Detailed Review of Monoclonal Antibody Production and Its Advantage as a Role in Malignancy and Transplant Rejection

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Abstract - Monoclonal antibodies were discovered by Kohler and Milstein in early seventies this discovery leads to a huge revolution in immunology field. In addition to this Kohler and Milstein along with Niels Kaj Jerne were awarded noble prize in 1984. There are various production methods of monoclonal antibodies which includes hybridoma technique which is the most traditional method, phage display, transgenic mouse method also production of monoclonal antibodies from plants this procedure is under development. Hybridoma technique is considered to be the traditional or conventional technique which was used for the first MAb production in 1975. But the phage display is an advanced method which can be assured to produce MAb in a faster rate compared to the hybridoma technique. Phage display method can be used in order to obtain specific MAb even against toxic antigens as it does not require any immunizations but hybridoma technique cannot be used to obtain MAb against toxic antigens because it is harmful to the animals when immunized with it. Both hybridoma technique and phage display method is continued by in vitro or in vivo method. The monoclonal antibodies produced can be murine MAb which is made up of 100% mouse protein, chimeric MAb which is made up of 25% mouse protein, humanized MAb which is made up of 10% mouse protein or else can be fully human MAb. MAbs which are approved are recommended and available in the market mainly it needs FDA approval in order to achieve FDA approval MAbs should undergo different phases and clinical trials. MAbs play a main role in cancer and prevention of graft rejection other than this it also has many other therapeutic uses. MAbs are more preferred compared to polyclonal antibodies as it is more specific but it is also very expensive. The immunology field can be developed through further development of MAbs which can also lead to more life span as many diseases can be treated with MAbs.

Index terms - Cancer, graft rejection, hybridoma technique, monoclonal antibodies, phage display method.

1. INTRODUCTION

Antibodies are defined as specific molecules present in the blood and tissue fluids which is produced as the result of immune response in order to fight against infection [1]. In addition to this it's a basic spatial structure is made up of 4 chains which are of 2 heavy chains and 2 light chains which are identical to each other and it is joined by disulphide bonds to form Y shape [2]. Monoclonal antibodies are said to play an important role in the biomedical research and in molecular immunology investigation [3]. MAb has a strong impact on laboratory diagnostics as it is used in therapy of diseases such as cancer, which includes hematological malignancy, and in tumor identification. MAb was discovered by Kohler and Milstein at early seventies in molecular laboratory at Cambridge this discovery made a huge revolution in immunology [2]. MAb is defined as the antibodies produced by the cell lines or clones by the animals such as mice, which are immunized with the antigen for which the MAb is needed to be produced. The steps involved in the production of MAbs are immunization, fusion and selection, screening, characterization followed by cloning [1]. MAb is also known as monovalent antibodies, which bind to the same epitope or antigenic determinant [4]. The main principle in the production of MAb is fusion of B cell produced by the immunized animal with the myeloma cell, which results in hybridoma cell. This hybridoma technique is said to be the first technique of MAb production discovered by Kohler and Milstein in 1975 [5]. As the B cell does not have a long life span so the fusion with the myeloma cells provides immortality. The each MAb is said to possess a unique specificity also it has main applications other than in treatments they include their role as abzymes in finger printing and in drug development like cancer vaccines. Even though mAb is considered as an important agent in modern diagnosis but it has many ethical issues regarding the use of animals in the modern methods of MAb production methods [6]. Monoclonal antibody market had a successful growth after the discovery of the first MAb product which was approved. MAb is said to have a great impact in therapies as 30 MAbs were approved by FDA in order to treat many diseased conditions including transplant rejection in human [4].

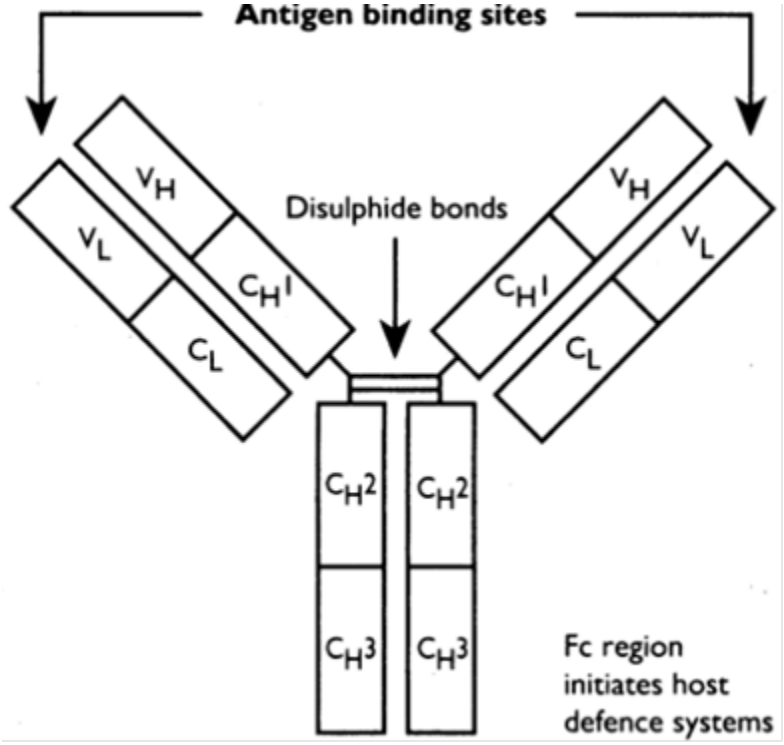

Figure 1: Structure of normal antibody. This diagram mentions the parts of antibody and its binding sites along with the sulfide bonds [7].

2. HISTORY

Normal antibodies which play an important role in immunology itself have a long history from 19th century about its discovery and characterization [2]. MAbs came into use in 1970s where, the first MAb was produced in 1975 using hybridoma technique by Georges Kohler of West Germany and Cesar Milstein of Argentina and then they developed the technique together in 1976. Their work was also published in same year in the journal nature. About 9 years later in the year of 1984 both were honored with noble prize along with Niels Kaj Jerne of Denmark for their work in the production of MAb using hybridoma technique. The phage display method is considered as an advanced technique developed in 1985 [8].

The therapeutic use of MAb on human took place for the first time around 1982 and around 2 years later in 1984 chimerization process was developed which results in development of chimeric MAb. [2]. Although the MAb produced was actually licensed in 1986 during this period mAb was commercially developed. The first licensed mAb was Orthoclone OKT3 which was widely used to prevent kidney transplant rejection. Also the process of MAbs to target tumour or cancer cells was proposed by Paul Ehrlich a century ago [4, 9]. In 1988 Greg Winter discovered humanized MAbs in order to avoid unwanted immune reactions. Then the approval for MAb products was very slow till 1990s when the chimeric mAb was approved for the first time [10]. Most of the therapeutic antibodies used to treat humans are the chimeric antibodies. Chimerization is the term that denotes the mAb whose variable regions are made up of murine but the remaining part that is the light and heavy chains are humanized. MAb was well developed commercially in 2013 over past twenty years where as it was the leading product which represented about 50% of the sales in the year 2013. Also the sales were statistically raised from $39billion in 2008 to $75billion in 2013. And currently there is more than 30 MAbs approved for therapeutic use for established markets in Europe and United states. The validation of the mAb technology was improved after the approval of panitumumab in 2006 [11]. In 2015 eight MAbs was approved for the sales in market by United States FDA. This is the timeline of the long way of the development of MAb in clinical field [2].

