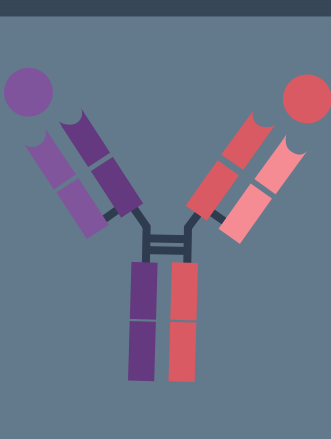
BioTechniques In Focus

Sino Biological

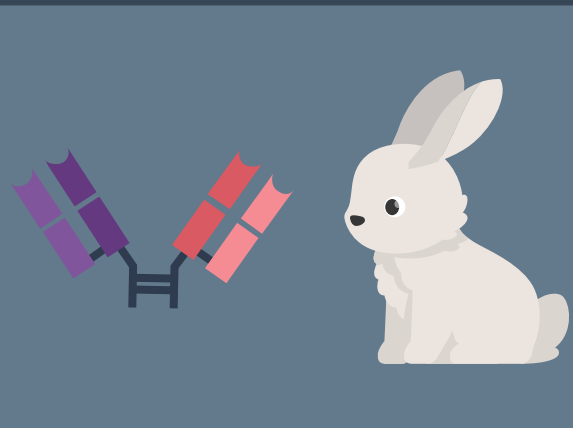
Next-generation antibodies for therapeutic applications

Advances in antibody engineering have led to the expansion of the use of a next generation of antibodies in therapeutic design, with some commercially approved and many more in preclinical and clinical development. Next-generation antibodies are designed to be more specific and more potent than conventional monoclonal antibodies and include bispecific antibodies (bsAbs), antibody–drug conjugates (ADCs) and nanobodies. This infographic reviews the structure, mechanisms, history and clinical performance of these next-generation antibodies, so you can compare, contrast and select the optimal approach for your drug discovery and development programs.

BsAbs



BsAbs consist of two distinct antigen-binding sites that recognize different epitopes from the same or two different antigens, enabling dual targeting.



They were conceptualized in 1960 and first demonstrated in 1964 by linking two antigen-binding fragments (Fabs) from rabbit antibodies.

They deliver enhanced specificity and higher potency compared to traditional monoclonal antibodies.

They enable novel approaches for drug design.

BsAb structure

There are two subgroups of bsAbs, differentiated by their possession of an Fc region. A selection of bsAb formats are shown below.

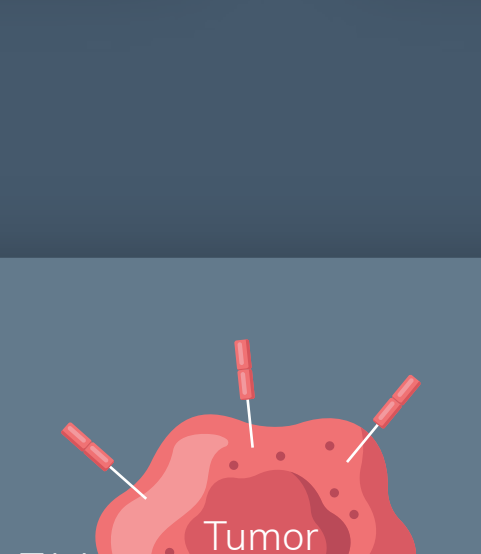
IgG-like constructs that possess an Fc region:

Asymmetric bsAbs possess heavy and light chains from two different parental antibodies and closely mimic the native Y-shaped IgG.

Knobs-into-holes (KIH)

Or

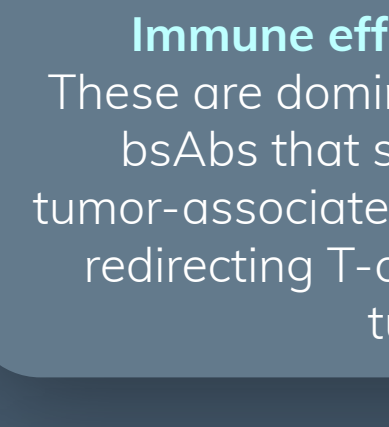
Symmetric bsAbs contain additional binding sites with an appended or modified IgG-like structure.



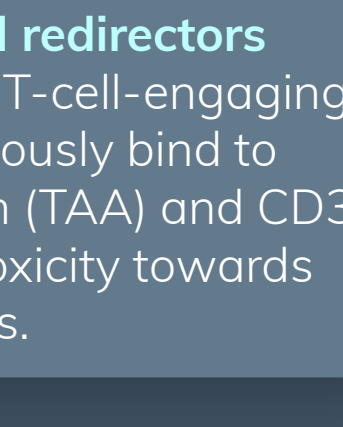
DVD-Ig

Non-IgG-like fragments that lack an Fc region:

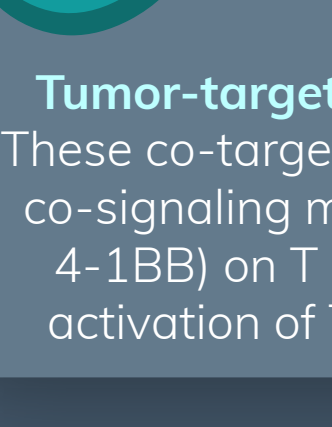
That are developed by combining multiple antigen-binding moieties (antibody fragments) in one molecule.



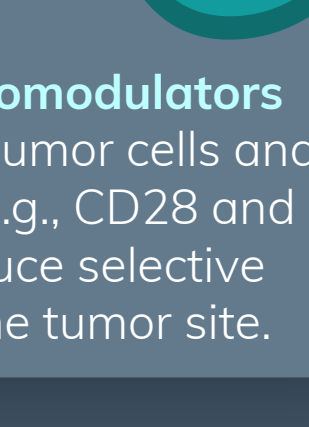
BiTE (Tandem scFv)



F(ab')$_2$

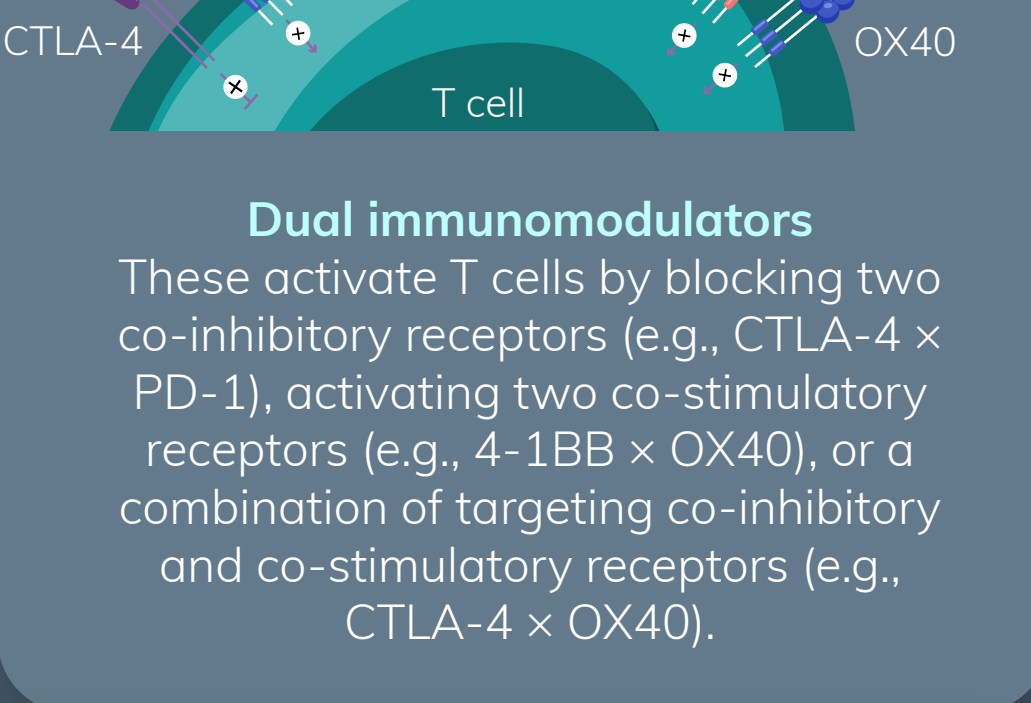


DART

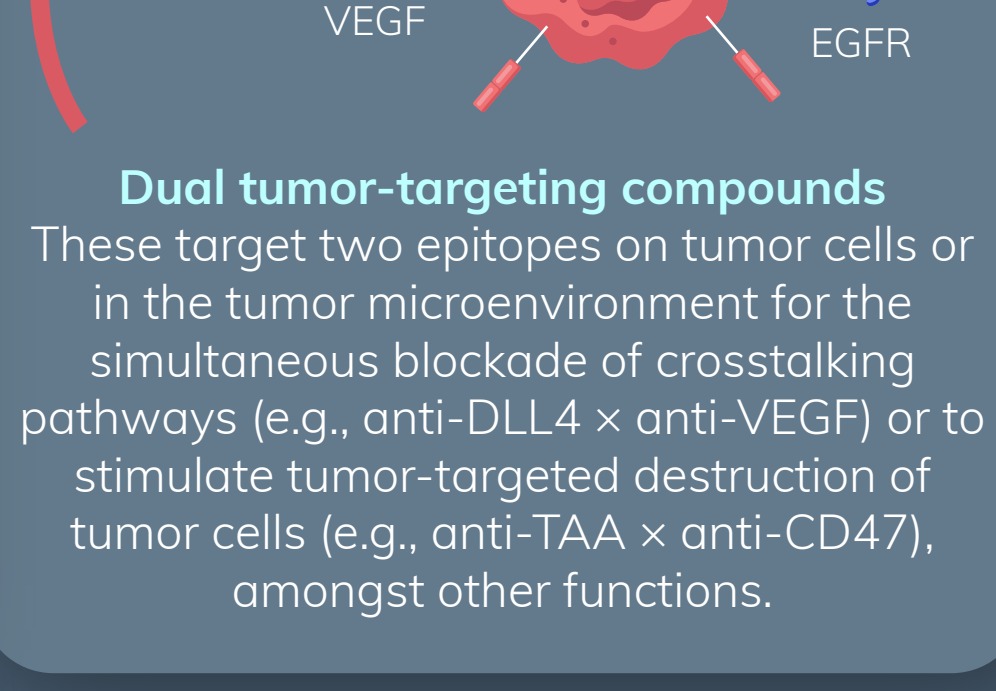


Diabody

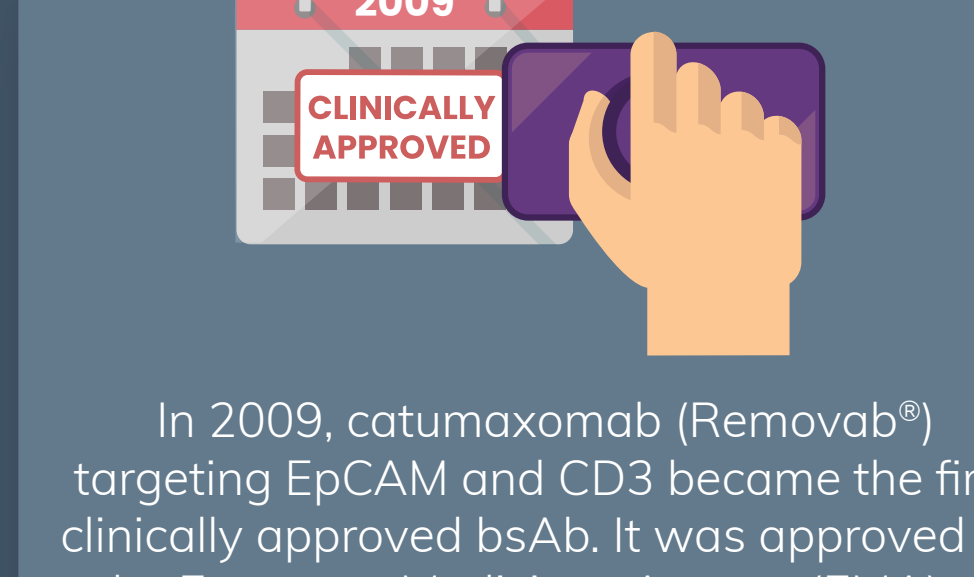
Mechanisms of action



Immune effector cell redirectors
These are dominated by T-cell-engaging bsAbs that simultaneously bind to tumor-associated antigen (TAA) and CD3, redirecting T-cell cytotoxicity towards tumor cells.



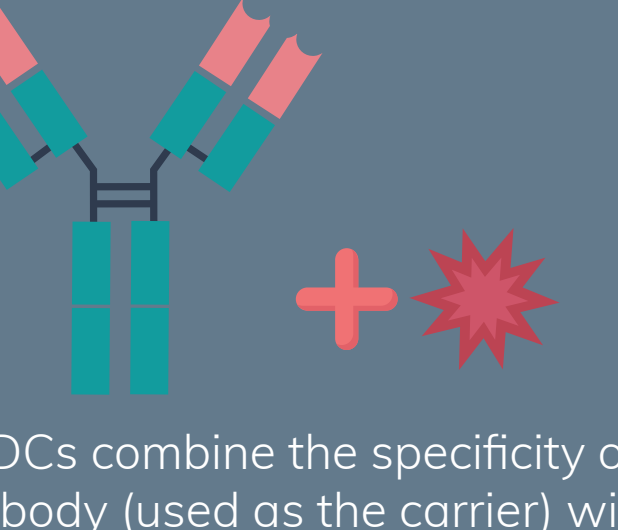
Tumor-targeted immunomodulators
These co-target TAAs on tumor cells and co-signaling molecules (e.g., CD28 and 4-1BB) on T cells to induce selective activation of T cells at the tumor site.



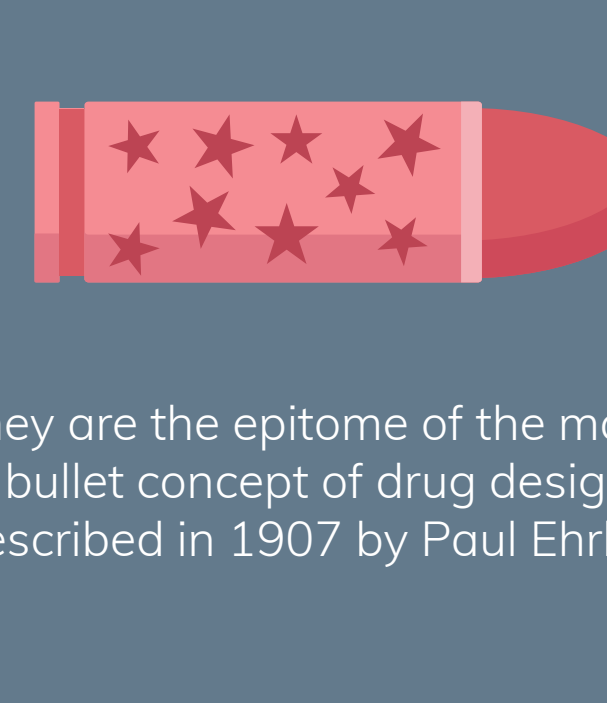
Dual immunomodulators
These activate T cells by blocking two co-inhibitory receptors (e.g., CTLA-4 × PD-1), activating two co-stimulatory receptors (e.g., 4-1BB × OX40), or a combination of targeting co-inhibitory and co-stimulatory receptors (e.g., CTLA-4 × OX40).

Dual tumor-targeting compounds
These target two epitopes on tumor cells or in the tumor microenvironment for the simultaneous blockade of crosstalking pathways (e.g., anti-DLL4 × anti-VEGF) or to stimulate tumor-targeted destruction of tumor cells (e.g., anti-TAA × anti-CD47), amongst other functions.

Clinical performance

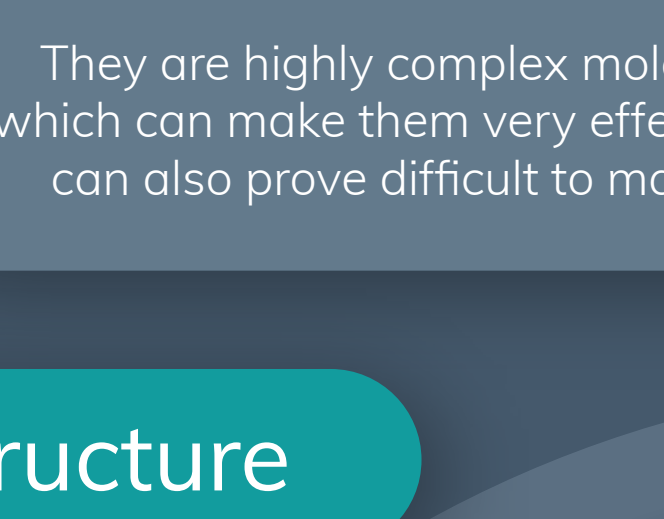


In 2009, catumaxomab (Removab®) targeting EpCAM and CD3 became the first clinically approved bsAb. It was approved by the European Medicines Agency (EMA) to treat malignant ascites. Malignant ascites describes the accumulation of ascitic fluid in the peritoneal space as a result of advanced cancer. It was withdrawn in 2017 for commercial reasons.

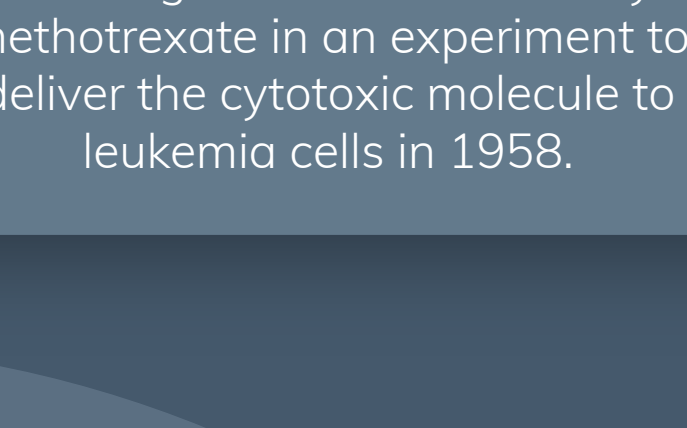


As of 2022, there were ten clinically approved bispecific antibody therapeutics worldwide.

ADCs

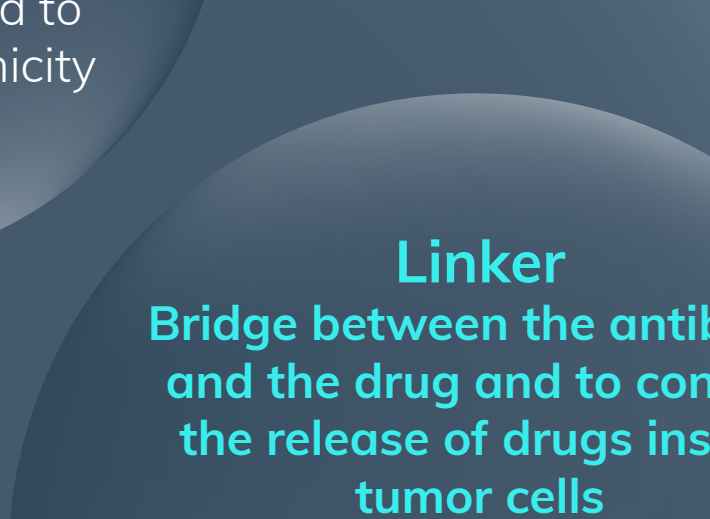


ADCs combine the specificity of an antibody (used as the carrier) with the potency of a highly cytotoxic chemical agent (known as the payload) to enable "targeted chemotherapy".



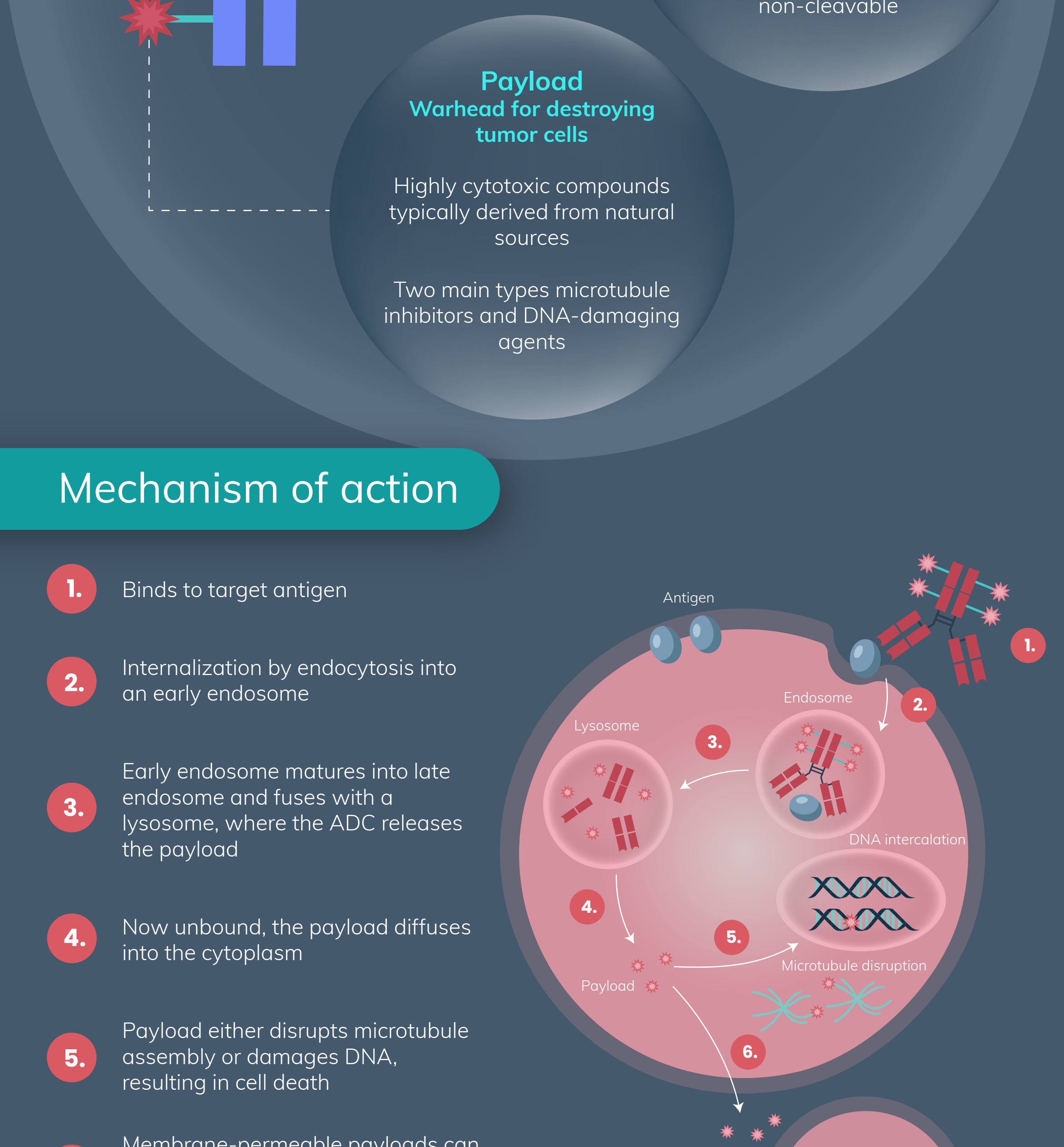
They are the epitome of the magic bullet concept of drug design described in 1907 by Paul Ehrlich.

They are highly complex molecules, which can make them very effective but can also prove difficult to manage.



The first drug linked to an antibody was methotrexate in an experiment to deliver the cytotoxic molecule to leukemia cells in 1958.

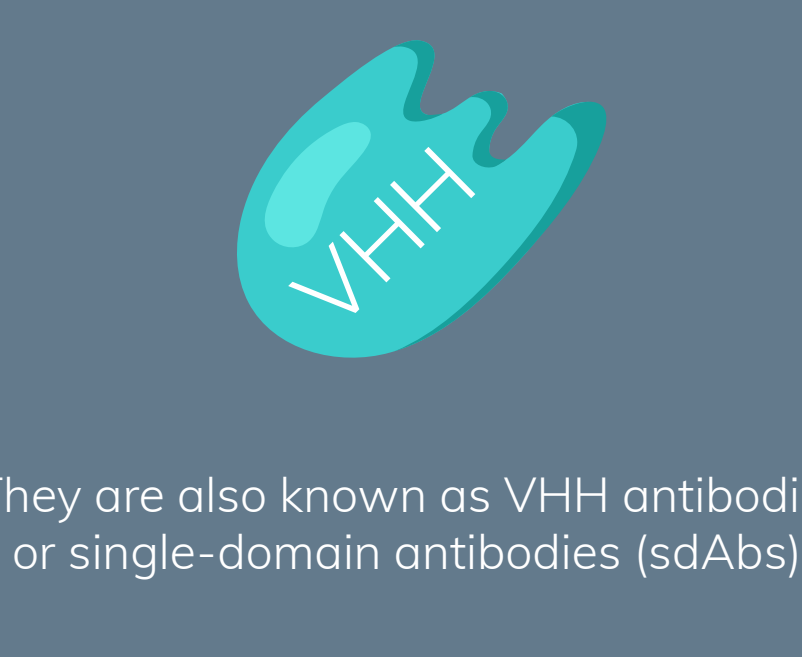
Structure



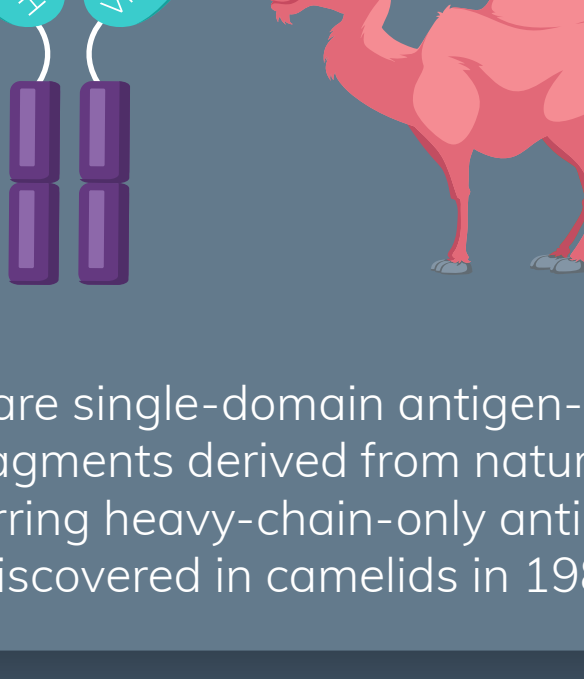
Mechanism of action

1. Binds to target antigen
2. Internalization by endocytosis into an early endosome
3. Early endosome matures into late endosome and fuses with a lysosome, where the ADC releases the payload
4. Now unbound, the payload diffuses into the cytoplasm
5. Payload either disrupts microtubule assembly or damages DNA, resulting in cell death
6. Membrane-permeable payloads can also diffuse into neighbouring cells and elicit bystander killing

Clinical performance

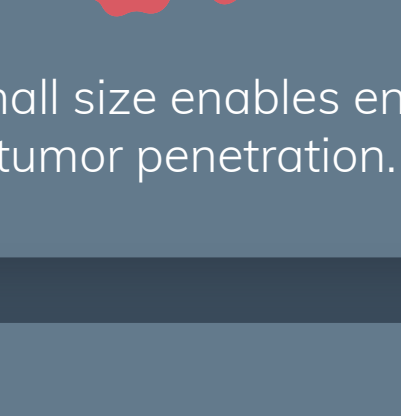


In 2000, gemtuzumab ozogamicin (Mylotarg®), which combines an anti-CD33 antibody with a toxic calicheamicin, became the first ADC approved. It received approval from the U.S. FDA for acute myeloid leukemia before being withdrawn in 2010 and re-approved in 2017.

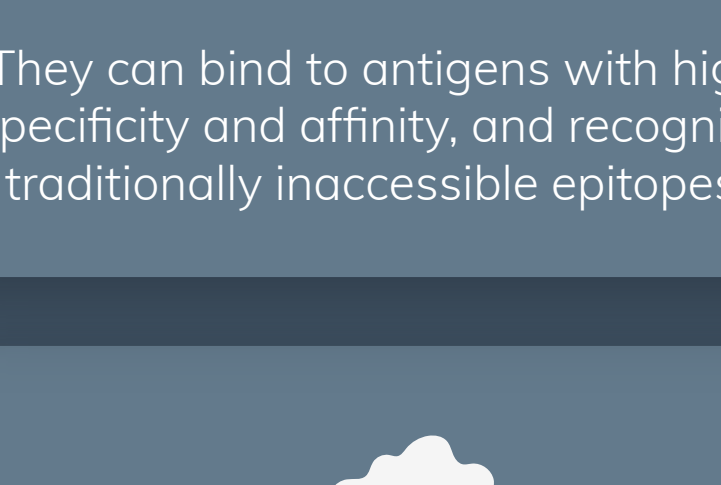


As of 2022, 15 ADCs have been approved worldwide.

Nanobodies

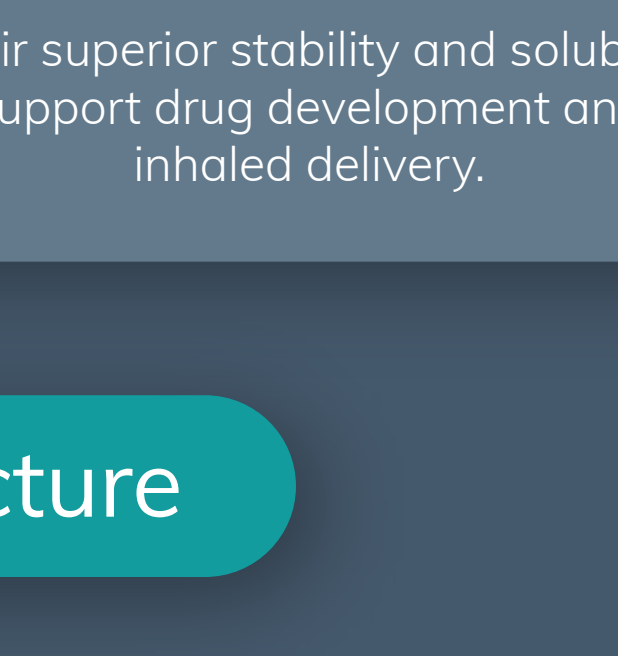


They are also known as VHH antibodies or single-domain antibodies (sdAbs).

