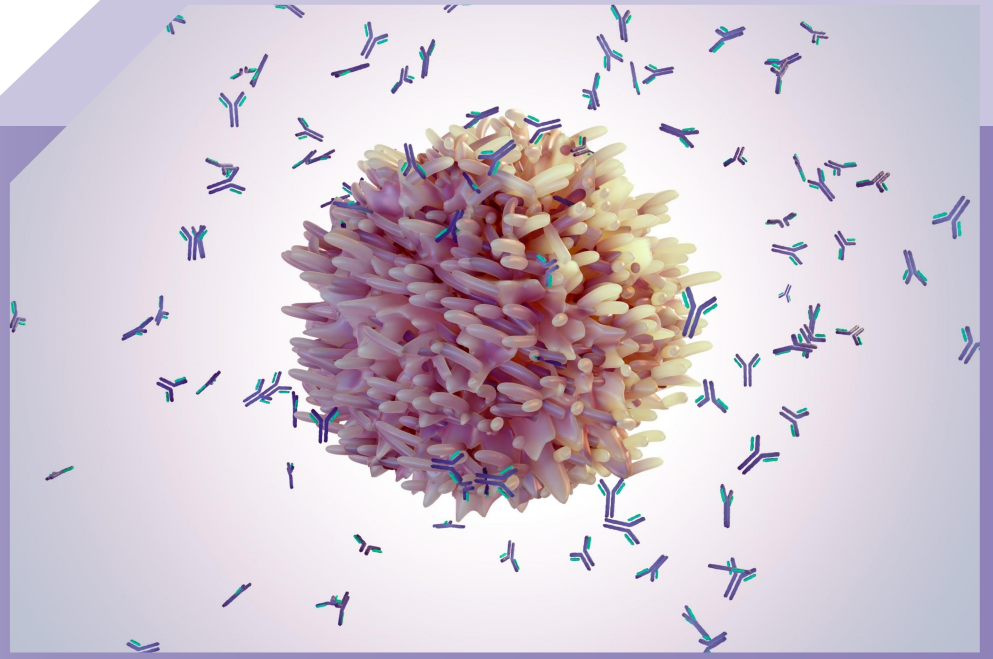
Protheragen
ImmuBridge™

# *ImmuBridge™*: Multispecific Antibody Platform and Next-Generation TCEs



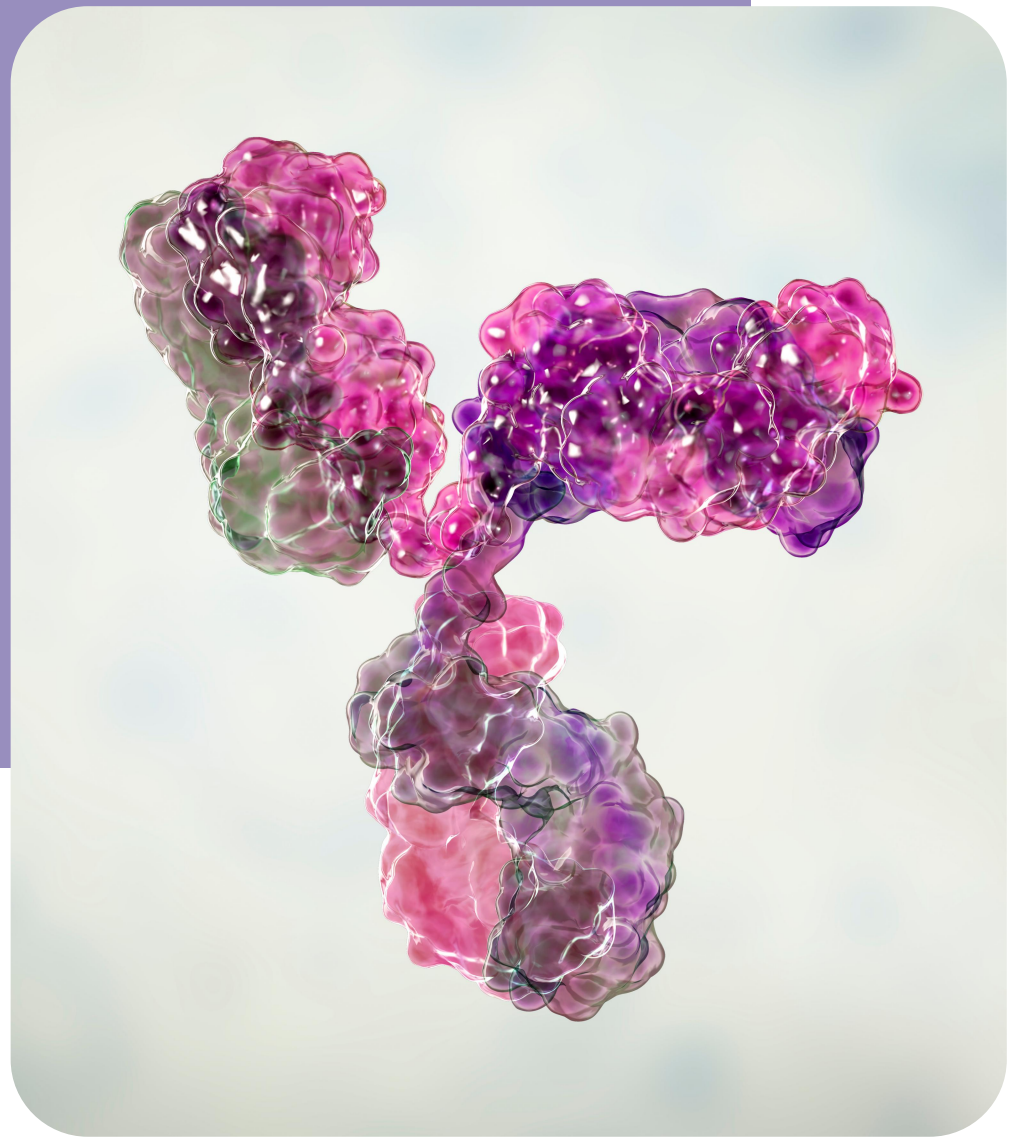
## Contact Us


info@protheragen.us

1-631-533-2057

www.immubridge.com

101-4 Colin Dr, Holbrook, NY 11741, USA



# Overview

# About Protheragen

As a leading research service provider pioneering advancements in the fields of oncology and immunotherapy, Protheragen stands at the forefront of innovation. Leveraging our *ImmuBridge™* platform, we are committed to customizing multispecific antibodies to precisely meet the unique requirements of your cancer immunotherapy research.

## Antibody Development

- Bispecific Antibody Development
- Trispecific Antibody Development
- Multispecific Antibody Development

## Our Advantages

- Innovative Platform
- Rich Expertise
- Customized Solutions
- Trusted Partner

