

Assay development and concept verification of a trispecific antibody

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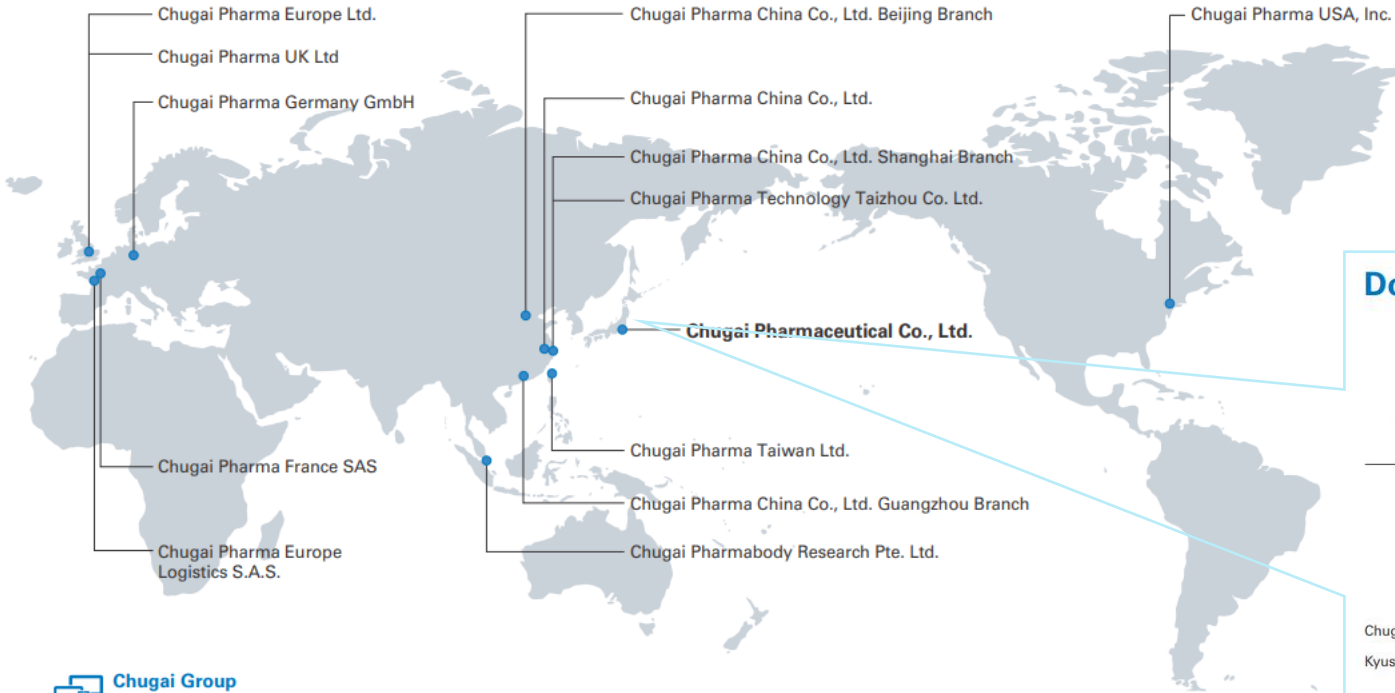
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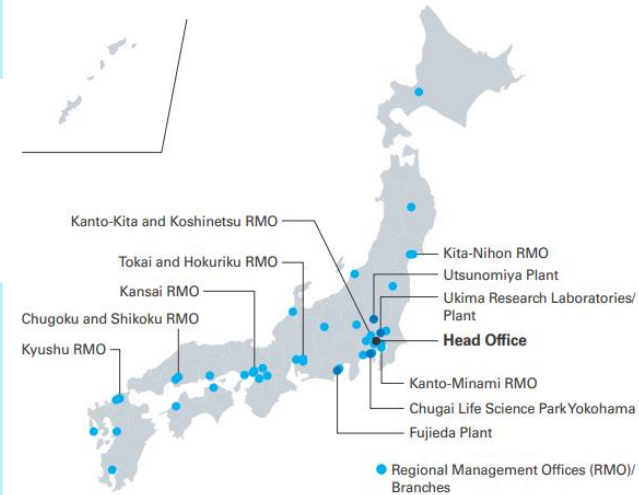
Chugai Group

Overseas Network (As of April 1, 2023)



 Chugai Group
<https://www.chugai-pharm.co.jp/english/profile/group/>

Domestic Network (As of April 1, 2023)



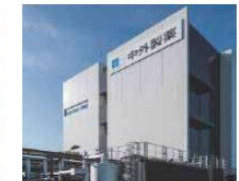
Head Office



Ukima Research Laboratories/ Plant



Utsunomiya Plant



Fujieda Plant

Chugai's New Research Center: Chugai Life Science Park Yokohama

By consolidating the functions of existing domestic research centers Fuji Gotemba and Kamakura Research Laboratories in one location, Chugai has built the foundation to maximize its drug discovery capabilities. In addition to the drug discovery research function, Chugai Life Science Park Yokohama also includes a facility that will mainly focus on the formulation of mid-size molecule compounds.



Chugai Life Science Park Yokohama

Agenda

01

Introduction of a trispecific Antibody

02

Phase appropriate approach

03

Method comparability study

04

Verification of specificity for three antigens in a single test system

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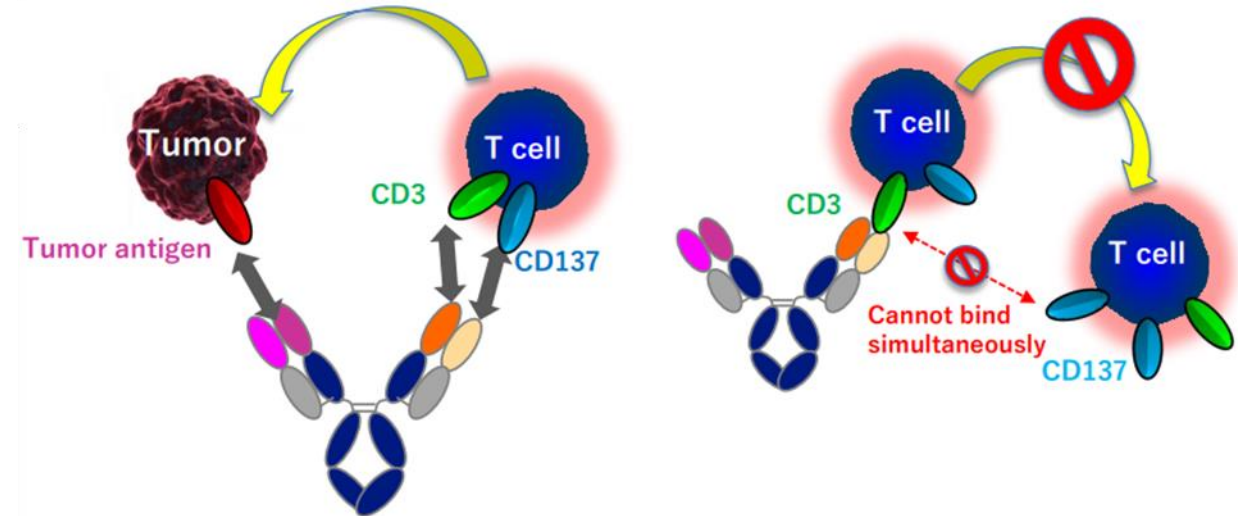
Antibody Engineering technology: Dual-Ig[®] (Dual effector/receptor redirecting-Immunoglobulin)

■ Characteristics of Dual-Ig[®]

- Dual-Ig[®] binds to CD3 and CD137 with T cell binding Fab. It is designed to avoid binding to CD3 and CD137 simultaneously.
- This would result in CD3-mediated activation and CD137-mediated co-stimulation of T cell only in the presence of tumor antigen.

