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POSSIBLE TREATMENT OF HIV VIRUS USING RECOMBINANT MONOCLONAL ANTIBODIES: AN EFFECTIVE TOOL AGAINST INFECTIOUS HIV

ABSTRACT

Acquired Immune Deficiency Syndrome (AIDS) is an immune disease caused by Human immunodeficiency viruses (HIV). Statistical data from World Health Organization (WHO) confirmed death rates of about 25 million people all around the world, there is no proper cure and treatment available for AIDS. In order to eliminate HIV by monoclonal antibody, several epitope regions on HIV were studied and found that conserved regions of gp120 and gp41 glycoproteins are the main cause for antigenicity of HIV that makes them potential target. Human antibody is not able to reach the deep cleft on gp120 and gp41 because of their higher molecular weight and long Y-shaped structure. Latest research by scientist show that camelids antibody VHH is smaller, show higher specificity and compete with soluble CD4 for binding at the gp120 site, leading to new possible light of treatment.

In this report, the possible way of treatment of HIV by using a mixture of antibodies from different species has been reported. This mixture of antibodies can more efficiently act on target gp120 site comparing with human antibodies. The proposed mixture of antibodies has to undergo preclinical and clinical trials before coming into the market.

KEY WORDS Infectious agent, Human Immunodeficiency Virus (HIV), Monoclonal antibodies (mAbs), Epitopes, Whole antibody (Ab), recombinant antibodies.

INTRODUCTION

The process of choosing the target infectious agent (IA)

Infectious agents are biological agents who are capable of causing an infection by the invasion of the host cell which can lead to a disease. These agents might be virus, bacteria and protozoa. The list of infectious agents (A-Z) can be obtained from Internet source: "The big picture book of viruses".[1] The following criteria were considered in order to choose the target infectious agent: Severity of the disease caused, Prevalence across the globe, Mode of transmission and susceptibility, Treatment and vaccine available from the above source and criteria the main focus on viruses because of their higher prevalence of the disease they cause over the other infectious agents. The virus diseases are usually more severe as well. Additionally, most viruses do not have treatment available while most bacteria could be easily treated using antibiotics. Thus, using Internet source and above criteria, for one virus, which will meet the selection factors the most. The proposed agent who was spread across the whole globe, causing disease, which will lead to death, being dispersed rapidly and for which the treatment available is only supportive was found to be the Human Immunodeficiency Virus (HIV).

Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus (HIV) causes Acquired Immune Deficiency Syndrome (AIDS) which is an opportunistic disease and collection of different diseases (Table 1). Antibodies provide immune resistance to the body against foreign infectious agents. When an individual is exposed to HIV virus, the immune system develops a response and produce antibodies against the virus. The HIV seems to overcome the immune response which leads to the collapse of the immune system. [2] The HIV virus was isolated in 1983. AIDS is one of the most common sexually transmitted diseases (STDs) worldwide. It can also be transmitted by transfusions of infected blood, breast feeding or by shared infected syringes. [3] Numbers of infected people are increasing every year. As until a date, there is no effective treatment available. Many pharmaceutical companies and governments across the world are investing the huge amount of funds into

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research to find an effective cure for AIDS disease. "The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that AIDS have killed more than 25 million people since it was first recognised on June 5, 1981, making it one of the most destructive epidemics in recorded history. In 2007 there were 2.7 million new HIV infections and 2 million HIV-related deaths". [4]

Structure of the HIV

Morphology of the HIV was studied extensively since its identification in 1982.[5] HIV is a retrovirus containing ssRNA as a nucleic acid and being able to integrate its genetic material into the host genome to cause the infection. The RNA is surrounded by nucleocapsid gag p24 protein, which is further covered by capsid gag p17 protein. All the proteins necessary for HIV infection are present inside the nucleocapsid core, which are: reverse transcriptase, integrase, protease and other viral proteins (Gag, Pol, Env). Spikes-like structures gp120 are presented to the surface of the double- layered envelope. The protein gp41 is integrated into the membrane of the HIV envelope as present in Fig 2. Nucleocapsid shapes can vary from spherical (isometric) to rod-shaped (a truncated cone). HIV can assume different shapes from spherical to hexamerical what makes it pleomorphic.[1]

Life cycle of HIV and its mechanism of infection

The HIV virus to replicate and survive requires a host cell therefore; it developed excellent way of entry to its target cells as shown in Fig 3. The most interesting stages of HIV life cycle for purpose of this report are all the stages happening outside of the host cell (in the blood stream) as the therapeutic agents of choice (antibodies) are not able to pass through the cell membrane, thereby, can only be effective at the stages: before the virus entry and release from the cell. HIV envelope consists of trimeric glycoprotein gp120, which binds non-covalently to transmembrane gp41. The entrance of HIV into the host cell is triggered by the association of the gp120 with the CD4 receptor present on T-cells. This binding triggers conformational changes that expose the co-receptor binding site. The fused gp120 and CD4 involve the co-receptor CCR5 or CXCR4 which then leads to the eventual entrance of the virus into the host cell by membranes fusion.[6] Through this gate HIV can access the white blood cells and alter the immune system of the patient.

