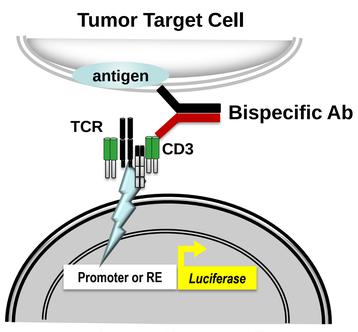
TCR/CD3 (NFAT) **TCR/CD3 (IL-2)**



Increasing concentrations of Abatacept were added to either TCR/CD3 (NFAT) or TCR/CD3 (IL-2) effector cells, as indicated. Abatacept induced a significant decrease in TCR-mediated luciferase activity in TCR/CD3 (IL-2) effector cells compared to TCR/CD3 (NFAT) effector cells. This is expected because CD28 functions independently of the NFAT response element (see Introduction).

5. Measurement of CD3 Bispecific Antibody Activity: Assay Design and Protocol

Assay Design for Measuring CD3 Bispecific Antibody Activity



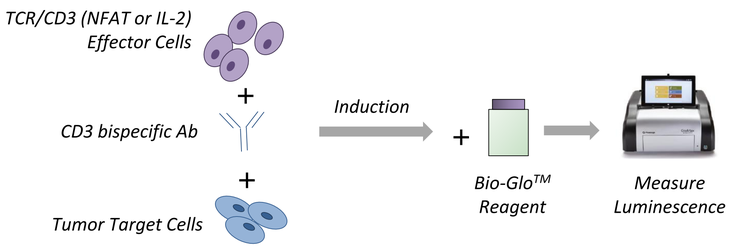
Experimental Design

(1) TCR/CD3 (NFAT or IL-2) effector cells are incubated with increasing concentrations of a CD3 bispecific Ab

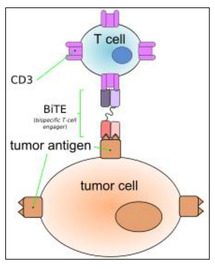
(2) The bispecific Ab simultaneously binds to TCR/CD3 on the effector cells and tumor antigen on the target cells

(3) Bispecific Ab binding stimulates IL-2 or NFAT luciferase activity

Assay Protocol for Measuring CD3 Bispecific Antibody Activity

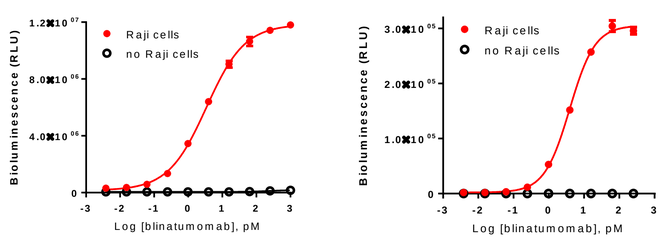


6. Analysis of Blinatumomab CD3 x CD19 Bispecific Antibody Activity



Blinatumomab belongs to a class of constructed monoclonal antibodies, bi-specific T-cell engagers (BiTEs), that exert action selectively and direct the human immune system to act against tumor cells. Blinatumomab specifically targets the CD19 antigen present on B cells.

TCR/CD3 (NFAT) **TCR/CD3 (IL-2)**



Increasing concentrations of Blinatumomab were added to either TCR/CD3 (IL-2) or TCR/CD3 (NFAT) effector cells, as indicated. Blinatumomab induced a dose-dependent increase in luciferase in both TCR/CD3 (IL-2) and TCR/CD3 (NFAT) effector cells in the presence of Raji (CD19+) target cells. No response was detected in the absence of Raji (CD19+) target cells.

7. Assay Qualification with Blinatumomab: Assay Precision, Accuracy and Linearity

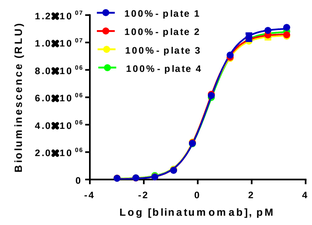
Accuracy and Intermediate Precision (N = 6)

Assay Qualification Design:
Two analysts
Three days
Four plates per day
- 100% vs 50%
- 100% vs 75%
- 100% vs 150%
- 100% vs 200%

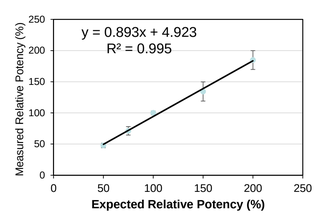
Data shown are generated using TCR/CD3 (NFAT) effector cells, binatumomab, and Raji (CD19+) target cells.