■ Effect of CD137 signal*

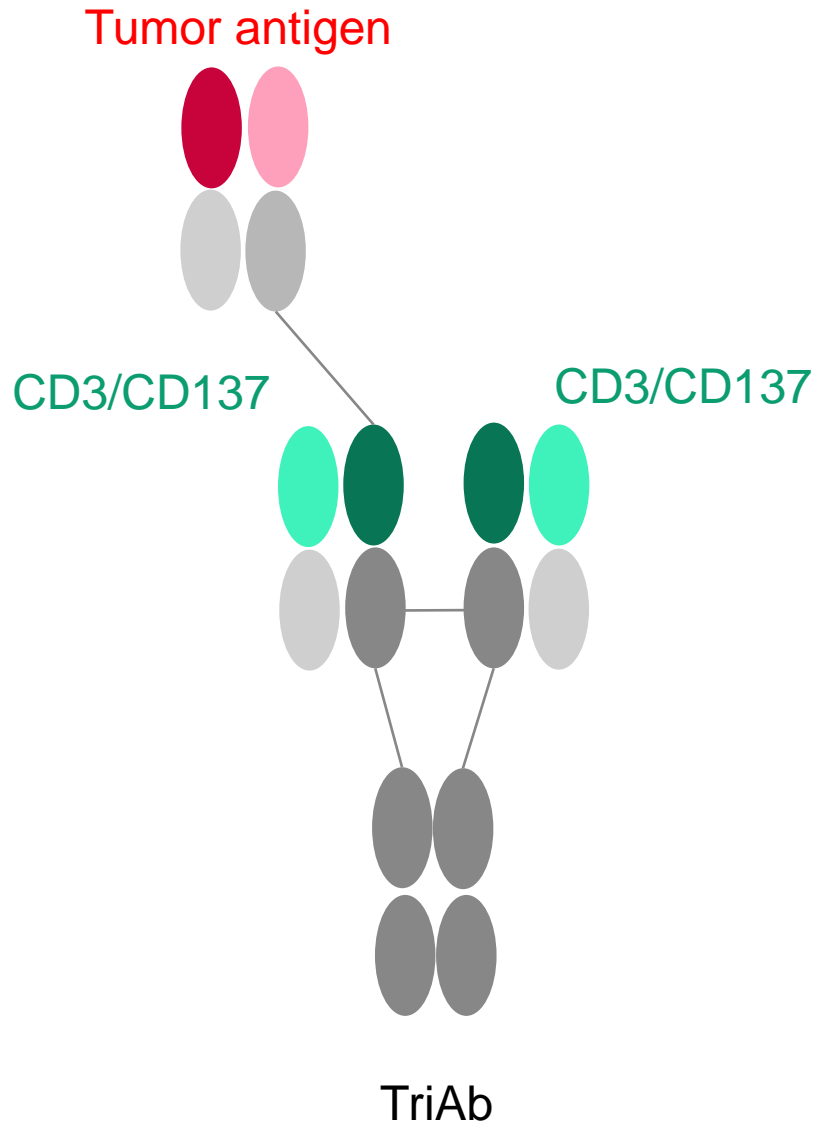
- T cell proliferation and survival
- Th1 cytokine production
- Prevention of T cell exhaustion



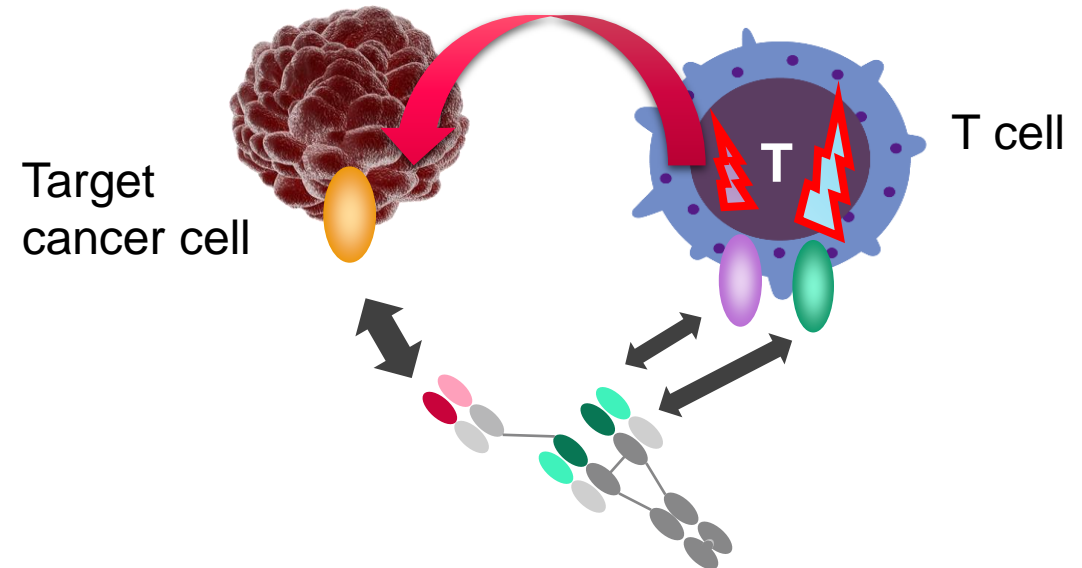
Conceptual illustration: Dual-Ig[®]

* Adrienne L, Nat Med. 2015 Jun; 21(6): 581-590.

Introduction of Trispecific Antibody (TriAb)



Mode of Action



- One antibody
- Two cell mediated reaction
- Three different antigens

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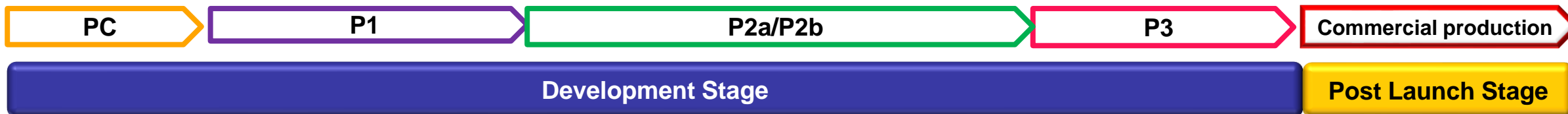
Method comparability study

04

Verification of specificity for three antigens in a single test system

Phase appropriate approach in potency assay

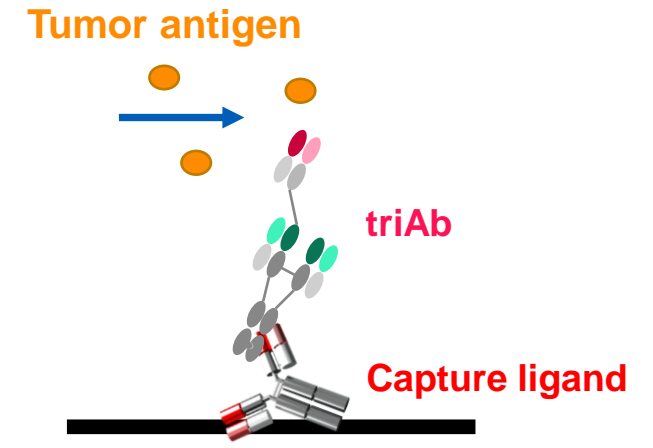
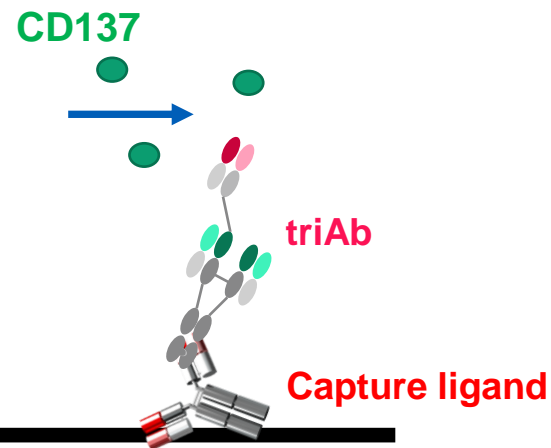
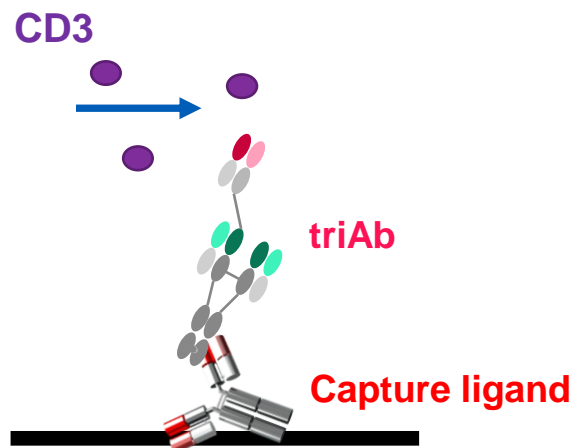
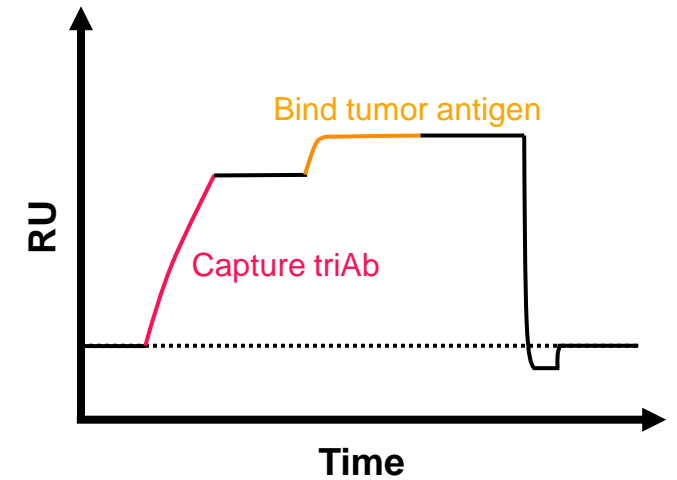
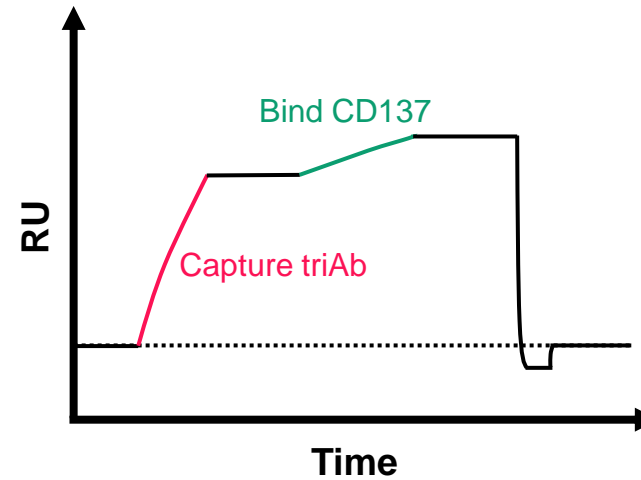
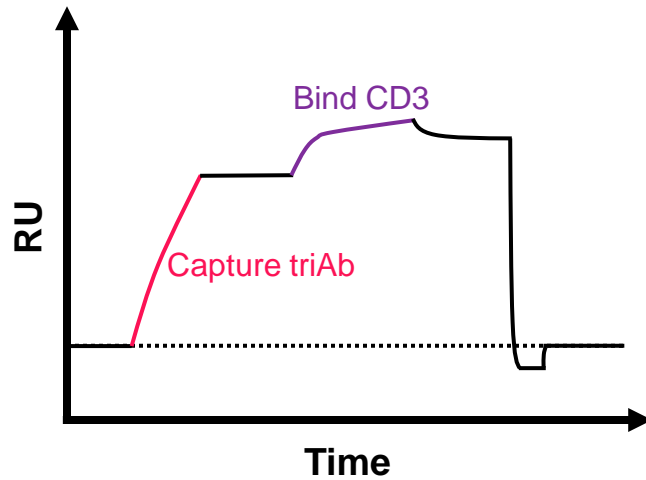
Bioassay development strategy in Chugai



