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GENE THERAPY FOR CANCER

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1. ABSTRACT

Cancer treatment has been the major goal of the gene therapy studies over the decades. Although there is no cancer gene therapy drug in the market yet, substantial progress has been made in defining potential targets and in developing viral and nonviral gene delivery systems recently. Numerous genes have been studied as the targets for cancer gene therapy so far. Various gene therapy strategies, including suicide gene therapy, oncolytic viral therapies, antiangiogenesis, and gene therapy vaccines have been developed. The combination of gene therapy with conventional methods, such as chemotherapy, radiotherapy, and immunotherapy, has further improved the therapeutic efficacy. Although the preclinical and experimental studies have yielded highly encouraging results, there are still few gene therapy agents at phase III trials. In the current chapter, we will review gene transfer systems, targets, gene targeting strategies, and cancer gene therapy in the clinic Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Safe methods have been devised to do this, using several viral and non-viral vectors. Two main approaches emerged: in vivo modification and ex vivo modification. Retrovirus, adenovirus, adenoassociated virus are suitable for gene therapeutic approaches which are based on permanent expression of the therapeutic gene. Non-viral vectors are far less efficient than viral vectors, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. To improve the function of non-viral vectors, the addition of viral functions such as receptor mediated uptake and nuclear translocation of DNA may finally lead to the development of an artificial virus. Gene transfer protocols have been approved for human use in inherited

diseases, cancers and acquired disorders. Although the available vector systems are able to deliver genes in vivo into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary.

2. INTRODUCTION

The improvements in the past 20 years in the molecular biology have evoked optimism in the treatment of cancer and yielded a number of targeted drugs in the market. However, the curative treatment of the cancer has still been possible with only the early diagnosis and early intervention in the vast majority of the solid tumors. Almost half of the cancer patients diagnosed each year have been dying of the disease throughout the world. In particular, the patients with distant metastasis have no hope of cure with the current treatment modalities. Cancer has been, from the beginning, a target of intense research for gene therapy approaches. Currently, more than 60% of all on-going clinical gene therapy trials worldwide are targeting cancer. Indeed, there is a clear unmet medical need for novel therapies. This is further urged by the fact that current conventional cancer therapies are frequently troubled by their toxicities.

Different gene therapy strategies have been employed for cancer

- Pro- drug activating suicide gene therapy
- Anti-angiogenic gene therapy, oncolytic virotherapy
- Gene therapy-based immune modulation
- Correction/compensation of gene defects, genetic manipulation of apoptotic and tumor invasion pathways
- Antisense
- RNAi strategies

Definition of cancer

A term for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems.

Cancer types, which have been targeted with gene therapy

- Brain
- lung

- breast
- pancreatic
- liver
- colorectal
- prostate
- bladder
- head and neck
- skin
- ovarian
- renal cancer

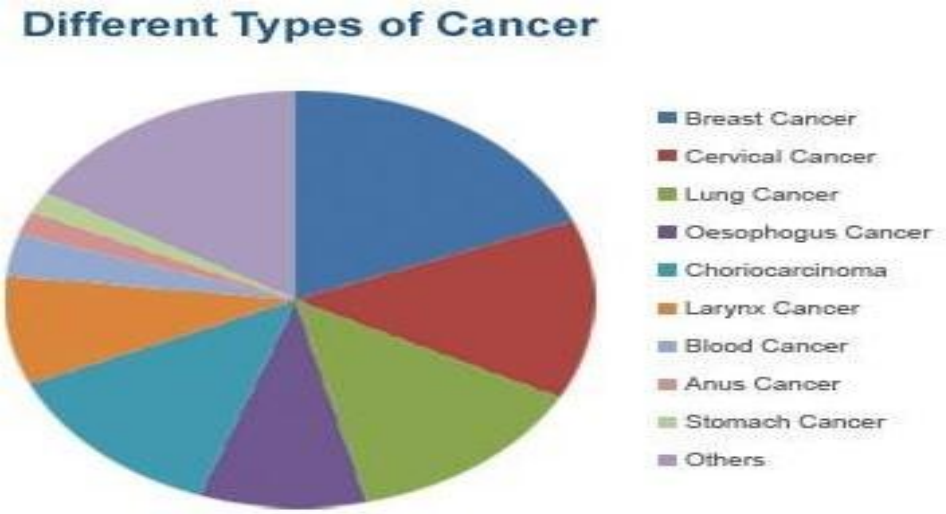


Fig: Distribution of common cancer types.

