Advanced protein design and engineering techniques have enabled the production of bispecific antibodies, unique antibody constructs that have the ability to bind two targets. This rapidly growing class of therapeutics is being investigated globally in clinical trials with several on the market for a wide variety of cancer types and a diverse spectrum of diseases and conditions such as rheumatoid arthritis, hemophilia, postmenopausal bone loss, plaque psoriasis, and idiopathic pulmonary fibrosis. Specially engineered antibodies with a variety of formats simultaneously bind to two different epitopes on the same antigen or different antigens, increasing selectivity and effectiveness. [1]

Despite the widespread use of monoclonal antibodies for passive cancer immunotherapy, patients often do not respond completely, exhibiting resistance to therapy or tumor recurrence. This is a result of the complexity involved in proliferation and survival of tumor cells, with multiple mutations and multiple and/or redundant pathways involved, including cross-talk between pathways. Bispecific antibodies are uniquely designed to overcome deficiencies in the effectiveness of monoclonal antibodies. By targeting two different receptors on the same cell or by targeting two ligands, the bispecific antibody can block two signaling pathways involved in cancer cell proliferation and the inflammatory response. This multi-targeted approach is highly effective in destroying tumor cells. Bispecific antibodies can also be engineered to guide immune effector cells such as Natural Killer (NK) cells or T-cells to bind tumor cells, leading to their destruction. [1, 2]

Currently in Clinical Development

There are more than 60 different bispecific antibody formats, developed to match a variety of clinical applications. There are 30 plus bispecific antibodies in clinical development as therapeutics for a wide variety of cancer types, as well as several diseases. Many of these bispecific antibodies are designed to redirect T cells to kill tumor cells, while others are designed to bind two different disease mediators (i.e., soluble ligands, cell surface receptors, and other proteins). For several non-oncology applications - rheumatoid
arthritis, idiopathic pulmonary fibrosis, and psoriasis - the bispecific antibodies function as a blockade of pro-inflammatory cytokines. [3]

One of the frontrunners, currently on the market for the treatment of refractory B-cell precursor acute lymphoblastic leukemia, is Amgen’s Blincyto® (blinatumomab). Blincyto simultaneously targets the T-cell surface protein complex CD3 and the tumor-associated antigen CD19, and so it serves as a mediator to redirect immune effector cells to tumor cells, in order to facilitate the destruction of the latter. [1]

Another bispecific antibody approved for therapy is Fresenius Biotech’s catumaxomab (Removab®), for the treatment of malignant ascites. Removab simultaneously targets the epithelial cell adhesion molecule (EpCAM) and CD3. This effectively brings into close proximity tumor cells, which express EpCAM, and T-cells which express CD3. Also, the Fc domain of Removab interacts with other immune system effectors such as natural killer cells, macrophages, and dendritic cells. The close proximity of the tumor cells with the immune cells promotes activation of immune cells, and subsequent destruction of cancer cells. [4]

The two major classes of bispecific antibodies are small single chain Fv (scFv)-based bispecific antibodies and the larger immunoglobulin-G (IgG)-like bispecific antibodies. [2] Each class has unique benefits, but also drawbacks with development potential. The more than 60 formats that fall within these two larger categories vary in many ways including their pharmacokinetic half-life, molecular weight, spatial relationship between different binding sites, number of antigen-binding sites, valency for each antigen, and ability to support secondary immune functions. [1] One of the benefits of the IgG-like bispecific antibodies is that they retain Fc-mediated effector functions, and the Fc region enhances purification, stability and solubility. The larger size of the IgG-like bispecific antibodies results in longer half-lives, while the smaller scFv-based bispecific antibodies have improved tissue penetration.[3]

Improvements Needed in Antibody Engineering and Development

With many companies jockeying for success in clinical trials, there are some critical hurdles to overcome and need for innovation and improvement. Throughout the antibody engineering and development process of new constructs, manufacturability issues such as low expression yields and product instability/short half-life have hindered development. [3]

A big challenge is in rapid discovery of potent lead bispecific antibodies and their targets, which requires a high-throughput approach. There is also a need for rapid purification of clinical-grade bispecific antibodies. Immunogenicity introduces complexities in drug design and development, and adverse effects from immunogenicity, mainly caused by a “cytokine storm,” can stifle clinical trials. [3]

Newer formats with greater potential for developability may overcome these problems. Here we’ll examine the most recent strategies in bispecific antibody engineering.

Optimizing Target/Ligand Interactions

In a powerful new strategy to aid engineering and selection efforts, Rhodin et al. of Lilly Research Laboratories developed mathematical modeling parameters to make predictions about how engineered antibody properties will affect binding to cell surface antigens. [5] Their model encompassed several parameters: antibody affinity, valency, and antigen density.

