WHITE PAPER

Antibody-based therapeutic modalities in oncology

Introduction

Cancer remains the leading cause of death worldwide—with estimates of 19.3 million new cases and nearly 10 million deaths due to cancer in 2020.[1] With over 200 different types of cancer, management relies on a variety of techniques, such as chemotherapy, radiotherapy, and surgery—with chemotherapy the most commonly used treatment strategy for cancer.[2,3] As chemotherapeutic drugs have limited selectivity for cancer cells over healthy cells, chemotherapy can be associated with severe side effects, such as systemic toxicity and drug resistance.[4] Finding alternative, safer, and more effective treatments is a priority in cancer therapeutics research. While researchers are exploring many different methods – such as cell therapy, gene therapy, and oncolytic viruses – one approach that has shown huge potential is monoclonal antibody-based cancer therapeutics—as highlighted by the steady stream of drug approvals in recent decades (Figure 1). Here, we explore the development and progress of this exciting area of anti-cancer research.

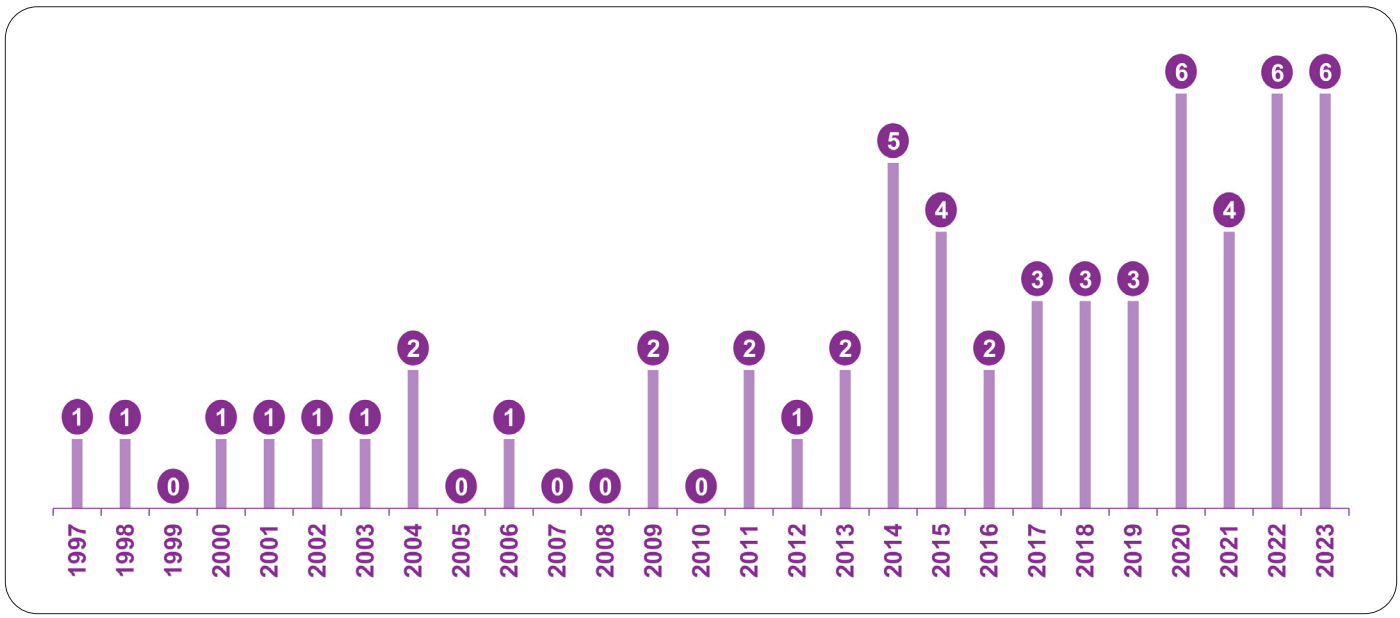

Figure 1. Number of antibody-based therapies approved for cancer per year in the EU or US since 1997. Source: The Antibody Society. Therapeutic monoclonal antibodies approved or in review in the EU or US. (10 March 2024); www.antibodysociety.org/resources/approved-antibodies.