1. Cancer is a complex genetic disease

It has long been suggested that the cancer has evolved from a single cell transformed by the influence of the environmental factors such as physical, chemical factors, and viruses. Changes in hundreds of genes, so- called mutations, are required to transform a normal cell into a cancer cell. The major functional changes that transform a cell are mainly the activation of oncogenes or inactivation of tumor suppressor genes.

The overexpression of oncogenes and loss of function of tumor suppressor genes usually induce malignant transformation. Those changes are also required for further growth of tumor cells.

A transformed cell usually gains some important biological properties to establish a malignant disease. Those properties, including uncontrolled proliferation, evasion of growth suppressors, inhibition of apoptosis, replicative immortality, angiogenesis, proliferative signals, invasion, and metastasis, are discussed in detail in a recent review of Hanahan and Weinberg. Although the conventional chemotherapy has mainly focused on direct tumor cell killing, a vast majority of current targeted therapies have aimed to eliminate one or more of the above-mentioned properties of cancer cells.

The targeting of angiogenesis, proliferation pathways, and immune system has yielded a number of drugs that are already in the market. Nodules of cancer cells cannot grow beyond 1–2 mm without expanding their blood supply to access every increasing need for oxygen and nutrients. In order to generate the additional blood supply, the tumor tissue stimulates the elaboration of its own vessel network, through a process called angiogenesis. If one could cut the blood supply of the tumor, it cannot grow beyond 1–2 mm, which means that they cannot grow enough to be diagnosed by the current diagnostic technology and cannot cause a clinical disease. The tumor vascular targeting therapy or antiangiogenetic therapies like bevacizumab and aflibercept targeting ligands of angiogenesis or small tyrosine kinase inhibitors of angiogenesis pathway receptors or signaling molecules have already emerged as standard therapeutic drugs in various tumors.

The overexpression of oncogenes and the loss of function of tumor suppressor genes are usually involved in both malignant conversion of the cells and further growth of tumor cells. A new generation of small molecules targeting proliferation pathways, like gefitinib, erlotinib, and imatinib, has been developed to block the cancer-causing signals within cancer cells and become standard treatments in those patients with mutations of EGFR or c-KIT. Antibody molecules, targeting the EGFR family of receptors like trastuzumab, cetuximab, and panitumumab also block the growth-promoting signals that push cancer cells into an unregulated pattern of growth. In contrast to standard chemotherapy, which is quite damaging to the normal tissues of the body as well as the cancer tissue, the targeted drugs are quite specific for the cancer cells and therefore relatively free of side effects.

Although majority of the cancer patients has a fairly intact immune system, the cells of the immune system do not usually respond to tumor cells because the immune system cannot differentiate the normal and cancer cells and therefore cannot fight against them. Immunotherapy or cancer vaccine therapy aims to activate immune system against tumors.

Recently, ipilumumab/tremelimumab and pembrolizumab/nivolumumab targeting checkpoints of immune response such as CTLA-4 or PD1 have also been approved. Likewise, a dendritic cell-based vaccine, sipuleucel T, for the treatment of metastatic prostate cancer has been approved 2 years ago.

2. Gene therapy history

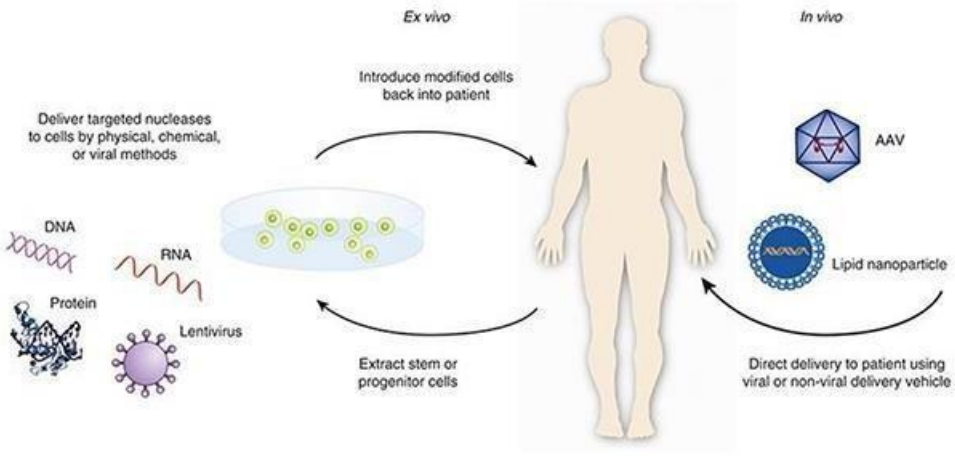
The first clinical study using gene transfer was reported. Rosenberg and his colleagues used a retroviral vector to transfer the neomycin resistance marker gene into tumor-infiltrating lymphocytes obtained from five patients with metastatic melanoma. These lymphocytes then were expanded in vitro and later re-infused into the respective patients. Since this first study showed that retroviral gene transfer was safe and practical, it led to many other studies. Indeed, since 1989, more than 900 clinical trials have been approved worldwide. What made gene therapy possible between 1963 and 1990 was the development of recombinant DNA technology. In 2003 as well as in November 2005 China approved the first gene therapy drugs for the treatment of certain malignanttumors.

