Pantimumab- Cancer Directed Fully Human Antibodies


Divya Dias
S.Y.B. (Pharm)

Sneha Potdar
S.Y.B. (Pharm)

Divya Dias : I enjoy reading and have a fascination for literature and science. I keep myself up to date with the latest research by reading science magazines and publications. I wish to work in the research field of Pharmacy and contribute to the development of new medicines.

Sneha Potdar : I am determined to make a name in the field of Pharmacy. I enjoy music, dance and am interested in genetic research. I am dedicated to my studies and plan to work in the area of genetic technology.

Abstract

The safety and efficacy of antibodies depends on their compatibility with the body system. Monoclonal antibodies having mouse sequences produce immunogenicity in humans, restricting their use. Genetically engineering transgenic mice with humanized humoral immune system is one of the possible solutions. They are obtained by merging human immunoglobulin loci with germ line of inactivated mouse antibody machinery. The hybrid mouse so obtained is called Xeno Mouse and is used as a source for high affinity high specificity human antibodies. Recently panitumumab (Vectibix) a completely human antibody directed against EGFR was successfully developed and approved in colorectal cancer treatment. This represents a milestone in the history and a herald for possible future applications of the Xeno Mouse technology.

1. Introduction- Antibodies and Immunity

When an antigen or foreign threat is intercepted by the body, specific cells- B and T cells of lymphatic system- recognize and respond or store the specific response to the particular agent. While T cells show a cell-mediated immunity wherein cells themselves travel and invade infected tissue, B cells show an antibody-mediated immunity wherein antibodies travel through blood stream and reach site of invasion. An antibody neutralizes, destroys or immobilizes the antigen. Its structure depends on the sequence of amino acids or 3 D structure of the epitope on the antigen that triggered its production.

Antibodies are also known as immunoglobulins since they belong to a group of glycoproteins called globulins. They mostly possess four polypeptide chains, two being light and two being heavy. The light chains are further differentiated in two classes called kappa and lambda while the heavy ones have five different classes-IgG1-4, IgA1-2, IgD, IgM, IgE, in humans. A heavy chain is connected by a disulphide bond to a light chain.

The mid regions of two heavy chains are connected by two disulphide bonds forming a hinge region whose flexibility yields a T shape or a Y shape. Beyond this parts of the two heavy chains form a stem region. The tips of the H and L chains are variable regions which bind to the antigens. The remainder of the H and L chains is the constant region (C) and determines the type of antigen-antibody reaction that occurs in a particular class of antibodies.

Antibodies are produced by the body itself but they may also be injected into humans to combat or prevent disease for e.g. vaccinations. Quite often, incompatible antibodies are recognized as antigens which then get destroyed by the body's defenses, producing allergic and other immune responses. Obviously, the more the antibody is accepted by the body itself, the more effective it will be.

2. Monoclonal Antibodies

Monoclonal antibodies (MAbs) are identical antibodies which are cloned from a single parent cell. Plasma cells or B cells are hard to grow in culture therefore a hybridoma methodology was developed. Hybridomas are hybrid cells consisting of B cells fused with tumor cells and are long term sources of MAbs. Tumor cells are used since they can grow easily and proliferate endlessly, thus making cell multiplication easier. B cells of animals like mice are mostly used in this procedure.

Monoclonal antibodies are used for various purposes including measuring levels of drug in a person's blood, diagnosis of strep throat, pregnancy, allergies and diseases like hepatitis, rabies and some sexually transmitted diseases. They can be used to detect cancer at early stages and ascertain the extent of metastasis. Vaccines to counteract rejection associated with transplants and for autoimmune diseases (perhaps even AIDS) can be formulated from these antibodies.

