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Overview on Gene Therapy Technology

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ABSTRACT

Gene therapy is a method of prevention or treatment specifically used to treat patients who are suffering from diseases due to defective genes. It is one part of gene based DNA technology and this therapy became possible through advance of genetics and bioengineering that enable manipulation of vector for delivery of gene. It has some requirements, which should be met such as genes of interest must be cloned; treatment should deliver sufficient copies of normal genes to target cells transferred genes should have stable expression. There are two gene therapy types, germ line and the somatic and are two basic delivery systems: *In vivo*, which involves direct vector injection into the body; and *ex vivo*, which involves genetic modification of cells in culture followed by transplantation. Gene cannot be directly inserted into an organism's cell. It must be delivered to the cell using a carrier, or vector. Vector systems can be divided into viral and non-viral. Gene delivery can be used for different purposes. The most common are: functional gene study, cancer therapy, in improving animal production through hormonal therapy like growth hormone and growth hormone releasing hormone therapy, infectious diseases therapies, and so on of the various challenges involved in the process, one of the most significant is the difficulty in releasing the gene into the stem cell which can causes cancer, immune activation and etc. Thus, there should be well established gene therapy research institute to further advancement of their usage.

Keywords: Gene therapy; Gene transfer; Technology; Vector; Growth hormone

INTRODUCTION

Gene is a structural, functional and mutational unit of DNA. Change in natural coding property of a gene is called mutation which is often lethal. Correcting that mutation is called gene therapy. This is a technique whereby the absent or faulty gene is replaced by a working gene, so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease.

The treatment of gene therapy involves researchers replacing the defective or faulty genes with a normal functioning gene. It involves detection of gene, determination of its role, cloning and introducing the gene by proper way. This is either germ line gene therapy (done in germ cells) or somatic gene therapy done in somatic cells [1-3].

Technological advances and the ever growing knowledge of molecular virology and virus-host cell relationships have constantly improved the safety profile of viral vectors that are now used i*n vitro* and *in vivo* to study cellular gene function, to correct genetic defects (gene therapy), express therapeutic proteins, vaccinate against infectious agents and tumors, produce experimental animal models, and for other purposes.

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One of the main focuses of this technique is the optimization of delivery vehicles (vectors) that are mostly plasmids, nano structured or viruses. The viruses are more often investigated due to their excellence of invading cells and inserting their genetic material.

While originally conceived as a way to treat life threatening disorders (inborn errors, cancers) refractory to conventional treatment, gene therapy now is considered for many nonlife threatening conditions, including those adversely affecting a patient's quality of life. The lack of suitable treatment has become a rational basis for extending the scope of gene therapy.

The potential therapeutic applications of gene therapy are vast. A major advantage of gene therapy over the use of conventional drugs is the prospect of curing disease rather than providing transient relief by suppression of disease symptoms. Replacing defective genes with functional genes through the use of gene therapy offers the prospect for long term therapeutic benefits without repeated drug application [4-7].

Thus, the objectives of this seminar are:

- To give an overview on types and methods of gene administration.
- To give highlight on current applications and future perspective of gene therapy.
- To summarize challenges toward application of gene therapy.
- To overview viral and physical agent used as gene transfer vehicle or vector.

LITERATURE REVIEW

Gene Therapy

Gene therapy is a strategy used to treat disease by correcting defective genes or modifying how genes they are expressed. The techniques used involve administrating a specific DNA or RNA sequence. Researchers hope that in the future, gene therapy will enable patients to be treated by inserting genes into their cells rather than administering drugs or subjecting them to surgery. Gene therapy is often aimed at achieving a long-lasting physiologically matched expression of the gene, without activating the immune system. The aim is even to integrate the genetic materials into the chromosome. Gene therapy promises to revolutionize agriculture as well as medicine. The early results on the clinical efficiency of gene therapies were disappointing, largely because the available gene transfer vectors provoked to be inadequate. Great progress was made in selecting and improving vectors and subsequently first positive results were reported.

Gene therapy has some requirements, which should be met. First of all, genes of interest must be cloned; treatment should deliver sufficient copies of normal genes to target cells; transferred genes should have stable expression; modified cells must have survival advantage over unmodified cells and finally gene expression must correct or reverse the disease.

