Gene therapy represents a potentially useful approach for the treatment of diseases refractory to conventional therapies. Various preclinical and clinical strategies have been explored for treatment of gynaecological diseases. Given the most severe unmet clinical need, much of the work has been performed with gynaecological cancers and ovarian cancer in particular. Although the safety of many treatment strategies has been demonstrated in early phase clinical trials, efficacy has been mostly limited heretofore. Major challenges include improving the vectors used with the aim of more effective and selective delivery. In addition, effective penetration into and spreading within advanced and complex tumour masses and metastases remains challenging. This review focuses on existing and developmental gene transfer applications for gynaecological diseases.

Keywords: cervical cancer, gene therapy, gene transfer, gynaecological disease, ovarian cancer, virotherapy

1. Introduction

An increasing understanding of the molecular biology and genetics of diseases has rationalised gene transfer as an approach for the treatment of diseases resistant to more conventional therapies. The approach was originally developed for correction of either hereditary or somatic genetic defects but because proteins are involved in nearly all pathological and physiological phenomena, gene transfer can be used for almost anything. Usually, genes are not the target of therapy but just used for coding of the proteins that mediate the desired effects. Vehicles for gene transfer include both nonviral and viral vectors. The former approach most commonly uses plasmid DNA or cationic liposomes. Virus vehicles, such as adenovirus, retrovirus, adeno-associated virus (AAV) and herpes simplex virus (HSV), have already been optimised by evolution for gene delivery to human cells, and are, therefore, generally more effective. Nonviral approaches might be pharmacologically more attractive as they are structurally and functionally simpler; well tolerated and repeated dosing typically does not produce strong immunological responses.

2. Gene therapy for ovarian cancer

Despite improvements in the treatment of ovarian carcinoma in the last three decades, it remains the leading cause of death from gynaecological malignancies in developed countries [1]. Worldwide, the estimated number of new cases and deaths are 204,499 and 124,860, respectively, in 2002 [2]. Median survival has improved due to multi-modality treatments often including a combination of primary cytoreductive surgery with subsequent platinum and taxane chemotherapy. Nonetheless, early diagnosis continues to be challenging, and ∼ 70% of patients present with disseminated disease for which the 5-year survival rate is ∼ 30%.
Further, treatment of metastatic disease eventually results in drug resistance and disseminated disease cannot be cured. Therefore, novel treatment approaches such as targeted therapies are needed. Ovarian cancer spreads preferentially throughout the peritoneal cavity (stage III) and, because the peritoneal cavity is easily accessible and offers a degree of compartmentalisation, these patients represent an attractive target for locoregional therapy.

2.1 Targeting adenoviruses to ovarian cancer

A number of approaches have been tested in Phase I clinical trials with impressive safety data (Table 1). The most commonly used vector platform is adenovirus serotype 5 (Ad5), which binds to the coxsackie-adenovirus receptor (CAR) [3]. Importantly, successful gene xenografts have been demonstrated in most trials where it was analysed. However, despite exciting preclinical data, ovarian cancer gene therapy approaches have displayed only few definitive clinical breakthroughs heretofore. In general, this result has been attributed to insufficient transduction of cancer cells in the context of heretofore. In general, this result has been attributed to insufficient transduction of cancer cells in the context of advanced solid tumour masses. The main conclusion is that tumour transduction has often been too low for significant therapeutic antitumour effect.

There is a growing body of evidence that expression of CAR on primary human tumour cells is highly variable and often low [3,4]. As CAR is ubiquitously expressed on most epithelial tissues, the imbalance between CAR expression in tumour versus normal tissues has probably been one reason for low tumour transduction and subsequent insufficient efficacy in clinical trials. Another reason might be the greater complexity of advanced solid tumour masses in comparison to relatively rapidly growing xenografts. Further, the efficacy of systemically administered gene delivery vehicle to patients could be compromised by unfavourable bioavailability and neutralisation by the immune system. By extension, this implies that it is crucial to perform extensive sampling and biopsies in Phase I trials to acquire material for correlative studies.