A first European application for the approval of a gene therapy drug for the treatment of an aggressive brain tumor was submitted to the European Agency for the Evaluation of Medicinal Products (EMEA) in 2005. Despite continued great difficulties in the technical implementation, the successes of gene therapy can doubtlessly be confirmed today. For example, successful therapies have been developed during the past five years for patients with severe hereditary immunodeficiency diseases. These treatments are visibly beneficial to these patients with life-threatening conditions. The death of a patient in the USA in 1999 as a result of a very high systemically administered dose of adeno

3. What is gene therapy?

Gene therapy can be defined as the delivery of genetic elements to the cancer cell or to the cells of the immune response in order to correct the abnormalities in the cancer tissue or to induce an immune response against the cancer cells. The corrective strategies can involve replacing missing or defective genes, i.e., tumor suppressor genes, suppressing the action of cancer promoting oncogenes , or programming normal or cancer cells to release into the systemic circulation molecules which suppress the growth of cancer cells or their vasculature There are some prerequisites for a successful gene therapy program in cancer, such as a suitable target to be replaced or modified, a carrier to reach the interest of gene to the cell, a successful targeting of the vector, and a sufficient expression of the therapeutic genes in the

target cells. Besides a strong therapeutic efficacy, safety isalso mandatory for the success of the treatment.



Unraveling the mystery of the genetic changes in the development of cancer has been proposed many genes as targets for gene therapy studies. The second step in gene therapy following the identification of a suitable gene is to introduce it into the target cell. Different vehicles (vectors) have been used to introduce the genes into the cells, such as viral vectors, nonviral vectors, and cell-based carriers. The mainly used viral vectors in cancer gene therapy are retroviruses, adenoviruses, and adeno-associated viruses. The gene therapist uses the capability of the virus to enter and reprogram the action of cells for purposes of therapy.

The therapeutic genetic element is first placed into a viral backbone to produce a complete therapeutic viral vector. Alternatively, the therapeutic genetic elements can be delivered into the cancer cells through droplets of fat called liposomes or nanoparticles. The genes themselves, in the form of naked DNA or DNA packed into particles can be administered locally or systemically.

A third way of delivering genes to the target tissues is accomplished byusing living cells such as irradiated tumor cells, blood cells, and mesenchymal or neuronal stem cells. All of these cells have the capability to home to particular types of target tissue through the blood stream. In this way, the therapeutic genes can be placed into the brain or other target tissues because of the homing properties of thosecells.

For the safety of the procedure and the increased therapeutic efficacy, the genes of interest should be expressed in only target cells or tissues. Sparing of the normal cells and tissues is one of the keystones in their clinical use. The target specificity of the vectors could be

achieved by the targeting of those specific to the tumor cells or tissues.

