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From Lab to Clinic: The Production of Different Types of Recombinant Antibodies as Cancer Treatments

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ABSTRACT

Cancer is one of the leading causes of death worldwide, with a wide variety of treatments targeting it but no definite cure. Within the category of immunotherapy, which is focused on using one's own immune system to target tumors, is monoclonal antibodies (mAbs). The expanding treatment of recombinant monoclonal antibodies made using recombinant DNA technology has proven that it provides several benefits over the traditional monoclonal antibodies made with hybridoma technology. There are multiple formats of recombinant antibodies defined by their structure, of which the most common are single chain variable fragment (scFv), fragment antigen binding (Fab), and bispecific (bsAb). Using literature review, this paper intends to evaluate and compare the production and structures of recombinant scFv, Fab, and bsAb antibodies, with minimal mention of the application of each. It is found that the production methods for each format are very similar, though bispecific antibodies differ the most due to their structure. There are also differences in the methods used to ensure soluble expression of antibodies in each format. New recombinant antibodies are still being developed with the goal of minimizing production time and labor while maximizing stability and specificity. This paper will help in future research dedicated to production methods that would minimize the cost of recombinant antibody therapy for cancer patients in the future.

Introduction

Cancer is a disease in which abnormal cell growth resulting from DNA mutations develops in a part of the body and has the potential to grow and spread throughout the body. It hinders the body's systems and is potentially lethal. Cancer is in fact one of the leading causes of death worldwide. In 2019, cancers resulted in 18 percent of deaths worldwide, only second to cardiovascular diseases (Roser). In 2022, cancers were the second leading causes of death in the United States (CDC and Prevention, 2024).

A definite cure to cancer is yet to be found, but various treatments have been developed, including immunotherapy. Immunotherapy is a treatment based on using one's own immune system to recognize and eliminate cancer cells. One common form of it is monoclonal antibodies (mAbs), which are antibodies derived from cloning a B cell that can be used to directly target a particular antigen either in free form or on certain cells, such as tumor cells expressing that antigen. MAbs are typically made in laboratories using the hybridoma method, in which B cells derived from initiating an immune response in an animal are fused with immortal myeloma cells. However, recombinant antibodies have gained relevance over traditional mAbs due to their increased efficiency and accuracy compared to hybridoma antibodies. They are produced by inserting synthetic genes into a B cell line in vitro, forming recombinant DNA that will cause the cells to produce recombinant antibodies that can be purified. Over time, this process has expanded, making it so that multiple formats of recombinant antibodies could be created, including recombinant bispecific antibodies (bsAbs) that can target two antigens at once. This study will focus on comparing the different recombinant antibody formats and their production, with an emphasis on bsAbs.

Myeloma is a type of cancer that affects plasma cells (antibody producing B cells), and recombinant antibodies have shown immense potential in treating it. Various recombinant antibodies have also been used to specifically

target myeloma. This includes recombinant bsAbs, which have enormous potential in treating cancer as a form of immunotherapy due to various benefits such as "defined structure, composition and biochemical, functional, and pharmacological properties" (Brinkmann & Kontermann, 2017). For example, teclistamab (commercially known as Tecvayli) has been used to treat myeloma ever since the FDA granted accelerated approval in 2022 (FDA, 2022). One recombinant antibody called bevacizumab (commercially known Avastin) was approved for treating various forms of cancer in the United States starting from 2004 (Roche, n.d.). Since then, it has been used in combination with chemotherapy to treat colorectal, lung, glioblastoma, kidney, cervical, and ovarian cancer (MedlinePlus, 2024). Such existing treatments show the wide application and importance of recombinant antibodies in modern cancer treatment, as will be asserted by various clinical studies later on.

Different types of bsAbs are all produced with the same general process. Two mAbs or antibody fragments meant to target different antigens are fused together with a linker. According to Brinkmann and Kontermann, the composition of these linkers varies between "short alanine linkers (Ala3), hydrophilic linkers, glycine-serine-rich linkers, linkers adopting a helical conformation, and linkers derived from various immunoglobulin and non-immunoglobulin molecules" (2017). After the main BsAb is created, it is purified using one of various techniques such as protein A chromatography (Zwolak et al., 2017). Though the first bsAbs were made using two different antibodies produced by hybridoma technology, recombinant technology grew and was used to greatly improve bsAbs. As a result, modern bsAbs are inherently recombinant since recombinant technology is used to fuse two mAbs or antibody fragments together.

There are many research articles that either focus on recombinant antibodies as a whole or single-chain fragment variable (scFv), fragment antigen-binding (Fab), or bsAbs individually. While there are also a number of papers comparing scFv and Fab antibodies, there seems to be a lack of comparison to bispecific antibodies. One reason to explain this may be that many bispecific antibodies are based around scFv or Fab antibodies, along with other molecules, so researchers have not felt the need to make a comparison. In the same fashion, it is also possible that researchers have only compared different types of bsAbs as seen in the previously cited article intended to provide a "comprehensive overview of the different bispecific antibody formats" (Brinkmann & Kontermann, 2017).

This paper intends to thoroughly compare recombinant scFv, Fab, and bispecific antibodies, with emphasis on their function and application. Slight differences in each antibody's production and structure will also be discussed briefly. There will also be a section to specifically compare the different types of bsAbs since they are made in various formats. Fulfilling this objective would be helpful within the relatively novel field of recombinant antibodies since it could truly quantify and show which type of recombinant antibody is optimal to use depending on specific criteria. Such information would make it easier for types of recombinant antibodies to relieve funding for production and approval, which would eventually help a large group of cancer patients that would benefit from mAb treatment.