3. RESEARCH ELABORATIONS ON PRODUCTION METHODS OF MAbs

MAb is used as a therapeutic drug so it requires a large scale production even though the laboratory research purpose requires only a small amount to be produced. There are various MAb production methods are being evolved along with it many other alternative methods for the production of MAb are being emerged and discovered. A therapeutic drug MAb is produced in a commercial level by using adherent or suspension cells and also some efficient procedures are followed to obtain a better culture of the cells. Whereas suspension cells method is considered to be an easier method compared to other methods [12].

The production methods of MAb are as follows which includes:

3.1 Hybridoma technique

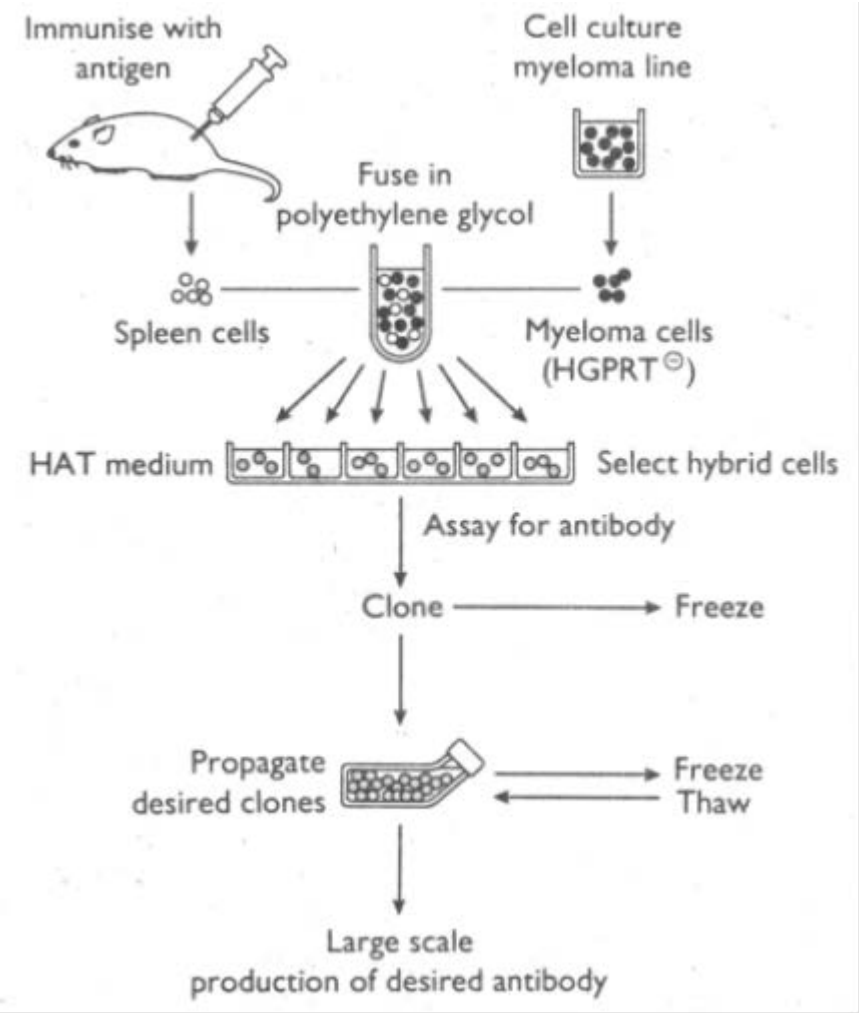

Figure 2: Procedure of hybridoma technique. Here the steps followed in hybridoma technique is mentioned and described at last the production of MAbs is shown [7].

3.1.1 Outline

Generation of MAb for the first time from mice in 1975 was done through hybridoma technique which is said to be a traditional technique. Combination of innate functions of both Immune cells (B lymphocytes) and cancer cells (myeloma cells) to produce hybridoma cell is the principle followed in hybridoma technology [4]. This type of production method of mAb involves the immunization of certain species like mice against the specific antigenic determinant on an antigen then the B lymphocytes are obtained from the spleen of the animal which was immunized. The B lymphocytes through chemical or virus induced methods is fused with immortal cancerous immune cells called myeloma cells in order to achieve immortality as the B lymphocytes has short life span.

These myeloma cell lines lacks in HGPRT gene along with immunoglobulin producing cells. Then the hybridoma cells produced requires a selective medium like HAT medium to be cultured [4]. In this medium only the hybridoma cells survive as they possess the immortality from myeloma cells and selective resistance from B lymphocytes inherited during the fusion. The myeloma cells do not synthesize nucleotides de novo as they lack HGPRT gene also this is inhibited by aminopterin present in the selective medium. The first culture of hybridoma cells does not contain only one specific antibody type it consists of different types of antibodies secreted by different B lymphocyte clones so these antibodies in the culture are termed as polyclonal [13]. Each individual clones are separated by dilution process into different culture wells then they are screened for the specific antibody required among the hundreds of different antibodies. The B lymphocyte clones in positive wells are selected and recloned and the specific antibodies produced by the selected one type of B lymphocyte are termed as monoclonal antibodies and these monoclonal antibodies along with the positive hybridoma cells are stored in liquid nitrogen for later purpose [14].

3.1.2 Detailed process

3.1.2.1 Immunization and selection of mice for generation of hybridoma cells

This is the first step whereas; the immunization is done for mice by injecting the particular antigen prepared by two methods. It includes emulsification of antigen by Freund's adjuvant or by using other adjuvants or else homogenization of antigen containing gel slice. The immunogen for injecting includes intact cells, whole membranes and microorganisms. Mice are mostly preferred for this method to produce the required MAb. According to the general protocol mice is immunized for every 2-3 weeks but the immunization schedule may differ based in the investigators conducting the experiments. The immunization schedule should be completed 6-10 weeks before fusion [14].

3.1.2.2 Screening of mice for production of antibody

After the immunization procedure is complete then a small volume of blood samples are collected from the mice by several humane techniques. In addition to this the serum antibody level is determined through various techniques like ELISA and flow cytometry. Further procedure is performed when the serum antibody level is high but, when its low then the mice is said to be boosted until an adequate immune response is achieved after the antibody titer of the mice is found to be high enough. All the mice are equally boosted by injecting the antigen without and adjuvants either intra-peritoneally or intravenously two weeks after first immunization but 3 days before fusion with myeloma cells. Then the spleen is removed to obtain B lymphocytes [3].

3.1.2.3 Myeloma cell preparation

Antibody producing B lymphocytes obtained from the spleen of the mice possess very limited life span so these cells are fused with myeloma cells which are derived from lymphocytes of immortal tumor. Myeloma cells are cultured in the presence of 8-azaguanine which gives the cell high viability i.e. immortality along with rapid growth [14].

3.1.2.4 Generation of hybridoma cells

This process involves the fusion of spleen cells from the mice with myeloma cells. Both of these cells are centrifuged in PEG, which is a substance that helps both the membranes of B lymphocyte and myeloma cells to fuse. Only the fused cells are incubated in the HAT medium where the unfused cells die after 7 days of fusion and only hybridoma cells survive [15]. Incubation of hybridoma culture supernatant, secondary enzyme labeled conjugate and chromogenic substrate is done where the formation of colored product indicates it as positive hybridoma. The cells are distributed in 96 wells plate which consists of feeder cells that are obtained from saline peritoneal washes of mice within 7-14 days of fusion. These feeder cells are used as a medium to supply growth factors such as interleukin to promote the growth of hybridoma cells [3].

3.1.2.5 Final process of cloning hybridoma cell lines

Supernatant of each well in the 96 wells plate is checked for the required antibody. Here the antibodies in a particular well is produced by same B cell and is also directed against same antigenic determinant so these kind of antibodies are termed as monoclonal antibodies. Cluster of hybridoma cells obtained is allowed to grow in tissue culture. Cloning process can be done later by mouse ascites method. The positive hybridoma cells obtained are cryopreserved in liquid nitrogen and stored for later use for production of MAbs. Other processes are done for purifying the mAbs [13].

