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Gene Therapies for Cancer: Strategies, Challenges and Successes

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Abstract

Gene therapy, which involves replacement of a defective gene with a functional, healthy copy of that gene, is a potentially beneficial cancer treatment approach particularly over chemotherapy, which often lacks selectivity and can cause non-specific toxicity. Despite significant progress pre-clinically with respect to both enhanced targeting and expression in a tumor-selective manner several hurdles still prevent success in the clinic, including non-specific expression, low-efficiency delivery and biosafety. Various innovative genetic approaches are under development to reconstruct vectors/transgenes to make them safer and more effective. Utilizing cutting-edge delivery technologies, gene expression can now be targeted in a tissue- and organ-specific manner. With these advances, gene therapy is poised to become amenable for routine cancer therapy with potential to elevate this methodology as a first line therapy for neoplastic diseases. This review discusses recent advances in gene therapy and their impact on a pre-clinical and clinical level.

Gene therapy in a global context involves correction of a genetic defect by introducing a normal version of a defective or missing gene thereby correcting an underlying disorder (Friedmann, 1992). Milestones on the path of developing gene therapies are presented in Figure 1. This concept was first advanced in the 1960s after observing that viruses could cause malignant transformation in cells by integrating their genetic information into the genomes of infected cells. In 1966, Edward Tatum proposed the use of viruses in the genetic manipulation of somatic cells and its possible therapeutic applications (Tatum, 1966). A few years later, initial proof-of-concept for gene therapy was demonstrated using tobacco mosaic virus as a vector to introduce a polyadenylate stretch to viral RNA (Rogers and Pfuderer, 1968). Encouraged by these results, gene therapy was attempted in the 1970s to correct a urea cycle disorder by administering wild type *Shope papilloma virus*, encoding the arginase gene, to two severely handicapped young girls suffering from hyperarginemia (Rogers et al., 1973; Terheggen et al., 1975). Unfortunately, the desired outcome was not achieved. The

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first successful therapeutic application of this gene therapy approach was evident in 1990 when a retrovirus-vector was used to mediate transfer of the gene encoding adenosine deaminase (ADA) into the T-cells of two children suffering from severe combined immunodeficiency (SCID) (Rosenberg et al., 1990). The response was positive for only one of the patients; however, debate arose since the patient simultaneously received enzyme replacement therapy alongside gene therapy. Another study was conducted in an 18-year-old patient suffering from ornithine transcarbamylase deficiency, a relatively mild form of nitrogen metabolism disorder. However, the application of gene therapy in patients was temporarily halted following the death of a patient due to vector-associated toxicity (Stolberg, 1999). In spite of the public backlash from this incident, in the last decades a growing body of evidences supported by positive human efficacy data confirmed that gene therapy could be used to correct certain debilitating conditions including Leber's congenital amaurosis (Maguire et al., 2009), β -thalassemia (Cavazzana-Calvo et al., 2010), X-linked severe combined immunodeficiency (SCID-X1) (Hacein-Bey-Abina et al., 2010) and cancer (Wirth et al., 2013). Additionally, an increased understanding of the genetic basis of many diseases, improvement of vectors to minimize unwanted toxicity, advances in approaches for manipulating DNA expression and delivery in a target-specific manner has raised expectations that a clinical breakthrough may be imminent. As a consequence, there have been over 1800 clinical trials already conducted or currently ongoing worldwide (Wirth et al., 2013).

Initially, gene therapy focused on rare (orphan) diseases mediated by detrimental monogenetic defects. However, with advances in the field, various chronic and progressive diseases such as heart failure, neurodegeneration or metabolic disorders were also evaluated using gene therapy approaches. These studies indicated that gene therapy techniques have broad potential applications, although cancer comprises over 60% of all ongoing clinical gene trials (Wirth et al., 2013). In this review, we focus on various gene therapy strategies that are currently employed, roadblocks and challenges in the field of cancer gene therapy, and a brief discussion of a few current successful applications of gene therapy for cancer.

Strategies in cancer gene therapy

Although the biological and clinical trial endpoints in monogenetic disease are well defined, technological and scientific advances over the past decades coupled with promising clinical data have made cancer one of the major disease targets for gene therapy. There are several prospective strategies currently being used for targeting cancer using gene therapy including: (a) expressing a gene to induce apoptosis or enhance tumor sensitivity to conventional drug/radiation therapy; (b) inserting a wild type tumor suppressor gene to compensate for its loss/deregulation; (c) blocking the expression of an oncogene by using an antisense (RNA/DNA) approach; and (d) enhancing the immunogenicity of the tumor to stimulate immune cell recognition. These strategies are briefly discussed below.

Deficient/defective apoptotic signaling stemming from mutations or imbalances in the expression of pro- and anti-apoptotic genes resulting in resistance to apoptosis in malignant cells provides the rationale for gene therapy to target the apoptotic machinery. Induction of apoptosis either by introducing genes encoding an inducer, mediator or executioner of

apoptosis is among the most common approaches employed in cancer gene therapy (Lebedeva et al., 2003). TNF-related apoptosis inducing ligand (TRAIL) is an example of an apoptosis-inducer used in cancer treatment that has been found to kill a wide variety of tumor cells with minimal toxic effects on normal cells (Griffith et al., 2009). Melanoma differentiation associated gene-7 (*mda-7*), also known as Interleukin-24 (IL-24), a member of IL-10 gene family, is another example of an apoptosis-inducer, which selectively provokes apoptosis in various cancers without showing any detrimental effect on corresponding normal tissue (Fisher, 2005). Apoptosis executioner genes, like caspases are effective on cancer cells regardless of the status of the apoptosis machinery (Jia et al., 2012). Selective *siRNA* or *microRNA*-mediated silencing of anti-apoptotic genes in malignant cells represents a strategy to sensitize them to pro-apoptotic reagents or radiotherapy, which are frequently used therapeutic approaches in cancer gene therapy (Jia et al., 2012).

There is now overwhelming evidence based on familial, epidemiological and cytogenetic studies supporting the hypothesis that cancer is a genetic disease which develops via a multistage process in which inherited and somatic mutations in two classes of genes—proto-oncogenes and tumor suppressor genes play major contributing roles (Heredity and Cancer, www.cancer.org). A large number of tumor suppressor genes including *p53* (which regulates cell cycle and apoptosis) (Matlashewski et al., 1984; Vogelstein et al., 2000), retinoblastoma gene *Rb* (which regulates cell cycle and differentiation) (Wiman, 1993), *p16INK/CDKN2* (which regulates cell cycle), *PTEN* (which regulates cell survival) have been identified and numerous attempts have been made to deliver these genes specifically to cancer cells to restore normal functions (Shanker et al., 2011). In 1996, retroviral vectors expressing human *p53* under the control of an actin promoter were used to treat non-small cell lung carcinoma (Roth et al., 1996). The biological activity of oncogenes can also be modulated and suppressed either at an RNA or DNA level and can be used for treating cancer. MYC, a transcription factor that regulates a variety of cellular processes is often deregulated in a wide range of human cancers (Henriksson and Luscher, 1996; Vita and Henriksson, 2006). Retardation in cell growth rate was achieved in melanoma cells treated with antisense oligonucleotides targeting the *c-myc* gene (Putney et al., 1999). Mutations in *K-Ras*, a member of the *RAS* gene family (*H-Ras*, *K-Ras*, *N-Ras*) commonly occurs in human colon cancers (about 40–60%) (Krens et al., 2010) and disruption of *K-ras* by antisense RNA leads to apoptosis and tumor growth suppression both in vitro and in vivo in animal models (Fleming et al., 2005). The apparent role of oncogenic microRNAs, a 20–22 small nucleotide that regulates specific genes post-transcriptionally, in tumor progression provides an entry point for gene therapy (Esquela-Kerscher and Slack, 2006). Recent advances in using antisense molecules to target oncomirs, which have been validated in both in vitro and in vivo animal studies, have established possible applications in the clinic (Liu et al., 2007; Broderick and Zamore, 2011).