Methods

The objective of this study is to evaluate and compare recombinant scFv, Fab, and bispecific antibodies, with emphasis on their function and application. Slight differences in each of their production and structure will also be discussed briefly. There will also be a section to specifically compare the different types of bsAbs since they are made in various formats. This research is done completely through literature review and analysis of previous clinical studies, so it does not involve the collection of any qualitative or quantitative data. Since this is completely online with no physical tools, ethical considerations will not be observed.

Cancer Incidence, Mortality, and Current Standard Treatments

As previously mentioned, cancer has one of the highest incidence rates in the world, and its mortality rate is also considerably high. According to the Global Cancer Observatory, over 19 million new cases were discovered globally,

and over 9 million died from cancer in 2022. According to the American Cancer Society, it was estimated that in 2022, the cancer incidence rate would be 1.9 million and the mortality rate would be over 609,360 in the United States (2022). The CDC report at the end of that year supported this death rate with a similar number of 607,790 deaths, showing that it made up around 18.57% of the 3,273,705 deaths that year and was the second leading cause of deaths in 2022. In fact, the CDC has stated in an earlier annual report on the United States in 2020-2021 that cancers have remained in the top 2 causes of death in the United States for over 75 years (2023). The same report shows that cancer deaths have been slowly decreasing, which can be attributed to various factors such as changing habits in the population, improved screenings and treatments.

Various types of cancer treatments have developed for different stages and types. The most common cancer treatments include surgery, radiation therapy, chemotherapy, and immunotherapy, and such treatments are typically used in conjunction (MedlinePlus, 2023). Surgery is used as local treatment, targeting a specific tumor and part of the body, to remove a tumor. The different types of surgery include curative surgery, which fully removes the tumor; debulking surgery, which only removes part of the tumor to avoid nearby organ damage; palliative surgery, which lessens the discomfort or problems of advanced cancer; supportive surgery, which is used to allow for another kind of treatment; and reconstructive surgery, which restores how something looks or function after a tumor is removed (American Cancer Society, 2019). Radiation is also a local treatment that uses radiation to break DNA within a cancer cell and cause it to die. This can be used to stop early-stage cancer, to stop or treat cancer that returned, and to lessen discomfort and problems from the size of tumors in advanced cancer (American Cancer Society, 2019). Chemotherapy, unlike the previous two treatments, is a systemic treatment, meaning it spreads through the entire body, that works by taking a drug or combination of drugs that kills cancer cells (American Cancer Society, 2019). Immunotherapy works by using the patient's own immune system. Other common cancer treatments include targeted therapy, hormonal therapies, and various other treatments using lasers, temperature, and light.

Out of these cancer treatments, this article will focus on immunotherapy. Immunotherapy works by using the patient's own immune system to target cancer cells. Despite its long-winded history, the field of immunotherapy began expanding recently as shown by the first immunotherapy treatment, sipuleucel-T, being approved by the FDA in 2010 (Dobosz & Dzieciątkowski, 2019). Sipuleucel-T is an autologous vaccine, meaning it is developed using immune cells from the individual patient being treated. It is also based on dendritic cells, which are groups of cells that patrol their environment and initiate immune responses when faced with pathogens or, in this case, cancer cells (Lee et al., 2023). Such cancer vaccines, which only work with prostate cancer as of 2022, work by identifying cancer cells that make up only one type of immunotherapy, the other common types include adoptive cell therapy, checkpoint inhibitors, immunomodulators, and monoclonal antibodies. Adoptive cell therapy is when a patient's immune cells are taken and grown or modified in a lab, before being put back into their body to kill cancer cells. One common form of this therapy is chimeric antigen receptor (CAR) T-cell therapy, which involves the genetic modification of T-cells to produce CARs on their surface that can identify antigens on the surface of cancer cells and initiate attack (National Cancer Institute, 2022). Another common form of T-cell therapy is tumor-infiltrating lymphocytes (TIL) therapy, which is done by increasing the strength and size of a patient's TILs in a laboratory so that they can effectively attack tumors and fight against their signals.

Checkpoint inhibitor therapy, which also involves the manipulation of T-cells using antibodies, is based around stopping the interaction between checkpoint proteins and other proteins which are meant to send signals that turn T-cells off. T-cells are white blood cells that can identify and attack pathogens and cancer cells, and checkpoint proteins are needed to turn them off, so they don't kill healthy cells and create tissue damage. However, proteins on cancer cells are capable of binding to checkpoint proteins, which stops T-cells from attacking them and protects the tumor (Pardoll, 2012). As a result, checkpoint inhibitors (antibodies that block the checkpoints) are useful in advanced cancer since they make sure checkpoint proteins are unable to bind to their partner proteins and the T-cells can continue to attack cancer cells. As of 2022, checkpoint inhibitors could be used to treat "bladder, cervical, colorectal, and triple negative breast cancer". Checkpoint inhibitors can be considered as a subcategory of immunomodulators, which are various types of treatments that boost the immune system in other ways. This includes using lab grown cytokines,

which slow cancer cell growth using signals through interferons, or drive communication between immune cells to attack cancer cells with interleukins. Immunomodulatory drugs are specific medications that can boost the immune system. This includes drugs such as thalidomide that "keep cancerous tumors from forming the new blood vessels the tumors need to grow" and use cytokines to increase white blood cell production (Cleveland Clinic, 2022). Aside from these forms, there are other immunotherapy treatments to directly target cancer cells that have developed and technologically expanded in the past few decades, including monoclonal antibodies, which will be further discussed in the next section.