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Monoclonal antibodies

The first type of antibody-based drug approved for cancer therapy involved monoclonal antibodies. Monoclonal antibodies offer a number of advantages as a therapeutic strategy, such as having high specificity and exerting immunological effects.[5] The classical approach relies on monoclonal antibodies binding to target antigens – such as antigens on the cancer cell surface – killing cancer cells through direct and indirect strategies.[6]

The development of effective antibody-based therapies relied on advances in antibody engineering technology. The first report of monoclonal antibodies was in 1975, where Köhler and Milstein generated monoclonal antibodies using their hybridoma technique.[7] This technique allowed researchers to generate pure monoclonal antibodies, paving the way for the development of antibody-based therapies.

The hybridoma technique involves collecting B cells from rodents immunized with a target antigen. These B cells are then fused with an immortal cancer cell line (such as myeloma cell lines), creating hybridoma cells that can continuously generate monoclonal antibodies against the target antigen.[8] While this technique allowed monoclonal antibodies to be developed as cancer treatments – offering a cost-effective approach to obtain highly specific antibodies with unlimited supply – these rodent monoclonal antibodies can also present challenges.[8] The hybridoma technique typically uses mouse cells for developing anti-cancer antibody therapies, generating murine monoclonal antibodies (Figure 2).[9] Murine monoclonal antibodies can trigger immunogenicity issues in humans (such as severe allergic reactions) and quick clearance from the body through the human anti-mouse antibody (HAMA) response.[10,11] Murine monoclonal antibodies can also lack ability to engage the human immune system for an effector or anti-cancer response.[11,12]

Aiming to overcome the challenges of murine monoclonal antibodies, genetic engineering advances allowed more human monoclonal antibody structures to be produced—including chimeric, humanized, and fully human monoclonal antibodies (Figure 2). Chimeric antibodies, developed