	P1	P2a	P2b	P3	Commercial
Methods	Binding method <ul style="list-style-type: none"> • SPR method • ELISA etc...		MoA-reflective method <ul style="list-style-type: none"> • Cell proliferation • Cytotoxicity assay • Reporter gene assay etc...		
	Easy-to-develop Good precision and robustness		More complex and mimics MoA Inherently variable and often lacks precision required skilled laboratory technique		

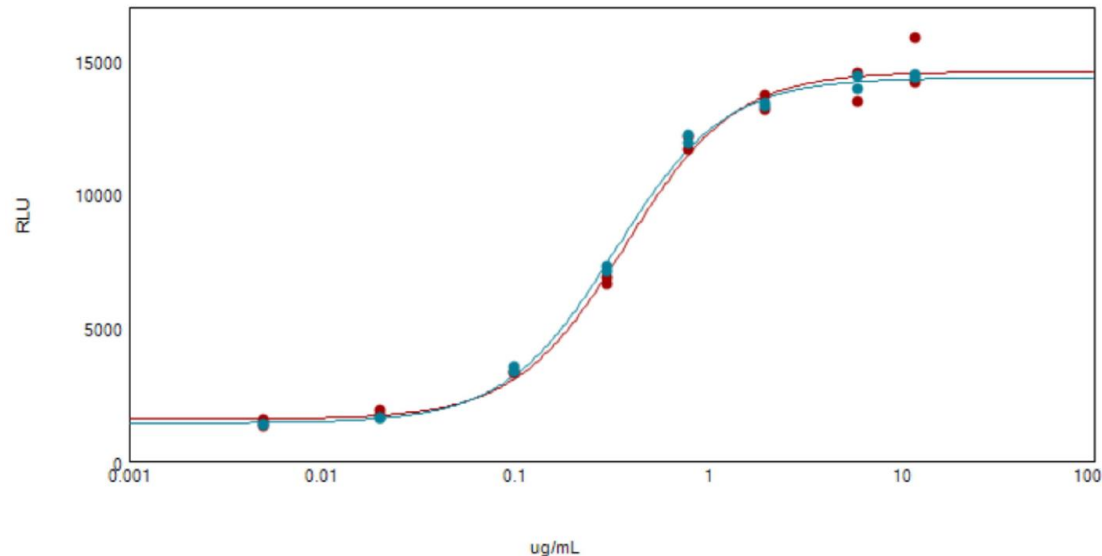
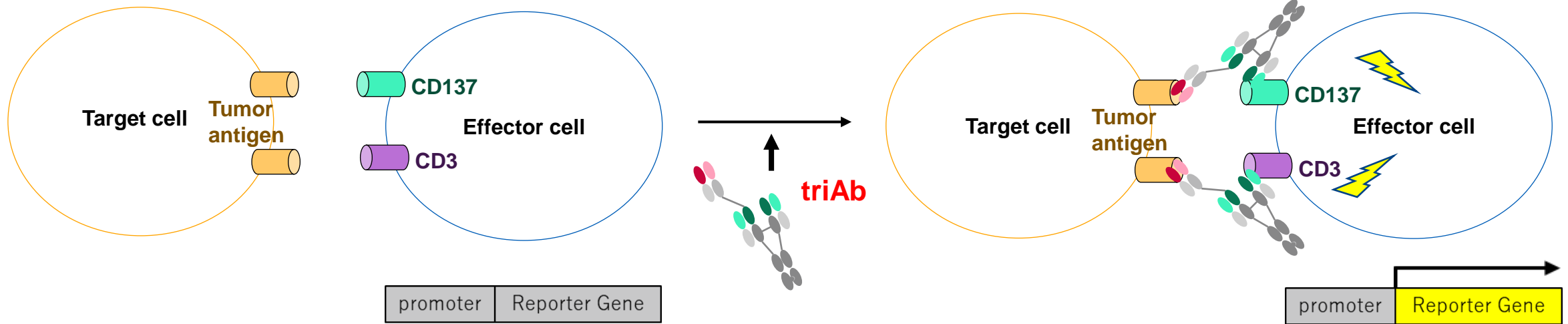
SPR binding assays for early clinical development

- Measure binding activities for each antigen using 3 independent assays
- Multi-point calibration curve method
- Accuracy: 99/102/96%recovery • Intermediate Precision: 3/1/1%RSD



Reporter gene assay for late clinical development

Effector cells express CD3 and CD137 and target cells express tumor antigen.



- 4-PL logistic analysis
- Accuracy: 109% Recovery
- Intermediate Precision: 5% RSD

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Verification of specificity for three antigens in a single test system

Method comparability study

1. Batch analysis

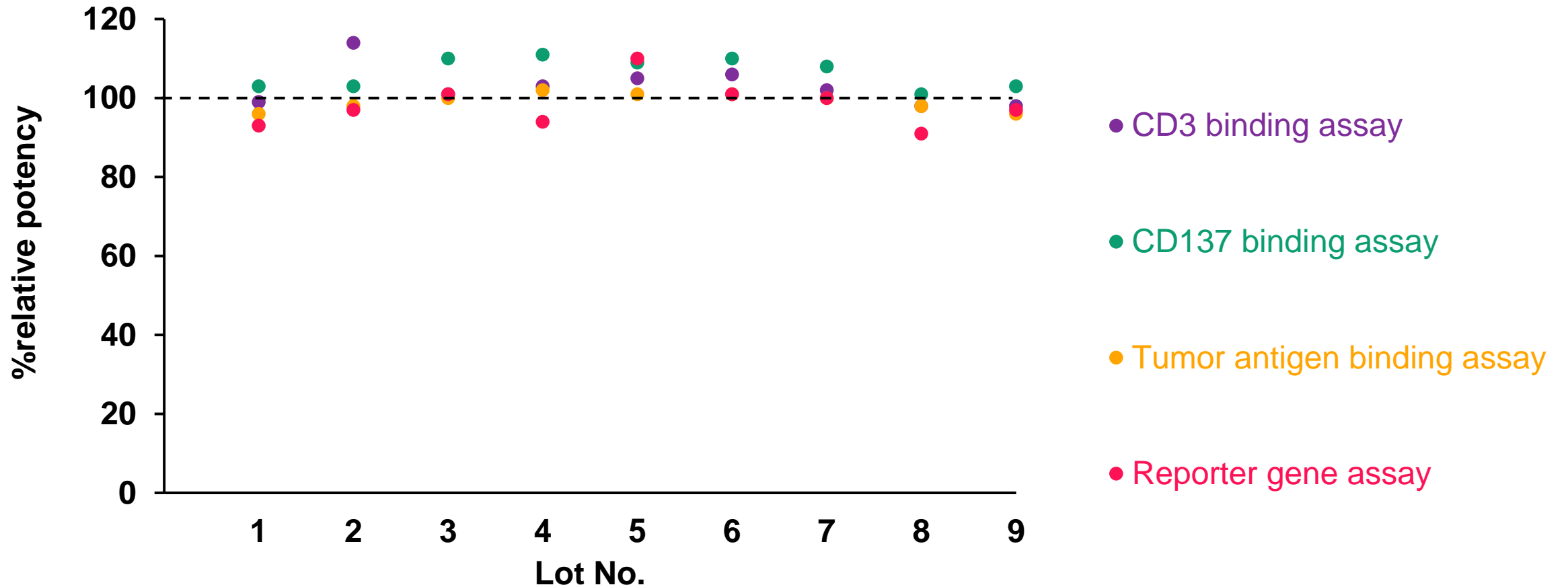
The results of batch analysis with each method were compared using a paired TOST (Two One-Sided Test method) on the log-transformed results.

2. Stress samples evaluation

40°C stressed TriAbs were used.

Batch analysis results

9 lots of TriAbs were used for the batch analysis.



Method comparability was shown (TOST)

90% CI of the ratio (the reporter gene assay to each binding assay) were within the range of MAD 12%.

