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Hybridoma Technology: A New Scientific Tool for AIDS Recovery

			
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ABSTRACT

Hybridomas (fused hybrid cells) technology allows the development of monoclonal antibodies (mAbs) to target antigen which caused to disease. mAbs can be prepared by fusing B-cells of animal spleen with respective antigen, and then placed in a suitable medium for development, then subject to characterized by physicochemical and structural properties, purity, impurities, and quantity of the mAbs, based on ICH guideline Q6B. Researchers found that gp120 and gp41 are the main cause for antigenicity of HIV that makes them target. Also, they present that camelids antibody VHH (variable domain heavy chain antibodies) show to compete with CD4 for binding & act on target gp120 site comparing with human antibodies, leads to new possible treatment. So, the actual goal of this review was to use of hybridoma technology to develop monoclonal antibodies from particular species for a treatment of HIV has been reported.



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INTRODUCTION:

As the population increases the number of infectious diseases also increased around the world. Even though causative agent found that by researchers but right now, also there is no proper treatment for complete cure. In such type of disease, AIDS is one of them.

History of AIDS caused by HIV

On 1981 June 5, the U.S. weekly report 'disease control and prevention' which published and brought notice to the world that the five homosexual men in Los Angeles, California suffered from this infection and caused by less immune activity leads to autoimmune diseases and this was known by GRID (gay-related immune deficiency). In July 1982, the name 4H disease (Homosexual, Haitian, Hemophiliac, and Heroin user related disease) came and it was later changed to AIDS. In 1983 The American Biomedical research group of Robert Charles Gallo and Jay Levy and French virologist Luc Antoine Montagnier, successfully isolated the infectious agent of AIDS i.e HIV.¹

Causes of HIV Transmission:

AIDS or HIV transmission is not caused by blind thoughts like hugging, dry kissing, body-body rubbing, massaging, bathing together, hand shaking, food eating or drinking with the patient. But it is then transmitted through the blood, semen, genital fluids or breast milk of a person infected with HIV, usage of patient used blade, scissor, having unprotected sex or sharing drug injection equipment with a person infected with HIV are the most common ways HIV is transmitted.

Fully developed causative HIV is known as virion, researchers found that it is about 100 nm in diameter and about 1/20th of the length of *E.coli* cell, and about 1/70th diameter of the white blood cells that the virus infects.²

AIDS

AIDS is one of the sexually transmitted diseases (STD). According to WHO Statistical analysis, there is peoples death rate is high around the world due to AIDS. Which results from retroviruses (HIV-1 and HIV-2), that destroy CD4⁺ lymphocytes and impair cell-mediated immunity, leads to nonspecific febrile illness, cancers can directly damage the brain, kidneys, and heart causing cognitive impairment, hypogonadism, and

cardiomyopathy. AIDS, which is defined by infections or a CD₄ count of < 200/μl. HIV infection can be diagnosed by nucleic acid (HIV RNA), antibody or antigen testing i.e ELISA test.³

Pathogenesis of AIDS

Two major systems affected by HIV are:

1. Immune system (T cells and B cells)
2. Central nervous system

1) Immune system:

The immune system is made up of T cells (cell-mediated immunity) and B cells (antigen-antibody) mediated immunity.

a) T-cells:

HIV mainly affects the cell-mediated immunity. The normal function of T-cells is recognition and binding to specific T cell receptors. T-cells express a variety of molecular protein complexes like CD₃, CD₄, CD₈, and CD₂ of all these protein complexes, CD₄⁺T Cells, and CD₈⁺T-cells are expressed as mutually exclusive subsets. CD₄⁺T cells are:

- The master regulator of the immune system.
- Secrete some soluble factors like cytokines that influence the other T cells of the immune system. Hence, if CD₄⁺T cells count is less, it then indicates that the regulation of immune system is not proper. The intensity of disease is measured by counts of CD₄ and CD₈ cells.

b) B cells:

The B cells are destroyed by glycoprotein (gp¹²⁰) of HIV.