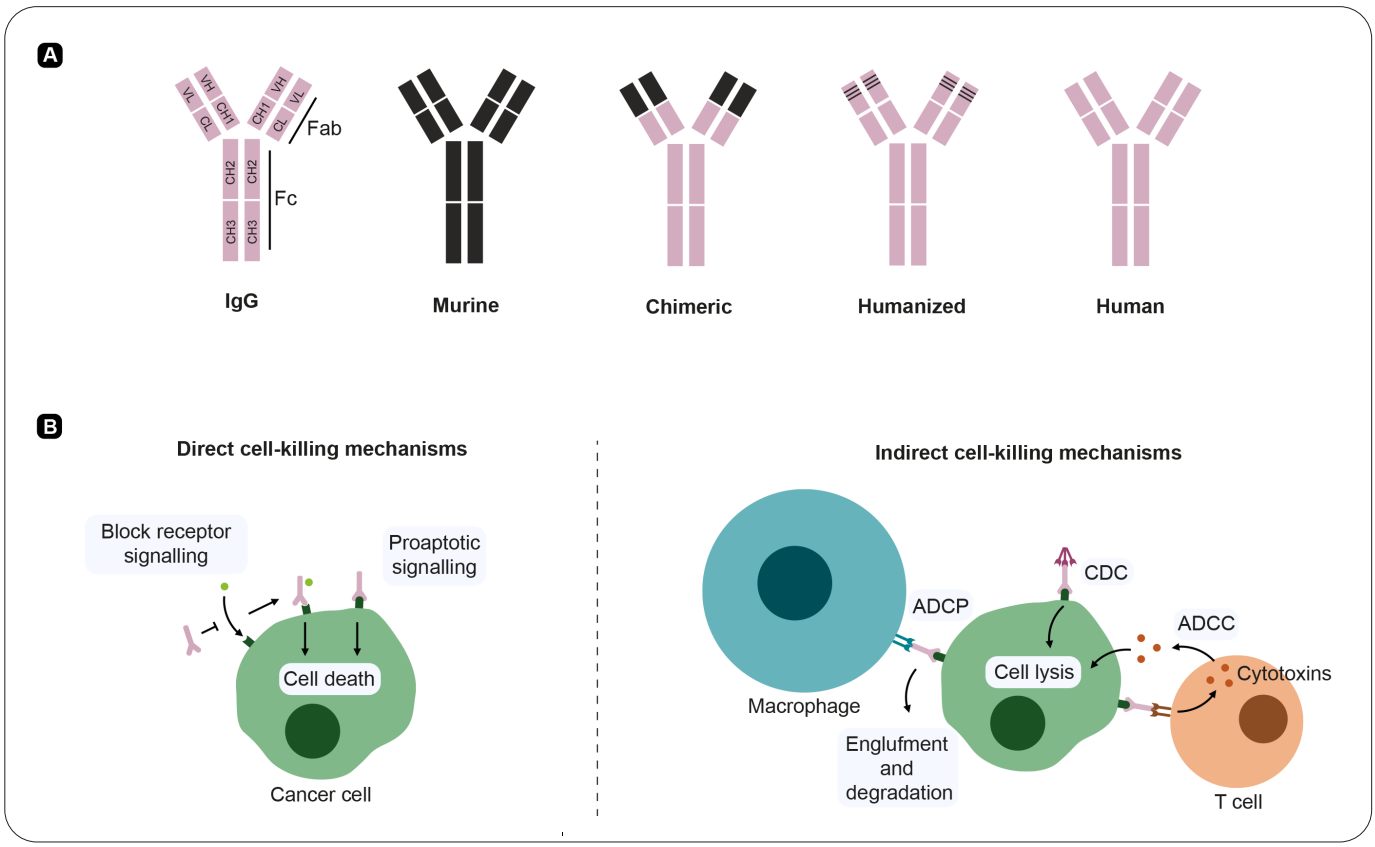

Figure 2: A) Overview of structure of antibody and composition of murine, chimeric, humanized, and human antibodies. Black sections represent murine regions and grey sections represent human regions; B) direct and indirect cell-killing mechanisms of monoclonal antibodies in cancer therapy.

in 1984, refer to monoclonal antibodies that have their murine constant (C) domains replaced with human IgG C domains.[11,13] Humanized antibodies, developed in 1986, refer to antibodies where non-human CDR domains are grafted into human antibodies.[14] Fully human antibodies were first generated using phage display.[15]

These developments in antibody engineering technology aided the development of monoclonal antibody-based therapies for cancer. The first monoclonal antibody treatment for cancer was a chimeric monoclonal antibody – rituximab (Rituxan) – which became available in 1997 to treat non-Hodgkin lymphoma, targeting the protein: cluster of differentiation 20 (CD20).[11,15] This transmembrane protein was targeted for non-Hodgkin lymphoma as >90% of B cell lymphomas express CD20; while it is found on developing B cells, early forms and plasma cells do not express CD20.[8]

Mechanism of action

In monoclonal antibody-based cancer therapies, antibodies attach to target antigens, which can kill cancer cells through (Figure 2):[6,16,17]

- Direct cell-killing mechanisms, such as blocking receptor signaling or inducing proapoptotic signaling
- Indirect cell-killing mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC); complement-dependent cytotoxicity (CDC); and antibody-dependent cellular phagocytosis (ADCP).