Expected Relative Potency	Assay	Analyst	Measured Relative Potency %	Mean %	SD%	Accuracy Recovery %	Precision RSD %
50%	1	1	45.3	47.8	3.6	95.7	7.6
	2	1	45.0				
	3	1	45.5				
	4	2	46.2				
	5	2	52.2				
	6	2	52.8				
75%	1	1	62.2	71.2	6.8	95.0	9.5
	2	1	73.9				
	3	1	63.3				
	4	2	78.4				
	5	2	75.9				
	6	2	73.6				
150%	1	1	144.5	134.4	15.5	89.6	11.5
	2	1	143.5				
	3	1	121.4				
	4	2	108.8				
	5	2	143.4				
	6	2	144.7				
200%	1	1	174.9	184.8	15.0	92.4	8.1
	2	1	195.5				
	3	1	179.8				
	4	2	162.0				
	5	2	198.9				
	6	2	198.0				
				Overall		93.2	9.2

Repeatability (%CV) = 3.01%

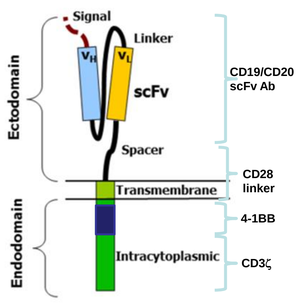


Linearity and Range (R^2=0.995)

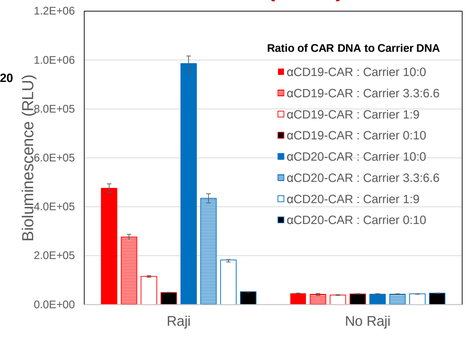


8. Measurement of Chimeric Antigen Receptor T (CAR-T) Cell Activity

CAR-T Design



TCR/CD3 (NFAT)

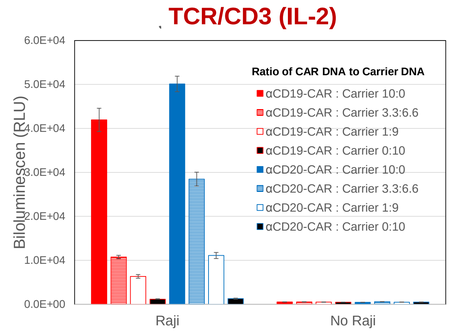


Experimental Design

(1) TCR/CD3 (NFAT or IL-2) effector cells were engineered to express anti-CD19/CD20 CAR-T receptors

(2) The effector cells were incubated in presence or absence or Raji (CD19/CD20+) target cells

TCR/CD3 (IL-2)



Serial dilutions of anti-CD19/CD20 CAR-T effector cells were incubated in the presence and absence of Raji (CD19/CD20+) target cells. Luciferase activity was detected in the presence of Raji cells, but not with the CAR-T effector cells alone.

9. Conclusions

We have developed a platform of T cell activation bioassays that incorporate a bioluminescent reporter-based readout of T cell activation via CD3 (NFAT-RE) or CD3 + CD28 (IL-2 promoter). These assays reflect the mechanisms of action of biologics designed to engage, recruit, and stimulate T cell activation to attack target disease cells. Specific applications include measurement of **anti-CD3 bispecific Ab** and **CAR-T cell** activity.

The bioassays provide the following:

Mechanism of action (MOA)-based measure of biologics activity

- Specific measure of CD3 or CD3 + CD28 T cell activation pathways
- Quantitative measure of anti-CD3 Ab and bispecific Ab potency

Consistent and reliable measure of biologics activity

- Demonstrated precision, accuracy, reproducibility, robustness
- All assays can be used as "Thaw-and-use" cell format, no cell culture required
- Functional performance suitable for development into potency, stability, and NAb assays (data not shown)

Easy-to-implement

- Rapid and convenient workflow
- Amenable to standard 96-well and 384-well plate formats

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