Heretofore, all published adenovirus studies have been performed with CAR-binding viruses. However, various strategies have been preclinically evaluated to modify adenovirus tropism in order to circumvent CAR deficiency, for increased transduction of tumour cells and reduced normal tissue tropism (Figure 1A–E) [4]. Increased understanding of the adenoviral cellular entry pathway gave rise to the strategy of transductional targeting, which can be achieved by, for example, retargeting complexes such as bispecific molecules that block the interaction with CAR and redirect the virus to a novel receptor. Such molecules include chemical conjugates between Fab fragments derived from an antiknob monoclonal antibody and another Fab directed against the target antigen. The initial in vitro proof of principle study was performed by Douglas et al., with a Fab–folate conjugate. This retargeting resulted in CAR-independent, folate receptor-mediated transduction of cancer cells that highly expressed the folate receptor [5]. Alternatively, the other end of the conjugate can be a naturally-occurring ligand such as basic fibroblast growth factor [6]. Another example is a fusion protein between the extracellular domain of CAR and a single chain antibody (sFv) against CD40 [7,8]. Also, a trimeric sCAR-fibritin-anti-erbB2-sFv has been used for increased gene transfer to c-erbB2-positive ovarian cancer cell lines in vitro [9].

Another strategy involves genetic modification of the viral capsid. Enhanced infectivity of ovarian cancer cells has been demonstrated by incorporating an αβ integrin-binding arginine–glycine–aspartic acid (RGD-4C) motif in the HI loop of the fibre knob [10,11]. αβ integrins are often highly expressed in tumour cells and tumour vasculature making the target attractive. Furthermore, adenoviruses with a C-terminal polylysine tail (pK7) binding to heparan sulfates, often expressed to high degree in cancer cells, display enhanced infectivity [12-17]. The complete knob can be deleted and replaced with a retargeting ligand. The Ad5 tail and immediately proximal part of the shaft can be fused with the C-terminal bacteriophage T4 fibritin, which allows for the incorporation of complex targeting ligands, such as CD40 ligand [18].

Fibre pseudotyping has also been evaluated with promising results. Substitution of the knob domain of Ad5 with the corresponding domain of serotype 3 (Ad3) allows binding and entry through the Ad3 receptor, which is expressed to a high degree on ovarian cancer cells [19,20]. Also, other serotype chimaeras have been evaluated. Specifically, Ad5/35 chimaeras displayed enhanced infectivity of cancer cells and a favourable biodistribution profile [21,22].

One way to increase the utility of viral gene therapy may be through cell-permeable peptides. Small polybasic peptides have been shown to cross the cell membrane through a receptor-independent, non-endocytic mechanism. These cell-permeable molecules have been used as carrier sequences to increase virally mediated gene delivery, and thus protein expression, in ovarian cancer cells in vitro [23].

High tolerability of adenoviruses in cancer trials has allowed administration of large doses. In most trials, the maximum tolerated dose has not been reached and the ‘maximum affordable dose’ has become limiting instead. Nevertheless, some trials have reported abdominal pain or liver enzyme elevation [24,25], suggesting that transduction of normal tissue has the potential for toxicity. Also, although very safe in comparison to, for example, chemotherapy, it is now well known that adenoviruses can cause even fatal immune reactions [26]. Therefore, it has become attractive to restrict expression of viral genes or transgenes to tumour cells by using tumour/tissue-specific promoters (TSPs) in a strategy called transcriptional targeting (Figure 1F,G). Several TSPs have been evaluated for ovarian cancer specificity, including L-plastin [27], midkine [28], COX-2 (cox-2) [28-30], ovarian-specific promoter-1 [31], secretory leukocyte protease inhibitor promoter (SLPI) [29,32], survivin [33] and mesothelin [34].
Although transcriptional targeting can reduce toxicity associated with transgene expression in nontarget tissues, it does not reduce immunological recognition of virus particles and infected cells. An immune response towards infected tumour cells can be useful for eradication of metastases and protection against relapse. In contrast, an acute immune reaction or clearance of infected nontarget cells can be harmful. Specific transductional targeting of viruses to target cells is a useful way to retain the potentially beneficial aspects of a vector-targeted immune response while reducing immunological toxicity.

Double targeting for ovarian cancer has been achieved in vitro and in vivo. Transductional targeting with a sCAR-fibrin-anti-erbB2-sFv adapter was able to increase gene transfer to target cells while reducing transduction of non-target cells [9]. When combined with transcriptional targeting with the SLPI promoter, a further increase in selectivity was seen [32].