2) Central nervous system:

After the attack of the immune system, the macrophages are then targeted. The HIV infected macrophages produce cytokines, which is very much toxic to the neuronal cells.⁴

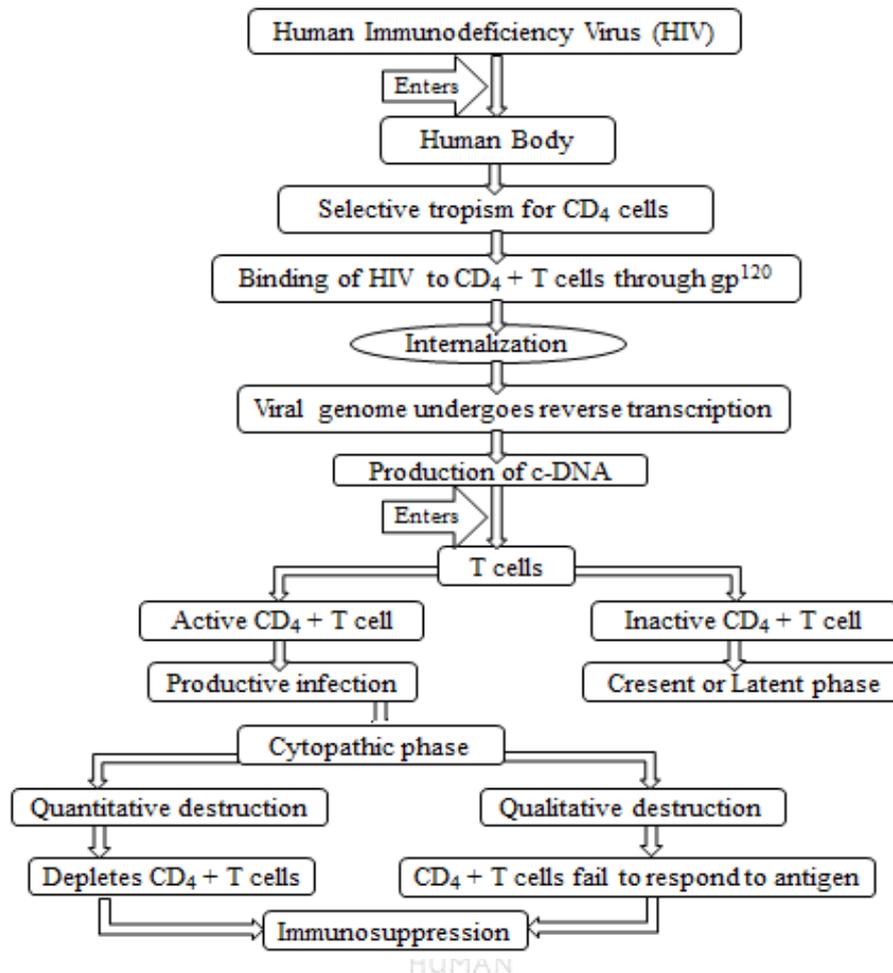


Figure 1 Stages of HIV development in human body

Available treatment for HIV:

For those affected by HIV, there is a treatment of life-long commitment. Once treatment has been started, strictly advise that the patient should continue his medicine. It sounds simple, but it's easier said than done. If when treatment is brake or stopped or interruption occurs, it may cause patients to become ill as they may lose their body's ability to contain the HIV virus.

According to WHO, to prevent HIV from multiplying and destroying infection-fighting CD4 cells, the combination of three drugs or more (a drug "cocktail"), which is known as ART or HAART i.e “highly active antiretroviral therapy”, is useful and effectively fight off infection and may observed the improve patient’s quality of life.⁵

Medicines of different classes are targeting the virus in different ways and at different stages of its replication cycle. The six classes of HIV medications are as follows:

Table 1 Classes of Anti-HIV drugs.⁶

Sr. No.	Classes	Examples
1	Non-nucleoside reverse transcriptase inhibitors	Delavirdine, Nevirapine, Efavirine, Etravirine
2	Nucleoside reverse transcriptase inhibitors	Abacavir, Zidivudine, Lamivudine, Stavudine
3	Protease inhibitors	Ritonavir, Darunavir, Indinavir, Nelfinavir
4	Fusion inhibitors	Enfuvirtide
5	CCR5 antagonists	Maraviroc
6	Integrase inhibitors.	Raltegravir

But health care may decide to pause or modify HAART. Because of AIDS.gov stated that the drug toxicity, that prevents patients from pursuing the treatment and regimen failure (results from the ineffectiveness of the prescribed drugs to keep the patient's HIV virus in check).⁷

However, this treatment is not much effective as it is able only to slow down the virus developing cycle but it does not offer a proper cure. Therefore many researchers have been working and found that the other possible areas for HIV treatment and recently turning their attention to the mAbs.

