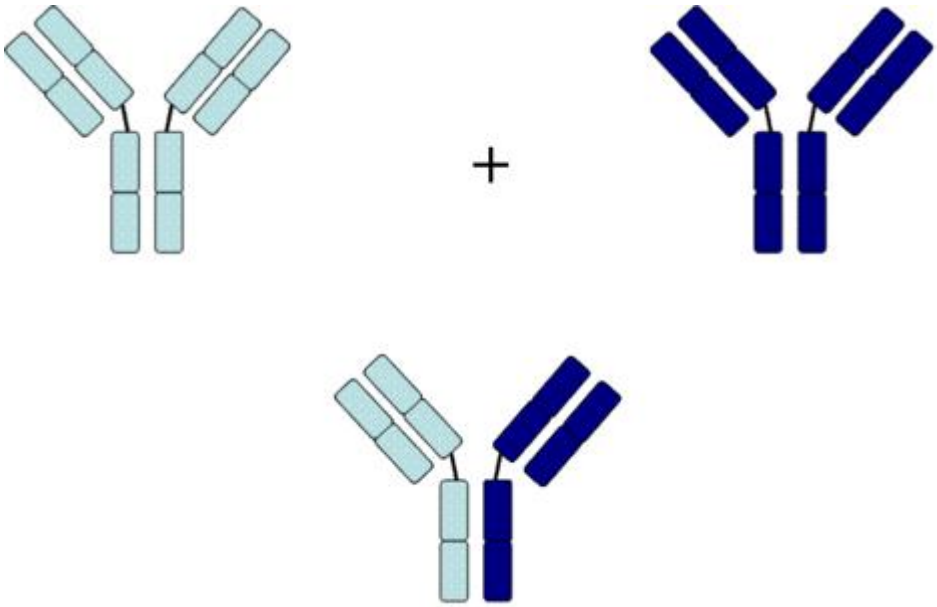
Bispecific antibodies and their potential for cancer immunotherapy

Bachelor Thesis



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Abstract

Immunotherapy for cancer is based on the principle of instructing your own body to fight the cancer off. To this end, most often monoclonal antibodies are being used. But because of increased ease of development there recently has been more interest in bispecific antibodies. These antibodies are capable of binding in two separate ways and therefore are able to direct immune cells to the tumour, deliver payloads, and inhibit two different pathways simultaneously. However, it remains unclear to what extent these bispecific antibodies can be used to treat cancer. This essay presents an overview of the development, treatment efficacy, and potential pitfalls of using bispecific antibodies in cancer immunotherapy.

There have been quite some struggles with the development of bispecific antibodies over the last decades. Most problems consisted of high immunogenicity, producing large quantities, and purifying them. Currently, most of these issues have been fixed, however the production is still suboptimal. There are still problems with the immunogenicity, but also with the short half-life time, and the non-human like structures of the antibodies.

Moreover, even though bispecific antibodies show great potential, the only approved ones are based on the mechanism of directing immune cells to the tumour, merely one of the many positive characteristics of bispecific antibodies. Adding to this is that the medicine that are approved are not the first preferred treatment for the diseases, due to low efficacy and many adverse effects. This shows that bispecific antibodies are far from their optimal potential and efficacy. Currently, there are several bispecific antibodies in clinical trials and many of them are using the inhibition of pathways as their mechanism and these antibodies seem very promising.

Another big problem is the cost of producing new medicine, which in turn leads to high costs of the medicine and it only being available to a select few. This is something that requires a lot of attention in the future by both the industry as well as the government. Overall, it was concluded that bispecific antibodies are very promising for the future, but a lot of work still needs to be done in their development but also in their efficacy. If this can be done the full potential of bispecific antibodies can be unleashed and this could possibly lead to curing some types of cancer.

Introduction

By definition, immunotherapy is 'a treatment of disease by inducing, enhancing, or suppressing an immune response'. In other words immunotherapy uses substances that stimulate your body to alter its immune response in such a way that it can fight diseases, like cancer. More specifically, according to the American Society of Clinical Oncology, immunotherapy has been used to stop or slow the growth of cancer cells, to stop the spread of cancer, and to enable the immune system to be more effective at destroying cancer cells. To this end there are several types of immunotherapy being used in cancer.

For the immune system it is essential to be able to differentiate between normal cells and foreign cells. Cancer cells are occasionally able to avoid detection by the immune system by abusing immune checkpoints. There are two well-known checkpoints, PD-1/PD-L1 and CLTA-4. To help the immune system there are substances, e.g. Nivolumab & Atezolizumab that target these checkpoints and by doing so the cancer cells will be recognized as foreign and subsequently destroyed. Another way of treating cancer is by using cancer vaccines. This can be via preventive vaccines to prevent cancer from developing, for example the vaccine against the human papillomavirus that can cause cervical cancer. However, there are also treatment vaccines, which strengthen the body's own immune response against the cancer. These vaccines can consist of dendritic cells, highly activated cancer specific T cells, or pure cancer specific antigens. But it has proven difficult to produce effective treatment vaccines.[1] Sipuleucel-T is an approved treatment vaccine for metastatic prostate cancer and it was found to increase the survival of men by 4 months.[2]