Conversion of pro-drugs into active compounds to exert in situ cytotoxic effects by introducing genes that encode the converting enzyme is an effective approach in cancer gene therapy. The most common example of this approach is the delivery of *Herpes Simplex Virus* (HSV)-thymidine kinase (TK), followed by treatment with ganciclovir (Freeman et al., 1993). This approach has translated into the clinic and several Phase I clinical trials are now

ongoing to treat patients with prostate cancer (Nasu et al., 2007) or malignant glioma (Colombo et al., 2005; Natsume and Yoshida, 2008). Development of chemoresistance during the therapeutic process is one of the major factors causing failure of many forms of chemotherapy (Gottesman, 2002). Unraveling the genetic basis and selectively silencing proto-oncogenes by gene therapy might help override such resistance. These approaches are well established on a pre-clinical level (Akada et al., 2005; Hegi et al., 2005; Modok et al., 2006), however technical innovations are necessary for effective translation into the clinic.

Despite the significant improvements made in targeting strategies, cancer gene therapy is still in its infancy, although during the past few years substantial successes have been achieved in other diseases such as severe immunodeficiency, lipid storage disorders, hereditary blindness, haemophilia B, hypercholesterolemia, etc. To appreciate the causes for this delay in achieving the full potential of gene therapy in the context of cancer, it is important to understand the challenges that impede success, and the approaches being used to overcome these impediments, which will be discussed in this review.

Challenges in gene therapy

To achieve the ultimate potential of gene therapy, that is, long-term therapeutic benefit or optimally a cure, a detailed understanding of the impediments to therapeutic intervention and developing approaches to circumvent these obstacles are essential. Optimal transgene expression for suppressing a cancer-associated gene or delivery of a cancer-therapeutic gene to diseased tissue at efficacious doses is a major barrier for successful cancer gene therapy. Identification of an appropriate therapeutic gene(s) capable of inhibiting disease progression will also influence success. Various approaches, which are discussed in the following sections, are being used to overcome these hurdles to effective gene therapy.

Optimal transgene expression

For efficacious gene therapy, it is essential to precisely regulate the expression of therapeutic transgenes based on clinical need and to also restrict any possible unwanted negative effects of the therapy. Additionally, it is important to switch gene expression off completely if adverse side effects should occur. Promoters and enhancers are two critical components that define the duration of and level of optimal transgene expression in specific cells or tissues. There are two types of promoters: constitutive and inducible. Constitutive promoters allow continuous transcription of its associated gene. Some constitutive promoters are tissue specific (Nettelbeck et al., 2000; Scanlon, 2004), such as tyrosinase for melanoma (Bentley et al., 1994), prostate specific antigen (PSA) for prostate cancer (Pang et al., 1995; Gotoh et al., 1998), while others retain functionality in a broad range of cancers, such as *hTERT* (Fujiwara et al., 2007) and progression elevated gene-3 (*PEG-3*) (Su et al., 1997, 2005b). Inducible promoters display transient expression and can be induced to express transgenes with hormones or small molecules that are provided exogenously. Both the specificity of a promoter and its' strength are relevant in order to obtain sufficient expression of effector proteins. This may be achieved by using enhancers either from different genes or a single gene. Using this strategy, Nettelbeck and others have developed a construct where a 200 bp enhancer element was inserted upstream of pigment cell specific human tyrosinase promoter to target malignant melanoma (Nettelbeck et al., 2002). Using

Prostate Specific Membrane Antigen (PSMA), an enhancer fragment placed upstream of the E1A transcription initiation site/TATA, generated a prostate cancer-specific adenovirus (Lee et al., 2002; Li et al., 2005). To limit expression in healthy cells, silencers with restricted expression in normal cells, can be used to maintain the vector in an inactive state in healthy normal cells (Xie et al., 2013). However, the regulation of transgene expression may not be required for every gene and is dependent on the nature of the encoded product and the cell's requirements. For example, a number of clinical trials have been conducted with Adenoviruses expressing *p53* with very limited side effects because of non-toxic effects when overexpressed in healthy normal cells. However, inducible controlled systems become preferable to enhance the intensity of expression over constitutive expression particularly when delivering a potentially toxic gene product.

Delivery of therapeutic genes

The clinical utility of any treatment modality is dependent on safety and efficacy. Tumor/target organ-restricted therapeutic gene expression provides a well-suited approach to maximize therapeutic outcomes. Accordingly, targeted delivery is one of the major goals for successful applications of gene therapy. There are two main targeting strategies. The first is physical targeting that allows gene delivery using catheters, gene guns, etc. These topics are extensively described (for example in Jain, 2009) and will not be discussed in this review. The second strategy uses viral or non-viral carriers of genes that are modified in such a way that they can selectively bind to tumor cells and not normal cells. In this review, we will discuss some of the strategies that are commonly used to achieve cell-specific targeting. In addition, we will also provide an overview of the approaches that are currently being evaluated in pre-clinical animal models and which hold promise for future potential clinical applications.

Viral-based gene delivery

Viral-based gene delivery provides one of the most efficient means of gene transfer relative to other current methods. However, toxicity of viral proteins to normal tissues, the possibility of random integration, host-induced neutralizing antibody or tissue-inflammation against viral vectors and adenovirus-associated liver toxicity are some challenging issues that compromise successful gene transfer (Larocca and Schlom, 2011). On the positive side, the robust expression of transgenes in diseased tissues, availability of methods for large-scale preparation, and comparative ease of genetic manipulation to make the vectors safe and non-immunogenic make them suitable for therapeutic applications. Adenoviruses (Ad), Retroviruses, Adeno-associated viruses (AAV) and Lentiviruses are the most commonly used therapeutic viral vectors, with specific advantages and disadvantages (reviewed in Das et al., 2012) as briefly described in Table I. The choice of a particular vector depends on various factors including packaging capacity, host specificity, gene expression profile, and tendency to elicit immune responses particularly when repeated administrations are required (reviewed in Das et al., 2012). In general, viral vectors fall into one of two main categories: integrating vectors (such as Retroviral and Lentiviral vectors), which insert into the recipient's genome; and non-integrating vectors (Adenoviral vectors and Adeno-associated vectors). As, Ads are among the most popular viral vectors used for gene therapy (Jia et al., 2012), our review will focus on recent progress made in the area of Ad gene therapy.