Mouse MAbs are produced by fusing mouse B cells with human tumor cells that grow easily and proliferate endlessly. This is done by a process called hybridoma which carried out as follows

1. The mouse is exposed to desired antigen
2. It produces antibodies against this antigen
3. Its spleen (producing antibodies) is removed
4. The antibodies are fused with cultured human myeloma (tumor) cells

5. Fused cells are grown briefly
6. Cells are injected into peritoneum (serous membrane) of another mouse
7. Monoclonal antibodies (cloned cells) are harvested from ascites fluid (abnormally accumulated serous fluid in peritoneum) of mouse

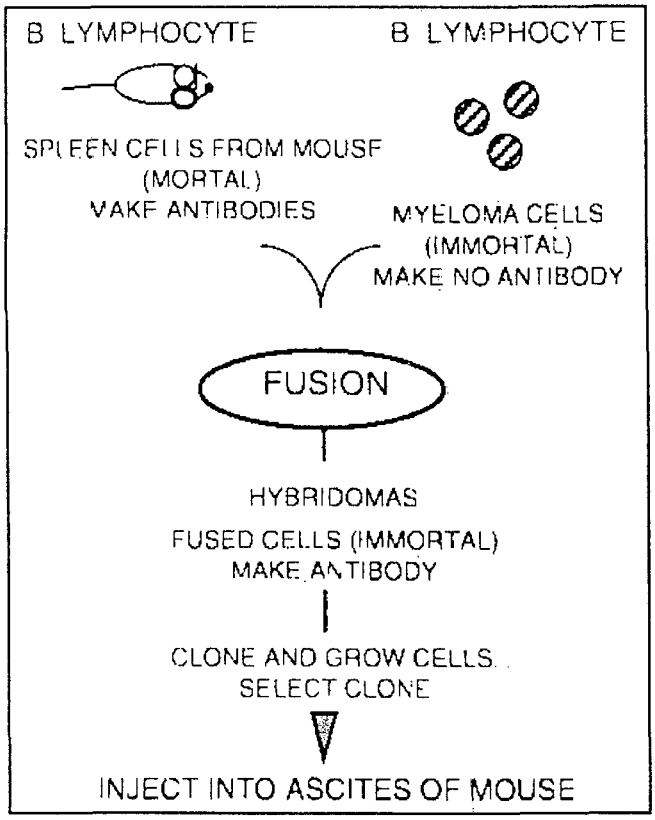

Figure 1 shows the process of production of these antibodies.

Hybridoma Technology in Mouse

The purity of the antigen, the mouse strain used, age and sex of the mouse, tolerance of the individual mouse; and many other factors may affect the outcome of Ab-producing procedures. Adjuvants are often used which hold the antigen at the site of immunization and release it over a long period of time. One of the more widely used- Freund's Complete Adjuvant kills mycobacteria. This serves to increase inflammation upon injection, and thereby enhances the mouse's immune response. It often results in painful lesions at the site of injection.

But mouse MAbs contain genes which belong to mice, and therefore are liable to be rejected by the human body. Rapid clearance, reduced efficacy, immunogenicity (ability to produce immune response) and allergic responses are some of the problems encountered. This hinders the treatment of chronic and recurring human diseases, which require frequent antibody administration.

3. Attempts to Produce Human MAbs

Difficulties experienced in the use of mouse MAbs generated the need to produce partially or completely human MAbs. Various routes were explored-

1. Generating MAbs from human B cells; much like the mouse B cells using the hybridoma technique- This was rejected due to the short lived nature and unavailability of the human B cells of desired specificity and affinity.
2. Replacement of parts of mouse MAbs with human sequences- This was not only tedious due to case-by-case molecular modeling and engineering but some part of mouse gene sequence was still retained. So chances of rejection were still high.
3. Manipulation of antibody genes to produce antigen-specific, high affinity human antibodies involves intensive in vitro processing. Here the bulk tissue is cultured in encapsulated or hollow fibre systems. Another method is through the expression of cloned antibody genes in high producing eukaryotes, using recombinant DNA technology.

4. Xenomouse

The mouse hybridoma technology being well established, transgenic mice genetically engineered with a human humoral immune system was considered next. Through recombination or joining of DNA strands from two different DNA molecules, and affinity maturation, or increased rate of response to antigen, different MAbs with required characteristics could be produced efficiently and conveniently.

In this method, Xeno Mouse strains are developed in which inactivated mouse antibody machinery is 'humanized' with megabase (Mb= 106 base pairs) sized human immunoglobulin loci (genes on human chromosome corresponding to mouse chromosome) to recreate human humoral immune system in mice.