The primary type of cancer immunotherapy is monoclonal antibodies, which are specifically designed to bind to an antigen, for example a receptor on a cancer cell. After binding of the antibody, it can recruit other parts of the immune system to attack the cell it bonded to. There are three types of monoclonal antibodies: naked, conjugated, or bispecific. The naked antibodies have no substances added and work by boosting the immune response or by inhibiting cancer growth. The conjugated antibodies have a drug attached that can be delivered specifically to the cancer cells. The last type are the bispecific monoclonal antibodies, these types of antibodies are specific to two types of antigens and are therefore able to bring together two types of cells. For example, it can bind to a cancer cell and to a cytotoxic T cell, this then leads to the destruction of the cancer cell by forcing an interaction between the two. However, the concept is wider than just binding two cells, it can also block two different signalling pathways, target different disease mediators, and deliver substances to targeted sites (same as conjugated antibodies).[3]

Of the naked, conjugated, and bispecific monoclonal antibodies most of the cancer immunotherapy currently consists of naked monoclonal antibodies. In 2013 their global sales were approximately 75 billion US dollars, and this number is expected to rise to 125 billion US dollars in 2020.[4] But these types of antibodies have several limitations, for example drug resistance or no response to the treatment.[5] The fact that bispecific antibodies are able to target two sites simultaneously makes them much more capable at fighting cancer. Currently there are many bispecific antibodies in testing and some have already been approved for general usage. The market has also noticed the great potential of bispecific antibodies and has therefore been investing tremendous amounts of money into Research & Development, as can be seen in a recent investment of 1 billion+ dollars by US biotech giant Amgen and Germany's Immatics Biotechnologies GmbH.[i] But the question with every new type of treatment is whether it is better than the current treatment. Even though bispecific antibodies seem to be more potent than monoclonal antibodies it is still unclear whether there are downsides, for example they can be unstable, difficult to produce, and expensive.

[i] http://www.biospace.com/News/this-german-biotech-just-inked-a-1-billion-cancer/443591

Therefore the aim of this thesis is to determine to what extent bispecific monoclonal antibodies can be used as cancer therapy. This will be done by looking at their development, efficacy, potential, and downsides.

Development

The discovery of monoclonal antibodies dates back to 1975.[6] Surprisingly enough, the first discussion about bispecific monoclonal antibodies priors this discovery.[7] So, even though only recently there has been an interest in bispecific antibodies it is not something new. Research dating back to 1984 was already describing the use of 'hybrid' antibodies that bind to both the targeted site and to the Fc-Gamma receptor, for use in immunotherapy.[8] During the year thereafter, it was already postulated that hybrid antibodies would be able to bind specifically to T cells.[9,10] Following these discoveries, it became obvious that these antibodies could be used for immunotherapy (fig. 1). Especially since monospecific antibodies have not been as successful as expected due to resistance.[11,12] Cancer is a multifactorial disease and usually reliant on several pathways so the notion that monospecific antibodies have not been very successful is not surprising. By blocking a single pathway the cancer cell is still able to survive. Because bispecific antibodies are able to bind to two targets they are able to redirect the immune system to the cancer cells to enhance the destruction of cancer cells. Moreover, they are able to target two pathways simultaneously inducing changes in the cell signalling. Unfortunately, bispecific antibodies do not occur naturally and therefore need to be artificially made. There are two classes of bispecific antibodies: small bispecific antibodies and IgG-like bispecific antibodies. The IgG-like bispecific antibodies are able to interact with the Fc receptor due to a conserved Ig constant domain. Small bispecific antibodies are mainly used for recruiting effector cells, for example guiding T-cells to the tumour cell.

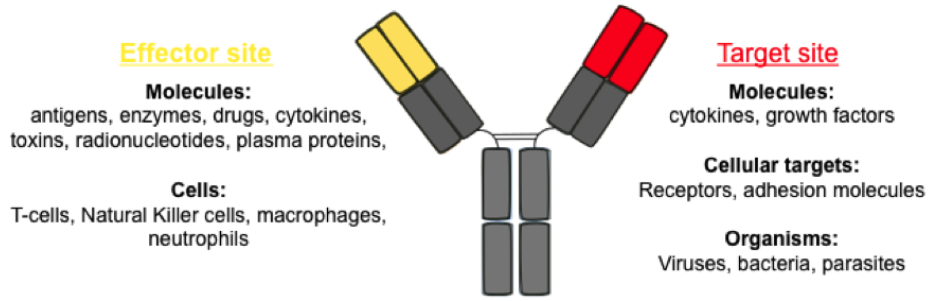

Figure 1. A bispecific antibody and its targets. This picture shows a bispecific antibody with in yellow the effector site and in red the target site. These two sites have different targets and are able to guide cells to targets, block pathways, and deliver compounds to targets.

The first described technique for developing bispecific antibodies is the hybridoma technology. However, due to miss-matching of the light and heavy Ig chains only a small fraction (1/10) of the produced antibodies are the correct ones, to isolate the correct one purification is needed.[13] Due to these downsides hybridoma technology could not be used produce large enough quantities for therapeutic use. To lower the amount of miss-matching with the hybridoma technology two different cell lines were used, from a rat and a mouse.[14] This method led to increased production of bispecific antibodies. A down-side of these bispecific antibodies is that they are more immunogenic due to rat/murine background.