# *ImmuBridge™*: Multispecific Antibody Platform and Next-Generation TCEs

## Platform Introduction

- Specializing in immunotherapy innovation, Protheragen has developed the *ImmuBridge™* multispecific antibody platform. Leveraging advanced protein engineering and antibody design, this platform enables the creation of multispecific antibodies that simultaneously target tumor antigens and immune cell receptors.
- The next-generation T cell engagers (TCEs) developed using the *ImmuBridge™* platform combine tumor-targeting domains with T-cell activation domains, linked through optimized linkers to achieve stability and flexibility. These TCEs are designed to redirect T cells to tumor cells with high specificity, promoting potent tumor cell killing while minimizing systemic toxicity.

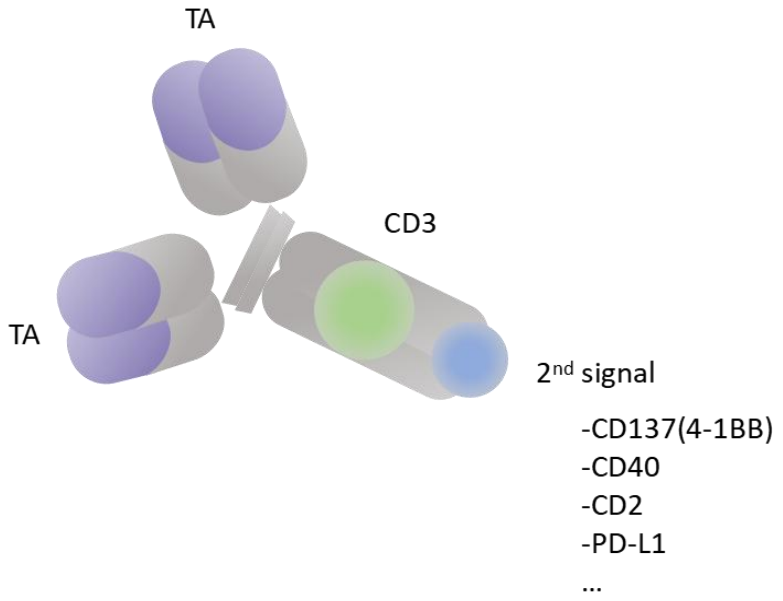



Fig. 1 Structure of the multispecific T cell engagers (TCEs). TA: Target antigens can be the same or different epitopes or two antigens; CD3: Part of the T-cell receptor (TCR) complex; 2nd signal: The binding site can be designed to target common surface proteins of T cells or tumor cells.