2.2 Replacement of tumour suppressor genes for treatment of ovarian cancer

Mutations of the p53 tumour suppressor gene are one of the most frequent genetic changes in cancer and they have been found in nearly 70% of advanced stage ovarian cancers [35]. Preclinical studies have demonstrated that adenovirus-mediated delivery of wild-type p53 inhibits growth of ovarian cancer cells both in vitro and in vivo (Figure 2A) [36,37]. p53 gene transfer to ovarian cancer cells using a cationic nonviral vector has also been reported, and re-establishment of wild-type p53 function restored the apoptotic pathway [38].
Adenoviral delivery with an Adp53 vector (SCH-58500) was evaluated in a Phase I/II trial and the treatment was well tolerated [24,39]. Importantly, gene transfer and biological activity were also demonstrated. Phase I patients received a single intraperitoneal injection of virus, whereas in Phase I/II they were treated with multiple doses, up to $7.5 \times 10^{13}$ viral particles (vp) on 5 consecutive days. Further, in the multiple dose schema, patients received three cycles of treatment, and last two of them were in combination with chemotherapy. Reverse transcriptase polymerase chain reaction (RT-PCR) transgene expression data were obtained from ascites fluid cell pellets and also tissue biopsies, although nonmalignant cells were not excluded. However, the presence of viral DNA in tumour cells was demonstrated with in situ PCR on a cancer sample from a single patient. Finally, 8 out of 16 patients in the multidose schema demonstrated $>50\%$ decrease in serum CA-125 level. In a similar study by Wolf et al., treatment was well tolerated and 1 out of 17 patients had $>50\%$ reduction in the CA-125 level [40].

Approximately 10\% of invasive ovarian cancers arise due to hereditary reasons, and many of these are caused by mutations in the BRCA-1 and BRCA-2 tumour suppressor genes. Retrovirus has been clinically studied for ovarian cancer therapy, for transfer of wild-type BRCA-1. A Phase I study using intraperitoneal delivery showed partial responses in 25\% of patients, and the majority had stable disease [42,43]. However, a subsequent Phase II study showed no responses and vector stability was discovered as poor [43].

### 2.3 Inhibition of growth factor receptors

Growth factor receptors such as HER-1 – 4 of the transmembrane receptor tyrosine kinase family can be...
targeted for replacement or inactivation. Deshane et al. constructed Ad21, an E1/E3-deleted adenovirus that encodes an intracellular single-chain antibody (intrabody) against erbB2/HER-2/neu [44,45]. This receptor is highly expressed in 10 – 30% of epithelial ovarian cancers with correlation to poor prognosis [46]. It was hypothesised that the intrabody traps HER-2 to the endoplasmic reticulum and therefore downregulates cell-surface expression of the protein. This approach resulted in induction of apoptosis and ovarian cancer cytotoxicity in vitro, and enhanced antitumour activity and survival in ovarian cancer animal models. In the setting of ovarian cancer, Ad21 was among the first clinically evaluated adenoviral gene therapy approaches [47]. The treatment was well tolerated up to $10^{11}$ plaque-forming units (pfu) without dose-limiting toxicity. Importantly, PCR and RT-PCR analyses from ascites samples demonstrated the presence of vector and expression of transgene, but the infected cell type was not studied. No responses were detected. The major disadvantage of this kind of an approach is the requirement of infecting each cancer cell to avoid rapid repopulation by uninfected cells.

Adenoviral E1A per se has been shown to downregulate HER-2 expression for tumour growth inhibition. Hortobagyi et al. evaluated cationic liposome-mediated E1A (DCC-E1A) transfer in a Phase I trial with breast and ovarian cancer patients [48]. DCC-E1A was administered as weekly intraperitoneal injections and expression of E1A and downregulation of HER-2 expression were demonstrated in peritoneal samples. Following dose escalation, abdominal pain eventually determined the maximum tolerated dose, and no responses were seen. This perhaps reflects the ineffectiveness of plasmid-based transduction in the context of advanced disease. A similar strategy was used in another Phase I trial with similar results [49].