Monoclonal antibodies (mAbs):

Monoclonal cells are defined as a group of cells produced asexually from a single ancestral cell by repeated cellular replication. Thus, they can be said to form a single "clone".⁸

Salient features of mAbs:

- Due to a large size of antibodies, it's easier to handle and binds to large targets.
- Due to their long half-life (10-20 days), so no need for daily administration.
- Ability to induce human immune system by its Fc effectors region against the invader.

Hybridoma Technology (Production of mAbs):

In 1975, Kohler and Milstein developed the production of mAbs. mAbs are important diagnostic reagents used in biomedical research, microbiological research in the diagnosis of

Hepatitis, AIDS, influenza, herpes simplex, infections and in the treatment of such diseases as infections and cancer.⁹ By fusing or cloning causative organism with B-lymphocytes, which allow producing only one type of specific antibodies hence called monoclonal antibodies which may cause to form hybrid cells (Hybridomas) the technique is called Hybridoma Technology.¹⁰

While the patient doesn't have enough antibodies against the disease, in such case researchers used to produce mAbs by using lab animals and use their activity against diseased antigens, and due to comparison to normal antibodies of human beings these mAbs may have active life cycle as well as life span, and does not allow to affection of antigens to normal cells (as it requires host cell for survive) by interrupting their life cycle and gives the relaxing life to the patient. According to HIV life cycle, before attachment and entry into the host cell placed mAbs into the system to act against the virus. For that, make and modify mAbs/ hybrid cells and are tested by the suitable method to check the activity and that activated mAbs can be placed into patient's body by intravenous infusion. Then assessing tolerability, pharmacokinetics and antiviral effects follow the subject.¹¹

MATERIALS AND METHODOLOGY:



Development of Monoclonal Antibodies:

Monoclonal antibodies were produced in mice transgenic for heavy and light chain genes or were isolated from a phage antibody library constructed or derived from non-immunized human donors or an immunized rabbit.¹²

Based on researchers work, mAbs are produced by one cell line and are fused or cloned to produce specific antibodies to a particular epitope. B-lymphocytes are the cells, which are able to produce antibodies and are obtained from the spleen. Both cells are usually taken from animal models. The fused cells are then placed in HAT medium and cloned. In HAT medium only the hybridomas can survive and reproduction occurs. Those can be maintained with in this culture and continue to produce monoclonal antibodies which can be separated and purified for actual clinical usage.¹³

Following are the steps of monoclonal antibody production are shown below.