# TA/CD3 Bispecific Antibody Development

With the *ImmuBridge™* platform, Protheragen has developed bispecific antibodies targeting a range of tumor antigens. A standout accomplishment is the targeted antigen (TA)/CD3 antibodies, which effectively bind to tumor-specific antigens on cancer cells and the CD3 receptor on T cells. This dual targeting mechanism activates T cells to specifically eradicate cancer cells expressing the antigen, facilitating precise destruction while safeguarding healthy tissues from harm.

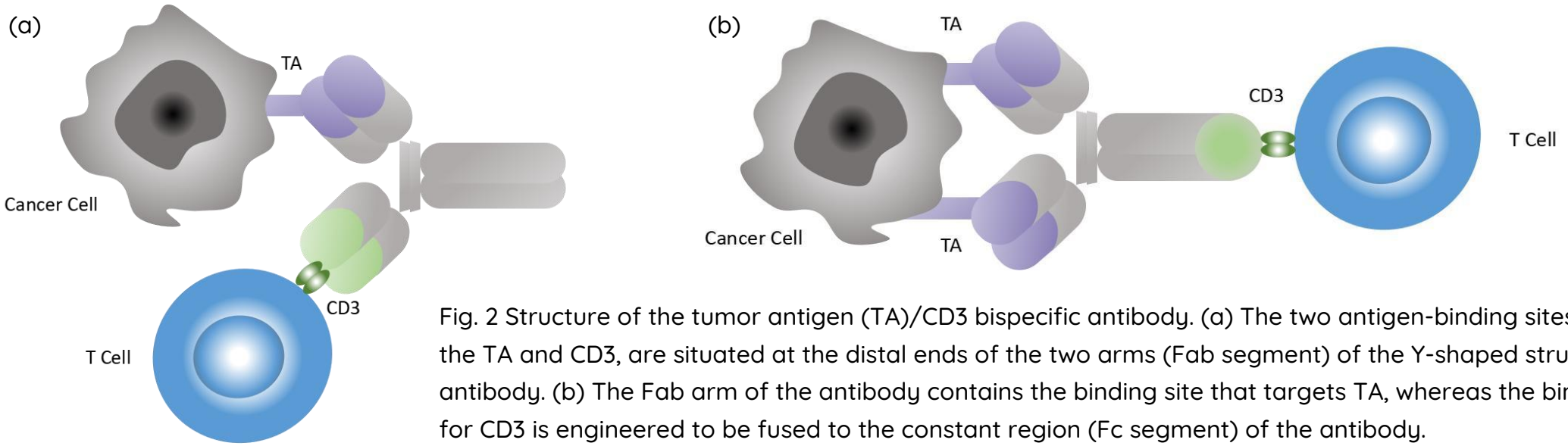



Fig. 2 Structure of the tumor antigen (TA)/CD3 bispecific antibody. (a) The two antigen-binding sites, targeting the TA and CD3, are situated at the distal ends of the two arms (Fab segment) of the Y-shaped structure of the antibody. (b) The Fab arm of the antibody contains the binding site that targets TA, whereas the binding site for CD3 is engineered to be fused to the constant region (Fc segment) of the antibody.

# TA/CD3/CD137 (4-1BB) Trispecific Antibody Development

Leveraging the *ImmuBridge™* platform, Protheragen has also developed the target antigen (TA)/CD3/CD137 (4-1BB) trispecific antibody. This antibody demonstrates a unique activation pattern dependent on tumor antigen recognition, stimulating both CD3 and CD137. CD137 is specifically expressed on cytotoxic CD8 T cells activated by tumor antigens, enhancing their tumor-killing function.