4. Gene transfer systems of cancer gene therapy

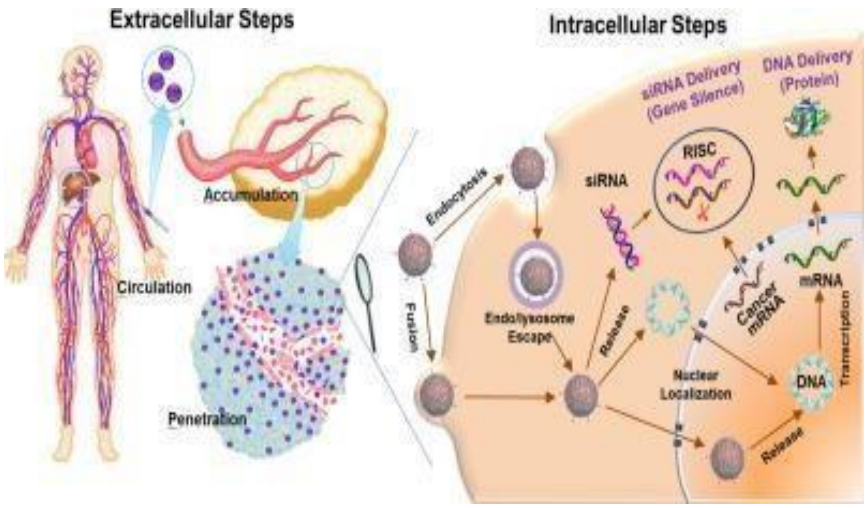
There are three main ways of transferring genes into the tumor cells

- Nonviral vectors
- Viral vectors
- Cell-based vehicles

The nonviral gene delivery vectors have usually been injected locally to the tumors. Although local injection is reasonable for tumors as melanoma, head and neck cancers, or peritoneal carcinomatosis; it is not suitable in patients with hematogenous metastases. The limitations of the viral vectors are also valid for the nonviral vectors for gene therapy. They have to survive through the blood stream to be arrested in the target tumor tissue, to extravasate, and to bind to specific cells and to enter the cells and then to reach the nucleus.

A. Nonviral vectors

Plasmid DNA, which is mostly used as nonviral gene therapy modality, is easily degraded by nucleases. Therefore, some strategies to reduce the size and prevent the degradation have been developed. The most commonly used agents for gene delivery are cationic lipids. The cationic head group of the lipids binds to DNA and the lipid tail enables the collapse of the DNA lipid complex. Cationic lipid DNA complexes (lipoplexes) (LPD/DNA) enter the target cell through an endosomal pathway. However, the transgene expression efficiency is very low with lipoplexes. It has been shown that only a very small portion of the systemically injected DNA could be reached to tumor tissue.



Lipid-based formulations of gene delivery have been predominantly limited to the intratumoral or local applications. The systemic administration carries the potential risk of adverse inflammatory and immune reactions. The development of systemic lipid delivery systems with the modifications to reduce the systemic toxicity could have the potential for clinical use in cancer gene therapy. In an animal model of breast cancer, folate-targeted lipid–protamine DNA complexes (LPD- PEG-folate) have been shown to reduce the tumor volume and increase the survival when administered systemically.

Neutral liposomes composed of DOPC (1,2-dioleyl-sn-phosphatidyl choline) and DOPE (1,2-dioleyl-sn-phosphatidyl ethanol amine) and polycationic carrier proteins as protamine, polylysine, polyarginine, polyhistidine, or polyethynilemine (PEI) are also suitable to carry the DNA. The hydrophobic polymers, such as polyethylene glycol (PEG), polyhydroxy propylmethacrylamide (pHPMA), and polyvinyl pyrrolidine (pVPyrr), have also been used to mask the positive charge of DNA to extend its half-life in the blood. Both the neutral liposomes and hydrophobic polymers yield less toxicity when administered systemically. The leaky nature of the blood vessels of the tumors allows the influx of macromolecules as polymer shielded DNA into the tumor. The PEGylation of plasmid DNA has been reported to circulate in the blood several hours and passively accumulate in the subcutaneous tumors in animals.

B. Viral vectors

Viruses have the natural ability to deliver the nucleic acids within its own genome to specific cell types, including cancer cells. This ability makes those attractive and popular gene-delivery vehicles. Retroviruses, adenoviruses, adeno- associated viruses, herpes simplex virus, poxviruses, and baculoviruses are commonly modified and used as gene therapy vectors in cancer. Additionally, chimeric viral-vector systems combining the properties of two or more virus type are also develop.

Retroviral vectors derived from retroviruses contain a linear single-stranded RNA of around 7–10 kb and have a lipid envelope. The viral particles enter the mammalian cells expressing appropriate receptors for retroviruses. After entering the cell, the viral reverse transcriptase transcribes the virus RNA into double- stranded DNA (dsDNS). The dsDNA transcribed in the cytoplasm forms a nucleoprotein preintegration complex (PIC) by binding cellular proteins. The PIC migrates to the nucleus and thereby integrates the host genome. The ability of transgene expression in only dividing cells is an advantage of retroviral vectors for cancer gene therapy to avoid undesired expression in nondividing cells of surrounding tissues.

