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BISPECIFIC ANTIBODIES: MECHANISM OVERVIEW & DEVELOPMENT CHALLENGES

Kishore Hotha, PhD, MBA

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Bispecific Antibodies: Overview

Traditional antibody therapies are designed to target a single antigen. However, many complex diseases are driven by multiple factors, so inhibiting a single target may fail to achieve significant efficacy. For example, in some diseases, cells may respond to the inhibition of one receptor by producing more of a second receptor to circumvent the impact of the drug.

Bispecific antibodies aim to treat multifaceted diseases by engaging two disease targets with one molecule. While natural antibodies have two targeting arms that bind to the same antigen, bispecific antibodies are engineered hybrid molecules with two distinct binding domains that target two different antigens.

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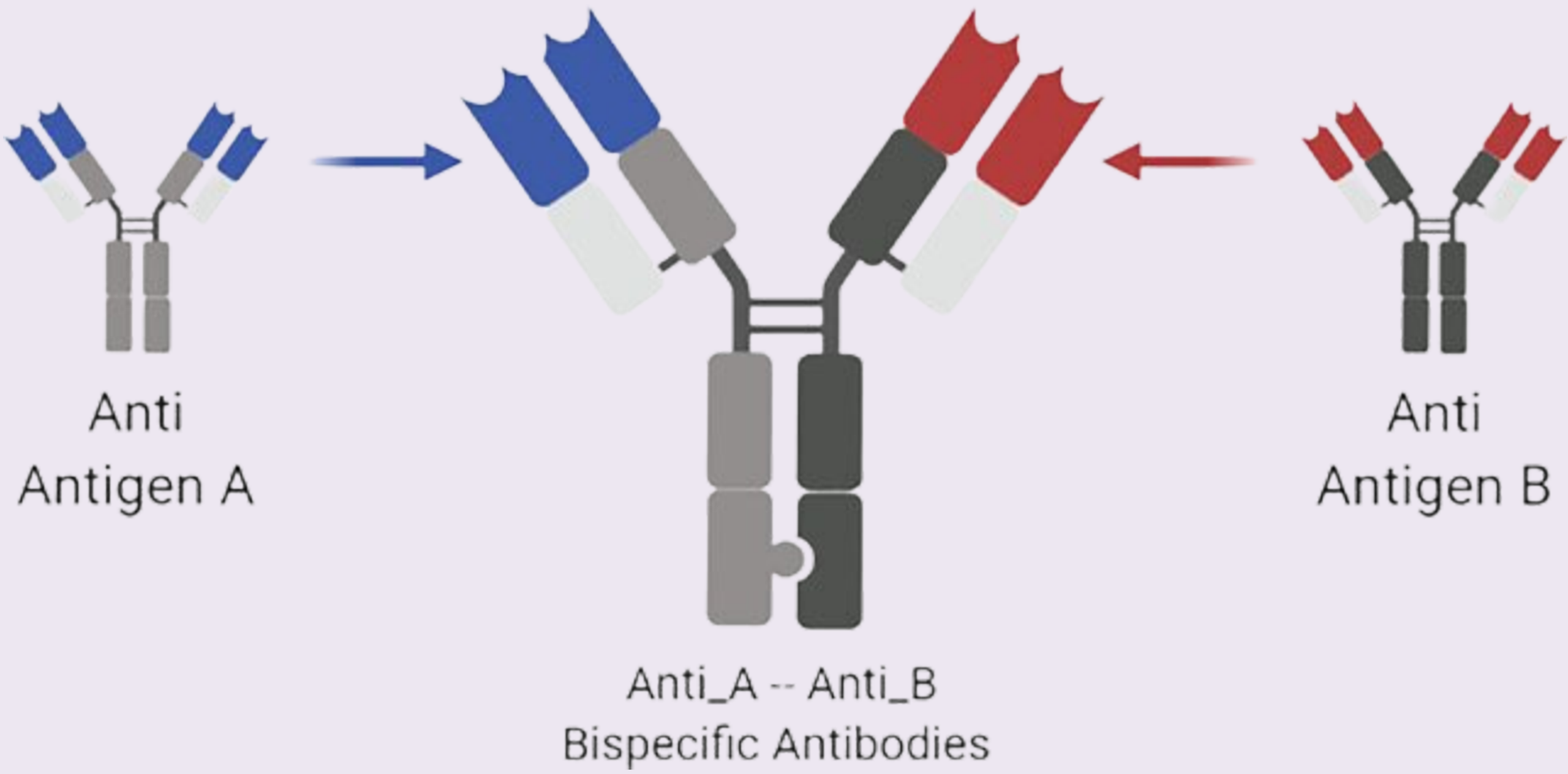
Mechanism of Action of Bispecific Antibodies