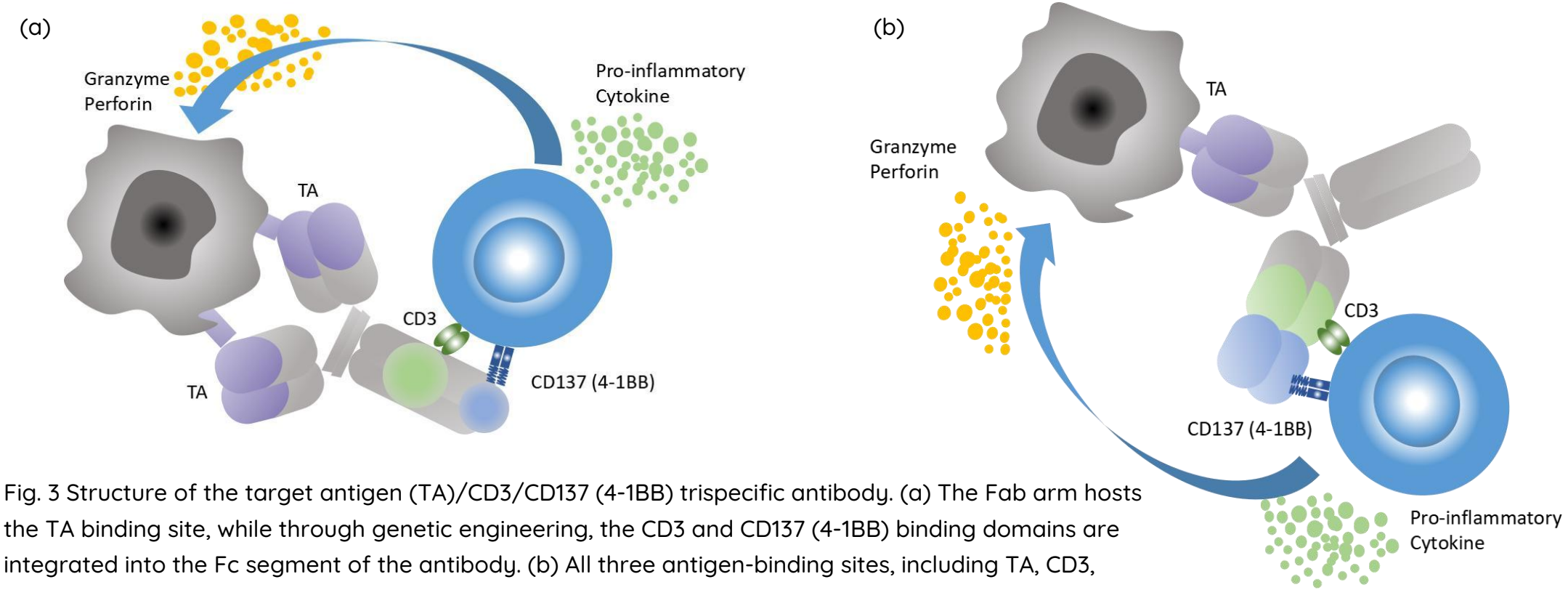



Fig. 3 Structure of the target antigen (TA)/CD3/CD137 (4-1BB) trispecific antibody. (a) The Fab arm hosts the TA binding site, while through genetic engineering, the CD3 and CD137 (4-1BB) binding domains are integrated into the Fc segment of the antibody. (b) All three antigen-binding sites, including TA, CD3, and CD137 (4-1BB), are expressed exclusively within the Fab segment of the antibody.

# TA/CD3/CD137/PD-L1 Multispecific Antibody Development

Protheragen has also developed the target antigen (TA)/CD3/CD137/PD-L1 multispecific antibody using the *ImmuBridge™* platform. The antibody is engineered to simultaneously target tumor-associated antigens, engage CD3 on T cells, activate CD137 for enhanced T-cell proliferation and survival, and block PD-L1 to reverse immune suppression, providing a potent, multi-functional approach to enhance anti-tumor immunity and reduce off-target effects.