Another technique is the 'Knobs into Holes' technique which was first described by Ridgway et al.[15] This technique is based on transfecting mammalian cells with modified human genes that code for the bispecific antibodies. A direct advantage of this technique is that there is less immunogenicity due to the use of mammalian cells and human genes. The technique works by improving heavy chain heterodimerization by substituting

a single amino acid in the opposing CH3 domains. In one heavy chain there is a 'knob', which means the amino acid has increased in size and in the other heavy chain there is 'hole', which means the amino acid has decreased in size. This optimizes the interaction between the two heavy chains. A downside of this technique is that there is still a problem with light chain miss-matching.

The third technique is the CrossMab approach which was first described by Schaefer et al.[16] This technique focuses on correctly binding the light chains by making the light chains different from one another. This then leads to immunoglobulin domain crossover. Because the chains are different association between partners that are unrelated is not possible. There are three proposed modifications, the modification is only done on one of the antibodies arms: 1) entire Fab region 2) VL-VH region 3) CH1-CL region. Due to this modification the modified light chain is now forced to bind correctly. This technique can be combined with the 'Knobs into Holes' technique to both bind the heaving and light chains correctly.[17]

The fourth technique is the Dual-Variable-Domain Immunoglobulin (DVD-Ig) approach which was first described by Wu et al.[18] DVD-Ig is able to maintain capabilities of its parental monoclonal antibodies and additionally is very stable. DVD-Ig's are developed using the variable domains of two parental monoclonal antibodies. These monoclonal antibodies are fused together via linkers that occur naturally. As mentioned before they maintain their parental affinities that indicate that both antigen sites of the bispecific antibodies are not affected by steric hindrance. There are many positives to DVD-Igs including: ease of production, ease of purification, and they are capable of targeting soluble proteins.

So far all techniques describe the production of IgG-like bispecific antibodies but there are also small bispecific antibodies, also called diabodies. They are made using DNA recombinant technology. Each chain of a diabody consists of a VH domain connected to a VL domain, the VL domain is linked with the VH domain of the other antibody.[19] This means that there are two opposing antibodies connected to each other with a short linker. Bispecific T-cell Engager Antibodies (BiTEs) are an example of a diabody. BiTEs are produced to specifically target CD3 on T cells, and they have a high potential to active them.[20] The other part of the antibody can then be used to target a cancer cell. This forces an interaction between the CD3 positive T cell and the cancer cell. This subsequently leads to a lytic synapse which can destroy the cancer cell.[21]

All of these techniques have led to a wide arrange of bispecific antibodies being developed, as is depicted in fig. 2. A commonly used way to produce bispecific antibodies is by using bacteria. It was found that *Escherichia Coli* could be used to produce bispecific antibodies.[22–24] More recently, a study has shown that a co-culture of two bacterial strains can be used to enhance the production of bispecific antibodies.[25] After many clinical trials this eventually led to the first BsMAb being approved in 2009. The next one was approved in 2014 and many more are now in clinical trials. These different bispecific antibodies will now be discussed in more detail to look at their efficacy and determine their potential.