Tost results

Criteria	Results	Acceptance
MAD [% (ratio)]: 12% (90% CI of the % ratio : 88%-112%)	RGA to CD3 binding	Lower limit 100%
		Upper limit 91%
	RGA to CD137 binding	Lower limit 96%
		Upper limit 89%
	RGA to tumor antigen binding	Lower limit 103%
		Upper limit 96%

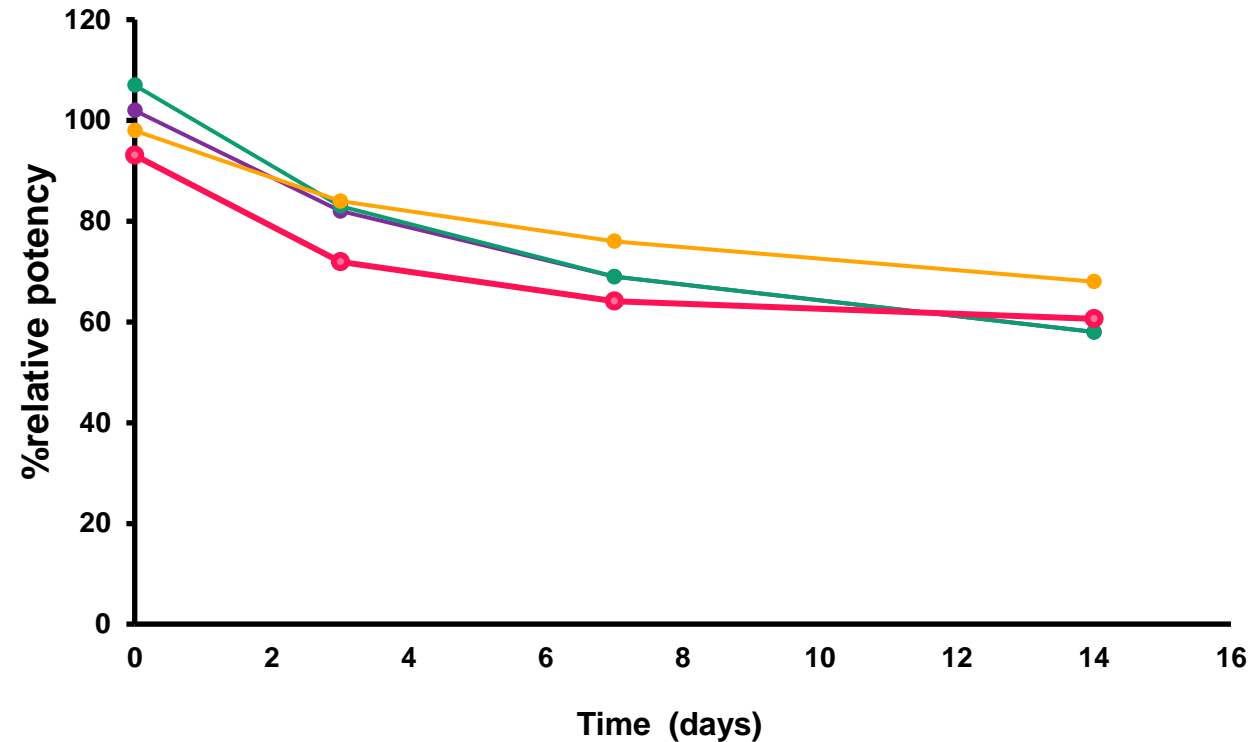
MAD: maximum allowable difference

RGA: reporter gene assay

Method comparability was shown (stress sample)

40°C stressed samples exhibited similar behavior between the binding assay for each antigen and reporter gene assay.

TriAb stressed at 40°C



—●— CD3 binding assay —●— CD137 binding assay —●— Tumor antigen binding assay —●— Reporter gene assay

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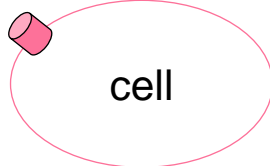

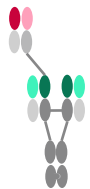
Method comparability study

04

Verification of specificity for three antigens in a single test system

How to verify the specificity for multiple antigens

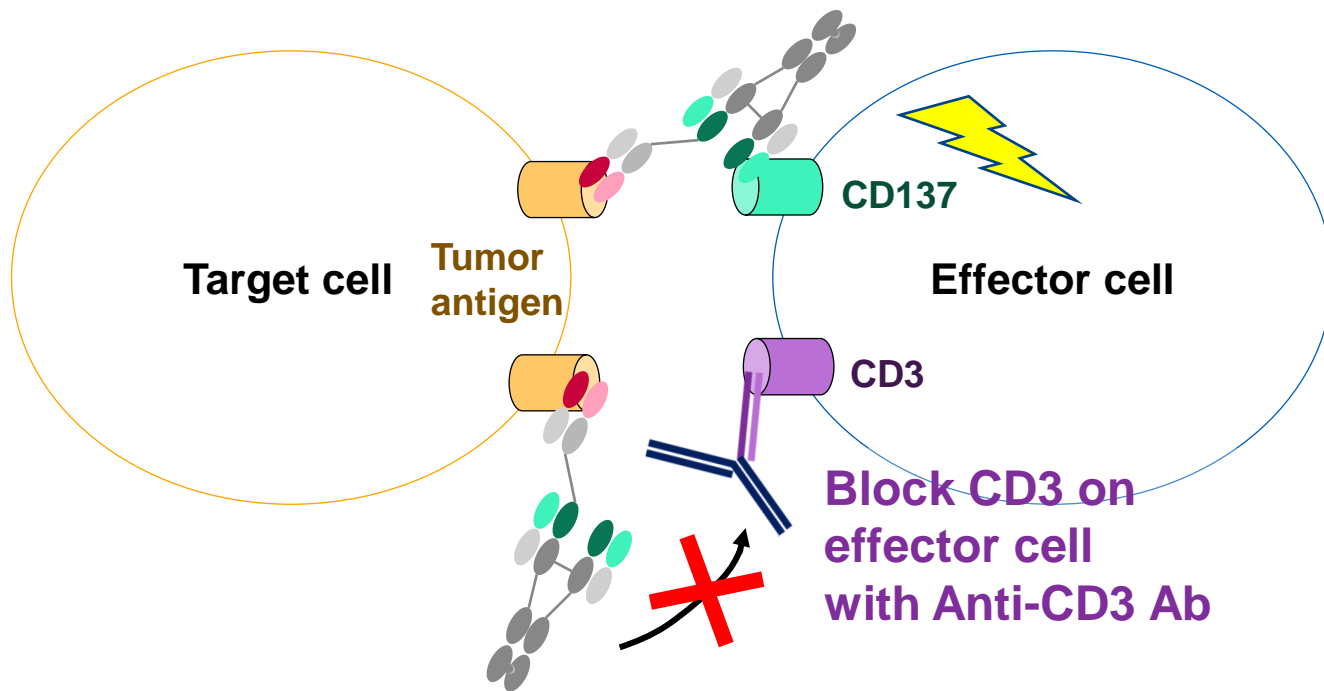
- Although cell assay can evaluate the bioactivity reflecting the overall MoA, it is generally difficult for one assay to evaluate each antigen in case of multiple targets.
- To overcome this challenge, two methods were tried: blocking antigens expressed on cells and blocking arms of triAbs.

Tools	Target	CD3	CD137	Tumor antigen
Blocking antibodies 	antigens 	✓	✓	✓
Antigens 	TriAb 	✓	✓	Not available





Experiment with blocking antibodies

Blocking antibodies added to the cells.

