

# Gene Therapy: The First Approved Gene-Based Medicines, Molecular Mechanisms and Clinical Indications

J.K. Rätty\*<sup>1,3</sup>, J.T. Pikkarainen<sup>1,3</sup>, T. Wirth<sup>1,3</sup> and S. Ylä-Herttuala<sup>1,2</sup>

<sup>1</sup>Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, <sup>2</sup>Department of Medicine and Gene Therapy Unit, University of Kuopio, FIN-70211 Kuopio, Finland and <sup>3</sup>Ark Therapeutics Oy, Neulanientie 2 L9, FIN-70210 Kuopio, Finland

**Abstract:** As gene therapy has matured from clinical trials to the first commercial products, understanding of the mechanisms of gene delivery has increased tremendously. This has also been reflected in viral vector development, creating a number of new approaches to tackle issues in transduction efficiency, biodistribution and viral safety. This review will highlight the most important issues and advancements in vector development, administration, surface modification, integration to host genome and safety. The gene therapy products currently available or near market approval, based on p53 expression (Gendicine™ and Advexin™), conditionally replicative adenoviruses (Oncorine™) and thymidine kinase + ganciclovir therapy (Cerepro®), are introduced with emphasis on the molecular mechanisms of action.

**Keywords:** Adenovirus, p53, conditional replication, thymidine kinase.

## INTRODUCTION

Gene therapy has been defined as the transfer of nucleic acids to somatic cells of a patient to result in therapeutic effect [1]. As compared to the traditional medicine, gene therapy offers unique possibilities to treat the genetic causes of diseases, such as fatal enzyme deficiencies. In addition, the use of gene therapy as a supportive method along with traditional treatments has gained increasing attention, for example in the treatment of malignant glioma [2]. Currently, the first gene based products have entered the market: in 2003 SiBiono GeneTech: Gendicine™ and in 2006 Shanghai Sunway Biotech: Oncorine™, hopefully facilitating further research and commercialization. Both of these products are based on adenoviral vectors, reflecting the fact that because of their efficiency over non-viral systems, the majority of the gene therapy clinical trials have been performed with viral vectors. The following sections will introduce possibilities to enhance properties of gene delivery vectors for future clinical needs.

## VECTOR ADMINISTRATION

Ideally, a gene therapy vector would target a specific tissue with high transduction efficiency and sustain a stable, regulated gene expression without any side effects or immunogenic responses. Currently, none of the most commonly used gene delivery vectors, introduced in Table 1, fulfill all these criteria. Yet, vectors and their administration methods can be extensively modified to decrease the injected dose and increase the transduction efficiency.

To improve patient safety and increase the gene transfer efficiency, the target cells can be removed from the patient, transduced with viral vectors and re-introduced into the patient [3, 4]. This *ex vivo* method increases the physical

proximity and limits the cellular target population thus increasing vector concentration and minimizing immunogenic responses. However, the method is limited to the cells which are available either by extraction or by growing from the stem cells *in vitro*, making it suitable for tissue engineering applications [5]. Excluding the *ex vivo* approach, the vectors need to be directly injected into the patient. Selection of the injection method also enables primary tissue targeting, as used with intracranial injections to the brain cavity after surgical removal of a tumor [6, 7]. Due to the limited diffusion of viral particles to the brain parenchyma, multiple injections with a small viral volume result in a higher overall transduction efficiency than a single injection with a larger volume. Similarly to multiple injections, a gene gun ejecting gold particles carrying plasmid DNA can penetrate with high velocity to cells in a wide area [8] but the transfection depth remains only a few millimetres [9]. Due to limitations in preparation of the ammunition, this method can only be used for plasmid DNA which has a poor overall transduction efficiency *in vivo*. However, the gene gun method has been used with success to transfer plasmids to foetuses [10] and in vaccination trials [8]. In cardiovascular gene therapy, catheters provide an alternative to surgery with a limited invasiveness and high targetability [11]. Catheter-based systems are also widely used in the clinic, providing a solid technical background for vector administration.

In addition to injections, gene delivery vectors can be delivered and retained in target areas with vector reservoirs, such as the periarterial collar used for gene delivery of vascular endothelial growth factor to the carotid artery of rabbits [12, 13] or a polymer capsule which may reduce vector immunogenicity [14]. Similarly, anatomical features, such as synovial capsules or the eye can be used to limit the escape of vectors [15].

In addition to the commonly used administration methods, magnetism has been utilized for gene delivery *in vitro* and *in vivo*. The use of paramagnetic particles enables physical concentration of the vectors *via* an applied magnetic field [16-19]. This has resulted in increased transduction efficiencies *in vitro* [20, 21] and better targeting [22, 23]. However,

\*Address correspondence to this author at the A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland; Tel: +358-17-162075; Fax +358-17-163751; E-mail: jani.ratty@uku.fi

**Table 1. Most Common Gene Delivery Vectors**

Vector	Advantages	Limitations	Reviewed in
<i>Adenoviridae</i> / Adenovirus	High efficiency Transduces quiescent and dividing cells >30 kb transgene capacity Easy to produce in high titers	Coxsackie adenovirus receptor-dependent transduction Immunogenic Existing humoral response to certain serotypes	[104]
<i>Retroviridae</i> / Murine leukaemia virus	Broad tropism Low immunogenicity Stable integration	Insertional mutagenesis Unable to transduce quiescent cells Inactivation by serum	[105]
<i>Retroviridae</i> / Lentivirus	Low immunogenicity Stable integration to quiescent cells	Insertional mutagenesis Potential risk of recombination of pathogenic vector (HIV)	[106]
<i>Parvoviridae</i> / Adenoassociated virus	Transduces quiescent and dividing cells Very long expression time Non-pathogenic, low immunogenicity Broad tropism	Very small transgene capacity Insertional mutagenesis may be problem	[107]
<i>Alphaviridae</i> / Semliki forest	High titer Broad host range Efficient transgene expression	Low transgene capacity Highly cytotoxic Short term expression	[108]
<i>Alphaherpesviridae</i> / Herpes simplex-1	Broad host range, High titer, Large capacity	Latent wild type-viral activation risk, Antigenic	[109]
<i>Baculoviridae</i> / Autographa californica multi-capsid nucleopolyhedrovirus	High titer Large transgene capacity Easy production Non-pathogenic	Limited transduction Production in insect cells Unstable genome	[110]
Non-viral vectors	Easy preparation, non-pathogenic	Limited transduction Bacterial contaminants	[111]

while *in vitro* a high magnetic field gradient of a few millimeters is sufficient to draw the magnetic particles into the monolayer cells, *in vivo* such a depth would be inadequate. In some experiments the typical depth of magnetic field of 0.1 T has been increased to 10 cm resulting in first pass targeting and retention for six days [24]. In any event, magnetic materials have been promising in several clinical studies including hyperthermia and magnetic drug targeting [25]. Although the idea of a highly specific 3D magnetic targeting *in vivo* is tempting, due to the properties of magnetic fields lateral targeting [26], localization to organs near skin [24, 27] and retention into the extremities [28, 29] have been the most feasible applications.