3.2 Phage display method

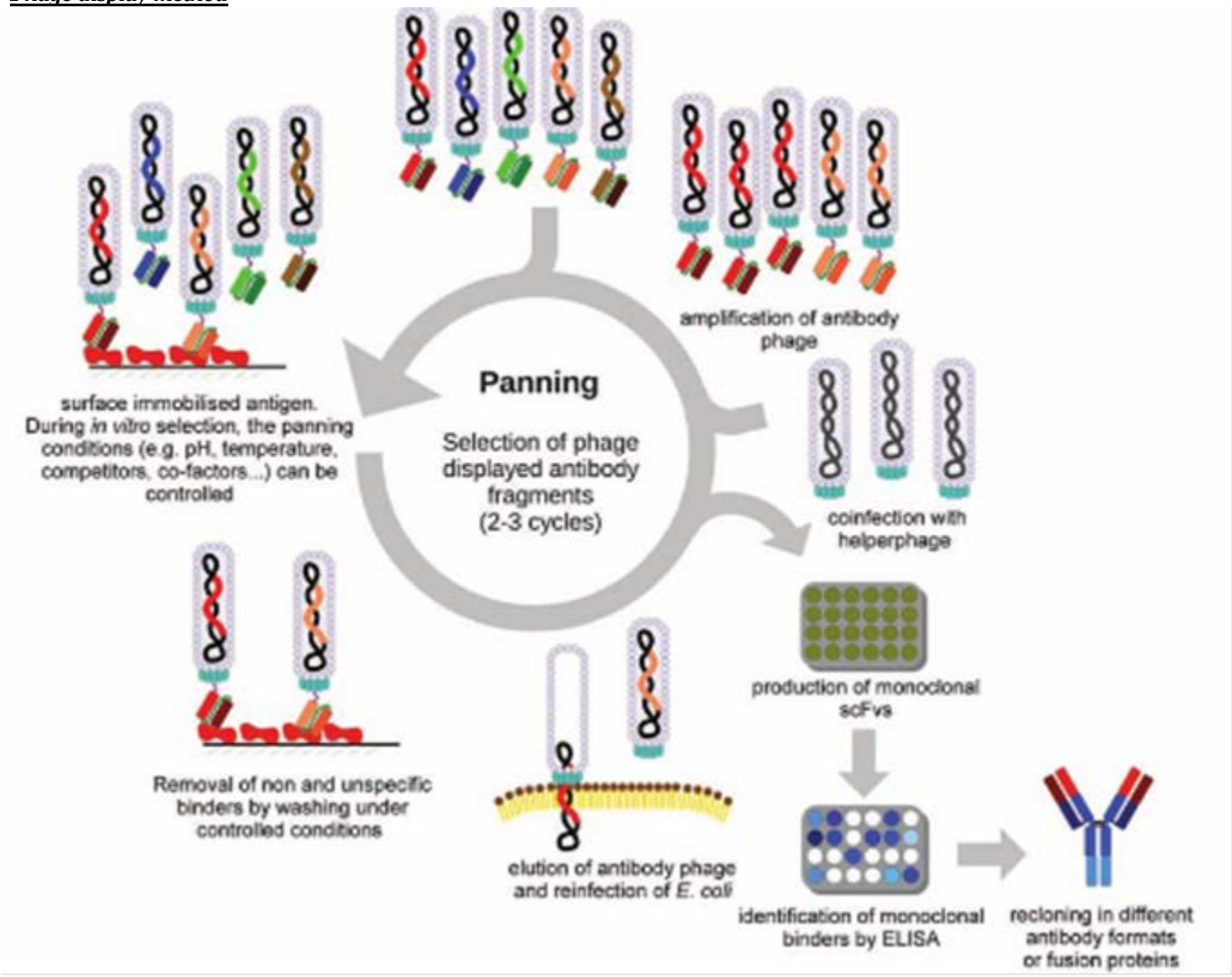

Figure 3: Phage display method. This diagram explains the phage display method through a flow chart about how the mAbs are obtained through phage display process [16].

3.2.1 Outline

Phage display is an advanced or alternative technology that was developed in 1985 also it differs from hybridoma technique [8]. This is a display method is used widely used now days after the work of Georg P. Smith on filamentous phage display also this method of human mAb generation is defined as an alternative method used in order to generate short peptides and protein molecules [8,16]. In addition to this for the first time lytic phage lambda was used for generation and screening of the antibody gene in phage [16]. The first step of this method is the isolation of B-lymphocytes from the blood of the human body continued by the isolation of mRNA and then converting it into cDNA through PCR. This method is combined with the use of PCR in order to amplify the variable region segments continued by cloning of the expressed immunoglobulin variable region of cDNA which results in phage displayed MAb variable region. A library of this phage displayed immunoglobulin variable region can be made. Also library with various kinds of human immunoglobulin variable region segments can be also produced [4, 8].

3.2.2 Detailed process

3.2.2.1 Isolation of B-lymphocytes
The B-lymphocytes from the human blood is isolated as a very first step [4].

3.2.2.2 Conversion into cDNA
Next step is proceeded where isolation mRNA from B lymphocytes is done continued by the conversion of the mRNA into cDNA [4].

3.2.2.3 Amplification of specific variable region
PCR is used in order to amplify the variable regions of both heavy and light chain and the expressed segments of the variable region are cloned as the further procedure [4, 8].

3.2.2.4 Cloning into vector
The bacteriophage (PIII protein) is used and the protein from the phage is used in order to infect the E.coli [4]. This protein is found in the surface of the phage but the resulting molecule after the fusion of the antibody fragments and the PIII protein is found to be presented on M13 phage [16]. E.coli is used as a vector it starts to produce bacteriophage variable regions of heavy and light chain. Then the variable regions of specific antigen are used for reinoculation of E.coli containing bacteriophage. [8, 17].

3.2.2.5 Library of phage displayed antibody
The isolation of cells containing plasmid is done followed by sequencing and the library of the specific phage displayed antibodies is produced as the result. [4, 8].

In this method for the expression of the antibody there are two different genetic systems available which includes, insertion of the antibody genes into phage directly which is fused to M13 phage protein III. Another method is phagemid system here, the genes encoding the antibody and PIII protein is provided on phagemid which is considered to be a separate plasmid that possesses the signal to assemble the phage particles for packaging the vector [16].

3.3 In vitro method
Phage display method is continued by in vitro process as no immunizations are involved in it also hybridoma technique also involves in vitro method [4]. In vitro procedure is said to be the method of mammalian cell culture fermentation done using bioreactors and continuous perfusion culture systems [18]. This method is defined as the technology of tissue culture for culturing the hybridoma cells in order to secrete mAb. The hybridoma cells are cloned for expansion in order to obtain higher concentration of mAb by multiplying them by in vitro procedure by involving various methods [3]. In addition to this in vitro method is used widely because there are no ethical issues as it does not require immunization procedures in addition to this in vitro method is preferred as the devices involved for this method is commercially available based on the operation followed depending on its complexity, cost and amount of antibody obtained. There are several kinds of containers used for both culturing of hybridoma cells and for accumulating the antibody produced [6]. Those containers includes static and agitated cell suspension culture systems, membrane based and matrix based culture system and also high density bioreactors which includes hollow fiber bioreactors [3,19]. The evaluation of in vitro MAb production procedure was done around 2000 and 2003 on GSK bio was mainly depending on suspension culture system and hollow fiber bioreactors [20]. This method is considered to be reasonable and practical one [21].

