



Targeted genetic and viral therapy for advanced head and neck cancers

Pin-I Huang^{1,2,6}, Ju-Fang Chang^{3,6}, David H. Kirn^{4,5} and Ta-Chiang Liu³

¹ Cancer Center, Taipei Veterans General Hospital, Taipei, Taiwan

² School of Medicine, National Yang-Ming University, Taipei, Taiwan

³ Department of Pathology and Immunology, Washington University in St. Louis, 660 South Euclid Avenue, Campus Box 8118, St. Louis, MO 63110, USA

⁴ Department of Clinical Pharmacology, University of Oxford, Oxford, UK

⁵ Jennerex Biotherapeutics, Inc., San Francisco, CA, USA

Head and neck cancers usually present with advanced disease and novel therapies are urgently needed. Genetic therapy aims at restoring malfunctioned tumor suppressor gene(s) or introducing proapoptotic genes. Oncolytic virotherapeutics induce multiple cycles of cancer-specific virus replication, followed by oncolysis, virus spreading and infection of adjacent cancer cells. Oncolytic viruses can also be armed to express therapeutic transgene(s). Recent advances in preclinical and clinical studies are revealing the potential of both therapeutic classes for advanced head and neck cancers, including the approval of two products (Gendicine and H101) by a governmental agency. This review summarizes the available clinical data to date and discusses the challenges and future directions.

Introduction

Despite the recent development of molecular-targeted therapeutics and other updated treatment regimens, head and neck squamous cell carcinoma (HNSCC) remains difficult to manage. At the time of diagnosis, only 15–30% of the patients present with early-stage disease, of which surgery or radiotherapy is the treatment of choice. The majority of head and neck tumor patients present with locally advanced disease (Stages III and IV) [1], and for these patients, surgery, followed by adjuvant concurrent chemoradiotherapy (CCRT) for resectable tumors and CCRT for unresectable tumors, remains the current standard regimen [1,2]. Most patients with advanced HNSCC die from locoregional progression, with local recurrence rate up to 40% even after CCRT [1,3]. The median overall survival ranges from six to eight months [1]. Re-irradiation, with or without chemotherapy for recurrent or second primary head and neck cancers, provides local control but usually results in significant treatment-related morbidity. Furthermore, tumors often develop resistance to chemotherapy and radiotherapy. Epidermal growth factor receptor (EGFR)-targeted monoclo-

nal antibody cetuximab has been recently approved for locally advanced HNSCC (concurrently with radiotherapy) [4,5]. It is still unclear, however, how cetuximab radiation compares with the standard of care (CCRT) in these patients and combination of cetuximab and chemotherapy showed increased grade 3/4 toxicities in some studies [6]. As the use of cetuximab becomes more common, it becomes clear that the incidence of cetuximab-related anaphylaxis and other severe toxicities is higher than had been previously reported [7,8]. Other anti-EGFR agents, including gefitinib and erlotinib, express only modest efficacy in HNSCC. Therefore, for locally advanced head and neck cancer, novel therapy with distinct antitumoral mechanisms, high tolerability, complementarity with existing therapies and the feasibility of repeat dosing is urgently needed.

With advanced knowledge and technology in cancer biology, genetics, microbiology and immunology, genetic therapy (a.k.a. 'gene therapy') and virotherapy have entered clinical trials and these studies provided insight on safety, efficacy, and biological activities of these agents. The first human clinical cancer gene therapy trial was approved in 1989 for melanoma and renal cell carcinoma [9]. Hundreds of trials were subsequently carried out worldwide. For head and neck cancers, clinical genetic therapy

Corresponding author: Liu, T.-C. (tliu@path.wustl.edu)

⁶ These authors contributed equally.

TABLE 1

Possible mechanisms of chemo-sensitization/radio-sensitization of *p53* gene therapy and oncolytic virotherapy.

Agent	Class	Possible mechanism(s)	Reference
Ad-p53	<i>p53</i> gene therapy	Functional <i>p53</i> sensitizes tumor cells to radiation or chemotherapy	[99,100]
E1B-55 kDa-deleted Adenovirus (Onyx-015, H101)	Oncolytic adenovirus	Viral protein sensitizes tumor cells to radiation or chemotherapy; microtubule modulation by chemotherapy enhances intracellular virus trafficking	[101–103]
γ 34.5-/ribonucleotide reductase-deleted HSV (G207)	Oncolytic HSV	Upregulation of cellular gene(s) and enhances viral replication	[38,104,105]

studies have focused on restoration of function of the *p53* tumor suppressor genes in tumor tissue [10]. Other approaches, such as prodrug activation, proapoptotic gene delivery, and antisense technology, have limited clinical data and will not be discussed here. On the contrary, oncolytic virotherapy has various antitumoral mechanisms and has been developed as a novel approach. Synergistic interaction between standard chemotherapy or radiation therapy and these treatment platforms have been demonstrated (Table 1). This review article summarizes the clinical results to date in targeting these tumors with genetic therapy and oncolytic viruses, including combination with radiation therapy and systemic chemotherapy. Unique challenges in this field and insights into future directions are also discussed.

Genetic therapy: *p53* gene restoration

About 40–60% of patients with head and neck cancer have mutated *p53* [11]. Inactivated, mutated *p53* tumor cells are associated with tumor invasiveness, disease recurrence and resistance to chemotherapy and radiotherapy [11]. Moreover, the mutational status of *p53* is associated with poor survival. The median overall survival in patients with mutant and wild-type *p53* were 3.2 and 5.4 years, respectively ($p < 0.01$) [12]. *p53* plays a dominant role in sensitizing tumor cells to chemotherapy and radiation therapy, and replacement of mutated *p53* reduces tumor growth and also increases radio-sensitivity/chemo-sensitivity [13]. It is, therefore, logical to attempt to restore/rescue functional *p53* by gene delivery.

Early Phase I trials focused on the safety and feasibility of utilizing nonreplicating adenoviral vectors for *p53* gene delivery to head and neck tumors (Table 2) [14–16]. The E1 region of serotype 5 adenovirus is replaced with the cDNA of the *p53* gene and is driven by a cytomegalovirus promoter. Patients were given intratumoral (IT) administration of adenovirus encoding human *p53* gene (Ad-p53) up to 10^{11} plaque-forming units (pfu; approximately equivalent to 10^{13} viral particles (vp)). No dose-limiting toxicity (DLT) was encountered and the most common adverse events (AEs) were fever and injection site pain. Clinical efficacy was also demonstrated. In one trial, 33 patients with locally advanced recurrent head and neck cancers were treated with IT Ad-p53 as a single agent [14,15]. Of these patients, 17 had unresectable tumors, and among them, two achieved partial response (PR), and six showed stable disease for up to 3.5 months. Of the remaining 15 patients with resectable disease, four remained disease-free with a median follow up of 18 months [15]. In a separate trial, Ad-p53 was administered to 12 patients with advanced laryngeal cancer [16]. There was no relapse in 11 of the 12 patients for more than five years after Ad-p53 treatment.