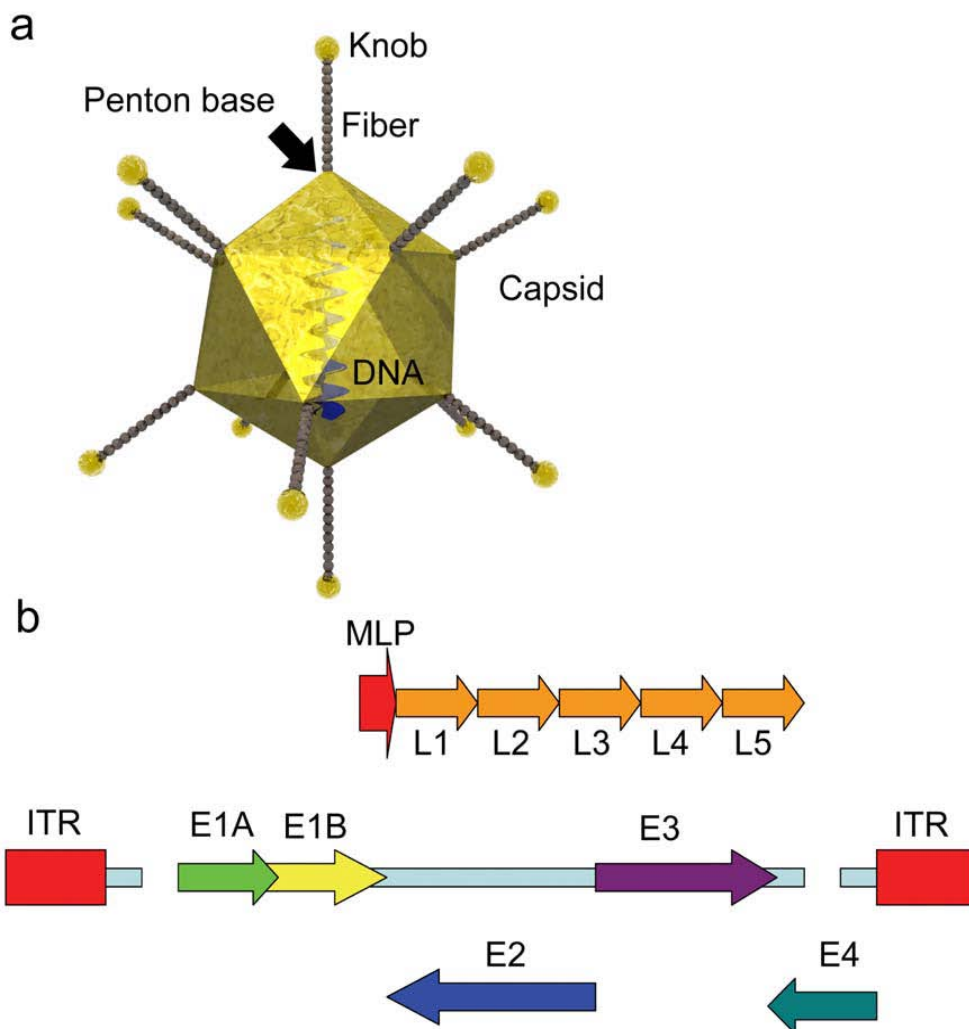
## VIRAL SURFACE MODIFICATIONS

The ability of viruses to transduce different cell types, cause immunological responses or resist complement activation depends greatly on the viral surface proteins. In order to influence the interactions between the virus and cell surface, the viral surface proteins can be modified, removed or replaced. Retroviruses and lentiviruses are frequently pseudotyped to widen their tropism, increase their yield in production and improve their safety, most often with *Vesicular Stomatitis* virus G-protein [30].

While changing the glycoproteins on the viral envelope is feasible, non-enveloped viruses require different ap-

proaches. Transduction of serotype 5 adenovirus is dependent on the adenovirus fiber knob protein (Fig. 1) binding to the coxsackie-adenovirus receptor expressed on the target tissue (Fig. 2). However the amount of this receptor is often low in tumour tissues, limiting the adenovirus transduction efficiency in various cancer cell types [31]. Therefore, various approaches have been utilized to increase their transduction efficiency [32]. Among the most successful have been the insertion of targeting peptides to the HI loop of the adenovirus fiber [33], binding moieties to the capsid [34] and chemical alteration of the capsid proteins [35]. Combinations of these techniques have also been used [36]. However, with the genetic methods the major limiting factor for recovering viable adenoviruses is the correct folding of the modified fiber. An incorrect folding of the fiber results in a dramatic decrease in the viral titer and therefore reduces transduction efficiency [37]. Adenoviruses chemically coupled with polyethylenglycol resulted in a reduced binding of neutralizing antibodies [38] and increased half-life after a systemic administration [39]. Adenoviruses have also been modified to block the binding to the native coxsackie-adenovirus receptor, resulting in lower toxicity and enabling targeting the virus to different cell types [40].

As a compromise between flexible chemical surface modification and robust genetic modification, display systems enabling coating of the virus with a wide selection of ligands have been introduced. Baculoviruses and adenoviruses displaying a synthetic immunoglobulin G binding



**Fig. (1).** Schematic representation of adenovirus structure.

**a)** Adenovirus consists of an icosahedral capsid (from 70 to 100 nm) with 240 hexons and 12 penton base proteins. Each penton bears a fiber and knob, which contribute to the viral tropism to coxsackie- adenovirus receptor. Inside the viral capsid is a double-stranded linear DNA with associated 55 kDa terminal proteins.

**b)** The ~36 kbp adenovirus genome is flanked by inverted terminal repeats (ITR). It contains five early functions (E1A to E4) associated with the first steps of viral replication and late functions (L1 to L5) which are driven by the major late promoter (MLP) and encode the viral structural proteins.

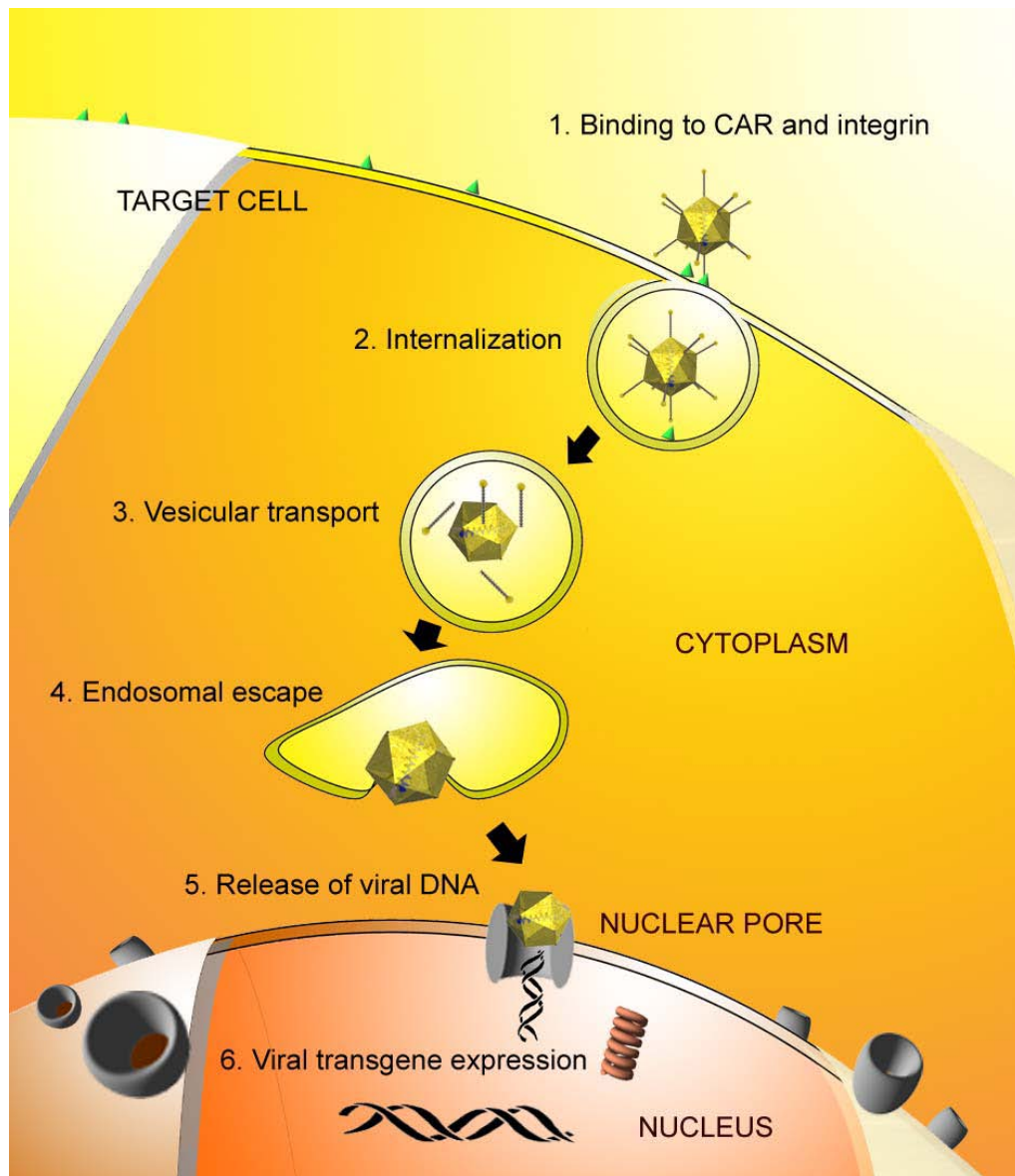
domain of protein A have been constructed and shown to be functionally active [41, 42]. These vectors enable a display of antibodies on the viral surface enabling the use of a wide selection of tissue-specific antibodies. Another method takes advantage of soluble crosslinking reagents, such as bispecific antibodies to provide a molecular bridge between the vector and targeting ligand [43].

Metabolically biotinylated vectors further widen the selection of different molecules by using avidin as a crosslinking reagent [34, 44]. Similarly, the display of avidin enables the use of different biotinylated ligands for an enhanced transduction, targeting and viral imaging [21, 44, 45]. While various capsid modifications for adenoviruses have been published, none of these modifications have yet entered

the market due to the lengthy approval process of gene medicine products.