Treatment available for HIV

To stop the HIV infectious expansion in the human system the supportive treatment therapy is available. Following pharmaceuticals are available on the market.[7] 1: Blockers of entrance to the T-cell (entry inhibitors) – Fuzeon . 2: Blockers of reverse transcriptase – nucleoside reverse transcriptase inhibitors or non-nucleoside reverse transcriptase inhibitors (NRTI or NNRTI) – Ziagen. 3: Blockers of viral assembly proteases inhibitors (PI) – Viracept. There are 20 US Food and Drug Administration (FDA) approved antiretroviral drugs which are currently available and used in US and Europe. Many of the drugs are used in combinations as this gives higher effectiveness and these are known as the cocktail treatment.[8] There are many drugs developed however, big part of them is not available worldwide because of following reasons:

- Fear before patients being addicted to the medications as these are strong painkillers,
- Restrictive national drug control policies,
- Problems in manufacturing, acquiring and distribution.

Due to regulatory barriers more than 80% of the world population has no access to these medications.[8] However, this treatment is not effective as is able only to slow down the infection but its not offering a proper cure. Therefore, many researchers have been extensively working on other possible areas of HIV treatment and recently turning their attention to the monoclonal antibodies (mAbs).[7]

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Monoclonal antibodies (mAbs)

Monoclonal antibodies (mAbs) are antibodies, which are produced artificially from one cell line and are cloned in order to produce a specific antibodies to a particular epitope. They are produced by fusing myeloma cells with B-lymphocytes to form the hybridoma cells which allow producing only on type of specific antibodies. [9] In 1975 the production of monoclonal antibodies was developed by Kohler and Milstein.

Production of monoclonal antibodies

The myeloma cells are constantly dividing tumour cells allowing continuous production of cell lines. B-lymphocytes are the cells which are able to produce antibodies and are obtained from the spleen.[5] both cells are usually taken from animal models. General model animals used for antibody production are mice and rats. The following steps of monoclonal antibody production are shown on the Figure no 5. Steps for monoclonal antibody production.[5]:

1) Immunization: the model animal is immunized against the antigen of interest, which stimulates the lymphocytes to produce an antibody against this antigen.
2) Isolation of B-lymphocytes: the spleen cells from the immunized animal are removed and cultured on the medium under sterile conditions. The B-lymphocytes are obtained from the culture medium.
3) Fusion: equal number of B-lymphocytes is fused with an equal number of myeloma cells. Dimethylsulphoxide (DMSO) may be used with polyethylene glycol PEG to enhance the fusion process.
   The fusion solution contains 3 types of cells:
   a) Unfused myeloma cells
   b) Unfused B-lymphocytes
   c) Hybrid cells (B-lymphocytes-myeloma cells) – cells of interest
4) Selection: hypoxanthine, aminopterine, thymidine (HAT) medium is used for selection of hybridoma cells. Hybrid cells will now possess two abilities first to grow continuously from myeloma cell and second salvage DNA synthesis thanks to hypoxanthine phosphoribosyl transferase (HPRT) gene from B-lymphocytes cells. Hypoxanthine – for purines synthesis, Aminopterine – for blocking the pathway of purine and pyrimidine synthesis, Thymidine – for pyrimidine synthesis
5) Cloning: single hybridoma cell is isolated and allow growing and forming clones using feeder layer.
6) Isolation of monoclonal antibodies for cultivation: the soluble antibodies can be isolated by use of insoluble cross-linked polyelectrolyte co-polymer.

Different types of monoclonal antibodies

First monoclonal antibodies were of murine origin which makes them limited in usage because of the incompatibility of human immune system with the antibodies from other species. Much effort has been made with the advent of recombinant DNA technology to enhanced hybridoma technology in order to obtain other more humanized monoclonal antibodies.[10] which would not induce and immune response. The four types of monoclonal antibodies have been developed and now are available on the market[5], that are-

1) Fully mouse antibody,
2) Chimaeric antibody – made by fusion of mouse variable regions and human constant regions,
3) Humanized antibody – made by fusion of mouse complementarity-determining regions (CDRs) and human antibody frame (variable + constant regions),
4) Fully human antibody.

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Advantages and disadvantages of monoclonal antibodies

There are many advantages of using monoclonal antibodies. One of them is their high specificity and strong affinity towards the targeted antigen and ability to induce the human immune system through its Fc effectors' region against the invader.5. To create a particular mAbs towards the target antigen that is not known the only things needed are its epitopes. The process of production of mAbs can give a variety of different antibodies, which might be useful in the future. Many labs working on development of new mAbs sometimes accidentally produced some mAbs towards unknown antigens; mAbs produced in this way are kept freezed in liquid nitrogen until they are needed and can be screened occasionally.10. The large size of antibodies could be both the advantage and disadvantage depending upon the situation. It could be beneficial because its large size allows easier handling and binding to large targets but on the other hand, is a disadvantage as it could be too big to reach the particular target sites and pass through the cell membrane.5.