Four approaches are now being used in gene therapy. These are gene augmentation therapy; targeted killing of specific cell, targeted mutation correction and the last is targeted inhibition of gene expression [8-11].

Types of Gene Therapy

In general gene therapy can be organized according to its cellular target, being called somatic gene therapy when the target is limited to somatic cells. This therapeutic method can also be considered an *ex vivo* system, since tissue samples or cells from the patient must be collected for biopsy with subsequent reimplantation after the cells are reprogrammed genetically allowing the correct synthesis of desired gene product. Another widely used method involves germ cell lineages generated after collection; the genes of interest are reprogrammed so that the new features will be perpetuated for future generations of cells from the patient.

Germ line therapy: This therapy involves the modification of the genes inside germ cells (sperm or ova). During reproduction, these gamete cells fuse to form a zygote, which would divide and pass on the modified gene into all other cells of the body during the development of offspring. In this way, the therapy alters the genome of future generations to come. Although theoretically this could counteract hereditary disease.

The scientific literature contains over forty reports of the successful *in vitro* uptake of exogene constructs (transgenes) by animal sperm cells. A majority of these reports provide evidence of post-fertilization transfer and maintenance of transgenes. Several of the studies report the subsequent generation of viable progeny animals, the cells of which contain transgene DNA sequences. While a minority of studies has used 'augmentation' techniques (electroporation or liposomes) to 'force' sperm to capture exogenesis, the standard methodology is very straightforward: Prior to *in vitro* fertilization or Artificial Insemination (AI), washed sperm cells are simply incubated in a DNA containing solution. As a potential tool for genetically manipulating animals, sperm mediated gene transfer has the advantages of simplicity and cost effectiveness, in contrast with more established methods of transgenes is such as pronuclear microinjection.

Somatic gene therapy: Unlike germ line therapy, somatic gene therapy only involves the insertion of therapeutic DNA into body cells and not the germ cells or gametes. This means any effects of the therapy are confined to the individual being treated and are not inherited by future offspring.

Several key steps appear to be involved in effective gene transfer to somatic cells:

- Type of delivery vehicle that may be composed of cationic liposomes, other types of liposomes, polymers, and their combinations, various types of viral or hybrid vectors and combinations of viral vectors with polymers or lipids.
- Interaction of the gene vehicle with serum components.
- Its circulation time in body fluids and bio distribution.
- Its escape from immune cells and macrophages.

- Its interaction with the surface of the cell.
- Its triggering of apoptotic pathways by this interaction.
- Its penetration through the cell membrane barrier.
- Its release from endoscopes or other sub cellular compartments and its escape from degradation by intracellular nucleases.
- Nuclear import.
- Ability of regulatory elements for driving the expression of the foreign gene in a particular cell type including DNA sequences that might determine integration versus episomally maintenance of a plasmid or viral vector.
- Persistence of the plasmid in the nucleus (or of the virus) as an extra chromosomal element for many cell cycles or integration into active chromatin loci.
- Maintenance of expression for long periods.
- Passage to progeny cells.
- Ability of the transcripts to be exported to the cytoplasm, translated, modified post-translation ally and transported through the end plasmatic reticulum and Golgi apparatus to the cell surface or extracellular [12-15].

Ways of Administration of Gene

There are two basic types of gene delivery systems: *In vivo*, which involves direct vector injection into the body; and *ex-vivo*, which involves genetic modification of cells in culture followed by transplantation. Most gene delivery strategies involve the former. The cells in culture are exposed to the virus, carrying the desired gene. After infection and integration of the desired gene in the cell's DNA, the cells are returned in the patient by injection into a vein. This technique is called *ex vivo* because the gene is transferred to the cells, while they are outside the patient's body. In the in *vivo* technique is the gene of interest is transferred to cells inside the patient's body by using of liposomes (fatty particles).

Gene Transfer

Gene cannot be directly inserted into an organism's cell. It must be delivered to the cell using a carrier, or vector. Generally, for full deployment, gene transfer technology needs to identify the appropriate target, either cell or gene, and strategy to pursue; deliver the genetic information solely into the right cell and in the right amount, *i.e.* selective and specific targeting and controlled/physiological expression of the therapeutic gene is a must; maintain the gene and its expression in the cell long enough to treat the disease or accomplish the task; restrain the gene from causing short or long term adverse effects, e.g. triggering autoimmunity, neoplastic transformation, or other disorders develop delivery systems with the least possible immunogenicity, and easy to produce and administer.