### VECTOR IMMUNOGENICITY AND INTEGRATION

Currently adenoviruses are the dominant gene delivery system in gene therapy. During vector development, the genes responsible for viral replication are the first to be removed to increase patient safety and the vector payload. The first generation adenoviruses have E1 or E1 and E3 deletions while the E2 and/or E4 deletions produced the second generation adenovirus vectors (Fig. 1). Currently the third generation consists of helper-dependent (gutless) adenovirus vectors, where all the viral replication genes have been re-



**Fig. (2).** Adenovirus transduction of target cell. CAR denotes coxsackie-adenovirus receptor.

moved to provide maximal capacity for transgenes (>30 kb). For latest review, see [46].

As adenoviruses are known to be highly immunogenic, limiting factors in their use are the innate and adaptive immune reactions; including antibodies from previous exposure to wild type adenoviruses, detectable in 97% of individuals [47]. Over 50 serotypes, divided into six subgroups (A-F), have been identified [48], of which serotypes 2 and 5 are the most common [49]. Adenoviral vectors used in clinical trials belong to serotypes 2 and 5. The presence of pre-existing immunity is a major factor decreasing the treatment efficiency [50] although in some cases the inflammatory responses may contribute to the treatment efficiency, such as in the treatment of malignant glioma, where the immune activation might intensify the elimination of the malignant cells [2, 6]. The first death related to gene therapy, a patient suffering from ornithine transcarbamylase deficiency, was at least partly due to the highly immunogenic nature of adeno-

virus, although the high dose of the vector ( $6 \times 10^{13}$  vp) together with a liver injection *via* the hepatic artery and the poor overall condition of the patient made a major contribution to the fatal outcome [51].

While the adenovirus transduction is transient, diminishing in a few weeks, retroviral and lentiviral vectors integrate their transgene into the host genome, resulting in enduring expression, unless the viral promoter is silenced [52]. It has been observed that the retroviral integration occurs often at an actively expressed site, presenting a possible threat to patients in the form of promoting uncontrolled cell division. An analysis of the adverse effects, i.e. leukaemia in X-linked severe combined immune deficiency trials using haematopoietic stem cells, has sparked considerable [53-55]. As a possible solution to the safety issues, it has been proposed that integration of the retroviral DNA could be directed to a predetermined site or chromatin insulators could isolate the viral promoter [56]. However, a study using a fusion protein

consisting of a HIV-1 integrase and a DNA-binding protein showed that random integration still occurs *in vitro* [57]. The advantage of lentivirus vectors to transduce non-dividing cells has led to the development of integrase-defective lentiviral vectors resulting in two months expression time *in vitro* [58] while still remaining efficient *in vivo* [59]. Additionally, transposon systems, such as *Sleeping Beauty* [60], provide methods to achieve integration and long-term expression without viral elements. Another possibility to avoid adverse effects deriving from integration into harmful sites could be targeting the integration into those actively transcribed sites in the genome, which are often related to fundamental cellular processes [61], but exist with a high copy number and use insulator elements [62] to restrict promoter activity only to viral transgene expression.

To further specify the target cells for gene expression, tissue-specific promoters and transcriptional activators, such as hypoxia-inducible factor, can be used to express transgenes in selected tissues [63, 64]. This method is often used as such to compensate for the lack of viral vector cell specificity, allowing the vector to penetrate into various cell types and yet express the desired transgene only in selected cells. As a drawback, the tissue-specific promoters and metabolic factors are often weaker than constitutive promoters, such as the cytomegalovirus immediate/early promoter. However, the tissue-specific promoters can be modified to result in stronger gene expression, for example by introducing a positive feed-back loop by using transcriptional activators [65] or by adding stabilization elements for labile mRNA, such as Woodchuck hepatitis virus posttranscriptional enhancer [66]. Remarkably, transient baculoviruses with the tissue specific glial fibrillary acidic protein/ cytomegalovirus hybrid promoter resulted in 90 day transgene expression, by which time the regular cytomegalovirus immediate/early promoter derived expression was already undetectable [67].

### p53 FOR CANCER TREATMENT

Several gene therapy approaches have been directed to treat cancer as it is one of the leading causes of death in Western countries with often poor prognosis. Several cancer types have been targets for new therapeutics approaches, based on the molecular differences between cancer and normal cells.

One such difference is a mutated p53 protein which is the most frequent alteration in human tumors, occurring approximately in 50 % of cancers [68] and contributing to tumor resistance to a variety of chemotherapeutics [69]. p53 maintains genetic integrity after DNA damage and functions as a gatekeeper of cellular growth, apoptosis and senescence. Normally cellular p53 exists in low concentrations and is relatively inactive, but during cellular stress the p53 amount is increased and the protein is activated. Depending on the cellular environment, the activation of the protein leads to either a cell cycle arrest or an apoptosis. In cancer therapy, several p53-approaches have been developed: introduction of small molecules reactivating the remaining wild type p53 activity in cells [70], reversion of the mutant p53 to the wild type conformation [71] and introduction of the *p53* gene by gene therapy e.g. in non-small cell lung cancers [72].

Recently, several studies have indicated that the restoration of p53 function in several different cancers induced growth arrest or obliterated the tumors (schematic mechanisms presented in Fig. 3). Ventura *et al.* observed that the restoration of p53 expression induced an apoptotic cell death in lymphomas and a cell cycle arrest in sarcomas in conditional *p53*-knockout mice, reflecting differences in cellular mechanisms in these tumours [73]. In hepatocellular carcinoma, transient p53 expression was enough to cause complete tumor regression by a combination of a cell cycle arrest and an induction of innate immune system activity in athymic nude mice [74]. However, Martins *et al.* observed that while the restoration of p53 increased the survival of E $\mu$ -myc lymphoma mice, the tumours developed alternative pathways to inactivate p53 [75]. Altogether, these studies suggest that p53 gene therapy could be useful in the treatment of various tumors.

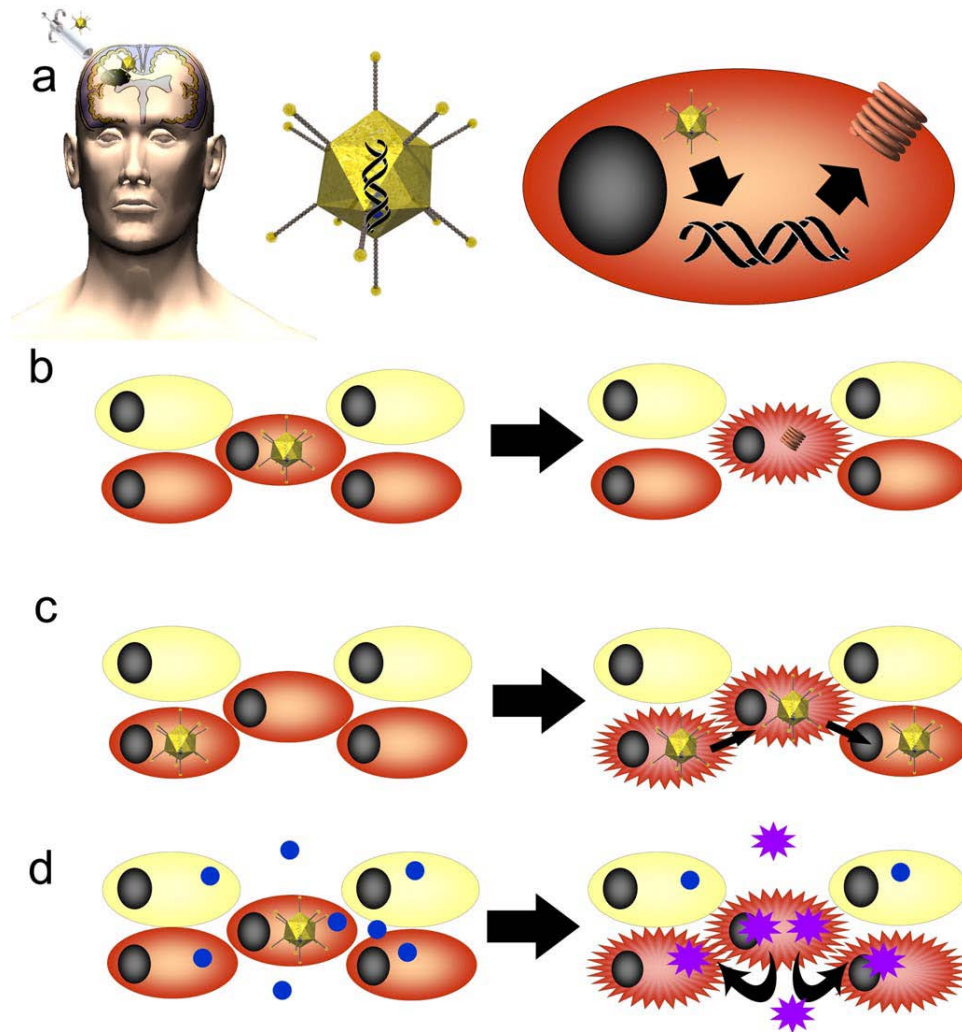
The first gene therapy virus approved for clinical use in China (2003), Gendicine™ (from Chinese Shenzhen SiBiono Genetechnologies) is a p53 adenovirus for the treatment of head- and neck squamous cell cancer in combination with radiotherapy [76, 77]. A similar product: Advexin™ (INGN 201; Introgen; Austin, TX, USA) [78] is pending approval from EMEA [79]. The approval of Gendicine™ by the Chinese SFDA, has lead to discussion about the efficacy of the treatment [80, 81].