Other advantages of mAbs is their relative long half-life (10-20 day) allowing them to stay within the system for long, hence no need for daily administration as other therapeutic substances. However, the only way of administration can be by injection as antibody is a protein and would be digested by the digestive enzymes if taking through oral route. It can accidentally bind to non-targeted antigen and mount an immune response towards healthy cells.10. The production process of monoclonal antibodies is expensive, laborious and the outcome is a mixture of distinct molecules consisting of different fragments of antibodies.

Another disadvantage one can think of is the single specificity of monoclonal antibody, which can exert pressure on the targeted viral particle to change its structure in order to escape from the therapeutic effect of mAbs. The virus has better genetic makeup and can undergo alteration in a short time while the process of production of single mAb can take years.10. These problems could be overcome by preparing a mAb mixture which has to take advantage of high potency of individual mAb and its ability to neutralize virus effectively even at a minimal dose. Mixture of mAbs should be able to aim at distinct non-overlapping epitopes in order to prevent escape .10.

Limitations of monoclonal antibodies

Few limitations should be considered while using the mAbs as therapeutics. These are listed below:

1. The purity of mAbs is not fully reliable. The immunological tolerance of every mouse who is used may vary and can affect the efficiency of the final mAbs outcome.[11]
2. A chance of triggering immune response in a patient who immune system can mistakenly recognise the mAbs as another foreign body thereby decreases its half-life and efficiency.[12]
3. Human mAbs are not Food and Drugs Administration (FDA) approved. because of their side effects like fever, weakness, low blood pressure, rashes, and in some cases it can affect the bone marrow leading to increased risk of bleeding (American Cancer Society, 2010).
4. mAbs therapy may results in severe suppression of the individual immune system.[12]

Therapeutic usage of mAbs

Monoclonal antibodies have been used as therapeutic agents in treatment of infectious agent such as respiratory synctial virus (RSV), hepatitis C virus and rabies virus (RV).[10] The introduction of humanized mAbs has led to a drastic decrease in immunogenicity. This immunogenicity could further be decreased when technique such as phage display or EBV-transformation are used to develop a full human mAbs.[13] For mAbs therapy to be effective, it has to aim towards the right epitope. It may be best to target at once multiple epitopes on the surface of the antigen for a particular pathogen.

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The possible target sites for mAbs in the treatment of HIV

The possible targets for monoclonal antibodies on the HIV-1 molecules are shown on figure no 6. The following are the possible ways by which antibodies can prevent HIV-1 – T-cell interaction by.[14]

a. Before attachment action by:
   - Inhibiting interaction of adhesion molecules and lectins on the virus envelop with their ligands that are present on the T-cell.
   - Inhibiting the interaction of virions with CD4 receptor and co-receptors on the surface of T-cell, and also preventing conformational changes of the virus envelope that is needed for subsequent steps in the virus life cycle.

b. After attachment action by:
   - Inhibition of conformational changes that occur in the virus envelope glycoproteins (glycosylation) which can create or expose the conserve domains involved in the fusion of the HIV with the T-cell.

c. The conserve protein domains that are involved in the fusion of the virus to the target cell can be blocked.

d. Viral uncoating, assembly and budding of immature HIV that occurs at the later stage of the life cycle can be inhibited.

e. Aggregation of the virus can be blocked.

From the above possibilities, monoclonal antibodies that will target the HIV on stages marked by an asterisk on the above list can be produced. Those target sites were chosen because of their great importance in interaction between the virus and the host cell which leads to the antigenicity (potency to antigen recognition). These targeted sites involve the conserve domains present on the HIV, the gp120, gp41, CD4 receptor and chemokine co-receptor.

Epitopes on the surface of HIV
Gp 120.

The main targets of HIV-1 and HIV-2 are CD4 receptors on the T lymphocytes cells. If those cells will be destroyed this ultimately will results in AIDS. The entry of this viral particle into the T-cell is mediated by gp120 and gp41 transmembrane glycoproteins which are in form of trimeric spikes.[15] Five different variable regions (V1- V5) were identified in HIV gp120.[16] Among these five variable regions four of them (V1 –V4) are exposed on the surface. The conserved region of gp120 has discontinuous structures that interact with the gp41 ectodomain and CD4 receptor. Variable and conserved gp120 regions are highly glycosylated what makes them protected. These variability and glycosylation of gp120 are main reasons of itsantigenicity. The binding of gp120 to CD4 receptor is associated with conformational changes in the gp120 glycoprotein. The co-receptors which are specific for chemokines act as secondary receptors for the virus entry helping by exposure and alterations. Chemokine receptors specificity is determined by the third variable (V3) region of gp120.[17] The main core of gp120 glycoprotein consists of 25 β strands, 5 α helices and 10 defined loop segments. All the polypeptide chains of gp120 are folded into two major domains which are parallel to each other as shown on Fig 7 above .[18]

