IMMUNE GENE THERAPY OF CANCER

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Running Title: Immune gene therapy of cancer

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IMMUNE GENE THERAPY OF CANCER

Abstract

Cancer gene therapy emerged as a promising treatment modality three decades ago. However, the failure of the first gene therapy trials in cancer has decreased its popularity. Likewise, immunotherapy has shown a similar course. While it was a popular and promising treatment with IL-2 and interferon and cancer vaccines in the 1980s, it later lost its popularity. Immunotherapy has become one of the main options for cancer treatment with the successful use of immune checkpoint inhibitors in the clinic approximately ten years ago. This success of immunotherapy has increased even more with the introduction of cancer gene therapy methods in this area. With the approval of oncolytic herpes simplex virus and CAR T-cells, immune gene therapy became a candidate to be one of the essential modalities in cancer treatment like surgery, radiotherapy, chemotherapy, and targeted therapies soon.

Key Words: Immunotherapy, Gene Therapy, cancer, cytosine deaminase, GM-CSF
**Introduction**

Recently, the use of new immunotherapeutic agents such as immune checkpoint inhibitors (ICIs), CAR T-cells, and oncolytic viruses have increased the median survival times in cancer. The unexpected long term remissions with the use of several monoclonal antibodies targeting immune checkpoints such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or programmed death 1 (PD1)/programmed death-ligand 1 (PD-L1) have raised the hope of a cure in advanced solid tumors (1). Combining immunotherapy agents with or without cytotoxic treatments has resulted in further synergistic activity (2).

Cancer gene therapy has long been studied since the late nineties as promising agents in cancer; however, only limited success has been achieved in humans. Talimogene laherparepvec (T-VEC), the first approved gene therapy product in cancer, has fueled the gene therapy studies aiming to induce tumor-specific immunity. T-VEC is an oncolytic herpes simplex virus modified to proliferate in only tumor cells and carry the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene (3). The GM-CSF produced in the tumor microenvironment induces tumor-specific immunity augments through the presentation of released tumor antigens by dendritic cells attracted via GM-CSF.

With the emerging role of immunotherapy in cancer, the conventional gene therapy methods that have been studied for about 30 years have started to be targeted to the immune system. Like the ICI’s the immune targeted gene therapy approaches may yield long term remissions in advanced cancer patients. Additionally, the combination of cytotoxic gene therapy treatments, such as suicide gene therapy and oncolytic vectors, aiming at tumor cell killing and immune-stimulation, might further increase therapeutic efficacy. In this paper, we will mainly discuss the immune system targeted gene therapy.
**Gene delivery systems**

Cancer gene therapy could be defined as the introduction of a therapeutic gene (transgene) into a tumor cell utilizing a delivery vehicle, called a vector. There are mainly two major categories of vehicles for transporting the transgenes as viral and non-viral vectors. Non-viral vectors include the physical and chemical transfer methods of genes, and bacterial and cellular vehicles. The non-viral transfer methods are usually safe and easy to use, but the transfection efficiency is usually lower than the viral vectors (4).

The electroporation, aiming at disrupting cell membranes using high voltage electrical pulses to facilitate the entry of DNA molecules into the cell, is one of the popular physical methods of non-viral transport of transgenes being tested in clinical trials (5). Likewise, nanoparticles carrying genetic material are also widely studied to deliver the genes into the cells. Some bacteria, like E. coli and S. typhimurium, are used to transfer the suicide genes to the tumor tissues and induce host immune responses against tumors (6). The genetically engineered bacteria are usually safe and cheaper compared to viral vectors (6). However, the use of bacteria as gene therapy vehicles are limited in immune-targeted gene therapies.

Viral vectors are the widely used gene delivery vehicles in cancer treatment. The clinical trials conducted have mainly utilized the adenoviral vectors, adeno-associated viral vectors, Herpes simplex viruses, alfa viruses, retroviral vectors, and lentiviral vectors. The widely used viral vectors and their features are outlined in Table 1. Because of the genomic integration, the retroviral vectors and lentiviral vectors are the less preferable vectors in cancer gene therapy trials. However, the lentiviral vectors are widely used for the ex vivo modification of immune
cells, such as DCs and T-lymphocytes (7). The adenoviral vectors and adeno-associated vectors are commonly used to introduce the therapeutic genes to the tumor cells.

The adenoviruses the most preferred viral vectors because they can express therapeutic genes episomally and have no risk of integration into the genome. The first-generation adenoviral vectors have been used as a carrier for the treatment of monogenic diseases by removing the E1 gene region of the vector (8). However, the first-generation adenoviral vectors are highly immunogenic, and a high prevalence of neutralizing antibodies in humans limits their clinical use. Besides, the first-generation adenoviral vectors may also produce replication-competent forms during and after the production process (9). To relieve the disadvantages as mentioned above, the second-generation adenoviral vectors were obtained by removing the E2 and E4 gene regions of the virus (10). Adenoviral vectors can transduce almost all cells and are safe because they do not integrate into the genome. Likewise, transient gene expression in cells seen in adenoviral vector transductions is not an issue for cancer treatment. The first and second-generation adenoviral vectors have a cargo capacity of fewer than 8 kb (10). In order to overcome the limited cargo capacity, the third-generation vectors have been obtained by further modifying the adenovirus. In this generation, all adenovirus genes have been removed except package signals, and the cargo capacity has been increased up to 30 kb and called gutless vectors (11). The vast cargo capacity of third-generation adenoviral vectors makes them attractable vehicles for cancer gene therapy. The gutless vectors have been tested in various in vitro cancer models (12). Nevertheless, they need further improvements to increase their therapeutic potential.

Adeno-associated viruses are small non-enveloped DNA viruses from the Parvovirus group that cause latent infection in cells. They can infect both dividing and non-dividing cells and integrate
the genes they carry into the host genome. Because of genome integration is site-specific in chromosomes, the risk of insertional mutagenesis is not as high as in retroviruses (13). Since the transient gene expression is usually sufficient for cancer treatment, adeno-associated vectors have not been studied much in cancer treatment. Besides, the limited capacity of less than 4 kb cargo is another obstacle for the transfer of big gene constructs (14).

Alphaviruses from the Togaviridae family are used in cancer gene therapy to stimulate cytotoxic T cell response (15). Semliki forest virus (SFV) and Sindbis virus (SIN) in this group are essential vectors that have the potential for cancer gene therapy (16).

Herpes simplex viruses have high cargo capacity with their complex genomes. As the genome size is as large as the app. 150kb, up to 30 kb, genetic material can be loaded easily (17). Not being integrated into the host genome is another advantage in terms of cancer gene therapy. Removing immediate early genes of the virus reduce the replication capabilities to prevent the possible toxicities of the virus (17). The modifications such as deletion of immediate early gene ICP47, which will enable them to reproduce in only cancer cells selectively make this vector as a preferable oncolytic viral agent (18). T-VEC, as mentioned before, acts as both an oncolytic vector and an immune-stimulating agent with its GM-CSF cargo (19).

Retroviral vectors are the second most studied vector group in cancer treatment. It is a small RNA virus and integrates into the host genome following cell entry. It is possible to load genetic material up to 10 kb by removing the capsid (GAG), reverse transcriptase (POL), and sheath (ENV) genes required for the replication of the virus (20). As it is stably integrated into the host genome, it provides very long-term gene expression and has the potential of insertional
mutagenesis. Despite their handicaps, such as low transduction efficiency and the inability to transduce non-dividing cells, they are frequently used in cancer gene therapy (21).

Lentiviruses are a select group of retroviruses and are more attractive because of their ability to transduce non-dividing cells. They also provide long-term gene expression and low potential for inflammation. However, they can integrate into the host genome and carry the potential for insertional mutagenesis. The lentiviral vectors are mainly used to modify the T-cells (22).

**The immunological targets in cancer gene therapy**

Cancer gene therapy mainly aims to transfer the therapeutic genes, gene segments, or oligonucleotides either with in vivo or ex vivo approaches to the target cells. The immune system is the most crucial target for the treatment of cancer. The main immunological targets for cancer gene therapy that are outlined in Table 2, are cytokine/chemokine genes, tumor-associated antigens, fusion proteins, including tumor antigens, genetically modified tumor cells, or immune cells.

Target cells are sometimes directly the tumor cells themselves in immune gene therapy of cancer. In this strategy, the gene therapy vehicles are directed against tumor cells to destroy or make them sensitive to the host immune system. Gene therapy could also target the host immune cells to make them specifically active against the tumor cells. The immune cells, such as cytotoxic T cells and dendritic cells, could also be modified ex vivo utilizing gene therapy methods before administering to the patients.
Tumor cells as targets for immune gene therapy

Gene therapy methods aiming at direct tumor cell killing, such as oncolytic vectors and suicide genes, could also induce tumor-specific immunity. Previously we and others have shown that tumor antigens shed from the dying tumor cells may induce anti-tumor immunity that further improve therapeutic results (23, 24). Viruses that have cytotoxic effects against human cells were suggested as a treatment modality decades ago (25). However, the natural cytotoxic viruses (oncolytic viruses) usually failed in the clinic. Herpes simplex virus (HSV), adenoviruses, paroviruses, and retroviruses have been modified so far to increase their therapeutic capacity and tested in clinical trials (26).

In a previous experimental tumor model, we have shown that replication-competent adenoviral vectors carrying L-plastin (Lp)-driven E1a adenoviral vectors yield significant anti-tumor specific immune cell killing when compared to control ones (24, 27). Likewise, the oncolytic viruses may also induce anti-tumor immune response via increasing the tumor antigen shedding.

Immune gene therapy methods have been tested in various cancer cells and experimental tumor models with success. We have previously designed various adenoviral vectors carrying either cytosine deaminase (CD) gene or immunostimulatory genes. Recently, we have tested if the combination of CD/5-fluorocytosine (5-FC) gene therapy, having the capability of killing tumor cells by converting 5-FC into 5-fluorouracil (5-FU) in the tumor tissue, with an immunostimulatory GM-CSF gene would further increase the therapeutic efficacy and augment the magnitude of the antitumor immune response induced by the adjuvant effect of dying tumor cells (Figure 1). We constructed an adenoviral vector carrying both CD and GM-CSF genes
driven by the cytomegalovirus (CMV) promoter to achieve this goal. The in vivo efficacy of the new adenoviral vector design of the bicistronic transcription unit of CD and GM-CSF and exogenous 5-FC tested in a syngeneic colon cancer model was successful (28). The suicide gene therapy and GM-CSF induced immunity have been found 5 times more than either CD or GM-CSF alone treatments along with the prolongation of survival times in mice. The above-mentioned adenoviral vector construct will soon be tested in a first-in-human clinical trial.

Combining cytotoxic treatments with immunostimulatory genes may increase therapeutic efficiency. The addition of IL-2 gene therapy to the suicide gene therapies such as TK has been shown to increase the anti-tumor response (29). The immune system plays a crucial role in the development of cancer. The tumor microenvironment (TME) provides an immunosuppressive milieu (30), where the tumor cells usually evade the immune system. The cytokines like IL-10, VEGF, IDO secreted by tumor cells suppress the cytotoxic T cells (31). The cells like MDSC, Tregs, M2 type macrophages in the TME also suppress the cytotoxic T lymphocytes. Likewise, the low pH established by the lactate from the tumor cells may further increase the immunosuppressive properties of the microenvironment (32). Therefore, modulation of tumor microenvironment through immunomodulating cytokines would be beneficial.

*Genetically modified tumor cell vaccines*

Gene therapy tools have long been used to modify the immune cells such as dendritic cells, cytotoxic T cells, and autologous or allogeneic tumor cells to induce anti-tumor immunity. GM-CSF gene is one of the prevalent immune cytokine genes to transduce tumor cells or dendritic cells (33). In animal models, CT26 colon cancer cells transduced with an adenoviral vector carrying GM-CSF have induced strong anti-tumor immunity against tumor cells and prevented
tumor regrowth (34). This strategy has been tested in various tumor models with success (35).

The clinical trials utilizing GM-CSF transduced autologous or allogeneic cancer cell vaccines have not yielded similar success as preclinical models. Though Tani et al. have reported two long term survivors out of 4 vaccinated patients with advanced renal cell carcinoma (36), no consistent results have been reported with the GM-CSF transduced autologous or allogeneic tumor cell vaccines (37). While no objective tumor responses were seen with those vaccines, a slight increase in overall survival was noticed.

Whole tumor cells or tumor antigens, either isolated from tumor lysates or synthetic ones, have been used for vaccination trials. Although some promising results reported in earlier trials that utilizing the vaccine as an adjuvant treatment, those strategies usually yielded a minimal success rate in advanced diseases (38). The leukemia cells cannot be recognized by the immune cells. Manipulation of those cells through gene therapy methods could increase their antigenicity. One such possibility is to express CD40 ligand (CD40L) on the leukemic cells to make them capable of antigen-presenting cells. The binding of CD40 expressing immune cells like T cells and nonimmune cells induces CD95 mediated apoptosis of the leukemic cells (39). In a phase I study of modified autologous chronic lymphocytic leukemia cells transduced with a replication-defective adenoviral vector carrying CD40L (ISF35), the transduced leukemic cells rendered the non-transduced leukemic cells to present antigen and induce death-receptor induced apoptosis. They yielded clinical responses (40, 41). Later on, tumor cells modified with viral vectors carrying especially immunostimulatory cytokine genes were studied in the clinical trials. In this strategy, the modified tumor cells behave as cellular vaccines via increasing the tumor antigenicity and inducing the immune response. Comparative analysis of a modified vaccinia virus strain Ankara (MVA) encoding CD40L, or TRICOM infected chronic
lymphocytic leukemia (CLL) cells, has shown the increased immunogenicity of those infected cells (42). Previously the combined expression of CD40L and IL-2 or OX40L by CLL cells transduced with adenoviral vectors has shown antileukemic immune response (43). Likewise, the malignant B cells from CLL patients behave as antigen-presenting cells when infected with the vectors carrying B7.1, ICAM-1, and LFA-3 co-stimulatory molecules (44). A therapeutic melanoma vaccine (AGI-101H) transduced with a fusion protein consisting of soluble IL-6 receptor and IL-6 linked by a flexible peptide chain was used in the adjuvant setting in melanoma patients (44). In two single-arm phase II trials, the AGI-101H yielded a significant prolongation in DFS and OS of stage IIB-IV resected melanoma patients compared to historical controls (45). Accordingly, in an advanced melanoma cohort of seventy-seven patients, the same vaccine yielded an app 50% disease control rate with a median OS of 17.3 months (46).

**Immune cytokines as immune gene therapy tools**

Cytokine and chemokine genes are widely studied in cancer gene therapy. The GM-CSF, interferon-gamma, interferon-alpha, IL-2, IL-4, IL-24, and IL-12 are the best-known examples of cytokines used in gene therapy studies (47). The systemic use of cytokines, such as interferon-alpha and IL-2, has caused significant toxicity in the clinic and they are no longer in use in the clinic (48, 49). However, the production of those cytokines in the tumor microenvironment would decrease the toxicity. The combination of the cytokine genes is also found to be effective in tumor models. Choi et al., showed that the co-expression of IL-12 and GM-CSF in the same oncolytic adenoviral vector could significantly increase the anti-tumor immunity and could be used as a potential treatment agent in cancer (50).

Hwang et al. have tested the co-administration of an adenovirus-mediated IL-12 gene transfer
and a cytosine deaminase-based suicide vector followed by 5-FC treatment (51). The co-administration of both vectors has yielded significantly higher tumor growth inhibition and prolonged median survival time in RENCA tumor-bearing mice.

4-1BB (CD137), an activation-induced costimulatory molecule expressed on activated T cells, is an essential immune checkpoint regulator. Targeting of 4-1BB or its ligand (4-1BBL), a member of the tumor necrosis factor (TNF) superfamily, may have the potential of inducing anti-tumor immune T cell responses. A replication-deficient adenoviral vector construct carrying 4-1BBL caused significant tumor growth inhibition in a cholangiocarcinoma bearing mice (52). Likewise, the co-administration of two different adenoviral vector constructs carrying either IL-12 or 4-1BBL yielded significant anti-tumor T-cell response and prolonged survival time in a mouse model of bearing a colon cancer (MCA26 cells) (53).

Chemokines recruiting the immune effector cells to the tumor microenvironment have also been used as immunostimulatory targets in gene therapy. Lapteva et al. have tested the delivery of RANTES (CCL-5) via an adenoviral vector. The intratumoral injection of Ad-RANTESE1a resulted in significant tumor reduction through increasing the infiltration of macrophages, CTLs and dendritic cells in the tumor microenvironment (54).

Tumor-associated antigens have long been tested as peptide vaccines for the treatment of cancer. However, the efficacy of those vaccines has been highly limited in the clinic. Likewise, gene therapy vectors carrying tumor-associated antigens have been tested with limited success even in tumor models (55, 56). However, combining the immune cytokine genes or checkpoint regulator genes with TAA would increase the immune response. Our group has previously designed an adenoviral vector carrying a fusion gene encoding CD40L and MUC1 antigen. The
fusion protein yielded significant anti-tumor immune response in preclinical models (57, 58). We then combined this vector vaccination with a prodrug/enzyme system. The combination therapy further increased the efficacy (57).

The combination of cytokine genes and TAA have also been tested in clinical trials (59). An attenuated vaccinia vector carrying IL2 and MUC1 has been reported to be effective in patients with advanced prostatic cancer (60). Von Mehren et al. have tested a vector vaccine of canarypox virus encoding B7.1 and CEA in patients with epithelial tumors expressing CEA in a phase I trial (61). Thirty-nine patients with CEA-expressing tumors were immunized with the vector intradermally every other week for eight weeks. Eight out of 30 patients completing eight vaccination cycles had stable disease. Although hundreds of different DNA vaccines have been tested so far, no DNA vaccine is available in the market yet.

Although the oncolytic viruses have long been studied as a cytotoxic treatment modality for cancer gene therapy, they have yielded only limited success in the clinical trials. The attempts to engineer those viruses to modulate the immune system have produced better response rates than the previous ones. Herpes simplex virus (HSV) has been modified to selectively proliferate in only tumor cells by deleting thymidine kinase (TK), ribonucleotide reductase (RR), or ICP34.5 genes alone or in combination (62). However, the addition of a copy of the GM-CSF gene to the HSV vector further significantly increased the therapeutic efficacy (63, 64). Likewise, the addition of IL-12 gene to an oncolytic HER2 targeted HSV showed improved efficacy for metastatic tumors (65).
CTLA4 and PD1 are the best-known inhibitor molecules that appeared on activated T cells. Upon binding of the B7.1 or PD-L1 molecules expressed on either tumor cells or macrophages in the TME to the CTLA-4 or PD1 receptors on activated T cells, the T cell responses are inhibited and regressed (66). The anti-CTL4, anti-PD1 or anti-PD-L1 antibodies called immune checkpoint inhibitors (ICI), bind either receptor or ligands to augment the previously acquired T cell responses against tumor cells. More than ten monoclonal antibodies have already been approved for the treatment of various solid tumors like melanoma, lung cancer, kidney cancer and they are already in the market (67).

The manipulation of immune checkpoint ligands or receptors with gene therapy methods is also being developed. One strategy is to introduce immune checkpoint inhibitor genes to the viral vectors. Reul et al. have constructed an AAV vector carrying the anti-human PD1 gene (68). The AAV-anti-PD1 vector has successfully produced monoclonal antibodies in tumor cells, both invitro, and in vivo. Likewise, Wu et al., have placed the scFv of anti-PDL1 gene into a vesicular stomatitis virus (VSV), which preferentially replicates in tumor cells (69). The VSV carrying scFv-PDL1 has shown potential therapeutic effects in a lung cancer mouse model with PD-L1/LLC cells. This strategy can be easily used with other checkpoint inhibitor molecules. Furthermore, the combination of ICIs with immune gene therapy tools might further increase therapeutic efficacy.

*Genetically modified T-lymphocytes*

T cells are the primary effector cells fighting against tumor cells. The tumor-infiltrating lymphocytes have long been suggested as the major effector population against tumor cells. However, some reports regarding the unfavorable prognostic role of the T cell infiltrated tumor
tissues have raised doubts about the use of those cells in patients (70). Further characterization of the TILs revealed that the Treg subpopulation of T cells in those patients having unfavorable prognosis (71). The patients with CD8 infiltrated cells usually had a favorable prognosis (72). The isolation of CD8+ TILs from fresh tumor tissues and infusion to the patient following the expansion of the cells so-called adoptive transfer of T cells has emerged as a promising immunotherapy modality. Rosenberg SA et al. have shown that the administration of TIL prepared from the fresh surgical specimens from melanoma patients in conjunction with IL-2 and lymphodepletion yielded a 29% 5-year remission rate (73). Adoptive transfer of TILs is found to be effective in heavily treated patients even with prior immunotherapies (74, 75). The number of T-cells, the proportion of CD8+ cells, and also the more differentiated form of those CD8+ cells might affect the therapeutic yields (76). However, in a small randomized study with 36 patients, the enrichment of CD8+ TILs did not increase the response rates compared to unselected ones (77).

Although the adoptive transfer of TILs has resulted in promising results in melanoma, the low yield of cells isolated from fresh tumor samples and their exhausted nature makes it challenging to use those cells in other solid tumors. To augment the amplitude of T cells’ activity, they are engineered to express tumor-specific T cell receptor (78). Clay et al. have shown the efficient transfer of the tumor-associated antigen-MART-1 reactive T cell receptor to human lymphocytes exert significant anti-tumor activity in vitro on MART-1 expressing melanoma cells (79). Later on, this strategy has been translated into a clinical trial in melanoma patients by Morgan et al. (80). Although a modest clinical activity has been achieved in that first-in-human trial, the durable objective responses in 2 patients were to herald the success of current immunotherapies. In another first-in-human trial of the T-cell receptor-targeted against E6
antigen of human papillomavirus in patients with advanced cervical cancer, sustainable objective responses have been reported (81).

Due to the limited success of TILs in clinical trials, new strategies are being developed that can ensure that T cells bind more tightly to tumor antigens. The most popular of these are CAR T-cells that are already approved for some indications in the clinic. The T cell receptor (TCR) loosely binds to the target antigen, and especially the tumor antigens need to be recognized by antigen-presenting cells before they can react. Since antibody-antigen binding is more specific than TCR-antigen binding, and there is no need for prior presentation, the TCR has been replaced by the antigen- binding site of an antibody to develop potent T cells. A chimeric antigen receptor (CAR) was obtained by using the antigen-binding region of a tumor-specific antibody fused with costimulatory molecules involved in signal transduction (82). The resultant chimeric antigen receptor gene is introduced into the T-cell through a viral vector to obtain more potent cytotoxic T cells expressing a large number of TAA specific receptors (82). These cells are then amplified in the laboratory and administered to patients. In the first-generation CAR T-cells, the CD3ζ chain, which plays a role in signal transduction and T cell activation, has been added next to the scFv molecule that binds to the antigen (83). The antitumor effect of first-generation CAR T-cells was limited and the cells underwent apoptosis after a while (84). Costimulatory genes such as CD28, CD134, 4-1BB have been added to the receptor in second and third-generation CAR T-cells (82). Thus, the antitumoral activities of the cells increased by their ability to proliferate and secrete cytokines. Currently, CAR T-cells targeting CD19 antigens of malignant lymphocytes have been approved for cancer treatment and have started to be used successfully in hematological malignancies, especially lymphoma and leukemia (85, 86). Although the CAR T-cell therapy has limited use in solid tumors due to the shortage of unique tumor specific antigens, recently promising results have been reported in in
vivo studies (87, 88). Xia et al have successfully used EGFR CAR T with potent and specific antitumor activity against a triple-negative breast cancer model (89). Likewise, CEA targeted CAR T-cells are also being tested for tumors expressing CEA (90).

**Genetically modified dendritic cells**

Dendritic cells have long been used as the central effector cells for cancer vaccines. The most critical antigen-presenting cells of the body are DCs. The antigen-presenting DCs could be produced through the stimulation of peripheral blood monocytes or CD34+ cells by GM-CSF and IL-4 within 3-6 days (91). In order to further activate and to increase the maturation of the DCs against specific antigens, the DCs are exposed to specific tumor antigens with either synthetic antigenic peptides or irradiated tumor cells for a few days. The tumor antigens-exposed DCs become fully activated and ready to present the tumor antigens to the immune cells. DCs have been safely tested in numerous clinical trials with some limited local inflammatory reactions and flu-like symptoms (92-94). However, the efficacy of those trials was modest. Especially the administration of DCs following surgery or cytotoxic treatment as chemotherapy and radiotherapy are the most widely implemented strategies to augment the immune response while the tumor burden at the lowest level. Accordingly, cytotoxic therapy and DCs vaccines have yielded synergistic activities (94).

Dendritic cells, ex-vivo transduced with either immunostimulatory genes or tumor antigens and synthetic peptides, have been administered to induce an anti-tumor immune response. The dendritic cells activated ex-vivo migrate to the lymph nodes when injected subcutaneously, and present the tumor antigens to CD8+ cytotoxic T cells and induce an immune response. The viral
vectors carrying tumor-associated antigens have been used so far to activate DCs. We have designed an adenoviral vector carrying a fusion protein of CD40L and MUC1 tumor antigen (95). We transduced the dendritic cells with the vector carrying the CD40L-MUC1 fusion gene and tested in a syngeneic mouse model of breast cancer via intratumorally. The intratumoral injection of dendritic cells loaded with the vaccine vector induced a potent anti-tumor CD84 T cell response and yielded a significant objective response. Furthermore, we have also achieved even an increased immune response and tumor response, combined with the DC vaccination with suicide gene therapy of a CD/5-FC system compared to vaccination alone (95). Adenoviral vectors, retroviral vectors, lentiviral vectors, and adenoassociated viral vectors have also been used to transduce DCs in-vitro (96). A DC-based vaccine, based on the ex-vivo activation of blood mononuclear cells by a fusion protein consisting of GM-CSF and prostatic acid phosphatase (Provenge®, Dendreon, USA), has been approved by FDA for metastatic prostatic carcinoma (97). Provenge has significantly extended the overall survival time for castration-resistant metastatic prostate carcinoma patients by four months (98). In the case of Sipuleucel-T, as well as in most of the clinical trials with other DC-based vaccines, autologous monocyte-derived DCs (moDCs) are used. The moDCs are not enough for recapitulation of the natural diversity of DCs. They usually mimic inflammatory DCs. Therefore, moDCs seem not to be ideal candidates for cancer vaccination.

The main problem with the ex-vivo activation of DCs is the selection of the useful cell subset of DCs. Therefore, the strategies aiming at the in vivo induction of DCs via powerful antigenic constructs seem much better than the ex vivo loading of DCs. The type and delivery methods of antigens used and the protocols might affect the activity of the DC-based vaccines (99).
CONCLUSIONS

Immunotherapy, which started with IL-2 and interferon-alpha in the late eighties, later accelerated with the use of ICIs and became one of the main elements of cancer treatment today. Immunotherapies have provided more extended survival periods for more than five years in many metastatic tumors, such as melanoma and lung cancers. A combination of immunotherapies with conventional therapies such as cytotoxic chemotherapy and radiotherapy further improved the treatment outcomes. Serious side effects seen in current immunotherapeutic drugs have fueled the research efforts. The application of gene therapy methods to this field has improved the side effect profile of immunotherapy to more acceptable levels and increased the treatment efficiency. Suicide gene therapies, which have cytotoxic effects on tumor cells, and the oncolytic treatments achieved with immune gene treatments, seem to be significant candidates to increase further the success rate of cancer treatment that we have reached in 2020 with ICIs and targeted therapies.
FIGURE LEGENDS

Figure 1. The adenoviral construct carrying cytosine deaminase and GM-CSF genes under the control of a CMV promoter produces CD and GM-CSF in tumor cells. The 5-FU produced in the tumor cell with the help of CD from 5-florocytosine, an anti-mycotic drug, kills the tumor cell and cause tumor antigen shedding. At the same time, the GM-CSF produced by the vector in the tumor cell attracts dendritic cells nearby. The immature DCs uptake tumor antigens and present to T-cells in lymph nodes. The armed T-cells then enter the systemic circulation and fight against tumor cells wherever they meet. (🌐: Naïve T-cell, 🕊: Armed tumor-specific T-cell, 🌿: Immature Dendritic Cell, 🌟: Mature Dendritic Cell, 🎯: Tumor Cell, 🍀: Adenoviral vector carrying Cytosine Deaminase (yellow) and GM-CSF (blue) genes.)
Table 1. Viral vectors commonly used in gene therapy studies.

<table>
<thead>
<tr>
<th>Viral vector</th>
<th>Packaging capacity (kb)</th>
<th>Features</th>
</tr>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>≤7.5</td>
<td>Transient expression in most of the cells, immunogenic.</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>≤4</td>
<td>Long-term expression in dividing and non-dividing cells.</td>
</tr>
<tr>
<td>Herpes Simplex virus</td>
<td>≥30</td>
<td>Long-term expression in most of the cells; low toxicity.</td>
</tr>
<tr>
<td>Alphaviruses</td>
<td>≤7.5</td>
<td>Transient gene expression in most of the cells including neurons and glial cells; low immunogenicity.</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>8</td>
<td>Long-term expression in dividing cells; genome integration.</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>8</td>
<td>Long-term expression in both dividing and non-dividing cells; genome integration.</td>
</tr>
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Table 2. The main immunological targets in the treatment of cancer gene therapy.

<table>
<thead>
<tr>
<th>Tumor cells</th>
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<tr>
<td>Immunostimulatory cytokines (GM-CSF, IL-12, CD40L, IL-12)</td>
</tr>
<tr>
<td>T cells</td>
</tr>
<tr>
<td>NK cells</td>
</tr>
<tr>
<td>Suicide genes (Cytosine deaminase, Thymidine kinase)</td>
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<tr>
<td>Oncolytic vectors</td>
</tr>
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