The replication-incompetent adenovirus contains the *p53* transgene in place of the viral E1 region (Fig. 1b), under a Rous sarcoma virus promoter together with a bovine growth hormone poly(A)-tail, produced in a bioreactor using human embryonic kidney cells and purified with chromatographic methods. In a phase I clinical trial with 12 laryngeal cancer patients, only one patient experienced self-limited fever and none of the patients had tumor relapse during the 5 year follow-up after the treatment [82]. Similarly in phase II/III trials with 132 head and neck squamous cell carcinoma patients, 32% showed fever as the only side-effect of the treatment. When Gendicine™ was used in combination with radiotherapy, 64 % of the patients responded with a complete regression and 29 % with a partial regression while with radiotherapy alone, 19 % showed a complete regression and 60 % a partial regression, suggesting a synergistic effect of the combination treatment [76]. The tumor regression was evaluated by tumor shrink rates according to WHO response criteria [76, 83]. However, the published details of these clinical trials are limited, making comparisons to other cancer trials difficult [80].

Due to the various roles of the p53 protein, the authors suggested that the results derive from the role of p53 in sequence-specific transcription regulation inducing apoptosis in the nucleus, mitochondria and Golgi apparatus; activating the immune system, inhibiting DNA repair and downregulating drug resistance-genes and vascular endothelial growth factors [76].

### ONCOLYTIC CANCER THERAPY

In Gendicine™ and Advexin™, the deletion of the viral E1 gene results in a non-replicative virus. However, by deleting the adenovirus *E1B 55K* gene coding region (Fig. 1b),



**Fig. (3).** A schematic representation of different approaches in currently available gene therapy agents. Cancer cells in darker shade, apoptotic cells with rugged surface.

**a).** Gene delivery starts with administration of the viruses, which carry a therapeutic gene. In the target cells, viruses release the therapeutic genes, which are then processed by the host cells and translated into therapeutic protein, causing the desired treatment effect.

**b).** In p53 therapy, therapeutic gene is a tumor suppressor gene *p53* whose deactivation is known to promote cancer. Reintroduction of p53 protein to cancer cells suppresses cancer activity, prevents cell growth and proliferation and induces apoptosis. However the effect is limited only to the transduced cells.

**c).** Conditionally replicating adenoviruses contain alterations in their genome, which direct virus replication only to p53- deactivated cells. After the initial transduction, replication continues into neighbouring p53-deficient cells, increasing the treatment effect and reducing the initial amount of virus needed to obtain a therapeutic effect.

**d).** With thymidine kinase gene therapy, the ganciclovir prodrug is administered (ball) when the thymidine kinase expression levels peak. The enzyme converts ganciclovir to a guanine analogue (star), which will be incorporated in cellular DNA, thus preventing cell proliferation and promoting apoptosis in actively dividing cancer cells. When cells die, guanine analogues are released into neighbouring cells and spread the effect (bystander effect), increasing treatment efficacy. Also, uptake of the prodrug *via* gap junctions helps to spread the treatment effect to the neighbouring cells.

the ability of the virus to bind and inactivate wild-type p53 protein is restricted [84]. Inactivation of the host cell p53 is essential for wild-type adenoviruses to disable the activation of the apoptotic pathway when the host cell shifts to S phase in the lytic infection. When E1B 55K activity is removed, replication in normal cells is blocked, allowing only replication in p53-deficient cells. In malignant cells the viral proliferation leads to oncolysis, used as a cancer therapy to treat

solid tumours. Although replication increases the concentration of viruses in target tissue, the viruses still suffer from neutralizing antibodies and dependency on the coxsackie-adenovirus receptor. Therefore, several modifications of oncolytic viruses have been constructed, [85], including alterations in viral pathways (e.g. [86]), transductional targeting (e.g [87]) and transcriptional targeting (e.g [88]). In addition to oncolytic adenoviruses, there are a number of other vi-

**Table 2. Some Naturally Oncolytic Viruses**

Oncolytic Viruses	Advantages	Limitations	References
<i>Paramyxoviridae</i> / Measles virus	Oncolytic	Pathogenic	[112, 113]
<i>Paramyxoviridae</i> / Newcastle disease virus	Non-pathogenic in humans, moderate efficiency, oncolytic	Unclear mechanism, not well studied, non-recombinant viruses used	[114, 115]
<i>Paramyxoviridae</i> / Mumps virus	Oncolytic	Pathogenic	[116]
<i>Parvoviridae</i> / Rat virus H1 and Minute virus of mice	Tumor tropism, autonomous replication, low immunogenicity	Low transgene capacity, low titers, replication competent viruses	[117]
<i>Reoviridae</i> / Respiratory enteric orphan virus	Mild pathogen, specific oncolytic activity	Previous antigens exist	[118, 119]
<i>Picornaviridae</i> / Poliovirus	Oncolytic	Narrow tropism, pathogenic, difficult manipulation	[120]
<i>Rhabdoviridae</i> / Vesicular Stomatitis virus	Relatively non-pathogenic, oncolytic	Difficult manipulation, Vesicular stomatitis G-protein inactivation	[121]

ruses that are naturally oncolytic and are currently being evaluated for oncolytic therapy (Table 2 and [89]).

Oncorine™ (H101; similar to Onyx Pharmaceutical's discontinued Onyx-015) from Chinese Shanghai Sunway Biotech is a conditionally replicative adenovirus, which gained marketing approval in China in 2006 for treating head and neck cancer and is in clinical trials for non-small-cell lung cancer. Oncorine™ contains a deletion in the *E1B 55K* region and together with Onyx-015 has provided a plethora of data in various types of cancer e.g glioma, head and neck, pancreatic and ovarian cancers [89-91]. In phase I trials, Oncorine™ showed safe and effective tumor shrinking in 3 patients out of 15 (1 partial regression and 2 with minor response). In phase II trials in 53 patients, the treatment group (two cycles of intratumoral injection of Oncorine™) showed a 28 % response rate against the 12 % rate seen in controls (no data available). The agent was well tolerated in the responsive patients (3 with complete regression and 11 with partial regression). Finally, in a phase III trials with 123 patients in the treatment group (combination of chemotherapy and Oncorine™) or control (chemotherapy alone), the response rate in the treatment group was 72.7 %, as compared to 40.4 % in the control group. Similarly with nasopharyngeal cancer, combination treatment with chemotherapy and Oncorine™ resulted in a response rate of 75.6 % against 57.1 % in the control group. The response was evaluated by the shrinkage of the tumor according to the WHO criteria.

The complications included fever, injection site pain, nausea, alopecia, leucopenia and flu-like symptoms. The authors speculate that since patients with fever showed an increased response rate, the elevated temperature might improve viral replication through heat shock protein assisted late mRNA export [90].

### TREATING MALIGNANT GLIOMAS WITH THYMIDINE KINASE GENE THERAPY

In malignant glioma, the tumour infiltrates into normal brain tissue, making a complete surgical removal very diffi-

cult. Several direct applications of the thymidine kinase expressing adenovirus to the walls of the tumor cavity after a surgical removal has been shown to be the most promising new treatment for malignant gliomas [2, 6, 92]. *Herpes simplex* virus thymidine kinase is an enzyme capable of converting a prodrug ganciclovir to a monophosphate form. Cell kinases continue the phosphorylation further and convert the monophosphate through the diphosphate form to a toxic ganciclovir triphosphate. This results in an inhibition of the activity of the DNA polymerase thus preventing DNA replication [93, 94]. However, the problem with this approach is the poor bioavailability of ganciclovir within the central nervous system. This is due to the blood brain barrier and the disadvantageous physicochemical properties of ganciclovir. Due to poor aqueous solubility and low lipophilicity, the hydrophilic ganciclovir possesses poor transcellular permeability across membranes [95]. Additionally, an effective influx and efflux transport system prevents accumulation of most antiviral drugs within the central nervous system [96]. After ganciclovir administration, the cells undergo apoptosis and phosphorylated analogues are released from the cells, taken up by the neighboring cells and the lethal effect spreads further without the need of transducing all cells with the thymidine kinase (so called bystander effect) [97, 98]. Due to the small size of the adenovirus and the bystander effect following the enzyme activity, the agent is diffused several millimetres from the injection site, thus potentially reaching distant tumor cells. It is also noteworthy, that since the ganciclovir administration is separate from the gene transfer, safety of the treatment is improved in the case of accidentally widespread or undesirably excessive transgene expression. As current gene therapy vectors cannot achieve 100% gene transfer in low concentrations, the bystander effect is essential in solid tumour eradication through increasing the treatment efficacy [99]. It has been estimated that a 10 % transduction efficiency is enough for tumor eradication in some models [100]. Also, the number of gap junctions plays a vital role in this effect [101], since the number of gap junctions seems to correlate with the efficiency of the bystander

effect in different cell lines [102]. The key component in gap junctions, connexin-43, is often downregulated in tumors. It has been shown that a chemically induced increase in connexin-43 intensified the bystander effect [102]. By enhancing the levels of connexin or developing 2'-deoxy-guanosine analogues with improved efficacy at lower concentrations, the cancer treatment efficacy could be further improved.