Two Phase II studies were consequently conducted to explore the efficacy of Ad-p53 gene therapy in conjunction with radiation therapy [17–19]. Enhanced efficacy was demonstrated. The dosing of Ad-p53 and radiation were similar in both studies (Ad-p53: 1×10^{12} vp IT weekly $\times 8$; radiation: 60–70 Gy total in seven to eight weeks). The most common AE in both studies was fever. An extended follow-up on a subset of nasopharyngeal carcinoma (NPC) patients has been recently published [19]. Eighty-two patients were randomized to receive Ad-p53 and radiotherapy (GTRT; $n = 42$) or radiotherapy alone (RT; $n = 40$). Transient low grade fever after Ad-p53 administration was the most frequent AE observed (81%) but resolved spontaneously. The overall response rate (ORR) was 97.6% in the GTRT group (66.7% CR and 30.9% PR) and 85.4% in the RT alone group (24.4% CR and 61% PR, $p < 0.01$). The median time to recurrence was also significantly longer in the GTRT group than in the RT alone group (73 months vs 59 months, $p = 0.002$). Combination therapy did not, however, significantly prolong survival and disease-free survival (59 and 58 months in GTRT vs 54 and 49 months in RT). In a separate report on a Phase III trial, a total of 69 patients with Stages III and IV head and neck cancers were randomized to receive Ad-p53 and radiotherapy (GTRT, $n = 36$) or radiotherapy alone (RT, $n = 33$). The ORR in the GTRT group was 96% (64% CR and 32% PR) compared with 80% in RT alone group (19% with CR and 61% with PR; $p < 0.01$) [20,21]. Ad-p53 (Gendicine) has since been approved by the Chinese government to be used in conjunction with radiation therapy in head and neck cancers and other solid tumors (advanced hepatocellular carcinoma, advanced lung cancer, soft tissue sarcoma, and so on) [21].

INGN 201 is another *p53* expressing, nonreplicating adenovirus, developed by Introgen (Houston, TX). INGN 201 has been tested in a number of HNSCC clinical trials, including a Phase II study in 217 recurrent HNSCC patients, followed by a Phase III comparative study (INGN 201 vs methotrexate) in 240 patients with refractory HNSCC, and another Phase III study exploring standard chemotherapy in combination with INGN 201 in 288 recurrent HNSCC patients [22,23]. The results of these trials are eagerly awaited. Importantly, a recent study has also indicated that Ad-p53 can be safely and repetitively administered intravenously (IV) up to 1×10^{12} vp daily for three consecutive days [24]. Of note, *p53* DNA is detectable in tumor tissue [24]. Further studies will determine the optimal administration route and combination regimen for head and neck cancer.

Oncolytic virotherapy: a novel therapeutic approach

Targeted oncolytic viruses replicate in and kill cancer cells (oncolysis) selectively; the new viruses produced within the dying

TABLE 2
Ad-p53 gene therapy clinical trials in head and neck cancers.

Route	Cancer type/phase	Patient number	Doses	Schedule	AE (G3/G4 episodes; DLT; most freq. AE)	ORR	PD	Gene expression	Reference
Single agent									
IT (pre-, intra-, post-op)	HNSCC I	33	$1 \times 10^6 - 1 \times 10^{11}$ pfu	3/week \times 2 till PD (unresectable); 3/week + intraop + postop (resectable)	No DLT; fever, injection pain, headache	2/17 (unresectable)	9/17 (unresectable)	p53, p21 IHC+ (bx)	[14,15]
IT (pre-/post op)	Laryngeal I	12	$1 \times 10^{10} - 1 \times 10^{12}$ vp	2 \times (qod \times 5)	No DLT; fever	N/A	N/A	p53 IHC+ (bx)	[16]
Combination therapy									
IT* (+R/T)	NPC II	82 (GTRT n = 42; RT alone n = 40)	1×10^{12} vp	qw \times 8 + R/T 70 Gy/35f/7–8w	Fever	41/42* (GTRT); 35/41* (RT alone)	0* (GTRT); 0* (RT alone)	p53, p21 Bax, VEGF, IHC + (Bx)	[19]
IT* (+R/T)	HNSCC II	42 (GTRT n = 20; RT alone n = 22)	1×10^{12} vp	qw \times 8 + R/T 70 Gy/35f/7–8w	Fever	16/17* (GTRT); 22/27* (RT alone)	0* (GTRT); 0* (RT alone)	N/A	[17]
IT* (+R/T)	NPC II	29 (GTRT n = 16; RT alone n = 13)	1×10^{12} vp	qw \times 8 + R/T 60–70 Gy/7–8w	Fever	100%* (GTRT); 100%* (RT alone)	0* (GTRT); 0* (RT alone)	N/A	[18]
IT* (+R/T)	HNSCC III	69 (GTRT n = 36; RT alone n = 33)	1×10^{12} vp	qw \times 8 + R/T 70 Gy/7–8w	Fever	27/28* (GTRT); 29/36* (RT alone)	0* (GTRT); 1/36* (RT alone)	p53 IHC + (Bx)	[20]

Abbreviations—AE: adverse event; Bx: biopsy; DLT: dose-limiting toxicity; HNSCC: head and neck squamous cell carcinoma; IHC: immunohistochemistry; IT: intratumoral; N/A: not applicable; NPC: nasopharyngeal carcinoma; ORR: overall response rate; PD: progressive disease; op: operation pfu: plaque-forming unit; qod: every other day; qw: per week; R/T: radiation; vp: viral particle; *: the efficacy of these trials were reported per injected tumor instead of per patient. The subset of patients with NPC were followed up and reported in [19].

cancer cells are then released and subsequently infect adjacent and/or distant cancer cells. This cycle can be repeated numerous times, with spreading waves of virus engulfing the tumor mass. Thus, cancer cell killing can occur through apoptosis-independent mechanisms. By contrast, normal tissues are highly resistant to cancer-targeted viruses. Complementary oncolysis-induced mechanisms include induction of tumor-specific immunity and anti-vascular effects [25]. In addition, oncolytic viruses can be 'armed' to express therapeutic proteins with diverse mechanisms. The development of targeted virotherapeutics has evolved from the use of *in vitro* passaged strains (first-generation), to genetically engineered selectivity-enhanced viruses (second-generation) and finally to genetically engineered transgene expressing, 'armed' oncolytic viruses (third-generation) [26]. Clinical development of oncolytic virotherapeutics has followed a staged approach [27]: intratumoral (IT) administration was first tested (for head and neck cancers, pancreatic, and liver metastases), followed by intracavitary (intraperitoneal), intraarterial, and finally to IV delivery. These administration routes have been extensively studied and optimized. Safety of this therapeutic platform has been confirmed, and systemic efficacy has been demonstrated in various tumor types [26,28–31].