Developments in monoclonal antibody therapies

While monoclonal antibodies typically target cell-surface antigens, a variety of intracellular tumor antigens have been identified. Many tumor-associated antigens inside cells have been difficult to target with conventional monoclonal antibodies, such as the antigen: preferentially expressed antigen in melanoma (PRAME).[18] One strategy to overcome this is through the development of T cell receptor mimic (TCRm) monoclonal antibodies. These antibodies can bind to peptides displayed by human leukocyte antigen (HLA) on the surface of cells. PRAME is expressed in a variety of solid tumors and leukemia and is reported to have low to absent expression in most healthy tissues—with a few exceptions, such as the testes and ovaries.[18,19] PRAME peptides (after proteasomal processing) can be displayed by HLA on cancer cells. TCRm antibodies have been shown to target a PRAME peptide displayed by HLA in leukemia cells and showed anti-tumor effects in a mouse xenograft model of human leukemia.[18]

Challenges of monoclonal antibody therapies

For effective antibody-based therapies with limited off-target effects, the therapeutic should reach and act at the target cell/location. This relies on selecting an appropriate antigen that is specific for the target cell. However, target antigens are not always identified in cancers. Even when target antigens are selected, the heterogeneity of some cancers can mean target antigen expression may vary between cells.[20] In addition, cancers can develop resistance to antibody-based therapies by downregulating or stopping expression of target antigens—rendering these therapies ineffective.[21] A variety of additional strategies have been explored for overcoming limitations of conventional monoclonal antibody therapies, such as antibody–drug conjugates and multispecific antibodies—which are discussed below.[21]

Antibody–drug conjugates

Antibody–drug conjugates (ADCs) involve a monoclonal antibody attached to a cytotoxic drug – or 'payload' – through a linker (a chemical structure that links two molecules together).[6] ADCs look to combine the tumor selectivity of monoclonal antibodies with the destructive effects of cytotoxic drugs, aiming to offer a class of anti-cancer drugs with reduced toxicity compared to conventional chemotherapeutic agents.[20]

The concept of targeted therapy was proposed by Paul Ehlrich, where he described the idea of a "magic bullet"—a therapeutic approach where target cells are killed, sparing healthy cells.[7,20] The first report of an ADC was in 1958, where methotrexate was conjugated to a polyclonal rat antibody that targeted leukaemia cells.[22] After decades of development, partly aided by the advances in monoclonal antibody technology discussed above, the first ADC – gemtuzumab ozogamicin (Mylotarg) – was approved in 2000.[20] Gemtuzumab ozogamicin targets CD33 expressed on CD33+ acute myeloid leukemia blast cells.[23] Since then, a total of 15 ADCs have been approved for both solid and hematologic cancers, with many more under clinical investigation.[20]

Mechanism of action

The ADC gains access intracellularly by first attaching to the target cell antigen through the antibody on the ADC, forming an antigen–ADC complex. The antigen–ADC complex is then taken up by the cell through receptor-mediated endocytosis

(Figure 3). During cellular uptake, the antigen–ADC complex is encapsulated within an endosome, which then fuses with a lysosome. The payload is then released from the ADC by either enzymes (such as proteases) breaking down the linker or the antibody, or the linker being cleaved by chemical action (such as hydrolysis or redox reactions). The payload then moves into the cell where it typically causes DNA damage or inhibits or disrupts microtubule polymerization, leading to cell death.[6,7,24]

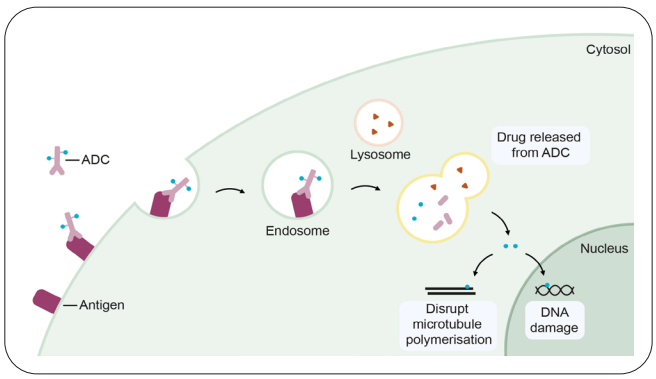

Figure 3: Overview of mechanism of action of ADCs.