Cerepro® from the Anglo-Finnish company Ark Therapeutics Group PLC is an adenovirus containing a *Herpes simplex* type-1 thymidine kinase transgene, under the cytomegalovirus promoter, for the treatment of malignant glioma together with ganciclovir. In a phase IIa clinical trial with Cerepro®, the mean survival time of patients increased to 15 months as compared to that in patients treated with retroviral therapy (7.4 months) or a non-effective adenovirus (8.3 months) [6]. In a continued phase IIb study, Cerepro® treatment increased survival to 17.65 months as compared to standard care groups (9.75 months) [2]. In all studies Cerepro® was well-tolerated and adverse effects were mostly limited to transient fever [103]. A phase III trial with 250 patients is currently underway.

## FUTURE DIRECTIONS OF GENE THERAPY

With the first gene therapy products in the market in China, the future of gene therapy seems promising. In addition to treating fatal monogenic diseases, the use of gene therapy as an adjuvant treatment with radiotherapy and chemotherapy has shown promising results. The transfer of gene therapy from rare genetic conditions to more common disorders, such as cancer or cardiovascular diseases is likely to benefit clinical medicine with a wider choice of treatments available for the patients.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge Ms. Minna Kaikkonen and Ms. Hanna Lesch for their valuable input for this review. This study was supported by Ark Therapeutics Ltd.

## ABBREVIATIONS

CAR	=	Coxsackie-adenovirus receptor
DNA	=	Deoxyribonucleic acid
EMA	=	European Agency for the Evaluation of Medicinal Products
HIV	=	Human immunodeficiency virus
ITR	=	Inverted terminal repeats
MLP	=	Major late protein
mRNA	=	messenger ribonucleic acid

## REFERENCES

[1] Ylä-Herttuala, S.; Alitalo, K. Gene transfer as a tool to induce therapeutic vascular growth. *Nat. Med.* **2003**, *9*(6), 694-701.  
 [2] Immonen, A.; Vapalahti, M.; Tyynelä, K.; Hurskainen, H.; Sandmair, A.; Vanninen, R.; Langford, G.; Murray, N.; Ylä-Herttuala, S. AdvHSV-tk gene therapy with intravenous ganciclovir improves

survival in human malignant glioma: a randomised, controlled study. *Mol. Ther.* **2004**, *10*(5), 967-972.  
 [3] Cavazzana-Calvo, M.; Hacein-Bey, S.; de Saint, B.; Gross, F.; Yvon, E.; Nussbaum, P.; Selz, F.; Hue, C.; Certain, S.; Casanova, J.; Bouso, P.; Deist, F.L.; Fischer, A. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* **2000**, *288*(5466), 669-672.  
 [4] Harrington, K.; varez-Vallina, L.; Crittenden, M.; Gough, M.; Chong, H.; Diaz, R. M.; Vassaux, G.; Lemoine, N.; Vile, R. Cells as vehicles for cancer gene therapy: the missing link between targeted vectors and systemic delivery? *Hum. Gene Ther.* **2002**, *13*(11), 1263-1280.  
 [5] Heyde, M.; Partridge, K. A.; Oreffo, R. O.; Howdle, S. M.; Shakesheff, K. M.; Garnett, M. C. Gene therapy used for tissue engineering applications. *J. Pharm. Pharmacol.* **2007**, *59*(3), 329-350.  
 [6] Sandmair, A. M.; Loimas, S.; Puranen, P.; Immonen, A.; Kossila, M.; Puranen, M.; Hurskainen, H.; Tyynelä, K.; Turunen, M.; Vanninen, R.; Lehtolainen, P.; Paljärvi, L.; Johansson, R.; Vapalahti, M.; Ylä-Herttuala, S. Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. *Hum. Gene Ther.* **2000**, *11*(16), 2197-2205.  
 [7] Wirth, T.; Ylä-Herttuala, S. Gene technology based therapies in the brain. *Adv. Tech. Stand. Neurosurg.* **2006**, *31*, 3-32.  
 [8] Chen, D.; Maa, Y. F.; Haynes, J. R. Needle-free epidermal powder immunization. *Exp. Rev. Vaccines* **2002**, *1*(3), 265-276.  
 [9] Wells, D. J. Gene therapy progress and prospects: electroporation and other physical methods. *Gene Ther.* **2004**, *11*(18), 1363-1369.  
 [10] Yoshizawa, J.; Li, X. K.; Fujino, M.; Kimura, H.; Mizuno, R.; Hara, A.; Ashizuka, S.; Kanai, M.; Kuwashima, M.; Kurobe, M.; Yamazaki, Y. Successful in utero gene transfer using a gene gun in midgestational mouse fetuses. *J. Pediatr. Surg.* **2004**, *39*(1), 81-84.  
 [11] Rutanen, J.; Rissanen, T. T.; Markkanen, J. E.; Gruchala, M.; Silvennoinen, P.; Kivela, A.; Hedman, A.; Hedman, M.; Heikura, T.; Orden, M. R.; Stacker, S. A.; Achen, M. G.; Hartikainen, J.; Ylä-Herttuala, S. Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmurular angiogenesis in porcine heart. *Circulation* **2004**, *109*(8), 1029-1035.  
 [12] Bhardwaj, S.; Roy, H.; Karpanen, T.; Hi, Y.; Jauhainen, S.; Hedman, M.; Alitalo, K.; Ylä-Herttuala, S. Periadventitial angiopoietin-1 gene transfer induces angiogenesis in rabbit carotid arteries. *Gene Ther.* **2005**, *12*(5), 388-394.  
 [13] Hiltunen, M. O.; Turunen, M. P.; Ylä-Herttuala, S. Gene therapy methods in cardiovascular diseases. *Methods Enzymol.* **2002**, *346*, 311-320.  
 [14] Sailaja, G.; HogenEsch, H.; North, A.; Hays, J.; Mittal, S. K. Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector-specific immune response. *Gene Ther.* **2002**, *9*(24), 1722-1729.  
 [15] Schopf, B.; Neuberger, T.; Schulze, K.; Petri, A.; Chastellain, M.; Hofmann, M. Methodology description for detection of cellular uptake of PVA coated superparamagnetic iron oxide nanoparticles (SPION) in synovial cells of sheep. *J. Magn. Magn. Mater.* **2005**, *293*(1), 411-418.  
 [16] Nesbeth, D.; Williams, S. L.; Chan, L.; Brain, T.; Slater, N. K.; Farzaneh, F.; Darling, D. Metabolic biotinylation of lentiviral pseudotypes for scalable paramagnetic microparticle-dependent manipulation. *Mol. Ther.* **2006**, *13*(4), 814-822.  
 [17] Hughes, C.; Galea-Lauri, J.; Farzaneh, F.; Darling, D. Streptavidin paramagnetic particles provide a choice of three affinity-based capture and magnetic concentration strategies for retroviral vectors. *Mol. Ther.* **2001**, *3*(4), 623-630.  
 [18] Haim, H.; Steiner, I.; Panet, A. Synchronized infection of cell cultures by magnetically controlled virus. *J. Virol.* **2005**, *79*(1), 622-625.  
 [19] Kadota, S. I.; Kanayama, T.; Miyajima, N.; Takeuchi, K.; Nagata, K. Enhancing of measles virus infection by magnetofection. *J. Virol. Methods* **2005**, *128*(1-2), 61-66.  
 [20] Chan, L.; Nesbeth, D.; Mackey, T.; Galea-Lauri, J.; Gaken, J.; Martin, F.; Collins, M.; Mufti, G.; Farzaneh, F.; Darling, D. Conjugation of lentivirus to paramagnetic particles via nonviral proteins allows efficient concentration and infection of primary acute myeloid leukemia cells. *J. Virol.* **2005**, *79*(20), 13190-13194.