Bispecific antibodies (BsAbs) are engineered proteins capable of simultaneously binding to two distinct antigens or epitopes. This dual-binding ability enables them to perform unique therapeutic functions, particularly in oncology, by directing immune cells to target and eliminate cancer cells.

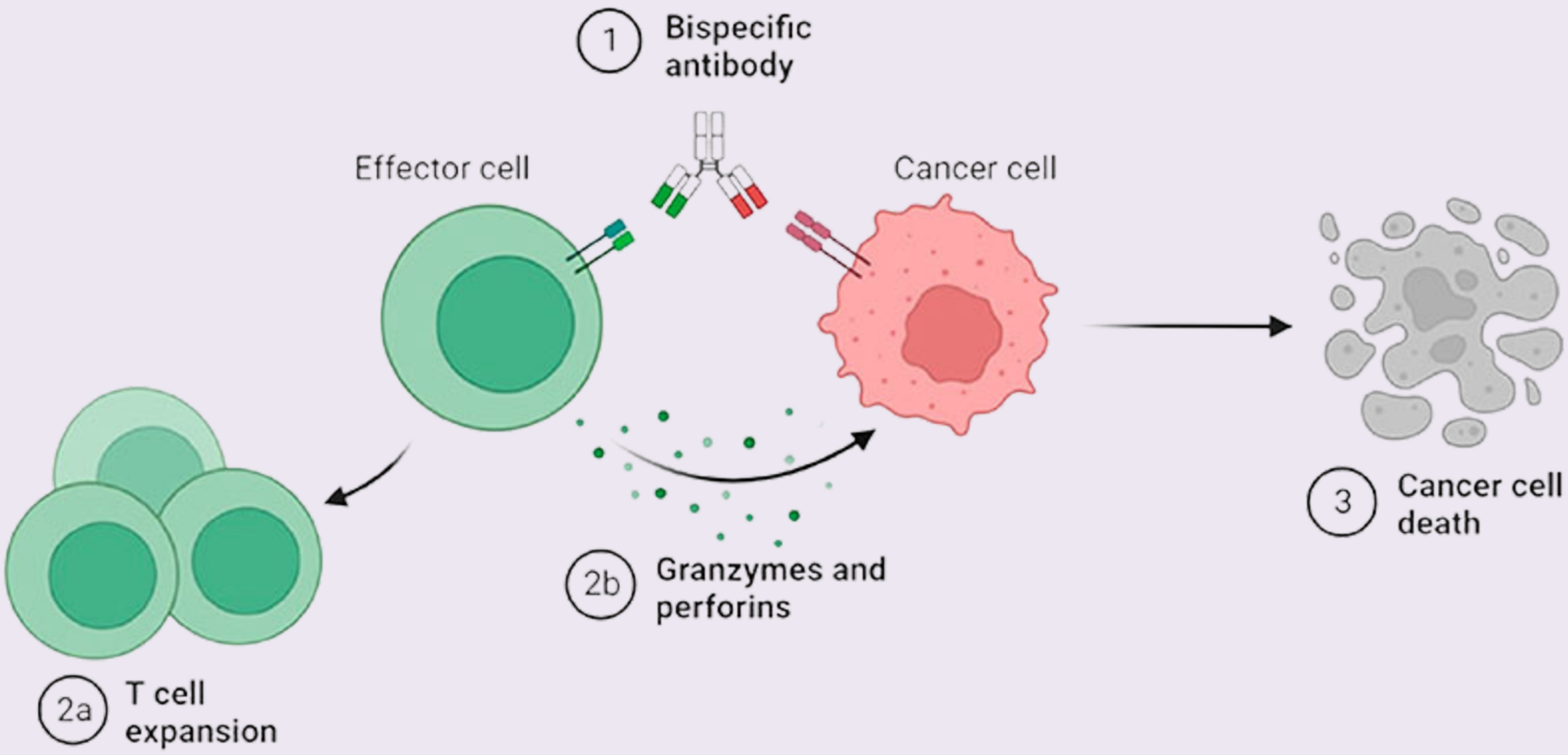


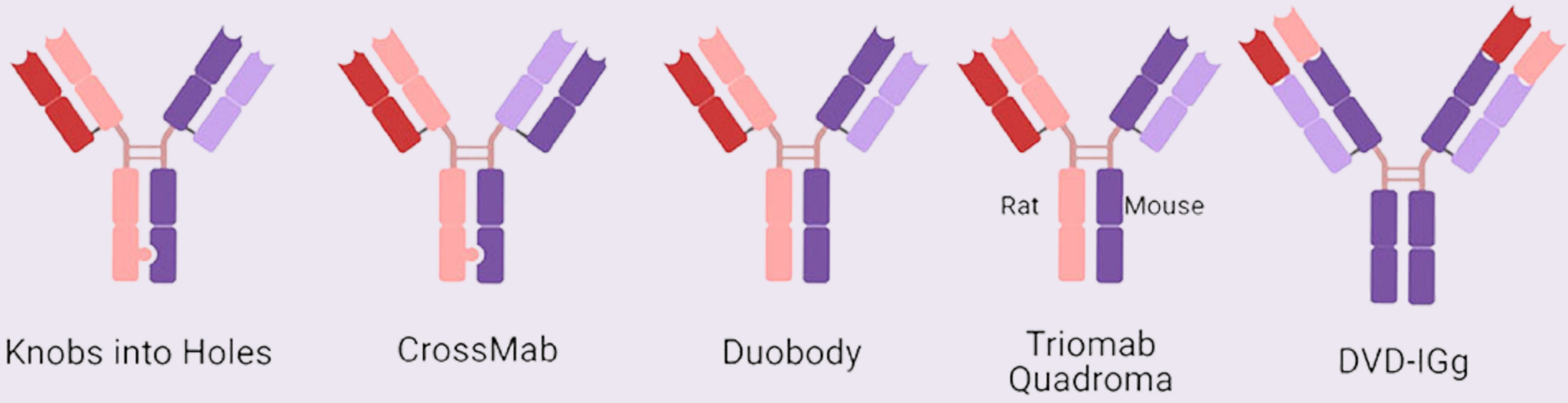
June 2022. Leukemia Research Reports. DOI: 10.1016/j.lrr.2022.100335. Licensed under CC BY-NC-ND 4.0. Lab: Sami El Khatib's Lab.

Mechanism of Action:

BsAbs can engage two different targets, such as a tumor-associated antigen on cancer cells and a receptor on immune cells. This dual engagement facilitates the formation of a bridge between immune cells and cancer cells, promoting the immune system's ability to recognize and destroy malignant cells. For instance, bispecific T-cell engagers (BiTEs) connect T cells to tumor cells, leading to T-cell-mediated cytotoxicity.

Mechanism of Action of Bispecific Antibodies





Reference:
Biointron. (n.d.). Bispecific Antibodies: Design, Development, and Therapeutic Potential. Retrieved from https://www.biointron.com/blog/bispecific-antibodies-design-development-and-therapeutic-potential.html

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FDA-Approved Bispecific Antibodies

TRADE NAME (GENERIC NAME) - APPROVAL YEAR	LINKER	PAYLOAD	MECHANISM OF ACTION
Blincyto (blinatumomab) - 2014	Non-cleavable peptide linker	T-cell engager	Redirects T-cells to target CD19+ leukemia cells
Hemlibra (emicizumab-kxwh) - 2017	IgG-like Fc region	None	Mimics Factor VIII for blood clotting
Rybrevant (amivantamab-vmjw) - 2021	IgG1-based bispecific format	None	Inhibits EGFR and MET signaling in cancer cells
Kimmtrak (tebentafusp-tebn) - 2022	Non-cleavable peptide linker	T-cell engager	Directs T-cells to gp100+ melanoma cells
Vabysmo (faricimab-svoa) - 2022	IgG-like bispecific format	None	Dual inhibition of VEGF-A and ANG-2

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FDA-Approved Bispecific Antibodies