The incorporation of retroviral genes into the host genome provides long-term expression of transgenes. Although this is advantageous, a nonspecific incorporation of viral DNA could impair the function of host gene or induce aberrant expression of a cellular oncogene. Although retroviral vectors have been the most widely used gene transfer vehicles in the clinic, the risk of insertional oncogenesis seen in the trial of X-SCID infants in 2003 has limited the use of retroviral gene transfer systems in humans. The possibility of generating replication-competent retroviruses is another safety issue regarding the clinical use of those vectors.

Lentiviral vectors derived from retroviruses can cause stable integration of the transgene into the host genome with long-term gene expression. The ability of transducing both dividing and nondividing cells make those vectors more suitable and efficient gene transfer vehicle over retroviruses. Targeting strategies of vectors at the level of cell entry and transgene transcription improved the use of lentiviral vectors in gene therapy trials. However, the biosafety concerns of random integration to the host genome as in retroviruses are the limitations of those vector.

Adenoviral vectors are widely used to introduce the therapeutic genes into the tumor cells. They can infect a broad range of cell types, transfer the genes being not dependent on cell division, and have high titers and high level of gene expression. The most widely used serotypes of adenoviruses to develop vectors in human cancer gene therapy studies are type 5 (Ad5) and type 2 (Ad2). They have the capacity of approximately 8–10 kb of therapeutic genes with first-generation vectors and up to 36 kbp with gutless third generation adenoviral vectors. However, along with the immunogenic potential, the broad range of host cells by adenovirus limits its systemic use in human cancer gene therapy trials. Targeting strategies have enabled the use of adenoviral vectors in human gene therapy trials. Adenoviral vectors cannot integrate to cellular genome and express the transgene episomally. They cannot induce random mutations.

However, the transgene expression is limited to 7–10 days postinfection. Therefore, repeated administrations of the vector are needed to achieve sustainable responses in cancer treatment. Adenoviruses could be engineered either as replication deficient by deleting the immediate early genes of E1 or replication- competent keeping the E1 region. Replication-competent adenoviral vectors will be further discussed in the section of oncolytic viruses.

Adeno-associated viruses (AAV) are simple viruses with approximately single-stranded DNA of 4.7 kb in size. They belong to parvovirus family and require a helper virus such as adenovirus or herpes virus for lytic replication and release from the cell . They can infect a wide variety of cells independent of cell cycle. This property makes AAV as suitable vectors for cancer gene therapy. Furthermore, unlike adenoviruses, they elicit little immune response when infect the normal host cells. Another advantage of AAV over adenoviruses is their ability to integrate the transgene into a particular spot on the 19th chromosome of human cells. Unlike retroviruses, AAV cannot induce mutations.

5. Cells as the carriers of cancer gene therapy vectors

The systemic administration of the gene therapy vectors usually failed because of low titer achieved in the target tissue and insufficient transgene expression. The clearance of the vector by the immune system, sequestration, and nonspecific binding to nontarget tissues are the major drawbacks of viral and nonviral vectors. In general, in vivo targeting has relied mainly upon the enhanced leakiness of the tumor vessels, allowing the extravasation and access to tumor cells. Besides, the target tropism, extravasations in tumor site, and poor penetration of the vectors into the tumor tissue are the major problems for the vectors to eradicate the metastatic tumor deposits.

Cell carriers have the potential of eliminating those problems. They are stable and most of them have tumor homing properties and can be administered locally, such as intraperitoneal or intratumoral injections or systemically. In case of the use of autologous cells, they will not be cleared by the immune system. Macrophages, bone marrow mesenchymal stem cells (MSC), T cells, NK cells, and eosinophils are the known cells infiltrating the tumor tissues. Also, the tumor cells themselves naturally have the potential of homing to the tumor deposits throughout the body.

Macrophages have been used to deliver therapeutic genes because of their naturally trafficking ability to sites of neoplastic diseases. Further refinement of the targeting of these cells by using transcriptional promoters could avoid the transgene expression in other parts of the body where the macrophages naturally traveled.