Vector-associated immune responses: Overcoming immune barriers

A major hurdle for viral vectors, including Ads, is pre-existing immunity to specific Ad serotypes in the human population either due to natural infection or vaccination. Pre-existing antibodies towards wild type Ad viral vectors are problematic for systemic administration. However, this problem can potentially be overcome by using a heterologous prime-boosting regimen, an approach where similar antigens are delivered through different vectors (Fournillier et al., 2013). Additionally, neutralizing antibody responses following repeated viral vector administrations can also reduce therapeutic efficiency (Kaufmann and Nettelbeck, 2012; Choi and Chang, 2013). This problem can be ameliorated by temporary immunosuppression (Ungerechts et al., 2007). In an alternative approach, use of Ad capsid chimeras, developed by pseudotyping the adenoviral type 5 (Ad.5) vector with fibers from less-immunogenic Ads, such as Ad serotype 45, may be employed to reduce serological recognition (Parker et al., 2009). In addition to humoral responses, virus-specific memory immune reactions are also evident in most individuals as a result of previous exposure to the peptide H910–924, a major capsid protein, which is highly conserved in different Ad serotypes, and might hamper the efficacy of Ad-based therapies (Olive et al., 2002; Tang et al., 2004). Hexon chimeric vectors generated via replacement of immunodominant hypervariable regions (HVRs) have been developed and the immunogenicity and safety of these vectors are currently under investigation (www.Clinicaltrials.gov; NCT00695877).

Another strategy to ablate the innate immune response to vectors is by using synthetic polypeptides such as polyethylene glycol (PEG) incorporated in the capsid, which reduces vector uptake by tissue macrophages and Kupffer cells (Abel et al., 2009). Similar to PEGylation, coating with poly(lactic-glycolic acid) (PLGA), poly-[N-(2-hydroxypropyl) methacrylamide], has also been shown to de-target the Ads and reduce host-vector interactions. Complement activation by viral particles is one of the barriers for viral-mediated gene delivery. Incorporation of host proteins within membranes helps to prevent complement activation (Vanderplasschen et al., 1998). The secretion of viral proteins such as *Vaccinia complement control* protein (VCP) binds and inactivates C4b and C3b, thus inhibiting the classic and alternative pathways of complement activation. Pushpakumar and colleagues have successfully used VCP to perturb complement activation and established the potential of using VCP in combination with other vectors to block complement activation (Pushpakumar et al., 2011). Shielding Ads with polyethylene glycol or genetic modification to express soluble CD59 are used to prevent deposition of the membrane-attack complex (Gandhi et al., 2011).

Initially, the first generation of therapeutic Ads was developed by deleting the early *E1* genes (rendering the virus replication deficient) without or with partial *E3* deletion (Das et al., 2012). To make these Ads non-immunogenic or less immunogenic, the next generation of “gutless” Ad vectors have been created by removing all viral coding sequences, which showed reduced cytotoxic T-lymphocyte and/or cytokine-mediated inflammatory responses (Alba et al., 2005). Use of organ-specific promoters that reduce expression of the transgene in professional antigen presenting cells (APCs) may also be helpful to minimize inflammatory responses (Nayak and Herzog, 2010b).

Other vector systems such as Lentiviruses and Adeno-associated viruses are inherently less inflammatory and immunogenic than Ads (Thomas et al., 2003). Indeed, T-cell responses may be elicited against the transgene product, in particular when the vectors transduce cells that are robust for antigen presentation, including dendritic cells. However, the degree of the response depends on the target organ, route of administration and dosing schedule. Several immune suppression protocols, such as the prophylactic use of an immunosuppressant drug such as rapamycin in combination with IL-10 (Nayak et al., 2009) have been successfully used in various in vivo animal models to block immune responses to the transgene product. Similar to Ad vectors, use of specific promoters also reduces the risk of immune responses to transgene products (Wang et al., 2006). Incorporation of several repeats of a target sequence is highly effective in blocking transgene expression in professional APCs (Nayak and Herzog, 2010a).

Clinically, the severity and risk of eliciting harmful side effects as well as failure to produce anticipated biological activity is intimately connected with target-specificity of vectors. Indeed, during natural infection, wild type viruses are restricted to particular organs, however, recombinant viruses are not subject to similar physical restraints when systemically delivered. Even following local administration, viral vectors may disseminate to other tissues due to the leaky vasculature of tumor tissue and promote undesirable outcomes. In this context, emphasis has been placed on developing approaches to restrict the vectors to the target site either by modifying the viral vector (Transductional or Transcriptional targeting) or by strategies designed to enhance delivery.

Targeted Transduction: Reducing the risk of non-specific delivery

Generally, virus-mediated gene therapy relies on high-affinity binding of viral fiber proteins with strain/serotype-specific host receptors. Low levels or absence of receptor expression in target tissue results in reduced infection efficiency. Additionally, one of the safety concerns for using viruses as vectors are their natural tropism that results in the uptake of therapeutic genes by non-targeted cells (Beatty and Curiel, 2012; Curiel and Fisher, 2012). To overcome these limitations, transductional retargeting, which involves modification of viral surface proteins to express particular ligands that bind to receptors preferentially or exclusively expressed on tumor cells, is a common practice in viral-mediated gene therapy. For example, Ad5 vectors, the most commonly used serotype in the clinic, employ coxsackie and adenovirus receptors (CAR) (Philipson and Pettersson, 2004; Coyne and Bergelson, 2006) to infect target cells, which are not well-expressed in human cancer cells thereby reducing efficacy of a variety of cancer therapeutic strategies. To ensure CAR-independent cell entry, tropism-modified Ads have been engineered by inserting an integrin-binding RGD (Arg-Gly-Asp) motif on their HI loop. An enhanced transductional efficacy (2-to 3-orders of magnitude) and gene delivery was observed in ovarian cancer cell lines and primary tumors (Dmitriev et al., 1998) suggesting possible utility of this Ad in the clinic. In a different strategy, a chimeric adenovirus vector was developed containing the non-CAR targeting serotype Ad3 fiber proteins incorporated in an Ad5 capsid (Krasnykh et al., 1996). The resulting virions (Ad.5/3) have shown great transductional efficacy in a variety of Ad.5-refractory cell types with low CAR expression including melanoma, ovarian cancer, renal cancer, squamous cell carcinoma, glioma, prostate, pancreatic and colorectal cancer (Haviv

et al., 2002; Kanerva et al., 2002; Kawakami et al., 2003; Rein et al., 2005; Dash et al., 2010b; Eulitt et al., 2010; Hamed et al., 2010; Dash et al., 2011a; Park et al., 2011; Azab et al., 2012; Hamed et al., 2013a,b; Sarkar et al., 2013; Azab et al., 2014) and enhanced cytopathogenicity towards primary melanoma cells (Volk et al., 2003; Rivera et al., 2004). To prevent CAR-dependent native tropism while simultaneously achieving target-specific infection, Kashentseva et al. (2002) engineered Ads expressing a bi-specific adapter protein comprised of sCAR (soluble CAR) and single-chain fragment variable region (scFv) against the *c-erbB-2* oncoprotein, highly expressed in breast cancer. The resulting protein in addition to limiting Ad infection to CAR-expressing cells also binds to cellular *c-erbB-2* oncoprotein and therefore, enables Ad-targeting via a CAR-independent pathway. However, cancers do not comprise a homogenous population. As such, targeting a single cellular surface receptor may not achieve maximum benefit. Thus, developing Ad-targeting strategies that utilize multiple ligands within the same virion is clearly advantageous. One of the first vectors reported was an Ad.5 containing both an RGD motif and a polylysine ligand, which targets both cell surface integrin and heparin sulfate proteoglycans (Wu et al., 2002). Following that scheme, using multiple fibers within the same Ad virion, Pereboeva et al. (2004) developed an Ad vector composed of both a wild-type fiber and a knob-less fiber (fibrin) presenting a 6-His motif, which showed both CAR- and artificial 6-His receptor-specific gene transduction. Insertion of a cellular-specific targeting peptide (such as SY-GYLPLP, vascular endothelial cell-targeting peptide) or “affibody,” a small antibody mimetic, in the HI loop has also provided increased transduction in a variety of cancer cell lines. To target epithelial cell adhesion molecule (EpCAM), present in tumor cells, a neutralizing anti-fiber antibody conjugated to an anti-EpCAM antibody-expressing Ad was developed (Haisma et al., 1999). This dual-specific antibody successfully mediated gene transfer to primary human colon cancer cells (Haisma et al., 1999).