3.3.1 Cell Suspension culture system
This type of culture systems is also known as static and agitated cell suspension culture which involves plastic cell culture equipment that contains T-flasks in order to maintain the cells available in its stationery state and also possess roller culture and spinner culture which allows the agitation of the medium [3,19]. Cell suspension culture system has two types involved namely stationary suspension culture system and rotation suspension culture system [20]. This method has an advantage as it can be used for both small group of individuals conducting research who need only a small spectrum of MAb and also for the professionals who need large spectrum of MAb and also for biopharmaceutical purposes [19, 20].

3.3.2 Membrane and matrix based tissue culture system

This culture system produce high amount of MAb when compared to cell suspension culture system [19]. In this system there are separated compartments where cells are in one compartment and nutrients are in another compartment so semipermeable membranes are available for the transfer of nutrients [20]. In addition to this there is gas permeable membrane which is a special gassing membrane available for the oxygen supply and carbon dioxide diffusion in membrane based tissue culture system. For the efficient production of MAb the entire nutrient medium and three fourth of the production medium is replaced with fresh medium twice a week

in matrix based tissue culture system [6, 20]. The replacement of production medium may differ based on the methodology. As the result secretion of higher amount of mAb occur but in lower volume is the advantage of this system [19]. This method also has a disadvantage as there is a chance for contamination to occur in MAb produced due to the contact with the dead cells. Now a days frequently used membrane based systems are CELL line and mini PERM [6].

3.3.3 Hollow fiber bioreactor

This is a method under the category of high density bioreactor which is developed in order to culture secretes higher amount of MAb by supporting the culture of high density of the cell [3]. The hollow fibers present in hollow fiber bioreactors is defined as the small and tube like filters that possess predefined cutoff and it is said to have the nature of semipermeable membranes present in the membrane based tissue culture system and are arranged and packed in cylindrical modules [6, 20]. The hollow fibers formed by the packing of fibers provide large surface area for culture also there is hollow fiber cartridge to provide aeration to the cells cultures. This system has both intra capillary and extra capillary space whereas the cells are cultivated or cultured only in the extra capillary space. In addition to this intra capillary space is also important as the essential metabolites and a nutrient gets freely perfused between the extra capillary and intra capillary space [20]. In this culture system cells are cultured in a three dimensional porous matrix on an individual component which is replaced with fresh medium continuously. As the result the secreted antibody is obtained from the supernatant which can be used as it is or else can be purified and used [3].

The in vitro method can be proceeded through two methods namely single harvest production method and multiple harvest production method. In single harvest production method the generation of MAb is based on the stability of the cell line cultured and is also near to or beyond the level of production. In this case only maximum permitted number is being generated [22].

Whereas, in multiple harvest production method continuous cultivation is done this should be specified and also be based on the stability and consistency of the product. It also depends on many factors like type of monitoring and required frequency, expression system and MAb's nature along with the length of the continuous cultivation period [22].

3.4 In vivo method

In vitro technique is the method used widely but there are some conditions where the in vitro method is found to be ineffective as the characters and properties of hybridoma cell vary as the MAb production is diverse [19]. In spite of using in vivo methods some other alternative methods are considered after that only in vivo method is applicable due to a valid reason. As it is considered to be a more painful method to the animals there is a need of scientific justification for the reasons like inability of the cells to adapt on in vitro conditions, the cell line's inability to maintain the MAb production, and the conditions where purification lead to decreased antibody level and also the loss of utility of cultured cell due to contamination [23]. In vivo method is a procedure somewhat similar to the in vitro method where the hybridoma cells produced are inserted into the peritoneal cavity of the mice through injection for the multiplication of those cells at last the fluid gets collected in the abdomen of the mice. As the multiplication of hybridoma cell increase more volume of fluid gets produced this accumulation cause's pain to the mice. The fluid is known as ascites fluid so this method can be also named as ascites method of MAb production [15]. Mostly the use of mice is reduced as this method cause's distress to mice also in this method based on the amount of antibody required the necessity of mice also increases [3].

The methodology for in vivo production method is as follows:

3.4.1 Priming

Priming is done for the peritoneal cavity of the mice used in order to inject the hybridoma cells into it [23]. This procedure is done by using priming agents like Pristane or Freund's agent which is administered via peritoneum (IP). There is particular volume to be used for example in case of Pristane it should not exceed above 0.3ml if it exceeds the limit it results in distress as it is irritating in nature. Freund's agent is not much recommended as it is more painful [1, 3].

3.4.2 Inoculation of hybridoma cells

In this step hybridoma testing is done where the hybridoma cells are tested for production of specific antibodies and then the cells are inoculated into peritoneal cavity of the mice. The range of the hybridoma cells to be inoculated is around 105 - 107 cells this should not exceed above 0.5ml [3, 15].

3.4.3 Production of ascites fluid

Injection of hybridoma cell results in the formation of a tumor which leads to accumulation of ascetic fluid. This ascetic fluid is formed along with large cell inoculum that leads to short life span or survival rate of the mice [3]. This is the step that leads to distress and uncomforted feeling in mice as the production of ascetic fluid is very painful so the mice should be monitored regularly by the investigator for two times a day [23]. During this stage there are some symptoms in the mice due to distress which includes unusual breathing like rapid breathing or even shallow breathing, hyperthermia, hypothermia, rapid loss of weight and also diarrhea and constipation occurs so if there are any serious symptoms then medical help is recommended [3, 15]. The abdominal distension caused is greater compared to the pregnancy time so the mice undergoes an inability to reach the food or water [23].

3.4.4 Collection of ascites fluid

The harvesting of ascites fluid should be done as soon as possible in order to reduce the mortality rate of mice because the survival rate decreases as the production of ascetic fluid increases [3]. The weight must not be above 20% based the normal weight considering the age and sex. So the collection process should be done before abdominal distress occurs. The mice is given anesthesia by a well-trained person before tapping as it is a painful process then the ascetic fluid is collected in a collection tube by holding the mice and tapping its abdomen with the help of 20 – 21 gauge needle. Abdominal taps done should be 2 but it can be 3 in case of euthanasia also regular observation is required after each tap if there is any severe distress observed then veterinary staff is recommended [15].

3.4.5 Purification of MAb

The ascetic fluid contains the secreted antibodies in order to obtain it purification process is done because there might be some other substances like cytoquines released by hybridoma cells and also some contaminants in it [3, 19]. Purification is done by centrifugation and filtering process through which larger debris of the cell and whole cells are being removed then the nucleic acids present are removed by ion exchange chromatography and at last the specific antibodies are obtained through affinity chromatography. There are some alternative methods to obtain the antibodies like precipitation where the MAb is precipitated or electrophoresis is used [19]