T cells can be used to transfer the therapeutic genes to target tissues because of their ability to circulate through the body and arrest in tumor tissues. T cells have the advantage of the release of vectors that they carry in an antigen-binding-specific manner. The T cells could also

provide further antitumoral activity by their cytotoxic effects. Tumor infiltrating lymphocytes (TIL) are the first example of cell-based carriers in cancer therapy in which they were transfected with cytokine genes.

Mesenchymal progenitor cells from either bone marrow (MSC) or adipose tissue (PLA) have the potential to expand in culture and the differentiation along the adipogenic, osteogenic, chondrogenic, and myogenic lineages. It has been shown that lentivirally transfected mesenchymal progenitors from the adipose tissue have sustained transgene expression, even after the differentiation into adipogenic and osteogenic lineages. Further modifications of PLA cells transfected ex vivo in order to target tumor tissues of their natural potential differentiation would provide an efficient gene delivery vehicle.

Some other cells such as fibroblasts and allogeneic cells have also been used as cell carriers for gene therapy vectors. Because of their homing properties to the tumor cell deposits, tumor cells could be good candidates to target the established metastases. An animal model of MDA-MB-231 cells, transduced ex vivo by a CD carrying Ad vector, has been shown to reduce the tumor volumes in the established metastases of the tumor.

6. Gene targeting in cancer gene therapy

In order to maximize the therapeutic index of cancer gene therapy, the expression of therapeutic genes could be restricted to the target tissues. Therefore, the targeting of gene therapy vectors is the major key for the success of those treatments.

There are two main targeting strategies

- Physical targeting
- Biological targeting.

A. Physical targeting

The first one is physical targeting by means of some physical methods such as local injections, catheters, gene guns, and electroporation. This strategy is usually used for local delivery of gene therapy vectors and is therefore not suitable for most of the cancer patients who may have cancer spread throughout the body. Supercoiled DNA molecules and oligonucleotides are also successfully delivered to the cells of the skin following intradermal injection to the tumor deposits accessible by local injections.

However, intratumoral injection might have only the transducing capacity of the cells neighboring the needle. The tumor deposits in the body cavities such as peritoneum, pleura, and meninges and in subcutaneous tissues are the potential targets for the physical targeting of the gene therapy vectors in the clinic.

B. Biological targeting

In a second strategy, the viral or nonviral carriers of the genes are modified in such a way that they can only bind to tumor cells but not the normal cells. Because of the low transduction efficiency of the currently used gene therapy vectors in distant tissues when administered systemically, the specific transgene expression or viral replication in target tissues could provide an opportunity to achieve sufficient antitumor activity. To achieve this goal, transcriptionally and transductionally targeted vectors have been developed. For safety reasons, mostly the replication defective vectors have been used to transfer the therapeutic genes into tumor cells. However, because of limitations of vector delivery and relatively low levels of gene transfer capacity, replication-deficient vector systems are usually inefficient for the treatment of large solid tumors.

Therefore, replicating vectors could efficiently transfer genes and also increase the therapeutic efficiency by means of its oncolytic effect. Such vectors could be targeted in such a way that they can replicate within the tumor cells but not in normal cells and cause no local or systemic toxicity.

7. Cancer gene therapy in the clinic—Future prospects

The vast majority of the clinical trials of gene therapy have been devoted to the treatment of cancer so far. The gene therapy agents have been tested in many types of cancer in the clinic. Almost 1200 clinical trials (approximately 64% of all gene therapy trials) in cancer have been started, conducted, or completed. Less than 4% of those are phase II or III and only few of them are phase IV trials. Although the preclinical and experimental studies have yielded highly encouraging results, the progress in the clinic is not so remarkable. There is no gene therapy agent available in the market yet.

The most important factor that has limited the success of clinical gene therapy trials in human subjects is the delivery of the vector genetic elements or their products to the target cancer cells and their vasculature. A second problem has been toxicity. Recent advances on improving the delivery and specificity of gene therapy vectors have suggested these trials

may be more successful in the coming years. This is especially true of the attempts to use vectors to activate the immune response against the tumor tissue. Continued testing of these strategies in the context of clinical trials may lead to new opportunities for individuals engaged in a personal struggle with cancer to control their disease.

8. Risks

Gene therapy has some potential risks. A gene can't easily be inserted directly into your cells. Rather, it usually has to be delivered using a carrier, called a vector.

The most common gene therapy vectors are viruses because they can recognize certain cells and carry genetic material into the cells' genes. Researchers remove the original disease-causing genes from the viruses, replacing them with the genes needed to stop disease.