TRADE NAME (GENERIC NAME) - APPROVAL YEAR	LINKER	PAYLOAD	MECHANISM OF ACTION
Tecvayli (teclistamab-cqyv) - 2022	Peptide linker	T-cell engager	Targets T-cells to BCMA+ myeloma cells
Lunsumio (mosunetuzumab-axgb) - 2022	Peptide linker	T-cell engager	Directs T-cells to CD20+ lymphoma cells
Epkinly (epcoritamab-bysp) - 2023	Peptide linker	T-cell engager	Engages T-cells to attack CD20+ lymphoma cells
Columvi (glofitamab) - 2023	Peptide linker	T-cell engager	Engages T-cells to target CD20+ B-cell lymphoma cells
Talvey (talquetamab-tgvs) - 2023	Peptide linker	T-cell engager	Targets T-cells to GPRC5D+ myeloma cells
Elrexfio (elranatamab-bcmm) - 2023	Peptide linker	T-cell engager	Redirects T-cells to BCMA+ myeloma cells
Imdelltra (tarlatamab-dlle) - 2024	Peptide linker	T-cell engager	Directs T-cells to target DLL3+ small cell lung cancer cells

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Development Challenges

Target Selection and Screening:

Identifying appropriate target pairings is essential for bsAb effectiveness. Early-stage screening often uses high-throughput (HTP) methods to explore numerous bsAb candidates, but ensuring consistent quality across large panels remains challenging. A balance between speed and accuracy is necessary, as these screenings must efficiently eliminate non-viable candidates without compromising on depth of analysis.

Chain Pairing and Assembly:

Achieving correct chain pairing is a significant hurdle. Technologies like “knob-into-hole” mutations and CrossMab engineering are utilized to ensure accurate pairing of heavy and light chains, but these processes require careful optimization. Incorrect pairing leads to impurities and reduced yield, adding time and cost to the development process.

Modularity and Flexibility:

bsAbs often use modular DNA designs to create adaptable formats, but this approach increases the risk of structural instability and requires robust quality control measures. Fragment-based approaches facilitate flexibility but complicate the downstream purification steps

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Manufacturing Challenges

Purity &Yield:

BsAb manufacturing demands high purity levels, which are difficult to achieve due to the propensity for aggregation and the formation of undesired side products. Techniques such as mixed-mode size exclusion chromatography (mmSEC) are often employed, but these methods can be costly and time-intensive when scaling up

Cell Line Development:

Establishing robust cell lines that can produce bsAbs efficiently remains a bottleneck. Gene integration and protein expression in host cell lines are more complex for bsAbs than mAbs, often resulting in lower yields and heterogeneous purity profiles

Scalability & Cost:

Unlike traditional mAbs, bsAbs require additional purification steps to remove mispaired chains and impurities, driving up production costs. Automated processes, including robotic plate handling and integration of analytical techniques, are essential to make bsAb production scalable, yet these require significant upfront investment

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Testing Challenges for Bispecific Antibodies

Analytical Validation:

BsAbs require more comprehensive testing than mAbs due to their dual-target nature. High-resolution mass spectrometry (MS) and advanced biophysical characterization are essential for confirming bsAb stability and function. However, these methods are complex and may not always be compatible with high-throughput formats

Biophysical Stability:

Ensuring that bsAbs remain stable under physiological conditions is critical, especially for those targeting T-cells or engaging in immune-mediated cytotoxicity. Forced degradation studies and thermal stability testing are often needed to identify and address vulnerabilities early in development

Regulatory Compliance:

The FDA and EMA require detailed bsAb characterization that aligns with monoclonal antibody standards. Achieving this level of scrutiny involves extensive documentation and rigorous testing, especially for post-translational modifications and aggregation risks

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Innovative Strategies in Bispecific Antibody Production and Development

High-Throughput Production:

Modern strategies emphasize rapid, high-throughput (HTP) screening to evaluate large numbers of bsAbs at early stages. This method facilitates diverse selection, maximizing the chances of identifying effective therapeutic candidates

Combinatorial Screening:

By combining various antibody fragments into modular structures, researchers create extensive panels of bsAbs. This approach is resource-efficient, allowing large-scale testing with fewer starting materials

Chemical Conjugation:

New conjugation techniques, such as cysteine-specific linkages, improve bsAb stability without toxic reagents. This chemical method creates stable bonds between antibody fragments, streamlining the production process

Chain Pairing Technologies:

Techniques like the "knob-in-hole" approach are improving correct chain pairing in bsAbs. These advances enhance purity by driving specific heavy and light chain interactions, simplifying purification and making production more efficient

Recombinant DNA Methods:

Using recombinant DNA to link antibody fragments minimizes the need for separate expression of each bsAb. This "plug and play" approach increases stability and can produce diverse panels for early-stage testing

Automated Production Workflows:

End-to-end automated platforms integrate every stage of bsAb production, from expression to purification. These systems manage large sample volumes and improve production efficiency significantly

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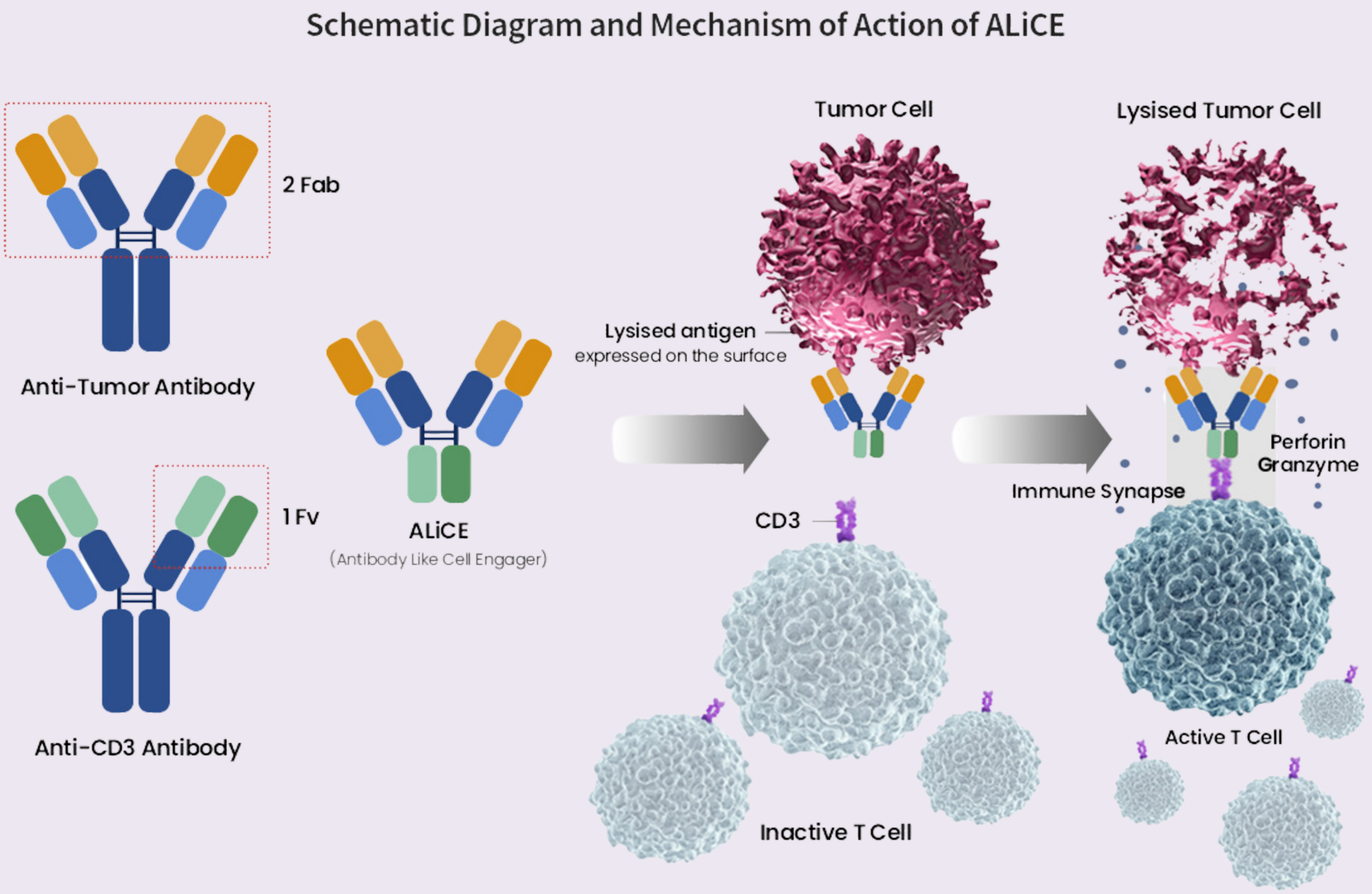
ALiCE Technology: A Novel Approach in Immuno-Oncology