Non-specific uptake of viral vectors by the liver and spleen is a principal problem resulting in a reduction of therapeutic virus availability, when viruses are delivered systemically. With respect to Ads, up to 90% of Ad.5 is sequestered from the blood by Kupffer cells in the liver, thus reducing the systemic availability of Ads. To avoid uptake by Kupffer cells, pretreatment with warfarin followed by multiple doses of replication-defective Ads is helpful and this strategy enhanced the antitumor efficacy in pre-clinical animal models (Shashkova et al., 2008). Development of liver-off profile by modifying the Ad5 hexon with Ad serotype 48 hexon, which only weakly binds factor X, is another way to reduce liver uptake (Zhang et al., 2011).

Tumor-specific expression by transcriptional targeting

The most important consideration when using viral vectors to deliver transgene(s) is to ensure tumor-specific expression. In the context of oncolytic viruses, this property needs to be strictly regulated. To achieve this objective, a number of distinct strategies are currently being evaluated (reviewed in Bhatia et al., 2013). Utilization of tumor- or cancer- selective promoters that restrict expression to specific cellular subsets is an approach currently being evaluated in vitro and in vivo. The critical step for this approach is to identify an appropriate tumor-selective/specific promoter. Recent advances in molecular biology and bioinformatics

including sequence analysis tools and database searches have proven useful in identifying such promoters.

Currently, various promoters are being used to maintain viral replication or viral-mediated gene expression in a tumor-selective manner. For example, prostate-specific antigen (PSA) promoter/enhancer element was inserted upstream of *EIA* to create a prostate-specific oncolytic virus (Rodriguez et al., 1997). Human telomerase reverse transcriptase (*hTERT*) expression and strong promoter activity is observed in different cancer cells, which rationalize the use of the *hTERT* promoter to generate replication-competent recombinant viruses (Fujiwara et al., 2007). These viruses show restricted replication in telomerase-positive tumor cells and efficiently lyse these target tumor cells. The use of *hTERT* for tumor-selective gene silencing was also reported in various cancer cells (Huang et al., 2003; Kim et al., 2003; Lanson et al., 2003; Wirth et al., 2003; Irving et al., 2004; Fujiwara et al., 2011). A limitation of this approach is that some tumors do not express *hTERT* where this promoter-transduction effect is not evident. Moreover, the survivin promoter for glioma (Van Houdt et al., 2006), the β -catenin responsive promoter for colorectal and liver cancer (Fuerer and Iggo, 2002), and the tyrosinase promoter for melanoma (Nettelbeck et al., 2002) are a few examples of promoters that have been used to drive viral replicating genes in specific cancers. Similarly, a cyclooxygenase-2 promoter driven oncolytic virus was used to target cervical, ovarian and pancreatic cancer cells (Hoffmann and Wildner, 2006). Other cancer-selective promoters such as MUC1/DF3 (Fukazawa et al., 2010), alpha-fetoprotein (AFP) (Hallenbeck et al., 1999), and squamous cell carcinoma antigen-2 to target squamous cell carcinoma (Hamada et al., 2010), glial fibrillary acidic protein promoter for GBM (McKie et al., 1998) were also validated at the pre-clinical level and found to be effective in diverse animal models.

Another strategy for optimizing tumor selectivity for oncolytic viruses is to modify gene functions that are critical for viral replication in normal cells, but not required in tumor cells. Wild type Ad contains *E1B-55kd* gene, which is responsible for p53-binding and p53-inactivation. A mutant Ad with deletion of *E1B-55kd* (*dl1520*) was generated to facilitate viral replication in tumor cells where p53 activity is compromised (Bischoff et al., 1996). Although this modification showed promising effects, recent findings demonstrated that loss of *E1B-55kd*-mediated late viral RNA export, rather than p53 inactivation, is important for restricting viral replication in normal cells (O'Shea et al., 2004).

Although target-specific oncolytic viruses have shown efficacy in clinical trials, the addition of a second therapeutic arm with a therapy agent in the same virus has shown additional significant clinical benefit. Ad.TK^{RC}, an *E1B-55k* deleted Ad with HSV-TK, showed a better outcome in a colon xenograft model in the presence of ganciclovir (Wildner et al., 1999). Various structural permutations and combinations with diverse therapeutic agents were developed and validated for their efficacy in preclinical studies. Development of a *Cancer Terminator Virus* or *CTV* (Fisher, 2005; Sarkar et al., 2005a,b, 2006a,b, 2007b, 2008; Greco et al., 2010; Das et al., 2012; Hamed et al., 2013b; Sarkar et al., 2013; Azab et al., 2014), is an example of this approach. In one type of *CTV*, the Ad employs the rodent promoter from *progression elevated gene-3*, PEG-Prom (Su et al., 1997), to regulate oncolysis and simultaneously express a cancer-specific therapeutic cytokine gene, *mda-7*/

IL-24 (Jiang and Fisher, 1993; Jiang et al., 1995). Significant therapeutic potential of the CTV was established in various cancer models including, breast (Sarkar et al., 2005a), prostate (Sarkar et al., 2007b; Azab et al., 2014) and melanoma (Sarkar et al., 2008) supporting its broad potential applications for treating human cancers. To obtain improved viral transduction and anti-tumor efficacy, a serotype chimera CTV, Ad.5/3-CTV, was developed and shown to have enhanced activity in low CAR human tumor cells, while retaining high activity in high CAR human tumor cells. The enhanced activity of the Ad.5/3-CTV was documented in human xenograft studies in nude mice employing human prostate (Azab et al., 2014), pancreatic (Sarkar et al., 2013) and GBM (Hamed et al., 2013b) tumor cells. In a complementary strategy, a human interferon gamma-producing CTV was generated in an Ad5 background and found to have profound activity against therapy-resistant human pancreatic cancer cells using an in vivo nude mouse human pancreatic cancer xenograft model (Sarkar et al., 2005b).

Delivery of viral vectors: Need for safety and efficiency

Even with considerable success in retargeting vectors for enhanced activity in tumor cells, non-specific uptake by other organs such as the liver or spleen can still result in unwanted side effects or loss of efficacy, principally when the viruses are delivered systemically. Vector neutralization, nonspecific adhesion, sequestration, and inability of vectors to locate tumor cells behind endothelial-matrix barriers are some common factors that limit positive clinical outcomes. Considering these impediments, appropriate methods are required that allow delivery of an effective dose into diseased tissue. However, this is challenging, particularly for organs that are potentially inaccessible for localized catheter-based methods or operative bed injections (Howard et al., 2006). In such cases, systemic administration is the only option for efficient target site delivery.