- [21] Raty, J. K.; Airene, K. J.; Marttila, A. T.; Marjomaki, V.; Hytonen, V. P.; Lehtolainen, P.; Laitinen, O.; Mähönen A.; Kulomaa, M. S.; Ylä-Herttua, S. Enhanced gene delivery by avidin-displaying baculovirus. *Mol. Ther.* **2004**, *9*(2), 282-291.
- [22] Plank, C.; Scherer, F.; Schillinger, U.; Bergemann, C.; Anton, M. Magnetofection: enhancing and targeting gene delivery with superparamagnetic nanoparticles and magnetic fields. *J. Liposome Res.* **2003**, *13*(1), 29-32.
- [23] Rudge, S.; Peterson, C.; Vessely, C.; Koda, J.; Stevens, S.; Catterall, L. Adsorption and desorption of chemotherapeutic drugs from a magnetically targeted carrier (MTC). *J. Control. Release* **2001**, *74*(1-3), 335-340.
- [24] Goodwin, S.; Peterson, C.; Hoh, C.; Bittner, C. Targeting and retention of magnetic targeted carriers (MTCs) enhancing intra-arterial chemotherapy. *J. Magn. Magn. Mater.* **1999**, *194*(1-3), 132-139.
- [25] Lubbe, A. S.; Alexiou, C.; Bergemann, C. Clinical applications of magnetic drug targeting. *J. Surg. Res.* **2001**, *95*(2), 200-206.
- [26] Li, A. M.; Zhang, C. X.; Fu, X. P.; Zhang, Z. W.; Xue, Q. H.; Yan, R. M.; Yi, L.H. Localization and distribution of magnetic chemotherapeutic drugs with magnetic targeting in rat brain. *Chin. Med. J. (Engl.)* **2005**, *118*(10), 824-827.
- [27] Arbab, A. S.; Jordan, E. K.; Wilson, L. B.; Yocum, G. T.; Lewis, B. K.; Frank, J. A. *In vivo* trafficking and targeted delivery of magnetically labeled stem cells. *Hum. Gene Ther.* **2004**, *15*(4), 351-360.
- [28] Alexiou, C.; Jurgons, R.; Schmid, R. J.; Bergemann, C.; Henke, J.; Erhardt, W.; Huenges, E.; Parak, F. Magnetic drug targeting--biodistribution of the magnetic carrier and the chemotherapeutic agent mitoxantrone after locoregional cancer treatment. *J. Drug Target* **2003**, *11*(3), 139-149.
- [29] Jiang, H.; Zhang, T.; Sun, X. Vascular endothelial growth factor gene delivery by magnetic DNA nanospheres ameliorates limb ischemia in rabbits. *J. Surg. Res.* **2005**, *126*(1), 48-54.
- [30] Cronin, J.; Zhang, X. Y.; Reiser, J. Altering the tropism of lentiviral vectors through pseudotyping. *Curr. Gene Ther.* **2005**, *5*(4), 387-398.
- [31] Mizuguchi, H.; Hayakawa, T. Targeted adenovirus vectors. *Hum. Gene Ther.* **2004**, *15*(11), 1034-1044.
- [32] Rein, D. T.; Breidenbach, M.; Curiel, D. T. Current developments in adenovirus-based cancer gene therapy. *Future Oncol.* **2006**, *2*(1), 137-143.
- [33] Work, L. M.; Nicklin, S. A.; Brain, N. J.; Dishart, K. L.; Von Seggern, D. J.; Hallek, M.; Buning, H.; Baker, A.H. Development of efficient viral vectors selective for vascular smooth muscle cells. *Mol. Ther.* **2004**, *9*(2), 198-208.
- [34] Parrott, M. B.; Adams, K. E.; Mercier, G. T.; Mok, H.; Campos, S. K.; Barry, M. A. Metabolically biotinylated adenovirus for cell targeting, ligand screening, and vector purification. *Mol. Ther.* **2003**, *8*(4), 688-700.
- [35] Turunen, M. P.; Puhakka, H. L.; Koponen, J. K.; Hiltunen, M. O.; Rutanen, J.; Leppanen, O.; Turunen, A. M.; Närvänen, A.; Newby, A. C.; Baker, A. H.; Ylä-Herttua, S. Peptide-retargeted adenovirus encoding a tissue inhibitor of metalloproteinase-1 decreases restenosis after intravascular gene transfer. *Mol. Ther.* **2002**, *6*(3), 306-312.
- [36] Kreppel, F.; Gackowski, J.; Schmidt, E.; Kochanek, S. Combined genetic and chemical capsid modifications enable flexible and efficient de- and retargeting of adenovirus vectors. *Mol. Ther.* **2005**, *12*(1), 107-117.
- [37] Magnusson, M. K.; Hong, S. S.; Henning, P.; Boulanger, P.; Lindholm, L. Genetic retargeting of adenovirus vectors: functionality of targeting ligands and their influence on virus viability. *J. Gene Med.* **2002**, *4*(4), 356-370.
- [38] Chillon, M.; Lee, J. H.; Fasbender, A.; Welsh, M. J. Adenovirus complexed with polyethylene glycol and cationic lipid is shielded from neutralizing antibodies *in vitro*. *Gene Ther.* **1998**, *5*(7), 995-1002.
- [39] Ogawara, K.; Rots, M. G.; Kok, R. J.; Moorlag, H. E.; Van Loenen, A. M.; Meijer, D. K.; Haisma, H. J.; Molema, G. A novel strategy to modify adenovirus tropism and enhance transgene delivery to activated vascular endothelial cells *in vitro* and *in vivo*. *Hum. Gene Ther.* **2004**, *15*(5), 433-443.
- [40] Koizumi, N.; Kawabata, K.; Sakurai, F.; Watanabe, Y.; Hayakawa, T.; Mizuguchi, H. Modified adenoviral vectors ablated for coxsackievirus-adenovirus receptor, alphav integrin, and heparan sulfate binding reduce *in vivo* tissue transduction and toxicity. *Hum. Gene Ther.* **2006**, *17*(3), 264-279.
- [41] Mottershead, D. G.; Alfthan, K.; Ojala, K.; Takkinen, K.; Oker-Blom, C. Baculoviral display of functional scFv and synthetic IgG-binding domains. *Biochem. Biophys. Res. Commun.* **2000**, *275*(1), 84-90.
- [42] Volpers, C.; Thirion, C.; Biermann, V.; Hussmann, S.; Kewes, H.; Dunant, P.; von der Mark, H.; Herrman, A.; Kochanek, S.; Lochmüller, H. Antibody-mediated targeting of an adenovirus vector modified to contain a synthetic immunoglobulin g-binding domain in the capsid. *J. Virol.* **2003**, *77*(3), 2093-2104.
- [43] Choi, V. W.; McCarty, D. M.; Samulski, R. J. AAV hybrid serotypes: improved vectors for gene delivery. *Curr. Gene Ther.* **2005**, *5*(3), 299-310.
- [44] Purow, B.; Staveley-O'Carroll, K. Targeting of vaccinia virus using biotin-avidin viral coating and biotinylated antibodies. *J. Surg. Res.* **2005**, *123*(1), 49-54.
- [45] Raty, J. K.; Liimatainen, T.; Wirth, T.; Airene, K. J.; Ihalainen, T. O.; Huhtala, T.; Hamerlynck, E.; Vihinen-Ranta, M.; Närvänen, A.; Ylä-Herttua, S.; Hakumäki, J. Magnetic resonance imaging of viral particle biodistribution *in vivo*. *Gene Ther.* **2006**, *13*(20), 1440-1446.
- [46] Campos, S. K.; Barry, M. A. Current advances and future challenges in Adenoviral vector biology and targeting. *Curr. Gene Ther.* **2007**, *7*(3), 189-204.
- [47] Chirmule, N.; Propert, K.; Magosin, S.; Qian, Y.; Qian, R.; Wilson, J. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther.* **1999**, *6*(9), 1574-1583.
- [48] Douglas, J. T. Adenovirus-mediated gene delivery: an overview. *Methods Mol. Biol.* **2004**, *246*, 3-14.
- [49] Parks, R.; Eveleigh, C.; Graham, F. Use of helper-dependent adenoviral vectors of alternative serotypes permits repeat vector administration. *Gene Ther.* **1999**, *6*(9), 1565-1573.
- [50] Bessis, N.; GarciaCozar, F. J.; Boissier, M. C. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. *Gene Ther.* **2004**, *11*(Suppl. 1), S10-S17.
- [51] Raper, S. E.; Chirmule, N.; Lee, F. S.; Wivel, N. A.; Bagg, A.; Gao, G. P.; Wilson, J. M.; Batshaw, M. L. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol. Genet. Metab.* **2003**, *80*(1-2), 148-158.
- [52] Ellis, J. Silencing and variegation of gammaretrovirus and lentivirus vectors. *Hum. Gene Ther.* **2005**, *16*(11), 1241-1246.
- [53] Woods, N. B.; Bottero, V.; Schmidt, M.; Von, K. C.; Verma, I. M. Gene therapy: therapeutic gene causing lymphoma. *Nature* **2006**, *440*(7088), 1123.
- [54] Thrasher, A. J.; Gaspar, H. B.; Baum, C.; Modlich, U.; Schambach, A.; Candotti, F.; Otsu, M.; Sorrentino, B.; Scobie, L.; Cameron, E.; Blyth, K.; Neil, J.; Abina, S. H.; Cavazzana-Calvo, M.; Fischer, A. Gene therapy: X-SCID transgene leukaemogenicity. *Nature* **2006**, *443*(7109), E5-E6.
- [55] Pike-Overzet, K.; de, R. D.; Weerkamp, F.; Baert, M. R.; Versteegen, M. M.; Brugman, M. H.; Howe, S. J.; Reinders, M. J.; Trasher, A. J.; Wagemager, G.; van Dongen, J. J.; Staal, F. J. Gene therapy: is IL2RG oncogenic in T-cell development? *Nature* **2006**, *443*(7109), E5-E7.
- [56] Yi, Y.; Hahm, S. H.; Lee, K. H. Retroviral gene therapy: safety issues and possible solutions. *Curr. Gene Ther.* **2005**, *5*(1), 25-35.
- [57] Tan, W.; Dong, Z.; Wilkinson, T. A.; Barbas, C. F., III; Chow, S. A. Human immunodeficiency virus type 1 incorporated with fusion proteins consisting of integrase and the designed polydactyl zinc finger protein E2C can bias integration of viral DNA into a predetermined chromosomal region in human cells. *J. Virol.* **2006**, *80*(4), 1939-1948.
- [58] Vargas, J., Jr.; Gusella, G. L.; Najfeld, V.; Klotman, M. E.; Cara, A. A novel integrase-defective lentiviral episomal vectors for gene transfer. *Hum. Gene Ther.* **2004**, *15*(4), 361-372.
- [59] Philippe, S.; Sarkis, C.; Barkats, M.; Mammeri, H.; Ladroue, C.; Petit, C.; Mallet, J.; Serquera, C. Lentiviral vectors with a defective integrase allow efficient and sustained transgene expression *in*

- vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA* **2006**, *103*(47), 17684-17689.
- [60] Izsvak, Z.; Ivics, Z. Sleeping beauty transposition: biology and applications for molecular therapy. *Mol. Ther.* **2004**, *9*(2), 147-156.
- [61] Bushman, F. D. Targeting survival: integration site selection by retroviruses and LTR-retrotransposons. *Cell* **2003**, *115*(2), 135-138.
- [62] Brasset, E.; Vaury, C. Insulators are fundamental components of the eukaryotic genomes. *Heredity* **2005**, *94*(6), 571-576.
- [63] Sadeghi, H.; Hitt, M. M. Transcriptionally targeted adenovirus vectors. *Curr. Gene Ther.* **2005**, *5*(4), 411-427.
- [64] Lee, J. W.; Bae, S. H.; Jeong, J. W.; Kim, S. H.; Kim, K. W. Hypoxia-inducible factor (HIF-1)α: its protein stability and biological functions. *Exp. Mol. Med.* **2004**, *36*(1), 1-12.
- [65] Nettelbeck, D. M.; Jerome, V.; Muller, R. A strategy for enhancing the transcriptional activity of weak cell type-specific promoters. *Gene Ther.* **1998**, *5*(12), 1656-1664.
- [66] Lee, Y. B.; Glover, C. P.; Cosgrave, A. S.; Bienemann, A.; Uney, J. B. Optimizing regulatable gene expression using adenoviral vectors. *Exp. Physiol.* **2005**, *90*(1), 33-37.
- [67] Wang, C. Y.; Wang, S. Astrocytic expression of transgene in the rat brain mediated by baculovirus vectors containing an astrocyte-specific promoter. *Gene Ther.* **2006**, *13*(20), 1447-1456.
- [68] Shiraishi, K.; Kato, S.; Han, S. Y.; Liu, W.; Otsuka, K.; Sakayori, M.; Ishida, T.; Takeda, M.; Kanamaru, R.; Ohuchi, M.; Ishioka, C. Isolation of temperature-sensitive p53 mutations from a comprehensive missense mutation library. *J. Biol. Chem.* **2004**, *279*(1), 348-355.
- [69] Luqmani, Y. A. Mechanisms of drug resistance in cancer chemotherapy. *Med. Princ. Pract.* **2005**, *14*(Suppl. 1), 35-48.
- [70] Vassilev, L. T. MDM2 inhibitors for cancer therapy. *Trends Mol. Med.* **2007**, *13*(1), 23-31.
- [71] Bykov, V. J.; Issaeva, N.; Selivanova, G.; Wiman, K. G. Mutant p53-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute database. *Carcinogenesis* **2002**, *23*(12), 2011-2018.
- [72] Huang, C. L.; Yokomise, H.; Miyatake, A. Clinical significance of the p53 pathway and associated gene therapy in non-small cell lung cancers. *Future Oncol.* **2007**, *3*(1), 83-93.
- [73] Ventura, A.; Kirsch, D. G.; McLaughlin, M. E.; Tuveson, D. A.; Grimm, J.; Lintault, L.; Newman, J.; Reczek, E.E.; Weissleder, R.; Jacks, T. Restoration of p53 function leads to tumour regression *in vivo*. *Nature* **2007**, *445*(7128), 661-665.
- [74] Xue, W.; Zender, L.; Miething, C.; Dickins, R. A.; Hernandez, E.; Krizhanovskiy, V.; Cordon-Cardo, C.; Lowe, S.W. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **2007**, *445*(7128), 656-660.
- [75] Martins, C. P.; Brown-Swigart, L.; Evan, G. I. Modeling the therapeutic efficacy of p53 restoration in tumors. *Cell* **2006**, *127*(7), 1323-1334.
- [76] Peng, Z. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum. Gene Ther.* **2005**, *16*(9), 1016-1027.
- [77] Wilson, J. M. Gendicine: the first commercial gene therapy product. *Hum. Gene Ther.* **2005**, *16*(9), 1014-1015.
- [78] Merritt, J. A.; Roth, J. A.; Logothetis, C. J. Clinical evaluation of adenoviral-mediated p53 gene transfer: review of INGN 201 studies. *Semin. Oncol.* **2001**, *28*(5 Suppl 16), 105-114.
- [79] Anonymous. INGN 201: Ad-p53, Ad5CMV-p53, Adenoviral p53, p53 Gene Therapy - Introgen, RPR/INGN 201. *Drugs R. D.* **2007**, *8*(3), 176-187.
- [80] Xin, H. Chinese gene therapy. Gendicine's efficacy: hard to translate. *Science* **2006**, *314*(5803), 1233.
- [81] Guo, J.; Xin, H. Chinese gene therapy. Splicing out the West? *Science* **2006**, *314*(5803), 1232-1235.
- [82] Han, D. M.; Huang, Z. G.; Zhang, W.; Yu, Z. K.; Wang, Q.; Ni, X.; Chen, X. H.; Pan, J. H.; Wang, H. [Effectiveness of recombinant adenovirus p53 injection on laryngeal cancer: phase I clinical trial and follow up]. *Zhonghua Yi Xue Za Zhi* **2003**, *83*(23), 2029-2032.
- [83] Peng Z. <http://www.biopharminternational.com/biopharm/Featured+Article/The-Genesis-of-Gendicine-The-Story-Behind-the-Firs/ArticleLong/Article/detail/95485?contextCategoryId=432> 2004 May 1.
- [84] Bischoff, J. R.; Kirm, D. H.; Williams, A.; Heise, C.; Horn, S.; Muna, M.; Ng, L.; Nue, J. A.; Sampson-Johannes, M.; Fattaey, A.; McCormick, F. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* **1996**, *274*(5286), 373-376.
- [85] Sonabend, A. M.; Ulasov, I. V.; Lesniak, M. S. Conditionally replicative adenoviral vectors for malignant glioma. *Rev. Med. Virol.* **2006**, *16*(2), 99-115.
- [86] Fueyo, J.; Gomez-Manzano, C.; Alemany, R.; Lee, P. S.; McDonnell, T. J.; Mitlianga, P.; Shi, Y. X.; Levin, V. A.; Young, W. K.; Kyriltis, A. P. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. *Oncogene* **2000**, *19*(1), 2-12.
- [87] van, B. V.; Mastenbroek, D. C.; van den Doel, P. B.; Lamfers, M. L.; Grill, J.; Wurdinger, T.; Haisma, H. J.; Pinedo, H. M.; Gerritsen, W. R. Conditionally replicative adenovirus expressing a targeting adapter molecule exhibits enhanced oncolytic potency on CAR-deficient tumors. *Gene Ther.* **2003**, *10*(23), 1982-1991.
- [88] Stoff-Khalili, M. A.; Rivera, A. A.; Nedeljkovic-Kurepa, A.; De-benedetti, A.; Li, X. L.; Odaka, Y.; Poddaturi, J.; Sibley, D. A.; Siegal, G. P.; Stoff, A.; Young, S.; Zhu, Z. B.; Curiel, D. T.; Mathis, J. M. Cancer-specific targeting of a conditionally replicative adenovirus using mRNA translational control. *Breast Cancer Res. Treat.* **2007**, [Epub ahead of print].
- [89] Crompton, A. M.; Kirm, D. H. From ONYX-015 to armed vaccinia viruses: the education and evolution of oncolytic virus development. *Curr. Cancer Drug Targets* **2007**, *7*(2), 133-139.
- [90] Yu, W.; Fang, H. Clinical trials with oncolytic adenovirus in China. *Curr. Cancer Drug Targets* **2007**, *7*(2), 141-148.
- [91] Khuri, F. R.; Nemunaitis, J.; Ganly, I.; Arseneau, J.; Tannock, I. F.; Romel, L.; Gore, M.; Ironside, J.; MacDougall, R. G.; Heise, C.; Randlev, B.; Gillenwater, Am.; Brusio, B.; Kaye, S. B.; Hong, W. K.; Kirm, D. H.; A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* **2000**, *6*(8), 879-885.
- [92] Pulkkanen, K. J.; Yla-Herttuala, S. Gene therapy for malignant glioma: current clinical status. *Mol. Ther.* **2005**, *12*(4), 585-598.
- [93] Reardon, J. E. Herpes simplex virus type 1 and human DNA polymerase interactions with 2'-deoxyguanosine 5'-triphosphate analogues. Kinetics of incorporation into DNA and induction of inhibition. *J. Biol. Chem.* **1989**, *264*(32), 19039-19044.
- [94] Matthews, T.; Boehme, R. Antiviral activity and mechanism of action of ganciclovir. *Rev. Infect. Dis.* **1988**, *10*(Suppl 3), S490-S494.
- [95] Patel, K.; Trivedi, S.; Luo, S.; Zhu, X.; Pal, D.; Kern, E. R.; Mitra, A. K. Synthesis, physicochemical properties and antiviral activities of ester prodrugs of ganciclovir. *Int. J. Pharm.* **2005**, *305*(1-2), 75-89.
- [96] Strazielle, N.; Ghersi-Egea, J. F. Factors affecting delivery of antiviral drugs to the brain. *Rev. Med. Virol.* **2005**, *15*(2), 105-133.
- [97] Culver, K. W.; Ram, Z.; Wallbridge, S.; Ishii, H.; Oldfield, E. H.; Blaese, R. M. *In vivo* gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. *Science* **1992**, *256*(5063), 1550-1552.
- [98] Ishii-Morita, H.; Agbaria, R.; Mullen, C. A.; Hirano, H.; Koepf, D. A.; Ram, Z.; Oldfield, E. H.; Johns, D. G.; Blaese, R. M. Mechanism of 'bystander effect' killing in the herpes simplex thymidine kinase gene therapy model of cancer treatment. *Gene Ther.* **1997**, *4*(3), 244-251.
- [99] van, D. I.; Mulder, N. H.; Vaalburg, W.; de Vries, E. F.; Hospers, G. A. Influence of the bystander effect on HSV-tk/GCV gene therapy. A review. *Curr. Gene Ther.* **2002**, *2*(3), 307-322.
- [100] Freeman, S. M.; Abboud, C. N.; Whartenby, K. A.; Packman, C. H.; Koepf, D. S.; Moolten, F. L.; Abraham, G. M. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res.* **1993**, *53*(21), 5274-5283.
- [101] Mesnil, M.; Piccoli, C.; Tiraby, G.; Willecke, K.; Yamasaki, H. Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins. *Proc. Natl. Acad. Sci. USA* **1996**, *93*(5), 1831-1835.
- [102] Matono, S.; Tanaka, T.; Sueyoshi, S.; Yamana, H.; Fujita, H.; Shirouzu, K. Bystander effect in suicide gene therapy is directly pro-