Monoclonal Antibodies in Cancer Therapy

Monoclonal antibodies (mAbs) are another form of immunotherapy, based around using a patient's own antibodies to create a line of lab grown antibodies that are injected into a patient's vein that can help them target antigens or cancer cells. MAbs are most commonly produced using hybridoma technology, which means they are formed from fusing activated B lymphocytes and immortal myeloma cells (Carvalho et al., 2017). However, this has various disadvantages such as long generation time and inconsistent yield. Newer recombinant technology that produces antibodies through inserting synthetic genes into a B cell line in vitro to generate recombinant DNA, fixes these problems.

MAbs were first invented using hybridoma technology and have since expanded into various types defined by how each is produced. In the immune system, there is a group of antigen presenting cells made up of dendritic cells, macrophages, and B lymphocytes, that are able to present an antigen to a T lymphocyte to initiate an immune response. T lymphocytes and B lymphocytes, also known as T cells and B cells, are both white blood cells. T cells direct B cells to attack antigens since B cells can create antibodies that can bind to a foreign substance and neutralize it with toxins (Carter, 2021). This is done through plasma cells that "develop from antigen-activated B lymphocytes in lymphoid organs" that are larger than B cells and secrete large amounts of antibodies when fully mature (Allen & Sharma, 2022). Antibodies are "modular protein defense systems possessing a paratope (variable domain), an antigen-binding site located at the upper tips of the "Y"-shaped structure" (Bashir & Payshuyse, 2020). Hybridoma cells are hybrid cells capable of producing a single type of antibody to defend against a specific antigen. Before the first mAbs were made, the first hybridoma cells were made in mouse-rat and mouse-mouse myeloma cell fusion experiments that resulted in "antibodies carrying the genes of both parental cells" (what is biotechnology? 2024). Over time, these fusion experiments were modified to improve the specificity of the antibodies, but there was still a major problem of the cells being short-lived. In 1975, they were finally made immortal by Georges Köhler and César Milstein after they tried fusing "an antibody-producing plasma cell with a myeloma cell," and this marked the invention of mAbs (Kaunitz, 2017). To elaborate, the plasma cell mentioned before is a specifically selected B-cell that comes from an immune response initiated by exposing a host animal to an antigen. The process of fusing specific antibody-producing B cells and malignant myeloma cells is still the main process for creating hybridoma mAbs in the modern day. MAbs are highly specific since they only bind to one epitope, unlike the previously discovered polyclonal antibodies which have a broad specificity from recognizing multiple epitopes, and they get their immortality from myeloma cells (Thermo Fisher Scientific, n.d.). MAbs started out as murine, with fully mouse/rat components, but production slowly evolved to the point where chimeric, humanized, and fully human mAbs could be made. Chimeric mAbs have human constant regions instead of murine, while humanized mAbs result "in an antibody where only the complementarity determining regions (CDRs) of the variable (V) regions are of mouse-sequence origin" (Harding et al., 2010). Fully human hybridoma mAbs are now common and are made where "antigen specificity has been selected either in vivo by use of genetically modified mice or by antibody engineered processes combined with screening" (Harding et al., 2010). Fully human mAbs are optimal in avoiding immune responses, such as human anti-mouse antibodies (HAMA) and allergic reactions, that are more likely when using murine, chimeric, or possibly humanized mAbs (Alfaleh et al., 2020). Though hybridoma technology is still a traditional way of producing mAbs, other production methods have developed over time.

Phage display, single B-cell technology, and recombinant DNA technology are each developing methods of mAb production that sometimes overlap in their usage. In phage display technology, genetic information is inserted into a bacteriophage's coat protein gene, allowing the protein the gene encodes to be displayed while the gene is still inside. This makes it easy to test for interactions between the displayed protein and other proteins, peptides, or DNA while having a direct connection between a protein's genotype and phenotype. Fully human mAbs can also be used in phage display, which is useful since it allows for screening of mAbs through exposure to an antigen. In the process of biopanning, mAbs can be repeatedly screened by exposing a mAb phage display library to a specific immobilized antigen and washing away non-binders. This isolates the specific mAb and allows them to be amplified and rescreened. Single B-cell technology is a novel and efficient method of generating mAbs using human B cells while directly amplifying their original heavy (VH) and light (VL) chain pairings which are critical for antibody function (Tiller, 2011). They are made from isolating a B cell from peripheral blood or from lymphoid tissues, which is usually done through fluorescence-activated cell sorting (FACS) due to the ease it provides in distinguishing and sorting different cells based on surface markers. The B cells are then analyzed using one of many possible methods and sequenced and amplified for insertion into mammalian cells or bacterial systems for in vitro production of mAbs. The resulting mAbs or fragments are screened to be tested and purified. Finally, recombinant DNA technology is a growing in vitro alternative to hybridoma technology. A synthetic gene library is generated and screened for strongly binding antigens, and recombinant DNA formed by inserting the remaining genes of interest into a cell line is used to produce antibodies or fragments. These are purified and tested to form the resulting recombinant antibodies. This is different from mAbs that are typically derived from animals in vivo, with added benefits of increased control over the antibody's characteristics, lower production time, and "high specificity, sensitivity, and reproducibility" (Azenta, 2022). Production can involve usage of the previously mentioned phage display or similarities with single B-cell technology. Recombinant technology allows for various antibody formats, including the common single chain variable fragment (scFv), fragment antibody (Fab), and bispecific antibody (bsAb).