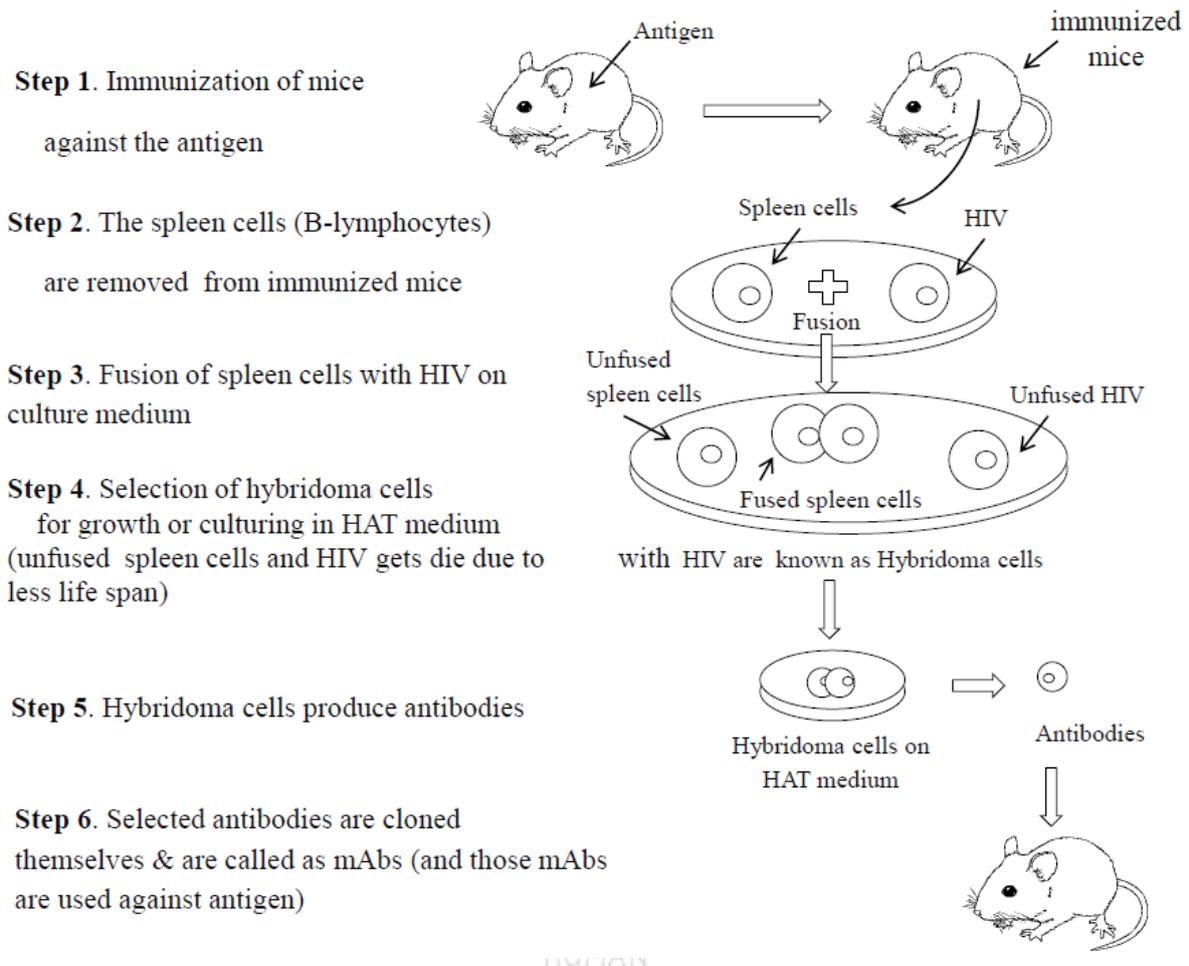


Figure 2 Production of monoclonal antibodies

Step 1: Immunization of mice and selection of mouse donors for generation of hybridoma cells:

The model animal is immunized against the antigen i.e HIV, which stimulates the lymphocytes to produce an antibody against this antigen. In general, mice are immunized every 2-3 weeks.

Step 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 obtained from the culture medium.

Step 3: Fusion:

An equal number of B-lymphocytes is fused with an equal number of HIV cells by using polyethylene glycol (PEG). The fusion solution may contain three types of cells:

- a) Unfused HIV cells
- b) Unfused B-lymphocytes
- c) Hybrid cells (B-lymphocytes-HIV cells) – cells of interest

Step 4: Selection:

HAT (Hypoxanthine, aminopterin, thymidine) medium is used for culturing of hybridoma cells. Hybrid cells are possessed to grow continuously from HIV cell and salvage DNA synthesis gene from B-lymphocytes cells. Hypoxanthine for purines synthesis, aminopterin for blocking the pathway of purine and pyrimidine synthesis, thymidine for pyrimidine synthesis.