- portional to the degree of gap junctional intercellular communication in esophageal cancer. *Int. J. Oncol.* **2003**, *23*(5), 1309-1315.
- [103] Wirth, T.; Hedman, M.; Mäkinen, K.; Manninen, H.; Immonen, A.; Vapalahti, M.; Ylä-Herttuala, S. Safety profile of plasmid/liposomes and virus vectors in clinical gene therapy. *Curr. Drug Saf.* **2006**, *1*, 253-257.
- [104] Ghosh, S. S.; Gopinath, P.; Ramesh, A. Adenoviral vectors: a promising tool for gene therapy. *Appl. Biochem. Biotechnol.* **2006**, *133*(1), 9-29.
- [105] Yamashita, M.; Emerman, M. Retroviral infection of non-dividing cells: old and new perspectives. *Virology* **2006**, *344*(1), 88-93.
- [106] Loewen, N.; Poeschla, E. M. Lentiviral vectors. *Adv. Biochem. Eng. Biotechnol.* **2005**, *99*, 169-191.
- [107] Wu, Z.; Asokan, A.; Samulski, R. J. Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol. Ther.* **2006**, *14*(3), 316-327.
- [108] Yamanaka, R. Alphavirus vectors for cancer gene therapy (review). *Int. J. Oncol.* **2004**, *24*(4), 919-923.
- [109] Post, D. E.; Fulci, G.; Chiocca, E. A.; Van Meir, E. G. Replicative oncolytic herpes simplex viruses in combination cancer therapies. *Curr. Gene Ther.* **2004**, *4*(1), 41-51.
- [110] Hu, Y. C. Baculovirus vectors for gene therapy. *Adv. Virus Res.* **2006**, *68*, 287-320.
- [111] Li, S. D.; Huang, L. Gene therapy progress and prospects: non-viral gene therapy by systemic delivery. *Gene Ther.* **2006**, *13*(18), 1313-1319.
- [112] Fielding, A. K. Measles as a potential oncolytic virus. *Rev. Med. Virol.* **2005**, *15*(2), 135-142.
- [113] Phuong, L. K.; Allen, C.; Peng, K. W.; Giannini, C.; Greiner, S.; TenEyck, C. J.; Mishra, P. K.; Macura, S. I.; Russell, S. J.; Galanis, E. C. Use of a vaccine strain of measles virus genetically engineered to produce carcinoembryonic antigen as a novel therapeutic agent against glioblastoma multiforme. *Cancer Res.* **2003**, *63*(10), 2462-2469.
- [114] Lorence, R. M.; Pecora, A. L.; Major, P. P.; Hotte, S. J.; Laurie, S. A.; Roberts, M. S.; Groene, W. S.; Bamat, M. K. Overview of phase I studies of intravenous administration of PV701, an oncolytic virus. *Curr. Opin. Mol. Ther.* **2003**, *5*(6), 618-624.
- [115] Lorence, R. M.; Roberts, M. S.; O'Neil, J. D.; Groene, W. S.; Miller, J. A.; Mueller, S. N.; Bamat, M. K. Phase 1 clinical experience using intravenous administration of PV701, an oncolytic Newcastle disease virus. *Curr. Cancer Drug Targets* **2007**, *7*(2), 157-167.
- [116] Russell, S. J. RNA viruses as virotherapy agents. *Cancer Gene Ther.* **2002**, *9*(12), 961-966.
- [117] Cornelis, J. J.; Lang, S. I.; Stroh-Dege, A. Y.; Balboni, G.; Dinsart, C.; Rommelaere, J. Cancer gene therapy through autonomous parvovirus-mediated gene transfer. *Curr. Gene Ther.* **2004**, *4*(3), 249-261.
- [118] Norman, K. L.; Lee, P. W. Not all viruses are bad guys: the case for reovirus in cancer therapy. *Drug Discov. Today* **2005**, *10*(12), 847-855.
- [119] Yang, W. Q.; Lun, X.; Palmer, C. A.; Wilcox, M. E.; Muzik, H.; Shi, Z. Q.; Dyck, R.; Coffey, M.; Thompson, B.; Hamilton, M.; Nishikawa, S. G.; Brasher, P. M.; Fonseca, K.; George, D.; Rewcastle, N. B.; Johnston, R. N.; Stewart, D.; Lee, P. W.; Senger, D. L.; Forsyth, P. A. Efficacy and safety evaluation of human reovirus type 3 in immunocompetent animals: racine and nonhuman primates. *Clin. Cancer Res.* **2004**, *10*(24), 8561-8576.
- [120] Gromeier, M.; Lachmann, S.; Rosenfeld, M. R.; Gutin, P. H.; Wimmer, E. Intergeneric poliovirus recombinants for the treatment of malignant glioma. *Proc. Natl. Acad. Sci. USA* **2000**, *97*(12), 6803-6808.
- [121] Barber, G. N. Vesicular stomatitis virus as an oncolytic vector. *Viral Immunol.* **2004**, *17*(4), 516-527.