Different Recombinant Monoclonal Antibody Formats

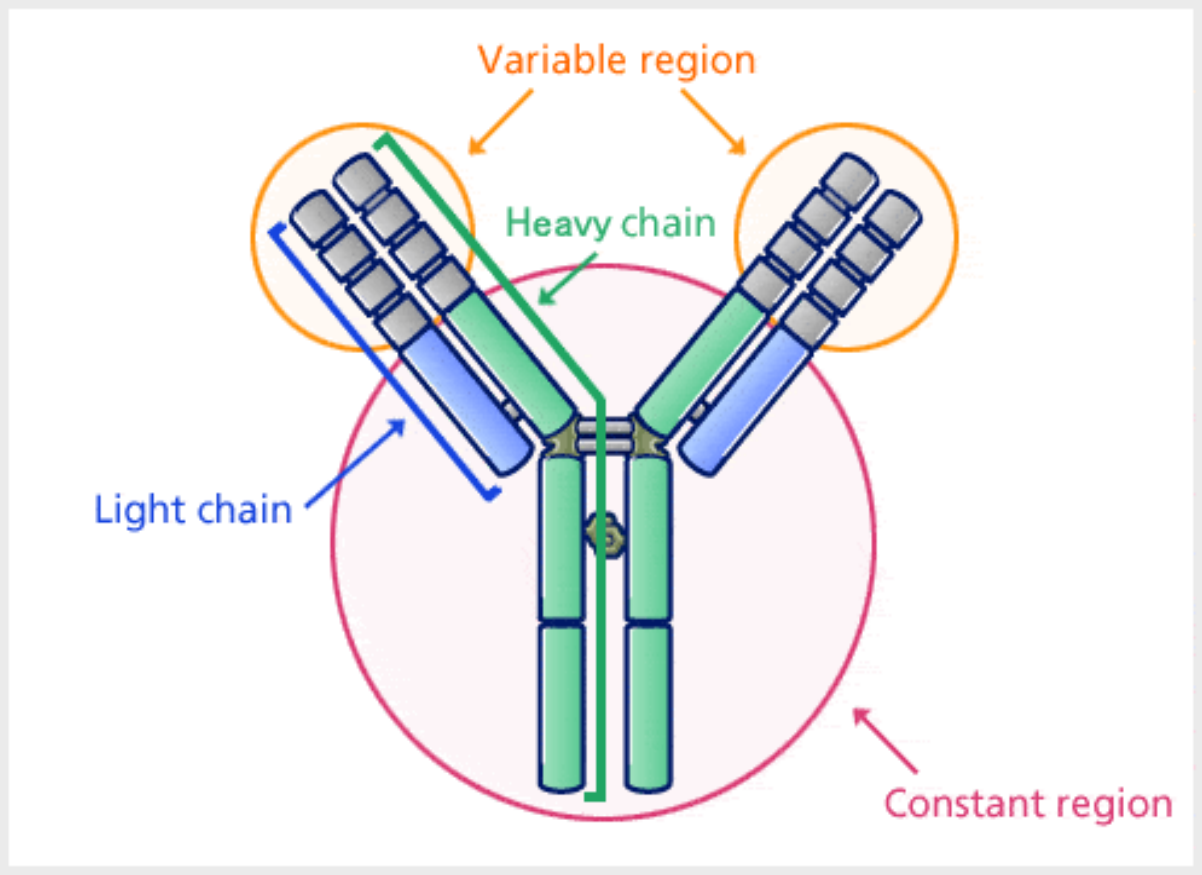

Figure 1. Immunoglobulin G Antibody Structure

Note. The image depicts the detailed structural composition of an immunoglobulin G (IgG) mAb. Reprinted from *Antibody- Structure, Classes and Function*s, by S. Aryal, 2024, https://microbiologyinfo.com/antibody-structure-classes-and-functions/.

Out of the various types of mAbs, immunoglobulin G (IgG) antibodies are the most common type of antibody produced in the human body and make up the basic structure of all other types of antibodies. The structures of scFv, Fab, and bsAb formats are also based on IgG mAbs, so they will be the basis of the antibody structures discussed in this article. Unlike IgG antibodies, however, scFvs and Fabs are typically produced through bacterial cell expression, specifically E. coli. The variable region of an antibody changes based on the type of antigen region being targeted (Figure 1). As mentioned earlier, the variable heavy (VH) and variable light (VL) chains are paired together and the constant heavy (CH) and constant light (CL) chains are also paired, which forms two Fab regions (Figure 1). The remaining CH chains forming the tail of the antibody make up the crystallizable region (Fc) that attaches to cells using the antibody (Zheng et al., 2011). The stability of an antibody "depends on the presence of intramolecular bonds within the light and the heavy chains and sugar groups located on the Fc fragment" (Pirkalkhoran et al., 2023). This explains why glycosylation, a modification in which a sugar molecule is added to a molecule, that occurs in the Fc plays an important role in antibody production and stability.