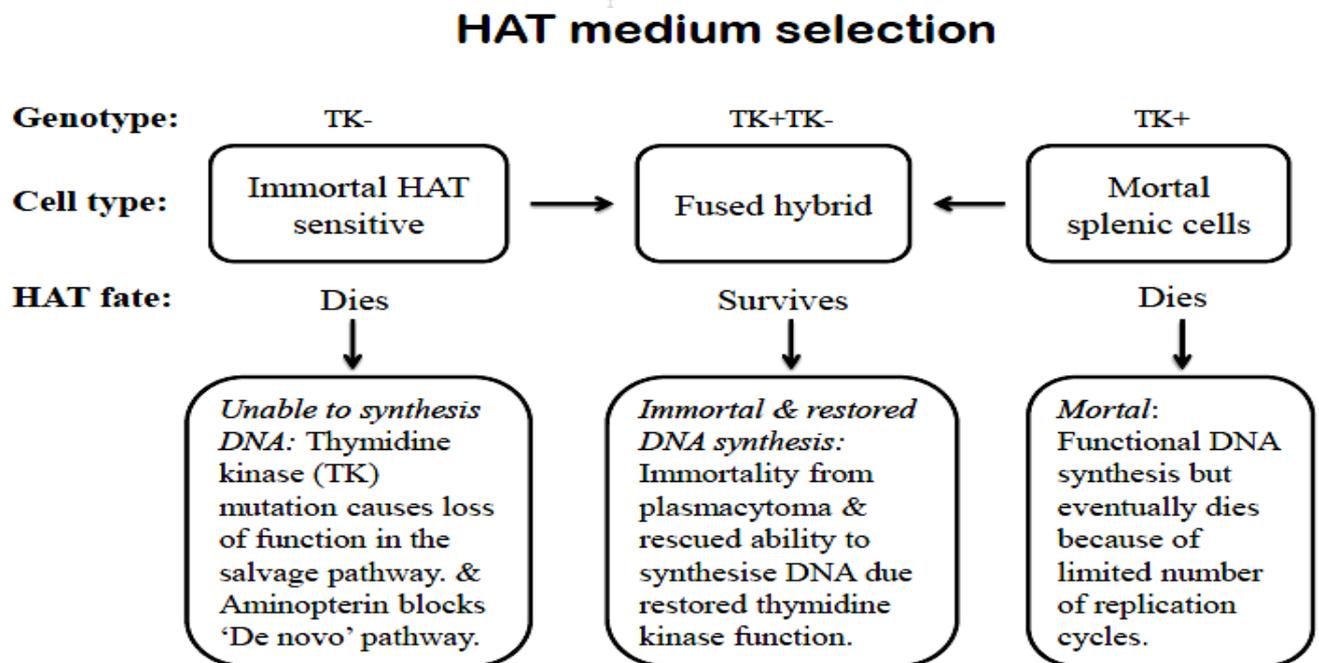


Figure 3 HAT Medium containing Thymidine kinase mutants (TK+/TK-) used for a preparation of mAbs for surviving.

Step 5: Isolation of monoclonal antibodies for cultivation:

The soluble antibodies can be isolated by use of insoluble cross-linked polyelectrolyte copolymer.

Step 6: Cloning:

Single hybridoma cell is isolated and allow growing and forming clones using feeder layer.^{14,15}

CHARACTERIZATION:

After preparation of mAbs, the characterizations include the determination of physicochemical and structural properties, purity, impurities, the quantity of the mAb, and stability in accordance with ICH guidelines Q6B.

➤ Structural characterization

- Amino acid sequence
- Amino acid composition
- Terminal amino acid sequence
- Peptide map
- Sulfhydryl group(s) and disulfide bridges
- Carbohydrate structure



The amino acid sequence should be verified by DNA sequencing and experimentally by using methods like peptide mapping, amino acid sequencing, and mass spectrometry analysis. Analyze the variability of N- and C- terminal amino-acid sequences, disulfide bridge integrity and mismatch, also structure of the carbohydrate chains, the oligosaccharide pattern, the glycosylation site (s) and occupancy, and determine carbohydrate content (neutral sugars, amino sugars), free Sulfhydryl group(s) and disulfide bridges.

➤ Physicochemical analysis

- Molecular weight or size

- Isoform pattern
- Extinction coefficient or molar absorptivity
- Electrophoretic patterns
- Liquid chromatographic patterns^{16,17}

Applications of monoclonal antibodies:

- mAbs applied to the cardiovascular, respiratory diseases, cancer, malaria, hormonal disorders, autoimmune diseases, treatment of septic shock.
- Used to reduce the risk of mother to child HIV transmission.
- Used as diagnostic and research reagents, as ELISA, immunofluorescence
- Used in identification of blood groups in the UK (UK blood typing)
- Immunopurification
- The use of mAbs gives opportunities to fighting the HIV.¹⁸

Limitations of monoclonal antibodies:

Few limitations should be considered while using the mAbs as therapeutics.

1. The purity of mAbs is not fully reliable.
2. The immunological tolerance of every mouse who is used may vary and can affect the efficiency of the final mAbs outcome.
3. A chance of triggering the immune response in a patient whose immune system can mistakenly recognize the mAbs as another foreign body thereby decreases its half-life and efficiency.
4. 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 the bleeding report by American Cancer Society, 2010.

5. mAbs therapy may result in severe suppression of the individual immune system.¹⁹

Table 2. Commercial development of HIV mAbs.²⁰

Type	Virus	Stage of development	mAb	Isoform	Target	Development technology	Development technology	Indication
Chronic, noncytopathic	HIV	Phase 2	TNX-355	IgG4	CD4	Humanized	Genentech	Treatment
	HIV	Phase 1	KD-247	IgG1	gp120-V3 tip	Humanized	Kaketsuken-Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan)	Treatment
	HIV	Phase 1	PRO140	IgG4	CCR5	Humanized	Progenics Pharmaceuticals (Tarrytown, NY, USA)	Treatment