4.1 Generation of Xeno Mouse strains

Xeno mice are basically transgenic mice, those who have one or more of their genes modified or inactivated. The basic aim of the research was to inactivate the antibodies produced by the mice and develop an immune system within the mice which is similar to the human humoral (antibody mediated) immune system so that the antibodies produced by the mice are not rejected by the human body. This was made possible by the generation of Xenomice strains. The genetic modifications were carried out in the embryonic stem cells (ES) cells which are pluripotent and can give rise to many different types of specialized cells. The modifications were to inactivate the mouse immune system which produces the antibodies and introduction of the human immunoglobulin heavy and light chains.

The process involved deletion of the heavy and light kappa genes in the ES cells by gene targeted deletion. The deletion of JH chain in mice (a kind of heavy chain) inhibited the the heavy chain recombinant machinery and therefore totally eliminated the immunoglobulin production. The C region (light chain region) was deleted and inactivated the Ig locus. These two strains were cross bred to give rise to mice where the production of antibodies and therefore the B-cell development was completely inhibited. The gene deletion targeted only the cis-acting sequences while the trans-acting factors for antibody rearrangement were maintained. This was significant as because of these factors the human immunoglobulin loci could be introduced in the mice.

The human immunoglobulin loci also had to be cloned and a large

amount transferred to preserve the genetic diversity and proper regulation of antibody maturation and expression. The genes which code for human immunoglobulin heavy and light K light chains each span over 1.5 Megabases on chromosomes 14 and 2 respectively and contain hundreds of segments that encode and control the expression of huge diversity of humoral immune response. These loci consisted of a variable segments encoding the variable (75 VH, 76 VK genes), diversity (30 DH genes) and joining (6 JH and 5 JK genes) domains which control antibody expression. Cloning of the large human heavy and light chain loci was facilitated by the yeast artificial chromosome (YAC) technology which permitted the stable isolation and efficient genetic manipulation of MB-sized DNA fragments.

A yeast artificial chromosome (YAC) is a vector used to clone large DNA fragments (100 kb to 3000 kb). It is a synthesized chromosome containing telomeric, centromeric and replication origin sequences needed for replication and preservation in yeast cells. Built using an initial circular plasmid, they are linearised with the help of restriction enzymes, and then DNA ligase is used to add a sequence of gene interest within the linear molecule by the use of cohesive ends. They were first described in 1983 by Murray and Szostack.

A 970 Kb human heavy chain YAC was created with the help of this technology. It consisted of the VH genes, all 30 DH and 6 JH genes and the Cμ and Cδ constant regions. The Cδ region was modified with either human γ1, γ2 or γ4 constant region, in conjunction with the mouse ʒ enhancer to generate three different yH2 YACs each equipped with a different heavy-chain isotype. Similarly an 800 kb yK2 YAC was reconstructed.

These large yH2 and yK2 YACs were introduced in the mouse genome y fusion of YAC containing yeast spherolats and ES cells. It yielded a high frequency of ES cells in which the large DNA fragments are integrated in the mouse genome in intact and unrearranged form and are transmitted through the mouse germ line. The yH2 or yK2 YACs were used to transmit the human immunoglobulin fragments and express human heavy or K chain protein respectively. The cross breeding of these mice strains with the Xeno mice strains which have their antibody mechanism inactivated doubly gives rise to mice which produce fully human antibodies. The research led to the generation of three different Xenomice strains each producing fully human antibodies with only one of the three isotypes- IgG1K, IgG2K, IgG4K which allowed the generation of antigen-specific MAbs for specific disease conditions. Subsequently the entire human gene locus Igγ was also introduced on YAC to generate three mouse strains XMG1-KL, XMG2-KL, XMG3-KL each producing both human IgGK and IgGλ antibodies in the ration 60:40.