Gp 41

The ectodomain of gp41 is the most conserved region in HIV-1 Env. The gp41 consists of 3-4 hydrophobic repeat sequences in the ectodomain which forms coiled coil. The gp41 N-terminal has 3-4 hydrophobic repeats and fusion peptides that are essential for membrane fusion, and at the C-terminus is the transmembrane segment. There are two protease resistance complex formed within gp41, which is N- 51 and C- 43 from N and C terminal regions of ectodomain respectively, which are stable α- helical trimeric complex of heterodimers. They both are oriented in an antiparallel fashion. These formations of coiled coil structure are critical for HIV-1 Env mediated membrane fusion.[19]

CD4 receptors on T-cells

The CD4 receptor is involved in amplification of signal of the immune response hence, it plays an important role in an immune system. Blocking of the CD4 receptor could stop its function and make the individual immune deficient. By targeting the sites of HIV binding to

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CD4 receptor (neutralization) unwanted side effects could be avoided. The site which takes part in these binding is a conserved region on gp120 glycoprotein on the HIV.[20]

Effective monoclonal antibodies.

In order to develop effective monoclonal antibodies towards chosen targets introduced on page 21, the following approaches have been considered. To neutralize the conserved region on the gp120 through which the virus binds with the CD4 receptor to neutralize gp41 binding. Another approach taken is to mimic the CD4 receptor and chemokine co-receptor in order to distract the virus from its real target.[21] Several types of human or humanized monoclonal antibodies could be used. We can choose from the following possibilities: a) Whole antibodies – native antibodies. B) Recombinant variety of different fragments of antibodies with improved features.

Whole antibody (Ab).

The whole native antibody could be of choice as it's the one with the easiest production. However, considering their big size of 150kDa they might not be able to reach the target sites on gp120 and gp41 which are not exposed as those are important conserved regions of the virus which aid it's binding with the target receptor. [2] thus discrediting the use of above because of these obstacles.

Fragment of Ab - recombinant antibodies

To overcome the problem associated with native mAbs, recombinant antibodies were recently developed and used. The engineered mAbs could be made of any fragment of the antibodies.[2] Engineered antibodies are competitive against native antibodies and are already succeeding in clinical trials. The size of the recombinant antibodies is reduced; the efficiency can be increased, and it is possible to conjugate them with enzymes, liposomes, viruses. What will increase their effectiveness. Overall 30% of biopharmaceuticals which are in clinical trials are engineered antibodies.[22] In 2002 the food and drug administration (FDA) approved the first radiolabel antibody for cancer immunotherapy, and about 30 antibodies are currently in the late-phase of clinical trials. The first FDA approved human derivative (D2E7) was a complete human anti-inflammatory antibody. Chimaeric antibodies can be produced by combining the mouse variable region with the human constant region. Proteolysis cannot generate molecules smaller than Fab fragment thus, the microbial expression of single chain variable domain antibody fragment (scfv) is a current method of antibody production. Variable regions from heavy and light chains are joined together by polypeptide linker.[23] In this method smaller molecules of antibodies can be produced, and further combination of several different small molecules can produce an efficient antibodies. Mutation in CDR regions can produce an antibody of higher specificity. The scfv fragment with variable domain region (VDR) loop can be used to target molecules, and that can improve specificity of binding and penetration. Multivalent scfv fragments can be produced such as dimeric, tertameric that are of very high specificity and were successful in clinical trials.[22] The illustration of different such a recombinant mAbs is presented on Fig 8.

Antibodies from different species (Shark, Camel)

Another very promising option is an application of antibodies derived from different species. The different species can bring different features of the antibody which could be highly beneficial.

A mammalian antibody does not penetrate in deep cleft due to their Y-shaped structure with heavy and light chain. However, native antibodies of camelids or shark show single variable domain with cavity penetration power that makes them possibly very important in treatment of HIV targeted against gp120 and gp41.[24]

Cartilaginous fishes like shark, skates, rays and chimeras antibodies consists of heavy chain, homodimer called as IgNAR's new antigen receptors.[25] Shark antibodies are smaller in size, have high penetration capacity, high rate of solubility, scfv linker is not required. They are highly stable. Sharks can be immunized with lethal mammalians proteins and amount of required antibodies can be obtained from

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them. Shark antibodies are produced by many companies; Gen Way is one of them. They estimated some of the basic criteria for production of shark antibody.

IgNARs are found in serum of shark (13kDa). It has been found that variable domains work independently.[26] They show that bivalent proteins of higher specificity and functional affinity could be produced by using only antibody fragments. Bivalent or bispecific IgNARs could be used in future diagnostic and therapeutic form. Other natural sources of antibody are camelids. Camelid antibodies have 2 domains and contain only heavy chain and its antigenic binding property is within variable region of heavy chain called as VHH.[24] as shown on fig 10. VHH is directly joined to CH2 domain via hinge region, CH1 domain is absent. VHH are very stable, could be produced at low cost, may be used as anti-microbicide, anti-gp120 VHH may be formed and used for treatment.[24]
In the thesis [24] author used camelids antibody and their fragments and applied them to gp120 binding sites. It was showed that camelids antibody VHH bound to gp120 with high affinity and specificity and competed with soluble CD4 for binding site. Thus, camelids VHH can be used for HIV-1 inhibition.