Oncolytic virotherapy has several unique features that are suitable for the treatment of head and neck cancers [32–34], including the capability to target multiple molecular pathways in these tumors, ability to compromise tumor blood flow and angiogenesis, multiple clinically optimized administration routes, and favorable safety/toxicity profiles (Table 3). Preclinical studies with oncolytic viruses have demonstrated that this treatment platform also synergizes with chemotherapy, radiation therapy and various other therapies, and the molecular mechanism(s) have been elucidated. For oncolytic adenoviruses, expression of the viral protein E4orf6 sensitizes the infected tumor cells to radiation [35,36], whereas E1A sensitizes tumor cells to chemotherapy [99,100]. For oncolytic herpes simplex viruses (HSV), chemotherapy and radiation enhances the expression of certain cellular DNA repair genes, which in turn enhances viral replication [37–39]. So far three oncolytic adenoviruses (E1B-55K-deleted *dl1520*, H101, and telomerase-driven KH901) and two HSV mutants (1716, HF10) have been tested in head and neck cancer trials.

dl1520 (Onyx-015)

dl1520 (a.k.a. Onyx-015; Onyx, CA) was the first engineered replication-selective virus to be used in humans. *dl1520* is an Ad2/Ad5 hybrid with deletions in E1B-55K and E3B regions [40]. *dl1520* was hypothesized originally to be replication-selective exclusively in cells with inactive *p53* [40]; subsequent studies revealed that the selectivity is also based on other genetic components, such as p14^{ARF} activation and late viral mRNA transport [41–43]. Onyx-015 has been tested in more than 15 clinical trials [26].

Initial trials of *dl1520* in head and neck cancers explored direct IT injection (up to 5×10^9 vp). The most frequent AEs were flu-like symptoms and injection site pain. Viral replication was demonstrated *in vivo* for up to 10 days; clearance may have been accelerated by the deletions of E3 immune avoidance genes [44–47]. Adjacent normal tissues did not support viral replication [44–47]. Transient antitumoral effects were demonstrated (objective response rate 14%); saline injection had no effect. Importantly,

TABLE 3
Oncolytic virotherapy clinical trials in head and neck cancers.

Virus	Route/ phase	Cancer type/patient number	Doses/schedule (viral particles)	AE (G3/G4 episodes; DLT; most freq. AE)	Toxic deaths	Antitumoral PD response†		Viral endpoints: gene expression, replication, shedding	Immune response	Reference
IT			Single agent							
d/1520 (Onyx-015; Adenovirus)	I	HNSCC/22	$5 \times 10^5 - 5 \times 10^9$ vp/single; q4w if SD	No DLT; fever, nausea, chills, injection site pain	0	3/22 (14%)	9/22 (41%)	4/22 ISH + (Bx);—in plasma and oropharyngeal swab	21/22 Ab ↑ (60%+ at baseline)	[44]
	II	HNSCC/40	5×10^8 vp*/qd × 5/q 3w; bid × 5/q2w	38 G3/G4 AE; injection site pain, asthenia, fever	0	5/37 (14%)	19/37 (52%)	7/11 ISH, HE, EM+ before d14 (Bx); 12/29 serum PCR+ at 24 h pi (cycle 1); 6/21 (cycle 2); 2/8 (cycle 3)	All Ab+ after cycle 2 (60%+ at baseline)	[45,46]
	I/II (pre-op)	Oral SCC/15	5×10^8 vp*/single	No attributable AE	0	0	N/A	Viral replication preferentially in tumor tissues (ISH, IHC)	N/A	[47]
H101 (Adenovirus)	I	Solid/15	$5 \times 10^7 - 1.5 \times 10^{12}$ vp/qd × 5	No DLT; injection site pain, fever	0	N/A	N/A	PCR+ (blood, urine, oropharynx)	N/A	[54]
KH901 (Adenovirus)	I	HNSCC/23	$3 \times 10^{11} - 1 \times 10^{13}$ vp; 1 or 3×10^{12} vp/biw × 3	No DLT; flu-like	0	N/A	N/A	PCR+ (blood, urine, feces)	GM-CSF+	
1716 (HSV)	I	Oral SCC/20	1×10^5 or 5×10^5 pfu; 1, 3, or 14 days before op	Tolerable	0	N/A	N/A	PCR+ (tumor)	N/A	[61]
HF10 (HSV)	N/A (case report)	HNSCC skin metastases/2	1×10^5 pfu × 3	Tolerable	0	N/A	N/A	N/A	Increased CD4, CD8 infiltration	[60]
IT			Chemo-combination							
d/1520 (Onyx-015)	II	HNSCC/37	5×10^8 vp*/qd × 5; <i>cisplatin</i> 80 mg/m ² day 1 i.v.b, 5-FU 800–1000 mg/m ² days 1–5 c.i./q3w	42% pts G3/G4 AE; asthenia, fever, chills	0	19/37 (53%) [¶]	N/A	4/7 ISH+ at d5–15 (Bx)	All Ab ↑ (56%+ at baseline)	[48]
H101	II	Solid/50	5×10^{11} vp/qd × 5/q3w × 1–5 weeks + C/T (drugs and doses N/A)	10 G3/G4 AE; fever, injection site pain, leukopenia, nausea, vomiting	0	14/50 (28%)	12/50 (24%)	2/3 IHC+ for hexon at d22 or d44 (bx)	9/14 Ab+ on d22 (21%+ at baseline)	[55,56]
IV			Chemo-combination							
d/1520 (Onyx-015)	I	Metastatic solid tumors/10	$2 \times 10^{10} - 2 \times 10^{13}$ vp/single/qw × 3; <i>carboplatin</i> (AUC 2), <i>taxol</i> 425 mg/m ² from cycle 3	No DLT; 11 G3/G4 AE; fever, chills	0	0 (1 MR)	1/10 (10%)	17/37 Q-PCR+ (blood) on d7	All Ab ↑ (20%+ at baseline); TNF, IFN-γ, IL-6, IL-10 induction	[52]

Abbreviations—Ab: antibody; AE: adverse event; Bx: biopsy; C/T: chemotherapy; DLT: dose-limiting toxicity; HNSCC: head and neck squamous cell carcinoma; IFN: interferon; IHC: immunohistochemistry; IL: interleukin; ISH: *in situ* hybridization; IT: intratumoral; IV: intravenous; MR: moderate response; N/A: not applicable; NPC: nasopharyngeal carcinoma; ORR: overall response rate; PD: progressive disease; op: operation pfu: plaque-forming unit; qd: per day; qod: every other day; qw: per week; R/T: radiation; SCC: squamous cell carcinoma; TNF: tumor necrosis factor; vp: viral particle; *: pfu reported; based on estimated vp: pfu ratio = 100:1.

neutralizing antibodies did not impact virus replication or anti-tumoral activity. Durable CRs were not reproducibly reported and efficacy at distant, non-injected sites was not seen.