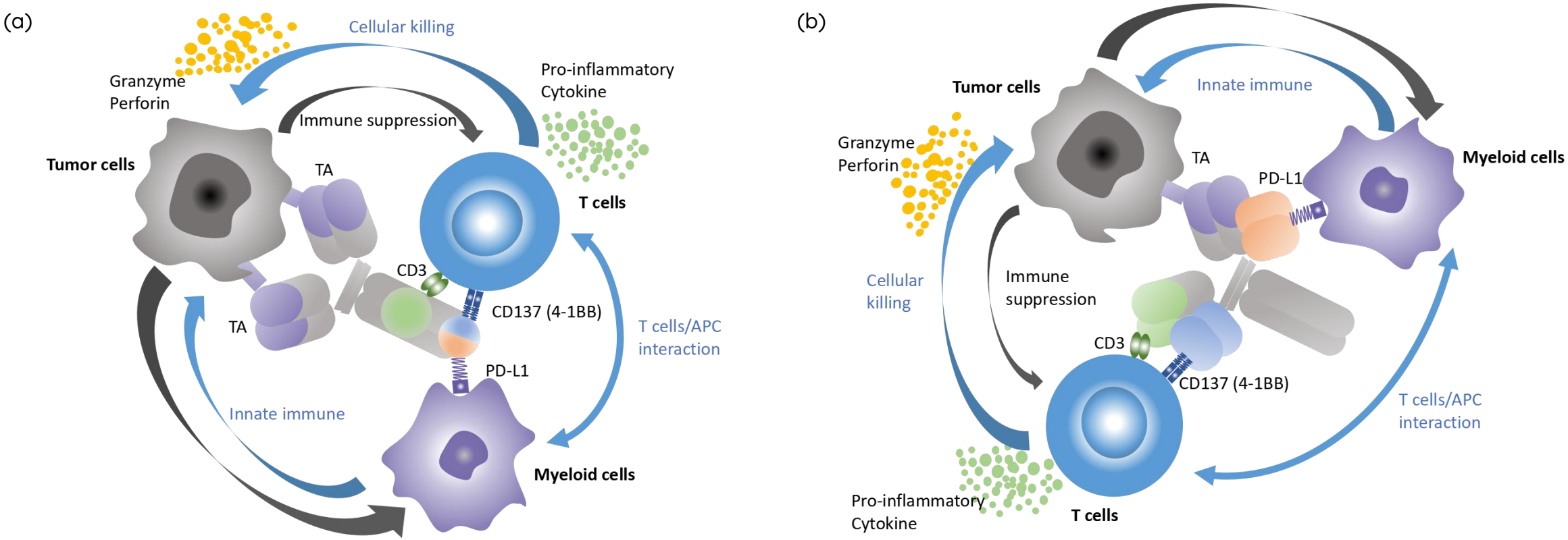



Fig. 4 Structure of the target antigen (TA)/CD3/CD137/PD-L1 multispecific antibody. (a) The Fab arm exclusively binds to the TA to maintain precise targeting, while the binding domains for CD3, CD137, and PD-L1 are fused to the Fc segment through genetic engineering. (b) Using a symmetrical design, all antigen-binding domains are integrated into the two Fab arms of the antibody.

# *ImmuBridge™* Platform Advantages

### IgG-like Antibody Structure

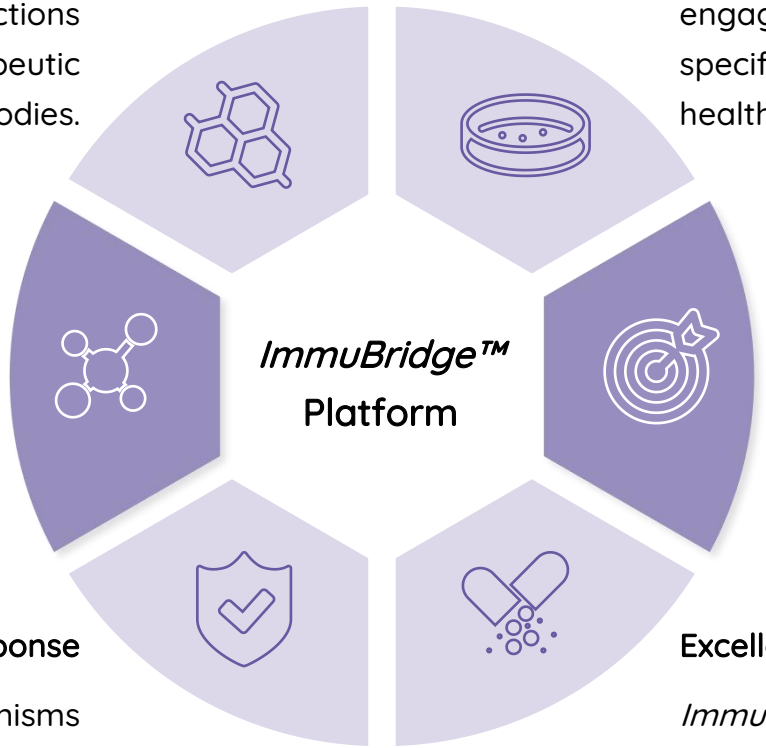
IgG-like structure design ensures stability, pharmacokinetic properties, and effector functions similar to IgG antibodies, enhancing the therapeutic potential of multispecific antibodies.