Developments in ADCs

Conventionally, ADCs have typically been designed to target cancer cell surface antigens, such as CDs and HER2. An alternative strategy for solid tumors that has recently emerged is targeting antigens in the tumor microenvironment (TME).[6] Tumors and their microenvironments are in constant cross-talk – with the TME promoting cancer cell survival, invasion, and spread – and the TME can present with dysregulated antigens on non-cancerous cells.[6,25] For example, LRRC15 is a protein overexpressed on cancer-associated fibroblasts in a variety of solid tumors, such as sarcoma.[6,26] An ADC, ABBV-085, that targets LRRC15 showed promising anti-tumor effects in pre-clinical data and was recently shown to be safe with anti-tumor activity against osteosarcoma and undifferentiated pleomorphic sarcoma in a phase I clinical trial (NCT02565758).[26,27]

Challenges of ADCs

Despite a key aim of ADCs being to improve on the toxicity observed in conventional chemotherapeutic drugs, ADCs do still face challenges in safety, efficacy, and specificity.[20] ADCs can also have variable drug loading, depending on the conjugation method used during manufacturing—such as amide coupling of the payload to lysine residues on an antibody through a linker. With around 40 lysine residues available for amide coupling, the number of drug molecules and the location of these molecules attached to the antibody can vary through this conjugation method. Variable drug loading can impact the pharmacokinetics and pharmacodynamics of an ADC. While alternative conjugation methods have been explored to develop more homogenous ADC structures, these can also have their own drawbacks. For example, one approach has been to modify antibodies with unnatural amino acids that have set functional groups, which allow site-specific conjugation of the payload. This approach can offer more homogenous ADC formulations but may also pose immunogenicity concerns arising from the unnatural amino acids present in the modified antibody structure.[7] In addition, manufacturing of ADCs can be complex and costly due to the multicomponent system of an ADC (antibody, linker, and drug).[20]

Multispecific antibodies

Multispecific antibodies are monoclonal antibodies that can bind to two or more epitopes.[28] As different forms of cancer signaling pathways can be activated through a range of molecules, multispecific antibodies offer a strategy to target multiple pathways—aiming to improve efficacy compared to classical monospecific antibodies.[17]

The first form of multispecific antibodies were bispecific (antibodies that bind two antigens) and developed in 1961. These antibodies were generated by reducing two different antibodies to release the Fab arms, followed by re-joining of fab arms (one from each antibody). After the hybridoma technique was developed, bispecific antibodies were also generated by fusing two hybridomas together. Obtaining a high yield of the desired bispecific antibody structure and in a purified form was difficult with these earlier formats. However, with technical advances, a wide array of multispecific antibody structures have now been developed.[21,29] These techniques can typically involve selecting polypeptide chains, expressing the polypeptides in a cell or organism, and then purifying and assembling the antibody structure. While a wide range of multispecific antibody structures have been produced, they can be grouped into IgG-based and fragment-based antibodies—where IgG-based antibodies have an Fc region and fragment-based antibodies do not.[17] A variety of antibody fragment “building blocks” are used, such as:[29,30]

1. Single domain antibodies (sdAb or nanobodies)
2. Single-chain variable fragments (scFv)
3. Fragment antigen binding (Fab).

Antigen-binding domains and non-immunoglobulin molecules (such as T cell receptors) can be joined together and/or with IgG molecules, offering a wide variety of structures.[29]

Mechanism of action

With the wide range of multispecific formats that have been generated, a variety of cancer targeting mechanisms have been explored. Multispecific antibodies can target many molecules and pathways—such as tumor-associated antigens, immune effector cells, cosignalling molecules, and TME functional pathways—leading to these antibodies to be classified into four groups (see Figure 4):[21]

1. Immune effector cell redirectors – target tumor-associated antigens and immune cells, activating and redirecting immune cell action to cancer cells
2. Tumor-targeting immunomodulators – target cosignalling molecules on cancer cells and immune cells, activating immune cell action to the cancer cells
3. Immunomodulators – target cosignalling molecules to modulate immunomodulatory pathways
4. Tumour-targeting compounds – target multiple pathways, such as TME function pathways and cell lysis pathways, and may also improve payload delivery.[21]