Mixture of mAbs

As previously introduced the idea of mixture of individual monoclonal antibodies will help to target better the sites of action. This concept is to put all the possible designed monoclonal antibodies into a solution so as to reduce the effect of a single monoclonal antibody that exert selective pressure on the target site making the virus to undergo escape and becoming more variable. Also using the mixture will enable different site to be targeted at once as well as binding of one antibody to one of the epitopes could induce conformational changes which could expose other site for antibody binding.[14]

Monoclonal antibodies used for treatment of HIV

Different mAbs targeting various regions of HIV molecule has been developed, some examples are collected in Table 2 .

The process of approval of proposed mAb
Pre-clinical trials

Before processing the new product/therapeutic to clinical trials the pre-clinical evaluation needs to take place. Pre-clinical trials are carried on the animal models as mice, rats and chimpanzees. After successful results of these experiments the product can be accepted for clinical trials assessment.[27]

Clinical trials.

The figure below shows the stages of approval of new monoclonal antibody which needs to be accomplished in order to successfully introduce it on the market. Below are the steps proposed by the HIV Vaccine Trials Network for the clinical trials of a new therapeutic.[27]:
Stages of HIV vaccine testing in humans include three phases:
Phase- I:

- 20-200 HIV negative volunteers should be selected.
- Phase - I mainly targets on HIV vaccine related side effects.
- Phase - I also provides the information of dose to be given and administration schedule for its optimum immune response.
- It lasts about 12- 18 months.

Once the baseline safety is achieved in phase - I of trails, it is continued to phase - II.

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Phase - II:

- Several hundreds of volunteers are participated in this phase.
- Still focused on safety additionally, it is focused on human immune response.
- Most effective dose and administration estimated.
- It lasts about two - three years.

Phase - III:

The most promising vaccines move to this phase, those which had greatest successes in phase - I and phase - II.

- Several thousands of HIV negative volunteers are selected.
- Phase - III should provide sufficient information of effectiveness of the vaccine in preventing the HIV infection.
- The data from phase - III supports application for licence and marketing of the product.
- Licence is issued from Food and Drug Administration (FDA).
- It lasts for three-five years.

The Experimental vaccines used in all the above three phases are not live viruses. Volunteers should not get HIV infection or AIDS. In order to introduce a safe and effective vaccine/therapeutic for HIV it should be tested in human volunteers to record and check the working nature as the product. Volunteers should be healthy and HIV negatives both genders that are immunologically active in HIV. Since HIV vaccines are genetically cloned parts of HIV proteins and do not contain real viruses it is highly safe for clinical trials. These human clinical trail help in finding out the exact reaction to the vaccine when used on volunteers.[27]

DISCUSSION

The aid of antibodies is human immune system. The HIV-1 has developed many ways to escape the humoral immunity by protecting some conserved regions that are important for targeting by antibodies.[2] However; effort has been made to reduce the viral load by producing monoclonal antibodies against the virus. The monoclonal antibodies produced are of high molecular weight of about 150kDa and mostly in the IgG1 class and are usually directed against the envelope protein to the surface of the virus or against the cell-surface receptors that the virus binds.[2]

The major challenge of using monoclonal antibodies in treatment of HIV is that the virus keeps undergoing resistance as a result of the selective pressure the monoclonal antibody exerts on the virus leading to its escape.[10] Also because of the steric hindrance that the HIV has developed against the antibodies thus, making it difficult for the antibodies to reach the conserved epitopes within the CD4 binding region.[2] To overcome these challenges, it would be best to use an antibody with small size, able to target those conserved domains within the HIV. Smaller antibodies such as scfvs and Fabs have been shown to have a higher neutralizing effect when compared to the larger monoclonal antibodies available.[2] Though these antibodies lack the Fc region and also tend to have a shorter half life, and they are not able to induce opsonization; they can only neutralize the target.

The attention could be directed towards the “domain antibodies” (dAbs) which are even smaller than the Fab and scfvs antibodies. These domain antibodies lack the V_L or V_H making the antigenic site concentrated over a smaller region so that it can interact with epitopes on the surface of the virus that are inaccessible to conventional antibodies. It has been shown that most envelops on the surface of HIV-1 are hidden by glycosylation and oligomeric occlusion. [2]

Targeting the CD4 binding site (CD4i) and the co-receptor binding site on gp120 seems to be difficult using a large antibody. This CD4 binding site on gp120 is seen to be highly conserved than is surrounded by regions that are variable. This site is surrounded by V1/V2 loops, and also the CD4i epitope is surrounded by V2 and V3 loops.[2] it is best that smaller antibody or one with protruding paratopes that can access this region are used such as dAbs or shark’s antibodies respectively.