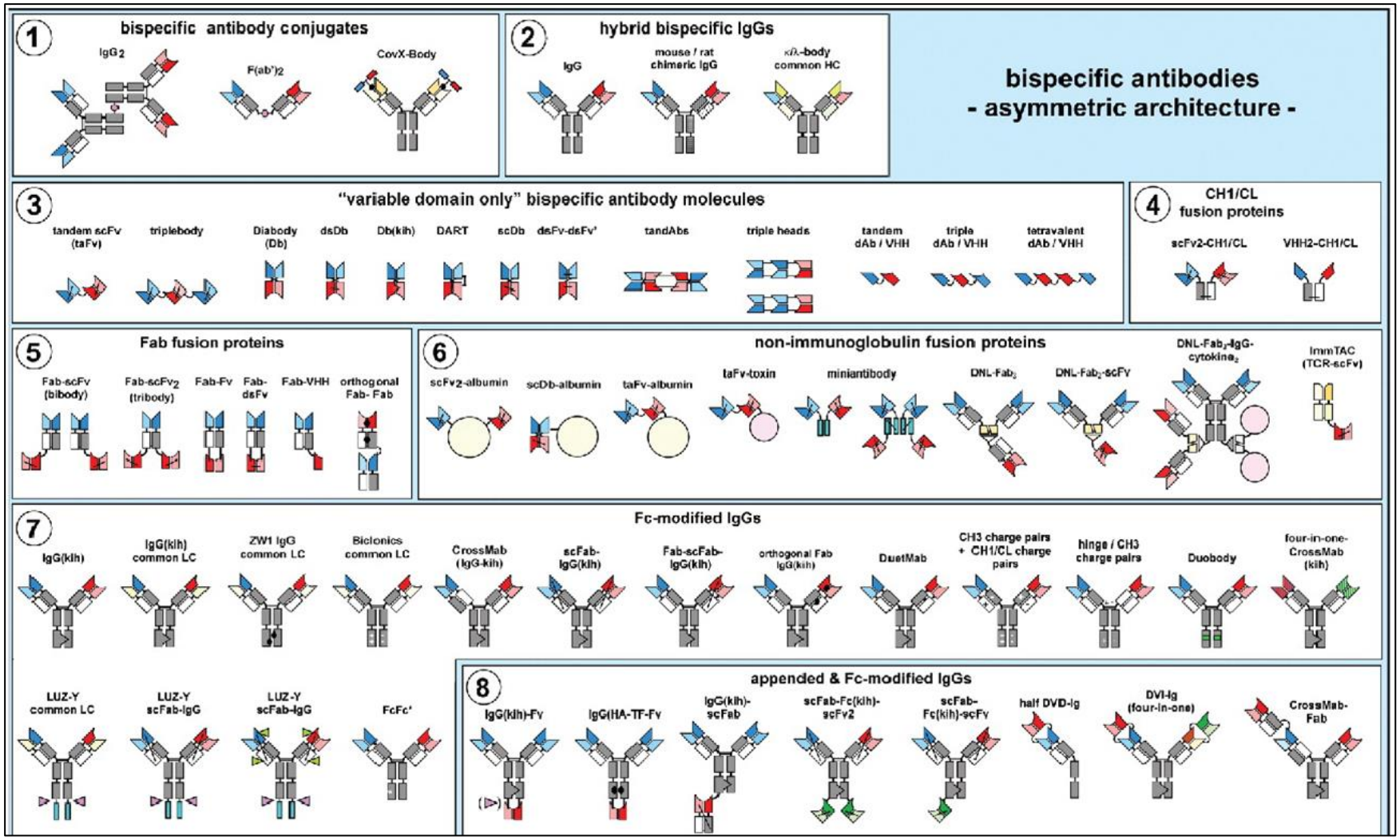

Figure 2A. Overview of all types of bispecific antibodies.[26] In this picture an overview of all known bispecific antibodies is given, as can be seen there are many different conformation and each functions in a different way.

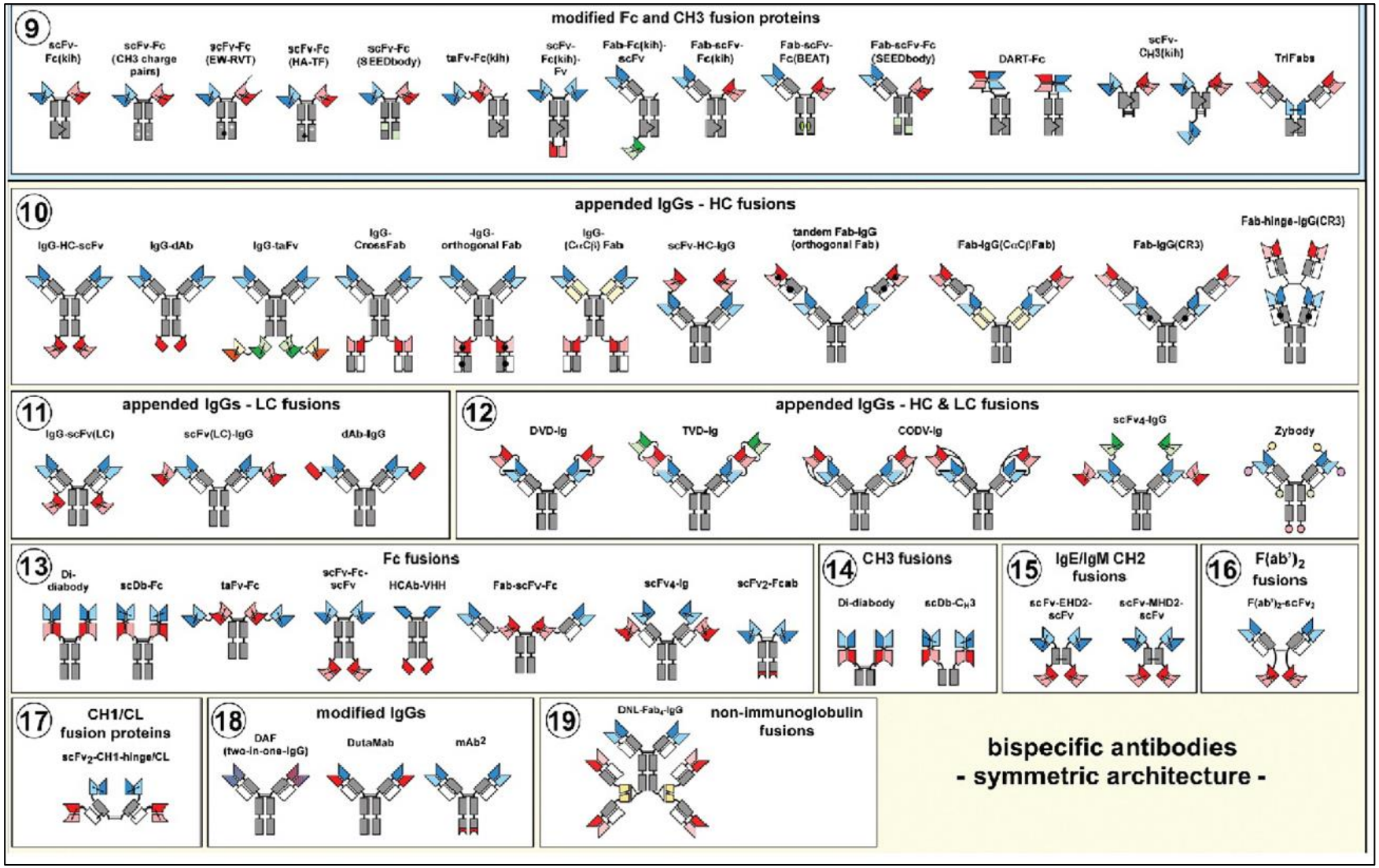

Figure 2B. Overview of all types of bispecific antibodies.[26] In this picture an overview of all known bispecific antibodies is given, as can be seen there are many different conformation and each functions in a different way.