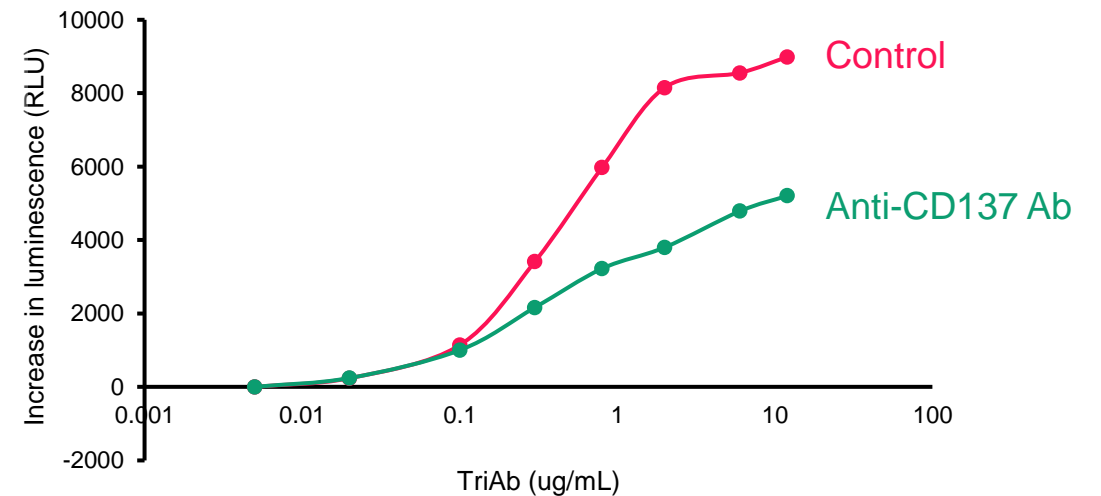
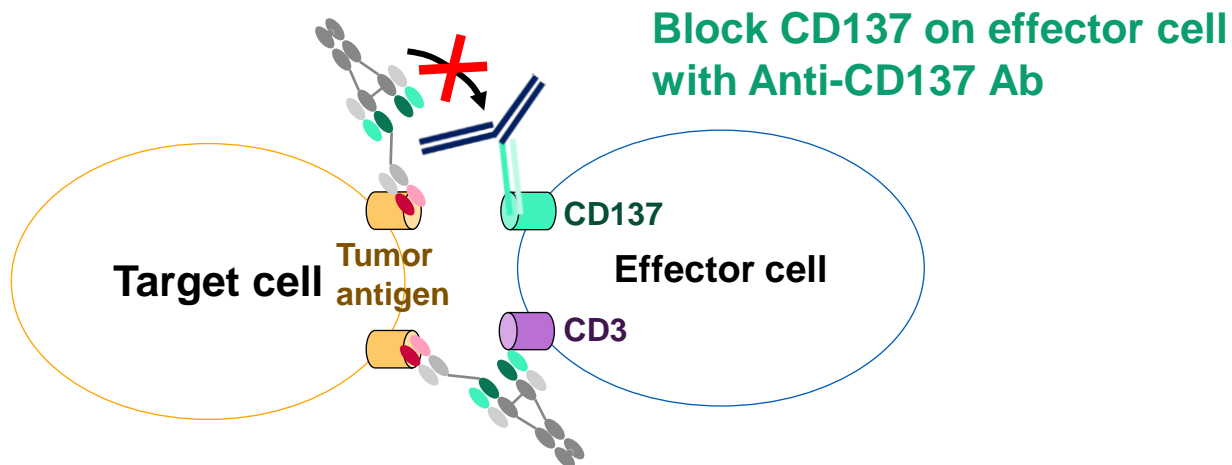
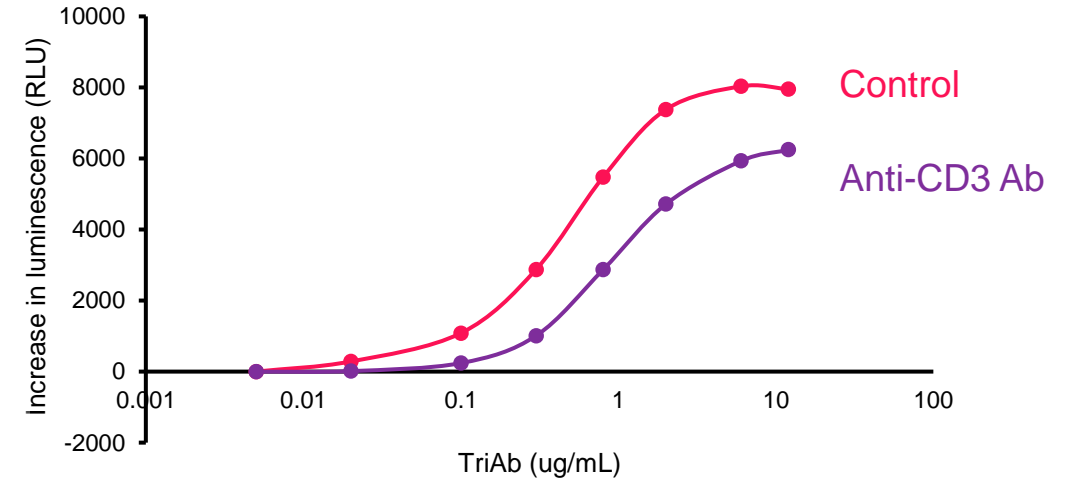
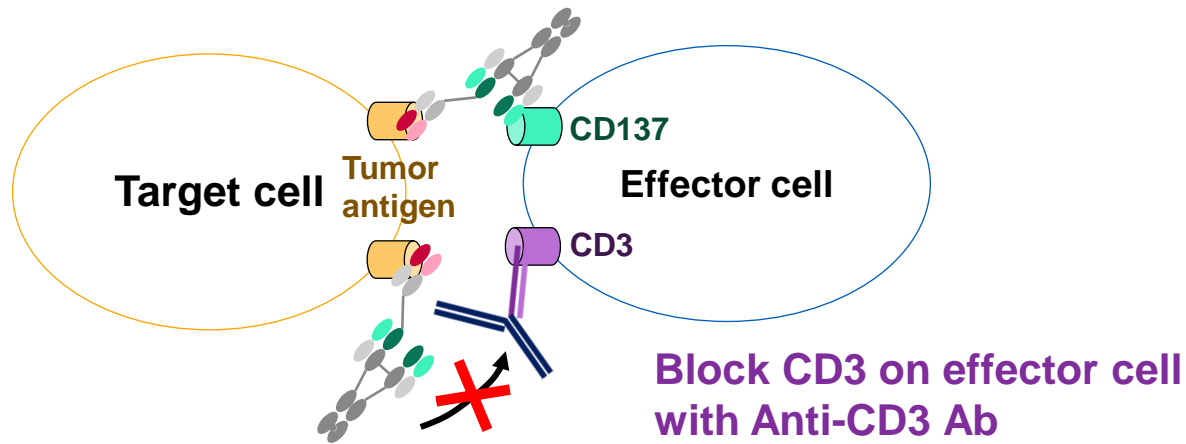
In case of condition 1



4 conditions

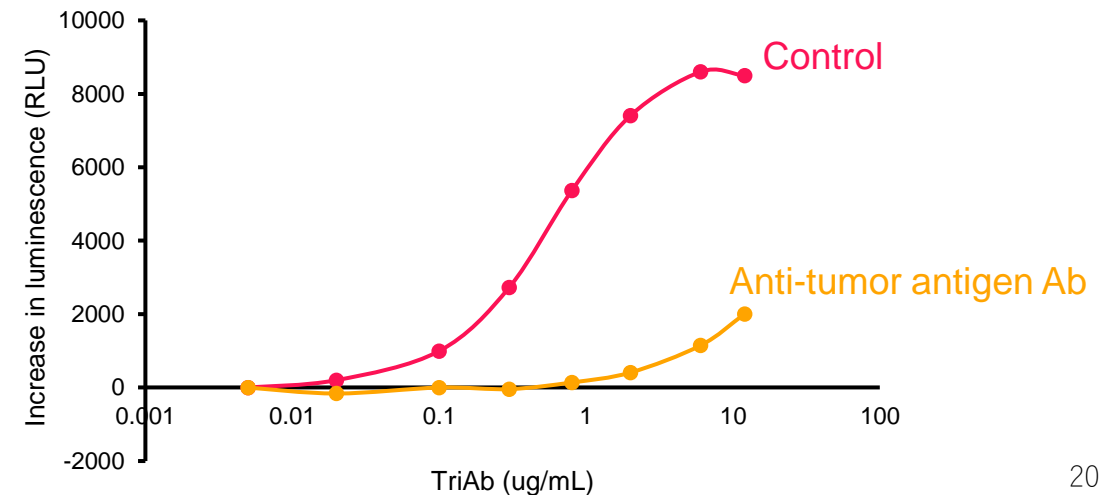
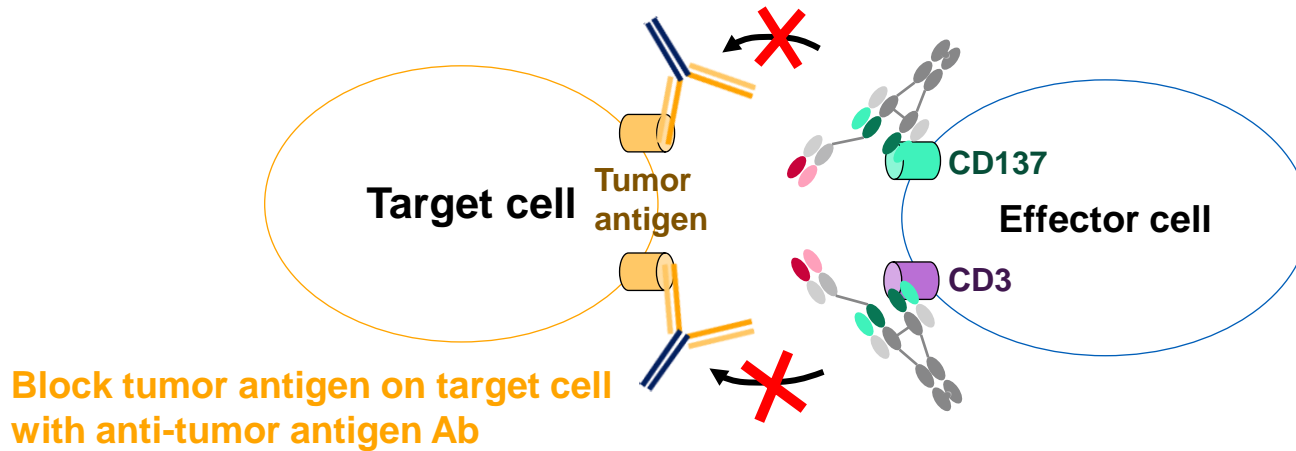
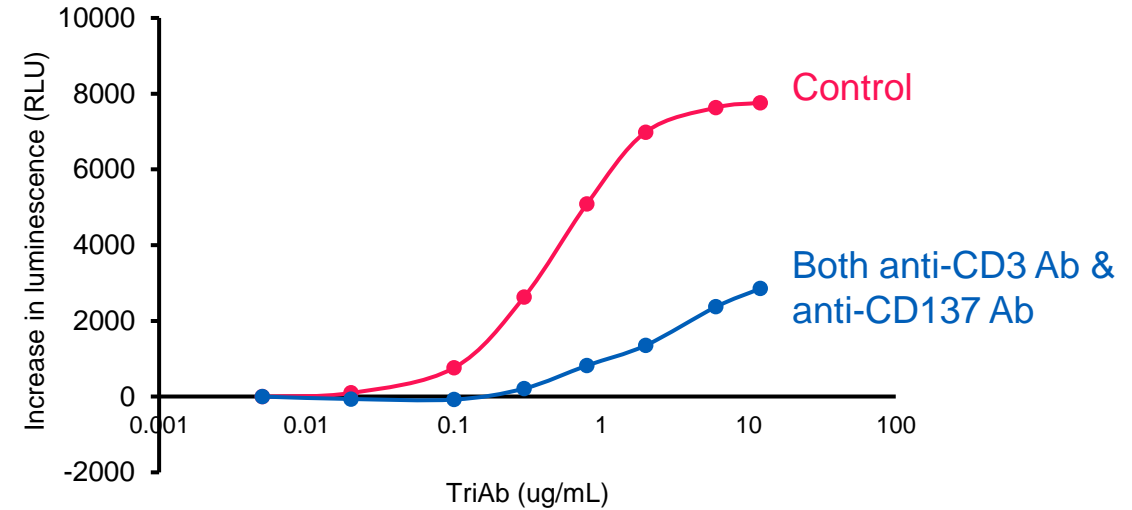
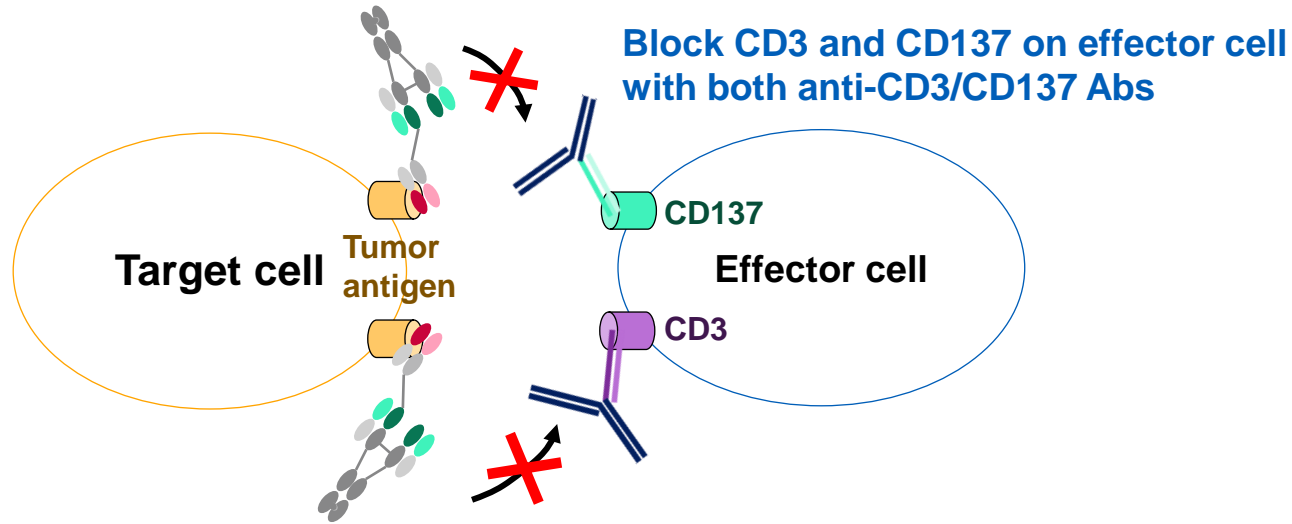
	Blocking antibodies		Expected result
1	Anti-CD3 Ab		Decrease in CD3 signal
2	Anti-CD137 Ab		Decrease in CD137 signal
3	Anti-CD3 Ab & Anti-CD137 Ab		Few signal
4	Anti-Tumor antigen Ab		Few signal