2.4 Molecular chemotherapy
Molecular chemotherapy (aka suicide gene therapy) is a strategy based on delivery of a gene encoding a prodrug-activating enzyme (Figure 2B). The most popular approach in the context of ovarian cancer has been herpes simplex virus type 1 thymidine kinase (HSV-TK), which converts the systemically administrable and relatively nontoxic prodrug...
ganciclovir (GCV) into a toxic metabolite, which can diffuse into neighboring cells for the 'bystander effect'. Thus, HSV-TK/GCV system has an advantage as compared with the intrabody or p53 approaches [50]. In a Phase I ovarian cancer trial 14 patients were treated intraperitoneally with AdHSV-TK with single doses ranging from $1 \times 10^9$ to $1 \times 10^{11}$ pfu, which was followed by 14 days of intravenous GCV [51]. Transient vector-associated fever was experienced by 4 out of 14 (29%) patients but there were no dose-limiting side effects. HSV-TK gene transfer and transgene expression were detected in peritoneal aspirates but no objective responses were reported, although 5/14 (38%) had stable disease. Even with the bystander effect, the antitumour effect mediated by this approach might be limited to surface layers of tumour masses.

Another Phase I study combined an intraperitoneally delivered HSV-TK-encoding adenovirus to intravenous acyclovir and concomitant topotecan [25]. Authors reported grade 3 – 4 trombocytopenia and neutropenia, which were most likely related to chemotherapy. Fever was frequently seen and was probably vector related. A total of 5 out of 10 patients underwent second-look exploration 20 – 40 days after adenovirus delivery, but none of the peritoneal biopsies showed residual adenviral DNA, as would be expected if infected cells were killed by the acyclovir. Median survival was 18.5 months, which compares favourably to previously reported second- and third-line chemotherapy trials.

Concurrent translational studies revealed variable expression of CAR on ovarian cancer clinical samples, which may have compromised the efficacy of gene delivery in these trials. Therefore, an α,β integrin-binding RGD-4C motif was incorporated into the fibre of an adenovirus coding for HSV-TK and also an imaging cassette and a trial is forthcoming [52,53]. In contrast to replication-deficient and poorly replicating oncolytic virus platforms, HSV-TK/GCV probably does not add efficacy to highly potent oncolytic virotherapy [54].

### 2.5 Antiangiogenic gene therapy

A prerequisite for tumour growth beyond a few millimeters is neovascularisation. Antiangiogenic approaches inhibit formation of neovascularure and can also collapse immature tumour-associated vascular structures. The efficacy of the approach has recently been validated in clinical trials [55-57]. Gene therapy might be able to improve on these data by achieving higher persistent local concentrations of antiangiogenic molecules (Figure 2C). Ovarian cancer cells have been shown to express high levels of proangiogenic growth factors such as vascular endothelial growth factor (VEGF) [58]. Effects of VEGF are mediated through endothelial receptors, such as Flt-1 [59]. Soluble FMS-like tyrosine kinase receptor 1 (sFlt-1) is a splice variant of Flt-1 and binds to VEGF for inhibition of angiogenic actions, and may also prevent dimerisation of wild-type Flt-1. Mahasteshi et al. evaluated the effect of adenovirus-mediated sFlt-1 transfer against ovarian carcinoma [60,61]. Intraperitoneal delivery of an α,β integrin targeted virus-encoding, sFlt-1-inhibited ovarian tumour growth and increased the survival of mice. However, intravenous delivery of the same construct resulted in hepatotoxicity.

Inhibition of angiogenesis was demonstrated after intraperitoneal injection of an AAV expressing sFlt-1 [62]. In addition, other antiangiogenic genes such as endostatin and angiostatin have been packaged into AAV [63]. Intraperitoneal delivery achieved stable transduction without evidence of toxicity in a murine model. Combination therapy with paclitaxel resulted in long-term tumour-free survival. Furthermore, AAV-mediated expression of a mutant P125A-endostatin either alone or in combination with carboplatin treatment was evaluated using noninvasive imaging. The combination treatment synergistically inhibited intraperitoneal tumour growth and AAV integration was increased by chemotherapy [64]. Lentiviruses have not been widely used for ovarian cancer therapy but transfer of IFN-α has been evaluated in a murine model [65]. Antitumour effects were associated with a decrease in the formation of hemorrhagic ascites and a reduction in microvessel density.