There are several innovative technologies in the field of immuno-oncology that aim to enhance the precision and effectiveness of cancer treatments. Among these, bispecific T-cell engagers represent a powerful class of therapeutics that bring immune cells directly into contact with cancer cells, promoting a targeted immune response. Unlike traditional antibodies, bispecific antibodies are engineered to simultaneously bind to two distinct targets – typically an antigen on a cancer cell and a receptor on an immune cell – enabling a coordinated attack on cancer cells while minimizing damage to healthy tissues. Technologies like ALiCE are at the forefront, designed to enhance binding selectivity, optimize immune synapse formation, and reduce side effects, thereby addressing unmet needs in cancer immunotherapy.



Reference:

https://ybiologics.com/en/sub/tech/alice.php
Satisfying Unmet Medical Needs by Preventing Immune Escape and Specifically Recognizing and Killing Only Tumor Cells

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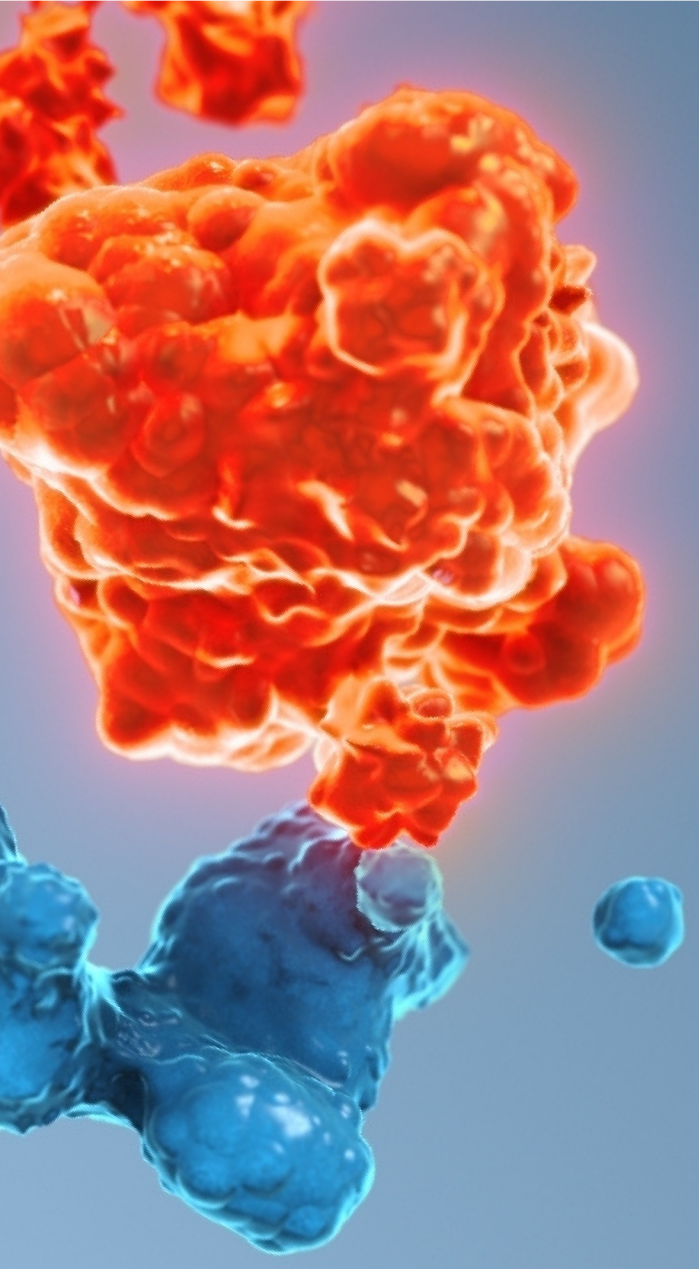

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email: contact@drhothas.com

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