Microbubbles (MBs), small gas-filled microspheres used as contrast agents for ultrasound, provide a safe method for delivering therapeutic agents, including viral vectors, transgenes, inhibitory siRNA/shRNA and therapeutic proteins, into target cells (Lawrie et al., 2000; Ng and Liu, 2002; Larina et al., 2005). MBs are filled with a heavy-molecular-weight gas such as perfluorocarbon or sulfur hexafluoride, which decreases their solubility, thus improving longevity and allowing passage through the microcirculation. MBs are metabolically inert, i.e., they do not cause an immune response in the host, and since viral vectors/transgenes/drugs are inside the bubbles this strategy permits the “stealth” delivery of therapeutics systemically. Moreover, ultrasound exposure generates microstreams or microjets, resulting in shear stress to cells thereby intensifying cellular permeability and enhancing transfection/transduction efficiency. Howard et al. (2006) initially evaluated the feasibility of microbubbles for site-specific gene delivery using an Ad vector carrying GFP marker gene in an in vivo system. Further studies experimentally confirmed that this approach could also be used to specifically deliver therapeutic viruses in both xenograft and spontaneous tumor models (Greco et al., 2010; Dash et al., 2011a).

MB size and the amplitude of ultrasound are two critical parameters that influence the behavior of MBs. The size of MBs should be within a range of 2.5 to 4 μm in diameter with adequate carrying capacity to entrap the viral vectors and prevent entrapment within the

pulmonary capillary bed. The frequencies employed need to be optimized based on the target organ in order to obtain enhanced transfer efficiency and to avoid tissue damage. Recently, an approach was developed to generate target-specific MBs or decorated MBs by addition of ligands to the surface of the MBs, usually an antibody *via* a biotin-streptavidin spacer or direct chemical coupling, for specific receptors of target tissues or organs (Ellegala et al., 2003; Anderson et al., 2010). For example, to target the tumor vasculature, single-chain VEGF molecules attached to the MB surface assures successful targeting to the tumor vasculature that overexpresses VEGFR2. The surface molecule VCAM-1, is also used to achieve targeted imaging in murine tumor models *in vivo*. The utility of this decorated MB to deliver therapeutic viruses is currently being evaluated (Unpublished data; Fisher lab, VCU).

Other vectors

Mesenchymal stem cells (MSCs), usually isolated from human bone marrow or adipose tissue are an alternative delivery vehicle for tumor-targeted gene therapy. Ease of generation, high proliferative capacity and better homing capacity to the tumor microenvironment are a few advantages of MSC-mediated gene delivery. Studeny et al. (2002) used hMSCs as vehicles to deliver Interferon- β to treat melanoma xenograft model. Subsequently, diverse therapeutic genes including apoptosis inducing agent TRAIL, chemokines CXCL1, iNOS, HSV-TK, and *mda-7/IL-24* have been engineered into MSCs to allow a targeted release in models of melanoma, breast cancer, glioblastoma, cervical cancer and prostate cancer, and have shown efficacy in inhibiting local tumor growth, suppressing metastasis and prolonging animal survival (Chan and Lam, 2013). Along with MSCs, neural stem cells (NSCs) also have been considered as vehicles for delivery of anticancer therapeutics (Hingtgen et al., 2012). Although remarkable progress has been made at the pre-clinical level, many questions remain before this approach becomes clinically applicable. Lack of a suitable method for ex-vivo expansion and poorly understood *in vivo* kinetics restricts MSCs and NSCs as a general platform for gene delivery.

Other methods for gene delivery

Despite accumulating data on improved viral vectors, issues related to insertional-transformation associated with the use of integrating vectors or viral-associated immunogenicity and toxicity have raised safety concerns in the clinic. Alternatively, chemical methods have attracted considerable attention in the area of gene therapy. These approaches have numerous advantages over their viral counterparts such as greater control of molecular composition, flexibility in the size of the transgene to be delivered, relatively lower immunogenicity and ease of generation. In this system, purified DNA is delivered with a complex with either cationic lipids (Lipoplexes) or cationic polymers (Polyplexes) and due to the anionic nature of the plasma membrane of mammalian cells, the complex is absorbed via electrostatic interactions. The efficient packaging of DNA before delivery into target cells is the first step for this process. Protonation is a common method of increasing the net positive charge of cationic polymers that facilitates DNA binding. However, strong binding does not ensure higher efficacy. Maintaining the natural DNA structure is critical during packaging. Serum stability, endocytosis by cells, endosomal escape, dissociation from lipo/polyplexes and nuclear internalization are other key factors that influence this

approach. Among various cationic polymers, such as diethylaminoethyl dextran, poly(l-lysine) (PLL), gelatin, polyamidoamine dendrimer, polybrene, poly(vinyl imidazole), poly(l-histidine-g-poly(l-lysine)), poly(β -amino ester), and chitosan that are commonly used for gene delivery purposes (Aied et al., 2013), polyethyleneimine (PEI) is one the most potent polymeric vectors because of its high pH-buffering capacity and endosomal escape abilities (Boussif et al., 1995; Aied et al., 2013). A number of next-generation PEIs with degradable cross-linkers for intracellular degradation, hydrolysis at low endosomal pH, enzymatic degradation, and cytosol-specific reduced degradation by glutathione have been developed. These agents displayed high transfection efficiency and low cytotoxicity as a result of their rapid in situ degradation of the polymer into small molecular weight and water-soluble fragments, which are processed easily and removed by the cells.

Binding to serum albumin and other proteins owing to the aggregation and accumulation of the complexes in fine capillary beds and clearing by phagocytic cells is an unwanted consequence of the chemical approach (Dash et al., 1999). Modification by addition of hydrophilic monomers/polymers such as PEG prevents aggregation of the complexes, increases complex stability and improves biocompatibility (Lai et al., 2012). To confer tissue-specificity, targeting-moieties, such as sugars, antibodies, peptides, and folates are conjugated to the complex. For instance, attachment of amino galactose to chemically modified poly β -amino esters results in a carrier having a ligand which targets the asialoglycoprotein receptor expressed on the surface of HepG2 cells (human hepatocarcinoma) and liver cells (hepatocytes) (Li et al., 2008).

Presently, as a chemical-based vector, gold nanoparticles, a non-oxidized state of Au (Aurum), which exhibits unique physiochemical properties including surface plasmon resonance (SPR), ability to bind amine and thiol groups, allowing surface modifications represent superior targeted transfection efficiency (Jain et al., 2012; Ahmad et al., 2013; Vigderman and Zubarev, 2013; Conde et al., 2014). Easy excretion through urine, insignificant toxicity on cells, excellent stability in the circulation, high gene carrying capacity represent some of the major advantages (DeLong et al., 2010; Jain et al., 2012). Although significant success in both in vitro cell culture and in vivo pre-clinical studies have been reported (reviewed by Jain et al. (2012); the application of nanoparticles remains relatively unexplored in clinical trials. CYT-6091, a citrate coated gold-nanoparticle carrying TNF- α (Paciotti et al., 2004; Farma et al., 2007; Goel et al., 2009) was used in an early phase clinical trial, which showed only partial response in one patient. Further enhancement in therapeutic approaches either by combination with a second clinical modality or using better therapeutics is under consideration for future applications of nanoparticles.