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Another approach that could be used is to produce monoclonal antibodies that will mimic the CD4 receptor, and the co-receptor that the HIV binds to. This mimicked antibodies will distract the HIV virus from binding to the real CD4 receptor hence, neutralises the effect of the virus.[21]

The final proposal is to introduce a mixture of possible effective monoclonal antibodies targeting discussed epitopes. The advantage of mixture will be greater over the individual mAbs as this will allow the target many sites with one delivery of the therapeutic, reduced the pressure on virus, make neutralization of the virus more effective and to provide an efficient cure for treatment for the HIV.[28]

The above possible monoclonal antibiotics to be able to reach the market need to go through the approval stages of pre-clinical and clinical trials.[27] Above proposal is very promising and brings hope to the people infected by the HIV virus. However, further evaluation of cost of this proposal needs to be studied.

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THE EXPERIMENT
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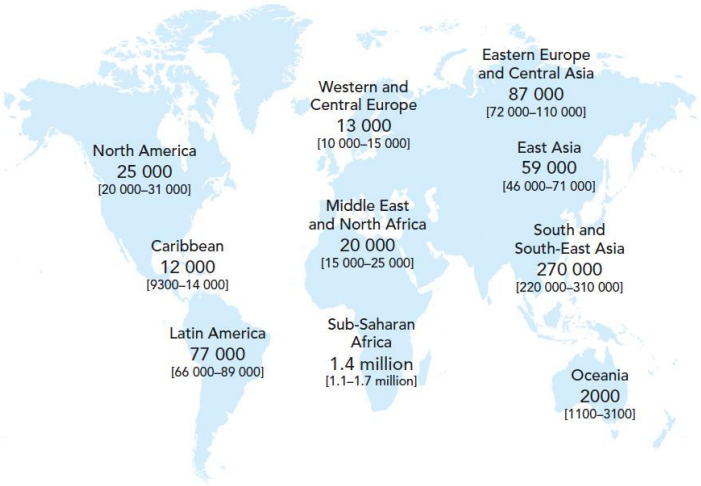

Figure 1: The map showing the estimated death rate due to AIDS in 2008.[29]

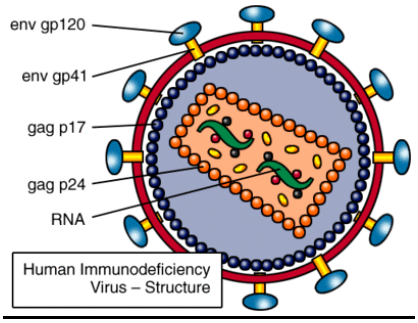

Figure 2: The structure of HIV.[30]

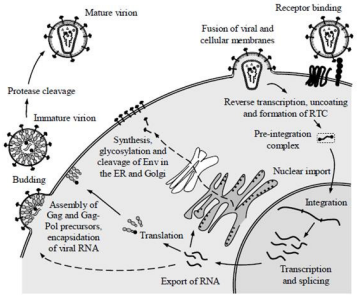

Figure 3. The systematic presentation of HIV life cycle. Showing all the stages starting from HIV binding to the CD4 receptor on the T-cells (host cells) through the intracellular steps to the viral assembly and release to the blood stream of new viral particles.[24]

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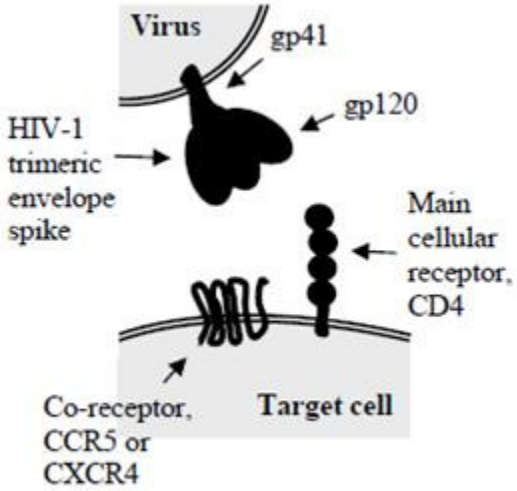

Figure 4: Cartoon showing the most important elements on the HIV and the T-cells involved in the process of viral entry to the host cell.[24]

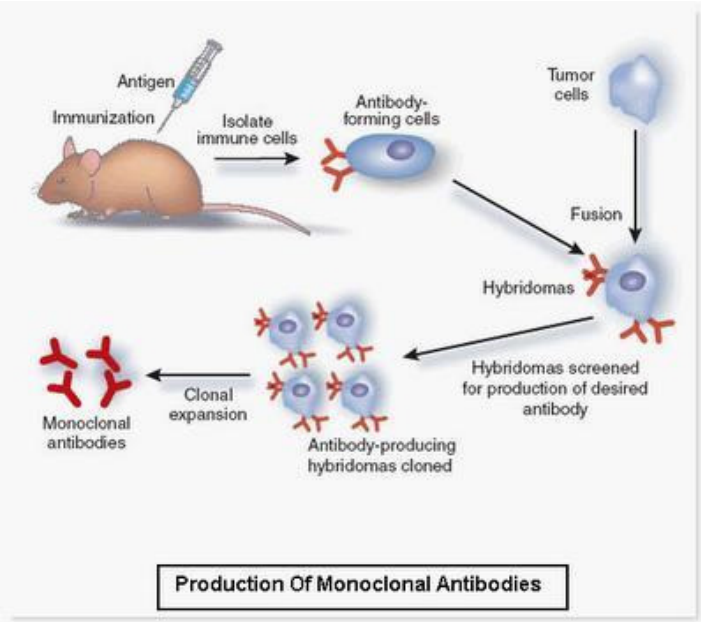

Figure 5: Systematic representation of monoclonal antibody production.[31]