Both scFv and Fab recombinant antibodies provide similar benefits since they are both fragments. This is useful because it allows the antibody to be smaller while still maintaining its paratope, meaning it still targets its specific antigen. Advantages from this include "better tumor penetration, more rapid blood clearance, and lower retention times in nontarget tissue" (Ahmad, 2012). Production is also less costly and more straightforward due to factors such as lesser glycosylation requirements being necessary (Pirkalkhoran et al., 2023). However, there are various

concerns regarding stability and safety in scFvs specifically, which are addressed through genetically modifying the VH and VL chains. As a result, only one scFv recombinant antibody has been FDA approved, brolucizumab, for macular degeneration, and none have been approved for cancer (Pirkalkhoran et al., 2023).

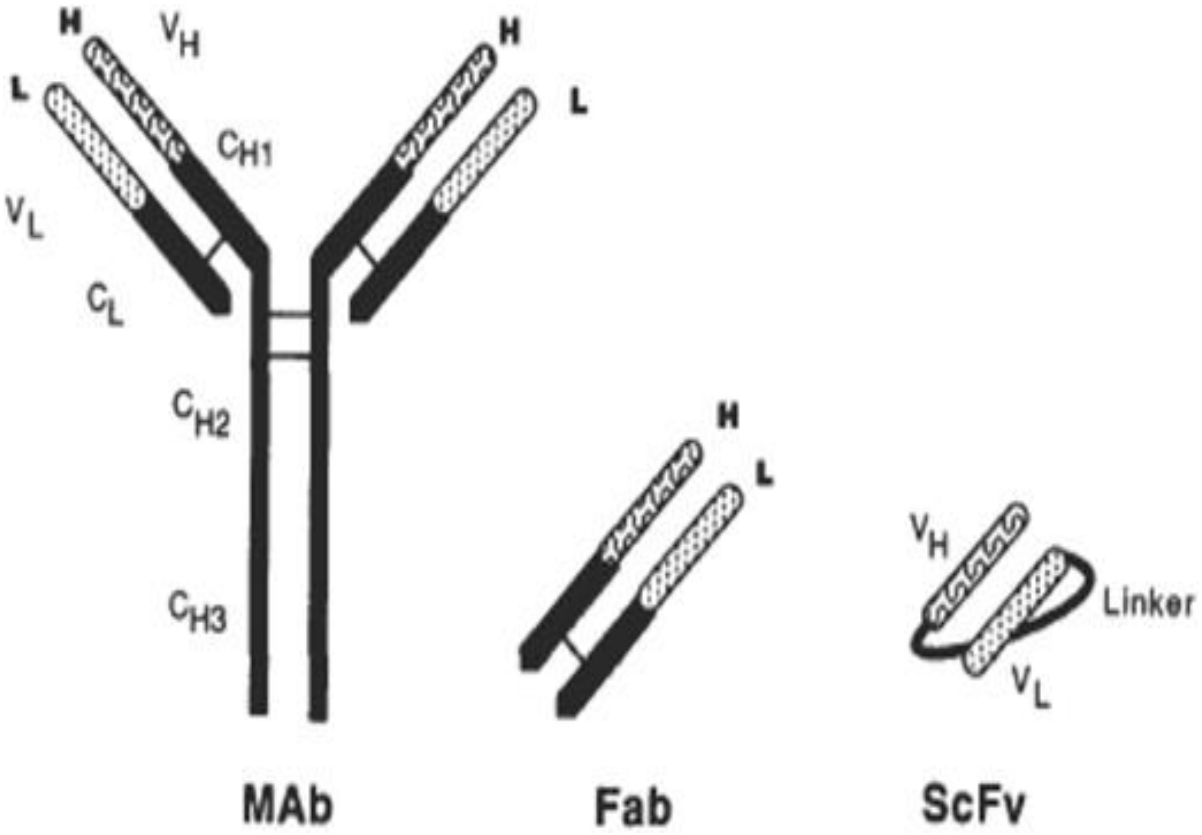

Figure 2. Structures of Different Antibody Formats

Note. The image depicts the structural makeups of a full IgG mAb, a Fab, and a scFv side by side. Reprinted from "Recombinant Antibody Technology" by A. E. Karu, C. W. Bell, and T. E. Chin, 1995, *ILAR Journal, 37(3),* General Principles Section, Figure 1. https://doi.org/10.1093/ilar.37.3.132. CC BY.

ScFv antibodies, which are the most often researched recombinant antibody format, are made from a VH and VL chain pair linked together with a flexible peptide linker (Figure 2). The structure of Fab antibodies is a VH VL chain pair and CH CL chain pair (Figure. 2). Though both scFv and Fab antibodies are typically produced through bacterial expression, any heterologous protein expression system using "host platforms such as bacteria, yeast, fungi, mammalian cells or even whole animals and plants" have been successful (Pirkalkhoran et al., 2023). Phage display is traditionally used in fragment antibody production, though the different strategies of biopanning may vary. A less complex alternative to this method is the usage of "transgenic mice with integrated human immunoglobulin loci," which can be done through various injection methods, to produce a varied population of expressed antibodies which can then target a specific antigen.