The first virotherapy–chemotherapy combination clinical trial was initiated in 1998 [48] following publication of promising pre-clinical data [49]. Patients with head and neck cancer were given IT injections (5×10^8 vp) together with IV cisplatin and fluorouracil (5-FU). Toxicities were similar to those seen with each treatment alone. The response rate was higher than in historical controls with chemotherapy alone (63% vs 30%). Patients with two injectable tumor masses had one injected and the other tumor left non-injected; patients therefore served as their own controls in this novel trial design [48]. Tumors that received combination treatment had a significantly higher response rate than tumors treated with chemotherapy alone ($p < 0.05$). Of note, in other trials, no overlapping toxicities were noted when *d11520* was given by HAI or IV injection in combination with chemotherapy [50–53].

H101

Following trial designs developed for *d11520*, H101 (Sunway; Shanghai, China), with an E1B-55 kDa deletion similar to *d11520*, was tested in patients with HNSCC or nasopharyngeal carcinomas (NPC) by IT injection [54]. Results were nearly identical to those with *d11520* described above [55,56]. Of note, the maximum dose given in these trials was up to 5×10^{12} vp, 1000-fold higher than that given in the *d11520* studies, and the toxicity profile is comparable to other trials with *d11520* despite a higher dose. A randomized Phase III trial of chemotherapy vs H101-chemotherapy in locally advanced head and neck cancers (with the same design as the *d11520* Phase III) was completed and demonstrated a statistically significant increase in the tumor response rate with the combination regimen [57]; results for survival have not yet been reported. H101 received marketing approval in China following these studies.

KH901

KH901 is an oncolytic adenovirus engineered with human telomerase promoter and armed with granulocyte macrophage-colony stimulating factor (GM-CSF) [58,59]. KH901 has been tested in a recent Phase I trial as IT administration in 23 patients with recurrent head and neck cancer. The trial was designed as a two stage study: the first stage explored the safety of single dosing of KH901 from 3×10^{11} to 1×10^{13} vp (13 patients total), whereas the second stage examined the safety, feasibility, and biological activity of multiple dosing (2/week \times 2 weeks) with 1×10^{11} or 3×10^{12} vp. Treatment with KH901 was well tolerated, with the most common AE being grade I/II flu-like symptoms. DLT was not defined. Virus shedding was detected temporarily in urine and little in feces. As was seen in *d11520* trials, secondary systemic peak was detected in the majority of the patients between two and four days post-injection. Serum GM-CSF levels increased as early as 12 h post-injection (indicating viral gene expression), and were undetectable after 15 days. As expected, all patients showed increase in neutralizing antibodies to adenovirus after treatment [59].

Oncolytic herpes simplex viruses (HSVs)

In addition to oncolytic adenoviruses, two oncolytic HSVs have been tested in head and neck cancer patients [60,61]. Oncolytic

HSV HF10 lacks the expression of U_L56 [62]. HF10 has been administered in two patients with HNSCC in an anecdotal report. Both patients had metastatic skin lesions that received intratumoral administration of 10^5 pfu HF10 \times 3, and the injected tumors were excised and examined two weeks post-injection. The injected tumors showed greater infiltration of CD4-positive and CD8-positive cells than the uninjected tumors [60]. The actual toxicity profile and clinical efficacy of HF10, however, can only be realized in a properly designed and executed clinical trial with expanded patient number. In the second study, oncolytic HSV 1716 (with deletion in $\gamma 34.5$ [63]) was administered IT (1×10^5 or 5×10^5 pfu) into 20 patients with oral SCC at days 1, 3, or 14 before surgery [61]. The primary endpoints were safety and intratumoral viral replication and associated tumor necrosis. Treatment with 1716 was well tolerated with no severe adverse events. There was, however, no evidence of intratumoral viral replication [61]. Of note, the administration dosage was significantly (1000-fold) lower than in two brain tumor trials with another oncolytic HSV (G207; $\gamma 34.5$ -ribonucleotide reductase-deleted) [64,65].

Summary of clinical studies to date

The clinical trials conducted on *p53* gene transfer and oncolytic virotherapies in advanced head and neck cancers have shown that both treatment modalities are safe and well tolerated, with maximal-tolerated dose/DLT not defined in most of the agents tested. Importantly, unlike those seen in chemotherapy, radiotherapy, and EGFR-targeted therapies, the majority of the AEs were transient flu-like symptoms and injection site pain and usually recovered within 72 h. Efficacy as single agents has been limited (with a few exceptions), whereas efficacy as combination therapy with chemotherapy and/or radiotherapy is significantly enhanced. Pre-clinical studies have also identified potential combination strategy with other therapies.

Attempts have been made to correlate *p53* mutation status and clinical efficacy in both Ad-*p53* and *d11520* studies. As *p53* expression in the tumors after Ad-*p53* administration lasts less than two weeks, it is perhaps not surprising that there is no definitive correlation between *p53* mutation and efficacy. Transient exogenous *p53* expression may act primarily as a radiosensitizer. Several preclinical studies were designed to explore whether the status of *p53* determines the efficacy of *d11520*. These studies showed that *p53* mutation is not the sole determinant of the selectivity of *d11520* [43,66–70]. Indeed, *p53* function can be lost in many cancers through mechanisms other than mutation, and therefore the *p53* pathway functionality may be a better determinant for viral replication and efficacy. As described earlier, the status of $p14^{ARF}$, downstream of *p53*, has been shown to determine the selectivity of *d11520* [41]. In addition, studies have also shown that late viral RNA export, rather than *p53* inactivation, determines the sensitivity of *d11520* [43]. Other adenoviral proteins also have *p53* inhibitory effects (e.g. E4ORF6) [71,72]. Clinical studies also showed no definitive evidence of correlation between efficacy and *p53* mutation status of the tumors.