Bispecific antibody targets & effects

Bispecific antibodies are capable of eliciting several immune responses and they are able to do this because of the two different antigen binding capability. An important feature of tumour cells is their mechanism to escape the immune system. Bispecific antibodies are capable of guiding immune cells towards the tumour cell (fig. 3a). Currently there are bispecific antibodies in development that are designed to direct T cells towards tumour cells.[27] These T cells are then able to interact with the tumour cell and destroy it. Other cells of the immune system can also be used for this goal, e.g. macrophages and natural killer cells.

Bispecific antibodies are also capable of blocking signalling pathways (fig. 3b); this is of importance because tumour cells frequently have altered pathways. An example being the receptor tyrosine kinases, which are critical for cancer.[28] Another advantage is the fact that bispecific antibodies can neutralize two different pathways simultaneously, which increases its treatment potential compared to monoclonal antibodies. An example being MM-111 which binds to the HER2/HER3 pathways, HER2 is a target for various cancers and HER3 is a signalling pathway that causes resistance to a HER2 inhibitor.[29] Because bispecific antibodies are able to bind to both pathways resistance poses less of an issue compared to monoclonal antibody use. In the case of HER2/HER3 this is because both pathways are targeted simultaneously, so resistance does not occur.

For tumours to grow it is important that they redirect blood vessels to the tumour (angiogenesis), by blocking angiogenesis tumours are unable to grow. There are many cancer therapies that disrupt angiogenesis by targeting angiogenic factors, e.g.: endothelial growth factor receptor 2/3, endothelial growth factor A, angiopoietins, and platelet-derived growth factors. It was found that targeting two factors simultaneously leads to a more efficient response.[30] An example is RG7221, made using the CrossMab technology, which was found to inhibit angiogenesis and by doing so inhibit tumour growth. This effect was found to be superior compared to only inhibiting a single pathway.[31]

Another important feature of bispecific antibodies is that they can deliver substances to tumour cells directly.[32] This enables them to, for example, deliver chemotherapy to the tumour cells, lowering the normal side effects. Bispecific antibodies can also be used in other diseases via the blocking of cytokines (which play a role in inflammatory and autoimmune diseases) and by forcing association of protein complexes (fig. 3c) (which play a role in coagulation diseases).

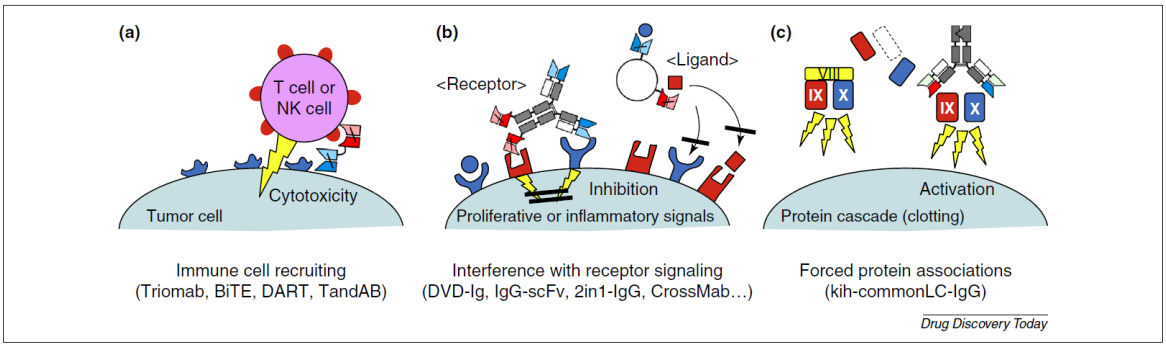

Figure 2. Bispecific antibody mechanisms.[33] Here three mechanisms are shown by which bispecific antibodies can perform their task. A shows the recruiting of immune cells, B shows the blocking of pathways, and c shows a forced protein association.

Bispecific antibody based medicine

Catumaxomab

Catumaxomab is a bispecific antibodies that was developed for the treatment of patients with malignant ascites, which is a medical condition where there is abnormal fluid accumulation in the peritoneal cavity. Malignant ascites are a symptom of advanced cancer and treatment is limited. Catumaxomab was the first bispecific antibody being approved for the treatment of malignant ascites in 2009 and it belongs to the class of BiTEs. It works by binding to epithelial cell adhesion molecule (EpCAM) on tumour cells and to T cells via CD3, it also capable of binding to Fcy receptors, specifically receptors 1, 2a, and 3 (fig. 4).[34] By binding to these Fcy receptors Catumaxomab is capable of activating accessory cells, for example macrophages and dendritic cells. EpCAM was chosen because it is frequently expressed on carcinomas. Catumaxomab enhances the patient's immune system against the tumour via T cell mediated lysis, phagocytosis, and antibody dependent cytoxicity.[35,36] These bispecific antibodies that have three functions are capable of inducing a long lasting immune response, adding to their efficacy.[37,38] Catumaxomab is produced using the hybrid hybridoma technique with both a rat and mouse cell line, by using two different cell lines there is less risk of miss-matching.[14]
Catumaxomab was shown to be highly efficient in reducing the amount of malignant ascites, increasing life-expectancy.[39,40]
A side-effect of catumaxomab seems to be a decrease in the amount of lymphocytes in blood, but this could be due to cytokines increasing the migration of T cells to the tissues.[41] Other side-effects include transient fever, nausea, and vomiting. This is most likely due to cytokine release which is a known side-effect of antibody therapy.[42] Since catumaxomab is made from a mouse-rat cell line there is a risk of immunogenicity but in a clinical trial no patients showed hypersensitivity reactions.[ii]Currently catumaxomab is being tested for ovarian cancer, gastric cancer, and epithelial cancer.[iii]

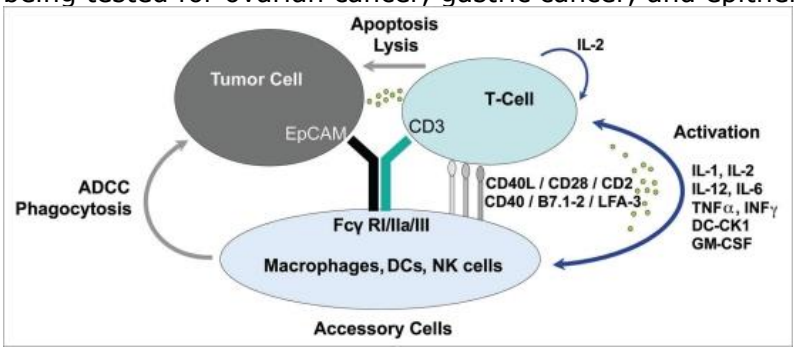

Figure 3 Catumaxomab mechanism.[43] Here the mechanism by which Catumaxomab works is shown, Catumaxomab binds to CD3 on T cells, EpCAM on the Tumour cell and via its Fcy receptor to accessory cells. This then leads to the destruction of the tumour cell.

[ii] http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000972/WC500051809.pdf

[iii] https://clinicaltrials.gov/ct2/results?term=catumaxomab&Search=Search

Developers	Lead molecule	Targets	Technology	Indications	Clinical stage
Trion Pharma, Neovii Biotech[a] (Munich)	Removab	Epithelial cell adhesion molecule (EpCam) × CD3	Triomab quadroma technology comprising hybrid mouse IgG2a × rat IgG2b antibody with intact immune effector functions	Malignant ascites	EU approval Apr. 23, 2009
Amgen (Thousand Oaks, California)	Blincyto	CD19 × CD3	Bispecific T-cell engager (BiTE) antibodies comprising different minimal antigen-binding domains from two single-chain Fvs (scFvs) arranged in tandem on a polypeptide chain	Philadelphia chromosome–negative acute lymphoblastic leukemia	FDA approval Dec. 3, 2014
Amgen	AMG-110	EpCam × CD3	BiTE antibody	Solid tumors	Phase 1
AbbVie	ABT-122	TNF-α x IL-17	DVD-Ig comprising tetravalent bispecific antibody with two additional variable domains, attached by linkers to the N-termini of the VH and VL domains of a conventional monoclonal antibody	Rheumatoid arthritis	Phase 2
AbbVie	ABT-981	IL-1α × IL-1β	DVD-Ig	Osteoarthritis	Phase 2
Affimed Therapeutics	AFM13	CD30 × CD16A	Tetravalent bispecific tandem diabody (TandAb) comprising four Fv domains joined by peptide linkers	Hodgkin lymphoma	Phase 2
Merrimack Pharmaceuticals (Cambridge, Massachusetts)	MM-111	Human epidermal growth factor receptor 2 (HER2) × HER3	Bispecific antibody fusion protein comprising two scFv domains linked by modified human serum albumin	Gastric cancer	Phase 2
Sanofi (Paris)	SAR156597	IL-4 × IL-13	Tetravalent bispecific tandem immunoglobulin (TBTI)	Idiopathic pulmonary fibrosis	Phase 2
Roche (Basel)	RG7221 (RO5520985)	Angiopoietin 2 (Ang-2) × vascular endothelial growth factor a (VEGF-A)	Bispecific IgG1 Crossmab antibody based on knob-into-hole mutations and Fab domain exchange	Colorectal cancer	Phase 2
Roche, Chugai (Tokyo)	RG6013 (ACE910)	Factor IXa × factor X	Asymmetric bispecific IgG4 antibody	Hemophilia A	Phase 2
Genentech (S. San Francisco, California)	RG7597 (MEDH7945A)	Epidermal growth factor receptor (EGFR) × HER3	Two-in-one IgG1 antibody	KRAS wild-type metastatic colorectal cancer; recurrent/metastatic head + neck cancer	Phase 2
Ablynx (Ghent, Belgium), Merck Serono (Darmstadt, Germany)	ALX-0761	IL-17A × IL-17F	Bispecific nanobody with albumin-binding domain	Autoimmune disease	Phase 2
Merus (Utrecht, the Netherlands)	MCLA-128	HER2 × HER3	BicIonics full-length bispecific antibody	Solid tumors	Phase 1/2
AstraZeneca (London), Amgen	MEDI-565 (AMG-211)	Carincoembryonic antigen × CD3	BiTE antibody	Gastrointestinal adenocarcinoma	Phase 1
Macrogenics, Servier (Suresnes, France)	MGD006	CD123 × CD3	Dual-affinity retargeting (DART) bispecific antibody based on two covalently linked Fv polypeptides	Acute myeloid leukemia	Phase 1
Regeneron (Tarrytown, New York)	REGN1979	CD20 × CD3	Bispecific antibody	Advanced malignancies	Phase 1

[a]Formerly Fresenius Biotech. Sources: company websites, PubMed

Table 1. Bispecific antibodies in clinical trials.[44]

Blinatumomab

Blinatumomab is bispecific antibody that was developed for the treatment of patients with acute lymphoblastic leukaemia (ALL). This form of leukaemia is characterized by the overproduction of cancerous and immature white blood cells, also called lymphoblasts. Death is caused by the lack of production of normal cells. ALL's main treatment consisted of chemotherapy but adverse events caused by this treatment are severe. Blinatumomab belongs to the class of BiTEs and works by specifically targeting CD3 on T cells and CD19 on B cells, as can be seen in fig. 5. CD19 was chosen as a target since it is frequently expressed on malignant B cells and it is presumed to be important for survival and proliferation.[45,46] In a study to determine targets for ALL treatment CD19 was found to be the most reliable target and found in almost 100% of malignant B cells.[47] Blinatumomab is made using recombinant DNA technology, this is done in Chinese hamster ovary cells.[20] Blinatumomab works by instructing T cells to lysis the CD19 positive B cells.[20] The mechanism by which Blinatumomab does this can be seen in fig 1. In a study with adult patients with relapsed or refractory ALL Blinatumomab showed anti leukaemia activity.[48] This activity means that 43% of patients went in complete remission and the median overall survival was almost doubled. In a study with paediatric patients with relapsed or refractor ALL Blinatumomab also showed anti leukaemia activity.[49]

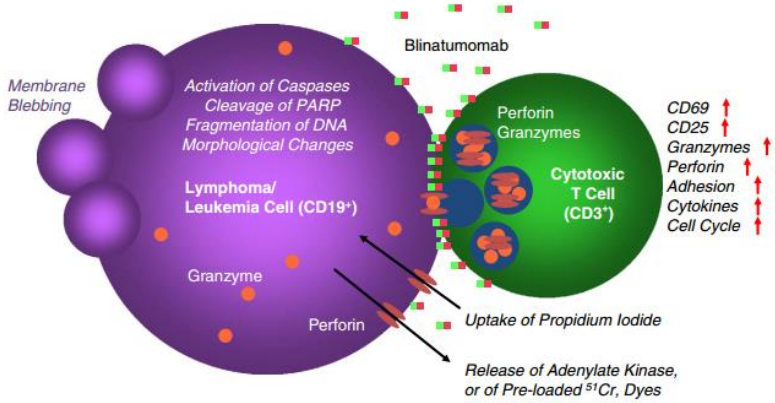

Figure 5 Blinatumomab mechanism.[50] Here the mechanism by which Blinatumomab works is shown. Blinatumomab binds to CD3 on T cells and CD19 on the leukaemic cell. This then leads to destruction of the leukaemic cell.

Side effects of Blinatumomab include pyrexia, rigor, fatigue, cytokine release syndrome, neurologic side effects. These neurologic effects include, aphasia, ataxia disorientation, and seizure.[50] These neurological effects could be due to inflammatory irritation caused by the activated T cells. All side effects were reversible. Blinatumomab attacks CD19+ cells but the long-term effect on depletion of these cells is unknown. An important step for Blinatumomab was when the way of admission changed from short-term to continuous infusion, this led to fewer side-effects, increased safety, and a more consistent T cell activation.[iv] Currently Blinatumomab is being tested for use in Non-Hodgkin's lymphoma, different forms of ALL, and Richter Transformation. Another downside of Blinatumomab is its price, when the drug hit the market it was priced at 178,000 USD per year. This made it only available to 1000 people in the United States and subsequently the most expensive cancer medicine available.[v]

Emicizumab

Even though only two bispecific antibodies are currently approved for use there are several more in development, for more than just cancer.[44] An example is a substance called Emicizumab, which is developed for the treatment of haemophilia A and works by forcing protein interaction. Haemophilia A is a genetic disease and the symptoms are increased bleeding. Emicizumab binds to factor nine and 10, which are associated with normal blood clotting. In a dose finding study it was found that Emicizumab decreased the bleeding rate in patients with haemophilia A, the side-effects that occurred were classified as mild.[51] Currently Emicizumab is being studied in several phase III studies.

Flotetuzumab

Another example is the substance called Flotetuzumab, which is developed for the treatment of myelodysplastic syndrome and acute myeloid leukaemia. These forms of cancer are resistant to normal agents, e.g. imatinib. Flotetuzumab binds specifically to CD3 on T cells and CD123 on leukemic stem cells and redirects T cells to kill the leukemic stem cells. In a pre-clinical study Flotetuzumab was found to be very efficient.[52] Currently phase I studies are being done for dose finding.

[iv] https://clinicaltrials.gov/ct2/show/results/NCT00274742

[v] http://www.fiercepharma.com/marketing/amgen-slaps-record-breaking-178k-price-on-rare-leukemia-drug-blincyto

Discussion

Because of the limited capability of monoclonal antibodies, bispecific antibodies seem to be a promising weapon to counter cancer. Therefore, in this essay I tried to answer the question: 'To what extent can bispecific monoclonal antibodies be used as cancer immunotherapy?' To this end I looked at how they are being developed, how efficient they are, their potential, and what their downsides are. This resulted in finding that their development has improved but is still limited and that they have not yet reached their full potential. Bispecific monoclonal antibodies do seem very promising on all areas but we still have a long way ahead of us before they will be widely available as cancer therapy.