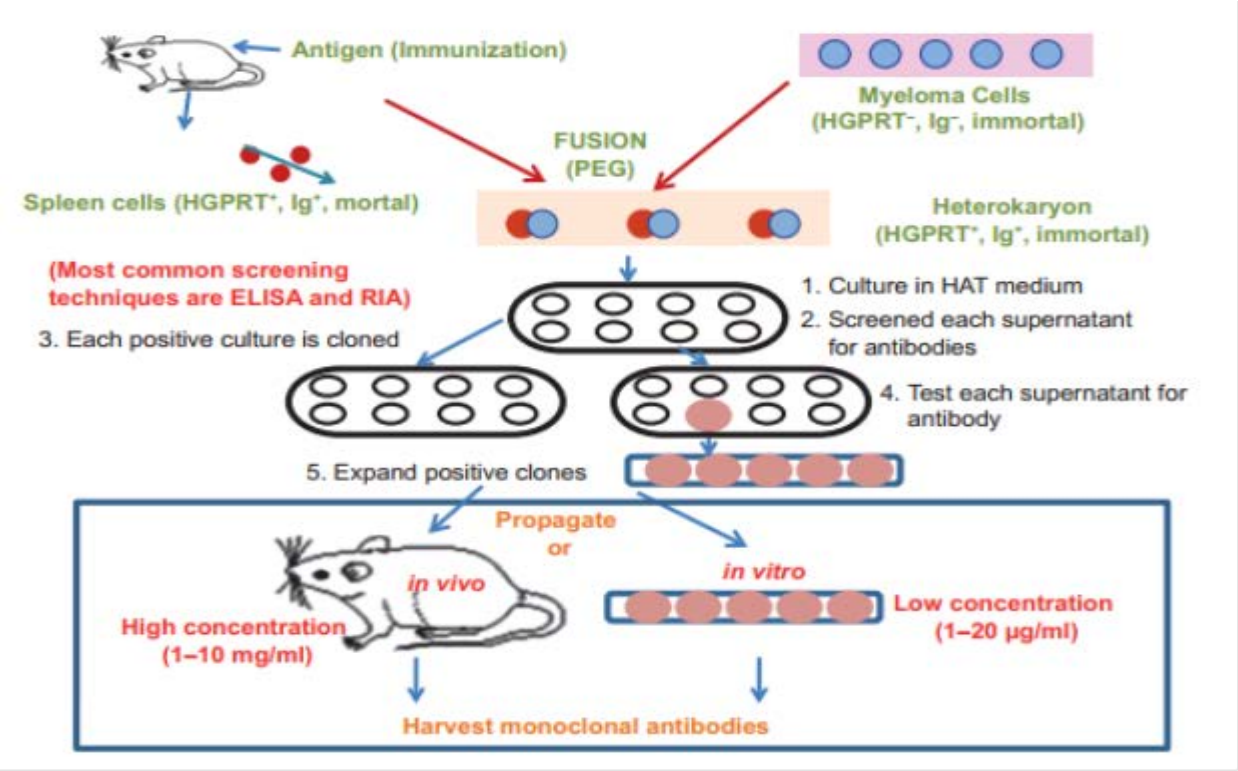

Figure 4: In vitro and in vivo methods. This diagram clearly differentiates in vitro and in vivo method and how the MAb is produced using both the techniques [14].

3.5 Transgenic mice method

Transgenic mice method is the most advanced method to produce human MAb using trans-chromosome mice as the immunoglobulin of transgenic mice has human immunoglobulin encoding genes in both heavy and light chain. In the further procedure the gene segments of variable region is made to express. As the immune response is low in the transgenic mice compared to normal mice more number of immunizations and antibody screening is done. By using this method human MAbs with different kinds of heavy chains are being produced for therapeutic purpose. Since 1998 about 10 human MAbs are entered into clinical trials and now about 33 human MAbs are in clinical use. This method must be well developed by improving the cell strains of transgenic mice in order to produce human MAbs in future [24].

3.6 Production of MAb from plants

Now a days there is a new record that MAbs are being produced from plants. So plants can be used for large scale production of MAbs where the transgenic plants can be also used for in vitro cultivation of MAbs as they play a role as bioreactors. It is grown through in vitro procedures most of the time. Comparing to other methods of derivation of MAbs the method of obtaining MAb from plants has low production cost, cheap maintaining cost, and high yield and is highly safe but the time period for production is medium and the therapeutic risk is unknown. This method also has some disadvantages as culture parameter is being uncontrollable and there is high chance for contamination due to bacteria or pollen. Purification process is essential which can be done through affinity chromatography based on protein A or protein G [25, 26].

4. TYPES OF MAbs

There are different kinds of MAbs available for therapeutic although their principle might be same but the targets differ. Particular MAb is chosen based on its availability, purpose, effectiveness and various other factors [8].

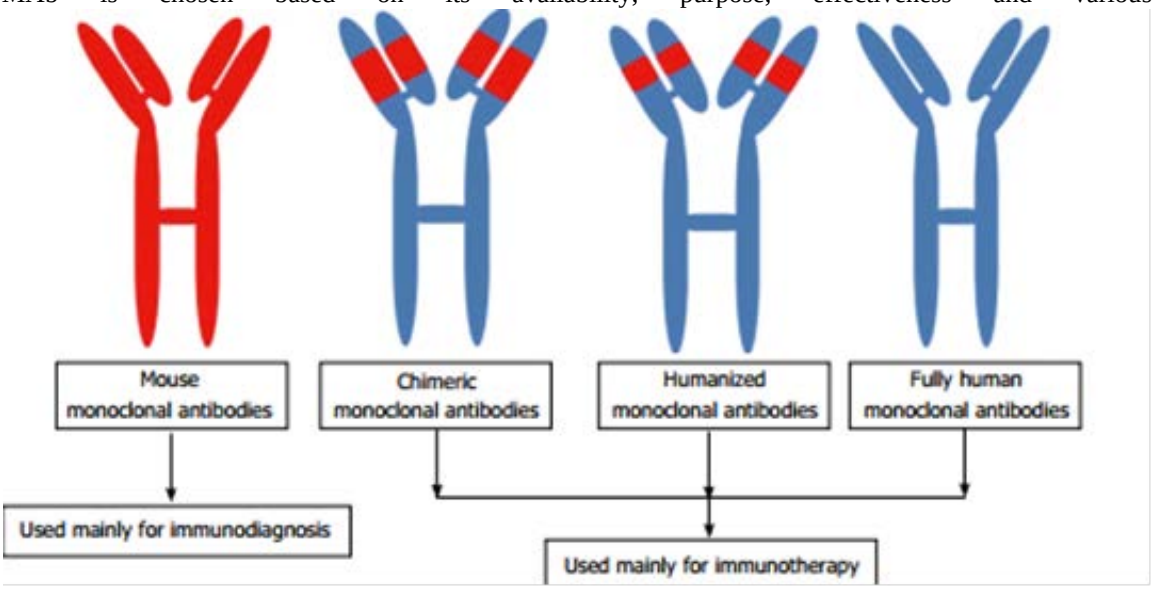

Figure 5: Types of MAbs. This diagram depicts different kinds of MAbs and their uses also with the structural differences [27].

4.1 Murine MAbs

Murine MAbs are defined as the antibodies produced by the murine hybridomas which are obtained as the result of fusion between B-lymphocytes and murine myelomas cells [22]. The use of murine MAb in clinical field has become limited. Use of murine MAb may cause some mild effects like cytotoxicity stimulation and continuous use may lead to severe symptoms like anaphylactic shock and allergies [8]. When these murine antibodies are introduced to an individual it results in the production of human anti murine antibody against the murine immunoglobulin this is known as HAMA response this causes allergic and adverse reactions so it does not serve as an effective MAb [8, 22]. To avoid the unwanted reactions in the body murine immunogenic components that induce immune response was removed. In spite of this OKT-3 which is the anti-CD3 MAb from murine hybridoma cell was the very first approved MAb for therapeutic use in human. Other examples: 90Y-ibritumomab, 131I-Tostitumab [8].

Table 1: List of murine MAbs in use and their year of FDA approval [12]

Name of MAb	Type of MAb	Therapeutic use	Year of FDA approval
Imciromab penlelale	Murine MAb	Detection myocardial injury	1996
Arcitumomab	Murine MAb	Diagnosis	1996
Orthoclone	Murine MAb	Immunological use	1992
FanolesomaB technetium Tc 99m	Murine MAb	Diagnosis	2004
Nofetumomab	Murine MAb	Diagnosis	1996
Ibritumomab tiuxetan	Murine MAb	Cancer	2002
Capromab penditide	Murine MAb	Cancer	1996

4.2 Chimeric MAbs

Chimeric MAbs are defined as the therapeutic antibodies produce as the result of combination of the genetic components of human and non-human here the non-human genetic component can be from the animals like mice [8]. The chimeric MAb is produced by the replacement of constant region of mouse immunoglobulin with constant region of human immunoglobulin so chimeric MAb is said to be a combination of constant region of human immunoglobulin and variable region of mouse immunoglobulin [24]. Chimeric MAbs when introduced into human there were some unwanted immune responses detected but the human genetic component is about 65% and non-human component is 30% approximately in chimeric MAbs. FDA has approved some MAbs of chimeric origin like Infliximab, Rituximab, Abciximab [8].