Challenges and future directions

Enhancing single agent efficacy

With a few exceptions, the overall response rate for cancer patients undergoing genetic or virotherapy as a single agent treatment has

been less than 20% [9,27,73]. For genetic therapy this can be attributed to vector-associated and/or transgene-associated issues. Short term/insufficient transgene expression can be caused by the host immune response to vectors and/or transgenes. In addition, poor penetration of the vectors through stroma and central necrotic areas in tumors can also limit transgene expression. In addition, cancers often contain disorders of multiple signaling pathways; hence transferring a single therapeutic gene may be unlikely to succeed. For example, a Phase III trial of Ad-p53 gene therapy in ovarian cancers did not show an adequate therapeutic benefit and was closed after the first interim analysis. Moreover, it was proposed that the multiple genetic changes in cancer and epigenetic dysregulations led to aberrant silencing of genes [74], and the recently identified dominant *p53* mutants, as well as *p63* and *p73* splice variants, could also seriously hamper the effect of *p53* gene therapy.

For oncolytic virotherapy, virus species that possess long replication cycles (> 24–48 h) are most likely to be eliminated by the host immune system within the first one to two rounds of replication. Recent studies on adenoviruses also showed that interaction with platelets and erythrocytes may lead to sequestration and inactivation, thus hampering systemic delivery and efficacy [75–77]. Immune evasion and modulation may therefore enhance efficacy. This has been achieved by several strategies. For example, coating vectors with polyethylene glycol or polycationic polymers, both resulted in extended systemic circulation, reduced toxicity and neutralizing antibody production, and prolonged half-lives of the viruses [78]. The utilization of less immunogenic vectors (e.g. recombinant adeno-associated viruses) may also reduce immune-mediated clearance [79]. On the contrary, co-administration of viruses with immunosuppressive agents has been explored; however, more safety studies are needed before implementing this strategy into clinical studies. Maintenance of virus-encoded immune response modifier genes may be crucial in avoiding rapid immune-mediated clearance, while deletion of such genes may increase the induction of tumor-specific immunity. Viruses have evolved several immunomodulatory genes to antagonize immune system-induced apoptosis signals [80]. With proper viral gene engineering, the host anti-viral immune response can be redirected to kill tumor cells. Examples include the adenoviral E3-gp 19 kDa and the HSV ICP47 genes, which both function to downregulate MHC I antigen presentation. Adenoviral and HSV mutants with a deletion in this gene have enhanced MHC I presentation, which correlates with enhanced cytotoxic T lymphocyte infiltration and enhanced antitumoral efficacy [81,82].

For viral vectors that utilize specific cellular surface virus receptors, it is possible to alter the virus tropism so as to enhance tumor infectibility [83]. Native viral fibers or capsular proteins can be engineered to recognize tumor-specific surface protein to achieve tumor-selectivity. This approach redirects the viruses to desired target cells. Before translating this technique to clinical use, the tumor-specific receptor(s) must, however, be thoroughly studied and confirmed, and safety and biodistribution be obtained from tropism-modified viruses to exclude the possibility of infecting normal tissues [27]. Moreover, one must take into account the fact that tumor cells are heterogenous and that the intensity of these 'tumor-specific' receptors, and hence their ability to be infected, might differ from cell to cell.

For oncolytic virotherapy, increased efficacy can potentially be achieved through the use of more potent virus species (e.g. vaccinia [30]), more potent strains (e.g. clinical isolates [84]), more potent viral gene modifications (e.g. adenovirus death protein overexpression or E1B-19 kDa deletion in adenovirus [85,86]), therapeutic transgene arming, and combination regimens with approved therapies. Potency is a function of virus spreading rate in tumors, cytopathic effects and virus receptor expression patterns on tumors.

Targeting more than one crucial regulator in oncogenic pathways may also enhance efficacy. This can be achieved by either incorporating multiple therapeutic transgene(s) targeting these pathways, or combination therapy with targeted molecular therapeutics. Other new therapies currently being developed for head and neck cancers include small molecule-based therapy (e.g. erlotinib) and monoclonal antibodies (e.g. cetuximab). Both of these classes are subject to resistance as a result of mutation [6]. Oncolytic virus targeting the EGFR pathway, for example (e.g. vaccinia virus vv-DD [87]), replicates in cancer cells with 'overall' abnormality in EGFR pathway, and its efficacy is not subject to cellular receptor epitope or tyrosine kinase mutation.

Several recent studies explore combination therapy of oncolytic virus with tumor stromal matrix-modifying approaches, both as co-administered agent (e.g. collagenase [88]), or incorporated as therapeutic transgene (e.g. relaxin [89]). For advanced head and neck cancers that have been heavily pretreated and are therefore fibrotic, these approaches are promising. Preclinical studies, however, have also indicated that modifying tumor stroma could lead to extensive bleeding and even tumor metastasis. Therefore, more studies are needed to confirm the safety of these approaches.

In addition, the likelihood of successful approval and clinical benefit for patients will be improved greatly if predictive factors for efficacy can be identified. Virotherapy efficacy might be predicted by histologic tumor type, cancer cell features (e.g. viral receptor levels, genetics) and/or patient immune status, for example. The role of gene or protein expression profiling in this setting should be explored.

Improving the understanding of biological mechanisms

To date, the selection of molecular and genetic targets for genetic therapy and virotherapy has been largely based on our knowledge of tumor biology, genetics and virology. However, little is known about the *in vivo* activities of these agents in humans. Whether the viruses and/or therapeutic transgenes behave in human as predicted in preclinical models is unclear. Restoring a functional therapeutic gene does not guarantee the restoration of the involved apoptosis induction pathway. *p53* pathway abnormality, for instance, can result from loss of *p53* and/or its upstream or downstream targets (*p14*, *p21*, MDM-2, and so on). It remains to be determined *in vivo* whether *p53* restoration can activate the downstream targets as shown *in vitro*. In addition to the failed ovarian cancer Ad-p53 Phase III trial, the early termination of retrovirus-BRCA1 trial in ovarian cancer patients also implies that a more thorough understanding of the mechanisms of the gene products and the interaction of these genes and vectors with the host is crucial [74,90].

Several approaches have been taken to improve our understanding of biological mechanisms of these agents. First of all, the

vectors can be designed to incorporate more sophisticated reporter genes to allow *in vivo* monitoring [91]. The use of radionucleotide imaging (e.g. positron emission tomography (PET) and single photon emission computed tomography (SPECT) has been shown to improve the detection of regional/spatial distribution of vector/transgene expression *in vivo* [91–93]. With the use of Na/I symporter system, PET imaging can reveal biodistribution as well as quantification of gene expression [91,93]. SPECT can also be used to image receptors, transporters, and other proteins expressed on cell surface. Similarly, vectors carrying bioluminescent reporter genes (e.g. luciferases) can be monitored for their biodistribution and gene expression *in vivo* in real time [94], although to date this approach is limited to animal models. Other examples of potential use in humans include magnetic resonance imaging for gene expression through reporters/enzymes, fluorescence imaging with green fluorescent protein introduced by the vector (for superficial tumors), as well as the somatostatin receptor gene used in combination with radio-labeled octreotide [95,96].