Contribution of both CD3 and CD137 signals confirmed with respective blocking Abs

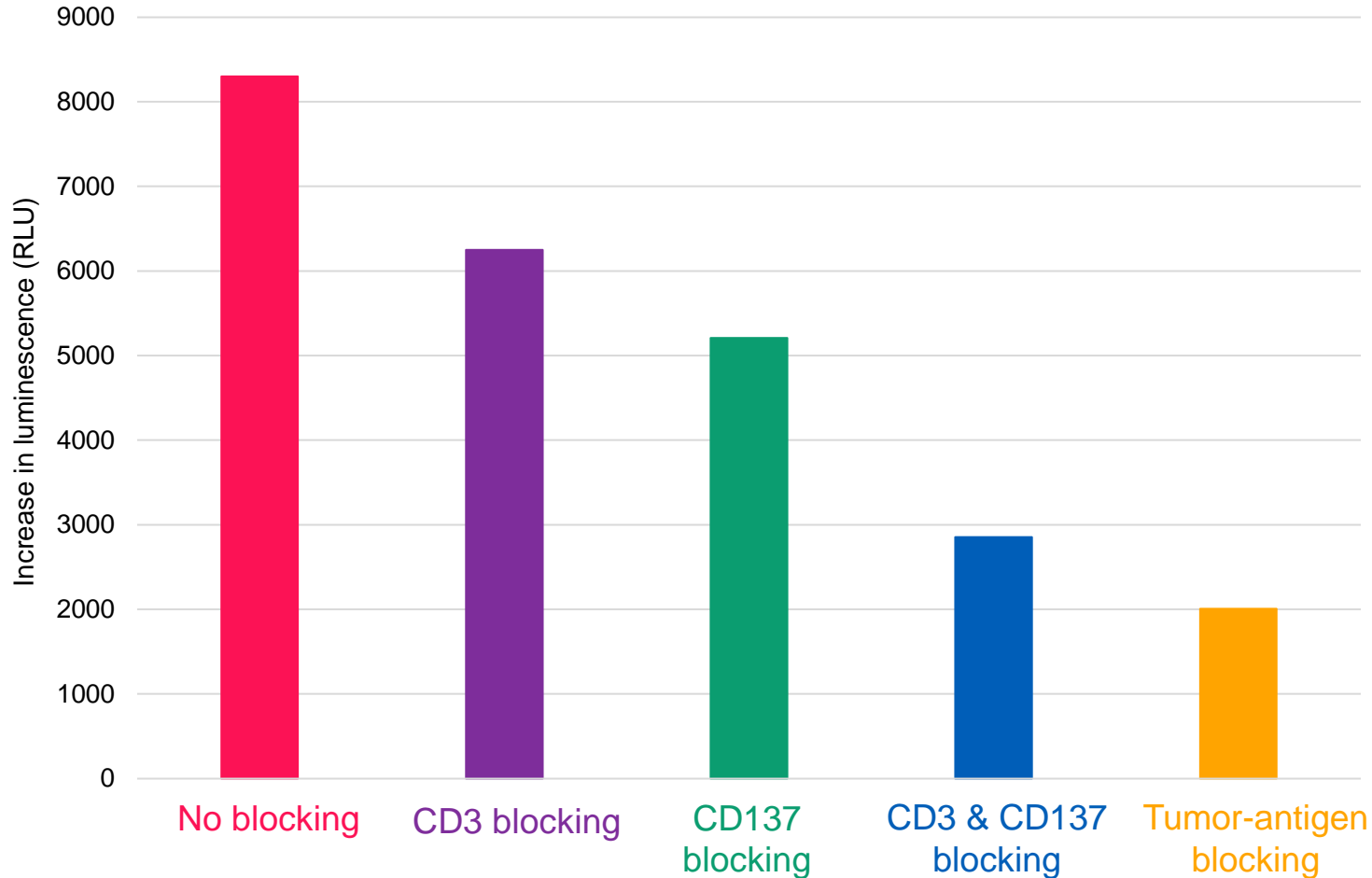


Cross-linking inhibition suggested with blocking Abs

The signal considerably decreased with both anti-CD3/CD137 Ab and anti-tumor antigen Ab .



Verified specificity for three antigens with blocking Abs



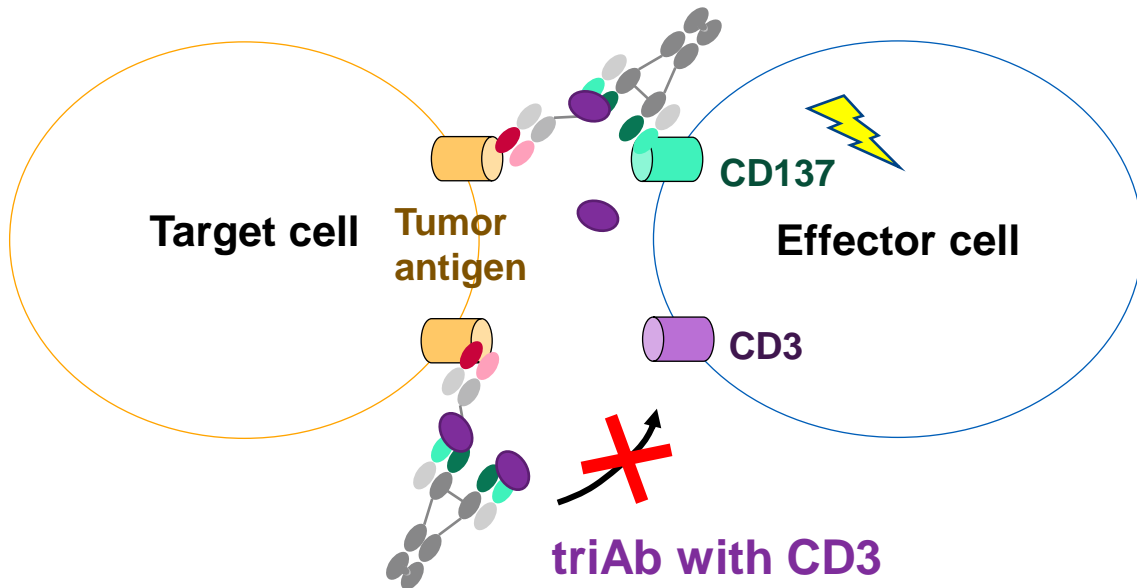
Assay condition	Block%
No blocking	0
CD3 blocking	21
CD137 blocking	42
CD3 & CD137 blocking	63
Tumor-antigen blocking	76

Block%: The decrease rates of luminescence compared to no blocking

Experiment with antigens

Antigen-bound triAb was added to cells.