### Versatile Target Antigen (TA) Design

TA allows for the targeting of the same or different epitopes on a single antigen or the simultaneous targeting of two distinct antigens. This flexibility enables tailored approaches for various cancers.

### Potent Immune Response

Next-generation TCEs enhance cytotoxic mechanisms and tumor cell destruction by simultaneously activating multiple molecular targets on the T cell surface, thereby improving anti-tumor efficacy.




### Cross-linking Dependent Activity

Activity is triggered only when multiple antigens are co-engaged, minimizing off-target effects, enhancing specificity for diseased cells and reducing toxicity to healthy tissues.

### High Tumor Targeting and Safety

Multispecific antibodies are designed to preferentially bind to tumor-specific antigens or to be activated only in the tumor microenvironment, ensuring maximum efficacy while protecting healthy cells.

### Excellent Drug Developability

*ImmuBridge™* platform facilitates the development of multispecific antibodies with different combinations of targets. This versatility enables the customization for various cancer therapies.

# *ImmuBridge™* Platform Applications

## Cancer Immunotherapy Development

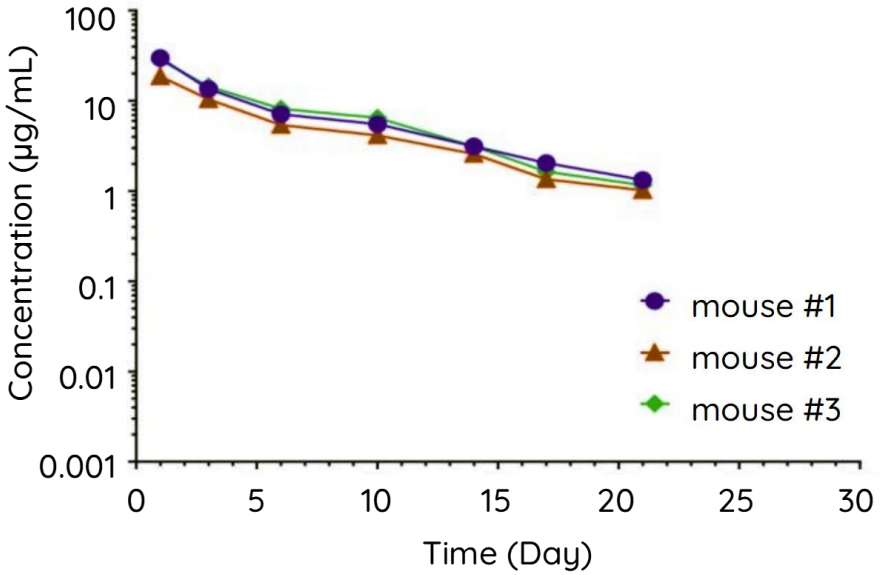
Multispecific antibodies have become pivotal in the realm of cancer immunotherapy, providing innovative approaches to target and stimulate the immune system in the fight against cancer cells. These antibodies are designed to simultaneously target cancer cells and immune cells, facilitating precise interactions between the immune system and tumor cells.

| Immune Cell Types | Targets | | |
|---|---|---|---|
| T Cells | • CD2<br>• CD3<br>• CD4<br>• CD8 | • CD25<br>• CD27<br>• CD28<br>• CD45 | • CD134 (OX40)<br>• CD137 (4-1BB)<br>• CD152 (CTLA-4)<br>• More |
| NK Cells | • CD226 (DNAM-1)<br>• NKp44<br>• NKp46 | • NKp30<br>• NKG2D<br>• CD244 (2B4) | • CD96<br>• CD40L<br>• More |

| Cancer Types | Targets |
|---|---|
| Breast Cancer | HER2, EGFR |
| Colorectal Cancer | CEA |
| Gastric Cancer | PD-1/PD-L1, CTLA-4 |
| Lung Cancer | PD-L1, EGFR |
| Lymphoma | CD20, CD19 |
| Ovarian Cancer | CA-125, Folate Receptor Alpha |
| Prostate Cancer | PSMA, PSCA |
| Pancreatic Cancer | MUC1 |
| Leukemia | CD19, CD22 |
| Glioblastoma | EGFRvIII, IL-13Rα2 |
| Renal Cell Carcinoma | CA-IX, PD-L1 |
| Bladder Cancer | PD-L1, Nectin-4 |

# Case Study- IgG-like Pharmacokinetics in Mice

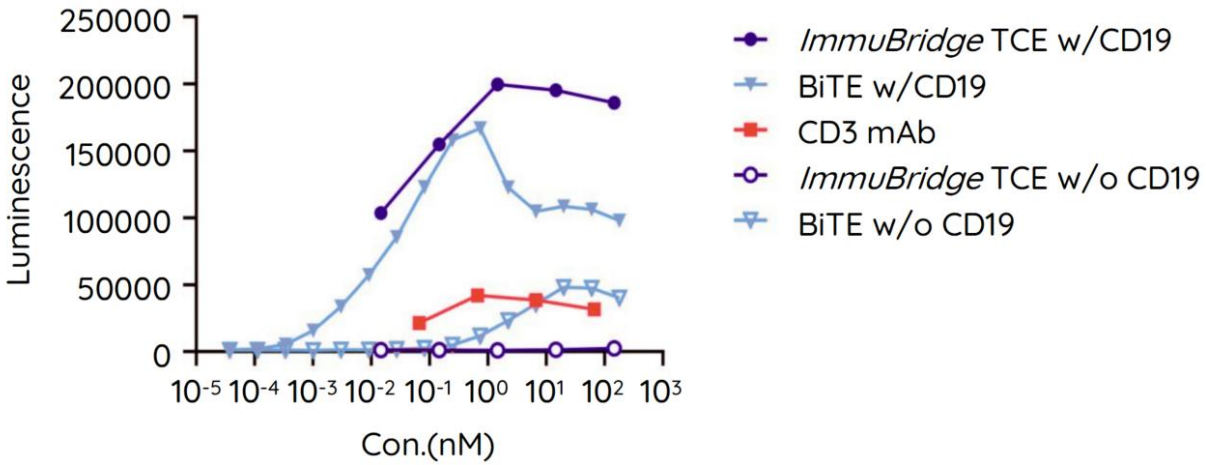
- *In vivo* pharmacokinetic studies of the TA/CD3/CD137 trispecific antibody involved administering a single intravenous dose of 5 mg/kg to mice, followed by collection of blood samples at predetermined time points over 21 days. Plasma was isolated from the samples, and antibody concentrations were quantified using validated methods such as ELISA or LC-MS. Pharmacokinetic parameters were analyzed using modeling software.
- Experimental results demonstrated IgG-like pharmacokinetics, with sustained plasma concentrations, a prolonged half-life, and low clearance, indicating favorable drug-like properties for therapeutic development.




| # | T1/2 (day) | Tmax (day) | Cmax (μg/mL) | AUC0-t (day*μg/mL) | AUC0-∞ (day*μg/mL) | Vss (mL/kg) | CL (mL/day/kg) |
|---|---|---|---|---|---|---|---|
| mouse 1 | 5.1 | 1 | 28.8 | 138 | 145 | 233 | 29 |
| mouse 2 | 5.3 | 1 | 17.6 | 97 | 101 | 336 | 41 |
| mouse 3 | 4.7 | 1 | 27.3 | 144 | 148 | 207 | 28 |
| Mean | 5.03 | 1.00 | 24.57 | 126.33 | 131.33 | 258.67 | 32.67 |
| SD | 0.25 | 0.00 | 5.91 | 25.11 | 26.11 | 69.84 | 7.23 |
| CV | 4.96% | 0.00% | 24.07% | 19.87% | 19.88% | 27.00% | 22.15% |

# Case Study-Robust TA-dependent CD3 Activation

*ImmuBridge* TCE was compared to a parental CD3 monoclonal antibody (mAb) and a bispecific T cell engager (BiTE) antibody to assess its functional efficacy. Experiments assessed T cell activation signals and TA-dependent CD3 engagement using *in vitro* assays such as flow cytometry or luminescence-based activation readouts.




The results demonstrated that the *ImmuBridge* TCE generated a **stronger activation signal** compared to both the parental CD3 mAb and the BiTE, indicating enhanced T-cell engagement.

Additionally, the *ImmuBridge* TCE exhibited **robust TA-dependent CD3 activation**, highlighting its specificity and potency in targeting tumor cells while minimizing off-target effects.