Trispecific antibodies typically have specificity for at least one tumor-associated antigen and at least one immune effector cell (T cell or NK cell).[31]

Developments in multispecifics

There are seven FDA-approved bispecific antibodies for cancer, with over a hundred more in clinical development.[28,32] As with the other antibody-based strategies discussed above, multispecific antibodies can also lack appropriate tumor-associated antigens—with only a small proportion of proteins found on the surface

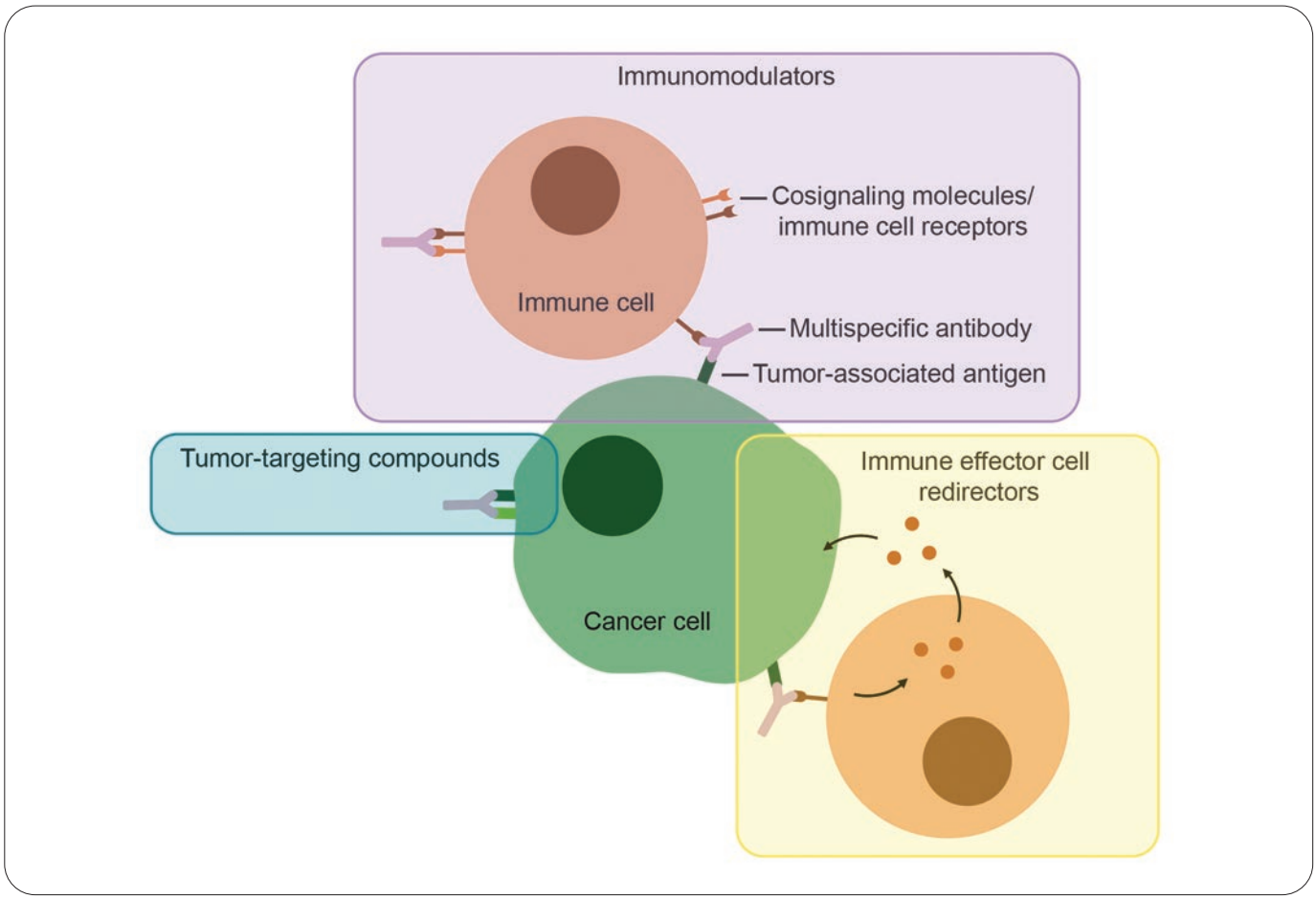

Figure 4: Overview of mechanism of action of multispecific antibodies in cancer therapy. Adapted from You et al.[21]