This technique presents the following risks

Unwanted immune system reaction. Your body's immune system may see the newly introduced viruses as intruders and attack them. This may cause inflammation and, in severe cases, organ failure.

Targeting the wrong cells. Because viruses can affect more than one type of cells, it's possible that the altered viruses may infect additional cells — not just the targeted cells containing mutated genes. If this happens, healthy cells may be damaged, causing other illness or diseases, such as cancer.

Infection caused by the virus. It's possible that once introduced into the body, the viruses may recover their original ability to cause disease.

Possibility of causing a tumor. If the new genes get inserted in the wrong spot in your DNA, there is a chance that the insertion might lead to tumor formation.

9. Safety of gene therapy

Despite the tragic case of Jesse Gelsinger, who died as a result of gene therapy using adenoviral vectors, the safety data collected from different human gene therapy trials have been uniformly satisfactory. However, it should be pointed out that viral vectors used in gene therapy are typically human pathogens, and hence, pre-existing antibodies against the viral vector may be present, which might result in an unwanted immune response. For example, an injection of adenoviral vectors will result in an initial non-specific immune response in the

host, i.e., release of a variety of cytokines followed by a specific antibody and cell- mediated immune response directed against transduced cells.

However, the response towards adenoviruses is serotype dependent. For example, a study by Thoma et al. demonstrated that the immediate cytokine response of macrophages following adenovirus stimulation differs between adenovirus serotypes, hence, is serotype-specific. Particularly, in a long-term study, wherein either adenovirus of the serotype 11 (Ad11) or 5 (Ad5) was administered intra peritoneally, Ad11 caused no/mild and Ad5 moderate/severe toxicity. Biomedicines 2014, Generally, there is still not much long-term safety data using viral vectors in humans. Nevertheless, several meta-analysis already exist for adenoviruses demonstrating an adequate safety profile in human.

The tolerability towards adenoviral vectors has been acceptable and the side effects have mostly been mild without any serious adverse events related to gene therapy. Different means with the intention of improving the safety of gene therapy have been implemented. One approach is to develop targeting strategies in order to enhance the delivery of gene transfer vectors, and hence, to improve the duration and efficacy of gene expression. Generally, one of the major shortcomings with gene therapy is their lack of specificity to their target cells and their low transduction efficiency. Improving specificity and/or transduction efficacy ultimately would result also in a better safety profile.

Consequently, the improvement of transduction efficacy of gene transfer vectors has come along with the development of vector technologies, including re- engineering of viral vectors using epitope insertion, chemical modification, and molecular evolution. An example for this was demonstrated in a phase I clinical trial by Kim et al., where they modified the RGD fiber knob on adenoviruses, thereby enhancing viral infectivity of cancer cells. The role of innate immunity, as well as the activation of T and B cells in response to the vector and its transgene product is a topic of intense research.

Particularly, the possible effects of gene transfer vectors and/or their expressed proteins on local lymph nodes are topics that require further evaluation. The pre- existence of neutralizing antibodies (e.g., against several adenovirus serotypes or AAVs) has been acknowledged already for quite some time and it is known that these pre-existing neutralizing antibodies can substantially diminish transduction efficiency. In order to improve specificity, as well as transduction efficiency, viral surface proteins have been modified, removed or

replaced. For example, lentiviral vectors have been generated, wherein a cell type specific ligand or antibody has been fused to the viral envelope (i.e., pseudotyping).

The downside of this has been that different modifications resulted in low vector titers during lentivirus production. Furthermore, it has been shown that targeting may also potentially compromise the entry of the vector into the cell. On the contrary to targeting viral vectors to specific cells, pseudotyping can also be used to broaden tropism of the viral vector to other cells. For example, retroviruses and lentiviruses are frequently pseudotyped with the Vesicular Stomatitis virus G- protein (VSV-G) to widen their tropism and to increase their yield in production. Another approach to increase specificity of viral vectors to their target cells is the use of tissue-specific or conditional promoters.

An example for conditional dependent gene expression is the use of hypoxia- specific regulatory systems, where gene expression is aimed to be induced and restricted to ischemic tissues. Commonly, these hypoxia-specific regulatory systems have been applied to various ischemic disease models, including ischemic myocardium, stroke, and injured spinal cord, but could also be used in cancer gene therapy. Gene expression can also be regulated based on a genotypic feature (e.g., a mutated TP53 gene in cancer cells), which has been discussed already above in case of Oncorine™.