Table 2: List of chimeric MAbs in use and their year of FDA approval [12]

Name of MAb	Type of MAb	Therapeutic use	Year of FDA approval
Brentuximab vedotin	Chimeric MAb	Cancer	2011
cetuximab	Chimeric MAb	Cancer	2004
infliximab	Chimeric MAb	Immunological use	1998
abciximab	Chimeric MAb	hemostasis	1993
rituximab	Chimeric MAb	Cancer	1997
basiliximab	Chimeric MAb	Immunological use	1998
siltuximab	Chimeric MAb	Immunological use	2014
dinutuximab	Chimeric MAb	Cancer	2015

4.3 Humanized MAbs

Humanized MAbs are obtained when the non-human variable region of antibodies are humanized in order to avoid the unwanted immune responses when introduced into human [8]. Here the non- human genetic ingredient is reduced to 5%-10% so the human origin is about 95%. The humanization procedure is done by the transfer of CDRs from mice MAb to human immunoglobulin through chain-shuffling randomization technique [24]. There are some humanized MAbs approved by FDA which includes daclizumab, omalizumab, alemtuzumab [8].

Table 3: List of humanized MAbs in use and their year of FDA approval [12]

Name of MAb	Type of MAb	Therapeutic use	Year of FDA approval
tocilizumab	Humanized MAb	Immunological use	2010
bevacizumab	Humanized MAb	cancer	2004
alemtuzumab	Humanized MAb	Immunological use	2001
certolizumab	Humanized MAb	Immunological use	2008
trastuzumab	Humanized MAb	cancer	1998
elotuzumab	Humanized MAb	cancer	2015
vedolizumab	Humanized MAb	Immunological use	2014
obinutuzumab	Humanized MAb	cancer	2013
Ado-trastuzumab emtansine	Humanized MAb	cancer	2013
Pembrolizumab	Humanized MAb	Cancer	2014
Alemtuzumab	Humanized MAb	Immunological use	2001
Ranibizumab	Humanized MAb	Ophthalmic use	2006
Gemutuzumab ozogamicin	Humanized MAb	cancer	2000
mepolizumab	Humanized MAb	Immunological use	2015
pertuzumab	Humanized MAb	cancer	2012
idarycizumab	Humanized MAb	hemostasis	2015
efalizumab	Humanized MAb	Immunological use	2003
eculizumab	Humanized MAb	hemostasis	2007
palivizumab	Humanized MAb	Antiviral	1998
natalizumab	Humanized MAb	Immunological use	2004
omalizumab	Humanized MAb	Immunological use	2003
daclizumab	Humanized MAb	Immunological use	1997

4.4 Fully human MAbs

The human MAb is generated through human hybridomas through phage display or trans chromosome mice technology to avoid the unwanted immune reactions when used for therapeutic purpose by expressing human genes only. Here the human antibody producing hybridoma can be derived fron transgenic mice through immunization [24]. This human MAb consists of only human genes so these are considered to be a safe drug for human. But this method is hard because the cell lines cultured should remain immortal but human MAbs was developed as an alternative for murine MAbs. Fully human MAb namely Humira® was discovered for the first time in 2003 to treat rheumatoid arthritis. MAbs like Adalimumab® and Panitimumab® are other MAbs which were approved but there are also some other human MAbs which are under clinical testing [8, 28].

Table 4: List of human MAbs in use and their year of FDA approval [12]

Name of MAb	Type of MAb	Therapeutic use	Year of FDA approval
Ofatumumab	Human MAb	Cancer	2009
Belimumab	Human MAb	Immunological use	2011
Secukinumab	Human MAb	Immunological use	2015
Ramucirumab	Human MAb	Cancer	2014
Daratumumab	Human MAb	Cancer	2015
Adalimumab	Human MAb	Immunological use	2002
Canakinumab	Human MAb	Immunological use, anti-inflammatory	2009
Nivolumab	Human MAb	Cancer	2014
Necitumumab	Human MAb	Cancer	2015
Alirocumab	Human MAb	Lipid-lowering	2015
Denosumab	Human MAb	Bone disorders	2010
Raxibacumab	Human MAb	Anti-toxin	2012
Evolocumab	Human MAb	Lipid-lowering	2015
Golimumab	Human MAb	Immunological use	2009
Ustekinumab	Human MAb	Immunological use	2009
Panitumumab	Human MAb	Cancer	2006
Denosumab	Human MAb	Cancer	2010
Ipilimumab	Human MAb	Cancer	2011

4.5 Engineered MAbs

This is an alternative approach as the chimeric MAbs is not much effective and the human MAbs production methods are problematic and humanized MAbs consists of small part of non-human sequence. Engineered MAbs can be guaranteed with decreased immune responses, increased half-life in in vivo circulation along with specificity [29].

5. PHASES IN MAB PRODUCTION AND TESTING

The time period of MAb production method is normally 4 to 6 months this period is split into three phases which are as follows:

5.1 Phase 1

Immunization of mice is done through pre-injection tail bleeds, antigen injections, all antigen boosts here about 5 mice is immunized so, around 1mg to 1.5 mg of the substance is essential for immunization. The mouse with high concentration is determined through ELISA for fusion process. The time period require for this phase is approximately about 2 months but the cost is $1,800 (US dollars) [13].

5.2 Phase 2

The B- lymphocytes obtained from mice is fused with the myeloma cells and the product obtained through fusion is plated on a 96 well plate and the wells expressing the antibody for the specific antigen is determined through ELISA assay. In addition to the antigen required in immunization 0.8mg to 1mg of antigen is required for ELISA screening procedure. The wells containing the antibody is

selected and isolated and plated into a 24 well plate. Then a portion of it is freezed and sent to the client for evaluation. This procedure requires a time period of 3 to 4 weeks approximately but it is very costly as it requires $2,900 (US dollars) [13].

5.3 Phase 3

The positive wells are selected and cloned. Then the clones obtained are expanded and 10 vials (vial is a small container which carries the liquid substance) of the clones is frozen. Then these frozen vials and the 10 ml of each clones are shipped to the desired client. This phase lasts for about 1.5 to 3 months and the cost is around $2,800 (US dollars) [13].

5.4 FDA approval

Then the MAbs produced goes for clinical testing and then after completing clinical trials it is approved mainly by FDA after this the MAb can be used for treatment [13].

Total Cost of the procedure including all phases can range up to $7,500 (US dollars).

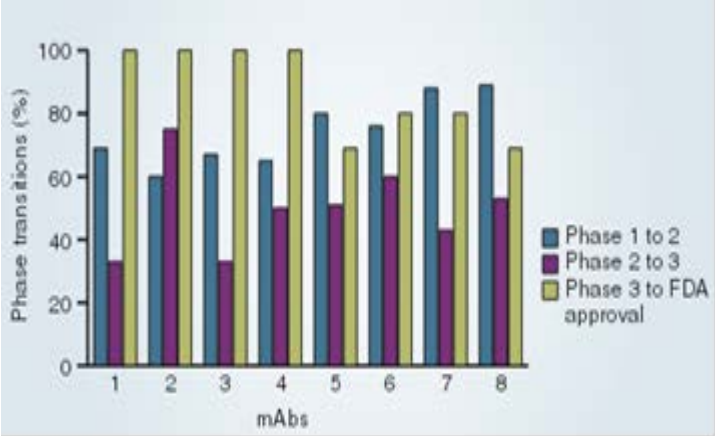

Figure 6: Clinical phase transition percentages. The above diagram shows a detailed graph of clinical phase transition percentages of therapeutic MAbs. 1- all products of chimeric MAbs, 2-oncological chimeric MAbs, 3-immunological chimeric MAbs, 4-chimeric MAbs from 1987 – 1997, 5-all products of humanized MAbs, 6-oncological humanized MAbs, 7- immunological humanized MAbs, 8-humanized MAbs from 1988 – 1997 [30].