In case of condition 1

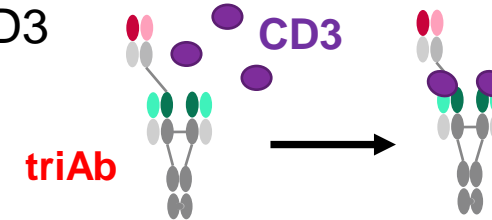


3 conditions

Antigen-bound triAb

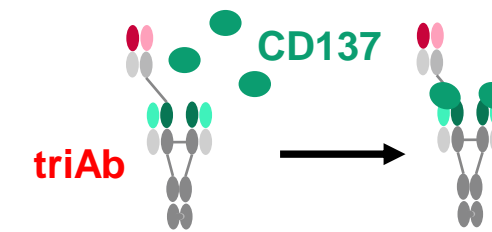
Expected result

1 TriAb with CD3



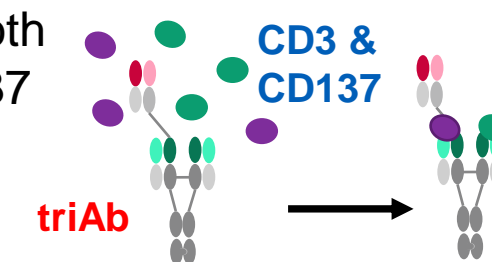
Mid signal
(due to Dual-Ig)

2 TriAb with CD137



Few signal

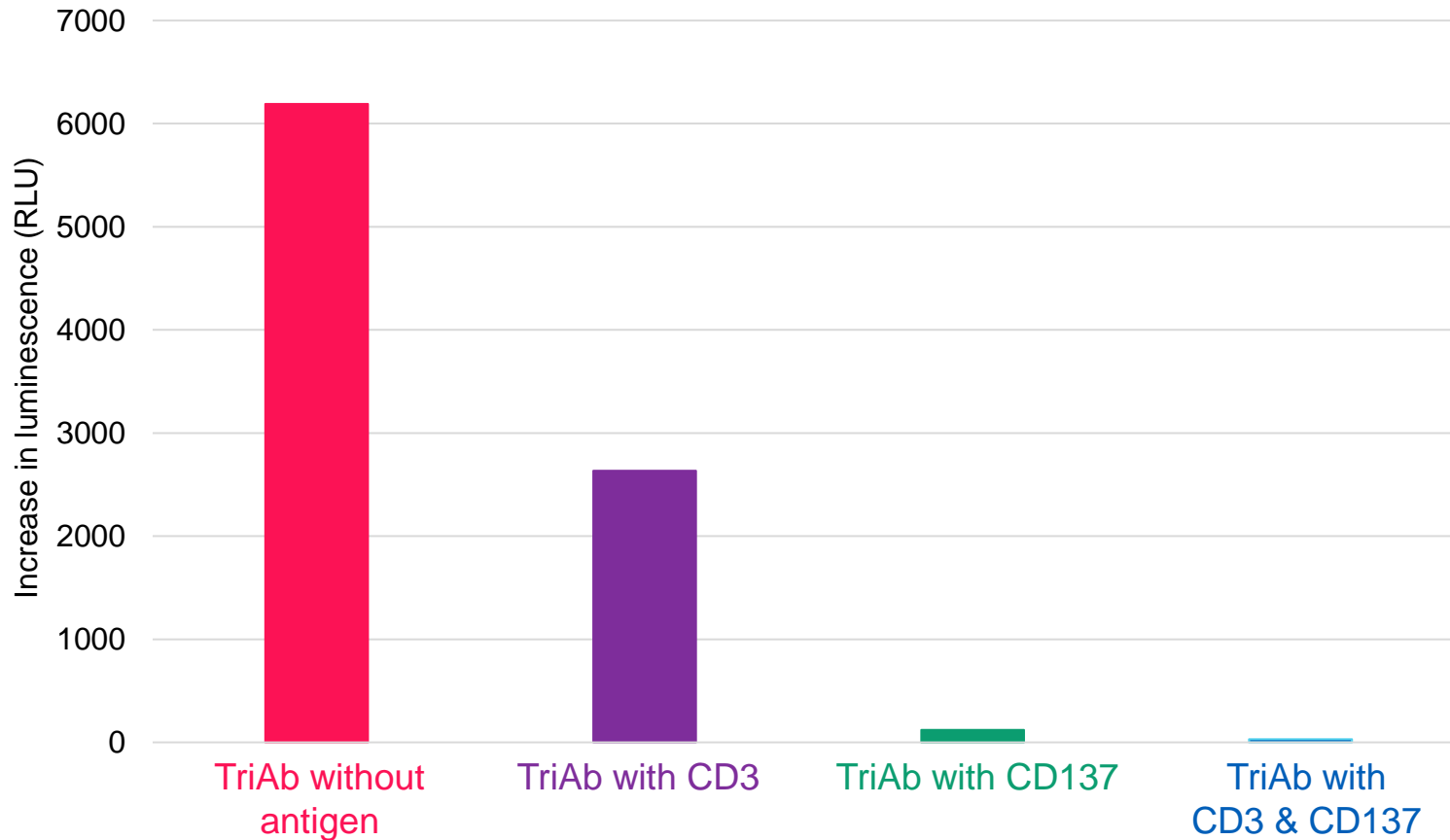
3 TriAb with both CD3 & CD137



Few signal

Verified specificity for CD3/CD137 with each antigen

The decreased signal was detected with both CD3/CD137.




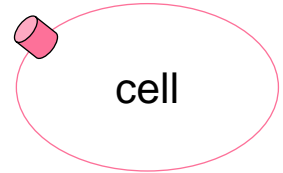

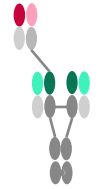
Assay condition	Block%
TriAb without antigen	0
TriAb with CD3	57
TriAb with CD137	98
TriAb with CD3 & CD137	100

Block%: The decrease rates of luminescence compared to triAb without antigen

Antigen	Kd (1/s)
CD3	6.84E-02
CD137	2.24E-04

Temperature: 37°C

Specificity for three antigens in a single test system was verified

	Tools	Target	CD3	CD137	Tumor antigen
Blocking antibodies		antigens 	Confirmed signal reduction	Confirmed signal reduction	Confirmed signal reduction
Antigens		TriAb 	Confirmed signal reduction	Confirmed signal reduction	Not available

Summary

- ◆ For trispecific antibody, three binding assays by SPR was applied for the early clinical development and one reporter gene assay was applied for late clinical development.
- ◆ The comparability between binding assay and reporter gene assay was confirmed through batch analysis and stability analysis of stress samples.
- ◆ To overcome the challenge, we have developed the verification method and shown the concept of multiple targets evaluation in single assay is appropriate.

Acknowledgements

Research Div.