10. Problems with gene therapy

The most frequent side effects following gene therapy include transient fever and flu-like symptoms. A grade-3 hypersensitivity reaction following intravenous administration is usually transient and managed with the usual supportive measures. Leukocytopenia, and in particular, lymphopenia, may represent cellular redistribution of white blood cells to target tissue such as tumors. Mild transient anemia has also been reported. However, toxicity, mutagenicity and immunogenicity associated with viral vector therapy have raised great concern. Retroviral (such as lentiviruses) mediated gene therapy leads to viral integration into host genome, thus, it may cause mutagenic events with possible second malignancies. This was reported in earlier studies on the murine leukemia retrovirus vector in the treatment of patients with severe combined immunodeficiency and five out of 30 cases developed leukemia, though, no second malignancy has been reported so far, in gene therapy for cancer.

Such mutagenicity depends on the site of viral insertion. For this reason, the FDA has required all clinical trials involving genomic integrated viral vectors to report and analyzes

viral vector insertion sites. Initial methodology was linear amplification mediated polymerase chain reaction, but lately, high-throughput DNA sequencing methods have been used. Clinical trials that initially or subsequently show evidence of higher mutagenicity are usually discontinued. Information obtained from such studies is of major significance in designing new and much safer therapeutic approaches. Another major problem with gene therapy for cancer is the resistance to treatment with subsequent tumorrecurrences and shorter survival.

A potential mechanism is intrinsic, and possibly acquired, tumor cell resistance to therapy-induced cell death (apoptosis) by dysregulation and release of anti- apoptotic inhibitor of apoptosis protein or Bcl-2 proteins. Recently, some pharmaceutical companies have developed several medications such as Novartis- LBH589, cIAP1, and cIAP2 which inhibit the Bcl-2 protein, thus promoting cell death (apoptosis) and tumor regression, prevent or delay tumor resistance, and prolong remission following gene therapy. These medications are presently in clinical trials.

11. REVIEW, CONCLUSIONS

Gene therapy for cancer has evolved relatively fast in the last two decades, and presently, few drugs are commercially available while others are still in clinical trials. Most reports on gene therapy have shown good safety profiles with transient tolerable toxicities. The lack of success in several clinical trials may partly be attributed to patient selection. Similar to initial chemotherapy outcomes thirty years ago, patients with advanced and therapy-resistant malignancies are presently enrolled in gene therapy trials. Perhaps, gene therapy maybe much more successful in patients with earlier stages of malignancies, or in those who have a lower tumor burden.

Alternatively, gene therapy may better be used after successful cancer therapy with maximum tumor load reduction, such as after radical surgery, following radiation therapy, or after successful chemotherapy. In the future, the wide use of patient and tumor genomic analysis as well as the assessment of host humoral and cellular immunity, will facilitate a better selection of the most appropriate gene therapy per patient. Recent progress in developing safe and effective vectors for gene transfer, such as with synthetic viruses and non-viral methods, as well as the success in using autologous and allogenic chimeric antigen receptor integrated T-lymphocytes, even from healthy individuals, as universal effector cells in mediating adoptive immunotherapy, will increase the effectiveness and safety profile of gene therapy.

Furthermore, with the advancement in biological research, much cheaper gene vectors will become commercially available, which will make gene therapy readily available to the majority of cancer patients, worldwide. This will transform the future of cancer therapy, from generalized cancer treatment strategies, based on tumor size, nature and location, to a more tailored, individualized cancer therapy, based on the patient's specific genomic constituents, host immune status, and genetic profile of the underlying malignancy. Treatment is expected to be fast, effective, relatively less toxic and inexpensive, with higher cure rates, and may even, cancer prevent.

12. RESULT

1. The gene therapy is effective for researchers introduce to foreign gene directly into cancer cells or into surrounding tissue.
2. The main goal for gene therapy is that the newly inserted gene will cause the cancer cells to die or prevent cancer cells and surrounding tissue from funneling blood to tumors, depriving them of nutrients they need for survival.
3. The gene therapy is also a way of treating or preventing disease by altering the genetic instructions within an individual's cells.
4. The gene therapy can involve replacing abnormal or absent genes with healthy ones that used to enable cells to produce useful proteins.

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I often wonder, if one gets to see god in the moral life, they might be like parents who shower their best fortunes always on me from the deepest depth of my heart to express my thanks.

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