THE EXPERIMENT
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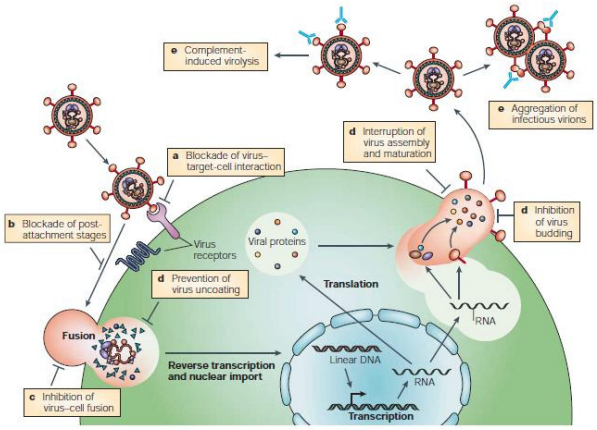

Figure 6: The HIV-1 and T-cell interaction with possible target sites for use of monoclonal antibodies as therapeutic .[14]

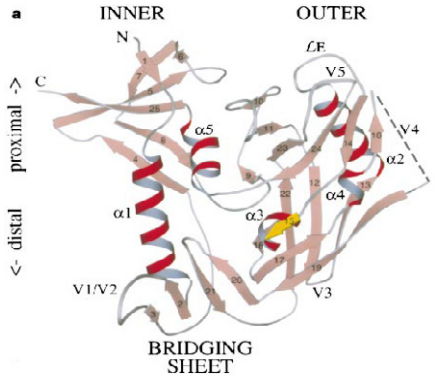

Figure 7: Tertiary structure of gp120 glycoprotein. V1-V5 – variable regions, α1- α5 – helixes, LE – loop.[32]

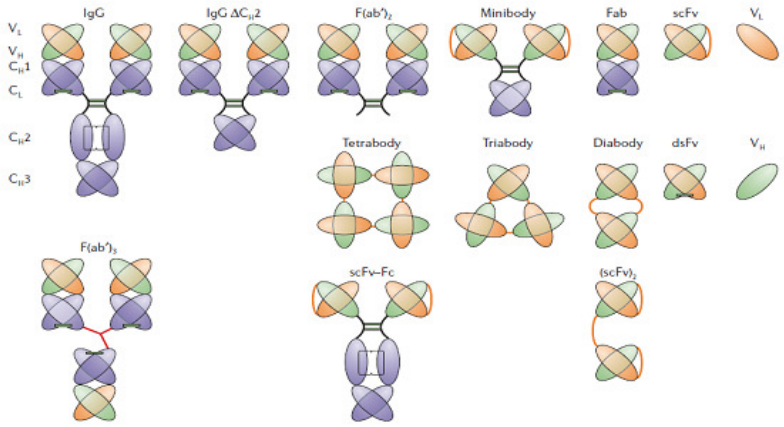

Figure 8: Variety of recombinant antibodies.[23]

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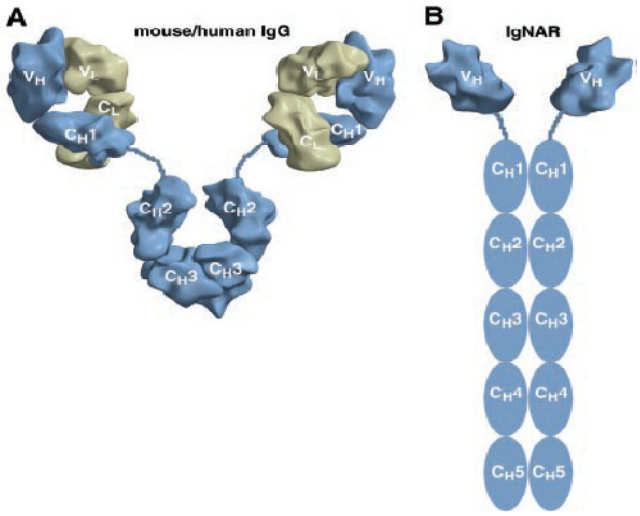

Figure 9: Representation of A) mouse/ human IgG and B) shark antibody IgNAR.[26]

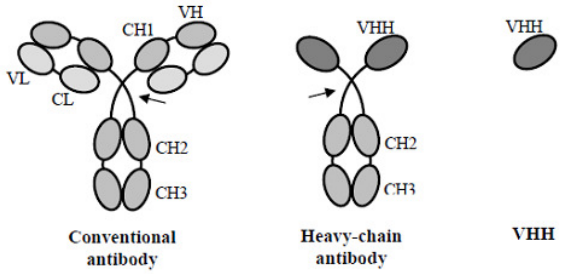

Figure 10: Comparison of structures of conventional antibody with camelid antibody.[24]