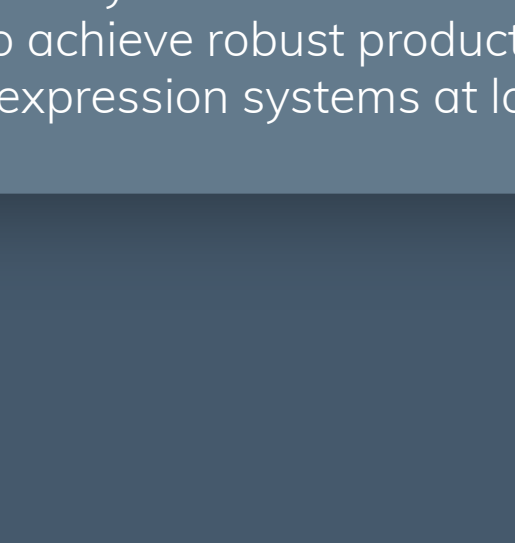


They are single-domain antigen-binding fragments derived from naturally occurring heavy-chain-only antibodies discovered in camelids in 1989.

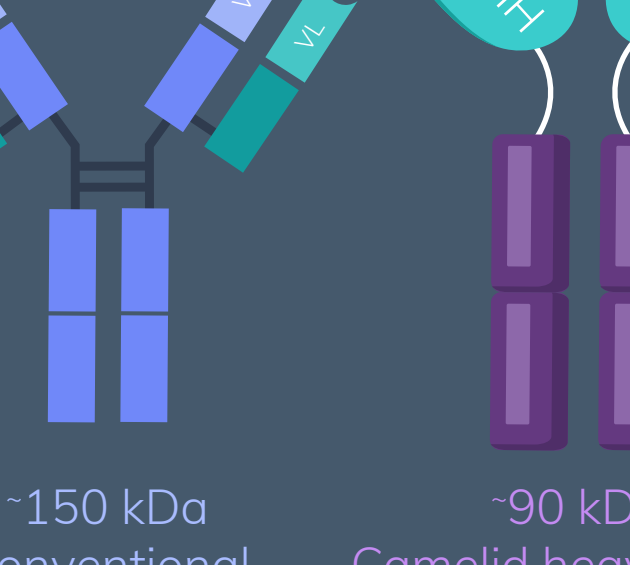
There are many advantages to nanobodies:



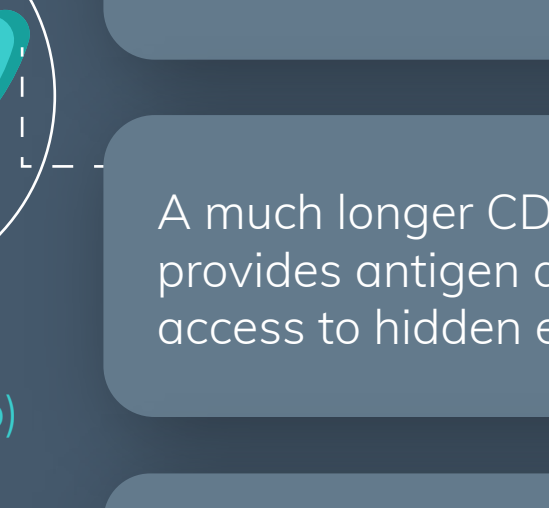
Their small size enables enhanced tumor penetration.



They can bind to antigens with high specificity and affinity, and recognize traditionally inaccessible epitopes.