4.2 Xeno Mouse strains produce high affinity fully human MAbs

Xenomouse lymphoid organs and serum on observation showed human antibody response from a broad primary immune response of a wider range to a proper secondary response displaying class switching and affinity maturation. The V, D and J genes were used for antibody transcription along the entire length of the YACs, as they would have been in humans. Efficient B cell development, higher levels of circulating antibodies utilization at high frequency of V genes, enabled better specificity, affinity and availability of antibodies. This was applied to creation of antibodies for numerous antigens, including interleukin-8, EGFR and tumor necrosis factor-α, IL-6, GROα and CD147, MUC18, platelet derived growth factor-D (PDGFR-D), insulin like growth factor receptor – 1(IGF-1R), cytotoxic T lymphocyte- associated antigen 4 (CTLA-4), CD40, hepatocyte growth factor (HGF), receptor activator of NF-xB (RANK) ligand and prostrate stem-cell antigen (PSCA).. In addition, the antibodies showed high potency in blocking in vitro and in vivo biological effects of their respective antigens on human cells, indicating their potential therapeutic use.

The pharmacokinetics of mABs are also affected by their targets and the related biology such that a MAb that targets circulating antigen may vary in pharmacokinetics profile than a MAb targeting a cell surface receptor that can mediate internalization of the receptor- mAb complex. For e.g. Administration of Xeno mouse derived anti-IL-8 mAb to subjects indicated pharmacokinetics similar to that of endogenous IgG2 which has a half life of 21 days and a Xeno Mouse derived mAb to RANK ligand has shown non linear pharmacokinetics and a very long half life allowing for dosing as infrequently as every 3 or 6 months.

4.3 Targeting EGFR by therapeutic antibodies

EGFR is a member of erbB family of receptor tyrosine kinases. They are type I membrane glycoproteins having an N-terminal extracellular ligand-binding domain, a single transmembrane domain and a C-terminal intracellular tyrosine kinase domain. EGFR binds to many ligands including EGF, transforming growth factor alpha, amphiregulin, betacellulin, heparin binding EGF and epiregulin. The high affinity receptors are shown to be biologically active. Dimerization of receptors catalyzes autophosophorylation of C terminal tyrosine residues which leads to recruitment of adaptor protein to those phosphorylated tyrosines and subsequent activation of one or more transduction pathways.

This mediates a variety of cellular responses including cell proliferation, differentiation, survival, motility, adhesion and angiogenesis. EGFR and other members of erbB family were initially found to be transmitted as oncogenes for the avian erythroblastosis virus.

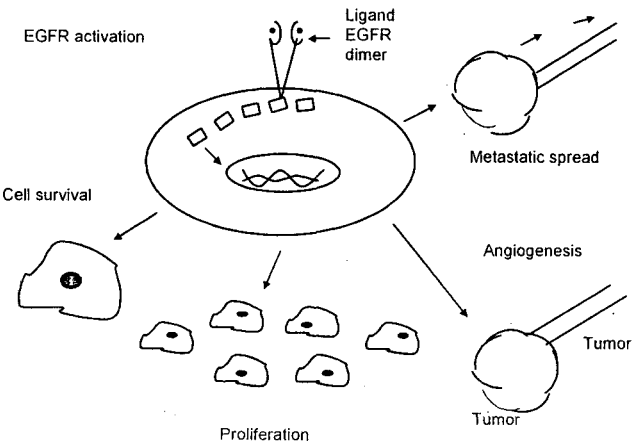

Fig2 The role of EGFR activation in tumorigenesis

EGFR having an important role in the development and progression of human solid tumors is surmised from some facts (Figure 2 shows the role of EGFR in tumorigenesis).

Role of EGFR in Tumorigenesis

Introduction and overexpression of EGFR induced transformed phenotypes in the recipient cell and excessive EGFR has been found in many human epithelial cancers including colon, lung, kidney, neck, breast, prostrate, brain and ovary. Overexpression of EGFR indicates poor clinical prognosis. Coexpression of EGFR and its ligands TGF-alpha or EGF was found in the same tumor tissues indicating autocrine regulatory loop may stimulate growth and progression of these tumors. A number of anti EGFR agents including small molecule inhibitors that block kinase activity and mouse EGFR neutralizing mABs that block ligand binding and EGFR activation, were able to inhibit tumor growth in xenograft (tissue grafted from different species) mouse model.

Hence an antibody targeting EGFR was considered for treatment of cancer.

The first approved therapeutic anti- EGFR mAb developed was cetuximab, a chimeric IgG derivative of mouse mAb. A fully human antibody was expected to minimize immunognecity and to improve safety profile and dosing schedule, which brought reearch focus on to panitumumab.