Vector systems can be divided into viral vectors and non-viral vectors. Vectors have several functions, including protecting the gene from degradation, facilitating entry into target cells, and securing stable gene transcription upon arrival in the nucleus. Ideally, a vector should be efficient in gene transfer and be safe. Safe transfer means that the vector introduces zero to minimal risk of infection or immunogenicity (immune response). In addition, a safe vector causes no mutation in the host cell or patient to patient transmission of a virus or other pathogens of the viral or non-viral methods of gene transfer, Retrovirus and Adenovirus based vectors had produced the best clinical results, but there remained always concern about the safety of these viruses that were used as vectors in the delivery of genes. Results also indicated that the somatic cell gene therapy would be practical and safer approach over germ line therapy.

Viral vectors for gene transfer: Viral vectors have been used in >70% of the clinical trials to date. Viruses are vehicles that efficiently transfer their therapeutic genes. This ability made them desirable for engineering virus vector systems for the delivery of therapeutic genes. The viral vectors currently used in genes into host cells research are based on RNA and DNA viruses processing. Genomic structures and host ranges. Particularly viruses were selected as gene delivery vehicles because of their capacities to carry foreign genes associated with efficient gene expression. These are the major reasons why viral vectors derived from retroviruses, adenoviruses, adeno-associated viruses and herpes viruses are employed in more than 70% of clinical gene therapy trial worldwide.

Gene transfer technology relies on, and attempts to exploit, the first step of replication and, at the same time, builds blocks to prevent production of infectious virus. In this context, transduction is defined as a non-replicative or dead end infection that allows heterologous (*i.e.* non-viral) genetic information to be delivered recise cell.

The first step of viral vector design is, therefore, to identify the viral sequences required for replication, assembly of viral particles, packaging of the viral genome, and delivery of the transgene into the target cells. Next, dispensable genes are deleted from the viral genome to reduce replication and pathogenicity, as well as expression of immunogenic viral antigens. The gene of interest together with transcriptional regulatory elements (referred to as the transgene) is inserted into the vector construct, and a Recombinant virus is generated by supplying the missing gene products required for replication and virion production. The more genes that are removed from the virus, the more replication defective the vector will be, and there is less chance of recombination to generate the infectious parental virus. The nature of the virus biology will usually determine the means of production. For example, Retroviruses are produced in packaging cell lines, and vector particles accumulate in the culture medium. In contrast, Adenovirus and Adeno-Associated Virus (AAV) vectors are generally produced from transfections, and cells must be lysed to liberate the viral particles reviews. Example of gene organization in AAV vector (**Figure 1**).

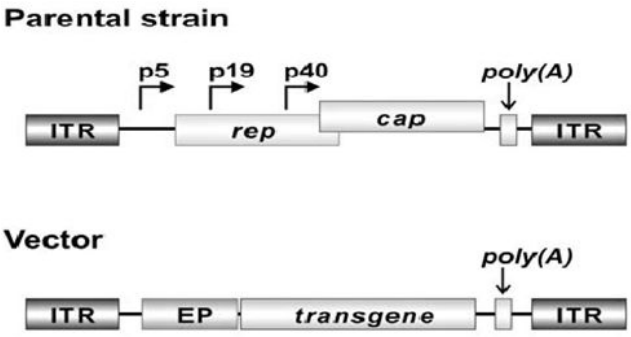

Figure 1: Genomic organization of a vector derived from an Adeno-Associated Virus (AAV).

The parental genome mostly consists of rep and cap encoding replicase and structural proteins. Expression of the AAV proteins is driven by promoter's p5, p19 and 40 and all transcripts share the same poly (A). Both poly (A) and Inverted Terminal Repeats (ITR) are maintained in the vector genome. By contrast, rep and cap are replaced by the transgene and the respective Eukaryotic Promoter (EP).

Adeno associated virus vector: Adeno Associated Virus (AAV) is a small less than 5 kb, single stranded DNA non enveloped Parvovirus. The natural route of AAV is the upper respiratory tract. Infection to occur, AAV requires coinfection with Adv For productive infection which allows the viral genome to replicate episomally and leads to synthesis of AAV proteins. The AAV requires an Adenovirus or a Herpes virus for viral replication. No pathology was linked to this virus.