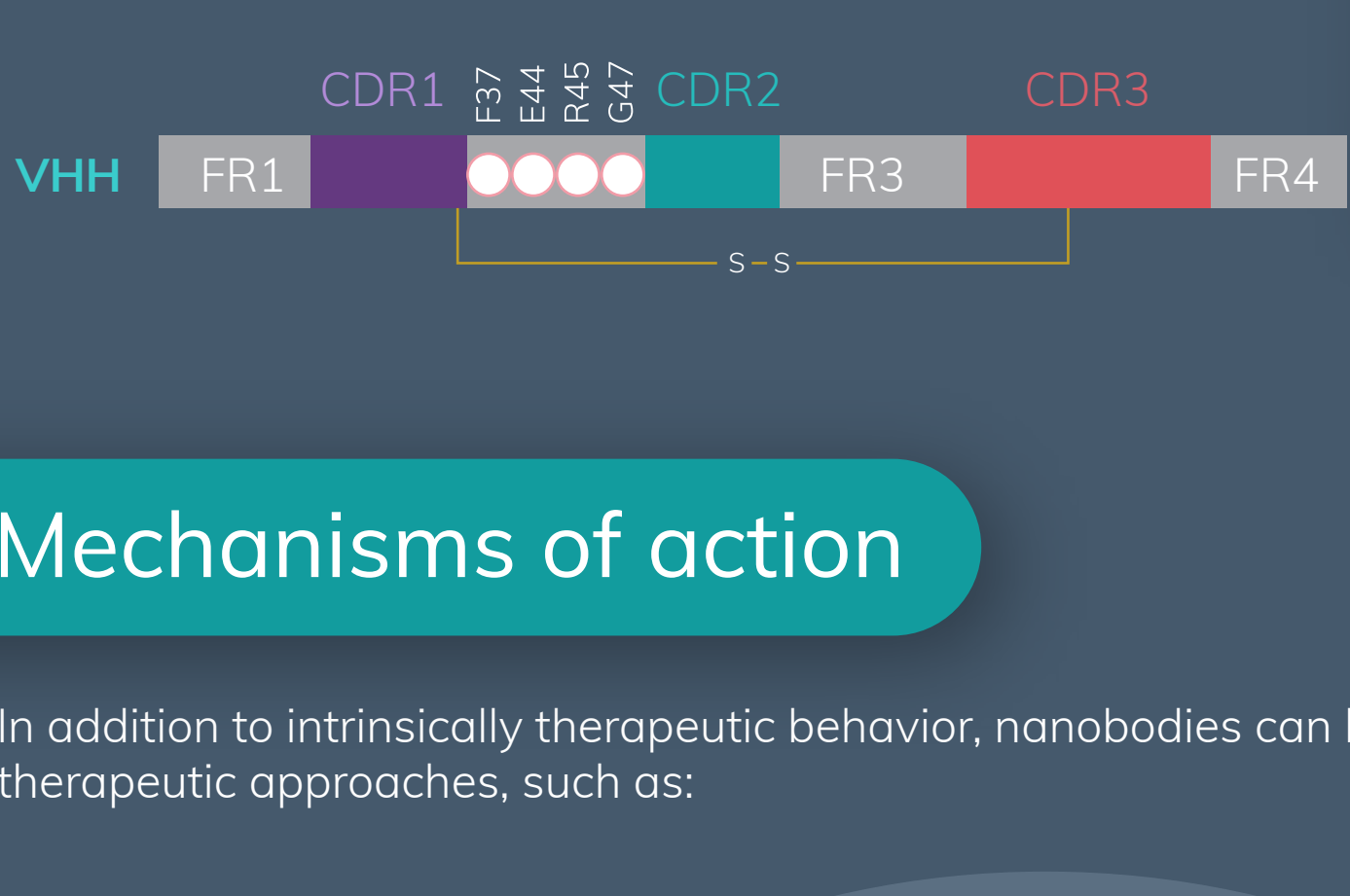


Their superior stability and solubility support drug development and inhaled delivery.



They are easy to manufacture and it is possible to achieve robust production from different expression systems at low cost.