Identification of therapeutic genes

For successful gene therapy, selection of an appropriate therapeutic gene to maximize therapeutic efficacy while minimizing toxicity is critical. The therapeutic gene families presently being utilized in clinical trials are listed in Table II. Currently, research is focused on identifying novel genes that are differentially expressed in cancer cells and potentially regulate transformed properties. In this respect, cancer genomic data is a powerful tool to

distinguish molecular changes in cancer cells. In these contexts, the ability to perform comprehensive molecular profiling of tumors, which facilitates the identification of target genes (Chin et al., 2011), has potential to identify novel targets for potential therapeutic intervention. Indeed, large-scale genome characterization and appropriate data interrogation are frequent bottlenecks of this approach. However, improvements in high-throughput technologies (Kircher and Kelso, 2010), such as whole-genome sequencing and array-based gene expression profiling (Sokhi et al., 2013, 2014), are improving the quality of the information generated and when combined with new computational tools is leading to meaningful and interpretable data. Additionally, the Gene Expression Omnibus (GEO) (www.ncbi.nlm.nih.gov/geo/), which is a repository of high-throughput gene expression data for various types of cancers, provides a resource with potential to lead to the discovery of novel genes for cancer treatment.

MDA-7/IL-24: A potential “magic bullet” for multiple cancers

As the process of cellular de-differentiation is important in oncogenesis (Leszczyniecka et al., 2001), it was postulated that genes upregulated during reactivation of defective differentiation programs might display therapeutic potential. Based on this hypothesis, Jiang and Fisher (1993) used subtraction hybridization to define genes upregulated when cancer cells, specifically melanoma cells, were induced to lose cancerous potential and terminally differentiate. Using this strategy, Jiang et al. (1995) identified and cloned melanoma differentiation associated gene-7 (*mda-7*) as a gene whose expression was upregulated following growth arrest and terminal differentiation in HO-1 human melanoma cells following treatment with recombinant fibroblast interferon and the protein kinase C activator mezerein (Fisher et al., 1985). During the last two decades following its discovery, research on MDA-7 (also known as Interleukin 24 (IL-24), a member of IL-10 gene family (Sauane et al., 2003; Pestka et al., 2004; Chada et al., 2006), referred to as MDA-7/IL-24, has expanded at an exponential rate resulting in an enhanced understanding of the mechanism underlying its’ tumor suppressing activity (reviewed in Lebedeva et al., 2007; Emdad et al., 2009; Dash et al., 2010a; Menezes et al., 2014), improving strategies to enhance therapeutic outcomes (Fisher, 2005; Bhatia et al., 2013) and developing innovative approaches for cancer-specific gene delivery (Dash et al., 2011b; Das et al., 2012; Bhatia et al., 2013) and expression (Su et al., 2005b; Bhang et al., 2011). In this section, we discuss improvements in using *mda-7*/IL-24 as a therapeutic for cancer based on overcoming the challenges that gene therapists are currently encountering (Fig. 2).

Properties that make *mda-7*/IL-24 a unique and effective cancer therapeutic gene

Genetic conditions or disorders that arise from mutations in a single gene are ideal candidates for gene therapy. Unfortunately, cancer is a multigenic disorder and restoration or manipulating a single gene may not produce a desirable outcome. In this context, tumor suppressor gene(s) may provide a more general means of inhibiting cancer through inhibition of functions that go awry as result of induction of the cancerous phenotype. An ideal therapeutic gene would be one which selectively promotes killing of cancer cells without harming normal cells and is capable of attacking cancer on multiple levels (Fisher, 2005; Sarkar et al., 2007a). A critical component for an effective tumor suppressor gene

would be an ability to be secreted and exert both direct and distant “bystander” anti-tumor effects (Fisher, 2005; Su et al., 2005a; Sarkar et al., 2007a).

The first demonstrations of cancer growth suppressive properties of MDA-7/IL-24 came from experiments in which *mda-7/IL-24* was ectopically expressed in human melanoma and other human and rodent cancer cells (Jiang et al., 1995, 1996). These studies also provided suggestive evidence that this novel cytokine did not harm normal cells. Differential activity of *mda-7/IL-24* in killing cancer vs. normal cells was shown definitively by delivering *mda-7/IL-24* using a serotype 5 replication incompetent adenovirus (Ad.5.*mda-7*) (Su et al., 1998). By the first decade after discovery the therapeutic potential of MDA-7/IL-24 was confirmed in a wide array of solid tumors, both in vitro and in vivo, including melanoma, malignant glioma, carcinomas of the breast, cervix, colorectum, liver, lung, ovary and prostate (reviewed in Fisher et al., 2003; Fisher, 2005; Lebedeva et al., 2007; Emdad et al., 2009). This led to its entry into the clinic where intratumoral injection of a replication incompetent adenovirus expressing *mda-7/IL-24* (Ad.*mda-7*; ING241) in patients with advance cancers resulted in clinically significant responses in 44% of treated patients (Fisher et al., 2003, 2007; Cunningham et al., 2005; Tong et al., 2005).

Elimination of a primary tumor is rarely adequate to obtain a complete cancer cure, since during cancer progression malignant tumor cells can spread and localize in multiple sites throughout the body rather than in a single tissue. Due to the limitation of diagnostic approaches to locate metastatic lesions (especially when in internal organs) and difficulties in efficient and selective drug delivery, metastatic disease is the most intractable clinical problem and a major cause of cancer-associated deaths. Ideally, a secretory therapeutic protein, a cytokine, which displays autocrine/paracrine functions and cancer-specific targeted toxicity, can remedy this situation (Pestka et al., 2004). The most intriguing property of MDA-7/IL-24 is its ability to evoke an autocrine/paracrine loop that allows it to be secreted from a primary target cell, normal or cancer, where it was introduced and to induce growth inhibition and apoptosis selectively in surrounding and in distant non-injected cancer cells (Fisher, 2005; Su et al., 2005a; Sauane et al., 2006, 2008). A series of experiments were performed in which tumor xenografts were implanted in both flanks and adenoviruses carrying *mda-7/IL-24* were delivered in tumors in a single flank (Emdad et al., 2009; Dash et al., 2010a; Menezes et al., 2014). Initial pre-clinical tumor xenograft studies were extremely provocative, since both the treated tumors and non-treated tumors significantly regressed indicating potent “bystander” anti-tumor activity in vivo (Fisher, 2005). In addition to direct cancer-specific killing and “bystander” antitumor activities, *mda-7/IL-24* also inhibits tumor angiogenesis (Ramesh et al., 2003; Lebedeva et al., 2007) and promotes tumor immune responses (Gao et al., 2008), making it a very attractive therapeutic gene for cancer therapy (Menezes et al., 2014).