In addition to imaging, the pharmacokinetics of the vectors, as well as transgenes, can also be monitored by incorporation of specific ‘marker’ genes, such as the tumor-associated antigen carcinoembryonic antigen (CEA). An oncolytic measles virus, encoding CEA, has been tested in preclinical models [97]. As the production of CEA correlates with virus replication, the amount of virus replication/persistence of this virus can be easily followed by measuring the blood level of CEA. This strategy enables more frequent and rapid measurement of the replicating virus. In terms of replication-competent oncolytic viruses,

pharmacokinetic monitoring can also be done by obtaining the quantity of viral genomes in bloodstream following treatment. Mathematical models can be used to calculate the number of virus particles produced and shedding into blood with each replication cycle [51].

Treatment of highly accessible tumors for biopsy will definitely increase our knowledge of the *in vivo* activity of these genes. There is little technical difficulty in obtaining biopsies from superficial tumors, such as head and neck cancers. For tumors that post-treatment biopsy is challenging, applying genetic or virotherapy as a neoadjuvant therapy will allow us to analyze the biological endpoints from the resected tumors. Administration of these agents into premalignant lesions will also provide precious insights into tumor biology [98]. Finally, the genetic determinants of clinical efficacy should be thoroughly studied.

In summary, genetic and virotherapy hold promise for the treatment of advanced head and neck tumors. Decades of pre-clinical and clinical studies have led to product commercialization. Future directions include validating and enhancing efficacy both as single agents and in combination regimens, identifying predictive markers and providing biologic and mechanistic insights. Translating preclinical findings into clinical development strategies will be key to success for both product classes.

Conflict of interest

DHK is founder and CEO of Jennerex Inc., a company dedicated for oncolytic virotherapeutics development. PIH, JFC, and TCL declare no conflict of interest.

References

- Argiris, A. *et al.* (2008) Head and neck cancer. *Lancet* 371, 1695–1709
- Forastiere, A.A. (2008) Chemotherapy in the treatment of locally advanced head and neck cancer. *J. Surg. Oncol.* 97, 701–707
- Brockstein, B. *et al.* (2004) Patterns of failure, prognostic factors and survival in locoregionally advanced head and neck cancer treated with concomitant chemoradiotherapy: a 9-year, 337-patient, multi-institutional experience. *Ann. Oncol.* 15, 1179–1186
- Bonner, J.A. *et al.* (2006) Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 354, 567–578
- Pfister, D.G. *et al.* (2006) Concurrent cetuximab, cisplatin, and concomitant boost radiotherapy for locoregionally advanced, squamous cell head and neck cancer: a pilot Phase II study of a new combined-modality paradigm. *J. Clin. Oncol.* 24, 1072–1078
- Burtneiss, B. (2005) The role of cetuximab in the treatment of squamous cell cancer of the head and neck. *Expert Opin. Biol. Ther.* 5, 1085–1093
- Chung, C.H. *et al.* (2008) Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose. *N. Engl. J. Med.* 358, 1109–1117
- O’Neil, B.H. *et al.* (2007) High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J. Clin. Oncol.* 25, 3644–3648
- Edelstein, M.L. *et al.* (2007) Gene therapy clinical trials worldwide to 2007—an update. *J. Gene Med.* 9, 833–842
- Wiman, K.G. (2006) Strategies for therapeutic targeting of the p53 pathway in cancer. *Cell Death Differ.* 13, 921–926
- Brennan, J.A. *et al.* (1995) Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 332, 429–435
- Poeta, M.L. *et al.* (2007) TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 357, 2552–2561
- Harrington, K.J. *et al.* (2005) Gene therapy for head and neck cancer. *Cancer Metast. Rev.* 24, 147–164
- Clayman, G.L. *et al.* (1998) Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. *J. Clin. Oncol.* 16, 2221–2232
- Clayman, G.L. *et al.* (1999) Adenovirus-mediated wild-type p53 gene transfer as a surgical adjuvant in advanced head and neck cancers. *Clin. Cancer Res.* 5, 1715–1722
- Han, D.M. *et al.* (2003) Effectiveness of recombinant adenovirus p53 injection on laryngeal cancer: Phase I clinical trial and follow up. *Zhonghua Yi Xue Za Zhi* 83, 2029–2032
- Zhang, S.W. *et al.* (2003) Treatment of head and neck squamous cell carcinoma by recombinant adenovirus-p53 combined with radiotherapy: a Phase II clinical trial of 42 cases. *Zhonghua Yi Xue Za Zhi* 83, 2023–2028
- Chen, C.B. *et al.* (2003) Recombinant adenovirus p53 agent injection combined with radiotherapy in treatment of nasopharyngeal carcinoma: a Phase II clinical trial. *Zhonghua Yi Xue Za Zhi* 83, 2033–2035
- Pan, J.J. *et al.* (2009) Effect of recombinant adenovirus-p53 combined with radiotherapy on long-term prognosis of advanced nasopharyngeal carcinoma. *J. Clin. Oncol.* 27, 799–804
- Zhang, S.W. *et al.* (2005) Recombinant adenovirus-p53 gene therapy combined with radiotherapy for head and neck squamous-cell carcinoma. *Zhonghua Zhong Liu Za Zhi* 27, 426–428
- Peng, Z. (2005) Current status of gene therapy in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum. Gene Ther.* 16, 1016–1027
- Gabrilovich, D.I. (2006) INGN 201 (Advexin): adenoviral p53 gene therapy for cancer. *Expert Opin. Biol. Ther.* 6, 823–832
- INGN 201: Ad-p53, Ad5CMV-p53, adenoviral p53, p53 gene therapy—introgen, RPR/INGN 201. *Drugs R&D* 8, 176–187
- Tolcher, A.W. *et al.* (2006) Phase I, pharmacokinetic, and pharmacodynamic study of intravenously administered Ad5CMV-p53, an adenoviral vector containing the wild-type p53 gene, in patients with advanced cancer. *J. Clin. Oncol.* 24, 2052–2058
- Breitbach, C.J. *et al.* (2007) Targeted inflammation during oncolytic virus therapy severely compromises tumor blood flow. *Mol. Ther.* 15, 1686–1693
- Liu, T.C. *et al.* (2007) Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nat. Clin. Pract. Oncol.* 4, 101–117
- Kirn, D. (2001) Clinical research results with dl1520 (Onyx-015), a replication-selective adenovirus for the treatment of cancer: what have we learned? *Gene Ther.* 8, 89–98