Even though the potential of bispecific antibodies was discovered in the 80's, scientists were limited by the difficulty of producing a pure product on a large scale. This caused the antibodies to be unstable and therefore unable to be tested in clinical trials. Though over the last few decades the development of bispecific antibodies has gone through many stages improving the production process. There are now many ways to produce bispecific antibodies on a large scale and these techniques can also be combined for even more efficiency, e.g. CrossMab and the 'Knobs into Holes' technique.[17] But there are still some downsides to the current development, for example the immunogenicity of the mouse/rat hybridoma developed bispecific antibodies. But also many of the IgG-like antibodies do not resemble human like IgG enough, for optimal tissue penetration and limited adverse effects it is necessary that IgG-like antibodies resemble human IgG as much as possible.[53,54] Whereas IgG like antibodies struggle with limited tissue penetration, diabodies are able to effectively penetrate tissue but they have a short serum half-life.[53] This requires them to be administered frequently. Overall bispecific antibodies can now be produced on a large scale and there are also many different arrangements possible, as can be seen in fig. 2. But the just mentioned downsides are also important since if we can improve them, bispecific antibodies will become more safe and easier to be produced.

Bispecific antibodies can perform their work via several pathways: they can guide immune cells towards the tumour cells, block signalling pathways, block angiogenesis, and deliver substances to tumour cells. Therefore bispecific antibodies seem very promising to fight cancer. But so far only two types have been approved for medical use, one being Catumaxomab which works by guiding T cells to the tumour and one being Blinatumomab which also works by guiding T cells to the tumour. So even though these antibodies are theoretically capable of working in many ways only one is being utilized. Adding to this is that Blinatumomab is the second-line treatment for ALL, which means that other medication is still preferred.[55] For Catumaxomab similar guidelines are set: it is only advised to be used when standard therapy is nog longer available or manageable.[43] Even though these medicines are not preferred for medical practice, both due to side effects and efficacy, they do increase the survival of patients, which shows some efficiency. Adding to this is that there are many bispecific antibodies currently in clinical development, as can be seen in table 1. These antibodies also make use of the other potential techniques, e.g. RG7221 which blocks two pathways associated with angiogenesis.[31,44] Overall this shows that bispecific antibodies are theoretically capable of a lot but clinically they are still limited, both in their ways of working but also in the amount of side effects.

The recent increased interest in bispecific antibodies has also led to an increase in review articles discussing the state of the antibodies. For example Zhang et al conclude that bispecific antibodies are very promising due to their unique mechanism of action but that it is urgently needed to increase their efficacy and reduce the adverse effects.[56] They also believe that the unique complexity of tumours requires combination therapy. Chames & Baty conclude that after many years of disappointments clinical trials are finally providing exciting results and they believe that for the first time, curing patients using bispecific antibodies seems possible.[57] Muller & Kontermann stress the importance of accessory

cells and co-stimulation for optimal efficiency but they expect to find more and more bispecific antibodies in the clinic in the near future.[33] Overall I believe that most of the review articles come to the same conclusion, that bispecific antibodies seem very promising but that a lot of work still needs to be done to find their true potential.

Most of the research has been done towards the use of bispecific antibodies in cancer immunotherapy and therefore most of this essay has also looked in this direction. However, these antibodies are capable of also being used in many other areas due to their broad range of mechanisms. This is because interfering in receptor-ligand system is not only important in cancer therapy but also in inflammatory disease, for example rheumatoid arthritis.[32] Therefore bispecific antibodies that can be used for interfering with multiple ligands and/or receptors are currently being tested in clinical trials. The fact that bispecific antibodies cannot only be used for cancer therapy but also for other diseases is another strong indication of their true potential.

Future perspectives

To unleash the true potential of bispecific antibodies, it is important to first improve the development: counter the immunogenicity of the IgG like antibodies, make them more human like, and also improve the serum half-life of diabodies. Together this would lead to increased efficacy and reduced adverse effects of the antibodies. It is also important to study the mechanisms by which bispecific antibodies can work in more depth so that these can also be utilized to develop new and efficient compounds. Finally, we need try and lower the costs of bispecific antibodies so that it will be more available and affordable to everyone. Currently there are many bispecific antibodies in clinically testing and these seem very promising, combine this with an increased market interest and the future seems promising regarding these antibodies.

Conclusion

Overall it seems that bispecific antibodies are a very promising medication for the treatment of not only cancer but also of other disease, e.g. inflammation diseases. This can also be seen in an increased interest from pharmaceutical corporations. But it is important to keep improving the development process and to make better use of the many positive characteristics that bispecific antibodies have. This can eventually lead to improving immunotherapy for many types of diseases.

Experience writing bachelor thesis

Writing this bachelor thesis was a good learning experience. First of all this thesis learned me to properly differentiate between important and less important literature, this was important because of the vast amount of literature available. It also learned me that making a proper framework of what you want to write is a crucial part of the writing process. This framework enables you to search in a more orderly fashion for your literature and it also avoids going off-track too much. Another part of this thesis was the writing in English which is always a really good exercise, and especially important since the academic life is mostly English. Finally, I learned that it is important to make a writing plan, set deadlines, and stick to your deadlines. This way you get the best results but also leave time for improvements. Overall the experience of writing a bachelor thesis was a good one.

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