of cells.[29] To combat this, bispecific and trispecific antibodies have also been designed with an epitope that recognises HLA-displayed peptides (from intracellular tumor-associated antigens). For example, tebentafusp is a bispecific antibody that is classed as an ImmTAC (immune-mobilizing monoclonal T-cell receptors against cancer) molecule. Tebentafusp involves a T cell receptor that binds to a glycoprotein 100 peptide–HLA complex on the target cell and an anti-CD3 single-chain variable fragment which triggers T cells to attack the target cell. Results from a recent phase III trial (NCT03070392) highlighted the promise of multispecifics, with tebentafusp treatment of patients with uveal melanoma improving overall survival compared to the control group.[33]

Challenges

As the structures of this class of antibodies are not typical of naturally occurring structures, these modified antibodies can pose an immunogenicity risk.[21,34] Multispecific antibodies may also present manufacturing challenges as the complex structures may be unstable or produce impurities/incorrect products during assembly. In addition, impurities generated during manufacturing can impact the immunogenicity of an antibody-based therapeutic—together, these points highlight the importance of good characterization and purification practices when developing these products.[21] Despite strategies being developed to target intracellular proteins (through binding to peptide–HLA complexes), identifying appropriate antigen targets is still a major challenge in monoclonal antibody-based therapies. In addition, many multispecific T cell-engaging antibodies target differentiation proteins, which can cause severe adverse effects.[34,35]

Conclusion

Monoclonal antibody-based therapeutics have shown great potential for managing and treating a variety of cancers—emphasized by the success of approved treatments over the past 30 years and the vast ongoing research efforts in developing new treatment strategies. Antibody-based treatments offer many strengths, such as high specificity for their target antigen/s and an ability to exert and act through multiple mechanisms. However, common challenges are found throughout the different antibody-based therapeutic strategies, such as finding suitable target antigens accessible to antibody-based drugs. Revvity offers a variety of immunoassays that can characterize antibody-based drugs and their interactions, helping identify drug targets and aiding development of effective antibody-based therapies.

About Revvity

Revvity – a science-based solutions company – is committed to advancing healthcare by expanding the boundaries of human potential through science. Revvity offers a variety of immunoassays and reagents to aid monoclonal antibody-based therapeutics research, such as:

- Ig detection assays – ready-to-use kits to quantify IgGs and biologics fragments, offering a homogeneous, easily miniaturized alternative to conventional ELISAs
- Fc gamma receptor (FcgR) and neonatal Fc (FcRn) binding assays cellular assays or biochemical binding assays to measure FcgR-specific antibody binding. These assays are ideal for predicting antibodies with strong ADCC activity and longer circulating half-lives
- *In vitro* assays to assay immune response – a range of highly sensitive immunoassays, including Cr51, TRF-based assay technology, and next-gen no-wash assays, to characterize the cytokine response, measure signaling events, and understand the ADCC of biologics
- Potency assays – bioassays, including cell-based assays, to help determine the potency of the drug substance and evaluate the reproducibility and stability of drug products
- Toolbox screening reagents – reagents that can aid characterization of protein–protein interactions (such as antibody–antigen binding efficiency). For example, Revvity offer reagents that can help run affinity maturation experiments and screen medium to large biologics libraries produced by hybridoma or in periplasmic crude extract
- Contamination assays – ready-to-use assays to detect host cell contaminants (such as from CHO and HEK cell lines, residual dsDNA, and mouse or human albumin), which is critical to obtaining reliable research results and ensuring the efficiency and safety of a drug product.

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