Obstacles to selective cancer therapy

It is now considered axiomatic that a combinatorial approach is more favorable as compared to a single therapy in achieving a clinically beneficial effect. Accordingly, to maximize MDA-7/IL-24-mediated therapeutic outcomes, we have applied two strategies: (a) conditionally cancer-specific oncolytic viruses armed with *mda-7/IL-24*, known as “Cancer

Terminator Viruses” or CTV (Sarkar et al., 2005a, 2006, 2007b, 2008; Das et al., 2012); and (b) by using *mda-7/IL-24* gene therapy in combination with conventional chemo- and radio-therapy (Sarkar et al., 2006; Dent et al., 2010; Menezes et al., 2014). In the CTV, cancer-specific oncolysis is stringently maintained using a cancer-specific promoter derived from progression elevated gene-3 (*PEG-3*) (Su et al., 1997, 2005b), and maximum production of MDA-7/IL-24 is ensured by using an efficient ubiquitously expressing promoter CMV (Sarkar et al., 2006). Pre-clinical animal modeling studies demonstrated the efficacy of the CTV in inducing tumor-specific oncolysis with MDA-7/IL-24-mediated anti-tumorigenic and “bystander” anti-cancer activity in different solid human tumors including breast (Sarkar et al., 2005a), prostate (Sarkar et al., 2007b), melanoma (Sarkar et al., 2008) and glioblastoma multiforme (Hamed et al., 2013b). The most important finding was a complete elimination of the primary treated tumor as well as the distant non-treated tumor (established in the opposite flank), thereby implementing a cure in these immune incompetent animals. In the second approach, where *mda-7/IL-24* gene therapy was combined with conventional therapy including radio/chemo-therapy, synergistic effects were evident. For example, in non-small cell lung carcinoma, MDA-7/IL-24 enhanced the efficacy of ionizing radiation and induced cell death (Kawabe et al., 2002; Nishikawa et al., 2004a,b). In another study, Chada et al. (2006) demonstrated that this combination improved the overall sensitivity of breast cancer cells to chemotherapy, biologic therapies and radiotherapy. In addition to radiation therapy, various chemotherapeutic agents are also efficiently used in conjunction with *mda-7/IL-24*. For example, Ad.*mda-7* when delivered with gefitinib, an EGFR inhibitor enhanced apoptotic death in non-small cell lung cancer by increasing the expression of a downstream effector molecule, RNA-activated protein kinase (PKR) (Emdad et al., 2007). Combinatorial effects were also observed when MDA-7/IL-24 was combined with Temozolomide in melanoma (Zheng et al., 2008) and glioblastoma (Germano et al., 2010) or HDAC inhibitors in renal carcinoma (Hamed et al., 2013a) and glioblastoma multiforme (Hamed et al., 2013b). In pancreatic cancer, perillyl alcohol (POH) and D-limonene enhanced conversion of *mda-7/IL-24* mRNA into protein resulting in significant cancer cell death both in vitro and in vivo (Su et al., 2001; Lebedeva et al., 2008a,b; Sarkar et al., 2013). Effective combinations were also demonstrated recently using MDA-7/IL-24 with a MCL-1-targeted small molecule inhibitor Sabutoclax in prostate and colon cancer (Dash et al., 2010c, 2011a; Azab et al., 2014) and an AKT inhibitor in breast cancer (Pal et al., 2014).

Methodologies ensuring enhanced cancer-specific delivery and expression

Despite successes using non-viral delivery of *mda-7/IL-24* (Ramesh et al., 2004; Hingtgen et al., 2012; Gu et al., 2014), the majority of MDA-7/IL-24-related research has employed viral vectors, particularly Adenovirus serotype 5 (Ad.5). As discussed previously, the utility of Ad.5 serotype is limited in the clinic due to its dependency on surface expressed CAR for virus uptake, which often are not abundantly expressed in tumors. To overcome this obstacle and expand the host range and enhance efficiency of infectivity, Dash et al. (2010b) produced a chimeric tropism modified adenovirus (a chimeric Ad.5 with an Ad.3 modified fiber, Ad.5/3) to deliver *mda-7/IL-24*. This modified virus transduced *mda-7/IL-24* more efficiently in low CAR human prostate cancer cells grown as tumor xenografts (Dash et al., 2010b) and in a spontaneous Hi-myc prostate cancer transgenic animal model (Dash et al., 2011a). Subsequent studies confirmed the superiority of Ad.5/3.*mda-7* over Ad.5. *mda-7* in

other cancer cells with low CAR including glioblastoma multiforme (Hamed et al., 2013b), renal carcinoma (Eulitt et al., 2010; Park et al., 2011), colorectal carcinoma (Azab et al., 2012) and breast carcinoma (Pal et al., 2014). Additionally, this modification enhanced the infectivity of the CTV, where the tropism modified CTV (Ad.5/3-CTV) displayed better transduction efficiency at a lower multiplicity of infection (m.o.i.) in various low CAR human cancer xenografts than the first generation Ad.5-CTV (Sarkar et al., 2013; Hamed et al., 2013b; Azab et al., 2014).

Even though tropism modified adenoviral-mediated *mda-7/IL-24* can induce apoptosis in diverse human cancers, it is apparent that the applicability of this therapeutic virus is restricted in a clinical context. Although the Ad.5/3-CTV displays enhanced efficacy and infectivity against a broad spectrum of human tumors, its applications clinically are limited when used systemically because of non-specific trapping in the liver, immune clearance and the limited options to efficiently deliver the virus to internal organs such as prostate, pancreas, kidney, etc. The multiple obstacles in using the Ad.5/3-CTV systemically are not unique to this virus, since these same problems are endemic to many viral-based therapeutic approaches. To circumvent these hurdles, a potentially useful approach employs MBs and ultrasound, i.e., ultrasound-targeted microbubble-destruction (UTMD) (Dash et al., 2011b; Das et al., 2012; Bhatia et al., 2013). This approach limits trapping of viruses in liver, shields the viruses from the immune system and permits release of the viruses in the tumor microenvironment and directly in internal organs as well as malignant tissues (Greco et al., 2010; Dash et al., 2011a,b; Das et al., 2012; Bhatia et al., 2013). UTMD was employed to target the release of a therapeutic virus (Ad.5/3.*mda-7*) at the tumor site (prostate gland) in immunocompetent Hi-Myc mice, a spontaneous prostate cancer transgenic mouse model which develops localized adenocarcinoma by 6 month of age (Dash et al., 2011a). MB loaded Ad.5/3.*mda-7* was injected intravenously three times per week for four weeks and ultrasound was used to ensure prostate-specific delivery. Target-specific delivery was confirmed by demonstrating the presence of MDA-7/IL-24 protein in tumor sections by immunohistochemistry (Dash et al., 2011a). Additionally, the study also emphasized the therapeutic potential of *mda-7/IL-24* gene therapy in combination with chemotherapy, i.e., an MCL-1 inhibitor (Sabutoclax). In a follow up study, Azab et al. (2014) used the UTMD approach to deliver Ad.5/3-CTV to Hi-Myc mice and confirmed and expanded on the previous findings of Dash et al. (2011b) using Ad.5/3-*mda-7* (a non-replicating adenovirus expressing *mda-7/IL-24*). These studies suggest that the UTMD approach can be used to selectively and efficiently deliver therapeutic genes (e.g., *mda-7/IL-24*) in internal target organs.

Gene therapy: Successes in the clinic

In the past two decades, fundamental and seminal advances in molecular biology and genetic engineering have provided opportunities for developing various gene therapies, however, in the vast majority of cases cancer is not cured using these approaches. However, a couple of successes resulting in long-term survival provide optimism that this approach might still fulfill its promise in the future. Below, we briefly describe a few gene therapy concepts that have successfully translated from the bench to the bedside.