6. FDA APPROVAL

FDA approval is essential for MAbs. There are many MAbs with therapeutic use but only very few among them are FDA approved those are very costlier but safe and recommended to use. There is a list of FDA approved MAbs in recent years and also the MAbs under clinical trials [8].

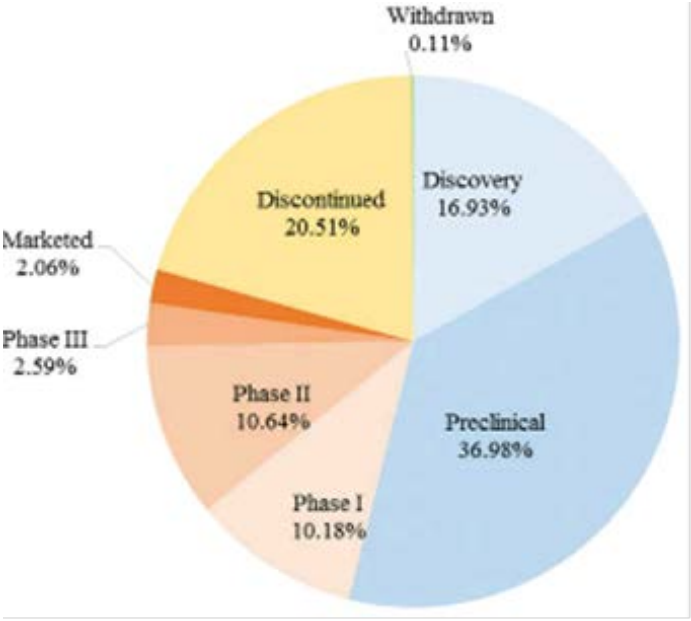

Figure 7: A pie chart of MAbs under trials and discovered. This pie chart clearly depicts the percentage of MAbs discovered, MAbs under trials i.e. in different phases and MAbs discontinued [31].

Table 5: The approved MAbs and their year of approval [30]

Generic name	Trade name	Approval date	Organisation of approval
Muromonab-CD3	Orthoclone OKT3	06/19/86 (US)	FDA
Abciximab	ReoPro	12/22/94 (US)	EMU
Rituximab	Rituxan	11/26/97 (US) 06/02/98 (EU)	FDA EMU
Daclizumab	Zenapax	12/10/97 (US) 02/26/99 (EU)	FDA EMU
Basiliximab	Simulect	05/12/98 (US) 10/09/98 (EU)	FDA EMU
Palivizumab	Synagis	06/19/98 (US) 08/13/99 (EU)	FDA EMU
Infliximab	Remicade	08/24/98 (US) 08/13/99 (EU)	FDA EMU
Trastuzumab	Herceptin	09/25/98 (US) 08/28/00 (EU)	FDA EMU
Gemtuzumab ozogamicin	Mylotarg	05/17/00 (US)	FDA
Alemtuzumab	Campath-1H	05/07/01 (US) 07/06/01 (EU)	FDA EMU

Ibritumomab tiuxetan	Zevalin	02/19/02 (US) 01/16/04 (EU)	FDA EMA
Adalimumab	Humira	12/31/02 (US) 09/1/03 (EU)	FDA EMA
Omalizumab	Xolair	06/20/03 (US)	FDA
Tositumomab-I131	Bexxar	06/27/03 (US)	FDA
Efalizumab	Raptiva	10/27/03 (US) 09/20/04 (EU)	FDA EMA
Cetuximab	Erbitux	02/12/04 (US) 06/29/04 (EU)	FDA EMA
Bevacizumab	Avastin	02/26/04 (US) 01/12/05 (EU)	FDA EMA
Natalizumab	Tysabri	11/23/04 (US)	FDA

7. THERAPEUTIC USE OF MABS

MAbs play an important role in cancer, graft rejection as clinical medicine. Detailed role is as follows:

7.1 Role of MAb in cancer

MAbs play an important role in cancer therapy where it binds to cancer cell by targeting them or else the drugs for the cancer like chemotherapeutic drugs are bound to the MAbs for clinical use. MAbs not only serve as a treatment to cancer it can be also used for histopathological diagnosis of cancer whereas specific types malignant conditions like leukemia and lymphoma and also solid tumor like carcinomas in lung, colon, breast, etc. is diagnosed other than this there are some special MAbs available for ovarian cancer and colorectal cancer. Samples of blood, sputum and biopsies are tested with MAbs to detect cancer cells [1,8,14]. It targets the specific cancer antigen MAbs are used to treat cancer where MAbs react in different ways through different mechanisms like MAbs are used along with complements to mediate cell cytotoxicity in cancer treatment as they are complement dependent or else it blocks the growth factors that promote the proliferation of tumor cells or through antibody mediated cytotoxicity and by inducing apoptosis [9].

The Mechanisms followed by MAb in the cancer therapy is as follows:

7.1.1 *Antibody dependent cytotoxicity*

In the ADCC mechanism the targeted cancer cell is bounded by MAbs that results in immune mediated destruction of cancer cell. Based on the Fc portion of MAb antibody dependent cytotoxicity occurs here the Fc portion of the Mab binds to FcgR present in the effector cells like macrophages, neutrophils, and NK cells. The Fab binds to the antigen of the tumor cell which results in a connection established between the cancer cell and the effector cell so the targeted tumour cell is identified and lysed through lytic attack [9, 33].

7.1.2 *Complement mediated cytotoxicity*

MAb binds to the targeted tumor cell results in lysis of tumor cell through the mechanism of CDC this reaction is mediated by complement proteins that are bound to MAbs [9].

7.1.3 Neutralization of soluble ligand

MAbs bind to the circulating proteins or factors that favor proliferation of the tumor cell by neutralizing those proteins. Example includes bevacizumab is a humanized MAb that binds to VEGF-A and inactivates it so it results in inhibition of angiogenesis. Due to this the proliferation of tumour cell gets blocked [9].

7.1.4 Cytotoxic agent delivery

By this process cytotoxic substances bound to MAb are sent to lyse the targeted specific tumor cell by delivering the cytotoxic agent. Example: trastuzumab-DM1 T-DM1 [9].

Also by inhibiting some essential signals cancer cell can be lysed or proliferation is prevented. MAbs are being used in radio immunotherapy and chemotherapy as a delivery vehicle or as homing device here the MAbs are said to be radiolabeled or chemo labeled antibodies. The MAbs are conjugated with radioactive atoms in case of radio immunotherapy or else conjugated with chemotherapeutic drugs in case of chemotherapy and they circulate in the body till the specific antigen of cancer cell is identified then the drug is delivered to the cancer cell and causes damage to it but it can also damage the normal tissues but in some cases MAbs are directly introduced to treat cancer. Brentuximab vedotin, ado-trastuzumab emtansine and Brentuximab vedotin are FDA approved MAbs that are conjugated with chemo drug. For example in order to treat Hodgkin lymphoma and anaplastic large cell lymphoma the MAb namely Brentuximab vedotin is conjugated to MMAE a chemodrug and targets CD30 antigen of cancer cell [14]. In total of 2441 diseases treated by using MAbs around 1324 was cancer [31].