Adenoviral vectors: Adenoviral vectors are derived from Adenoviruses (ADV), DNA viruses with a linear double stranded genome (36 Kilo base pairs, Kbp), a non enveloped icosahedral capsid with characteristic morphology replicating in the nucleus and producing thousands of progeny virion released by cell lysis. The viral genome encodes about 50 viral proteins, 11 of which are structural and used to physically build the virion. These viruses have been isolated from a large number of species, and in humans they primarily infect the respiratory airways and the gut causing mild and recurrent respiratory and gastro enteric diseases. Because of their low pathogenicity, infectious properties, wide tropism, high level of expression of viral proteins during replication, and the natural delivery of the viral genome in the nucleus, these viruses have been considered potential candidates for gene therapy since its inception [16-19].

Herpes virus vectors: Herpes virus vectors mainly derive from HSV type-1, a neurotoxic large DNA virus (152 Kbp, double-stranded DNA) that comprises more than 80 genes categorized into essential and non-essential genes according to their requirement for viral replication. In its natural life cycle, HSV-1 is spread by contact, infects and replicates in skin membranes, and is taken up by sensory nerve terminals where it establishes a latent state from which the virus can subsequently reactivate and spread to other individuals. These features, high infectivity and ability to transduce and persist in dividing and non-dividing cells make the HSV vector a good candidate for gene transfer. The virus contains essential genes involved in subtle interactions with the host cell, decoying the immune system, creating conditions for viral persistence in specific body sites and other functions that, from the vector point of view, are useless or even detrimental, and are therefore removed during vector construction. Removal of non-essential genes makes room for up to 50 Kb heterologous DNA thus making the HSV vector the largest carrier among the viral vectors.

Retrovirus vectors: Retroviruses are a group of RNA containing viruses characterized by the employment of the unique molecular mechanisms which is an efficient transfer system. By the reverse transcriptase enzyme activity, the viral RNA genome to be transcribed into double stranded DNA that stable integration into host DNA. The advantage of retroviral vectors are the stable integration into the host genome, generation of titers that allow efficient gene transfer into a broad variety of target t cells and the ability to carry foreign gene up to eighty kb. Vectors based on Lenti viruses such Human immune deficiency virus have the ability a variety of post mitotic tissues as heart, muscle or brain, but biosafety remains a major in production of such vectors. Somatic gene therapy consists in stable expression of a transgene product from an implanted group of cells that could be eventually removed if desired. The vectors used in these cases are Retroviruses because of their property of integrating into the transduced cells genome and express the transgene for the sequential generations in the cell line.

Non-viral vector for gene transfer: Non-viral gene therapy is the introduction of therapeutic genes via plasmid DNA into target cells without the use of a virus. In general, these techniques demonstrate low toxicity, immunogenicity, and pathogenicity, rendering these techniques safer than viral methods. However, they demonstrate low transduction efficiencies, which make them less desirable. These techniques include microinjection, electroporation, nonoperation, gene gun, controlled, and chemical delivery methods.

Comparative with viruses, the non-viral techniques for gene transfer *in vivo*, the direct injection of plasmid DNA is simple to use, easy to produce on large scale, inexpensive and safe, as it lacks specific immune response. In addition to expression of reporter enzymes, skeletal muscle is now used as a bioreactor to express therapeutic proteins having either a local or systemic effect. This methodology was limited by the relatively low expression levels due to inefficient DNA uptake into muscle fibers to ensure systemic physiological levels of secreted proteins.

Naked DNA microinjection: This method involves microinjection of purified circular DNA into target cells. This has been mainly performed using mouse skeletal muscle cells. In relative comparison terms, this method of delivery has shown to be more efficient when using mouse skeletal muscle tissue than with adenoviral and retroviral systems; however, the time course of expression is transient. This delivery system limits its efficient transfer characteristics only in the skeletal muscle because the muscle cells have an extensive tubular system, allowing the DNA delivery. It has been tried in vaccination

procedures, because a low and short expression is sufficient to induce immune response.

In gene gun system, micron-sized gold particles are coated with plasmid DNA and then accelerated at high speed toward target cells. Cells penetrated by the gold particles have high probability of being transfected by the DNA thus introduced. Micron gold metal particles are used as carrier of plasmid DNA containing desired gene.