4.4 Discovery and pre clinical developments of panitumumab

It is a fully human IgG2 mAb. It binds specifically to EGFR which play a role in cancer. Over expression of EGFR might lead to some form of cancer. Panitumumab plays a role in preventing this by combining with EGFR and preventing its expression and therefore preventing cancers like colorectal. It was raised by immunizing the IgG2 strain of Xenomouse using human epidermoid cervical carcinoma A341 cells which over express the EGFR cells on their surface.

Panitumumab has a high affinity and specificity towards the EGFR and its potent enough to block ligand binding and inhibit receptor phosphorylation. Its affinity is greater than 200 fold greater than the ligand to low affinity receptors and greater than 500 fold for high affinity receptors. EGFR is widely expressed in various epithelial tissues like skin, liver, kidney and lung. An IgG2 isotype was chosen to minimize the potential toxicity to EGFR expressing normal tissues from recruitment of antibody-dependent, cell-mediated cytotoxicity and complement-dependent cytotoxicity. This combination of fully human nature of the antibody anf the IgG2 isotype makes it less vulnerable to the immune system of the human body and can lead a longer life in the body. It also alleviates symptoms like fever , chills, difficulty in breathing which are normally associated with the rejection of a foreign object in the body.

4.5 Action of Panitumumab

It acts by binding with domain III of EGFR which is ligand binding domain. As soon as they bind, it leads to the disruption of the receptor binding action at the cell surface. Because the EGFR cannot bind to the receptor, the further process of metastasis does not occur. Panitumumab may also prevent phosphorylation reactionsof EGFR and cause cell cycle arrest and suppress production of pro-angiogenic factors like vascular endothelial growth factor and IL-8 by the tumour cells. It suggests that Panitumumab is capable of stopping tumour growth and progression by blocking cell proliferation and inhibiting angiogenesis, that is growth of new blood vessels which supply the tumour cells with nutrition.

In a research conducted, Panitumumab was able to completely eradicate the already established A431 tumour xenografts apart from preventing and inhibitng tumour growth. It also shows anti-tumour activity in tumours which express lower levels of EGFR such as pancreatic tumour BxPC3 and colon tumour HT-29. however not all the EGFR- expressing tumors examined were sensitive to this treatment exphasizing that EGFR expression merely is not enough to predict tumor response to these MAbs. Combination therapy has worked and combinations of panitumumab and germcitabine have resulted in enhanced anti-tumor efficacy compared to either agent alone.

4.6 Clinical Deveolpment

It began in 1999 with the phase I study evaluating safety and pharmacokinetics of panitumumab in people who were previously treated advanced EGFR-expressing solid tumors. Phase I enrolled 96 people with advanced solid malignancies and a range of doses (0.01-9mg/kg) were administered. It was generally well tolerated, skin toxicity being the most common dose-related adverse event. Phase 2 evaluation focussed on people with metastatic colorectal cancer whose disease had progressed during or after chemotherapy regimens. They investigated efficacy, safety and potential relationship between panitumumab efficacy and EGFR tumor membrane expression levels as measured by immunohistochemistry. Phase 3 consisted of a pivotal trial designed to study panitumumab monotherapy in people with previously treated metastatic colorectal cancer.

There was also a study comparing panitumumab plus best supportive care versus only best supportive care in people with metastatic colorectal cancer who had the disease progression after fluropyrimidine, irinotecan and oxaliplatin containing chemotherapy regions. The study was conducted in Europe, Canada and Australia. (Graph 1 illustrates these results).