- 28 Freeman, A.I. *et al.* (2006) Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol. Ther.* 13, 221–228
- 29 Liu, T.C. and Kim, D. (2007) Systemic efficacy with oncolytic virus therapeutics: clinical proof-of-concept and future directions. *Cancer Res.* 67, 429–432
- 30 Park, B.H. *et al.* (2008) Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a Phase I trial. *Lancet Oncol.* 9, 533–542
- 31 Vidal, L. *et al.* (2008) A Phase I study of intravenous oncolytic reovirus type 3 Dearing in patients with advanced cancer. *Clin. Cancer Res.* 14, 7127–7137
- 32 Tanaka, H. *et al.* (2005) A replication-selective adenoviral vector for head and neck cancers. *Arch. Otolaryngol. Head Neck Surg.* 131, 630–634
- 33 Kim, S.H. *et al.* (2005) Combination of mutated herpes simplex virus type 1 (G207 virus) with radiation for the treatment of squamous cell carcinoma of the head and neck. *Eur. J. Cancer* 41, 313–322
- 34 Reddy, P.S. *et al.* (2006) Enhanced gene transfer and oncolysis of head and neck cancer and melanoma cells by fiber chimeric oncolytic adenoviruses. *Clin. Cancer Res.* 12, 2869–2878
- 35 Hart, L.S. *et al.* (2005) The adenovirus E4orf6 protein inhibits DNA double strand break repair and radiosensitizes human tumor cells in an E1B-55K-independent manner. *J. Biol. Chem.* 280, 1474–1481
- 36 Hart, L.S. *et al.* (2007) The adenoviral E4orf6 protein induces atypical apoptosis in response to DNA damage. *J. Biol. Chem.* 282, 6061–6067
- 37 Jarnagin, W.R. *et al.* (2006) Treatment of cholangiocarcinoma with oncolytic herpes simplex virus combined with external beam radiation therapy. *Cancer Gene Ther.* 13, 326–334
- 38 Aghi, M. *et al.* (2006) Effect of chemotherapy-induced DNA repair on oncolytic herpes simplex viral replication. *J. Natl. Cancer Inst.* 98, 38–50
- 39 Advani, S.J. *et al.* (2007) Enhancing radiotherapy with genetically engineered viruses. *J. Clin. Oncol.* 25, 4090–4095
- 40 Bischoff, J.R. *et al.* (1996) An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274, 373–376
- 41 Ries, S.J. *et al.* (2000) Loss of p14ARF in tumor cells facilitates replication of the adenovirus mutant dl1520 (ONYX-015). *Nat. Med.* 6, 1128–1133
- 42 McCormick, F. (2000) ONYX-015 selectivity and the p14ARF pathway. *Oncogene* 19, 6670–6672
- 43 O'Shea, C.C. *et al.* (2004) Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer Cell* 6, 611–623
- 44 Ganly, I. *et al.* (2000) A Phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin. Cancer Res.* 6, 798–806
- 45 Nemunaitis, J. *et al.* (2000) Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55 kDa gene-deleted adenovirus, in patients with advanced head and neck cancer: a Phase II trial. *Cancer Res.* 60, 6359–6366
- 46 Nemunaitis, J. *et al.* (2001) Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J. Clin. Oncol.* 19, 289–298
- 47 Morley, S. *et al.* (2004) The dl1520 virus is found preferentially in tumor tissue after direct intratumoral injection in oral carcinoma. *Clin. Cancer Res.* 10, 4357–4362
- 48 Khuri, F.R. *et al.* (2000) 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.* 6, 879–885
- 49 Heise, C. *et al.* (1997) ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumor efficacy that can be augmented by standard chemotherapeutic agents. *Nat. Med.* 3, 639–645
- 50 Reid, T. *et al.* (2001) Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a Phase I trial. *Gene Ther.* 8, 1618–1626
- 51 Reid, T. *et al.* (2002) Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): Phase II viral, immunologic, and clinical endpoints. *Cancer Res.* 62, 6070–6079
- 52 Nemunaitis, J. *et al.* (2001) Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: safety, feasibility and biological activity. *Gene Ther.* 8, 746–759
- 53 Nemunaitis, J. *et al.* (2003) Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. *Cancer Gene Ther.* 10, 341–352
- 54 Yuan, Z.Y. *et al.* (2003) Safety of an E1B deleted adenovirus administered intratumorally to patients with cancer. *Ai Zheng* 22, 310–313
- 55 Xu, R.H. *et al.* (2003) Phase II clinical study of intratumoral H101, an E1B deleted adenovirus, in combination with chemotherapy in patients with cancer. *Ai Zheng* 22, 1307–1310
- 56 Lu, W. *et al.* (2004) Intra-tumor injection of H101, a recombinant adenovirus, in combination with chemotherapy in patients with advanced cancers: a pilot Phase II clinical trial. *W. J. Gastroenterol.* 10, 3634–3638
- 57 Xia, Z.J. *et al.* (2004) Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. *Ai Zheng* 23, 1666–1670
- 58 Shen, F.B. *et al.* (2007) Tumor-selective replication, cytotoxicity and GM-CSF production of oncolytic recombinant adenovirus in KH901 injection. *Sichuan Da Xue Xue Bao Yi Xue Ban* 38, 31–34
- 59 Chang, J. *et al.* (2009) A Phase I study of KH901, a conditionally replicating granulocyte-macrophage colony-stimulating factor: armed oncolytic adenovirus for the treatment of head and neck cancers. *Cancer Biol. Ther.* 8 [epub ahead of print]
- 60 Fujimoto, Y. *et al.* (2006) Intratumoral injection of herpes simplex virus HF10 in recurrent head and neck squamous cell carcinoma. *Acta Otolaryngol.* 126, 1115–1117
- 61 Mace, A.T. *et al.* (2007) Cytotoxic effects of the oncolytic herpes simplex virus HSV1716 alone and in combination with cisplatin in head and neck squamous cell carcinoma. *Acta Otolaryngol.* 127, 880–887
- 62 Takakuwa, H. *et al.* (2003) Oncolytic viral therapy using a spontaneously generated herpes simplex virus type 1 variant for disseminated peritoneal tumor in immunocompetent mice. *Arch. Virol.* 148, 813–825
- 63 MacLean, A.R. *et al.* (1991) Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17+ between immediate early gene 1 and the 'a' sequence. *J. Gen. Virol.* 72 (Pt 3), 631–639
- 64 Markert, J.M. *et al.* (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a Phase I trial. *Gene Ther.* 7, 867–874
- 65 Markert, J.M. *et al.* (2009) Phase Ib trial of mutant herpes simplex virus G207 inoculated pre- and post-tumor resection for recurrent GBM. *Mol. Ther.* 17, 199–207
- 66 Rothmann, T. *et al.* (1998) Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. *J. Virol.* 72, 9470–9478
- 67 Vollmer, C. *et al.* (1999) p53 selective and nonselective replication of an E1B-deleted adenovirus in hepatocellular carcinoma. *Cancer Res.* 59, 4369–4374
- 68 Georger, B. *et al.* (2002) Oncolytic activity of the E1B-55 kDa-deleted adenovirus ONYX-015 is independent of cellular p53 status in human malignant glioma xenografts. *Cancer Res.* 62, 764–772
- 69 Ries, S. and Korn, W.M. (2002) ONYX-015: mechanisms of action and clinical potential of a replication-selective adenovirus. *Br. J. Cancer* 86, 5–11
- 70 O'Shea, C.C. *et al.* (2005) Heat shock phenocopies E1B-55K late functions and selectively sensitizes refractory tumor cells to ONYX-015 oncolytic viral therapy. *Cancer Cell* 8, 61–74
- 71 Querido, E. *et al.* (2001) Identification of three functions of the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6-E1B55K complex. *J. Virol.* 75, 699–709
- 72 Querido, E. *et al.* (2001) Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes Dev.* 15, 3104–3117
- 73 Rainov, N.G. and Ren, H. (2003) Oncolytic viruses for treatment of malignant brain tumours. *Acta Neurochir. Suppl.* 88, 113–123
- 74 Zeimet, A.G. and Marth, C. (2003) Why did p53 gene therapy fail in ovarian cancer? *Lancet Oncol.* 4, 415–422
- 75 Stone, D. *et al.* (2007) Comparison of adenoviruses from species B, C, E, and F after intravenous delivery. *Mol. Ther.* 15, 2146–2153
- 76 Stone, D. *et al.* (2007) Adenovirus-platelet interaction in blood causes virus sequestration to the reticuloendothelial system of the liver. *J. Virol.* 81, 4866–4871
- 77 Carlisle, R.C. *et al.* (2009) Human erythrocytes bind and inactivate type 5 adenovirus by presenting Coxsackie virus-adenovirus receptor and complement receptor 1. *Blood* 113, 1909–1918
- 78 Fisher, K.D. *et al.* (2007) Passive tumour targeting of polymer-coated adenovirus for cancer gene therapy. *J. Drug Target.* 15, 546–551
- 79 Li, C. *et al.* (2005) Adeno-associated virus vectors: potential applications for cancer gene therapy. *Cancer Gene Ther.* 12, 913–925
- 80 Benedict, C.A. *et al.* (2002) To kill or be killed: viral evasion of apoptosis. *Nat. Immunol.* 3, 1013–1018
- 81 Wang, Y. *et al.* (2003) E3 gene manipulations affect oncolytic adenovirus activity in immunocompetent tumor models. *Nat. Biotechnol.* 21, 1328–1335
- 82 Todo, T. *et al.* (2001) Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. *Proc. Natl. Acad. Sci. U.S.A.* 98, 6396–6401
- 83 Wohlfahrt, M.E. *et al.* (2007) A capsid-modified, conditionally replicating oncolytic adenovirus vector expressing TRAIL Leads to enhanced cancer cell killing in human glioblastoma models. *Cancer Res.* 67, 8783–8790