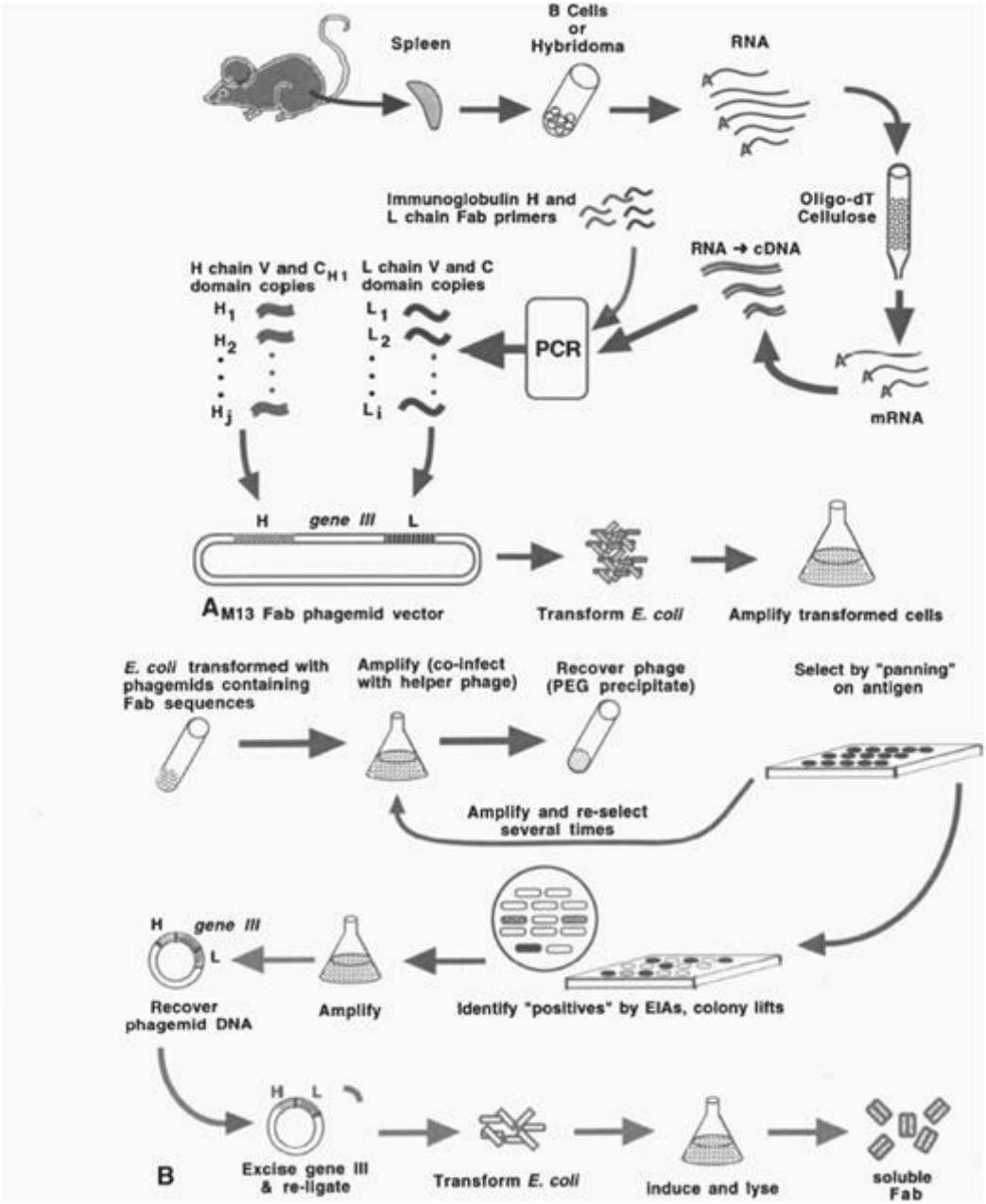

Figure 3. Fab Recombinant Antibody Production

Note. The image depicts the full process of recombinant Fab antibody production. Part A shows the derivation, and part B shows the selection and expression. Reprinted from "Recombinant Antibody Technology" by A. E. Karu, C. W. Bell, and T. E. Chin, 1995, ILAR Journal, 37(3), General Principles Section, Figure 1. https://doi.org/10.1093/ilar.37.3.132. CC BY.

Fab production can be divided into three parts, derivation, selection, and expression (Figure 3). Antibody genes are derived by getting mRNA from antibody producing cells, and this is used to produce complementary DNA (cDNA). Fab antibody gene sequences are isolated from the cDNA "by PCR using specific complementary oligonucleotide primers" and amplified. This DNA is purified and put into a cloning phagemid vector, and antibiotic resistance is used to select for cells that have said phagemid, which is used to directly insert DNA into E. coli (the bacterium

commonly used as host cells), which is known as transformation. The cloned antibody sequences are trascripted and produced as a "fusion protein with a minor coat protein (gIII, the phage gene III product)." Infection of the E. coli with the M13 helper phage makes it so the phagemid is "packaged into complete, infective bacteriophage" instead of a plasmid (Karu et al, 1995). In selection, phage display and biopanning are used to isolate phages with the desired antibody, which is done repeatedly along with amplification. In expression, the gIII that was fused in earlier should not be expressed for an antibody produced from a phage to be soluble. It is removed through digestion using restriction enzymes and the vector is re-ligated in new host cells. The resulting soluble Fab is isolated from the cell through gentle cell lysis (Karu et al, 1995). ScFv production is largely the same, with a few key differences. For one, the transcribed cloned antibody sequences result in expression as a single protein linked to a gIII where the fusion is incorporated into the phage, while only the H chain is fused with gIII in Fabs and free L chain associates with the fusion to form a functional Fab. Another difference is how the gIII is removed for the scFv antibodies to become soluble. In scFvs, the phagemid is initially grown in an E. coli strain that suppresses the stop codon, which means the scFv-gIII fusion ends up being expressed (Karu et al, 1995). To fix this, the phagemid is switched to a different strain that recognizes the stop codon, doesn't express the fusion, and instead expresses the soluble scFv antibody.

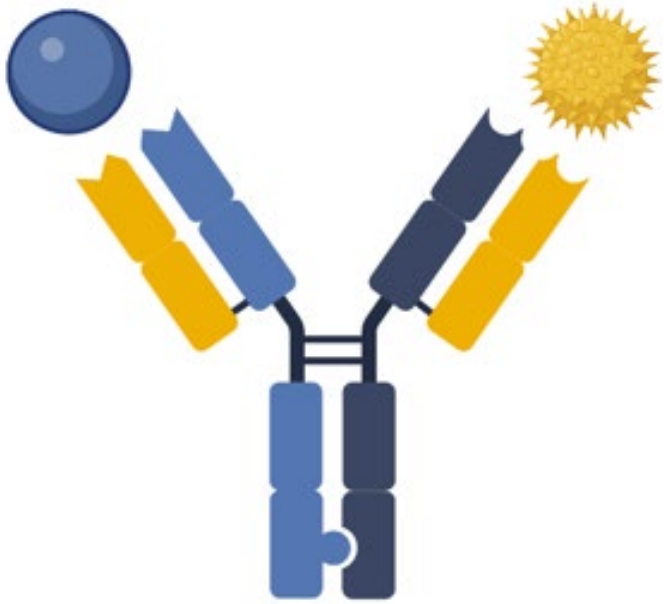

Figure 4. Possible Bispecific Antibody Structure

Note. The image depicts a bispecific antibody with the same structural composition as an IgG mAb, aside from the two distinct arms made for two distinct targets (which can either be separate antigens or epitopes on one antigen). Reprinted from *Bispecific Antibodies: A Dual Attack on Complex Disease* by C. Olsen, 2023, https://www.dotmatics.com/blog/bispecific-antibodies-complex-disease.

Recombinant bsAbs are similar to IgG mAbs, with VH and VL pairs and CH and CL pairs (Figure 4). The main difference is that the two antigen binding regions of the antibody are two distinct VH VL pairs, which means the antibody can target two separate antigens or epitopes. However, there are many types of recombinant bsAbs, which are separated into bsAbs with and without an Fc. Due to the complexity involved in evaluating and comparing the production methods for every type of bsAb, the processes described in this article will be largely generalized, though they are similar to the processes mentioned for other recombinant antibody formats. First off, the DNA sequences for two desired antibodies are synthesized and ligated into the chosen vector using PCR and primers. The plasmids resulting from this are transformed into the selected host cells, which are also traditionally E. coli, allowing for antibody

construction. After this step, a method is used to make sure there is soluble expression of the antibody, and this method varies based on the format the bsAb follows. The bsAb is characterized and evaluated for binding to the target antigen, proliferation, and apoptosis. An example of a recombinant bispecific scFv based antibody following this procedure is the pET32Ek/LIC-anti-VEGFR2/EPCAM bispecific antibody, where pET32Ek/LIC refers to the vector used and VEGFR2 and EPCAM are the two molecules being targeted due to their prominent role the expression of various cancers (Barzaman, 2021). Aside from this method of production, it is also common to form separate scFv fragments through either phage display or transgenic mice and link them together to create different types of scFv bsAb formats (Figure 5). In some cases, such as in tandem scFvs, expression ends up insoluble, so one of various possible methods must be applied to make the scFv soluble. This includes refolding protocols, using mammalian cell expression systems instead, and using different lengths and flexibilities for the peptide linkers (Kontermann, 2004). Modifications to the bonds between the VH and VL chains are sometimes applied as well to improve bsAb stability.

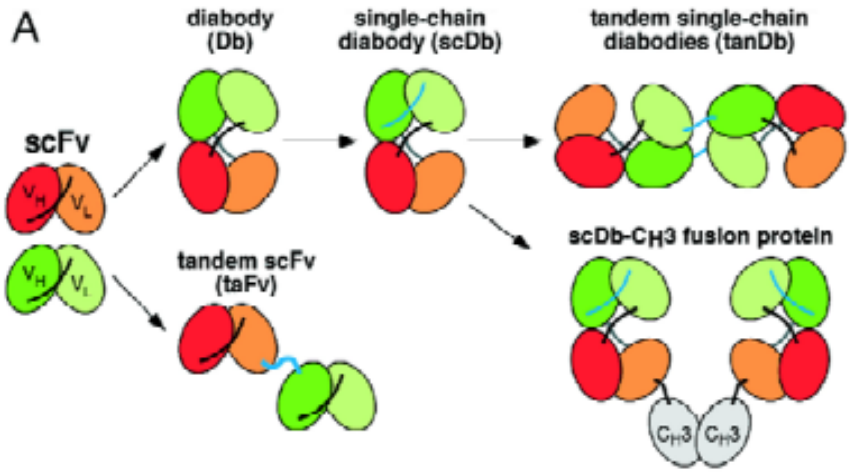

Figure 5. ScFv Based Bispecific Antibody Types

Note. The image depicts different types of recombinant bispecific antibodies derived from scFv antibody formats. Adapted from "Recombinant bispecific antibodies for cancer therapy" by R. E. Kontermann, 2005, *Acta Pharmacologica Sinica, 26(1)*, p. 2, https://doi.org/10.1111/j.1745-7254.2005.00008.x.

Clinical Studies with Recombinant Antibodies

Table 1. Clinical Studies on Recombinant Antibodies. The table shows the information from the clinical trials of five recombinant antibodies. All information was retrieved from ClinicalTrials.gov. The table was created by the author.

NCT Designation	Recombinant Antibody Format	Antibody Name	Antigen Type(s)	Cancer Condition	Use in Combination or Not
NCT00560794	Bispecific	MT103	CD19, CD3	Acute Lymphoblastic Leukemia	No
NCT04338659	Bispecific	IBI322	CD47, PD-L1	Advanced Malignant Tumor	No

NCT03804996	Bispecific	TG-1801	CD19, CD47	B-Cell Lymphoma	No
NCT03192202	Bispecific	AFM13	CD30, CD16A	Refractory/Relapsed Cutaneous Lymphoma	No
NCT02609776	Bispecific	JNJ-61186372	EGFR, MET	Non-Small-Cell Lung Cancer	Yes

Various studies have been done on recombinant antibodies, specifically bsAbs of different types. The lack of clinical studies on scFv or Fab recombinant antibodies that aren't bsAbs is due to there being no FDA approved antibodies of those types. Instead, there are various bsAbs made from scFvs that have been FDA approved. For example, bispecific T-cell engagers (BiTEs) are made using two scFv fragments connected with a flexible linker. One paratope targets a tumor and the other targets a T-cell to activate a response against the tumor.

An example of a BiTE is MT103, or blinatumomab, which was investigated in a clinical study to treat minimal residual diseases, or a presence of leukemia cells below the cytological detection limit (Table 1). It targets CD19, a protein with high expression on leukemia cells, and CD3, another protein that is a marker for T cells, allowing T cells to be in close enough proximity to the malignancies to attack. It was found that patients responded to the bsAb when rearrangements in their immunoglobulin or T-cell receptor was below a certain threshold. Another clinical study was done to evaluate the maximum tolerated dosage of IBI322 when treating advanced malignant tumors in patients who failed standard treatment, and no results were posted. IBI322 targets CD47, a protein that blocks the elimination of cancer cells, and PD-L1, a protein on tumor cells that binds to PD-1 T cells to stop them from eliminating the tumor. The recombinant bsAb of TG-1801, or ublituximab, was used in a clinical study to evaluate its effect on patients with B-cell lymphoma, and no results were posted. TG-1801 targets CD47 and CD19, which were also targeted by bsAbs in the previously mentioned clinical studies. In a clinical study evaluating the effects of recombinant bsAb AFM13 on lymphoma in patients where prior treatment has failed, it was found that certain dosages of AFM13 had varying types of adverse effects on patients, while a flat dose had none. Response rates also varied from 0/3 to 2/3, which may not be reliable due to the very low number of participants in the study . Lastly, an active clinical trial is evaluating the optimal Phase 2 doses of amivantamab, a recombinant antibody with a Fab arm, in combination with lazertinib on patients with non-small cell lung cancer that is metastatic or unresectable. Amivantamab targets MET receptors, which are overexpressed in many forms of cancers, with its Fab arm, and epidermal growth factor receptors, which perpetuate cancer growth when mutated. The existence of these clinical trials shows that various bsAbs have been FDA approved, are in usage, and are being researched. They also show examples of specific proteins bsAbs are used to target, and the reasons for doing so.

Conclusion

The processes for recombinant scFv and Fab production are largely identical despite their different structures. They both follow the same methods of derivation with PCR and primers, selection through phage display, and making sure there is soluble expression, so the specifics of that slightly vary between the two. The production process of recombinant bsAbs differs more from the other two formats, which is fitting considering how its structure differs from the other two. It is also worth considering that there are many different structures and associated production methods within the category of recombinant bsAbs which add more variation. One prominent difference between each recombinant format is the process used to ensure soluble expression of the antibody in E. coli. Regardless, there are still similarities in that the DNA is derived the same way and ligated into a vector. E. coli is also the most common host cell across the board. These similarities can be attributed to the necessary usage of recombinant DNA technology in creating such antibodies. Due to the amount of recombinant bsAbs that are made by linking scFv or Fab fragments,

there is also a large overlap in production methods between them because the fragments follow normal production before being linked. These findings have future significance because they allow researchers to easily find and compare the production methods of different recombinant antibodies. As a result, researchers can choose a specific recombinant antibody format to focus on in their own studies after carefully considering the methods and materials necessary to produce each. This paper has the potential to help researchers in the future be cost and material effective when performing studies on a recombinant antibody format. Hopefully, low-cost recombinant antibody treatments can be developed for cancer patients in the future because of this.

Limitations

This article purely focuses on comparing production methods of different recombinant antibody formats. As a result, there is minimal mention of the certain advantages each antibody format has or how they function. Since the paper is a literature review, no new experimental data was produced or analyzed regarding recombinant antibodies. While writing the paper, lack of access to various papers that required payment was a setback that may lessen the quality of the paper. Another such setback was that the author had minimal knowledge of monoclonal antibodies in advance, which significantly slowed the process of writing this paper.

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