	HIV	Phase 1 Preclinical	HGS004 HGS101	IgG4 IgG1	CCR5	AbgenixXen oMouse technology	Human Genome Sciences (Rockville, MD, USA)	Treatment 5.5-fold greater potency and a broader range of activity against HIV-1 viral strains than HGS004
	HIV	Preclinical	Tarvacin (Bavituxi mab)	IgG1	Aminoph ospholipi ds exposed on the surface of cells	Chimeric	Peregrine Pharmaceuti cals (Tustin, CA, USA)	Treatment
	HCV	Phase 1	Tarvacin (Bavituxi mab)	IgG1	Aminoph ospholipi d binds to phosphol ipids which are derived from the host cell	Chimeric	Peregrine Pharmaceuti cals	Treatment of HCV and HIV co- infection

	HCV	Phase 1	Helen-C (XTL-6865, cocktail of mAbs AB68 and AB65)	IgG1	Envelope protein E2	AB68, human hybridoma; AB65, EBV immortalization	XTL Biopharmaceuticals (Rehovot, Israel)	Treatment
	HCV	Preclinical	HuMax-HepC	IgG1	Envelope protein E2	EBV immortalization	Genmab (Copenhagen)	Prophylaxis

Table 3 List of Patents of China in USPTO (United States Patent and Trademark Office) on Production of Monoclonal antibodies.²¹

Number	Title	IPC (International Patent Classification)	Assignee (Applicants)	Publication date (MM/DD/YYYY)
1	Use of DKK-1 protein in the cancer diagnosis	A61B 5/00 (20060101); A61B 8/00 (20060101); A61B 10/00 (20060101)	Shanghai Cancer Institute (Shenzhen, CN)	12/27/2012
2	Monoclonal antibodies binding to avian influenza virus subtype H5 haemagglutinin and uses thereof	A61K 39/42 (20060101)	Xiamen University (Fujian Province, CN)	12/22/2011
3	Fully human anti-TNF-alpha monoclonal antibody, preparation method, and use thereof	A61K 39/395 (20060101); C07K 16/24 (20060101); C12N 5/10 (20060101); C12N 15/13 (20060101); C12N 15/00 (20060101)	Shanghai BiomAbs Pharmaceuticals Co., Ltd. (Shanghai, CN)	12/06/2012

4	Anti-EFGRv3 monoclonal antibody	A61K 39/395 (20060101); C12P 19/34 (20060101)	Shanghai Cancer Institute (Shanghai, CN)	02/14/2013
5	Use of DKK-1 protein in the cancer diagnosis	A61K 51/00 (20060101); A61M 36/14 (20060101)	Shanghai Cancer Institute (Shanghai, CN)	05/14/2009
6	Anti-VEGFR monoclonal antibody, method of making, and uses thereof	A61K 39/395 (20060101); C07K 16/00 (20060101); A61K 39/00 (20060101); C07K 16/28 (20060101)	Shanghai Aosaiersi Biotech Co., Ltd. (Shanghai, CN)	02/17/2011
7	Anti-human trail receptor DR5 monoclonal antibody (AD5-10), method thereof, and use of the same	C12P 21/08 (20060101); C12N 5/16 (20100101); C12N 5/07 (20100101); A61K39/395 (20060101); C07K 16/00 (20060101)	The Institute of Basic Medical Sciences of Chinese Academy of Medical Sciences (Beijing, CN) 04/01/2010	
8	Monoclonal antibody against hepatitis E virus or its fragment with binding activity and use thereof	C12P 21/08 (20060101); C07K 16/00 (20060101)	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. (Haikou, CN)	10/19/2006
9	Method and composition for diagnosis of melanocytic lesions	G01N 33/53 (20060101); G01N 33/574 (20060101)	Shanghai CP GuoJian Pharmaceutical Co., Ltd. (Shanghai, CN)	N/A

CONCLUSION

Hybridoma technology is a newer technique for a formation of monoclonal antibodies, it is known as one of the most challenging tools for various infectious diseases, for treat or

recover them by making hybridomas i.e hybrid cells, by combining B cells of animal spleen with respective antigen to form antigen antibody fusion, due to mAbs are specific for particular antigen which inhibits the antigens life cycle, thus virus activity in the body has been stopped. So this hybridoma technology is known to be the valuable tool in pharmaceutical and biotechnological science to treat the various dangerous diseases like AIDS, Cancer.

FUTURE PERSPECTIVES:

In the past two decades, there was a huge growth, development, and usage of maps in the field of pharmaceutical biotechnology by comparing among other human therapeutic products. And This type of hybridoma technology will promote number of research and development in coming future days by using advances like the immune system, recombinant DNA and the above type of monoclonal antibodies have led to rapid increase in the value of pharmaceuticals and biotechnological field for human use like the recovery of life challenging infectious diseases.

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