Gene therapies based on *p53*, a gene that is defective in many cancers, have been and are currently the subject of various clinical trials worldwide. In 2001, ONYX-015, also known as *dl1520*, a genetically modified Ad lacking a 55 kd gene in the *E1B* region (to restrict viral replication to *p53* mutant cells), was tested in patients with non-small cell lung carcinoma (Ahrendt et al., 2003). In Phase I clinical trials, the virus was well tolerated and partially effective in promoting tumor shrinkage, however the response rate was not as profound as anticipated from pre-clinical studies. Three out of fifteen patients showed a partial response. In a follow up Phase II clinical trial, two cycles of intratumoral injections resulted in a 28% response rate in the treatment cohort as compared to 12% response rate in the control group. In the Phase III clinical trial, ONYX-015 administered alone or in combination with a chemotherapeutic, showed a 72.7% response rate as compared to a 40.4% response in the control group. In a different clinical study, a prostate specific therapeutic adenovirus, CG7870 developed by regulating viral replication under a prostate cancer-specific promoter, was used. However, no objective clinical responses in terms of tumor regression were observed although five patients showed 25–49% reduction in their serum PSA levels (Reid et al., 2002).

The first commercialized gene therapy, Gendicine™, a recombinant adenovirus engineered to express wild type-*p53* under a *Rous sarcoma virus* promoter was used to treat head and neck squamous cell cancer (Raty et al., 2008). In a Phase III clinical trial (132 patients were enrolled), 64% of patients responded with complete regression when the virus was used in combination with radiotherapy. However, the published details of this clinical trial are limited. A similar product, Advexin® (INGN 201; originally produced by Introgen Therapeutics, Inc., Austin, TX) was also evaluated for clinical efficacy in various cancers and the results were reviewed (Gabrilovich, 2006).

In addition to *p53*, other tumor suppressor genes such as tumor necrosis factor alpha, TRAIL, interleukin-2, and *mda-7/IL-24* genes have also shown some promise in clinical trials. In 2005, studies published by Cunningham et al. (Cunningham et al., 2005) and Tong et al. (Tong et al., 2005), reviewed in Lebedeva et al. (Lebedeva et al., 2005) and Fisher et al. (Fisher et al., 2007), demonstrated that a replication incompetent adenovirus expressing *mda-7/IL-24*, Ad.*mda-7* (INGN-241), was well tolerated clinically and effective in patients with advanced cancers (both carcinomas and melanomas). Multiple intratumoral injections of Ad.*mda-7* induced apoptosis, which was closely correlated with MDA-7/IL-24 expression, and resulted in clinically significant responses (greater than 50% shrinkage of injected lesion) in 44% (2 patients out of 5 patients) without provoking any toxicities suggesting that this therapeutic is safe as well as efficacious. Interestingly, apoptosis demonstrated in patient tumors receiving MDA-7/IL-24 was significantly greater than what was observed following treatment with Ad.*p53* in non-small-cell lung cancer. The most dramatic response was observed in a 64-year old woman with metastatic melanoma. A less dramatic response was seen in another patient with squamous cell carcinoma of the penis in which one of several skin nodules were injected. Similar to preclinical studies (Fisher, 2005; Su et al., 2005a; Gao et al., 2008; Sauane et al., 2008), potent “bystander” antitumor and immune modulatory activity was also evident in clinical trials.

Gene directed enzyme pro-drug therapy relies on the conversion of non-toxic substances (pro-drug) into physiologically active agents. Most frequently, HSV-TK has been used to convert the pro-drug ganciclovir into the cytotoxic triphosphate ganciclovir. Utilizing this approach, the Anglo Finnish Company Ark Therapeutics developed Cerepro, an adenovirus containing HSV-TK under cytomegalovirus promoter to treat malignant glioma. Cerepro demonstrated a significant survival benefit when compared with patients treated with retroviral therapy or standard therapy. The adverse effects were mostly limited to transient fever. The findings from a Phase III clinical trial using Cerepro (sitimagene, ceradenovec) have recently been published (Westphal et al., 2013).

Concluding remarks

Currently, the success of cancer gene therapy is lagging behind that obtained in the treatment of monogenetic diseases. Although cancer is a genetic disorder, the abnormalities are generally polygenic and genetic variation between individuals or even tumors at different sites within the same patient are substantial. The major reasons for failure or limited success of cancer gene therapy are not only technical, which we have discussed in this review, but also related to ethical, policy and financial issues. The fear of insertional mutagenesis also raises further health risk concerns. The possibility of passing the genetic changes onto offspring is an argument used against human cancer gene therapy involving approaches that result in integration into the genome. Unlike chemotherapies, gene therapies are only effective in a subset of patients with any given cancer, making each gene therapy agent an orphan drug. Moreover, failure to target metastatic cells, the major driver of cancer-associated mortality has limited the general utility of cancer gene therapy. Targeting of metastases using gene therapy approaches has proven inefficient for many reasons including genetic and epigenetic heterogeneity, development of resistance and difficulty in locating metastases, which can frequently be disseminated throughout the body. Ideally, gene therapy should provide a means of treating primary and disseminated tumors, with a minimal effect on normal cells. This problem can be resolved by using a gene product with the ability to induce its own translation in a cancer cell-specific manner or stimulate the immune system to prevent colonization to a distant site. We are optimistic that as the field of gene therapy continues to advance, current impediments to effective systemic therapy, such as non-specific targeting, trapping in organs such as the liver, and neutralization by the immune system, will be overcome leading to further triumphs in treating cancer. Moreover, combinatorial approaches using gene therapy with chemotherapy, small molecule inhibitor therapy, radiation therapy and/or immunotherapy will lead to further incremental increases in the efficacy of this approach for the treatment of diverse human cancers.

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Fig. 1.
The timelines and milestones in developing gene therapy approaches: from conception to clinical applications.

Fig. 2.

Progression timelines for *mda-7/IL-24* development as a therapeutic gene: from “bench to bedside.” After discovery, multiple approaches were used to enhance therapeutic outcome of *mda-7/IL-24*-mediated gene therapy of cancer.

TABLE I

Overview of general characteristics of viral vectors commonly used in cancer gene therapy

Virus	Family	Genome/insert size/particle size/envelope	Advantages	Disadvantages
Adenovirus	Adenoviridae	dsDNA 36 kb/7.5 kb/90 nm/No	Infects dividing and non-dividing cells; low toxicity to host cells; efficient gene transfer; high-viral titers achievable.	Small insert size; immune response decrease infectivity of desired target cells.
Herpes simplex virus	Herpesviridae	dsDNA, 152 kb/40–50 kb/120–200 nm/Yes	Infects dividing and non-dividing cells; prolonged gene expression achievable; large insert size for transgene; high-viral titers achievable; sensitive to acyclovir/ganciclovir.	Immune response of desired target cells; potential for herpes encephalitis.
Retrovirus	Retroviridae	ssRNA, 7–1 kb/8 kb/10 nm/Yes	High-transduction efficiency; easy to design; chronic infection; integration into host genome resulting in prolonged gene expression; viral proteins not expressed in the host, thereby limiting the chances of reconstitution of replication-competent viruses.	Infects dividing cells only; low titers; random site of integration; possibility of latent diseases, including cancer and immune-mediated diseases.
Adeno-associated virus	Paroviridae	ssDNA, 5 kb/5 kb/20 nm/No	Infects dividing and non-dividing cells; lack of pathogenicity; nontoxic to host cells; stable integration into the host cells at a specific site in human chromosome 19.	Low insert size; provokes humoral immunity on infection; large-scale production is labor intensive.

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TABLE II

Different gene types delivered in gene therapy clinical trials

Gene type	% of Gene therapy trials
Antigen	20.5
Cytokine	18.4
Tumor suppressor	8.3
Suicide	8.1
Deficiency	8
Growth factor	7.5
Receptor	7.2
Replication inhibitor	4.3
Marker	2.9
Other categories	14.7