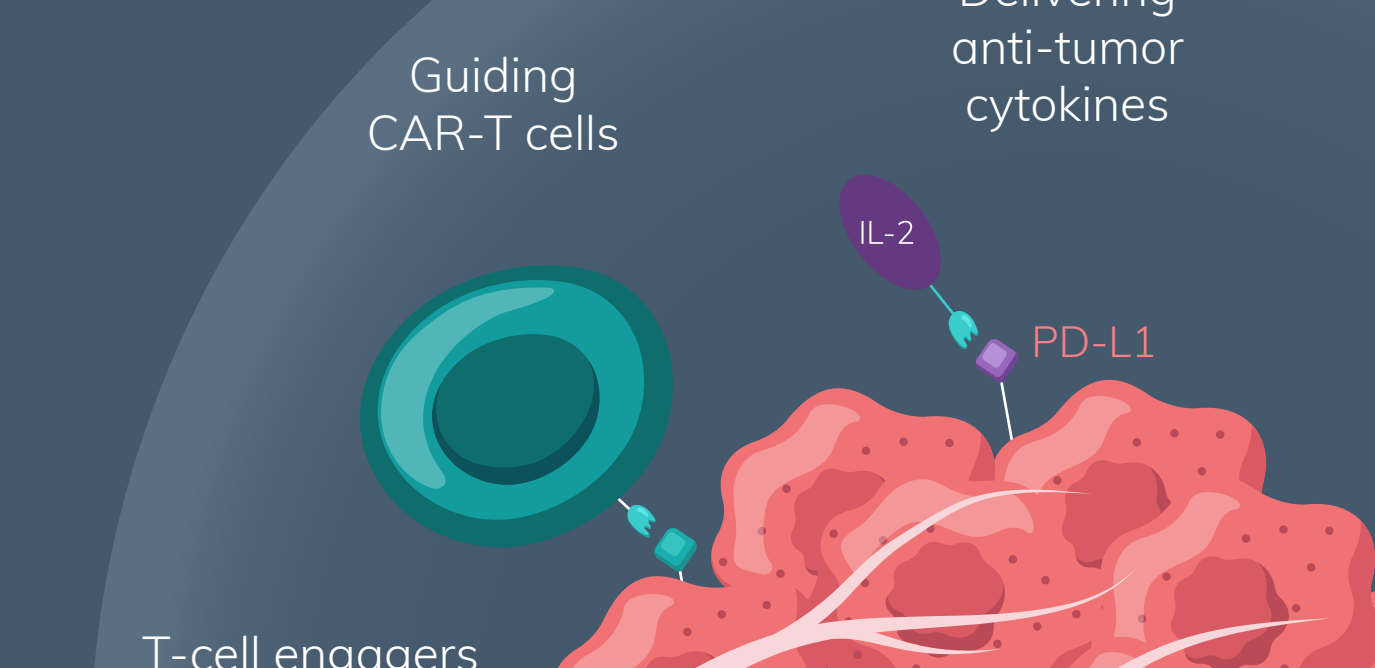
Structure



The heavy-chain variable domain of camelid hcAbs

A much longer CDR3 loop provides antigen affinity and access to hidden epitopes

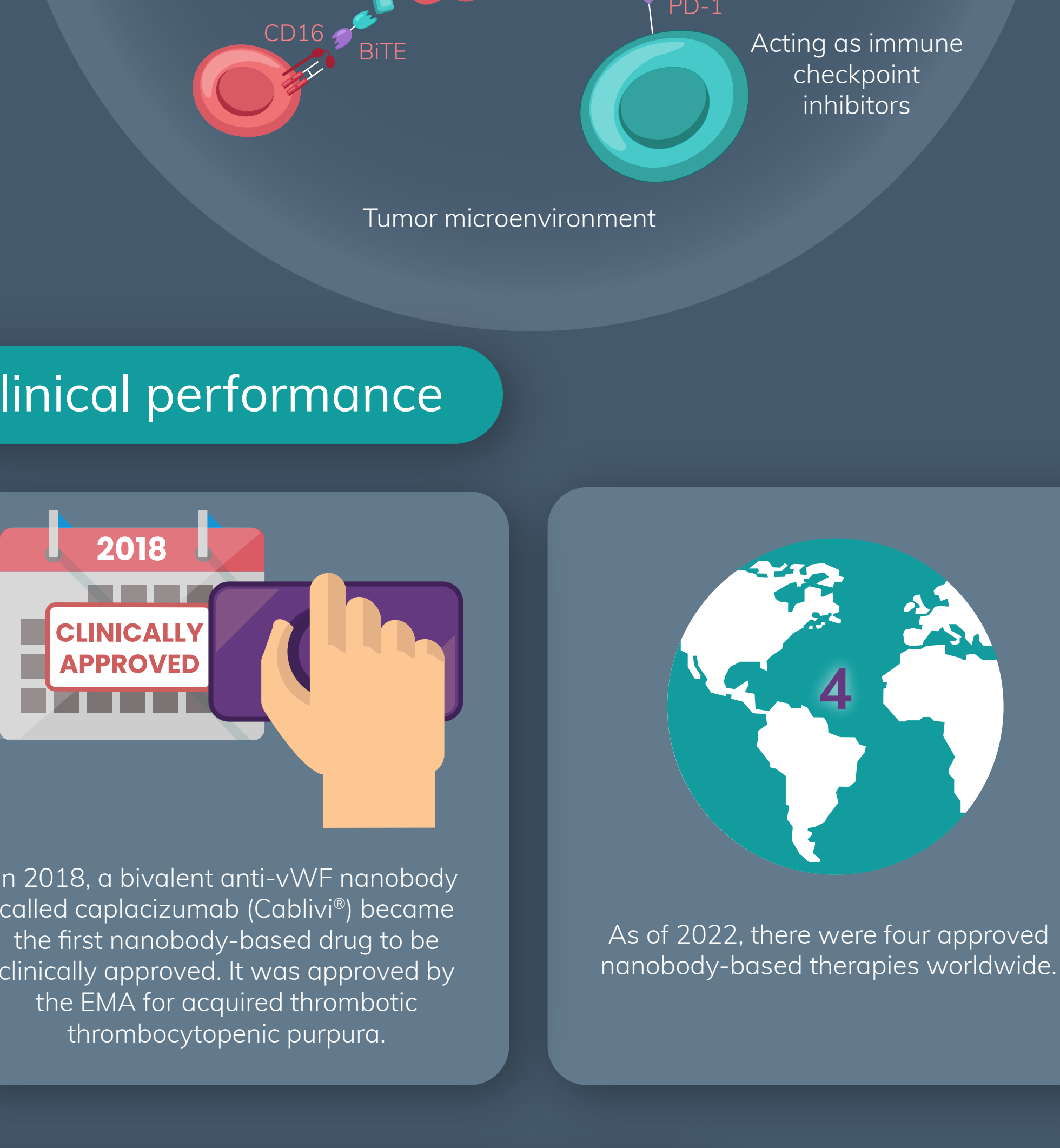
Smallest-known functional antibody fragments



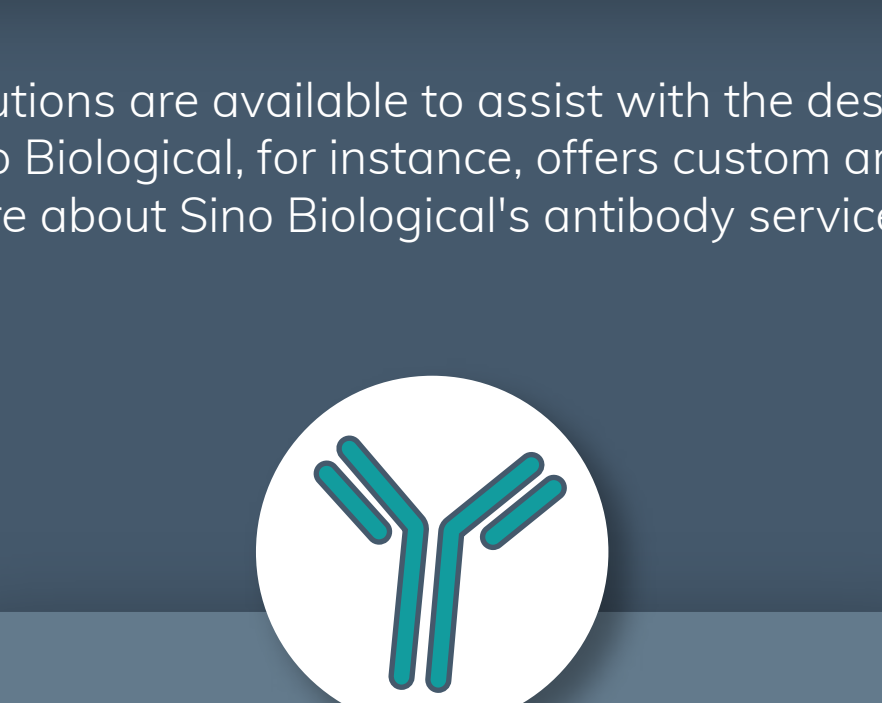
The four highly conserved hydrophobic residues found in the FR2 region of a human VH are replaced by hydrophilic residues, making the nanobody highly soluble.

Mechanisms of action

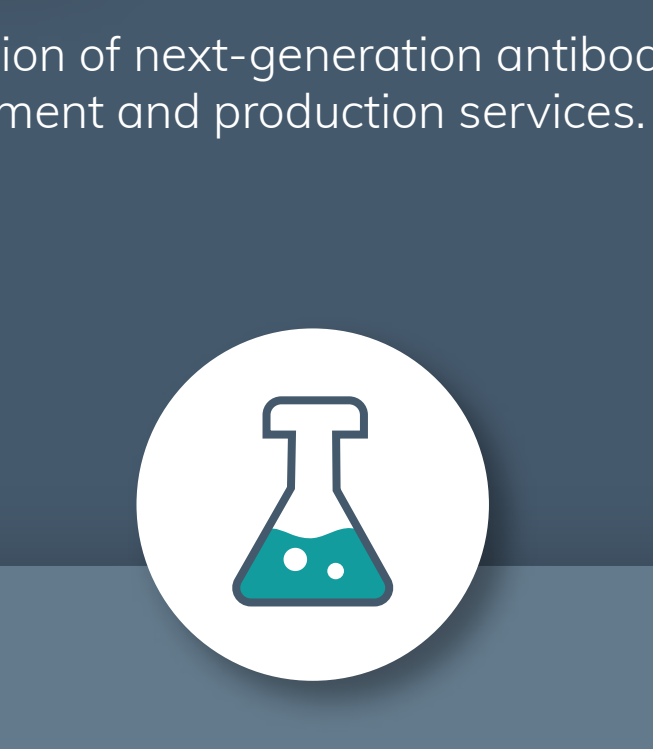
In addition to intrinsically therapeutic behavior, nanobodies can help augment other therapeutic approaches, such as:



Clinical performance



In 2018, a bivalent anti-vWF nanobody called caplacizumab (Cablivi®) became the first nanobody-based drug to be clinically approved. It was approved by the EMA for acquired thrombotic thrombocytopenic purpura.



As of 2022, there were four approved nanobody-based therapies worldwide.

What solutions are available?

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