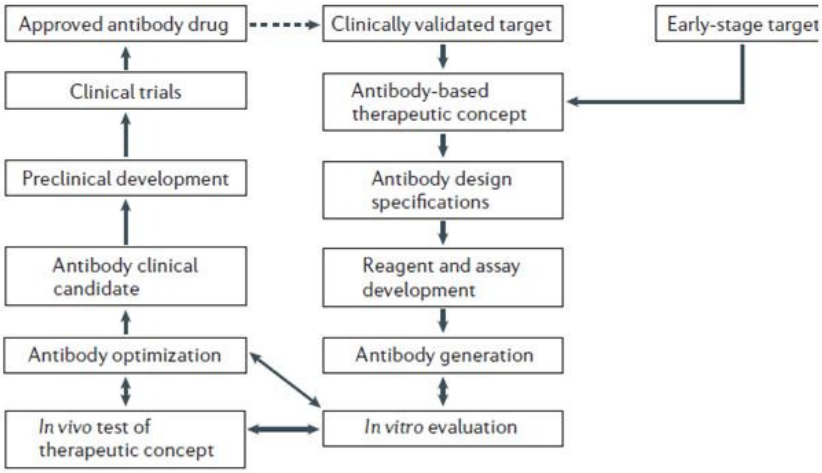

Figure 11: Stages of new antibody approval.[5]

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Opportunistic diseases caused with AIDS	Percentage (%)
Pneumocystis carinii pneumonia	63.6
Oral / pharyngeal candidiasis (thrush)	44.8
Kaposi sarcoma	20.8
Esophageal Candidiasis	10.6
Extra pulmonary cryptococcosis	6.8
Cytomegalovirus infection	5.0
Herpes zoster	4.8
Mycobacterium avium complex infection	4.2
Chronic mucocutaneous herpes complex	3.6
Chronic enteric Cryptosporidiosis	3.1
Toxoplasmosis of brain	2.5
Tuberculosis (at any site)	1.9
Immunoblastic sarcoma	1.0
Primary lymphoma of the brain	0.7
Disseminated histoplasmosis	0.6
leukoencephalopathy	0.6
Bronchopulmonary candidiasis	0.5
Burkitt's lymphoma	0.3
Chronic enteric isosporiasis	0.2
Mycobacterium kansasii infection	0.2
Salmonella septicaemia	0.2
Legionella pneumonia	0.1
Aspergillosis	0.1
Herpes simplex virus pneumonia	0.1
Coccidoidomycocis	0.1
Nocardosis	0.1

Table 1: Diseases caused by HIV.[33]

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Table 1 Representative neutralizing human monoclonal antibodies against HIV-1 used *in vivo*

Epitope	Antibody	Format	Animal/human	Virus	Infusion route	Challenge route	Efficacy
CD4-binding site on gp120	b12	IgG1	Macaque	$SHIV_{162P4}$	Intravenously	Vaginally	4/4 protected
	b12	IgG1	Macaque	$SHIV_{162P4}$	Vaginally	Vaginally	9/12 protected
	F105	IgG1	Human	NA	Intravenously	NA	No obvious inhibitory activity
Carbohydrate on gp120	2G12	IgG1	Macaque	$SHIV_{89.6PD}$	Intravenously	Intravenously	High-level plasma viremia; less decrease in $CD4^+$ T cells
gp41	2F5	IgG1	Macaque	$SHIV_{89.6PD}$	Intravenously	Intravenously	High-level plasma viremia
Combination	2F5/2G12	IgG1	Macaque	$SHIV_{89.6PD}$	Intravenously	Intravenously	1/3 with transient infection; 2/3 with marked reductions in viral loads
	2F5/2G12	IgG1	Human	NA	Intravenously	NA	5/7 with transient reductions in viral loads; 7/7 with transient increase in $CD4^+$ T cells
	2F5/2G12/4E10	IgG1	Human	Mostly clade B	Intravenously	NA	2/8 chronically and 4/6 acutely infected individuals with delay in viral rebound
V3 on gp120	Cβ1	IgG1	Chimpanzee	$HIV\text{-}1_{IIIB}$	Intravenously	Intravenously	1/1 protected
	KD-247	IgG1	Macaque	$SHIV_{C2/1}$	Intravenously	Intravenously	2/2 protected
	CGP 47439	IgG1	Human	NA	Intravenously	NA	7/12 with reductions in plasma viral RNA and p24
	hNM01	IgG1	Human	NA	Intravenously	NA	3/4 with decrease in plasma viral loads
CD4	TNX-355	IgG4	Human	NA	Intravenously	NA	Dose-related reductions in plasma viral loads in 30 individuals
CCR5	PRO 140	IgG1	Human	NA	Intravenously	NA	Dose-related reductions in plasma viral loads in 39 individuals

CCR5, chemokine (C-C motif) receptor 5; NA, not applied; SHIV, simian/human immunodeficiency virus.

Table 2: Different monoclonal antibodies used in the treatment of HIV virus .[2]

Bipin Deochand Lade

University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK.