Translational Approaches towards Cancer Gene Therapy: Hurdles and Hopes
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ABSTRACT

Introduction: Of the cancer gene therapy approaches, gene silencing, suicide/apoptosis inducing gene therapy, immunogene therapy and targeted gene therapy are deemed to substantially control the biological consequences of genomic changes in cancerous cells. Thus, a large number of clinical trials have been conducted against various malignancies. In this review, we will discuss recent translational progresses of gene and cell therapy of cancer. Methods: Essential information on gene therapy of cancer were reviewed and discussed towards their clinical translations. Results: Gene transfer has been rigorously studied in vitro and in vivo, in which some of these gene therapy endeavours have been carried on towards translational investigations and clinical applications. About 65% of gene therapy trials are related to cancer therapy. Some of these trials have been combined with cell therapy to produce personalized medicines such as Sipuleucel-T (Provenge®, marketed by Dendreon, USA) for the treatment of asymptomatic/minimally symptomatic metastatic hormone-refractory prostate cancer. Conclusion: Translational approach links two diverse boundaries of basic and clinical researches. For successful translation of genommedicines into clinical applications, it is essential 1) to have the guidelines and standard operating procedures for development and application of the genommedicines specific to clinically relevant biomarker(s); 2) to conduct necessary animal experimental studies to show the “proof of concept” for the proposed genommedicines; 3) to perform an initial clinical investigation; and 4) to initiate extensive clinical trials to address all necessary requirements. In short, translational researches need to be refined to accelerate the genommedicine development and clinical applications.

Introduction
While conventional cancer therapies (chemotherapy alone or in combination with immunotherapy and ionizing radiation modalities) are used as the approved modalities, undesired side effects within the normal cells cause many difficulties. Within the tumor microenvironment, for example, the acidic extracellular milieu can alter the uptake pattern of chemotherapeutic drugs, and interfere with the immune system activity. A complex framework of cellular metabolic and transport machineries underlie the pH homeostasis in mammalian cells and the tumor cells exploit such bio-machineries, dysregulating several transporters such as vacuolar-type (V-type) H(+) -ATPases, monocarboxylic transporters (MCT1 and MCT4), carbonic anhydrase (CA IX) and enzymes (e.g., indoleamine 2,3-dioxygenase). Hence, the genes involved in tumor alkalinization may represent a key target of future antitumor strategies (De Milito and Fais, 2005, Pinheiro et al., 2011).

Furthermore, aberrant cells overexpress various cell surface markers. The tumor-associated markers (TAMs) or tumor-specific markers (TSMs) can be capitalized for targeted gene therapy of cancer. It seems we need powerful modalities to impose specific impacts at the early stage of cancer development through combination of several advanced modalities such as gene based nanomedicine targeting various bioelements of cancer cells. Efficient gene transfer is a pivotal step, which continues to be one of the major barriers for successful gene therapy. In fact, there exist some hurdles that make gene therapy a formidable task. There are problems with delivery of sufficient copies of a gene (e.g., siRNA or antisense) to all tumor cells. The biology of malignancies is very complex and potentially all related genes must be covered. Another barrier is the lack of proper gene delivery system (GDS) and non-specificity of GDSs, which makes gene therapy strategy very uncertain.
Conceivably, direct administration of supercoiled DNA into tissues is considered as the simplest approach for in vivo gene transfer of plasmid vectors into cells. Early studies have shown that DNA can be directly transferred into the target cells in vivo through simple injection of the desired gene-based medicine into the target organs using virus DNA.

On the basis of such assumption, when polyoma virus or ground squirrel hepatitis viruses DNA were directly injected, the animals developed the related systemic infection, in which active virus particles were detected. This can be considered as viral based gene delivery, while direct injection of naked plasmid DNA was shown to yield significant levels of gene expression in rat skeletal and cardiac muscle, where gene expression in transfected cells appeared to be sufficient to produce antiviral immunity. Further, direct injection of plasmid DNA can provide much higher gene delivery efficient to subcutaneous tissues, developing greater activity of immune system. The efficiency of intradermal gene transfer can be improved using iontophoretic technique that can be literally used for the transdermal delivery of both ionic and nonionic medications. Alternatively, for delivery of plasmid constructs into human cells in vivo, gene gun can be used to deliver the DNA vector coated nanoparticles directly into tissues. This method may be successfully used for cancer vaccines; nevertheless its efficiency is limited to subcutaneous tissues. Overall, the current gene therapy approaches are capable of introducing genes into cells in vivo without discrimination within target and non-target cells. However, such unselective approach can impact both normal and aberrant cells. Thus, incorporation of a homing device (e.g., monoclonal antibodies (mAb), antibody (Ab) fragments, or target specific aptamers) with an appropriate delivery nanosystem encapsulating gene-based medicine may result in cell-specific targeting and greater clinical outcomes. Nevertheless, production and translation of such advanced targeted nanogenomedicine need integration and harmonization of several scientific dominions. For example, as shown in Fig. 1, for brain tumor gene therapies need to remain stable in blood circulation and efficiently circumvent blood-brain barrier (Omidi and Barar, 2012). The central aim of the current review is to provide necessary information upon the specific gene therapy strategies and gene targets. We will discuss impacts of oncogenes, tumor suppressor genes and apoptosis-inducing genes on cancer gene therapy strategies as well as methods that specifically reactivates pathways that render the mutated cells susceptible to antitumor agents and immunotherapy. Further, targeted nanogenomedicine therapy of cancer will be stated.

The arc of gene therapy

To date, more than 65% of the gene therapy trials have been devoted to the cancer diseases using various vectors (retrovirus (20%), adenovirus (18%), adeno-associated virusade (5%), lipofection (6%)) and naked/plasmid DNA (18.5%). Despite conducting more than 1186 cancer gene therapy trials (out of 1843), 45 have reached to phase III and only 1 is in phase IV; reader is referred to see (JGM-ClinicalTrials, 2012). At the moment, only 9 gene therapy clinical trials have been conditionally approved to be used in adjuvant therapies (Table 1).
The main basis of gene therapy is to fix the genomic defects. It seems the gene therapy along with cell therapy modalities have the potential to revolutionize treatment concepts. Genomic and epigenomic alterations can be literally targeted by smart genomedicines. The concepts. Genomic and epigenomic alterations can be exploited for specific targeted gene therapy (Weber, 2008).

Of gene therapy approaches, greater attentions have been given to some dominions implementing suppressor/suicide genes, apoptosis inducing genes, growth control genes, chemoresistance inducing genes and immuno-oncogenes. The immune-based gene therapies such as DNA vaccine harness the immune system potential (dendritic cells) to stimulate the immune system activities through mechanisms against cancer cells. DNA vaccines possess intrinsic ability to activate multiple pathways of innate immunity, that also provide a unique opportunity to guide defined antigens, accompanied by specific activator molecules, through a patient’s compromised immune system (Stevenson et al., 2010). The suicide gene therapy shuttle designated genes into the target cancer cells, in which the cancer cells have the capability to convert the nontoxic prodrugs into the active chemotherapeutics. This approach is a target gene therapy modality on the basis that the cancerous cells containing suicide genes are solely targeted through a systemic administration of prodrug. This approach is deemed to provide the maximal inhibition in the target cancer cells with trivial toxicity in normal cells (Vassaux and Martin-Duque, 2004).

Gene silencing
Gene suppression has been performed by gene based pharmaceuticals such as antisense RNA, siRNA, ribozymes, DNAzyme and aptamers, and their combination with other cancer therapy modalities including chemotherapy and immunotherapy can open other avenues for cancer therapy (Liu et al., 2010, Candolfi et al., 2009, Rachakatla et al., 2008).

Antisense and RNA interference
Antisense oligodeoxynucleotides (AS-ODNs) are used to suppress the expression of undesired genes such as VEGF, Ang-1, murine double minute 2 (MDM2), protein kinase C, c-Myb, integrin subunit b3, PKA-I, H-RAS, Bcl-2, c-RAF, R1/R2 subunits of ribonucleotidereductase (Wacheck and Zangemeister-Wittke, 2006). In contrast to AS-ODNs technology, the mechanism of silencing an endogenous gene through a homologous double-stranded RNA (dsRNA), which is termed post-transcriptional gene silencing (PTGS) or RNA interference (RNAi), is a natural mechanism by which mammalian cells can regulate expansion of genes. Accordingly, short interfering RNA (siRNA) can be used for gene silencing. It is currently the fastest growing sector for target validation and therapeutic (Devi, 2006).
Considering cancer cells ability to escape from the immune system within the tumor microenvironment, the immune targeted gene therapy may grant an effective modality to the activation of immune systems within the microenvironment (Dougherty and Dougherty, 2009). Fig. 2 and Table 2 respectively represent the mechanism of action of AS-ODNs and their applications. The inhibitory impacts of AS-ODNs have been assessed through alterations in growth rate, morphology and molecular analysis. Various oncogenes have been targeted by AS-ODNs. For example, using non-viral vectors as delivery system, we have previously used AS-ODNs to target the epidermal growth factor receptor (EGFR) and showed substantial inhibition of EGFR in A431 cells (Hollins et al., 2004) as well as A549 lung cancer cells (Nakhlband et al., 2010).

**Fig. 2.** Mechanism of action of AS-ODN. A) Inhibition of proteins by small molecule drugs after translation. B) Suppression of mRNA by AS-ODN before translation in the presence of RNase H.

**Table 2.** Selected oncogenes targeted by AS-ODNs

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>c-ErbB-2 AS-ODN Inhibit serum-induced cell spreading of ovarian cancer cells</td>
<td>(Wiechen, 2001)</td>
</tr>
<tr>
<td>(c-ErbB-2)</td>
<td>Inhibitory effects of c-ErbB-2 AS-ODN in uterine endometrial cancer Ishikawa cells</td>
<td>(Zhao et al., 2009)</td>
</tr>
<tr>
<td>BCL-2</td>
<td>BCL-2 AS-ODN inhibits sensitize small cell lung cancer cells (in vitro and in vivo) to radiation</td>
<td>(Loriot et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Phase I/II study of G3139 (Bcl-2 AS-ODN) combined with doxorubicin and docetaxel in breast cancer</td>
<td>(Moulder et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis and increased chemosensitivity in human prostate cancer cells by Bcl-2 AS-ODN</td>
<td>(Yamanaka et al., 2006)</td>
</tr>
<tr>
<td>c-RAF-1</td>
<td>Phase I study of the c-Raf-1 AS-ODN (ISIS 5132) combined with carboplatin and paclitaxel in patients with advanced non-small cell lung cancer</td>
<td>(Fidias et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Phase I study of the c-raf-1 AS-ODN (ISIS 5132) in patients with advanced cancer</td>
<td>(Rudin et al., 2001)</td>
</tr>
<tr>
<td>c-FOS</td>
<td>Tissue-targeted antisense c-Fos retroviral vector inhibits established breast cancer xenografts in nude mice</td>
<td>(Arteaga and Holt, 1996)</td>
</tr>
<tr>
<td></td>
<td>c-Fos AS-ODN control prostaglandin E2-induced upregulation of vascular endothelial growth factor in human liver cancer cells</td>
<td>(Li et al., 2005)</td>
</tr>
<tr>
<td>c-MYC</td>
<td>c-Myc AS-ODN sensitize human colorectal cancer cells to chemotherapeutic drugs</td>
<td>(Abaza et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of c-MYC by antisense phosphorodiamidatemorpholino oligomer in prostate cancer murine models and humans</td>
<td>(Iversen et al., 2003)</td>
</tr>
</tbody>
</table>
Fig. 3. Cleavage and degradation of mRNA expression by siRNA. Short interfering RNAs (siRNAs) basically consist of two 21-25 single-stranded RNAs forming double strand RNA with overhangs at 3' end. The antisense strand of the siRNA bound to RNA-induced silencing complex (RISC) can cleave the target mRNA.

The siRNA (also called as short interfering RNA or silencing RNA) are double-stranded RNA (dsRNA) molecules of 20-25 nucleotides. The siRNA gene-silencing mechanism is induced by dsRNA and it is largely sequence-specific. Fig. 3 represents mechanism of siRNA in controlling the expression of mRNA. RNA interference (RNAi) approach appears to be an extremely powerful tool for silencing gene expression in vitro (McManus and Sharp, 2002). And accordingly, huge researchers have been conducted to expand this technology towards in vivo applications (Gondi and Rao, 2009).

Basically, studies on RNAi can be categorized into two distinct methodologies, as 1) cytoplasmic delivery of siRNA to target cells, mimicking an active intermediate of an endogenous RNAi mechanism and 2) nuclear delivery of gene expression cassettes that express a short hairpin RNA (shRNA), mimicking the micro interfering RNA (miRNA) active intermediate of a different endogenous RNAi mechanism (Aigner, 2008).

Ribozymes and DNAzymes
Ribozymes were discovered in early 1980s. They are a class of RNA showing catalytic activity to cleave RNA molecules in a sequence specific manner and have been used for cancer gene therapy. They appear to impose excellent catalytic reactions with great precision, which can be encoded and transcribed from DNA. It was a decade later that DNAzymes (the so called deoxyribozyme) entered the scene of nucleic acid-mediated catalysis (Dass et al., 2008). They are special class of nucleic acid chains, which usually consist of both double and single stranded regions that fold into a specific three-dimensional structure performing catalytic functions. Fig. 4 schematically exemplifies the morphology and cleavage mechanism of a ribozyme and a DNAzyme. Various ribozyme formats (e.g., hammerhead, hairpin, axhead, group I intron, and RNase P) can be used as transacting catalysts. Of these, the hammerhead and hairpin ribozymes seem to be the most commonly used ones. For example, the efficacy of an anti-K-ras hammerhead ribozyme targeted against GUU-mutated codon 12 of the K-Ras gene was evaluated in a cell-free system and also in cultured pancreatic carcinoma cells (Tsuchida et al., 1998). It should be remarked that the catalytic ribozyme core is basically attached to the specific regions of the target transcript through flanking antisense sequences. They have been designed to effectively cleave the target transcripts resulting in suppressed gene sequences. They have been designed to effectively cleave the target transcripts resulting in suppressed gene expression. For inhibition of gene expression, it is deemed that ribozymes are more effective than AS-ODNs because they cleave the target transcripts catalytically. The DNAzymes consist of the 10-23 nucleotides, which bind to mRNA in a highly sequence-specific manner and cleave the RNA independent from RNase with the relatively stable chemistries used in oligodeoxynucleotide-based antisense reagents.

Oncogenes
Tumor epithelial and endothelial cells as well as tumor associated cells represent unique marker molecules that can be harnessed for targeted therapy of cancer. For example, tumor vasculature varies significantly from its normal counterpart, representing unique cancer marker molecules. This has been emphasized through recent technologies including: immunohistochemistry laser-capture microdissection (immuno-LCM), genome-wide high-throughput screening, and proteomics uncovered. It is deemed that the vast array of vascular bed-specific markers may provide an exceptional platform for discovery of new therapeutics that target tumor microvasculature in various malignancies (Li et al., 2010). It is the same for tumor epithelial cells and TACs. Regarding the epithelial cells, EGF receptors are the most studied CMMs, whose upregulation in cancer cells were shown to be substantially down regulated with gene-based medicines such as siRNA and AS-ODN. Likewise, vascular EGF and EGF-receptors have been shown to be
upregulated in tumor endothelial cells and they can also be suppressed by genomedicines (Wang et al., 2009). Malignant brain tumors (high-grade glioma), pancreatic cancer and malignant melanoma are among the most aggressive tumors known. Despite these facts, necessary translational steps are needed to be fulfilled for their clinical applications. For example, Antisense Pharma has recently taken an AS-ODN medication (i.e., Trabedersen or AP 12009) into several clinical trials. Trabedersen is a DNA-oligonucleotide that inhibits the synthesis of the cytokine transforming growth factor beta 2 (TGF-ß2) through specific binding to mRNA of TGF-ß2 that is overexpressed in many highly aggressive tumors suppressing the immune system activity (Jaschinski et al., 2011, Schlingsiepen et al., 2011, Vallieres, 2009). Table 3 represents some of these CMMs that have been exploited for cancer gene therapy.

Table 3. Selected oncogenes causing various malignancies and gene therapies.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name and Description</th>
<th>Gene ID</th>
<th>Overexpressed tumors</th>
<th>Gene-based Medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>v-Akt mouse thymoma viral oncogene homolog 1</td>
<td>207</td>
<td>Breast, colorectal, ovarian, NSCLC</td>
<td>shRNA (Jiang et al., 2011), antisense [Yoon et al., 2009]</td>
</tr>
<tr>
<td>AKT2</td>
<td>v-Akt mouse thymoma viral oncogene homolog 2</td>
<td>208</td>
<td>Ovarian, pancreatic</td>
<td>Antisense (Pu et al., 2006)</td>
</tr>
<tr>
<td>ALK</td>
<td>Anaplastic lymphoma kinase (Ki-1)</td>
<td>238</td>
<td>AML, NSCLC, Neuroblastoma</td>
<td>-</td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Familial breast/ovarian cancer gene 2</td>
<td>675</td>
<td>Breast, ovarian, pancreatic</td>
<td>rAV (Lazenhe and Katzennellenbogen, 1999)</td>
</tr>
<tr>
<td>CNOT1</td>
<td>Cyclin E3</td>
<td>988</td>
<td>Serous ovarian</td>
<td>-</td>
</tr>
<tr>
<td>CDK4/6</td>
<td>Cyclin-dependent kinase 8</td>
<td>1024</td>
<td>Colon cancer</td>
<td>siRNA (He et al., 2011)</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase 2</td>
<td>1001136456</td>
<td>Prostate cancer</td>
<td>Antisense (Dandekar and Lakeshwar, 2004)</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>Catenin (cadherin-associated protein), beta 1</td>
<td>1499</td>
<td>Colorectal, ovarian, hepatoblastoma, pleomorphic salivary adenoma</td>
<td>siRNA (Zeng et al., 2007)</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
<td>1956</td>
<td>Glioma, NSCLC</td>
<td>siRNA (Gao et al., 2012a), bispecific AS [Rubenstein et al., 2012], aptamer (Li et al., 2011)</td>
</tr>
<tr>
<td>ERBB2</td>
<td>v-erb-B2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)</td>
<td>2064</td>
<td>Breast, ovarian, NSCLC, gastric</td>
<td>Antisense (Klos and Yu, 2004)</td>
</tr>
<tr>
<td>KRAS</td>
<td>v-Ki-Ras2 Kirsten rat sarcoma 2 viral oncogene homolog</td>
<td>3845</td>
<td>Pancreatic, colorectal, lung, thyroid, AM6, others</td>
<td>Antisense (Shen et al., 2008), siRNA (Zhang et al., 2006)</td>
</tr>
<tr>
<td>HIF-1</td>
<td>Hypoxia inducible factor 1</td>
<td>3091</td>
<td>Various cancers</td>
<td>Antisense (Chung et al., 2006, Sun et al., 2010)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
<td>3569</td>
<td>Head and neck cancer</td>
<td>Antisense (Bran et al., 2011)</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus kinase 2</td>
<td>3711</td>
<td>ALL, AML, MPO,CMML</td>
<td>-</td>
</tr>
<tr>
<td>JUN</td>
<td>Jun oncogene</td>
<td>3723</td>
<td>Sarcoma</td>
<td>Antisense (Suga et al., 1999)</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>Manganese superoxide dismutase</td>
<td>100037831</td>
<td>Various solid tumors</td>
<td>Antisense (Benloch et al., 2006)</td>
</tr>
<tr>
<td>MDM2</td>
<td>Mdm2 p53 binding protein homolog</td>
<td>4193</td>
<td>Sarcoma, glioma, colorectal, other</td>
<td>Antisense [Bianco et al., 2005], siRNA (Chen et al., 2012)</td>
</tr>
<tr>
<td>MET</td>
<td>Met proto-oncogene (hepatocyte growth factor receptor)</td>
<td>4233</td>
<td>Head-neck squamous cell, glioma</td>
<td>Antisense (Chu et al., 2006, Salvi et al., 2007)</td>
</tr>
<tr>
<td>c-MYC</td>
<td>Mycmyelocytomatosis oncogene</td>
<td>24577</td>
<td>Prostate, colorectal cancer</td>
<td>Antisense (Steiner et al., 1998, Abaza et al., 2008)</td>
</tr>
<tr>
<td>v-MYC</td>
<td>v-mycmyelocytomatosis viral oncogene homolog (avian)</td>
<td>4609</td>
<td>Burkitt lymphoma, amplified in other cancers, B-CLL</td>
<td>-</td>
</tr>
<tr>
<td>H-RAS</td>
<td>H-Ras1 Harvey rat sarcoma virus Oncogene 1</td>
<td>154561</td>
<td>Advanced carcinoma</td>
<td>Antisense (Cunningham et al., 2001)</td>
</tr>
<tr>
<td>NRG1-1</td>
<td>NRG-3 homologue 1</td>
<td>7080</td>
<td>NSCLC</td>
<td>-</td>
</tr>
<tr>
<td>PKR1X</td>
<td>Phospholipid-3-kinase, regulatory subunit 1 (alpha)</td>
<td>5295</td>
<td>Glioblastoma, ovarian, colorectal</td>
<td>-</td>
</tr>
<tr>
<td>REL</td>
<td>v-Rel/reticuloendotheliosis viral oncogene homolog (avian)</td>
<td>5966</td>
<td>Hodgkin Lymphoma</td>
<td>Antisense [Perez et al., 1996]</td>
</tr>
<tr>
<td>SOX4</td>
<td>SRY (sex determining region Y) box 4</td>
<td>6659</td>
<td>Hepatocarcinoma</td>
<td>Antisense (Ahn et al., 2002)</td>
</tr>
<tr>
<td>TGF-beta 2</td>
<td>Transforming growth factor, beta 2</td>
<td>21808</td>
<td>Head and neck squamous carcinoma, Glioma</td>
<td>Antisense (Vallieres, 2009, Endo et al., 2000)</td>
</tr>
<tr>
<td>TP53(P53)</td>
<td>Tumor protein p53</td>
<td>7157</td>
<td>Breast, colorectal, lung, sarcoma, adrenocortical, glomus, multiple other tumor types p53 gene (Prabha et al., 2012, Swisher and Roth, 2002), siRNA (Berendan-Neagoe et al., 2009)</td>
<td></td>
</tr>
<tr>
<td>TYMS</td>
<td>Thymidylate synthase</td>
<td>7298</td>
<td>Various solid tumors</td>
<td>Antisense (Jason et al., 2007)</td>
</tr>
<tr>
<td>VHL</td>
<td>von Hippel Lindau syndrome gene</td>
<td>7438</td>
<td>Renal, hemangioma, pheochromocytoma</td>
<td>-</td>
</tr>
<tr>
<td>WTI(HP154)</td>
<td>Wilms tumor 1</td>
<td>7490</td>
<td>Ovarian cancer</td>
<td>Antisense (Huo et al., 2011)</td>
</tr>
<tr>
<td>WNT-1</td>
<td>Wingless-related MMTV integration site 1</td>
<td>22408</td>
<td>Breast cancer</td>
<td>siRNA (Wischerek et al., 2008)</td>
</tr>
</tbody>
</table>

Fig. 4. Schematic representation of morphology and cleavage mechanism of Ribozyme (A) and DNAzyme (B).
Tumor antigen-specific vaccines and DNA vaccines

Cancerous cells of different types of tumors often display expression of aberrant genes such as: 1) mutated genes (e.g., mutated p53, Ras, Bcr-abl), 2) unique genes resultant from viral oncogenesis (e.g., HPV E6 or E7), 3) overexpressed cancer specific genes (e.g., Her2, Transforming growth factor beta 2, carcinoembryonic antigen, mucin). These aberrant genes could be recognized by the host immune system, resulting in elimination of the cancerous cells expressing such oncogenes. However, cancer cells can circumvent from the anticancer activity of immunosystem within the permissive tumor microenvironment. Accordingly, the basis of the tumor antigen-specific vaccines is boosting the immune systems harnessing these aberrant antigens. Nevertheless, success of this approach depends on identification and appropriate use of tumor specific genes (Gomez et al., 2012, Keilholz, 2007, Bodles-Brakhop and Draghia-Akli, 2008). So far, over 730 DNA vaccines clinical trials have been undertaken. Of these, 156 are different types of challenging cancers. Although no DNA vaccine has been approved for human, a plasmid DNA encoding human tyrosinase (huTyr) has been approved by the US Department of Agriculture to treat canine melanoma (Grosenbaugh et al., 2011). The results supported the safety and efficacy of the huTyr DNA vaccine in dogs as adjunctive treatment for oral malignant melanoma.

To date, no DNA vaccine has been approved by the U.S. Food and Drug Administration (FDA) for human; however, there exist more than 150 trials for different types of cancers. Fig. 5 represents the pattern of DNA vaccines in clinical trials.

As the first personalized medicine, Sipuleucel-T (Provenge®, Dendreon, USA) was approved in 2010 by the FDA for treatment of asymptomatic/minimally symptomatic metastatic hormone-refractory prostate cancer (HRPC). Provenge® is the first personalized medicine, which is a cellular immunotherapy and its administration demands 3 steps, that are 1) extraction of patient’s antigen-presenting cells (APCs) through a leukapheresis procedure, 2) incubation with a fusion protein PA2024 consisting of the antigen prostatic acid phosphatase (PAP) and an immune signaling factor granulocyte-macrophage colony stimulating factor (GM-CSF) that helps the APCs to mature, and 3) infusion of the activated blood product (Huber et al., 2012, Di Lorenzo et al., 2011). Fig. 6 represents the cell therapy process of Sipuleucel-T modality.

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**Fig. 5.** Selected DNA vaccines in clinical trials. Only 4 trials have reached to the phase IV trial, in which 3 of them are targeting human papillomavirus (HPV) and 1 targeting HPV and hepatitis B. Data were obtained from (NIH-ClinicalTrials, 2012).

**Fig. 6.** Mechanism of action of Sipuleucel-T (A) and steps of the cell therapy (B). Treatment starts with isolation of dendritic cells (DCs) as antigen presenting cells (APCs) from patient’s blood, in vitro cultivation in the presence of fusion protein PAP–GM-CSF composed of prostate acid phosphatase (PAP) and granulocyte–macrophage colony-stimulating factor (GM–CSF) as immune responses enhancer (panel B). DCs expressing CD54 and PAP are re-infused into the patient to activate T-cell response against prostate cancer cells (panel A). For detailed information, reader is referred to see (Di Lorenzo et al., 2011).
It should be inferred that the risk of inadvertent immunogenicity against vaccine components is low because Sipuleucel-T is a personalized medicine that is composed of the patient's own dendritic cells (DCs), while immune stimulation against the target antigen is maximized (Di Lorenzo et al., 2011).

**Suicide gene therapy: a targeted genomedicine modality**

The notion behind the suicide gene therapy (SGT) is combined use of suicide gene medicine along with a prodrug that can be converted to a toxic metabolite solely in cancer cells that produce the metabolizing enzyme. Among various cancers, breast cancer is one of the cancers that show very high rate of prevalence worldwide. Genes associated with a high risk of developing breast cancer are BRCA1, BRCA2, p53, PTEN, CHEK2 and ATM. Suicide gene therapy through gene-directed enzyme/prodrug therapy (GEPT) were shown to improve the therapeutic efficacy of conventional cancer radiotherapy and chemotherapy without side-effects. Of the SGTs, the HSV-TK system gene can sensitize cells to the cytotoxic effects of designated drugs such as ganciclovir (GCV) and acyclovir (ACV). The herpes simplex virus thymidine kinase gene (HSV-TK) is a prototype gene, which can be transferred into tumor cells either by viral or non-viral vectors (Singhal and Kaiser, 1998). The HSV-TK-based SGT approach has resulted in promising outcomes in glioblastoma, showing that brain injections of M11 retroviral vector-producing cells for glioblastoma HSV-1 TK gene therapy were well tolerated and associated with significant therapeutic responses (Klatzmann et al., 1998). Other examples of this approach are cytosine deaminase/5-fluorocytosine (CD/5-FC) and carboxyl esterase/irinotecan (CE/CPT-11). Further, genetically engineered stem cells (GESTECs) have also been applied for GEPT (Yi et al., 2012a). Chemotherapy of brain tumors is often disrupted by the brain blood barrier (BBB) (Omidi and Barar, 2012), however GESTECs (consisting of neural stem cells (NSCs) expressing cytosine deaminase (CD) gene) were shown to be effective novel cell therapy modalities. For example, in a study, GESTECs were injected into xenograft mouse model of lung cancer metastasis to the brain, which was produced by the implantation of 549 lung cancer cells in the right hemisphere of the mouse brain. Two days post injection, 5-FC was administered via intraperitoneal injection and the histological analysis of extracted brain clearly revealed the therapeutic efficacy of these cells that were able to convert the 5-FC into 5-FU resulting in the decreased density and aggressiveness of lung cancer cells (Yi et al., 2012b). Likewise, in another study, the GESTECs expressing either CD or CE were harnessed to inhibit the ovarian cancer SKOV-3 cells through the conversion of prodrugs 5-FC into 5-FU (Kim et al., 2010). Table 4 represents the clinical trials for suicide gene therapy of cancer.

**Table 4. Clinical trials for suicide gene therapy of cancer**

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>US Trial ID</th>
<th>Malignancy</th>
<th>Intervention</th>
<th>Phase</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized trial of suicide gene therapy and prostate cancer</td>
<td>NCT00583492</td>
<td>Prostate cancer</td>
<td>Biological: yCD/mutTKSR39rep-ADP; Radiation: IMRT</td>
<td>II/III</td>
<td>Rg</td>
</tr>
<tr>
<td>Suicide gene therapy for donor lymphocytes infusion after allogeneic hematopoietic stem cell transplantation (ILD-TK01)</td>
<td>NCT01086735</td>
<td>Hematological malignancy</td>
<td>Biological: donor lymphocyte infusion</td>
<td>I/II</td>
<td>Rg</td>
</tr>
<tr>
<td>TK-based suicide gene therapy for hepatocellular carcinoma</td>
<td>NCT00844623</td>
<td>Carcinoma, hepatocellular</td>
<td>Genetic: TK99UN</td>
<td>I</td>
<td>Cd</td>
</tr>
<tr>
<td>A study of an infectivity enhanced suicide gene expressing adenovirus for ovarian cancer in patients with recurrent ovarian and other selected gynecological cancers</td>
<td>NCT00964756</td>
<td>Ovarian cancer</td>
<td>Genetic: Ad5.SSTR/TK.RGD; Drug: GCV</td>
<td>I</td>
<td>Rg</td>
</tr>
<tr>
<td>CASPALLO: Allodepleted T cells transduced with inducible caspase 9 suicide gene</td>
<td>NCT00710892</td>
<td>Lymphoblastic leukemia; non-Hodgkin's lymphoma</td>
<td>Biological: Allodepleted Cells</td>
<td>I</td>
<td>Active</td>
</tr>
<tr>
<td>Administration of donor T cells with the caspase-9 suicide gene</td>
<td>NCT01494103</td>
<td>Leukemia; lymphoma</td>
<td>Biological: Allodepleted cells; Drug: AP1903</td>
<td>I</td>
<td>Rg</td>
</tr>
<tr>
<td>Infusion of donor lymphocytes transduced with the suicide gene HSV-TK in patients with hematological malignancies</td>
<td>NCT00423124</td>
<td>Hematological malignancies</td>
<td>Genetic: HSV-TK</td>
<td>I/II</td>
<td>Active</td>
</tr>
</tbody>
</table>

Ad5-yCD/mutTKSR39rep-ADP: Replication-competent adenovirus; Ad5: Adenovirus; yCD: Yeast cytosine deaminase; ADP: Adenovirus death protein; Td: terminated; Rg: recruiting; Cd: completed; IMRT: Intensity-modulated radiation therapy; TK99UN: An adenoviral vector containing herpes simplex virus’s thymidine Kinase; GCV: Ganciclovir; HSV-TK: herpes simplex virus’s thymidine Kinase. Copyright © 2012 by Tabriz University of Medical Sciences
Immunogene therapy of cancer

Cancer immunotherapy is considered as an effective cancer therapy, arisen from the concept that the immune system plays a central role in the prevention of development/progression of tumors, and is also called immunosurveillance (Ben-Efraim, 1996). Perhaps the most compelling evidence for such tumor immunosurveillance is immune system activity in paraneoplastic diseases that are neurological disorders resultant from an anti-tumor immune response (Palucka and Banchereau, 2012).

Based upon innate and adaptive responses of immune system, immunotherapy modalities are performed either as “passive therapy” (using antibodies (Abs)/cytokines), “adaptive therapy” (in the form of the graft vs. leukemia (GVL) reaction associated with the graft vs. host (GVH) reaction) or “active therapy” by stimulating the immune system (Mathe, 1987).

Basically, autologous antigen-specific T cells can be expanded ex vivo and then re-infused into patients to boost T cells-based immune system activities. Of the immunotherapy approaches, the DCs as very potent antigen-presenting cells’ (APCs) vaccination are very promising. DCs play a central role in immune system activities because they control both the immune tolerance and the immunity. Thus, DCs have been extensively exploited for cell-based immunotherapy modalities (Palucka and Banchereau, 2012). Inherently, the aim of DCs-based immunotherapy is to induce the tumor-specific effector T cells (CD4⁺ T cells, CD8⁺ T cells and B cells) that can effectively reduce the tumor mass and can also induce immunological memory to control tumor relapse (Palucka and Banchereau, 2012).

The first step of DCs-based vaccination is to provide DCs with tumor-specific antigens. It can be performed through ex vivo cultivation of the patients-derived DCs with an adjuvant for DC maturation and the tumor-specific antigen. The processed DCs can then be injected back into the patient (Fig. 6). For example, for conducted consolidation therapy of advanced ovarian cancer, phase I/II randomized trial of DCs-based vaccination with or without cyclophosphamide have shown that the peptide-loaded DC vaccination induced modest immune responses with a promising survival rate (Chu et al., 2012).

Antiangiogenic gene therapy of cancer

Angiogenesis is essential for the growth, development and invasion of cancer. Therefore, antiangiogenic therapy is deemed to be an effective strategy for cancer therapy.

Table 5 represents some selected examples for angiogenic gene therapy trials.

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>US Trial ID</th>
<th>Malignancy</th>
<th>Intervention</th>
<th>Phase</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I - Pre-Radical Prostatectomy RTVP-1 Gene Therapy for Prostate Cancer</td>
<td>NCT00403221</td>
<td>Prostate Cancer</td>
<td>Genetic: RTVP-1 Gene</td>
<td>I</td>
<td>Cd</td>
</tr>
<tr>
<td>Trial of E10A in Head and Neck Cancer</td>
<td>NCT00634595</td>
<td>Head and neck squamous carcinoma; Nasopharyngeal carcinoma</td>
<td>Drug: E10A, Paclitaxel Cisplatin, Paclitaxel</td>
<td>II</td>
<td>NA</td>
</tr>
<tr>
<td>Safety and Efficacy of Adenoviral Endostatin in the Treatment of Advanced Solid Tumor</td>
<td>NCT00262327</td>
<td>Advanced solid tumor</td>
<td>Drug: antiangiogenic agents; Genetic: endostatin gene</td>
<td>I</td>
<td>NA</td>
</tr>
<tr>
<td>Gene Therapy in Treating Patients With Unresectable, Recurrent, or Refractory Head and Neck Cancer</td>
<td>NCT00004070</td>
<td>Head and neck cancer</td>
<td>Biological: interleukin-12 gene</td>
<td>I/II</td>
<td>NA</td>
</tr>
<tr>
<td>Interleukin-12 Gene Therapy in Treating Patients With Skin Metastases</td>
<td>NCT00028652</td>
<td>Metastatic cancers</td>
<td>Biological: interleukin-12 gene</td>
<td>I</td>
<td>Td</td>
</tr>
<tr>
<td>Interleukin-12 Gene and in Vivo Electroporation-Mediated Plasmid DNA Vaccine Therapy in Treating Patients With Merkel Cell Cancer</td>
<td>NCT01440816</td>
<td>Skin cancers</td>
<td>Biological: interleukin-12 gene; electroporation-mediated plasmid DNA vaccine therapy</td>
<td>II</td>
<td>Rg</td>
</tr>
<tr>
<td>Treatment of B-CLL With Autologous IL2 and CD40 Ligand-Expressing Tumor Cells + Lenalidomide</td>
<td>NCT01604031</td>
<td>Chronic lymphocytic leukemia</td>
<td>Biological: Vaccine; Drug: Lenalidomide</td>
<td>B-CLL</td>
<td>I/II</td>
</tr>
</tbody>
</table>

RTVP-1: related to testes-specific, vespid, and pathogenesis protein; Cd: completed; NA: not available; E10A: an adenovirus carrying humanendostatin gene (Zhao et al., 2008); Td: terminated; Rg: recruiting.
Cancer cells can secrete a number of "angiogenesis" factors such as vascular endothelial growth factor (VEGF) (Im et al., 2001), thrombospondin-1 (THBS1) (Xu et al., 1998), endostatin (Ning et al., 2009), tumstatin (Yao et al., 2005), canstatin (Wang et al., 2008), angiotatin (Ponnazhagan et al., 2004), 16 kDa human prolactin fragment (16K hPRL), interleukin-12 (IL-12) (Sangro et al., 2005), interleukin-18 (IL-18) (Hara et al., 2000), tumor necrosis factor-α (TNF-α) (Chung et al., 1998), and transforming growth factor (TGF) (Tandle et al., 2004).

Of these, VEGF is the most studied target. The effect of INF-β gene therapy on the growth of human prostate cancer was determined in nude mice bearing PC3MM2 cells. The intrallesional delivery of an AV-IFN-β was shown to suppress the growth of tumor by the inhibition of angiogenesis in a dose-dependent manner. Such impacts might be imposed through the induction of levels of INF-β and inducible nitric oxide synthase (iNOS) as well as lower levels of basic FGF and TGF-β1 (Cao et al., 2001).

There exist many investigations on the use of angiogenic gene therapy. For example, in a study, the rAAV vectors were constructed to express endostatin (rAAV-endostatin) or the antiangiogenic domain of thrombospondin-1 3TSR (rAAV-3TSR). Upon the implementation of these vectors in a mouse angiogenesis model, the rAAV-mediated gene delivery resulted in the inhibition of VEGF-induced angiogenesis. In fact, pretreatment of mice with i.m. or intrasplenic injection of rAAV-endostatin or rAAV-3TSR significantly inhibited tumor growth (Zhang et al., 2007).

**Targeted nanogenomedicines: nanotechnology and gene therapy integration**

To date, emergence of nanotechnology with gene therapy has resulted in great advancements in the production of nano-scaled smart gene-based medicines. The new class of the smart nanogenomedicines can specifically target the cancer cells through homing devices, resulting in efficient delivery of the genemedicine into the target cells harnessing both passive and active targeting mechanisms (Omidi et al., 2003, Omidi et al., 2005a, Hollins et al., 2007, Omidi et al., 2008, Barar et al., 2009, Omidi and Barar, 2011).

Lipids and polymers, depending on their end groups, can be conjugated with different moieties such as imaging devices (fluorescent dyes, quantum dots) and homing agents (antibody, peptide, aptamer). Post-formulation conjugation of NPs are basically performed through chemical grafting using homobifunctional crosslinkers (e.g., N-hydroxysuccinimide (NHS) esters, iminodesters, sulphydryl-reactive crosslinkers, hydrazides) or heterobifunctional crosslinkers (e.g., sulphydryl-reactive and photoreactive crosslinkers such as SPDP, LC-SPDP, and Sulfo-LC-SPDP) (Hermanson, 2008).

Decoration with homing devices can arm them to target cancer cells and deliver the cargo gene-based molecules directly to the tumor microenvironment and thereby cancer cells, but not normal cells/tissues.

Antibodies can be modified via amine groups using 2-iminothiolane (Traut's reagent) and conjugated to NPs. They can also be activated with, N-succinimidyl S-acetyltioacetate (SATA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), in which the active NHS ester end of SATA or SPDP can react to amino groups in proteins and other molecules to form a stable amide linkage.

Further, conjugation of the NPs with poly ethylene glycol (PEG) (the so called PEGylation) can favor the pharmacokinetics of these NPs prolonging the circulation periods that grant a proper time frame to NPs to be accumulated in the tumor microenvironment. Although PEG is the most effective method to reduce protein adsorption *in vivo* and to avoid the RES system clearance, several other polymers have successfully been implemented as alternatives to PEG, including poloxamer, polyvinyl alcohol, poly(amino acid)s, and polysaccharide. However, PEG is still the most widely used polymer to engineer stealth NPs (Guo and Huang, 2011).

For nanoliposomes, PEG-lipid (such as PEG-DSPE) is usually inserted into liposomes to form a hydrated layer on the liposome surface. These nanosystems can be used for simultaneous imaging and therapy (Omidi, 2011a). Fig. 7 represents schematic structure of the advanced nanogenomedicines.
Typically, tumor microvasculature displays discontinuous fenestrated morphology characteristics with gaps and pores between endothelial cells, in which the pore sizes are at a range of 100 nm to 1000 nm (Adiseshaiah et al., 2010). For instance, subcutaneously grown tumors were reported to have profound fenestration, shoeing pore sizes at a range of 200 nm to 1200 nm (Hobbs et al., 1998). Most tissues present tight junctions between cells with intercellular openings lower than 2 nm and around 6 nm in post-capillary venules, and tissues with discontinuous fenestrated endothelium such as kidney glomerular and sinusoidal endothelium of liver have larger junctions respectively with pore sizes of 40-60 nm and 70-150 nm (Seymour, 1992). As a result, NPs with size ranging 150-250 nm can substantially extravasate showing significant enhanced permeation and retention (EPR) effects within the tumor microenvironment (Li et al., 2012).

Since long circulation of NPs in blood is a pivotal requirement for their successful in vivo applications, they are basically grafted with PEG that provide greater hydrophilicity and longer circulation in blood resulting in greater accumulation within the tumor micro-environment (Shan et al., 2009).

The "naked gene based medicines such as AS-ODN and siRNA can simply be degraded and destroyed by nuclease enzymes within blood, thereby not taken up by the target cells. This may give a rise to undesired harmful immune reactions. Thus, nano-scaled protected gene medicines will provide desired canonical outcomes. Recently, it was shown that the siRNA protected by cyclodextrin-containing polymers (RONDEL) can literally get to the proposed target site and impose the intended impacts (Heidel and Schluep, 2012). Table 6 represents some selected gene therapy trials using liposomal nanoformulations.

Fig. 7. Schematic structure of the advanced nanogenomedicines.
Cell based gene therapy of cancer

Cell-based therapy of cancer has been considered as a promising personalized modality. Of these, mesenchymal stem cells (MSCs) are considered to hold great potential as targeted-delivery vehicle in cancer gene therapy (Hu et al., 2010). Their propagation in culture is simple; that also shows contingency toward genetic modification in order to express therapeutic proteins. Above all, MSCs possess inherent tumor-tropic and migratory properties that allow them to serve as robust cell based carriers and as targeted drug delivery systems for isolated tumors and metastatic diseases (Gao et al., 2012b).

In a study, the migration ability of MSCs towards prostate cancer cells (in vitro and in vivo and incorporating into the tumor mass) was investigated. The infected cells with HSV-TK gene were shown to incorporate into the tumor mass (Song et al., 2004). The tumoricidal bystander effect in the HSV-TK gene cancer xenografts in nude mice in the presence of GCV significantly inhibit the growth of subcutaneous PC3 prostate tumor cells (in vitro and in vivo) and maintaining their tumor tropism capabilities and significantly inhibit the growth of subcutaneous PC3 prostate cancer xenografts in nude mice in the presence of GCV application in vitro, they employed noninvasive nuclear imaging using a radioiodinated nucleoside (fraluridine) serving as a substrate for HSV-TK in BALB/C mice.

Tissue-specific promoters and inducible promoters

Tissue-specific promoters (TSPs), a powerful tool for decreasing the toxicity of cancer gene therapy to normal tissues, have been used as targeted gene therapy approach. TSPs have been utilized for specific mutation compensation or delivery of prodrug-converting enzymes and also for controlling crucial viral replication regulators and consequent restriction of replication to tumor cells (Saukkonen and Hemminki, 2004). The safety and contingency of this approach has been shown in some initial clinical trials (Shirakawa et al., 2000). Of these, the cytomegalovirus (CMV) immediate-early promoter is often harnessed in gene therapy since it can express target genes at high levels in tumor cells. Lin et al. (2001) examined the effects of the involucrin (INV), keratin 14 (K14) and CMV promoters on the expression of the reporter gene beta-galactosidase. They introduced the plasmid DNA to BALB/c mice using a gene gun, and looked at the skin biopsies. They found that the K14 and INV promoter constructs could induce the beta-galactosidase gene expression only in the epidermis, while the CMV promoter was able to elicit gene expression in both the dermis and epidermis (Lin et al., 2001).

To increase promoter strength while maintaining tissue specificity, Qiao et al. (2002) constructed a recombinant adenovirus encompassing a binary promoter system with a tumor-specific promoter “VISA” (VP16-GAL4-WPRE integrated systemic amplifier) and a pancreatic-cancer-specific promoter CCKAR (cholecystokinin type A receptor) (CCKAR-VISA or C-VISA) which drives expression of the gene BikDD, a mutant form of the potent proapoptotic gene Bik (Bcl-2 interacting killer).

Table 6. Gene therapy clinical trials using liposomal formulations

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>US Trial ID</th>
<th>Malignancy</th>
<th>Intervention</th>
<th>Phase</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Therapy in Treating Patients With Advanced Head and Neck Cancer</td>
<td>NCT0009841</td>
<td>Advanced head and neck cancer</td>
<td>Liposomal formulation of EGFR antisense</td>
<td>I</td>
<td>NA</td>
</tr>
<tr>
<td>FUS1-nanoparticles and Erlotinib in Stage IV Lung Cancer</td>
<td>NCT01455389</td>
<td>Lung cancer</td>
<td>DOTAP-Chol-fus1; Erlotinib; Dexamethasone</td>
<td>I/II</td>
<td>Active</td>
</tr>
<tr>
<td>Study to Determine the Maximum Tolerated Dose of LErafAON in Patients With Advanced Solid Tumors</td>
<td>NCT00024661</td>
<td>Advanced tumors</td>
<td>LErafAON</td>
<td>I</td>
<td>Cd</td>
</tr>
<tr>
<td>C-VISA BikDD: Liposme in Advanced Pancreatic Cancer</td>
<td>NCT00968604</td>
<td>Advanced pancreatic cancer</td>
<td>BikDD Nanoparticles</td>
<td>I</td>
<td>Active</td>
</tr>
</tbody>
</table>

EGFR: epidermal growth factor receptor; NA: not available; Cd: completed; LErafAON: liposomes carrying antisense oligonucleotide against the Raf-1 protein; siRNA: small interfering RNA; EphA2: ephrin type-A receptor 2; C-VISA BikDD: liposome consists of a pancreatic-cancer-specific expression vector “VISA” (VP16-GAL4-WPRE integrated systemic amplifier) and a pancreatic-cancer-specific promoter CCKAR (cholecystokinin type A receptor) (CCKAR-VISA or C-VISA) which drives expression of the gene BikDD, a mutant form of the potent proapoptotic gene Bik (Bcl-2 interacting killer).
Preclinical factors
Previous studies have highlighted the importance of the gene delivery systems in terms of the endpoint results. While the viral vectors may associate with inadvertent immunogenicity (Tomanin and Scarpa, 2004), the non-viral vectors may induce non-specific genotoxicity (Kafil and Omidi, 2011, Barar et al., 2009, Omidi and Barar, 2009, Omidi et al., 2005b, Omidi et al., 2005a, Hollins et al., 2007, Omidi and Barar, 2011, Omidi et al., 2003). Viral vectors result in much higher transfection efficiency, thus the chimeric viral vectors generated by combining favorable features of two or more different viruses into one may resolve this problem (Tomanin and Scarpa, 2004).

Despite great achievements in the cell based models, the differences between animal models and patients involved in the clinical trials for cancer gene therapy may cause problems. The biological responses within the young and healthy animals used in preclinical studies may differ from those of the human subjects. Some of these models are transgenic animals; as a result we do not have a complete picture for similarity of their responses to therapy in order to compare with human subjects. These models can only imitate the disease condition and may not reflect the exact situation in human.

Clinical factors
For success of any clinical trial, several issues need to be considered including: effective protocols for good clinical practice (GCP), ethics approval and informed consents, required documentation for trial master file, sponsorship and indemnity, monitoring and auditing, trial management, and trial reports. To ensure upon GCP, FDA has released tremendous information and guidelines for clinical trials that can be used for translational medicine; reader is directed to see FDA official website.

From pharmacokinetics viewpoints, the optimal dose, duration, and timing of the gene therapy modalities must be very carefully figured out. Pharmacokinetic parameters (ADME) are largely dependent on patient condition. While the short-term effects of gene therapy are studied, the long term consequences of the gene therapy need to be clearly addressed. For example, 5-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy (RCAV-SGT) for treatment of prostate cancer have shown the effect of gene therapy on prostate-specific antigen doubling time (PSADT) that is considered as a surrogate end point with significant prognostic power. It has been shown that the PSADT increased following the gene therapy from a mean of 17 to 31 months (median 16 to 22 months) (P=0.014). Once combined with androgen suppression therapy (AST), uniformly initiated at a PSA of 15 ng/mL, the gene therapy was shown to delay the projected onset of salvage therapy by an average of 2 years, indicating that the RCAV-SGT may provide a potential long-term benefit for patients (Freytag et al., 2007). However, we need to accelerate the whole process by integrating many aspects of the study from the designing stage to the application in human subjects, in which patient selection and follow-ups need to be pragmatically performed.

Concluding remarks
Cancer gene therapy continues to grow even though clinical applications of this approach demand further investigations. Trajectory of gene therapy shows great impacts of genomedicines (i.e., As-ODNs, siRNA, Ribozymes, DNAlzyme) both in cell based and animal models, while tumor antigen-specific vaccines and DNA vaccines appear to be the most promising modalities. While suicide gene therapy, immunogene therapy and angiogenic gene therapy continue to become a mature modality, integration of nanotechnology into development of multifunctional nanoparticles appear to provide a resilient, yet versatile platform for targeted cancer gene therapy as “nano-genoeuticals”. Rise of MSCs-based cancer gene therapies may also open a new chapter as “cyto-genoeuticals”.

In fact, the translational researches require effective rational protocols for knowledge and technology transfer and integration of several domains to meet the A to Z of the proposed researches (Omidi, 2011b). For successful translation of genomedicines into clinical applications, in fact, we need to re-meet the guidelines and standard operating procedures (e.g., protocols, ethics and consents, required documentation for trial master file, sponsorship and indemnity, approvals, monitoring and auditing, trial management, and trial reports).

Still many tumor suppressor and apoptosis-inducing genes can be evaluated for clinical applications. Attributable to intricate nature of malignant diseases, to achieve more effective gene therapy against cancer, genomedicines need to be advanced to be able to holistically target the most cancer causing genes. It is also essential to target both the tumor cells and other cancer associated players of the tumor microenvironment including: tumor microvasculature and tumor associated cells, stromal cells and CSCs.

Expert opinion
Gene and cell therapy modalities (e.g., Sipuleucel-T as the first approved personalized vaccine for cancer therapy) will literally change the directionality of the human diseases therapy toward much more mechanistic approaches. Up until now, over 65% of the gene therapy trials have been devoted to the cancer diseases. However, less than 3% of these trials have successfully been progressed to the phase II/III and only few to the
phase IV. This clearly emphasizes that, after decades of investigations upon gene therapy, our successes in clinical applications are marginal. There exist conceivable evidences on the effectiveness of gene therapy modalities in preclinical cell based and animal model studies. These findings, together with a large number of early clinical trials, are very persuasive confirming the efficacious plausibility of the gene therapy as personalized medicine for different diseases including cancer. Nevertheless, the rate of success in marking these genomedicines appears to be menial. One reason could be that the current translational approaches have not consistently been decisive and constructive. Needless to remark that, to be able to continue translating the basic outcomes of the promising gene therapy modalities toward successful clinical applications, much more integrative approach is essential harmonizing various aspects of gene therapy. Further, this trivial success rate clearly highlights that the whole process for discovery and development of genomedicines need to be revisited and refined to speed up the process and to enhance success rate, in which perfect interdisciplinary collaborative work is essential. Finally, the pitfalls and impairing factors in the clinical trials protocols and corollaries need to be pinpointed.

**Ethical issues**

No ethical issues to be articulated.

**Conflict of interests**

No conflict of interests to be declared.

**Acknowledgment**

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**References**


Translational approaches towards cancer gene therapy