Table 6: The oncological MAbs with the mechanism and type of cancer targeted [9, 33]

MAb name	Mechanism followed	Type of MAb	Therapeutic use
Cetuximab	Inhibition of EGFR signaling (targets EFGR), ADCC	Chimeric MAb	Colorectal, breast and lung cancer
Panitumumab	Inhibition of EGFR signaling (targets EFGR)	Human MAb	Colorectal cancer
Nimotuzumab	Blocks EGFR signaling (targets EFGR)	Humanized MAb	Head and neck cancer
Rituximab	ADCC, induction of apoptosis, CDC (targets CD 20)	Chimeric MAb	Non-Hodgkin lymphoma
Trastuzumab	Inhibition of HER2 signaling (targets HER2), ADCC	Humanized MAb	Breast cancer
Alemtuzumab	Induction of apoptosis (targets CD52), CDC	Humanized MAb	Chronic lymphocytic leukemia
Bevacizumab	Inhibition of VEGF signaling by (targets VEGF)	Humanized MAb	Colorectal and lung cancer
Ofatumumab	ADCC, CDC (targets CD20)	Human MAb	Chronic lymphocytic leukemia
Ipilimumab	Inhibition of CTLA-4 (targets CTLA-4)	Human MAb	Metastatic melanoma
Pertuzumab	Blocks HER2 signaling (targets HER2)	Humanized MAb	Breast cancer
Denosumab	It targets RANK ligand	Human MAb	Solid tumor bony metastases
Brentuximab vedotin	Delivers toxic payload, auristatin toxin (targets CD 30)	Chimeric MAb	Hodgkin's or systemic anaplastic large cell lymphoma
Gemtuzumab	Delivers toxic payload, calichaemicin	Humanized MAb	Acute myelogenous

ozogamicin	toxin (targets CD 33)		leukemia
90Y-Ibritumomab tiuxetan	Delivers the radio isotope yttrium-90 (targets CD 20)	Mouse MAb	Low grade or transformed B cell non-Hodgkin's lymphoma
Tositumomaband 131I-tositumomab	Delivery of radio isotope iodine-131, ADCC, induction of apoptosis (targets CD 20)	Mouse MAb	Lymphoma

7.2 Role of MAb in transplant rejection

Organ transplantation was possible only in 1960s but there were rejections of organs transplanted in some cases so immune suppressive drugs were used in late 1970s [34]. OKT3 is the first murine type of MAb used in allograft rejection [35]. Usage of MAb in organ transplantation process prevents the recognition of donor tissue as foreign so there is no antibody mediated or cellular mediated rejection [37].

Various stages of MAbs administration during organ transplantation are as follows:

7.2.1 Administration of MAbs before organ transplantation

For the prevention of the early rejection of the transplanted organ desensitization is followed as the patient is very sensitive. Here the blood samples obtained are tested for the antibodies against MHC in specific it is screened for antibody against HLA. Mainly the individuals who had faced pregnancy risk, blood transfusion or previous organ transplants has more risk of antibodies against HLA so when an organ transplantation is performed then the transplant gets rejected within minutes or hours. So MAbs are used for desensitization and the production of antibodies against the transplantation of organ does not occur [37].

7.2.2 *Administration of MAbs during organ transplantation*

There are drugs to prevent this condition like corticosteroids and calcineurin inhibitors but there are also severe side effects so MAbs are administered which can used as an alternative to limit the usage of corticosteroids and calcineurin inhibitors and also reduce the early rejection and loss of the transplant. For example MAb like Muromanab is used [37].

7.3.3 Administration of MAbs after organ transplantation

This is mainly followed for allograft rejection here the immune system gets interrupted so the individual has the risk of malignancy or other infections after the transplantation is performed so there should be careful assessment after transplantation [37].

Daclizumab is a humanized MAb and Basiliximab a chimeric MAb is used for renal transplantation process and OKT3 is used to prevent the rejection after transplantation within 3 months [35] [36]. Due to the discovery of MAbs for transplant rejection the renal transplantation is raised from 30% to 80% [37].

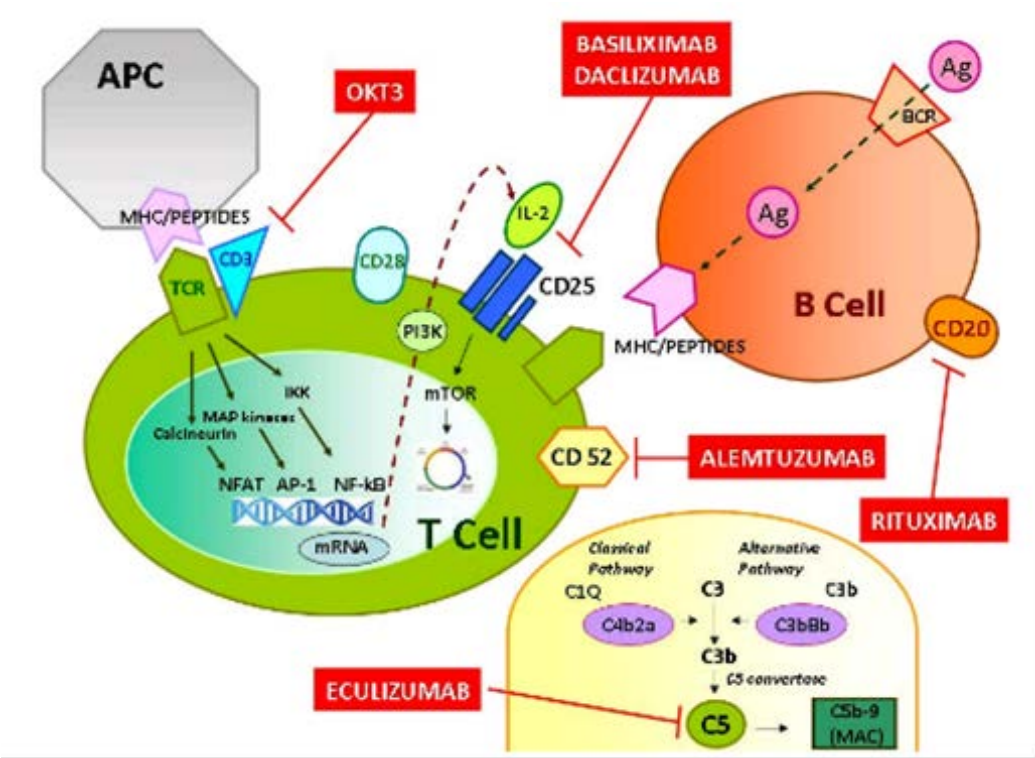

Figure 8: Role of MAbs in renal transplantation. The above diagram depicts the mechanisms handled by MAbs to prevent renal transplant rejection [37].

8. REASONS FOR PREFERRING MABS

MAbs are more preferred when compared to polyclonal antibodies as it is very specific and has the properties like mono specificity as all antibodies has sane antigen binding sites, homogeneity as all are from same class, and consistency but MAbs also has some disadvantages like it is more expensive, time consuming and requires well trained persons [38].

9. CONCLUSION

MAbs is considered as an alternative therapy for many diseases in clinical field and also it is very specific so it is preferred more than polyclonal antibodies. In addition to this MAbs are being developed in cancer field is considered as a major advantage because cancer is regarded as a disease affecting major population and it is mostly preferred by pharmaceutical companies for diagnosis of diseases and therapeutic uses. It also has some disadvantages but it can be faced by pooling and usage of mAbs of desired specificity also more development in MAbs can lead to revolution in the immunology field and medical field [4, 38].

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