- 84 Liu, B.L. *et al.* (2003) ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther.* 10, 292–303
- 85 Doronin, K. *et al.* (2000) Tumor-specific, replication-competent adenovirus vectors overexpressing the adenovirus death protein. *J. Virol.* 74, 6147–6155
- 86 Liu, T.C. *et al.* (2005) Functional interactions of antiapoptotic proteins and tumor necrosis factor in the context of a replication-competent adenovirus. *Gene Ther.* 12, 1333–1346
- 87 McCart, J.A. *et al.* (2001) Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. *Cancer Res.* 61, 8751–8757
- 88 McKee, T.D. *et al.* (2006) Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. *Cancer Res.* 66, 2509–2513
- 89 Kim, J.H. *et al.* (2006) Relaxin expression from tumor-targeting adenoviruses and its intratumoral spread, apoptosis induction, and efficacy. *J. Natl. Cancer Inst.* 98, 1482–1493
- 90 Tait, D.L. *et al.* (1999) Ovarian cancer BRCA1 gene therapy: Phase I and II trial differences in immune response and vector stability. *Clin. Cancer Res.* 5, 1708–1714
- 91 Raty, J.K. *et al.* (2007) Non-invasive imaging in gene therapy. *Mol. Ther.* 15, 1579–1586
- 92 Merron, A. *et al.* (2007) SPECT/CT imaging of oncolytic adenovirus propagation in tumours *in vivo* using the Na/I symporter as a reporter gene. *Gene Ther.* 14, 1731–1738
- 93 Winkler, A. *et al.* (2007) Switching on the lights for gene therapy. *PLoS ONE* 2, e528
- 94 Iyer, M. *et al.* (2005) Applications of molecular imaging in cancer gene therapy. *Curr. Gene Ther.* 5, 607–618
- 95 McCart, J.A. *et al.* (2004) Oncolytic vaccinia virus expressing the human somatostatin receptor SSTR2: molecular imaging after systemic delivery using ¹¹¹In-pentetreotide. *Mol. Ther.* 10, 553–561
- 96 Serganova, I. *et al.* (2007) Human reporter genes: potential use in clinical studies. *Nucl. Med. Biol.* 34, 791–807
- 97 Phuong, L. *et al.* (2003) Use of a vaccine strain of measles virus genetically engineered to produce carcinoembryonic antigen as a novel therapeutic agent against glioblastoma multiforme. *Cancer Res.* 63, 2462–2469
- 98 Rudin, C.M. *et al.* (2003) An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. *J. Clin. Oncol.* 21, 4546–4552
- 99 Lowe, S.W. *et al.* (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 74, 957–967
- 100 Hamada, M. *et al.* (1996) The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colorectal cancers. *J. Cancer Res. Clin. Oncol.* 122, 360–365
- 101 Liao, Y. and Hung, M.C. (2004) A new role of protein phosphatase 2a in adenoviral E1A protein-mediated sensitization to anticancer drug-induced apoptosis in human breast cancer cells. *Cancer Res.* 64, 5938–5942
- 102 Lee, W.P. *et al.* (2003) Adenovirus type 5 E1A sensitizes hepatocellular carcinoma cells to gemcitabine. *Cancer Res.* 63, 6229–6236
- 103 Cheong, S.C. *et al.* (2008) E1A-expressing adenoviral E3B mutants act synergistically with chemotherapeutics in immunocompetent tumor models. *Cancer Gene Ther.* 15, 40–50
- 104 Bennett, J.J. *et al.* (2004) Up-regulation of GADD34 mediates the synergistic anticancer activity of mitomycin C and a gamma134.5 deleted oncolytic herpes virus (G207). *FASEB J.* 18, 1001–1003
- 105 Stanziale, S.F. *et al.* (2002) Ionizing radiation potentiates the antitumor efficacy of oncolytic herpes simplex virus G207 by upregulating ribonucleotide reductase. *Surgery* 132, 353–359