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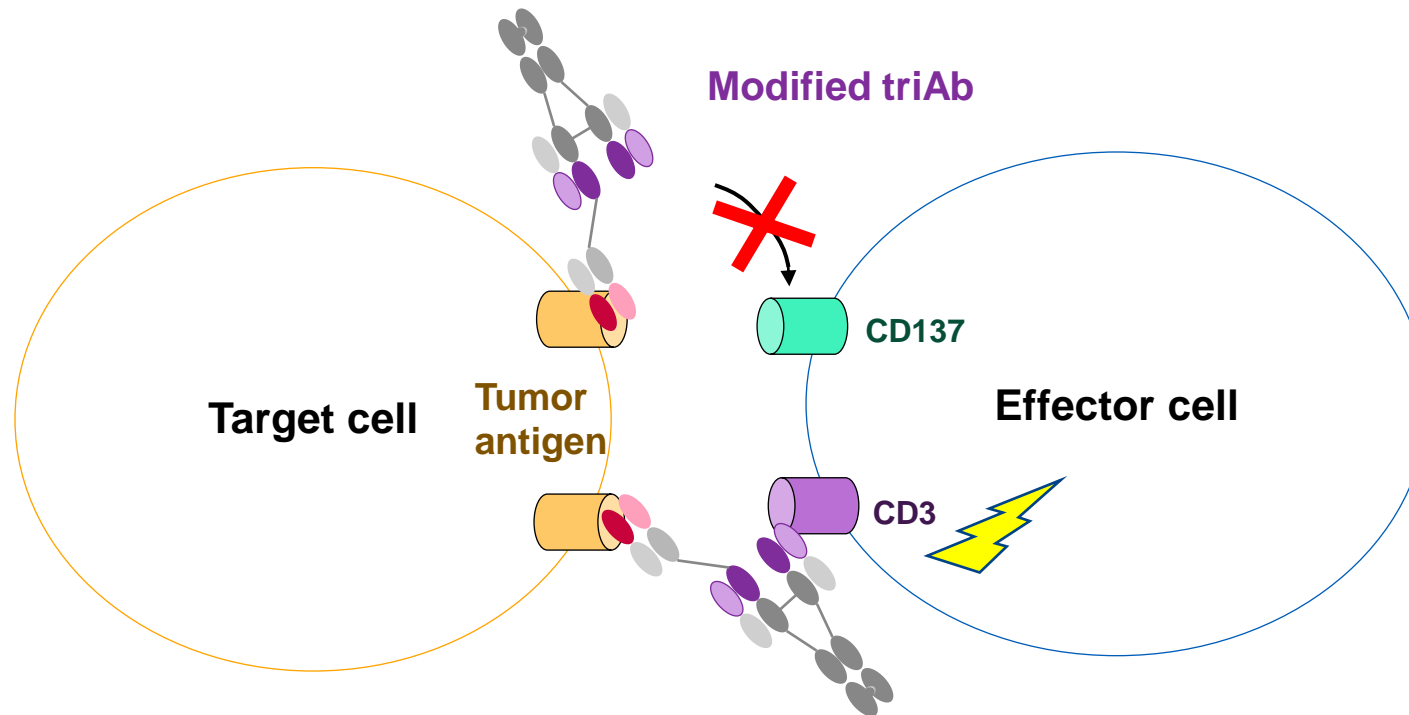


INNOVATION BEYOND IMAGINATION

Appendix

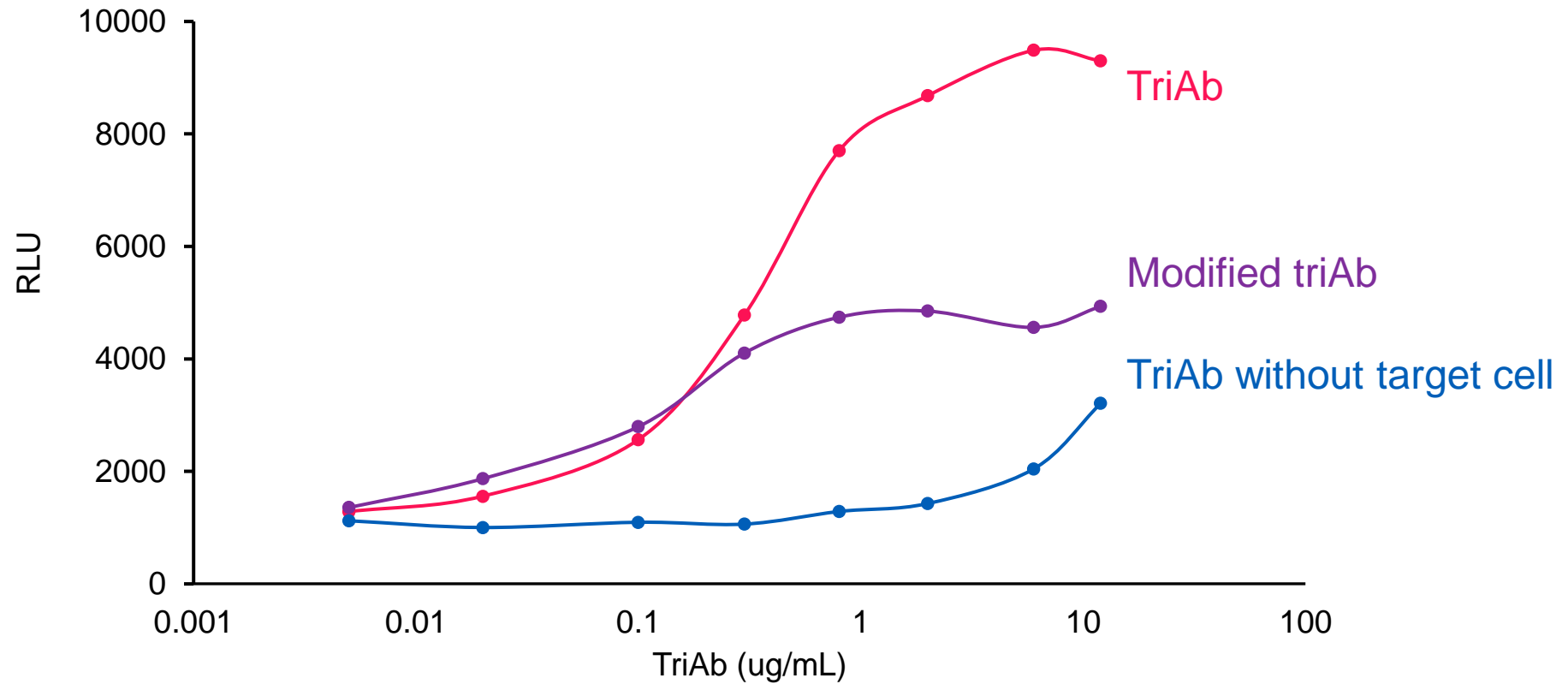
Experiment with modified antibody

The assay was performed using a modified triAb that can only bind to CD3 and tumor antigen and cannot bind to CD137.

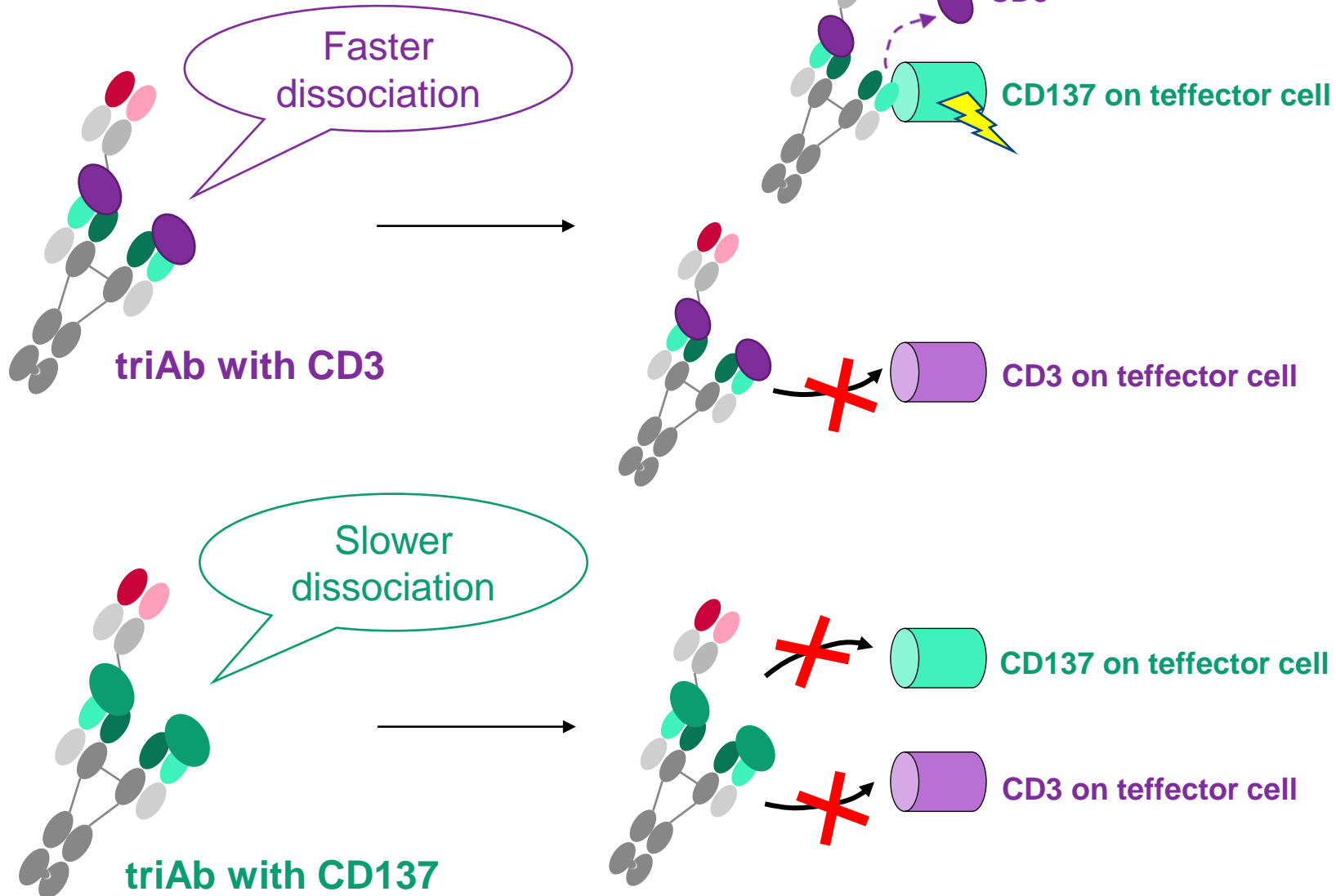


Result

Modified triAb (bind to CD3 and tumor but not to CD137) showed decrease signal.



CD3 vs. CD137



Antigen	Kd (1/s)
CD3	6.84E-02
CD137	2.24E-04