Intriguingly, the model predicted that for bispecific antibodies, the antigen density has a very strong effect on the binding affinity of the lowest expressing antigen. Rhodin et al. was able to predict the magnitude of this ratio based upon the expression ratio of the two antigens being targeted. To confirm their predictions, they validated experimentally using
bispecific antibodies with different valencies on a variety of cell surface antigens, including EGFR and MET. The authors developed a conceptual model through which antigen density has a strong effect on binding to the less predominant antigen. This occurs with the initial binding event, in which the antibody is more likely to encounter the more highly expressed antigen because of its elevated abundance. After this initial binding event, the second arm is located in a constrained position near the cell surface which allows rapid binding to its target antigen.

Another novel strategy for development and quality control is monitoring target/ligand binding of bispecific antibodies through surface plasmon resonance (SPR). While an enzyme-linked immunosorbent assay (ELISA) is more commonly used to assess ligand binding, it does not provide dynamic output, as only endpoint data can be obtained. In contrast, SPR, which is label-free, allows users to view the dynamics of bispecific antibody binding and dissociation events with two targets. SPR systems such as Biacore (GE Healthcare) are label free, the readout is continuous, and data analysis can be done at multiple time points, or by fitting entire binding curves to determine affinity and kinetic parameters. SPR can detect even weak binding interactions. [6]

**Half-life Extension Strategies**

Small chain (scFv)-based bispecific antibodies have some advantages over the larger sized IgG-like bispecific antibodies, including enhanced tissue penetration as well as easier manufacturing. Their smaller size affords the ability to bind epitopes that may be stERICally inaccessible to IgG-like bispecific antibodies, and they are less immunogenic due to the lack of an Fc region. However, the short half-life of scFv-based bispecific antibodies is a major drawback compared to that of IgG-like bispecific antibodies. A short half-life leads to issues in clinical applications such as fast off-rates, rapid blood clearance, and poor retention time at target sites, which limits potency and increases the number of therapeutic doses required. [3]

One strategy for increasing half-life is to attach flexible, hydrophilic molecules, such as a single PEG chain(s) using a site-directed approach. This effectively increases the hydrodynamic volume, which increases the half-life. [7]

A more widely adopted technique is to fuse scFv-based bispecific antibodies to human serum albumin or alternatively, an albumin-binding moiety. This increases half-life through multiple mechanisms: by increasing the molecule size and promoting the recycling of the bispecific antibodies. Another option for antibody engineers is to fuse an Fc fragment to scFv-based bispecific antibodies. This also has the dual effect of increasing the size of the antibody and promoting recycling. [3]

In another novel approach, human mesenchymal stromal cells (MSCs) can be genetically modified to produce and secrete bispecific antibodies continuously throughout the lifetime of the patient. [8] MSCs have a plethora of beneficial properties which make them uniquely adapted for this purpose. For one, they accumulate next to tumors, including metastatic tumors. They have low levels of immunogenicity. Other features of MSCs are easy transduction by viral vectors, in vitro expansion, and long life in vivo. [8, 9]

**A Wide Spectrum of Upcoming Applications for Bispecific Antibodies**

Beyond the current applications for bispecific antibodies discussed here, there are several other exciting applications now in preclinical development. One unique application for bispecific antibodies is in delivery of therapeutic antibodies across the blood-brain barrier for neurological conditions, as monospecific antibodies are unable to cross this barrier.[10] Another unique application for bispecific antibodies is in the delivery of drug, nanoparticle or radiolabel payloads to tumor sites. The bispecific antibody serves to concentrate these payloads at the tumor site, significantly enhancing serum retention time. [3]
Bispecific antibodies are being developed for use in diagnostic assays, functioning as a cross-linker to bind a reporter molecule and antigen simultaneously. As an example, a monospecific capture antibody can be attached to a solid surface, which is used to capture a target antigen in serum. As a next step, a reporter molecule and bispecific antibody are added, which bind the bound antigen, and thus identification can be made. These bispecific antibody-based immunoassays are in development for diagnosis of patients with various infectious diseases: SARS, hepatitis B, tuberculosis, as well as E. coli infections. [11]

Another application involves tackling the rising threat of antibiotic resistant bacteria through protein engineering. A new bispecific antibody was engineered to be effective against Pseudomonas aeruginosa infections, which causes pneumonia and mortality in cystic fibrosis patients, and is resistant to single epitope antibiotics. This novel bispecific antibody binds with one arm to an extracellular polysaccharide that is important for immune evasion and biofilm formation and with the other arm, to a virulence factor. It’s been proven effective in animal studies, and is now in clinical studies.[12, 13]

Bispecific antibodies have made a rapid entry into the antibody market, with major applications in oncology and a variety of diseases/disorders. The focus in oncology has been in either blocking multiple and redundant signaling pathways involved in oncogenesis or redirecting immune effector cells to be in close proximity to tumor cells. In non-oncology applications, a major developmental effort has gone into blocking pro-inflammatory cytokines. Improvements to the antibody design, engineering and manufacturing process are needed and are being addressed. A plethora of exciting applications are underway in this fast-moving arena.
References