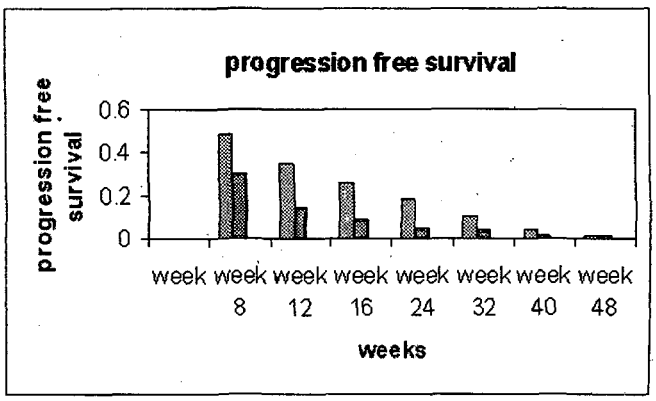

Fig. 3. Panitimumab vs Best supportive care

A safety analysis of 920 persons from ten panitumumab monotherapy

studies have shown that panitumumab is generally well tolerated with expected on- target effects related to EGFR inhibition like skin related events, hypomagnesemia and diarrhea. Complications like sepsis have been observed particularly in association with severe skin infections. Other skin related toxicities included paronychia and fissures after long term exposure specially. Fatigue, nausea and vomiting were also documented. However these are not very different from the complications involved in traditional chemotherapy drugs and the safety profile was considered good enough for marketing.

Benefits: These antibodies have the added advantage of facing very low immunogenicity and immune reactions as they are fully human antibodies without the presence of mice genes. The traditional drugs face risks of triggering the development of anti drug antibodies which elicit hypersensitivity, neutralize the antibody function and causes discomfort to the patients. Panitumumab has been found to elicit low immunogenecity response and in many cases no immune response at all.

4.8 Ongoing clinical trials

The PACCE (Panitumumab Advanced Colorectal Cancer Evaluation) is a phase 3b randomized open-label clinical trial evaluatinf oxaliplatin and irinotecan-based chemotherapy and bevacizumab with and without panitumumab for first-line treatment in people with metastatic colorectal cancer. The results as of now were not very promising as there is additional toxicity and a lack of bio-synergy between panitumumab and bevacizumab in combination with oxaliplatin-based chemotherapy. Additional studies in the field of head, neck and other cancers are currently going on. Two additional phase 3 randomized controlled trials are evaluating the role of panitumumb in combination with FOLFOX and FOLFIRI chemotherapy without bevacizumab in first and second line metastatic colorectal cancer. Other anti-EGFR MAbs like cetuximab, a chimeric IgG1 antibody was also approved in 2004 for treatment of colorectal cancer and in 2006 for treatment of SCCHN. There is a biological difference between the two antibodies in terms of half-life and dosing requirements. A number of additional MAbs targeting the EGFR ,like matuzumab, nimotuzumab, zalutumumab are currently under study.

Table I shows the newer and upcoming molecules which are in production and currently under development by various companies.

5. Conclusion

Panitumumab is the first fully human antibody developed from Xenomose technology. Antibodies which can be produced similarly are great potential drugs for reducing immune reactions from the human body and at the same time be effective enough to have a positive risk-benefit profile. They have longer half-lives confer potentially important safety benefits and dosing flexibility. It has opened up many avenues for exploration of other such molecules which can be used in lieu of traditional chemotherapy and be greatly effective for a variety of different cancers.

mAb	Target	Indication	Company	Clinical trial stage approved
Panitumumab	EGFR	Cancer, solid tumors	Amgen	2,3
Denosumab (AMG 162)	RANK ligand	Osteoporosis, treatment induced bone loss, bone metastases, multiple myeloma	Amgen	2,3
AMG 102	HGF	Cancer, solid tumors	Amgen	1
AMG 655	Trail Receptor 2	Cancer, solid tumors	Amgen	1,2
CP-675,206	CTLA-4	Cancer, solid tumors	Pfizer	3
CP-870,893	CD40 agonist	Cancer, solid tumors	Pfizer	1
CP-751,871	IGF-IR	Cancer, solid tumors	Pfizer	2
HCD122	CD40 antagonist	Cancer, hematologic tumors	Novartis/ Xoma	1
CR002	PDGFR	Kidney inflammation	CuraGen	1b
CRo11-vcMMAE	GPNMB	Cancer, melanoma	CuraGen	1
HGS004	CC chemokine receptor 5	HIV	Human Genome Sciences	2
AGS-PSCA/ MK-4721	PSCA	Cancer, solid tumors	Agensys/ Merck	1

Table. I Xenomouse- derived mAb product candidates in clinical development.

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