# Collaboration Opportunities

## Unlock the Future of Multispecific Antibody Therapeutics with *ImmuBridge™*

At Protheragen, we invite visionary partners and innovators to join us in revolutionizing the field of antibody therapeutics with our advanced *ImmuBridge™* platform. Whether you are looking to leverage our advanced platform for next-generation drug discovery or seeking custom development services for multispecific antibody candidates, we are here to help accelerate your research journey.


info@protheragen.us

www.immubridge.com

1-631-533-2057

101-4 Colin Dr, Holbrook, NY 11741, USA

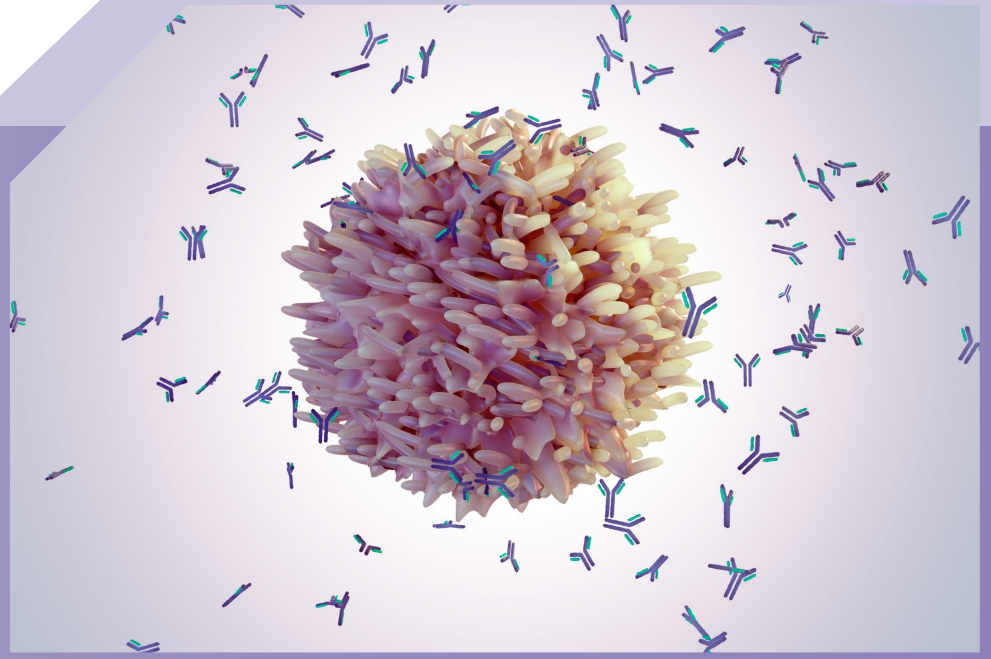
# Pipeline

Using the *ImmuBridge™* platform, Protheragen has established multiple research pipelines that can be licensed out, helping customers access rich research resources and accelerate the progress of breakthrough antibody therapeutic solutions.

| Projects | Antibody | Indication | Discovery | Preclinical | IND | Clinical |
|---|---|---|---|---|---|---|
| MSAD001 | HER2/CD3 | Breast and gastric cancers | | | | |
| MSAD004 | EpCAM/CD3 | Colorectal, breast, pancreatic, and ovarian cancers | | | | |
| MSAD007 | CD19/CD3 | Leukemias and lymphomas | | | | |
| MSAD009 | HER2/CD3/CD137 | Breast, stomach, and ovarian cancers | | | | |
| MSAD012 | EGFR/CD3/CD137 | non-small cell lung cancer and colorectal cancer | | | | |
| MSAD016 | CD19/CD3/CD137 | B-cell lymphomas, acute lymphoblastic leukemia | | | | |
| MSAD020 | CEA/CD3/CD137 | Colorectal, gastric and pancreatic cancer | | | | |
| MSAD023 | HER2/CD3/CD137/PD-L1 | breast, gastric, esophageal and ovarian cancer | | | | |
| MSAD025 | EGFR/CD3/CD137/PD-L1 | non-small cell lung cancer, colorectal cancer | | | | |
| MSAD028 | CEA/CD3/CD137/PD-L1 | colorectal, gastric, pancreatic and lung cancer | | | | |
| MSAD029 | MUC1/CD3/CD137/PD-L1 | breast, pancreatic, ovarian and lung cancer | | | | |
| MSAD031 | EpCAM/CD3/CD137/PD-L1 | colorectal, breast, pancreatic and ovarian cancer | | | | |
| MSAD034 | CD19/CD3/CD137/PD-L1 | non-Hodgkin lymphoma, chronic lymphocytic leukemia | | | | |

Protheragen
ImmuBridge™

# Your Trusted Partner in Next-Generation Multispecific Antibody Drug Development



## Contact Us


info@protheragen.us

1-631-533-2057

www.immubridge.com

101-4 Colin Dr, Holbrook, NY 11741, USA