Electroporation (EP) includes cell electro permeabilization, with the help of exposure to appropriate electric field pulses, is currently receiving much attention as a way to increase DNA delivery. In EP, brief electric pulses activate transient pores in the cell membrane and convey the agents into the cytosol. EP often does not induce any critical harmful end results, and therefore various veterinary clinical trials have demonstrated the safety and efficacy of Electro Chemotherapy (ECT), chemotherapy delivered *via* EP. The combination of cytotoxicity with chemotherapy along with the anti-tumour immune responses of immune modulatory therapy hinder tumour growth in multiple types of cancer and Electroporation (EP) appears as an attainable approach for cautiously and adequately combining this therapeutics. Electroporation is therefore a real technique for transfecting agents, such as chemotherapeutics and plasmid DNA (pDNA), into host cells. EP is increasingly being used among the scientific and the medical communities, as it is a safe and efficient technique to transfer a variety of material (e.g. nucleic acids, cytotoxicity drugs and ions) into target cells and tissues without harming them. However, the transfection efficiency assisted DNA delivery is still low compared to viral methods and there is a clear need to optimize this approach [20].

Liposomes: The liposome mediated delivery of genes relies upon the electrical charge properties of 3 components: The negatively charged DNA (attributed by the phosphate backbone of the double helix), the positively charged liposome, and the net negative charged cell surfaces, owing to the presence of sialic acid residues. The interplay of these components results in the liposome-DNA complex fusing with cell membrane plasmid DNAs degraded in the end lysosomal pathway, hence very little DNA actually reaches the nucleus.

Nanoparticles: Nanoparticles (NPs) offer an alternative to the use of viral vectors in gene therapy. NPs are particles that are 1 nm-100 nm in size. There are several different forms of nanoparticles and they typically contain a segment of DNA or RNA that is compacted with a polycationic polymer. Due to their small size, NPs can readily interact with molecules on the cell surface or inside cells. Unlike their viral vector counterparts, NPs do not introduce additional genes cells. They tend to be less immunogenic and cytotoxicity than viral vectors. Finally, NPs are able to incorporate numerous ligands such as DNA, antibodies, peptides, and probes and therefore present an array of therapeutic modalities. Pathways of cellular internalization of NPs include phagocytosis, micropinocytosis, clathrin or caveolae mediated endocytosis, and other clathrin and caveolae independent endocytic pathways.

DISCUSSION

Current Application and Future Perspectives of Gene Therapy

Current applications: Gene delivery can be used for different purposes. The most common are: Functional gene studies, correction of genetic defects expression of therapeutic proteins, and immunization against tumors and infectious agents.

In cancer treatment: Development of high technology pharmaceuticals against cancer based on gene therapy systems promise to elicit negligible side effects and to bring a major advancement and revolution in molecular medicine. For example, demonstration of tumor regression in animal xenograft models using virus liposome combination systems and their feasibility inhuman clinical trials would lead to the development of novel pharmaceutical products based on virus liposome complexes; endeavor, although it may appear to come from a science fiction movie, is feasible and has a potential in the 60 billion dollar anticancer pharmaceutical market.

In livestock production: Most important affecting factors are nutrition and disease. Animal diseases cause great reduction in their production. Use of gene therapy may significantly contribute in curing diseases and making animal healthier and more productive. Some examples of gene therapy are gene therapy of lysosomal storage diseases.

Growth enhancement: By using naked DNA porcine Growth Hormone (GH) treatment induces insulin resistance of protein metabolism and consequently reduces theoretical possibility for increased protein synthesis in the fed state. GH markedly reduces the amount of carcass fat; consequently, the quality of products increases Life tide® SW5 is the world's first and only approved growth hormone releasing hormone (GHRH) DNA therapy for food animals. It is an injectable DNA plasmid encoding for porcine GHRH and administered once for a life time treatment in sows of breeding age not only to increase the growth rate but also to increase number of piglets born alive and weaned. After intramuscular injection and electroporation, the active constituent Life tide® SW5 plasmid sequence enters to the skeletal muscle cell at the injection site and resides within the muscle cell. Then the treated muscle cells actively produce GHRH at physiological concentrations. The GHRH induces the animal to produce and secret indigenous growth hormone under the control of normal physiological feedback mechanism.

In vaccine production: By using plasmid DNA vaccination is one of the most effective and sustainable methods of controlling disease. A recent approach has been to use vaccines based on DNA. The use of DNA in vaccines is based on the discovery that injecting genes in the form of plasmid DNA can stimulate an immune response to the respective gene products. This immune response is a result of the genes being taken up and expressed by cells in the animal after injection. Compared to most traditional vaccines, which

preferentially elicit a humoral response, immunization by means of recombinant viral vector also triggers a robust Cytotoxic T Lymphocyte (CTL) response. That is particularly efficient in eliminating virus infected cells, intracellular pathogens, and cancer cells, and extending protection to other strains of the same pathogen by recognizing highly conserved epitomes. Vaccine approaches to prevent and treat prion infection. Molecular basis of pathogenesis of FMDV. Recombinant Adenovirus co-expressing capsid proteins of two serotypes of Foot and Mouth Disease Virus (FMDV), *in vitro* characterization and induction of neutralizing antibodies against FMDV in swine.

Future perspectives: Since the first clinical gene therapy trial was conducted, much attention and considerable promise has been given to the field. There has been substantial public and private sector investment, as well as increasingly higher levels of research activity. Numerous preclinical animal model studies have provided proofs of concept for multiple potential clinical applications. Also, major advances have been made in understanding vector biology and improving vector design and production.

RNA interference or gene silencing may be a new way to treat Huntington's. Short pieces of double stranded RNA (short, interfering RNAs or si RNAs) are used by cells to degrade RNA of a particular sequence. If a si RNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced. New gene therapy approach repairs errors in messenger RNA derived from defective genes. Technique has potential to treat the blood disorder thalassemia, cystic fibrosis, and some cancers.

Challenges of Applications of Gene Therapy

Gene delivery: The various challenges involved in the process, one of the most significant is the difficulty in releasing the gene into the stem cell. Thus, a molecular carrier called a “vector” is used to release the gene, which needs to be very specific, display efficiency in the release of one or more genes of the sizes necessary for clinical applications, not be recognized by the immune system and be purified in large quantities and high concentrations so that it can be produced and made available on a large scale. Once the vectors inserted into the patient, it cannot induce allergic reactions or inflammatory process; it should increase the normal functions, correct deficiencies, or inhibit deleterious activities. Furthermore, it should be safe not only for the patient, but also for the environment and for the professionals who manipulate it. Finally, the vector should be capable to express the gene, in general, for the patient’s entire life.

Immune response: Gene delivery vectors must be able to escape the host’s natural immune surveillance systems. The immune system may also prevent repeat administration of a vector, resulting in only short term gene correction. Host immune responses to vector proteins or to the transferred gene product (protein) have been a significant obstacle to achieving therapeutic levels of gene transfer and expression in some cases notably the hemophilias.

Disrupting important genes in target cells: A gene that is introduced into any group of cells ideally needs to remain intact and continue to function inside the cell. For this the freshly introduced gene must integrate into the cells existing nuclear DNA which is a random process *i.e.* not site specific that may lead to integration of foreign gene within the existing nuclear genes causing disruption of those genes in the cells. Integration into the host cell genome has resulted in insertional mutagenesis and oncogenic transformation in clinical trials for X-linked severe combined immunodeficiency disease.

CONCLUSION

Gene therapy is one of the recent gene based technologies that is obtaining progressive advance. Although several protocols have been successful, the gene therapy process remains complex, and many techniques need new developments. The specific body cells that need treatment should be identified and accessible. A way to effectively distribute the gene copies to the cells must be available, and the diseases and their strict genetic bonds need to be completely understood. Gene therapy has been named the medicine of the future. Besides it considered as it would revolutionize agriculture by applying effective and safe methods for animal production through using effective and efficient disease treatment and prevention methods in animals and applying hormones that enhance growth and carcass quality of animal.

RECOMMENDATIONS

Based on above conclusion the following recommendations are forwarded:

- It is important for government to adopt and develop this technology to country as it is essential in treatment and production improvement of animal.
- There should be essential environmental considerations up on production of vector especially viral vectors.
- There should be well established gene therapy research institute to further advancement of their usage.

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