### 2.6 Immunotherapy

Gene therapy can be used to induce immunity toward tumour antigens. One approach is to modify the tumour cells ex vivo and then return them into the patient (Figure 2D). If antigen presentation can be increased and energy broken, antitumour immunity may result. With regard to ovarian cancer, in vivo transfer of cytokines has been explored. Sterman et al. reported a case study with an adenovirus vector encoding for IFN-β. An ovarian cancer patient with pleural metastasis displayed partial response after a single intraperitoneal injection of vector although the disease progressed after four months [66]. Building on promising recent clinical data with monoclonal antibodies (mAb) [67], a full-length anti-HER-2 antibody was cloned into an adenovirus vector [68]. In a model of metastatic ovarian cancer, intravenous injection of virus resulted in measurable levels of trastuzumab in serum and antitumour efficacy was seen.

### 2.7 Virotherapy

For more than a century, it has been known that virus infection or vaccination with live viruses can result in tumour responses. Nevertheless, due to safety concerns, most gene therapy approaches have been based on viruses that are unable to replicate in infected cells. However, the main result from a generation of clinical cancer trials with these agents is that the utility of replication-deficient viruses may be limited when faced with advanced and bulky disease. The intratumoural diffusion of relatively large compounds such as viruses may be a limiting step. Although tumour targeting and infectivity enhancement have improved transduction rates of replication-deficient viruses preclinically, to the authors’
knowledge no trials have been initiated yet, although a number are in preparation [69].

Oncolytic viruses might prove useful for improving tumour transduction. These viruses have a cytolysic nature (i.e., the replicative life cycle of the virus results in host cell destruction). Infection of tumour cells results in replication, oncology and subsequent release of the virus progeny whereas normal tissues are spared due to lack of replication. Various naturally occurring, inherently tumour-selective or engineered oncolytic viruses have been used, including adenovirus, HSV, Newcastle disease virus, vaccinia, reovirus, measles and vesicular stomatitis virus. Oncolytic adenoviruses are the most widely studied members of this group (Figure 2E), and > 1000 cancer patients have been treated with them in clinical trials. The first oncolytic virotherapeutic agent H101 was recently approved based on promising data in head and neck cancer patients when given in combination with chemotherapy [70-72].

In type I oncolytic adenoviruses, tumour-specific replication is achieved by engineering deletions in genes critical for efficient viral replication in normal but not in tumour cells. The most widely studied one is ONYX-015 (aka dl1520 and closely related to H101), which carries deletions in E1B and has been postulated to replicate selectively in tumour cells [73]. ONYX-015 has been evaluated in a Phase I ovarian cancer trial [74]. A total of 16 patients received from 1 to 4 cycles of ONYX-015 on days 1 – 5 of every 3-week cycle at doses from 1 to 4 cycles of ONYX-015 on days 1 – 5 of every 3-week cycle at doses from 1 × 10^9 to 1 × 10^11 pfu. One patient developed dose-limiting grade 3 abdominal pain and diarrhoea but the maximum-tolerated dose as defined by protocol was not reached. Using PCR, the presence of ONYX-015 DNA in the cell-free fraction of peritoneal aspirates was demonstrated. In situ hybridisation of smeared ascites cell pellets demonstrated viral DNA in one case. Only 4 out of 16 treated patients demonstrated stable disease for at least 6 weeks and no responses were seen.

In addition to ONYX-015, many other deletion mutant oncolytic adenoviruses have been evaluated preclinically. α,β-integrin-targeted Ad5-Δ24RGD [75] and serotype 3-receptor targeted Ad5/3-Δ24 [76] contain a 24 base pair deletion in the retinoblastoma (Rb) binding site of E1A. Therefore, these viruses replicate selectively in cancer cells deficient in the Rb/p16 pathway (Figure 1H). Recent studies have demonstrated that both agents deliver a powerful anti-tumour effect to ovarian cancer cells in vitro, to clinical ovarian cancer specimens, and in orthotopic models of ovarian cancer, and both viruses are now proceeding towards clinical testing [76-79]. As compared to E1B mutated viruses, Δ24 agents are advantageous because they are not attenuated in tumour cells [80,81]. Ad5-Δ24RGD and Ad5/3-Δ24 and are, in fact, more oncolytic than wild-type virus because of the infectivity enhancement mediated by the capsid modifications [75,76,78].

Type II oncolytic adenoviruses are designed to achieve replicative specificity based on heterologous promoters placed to control the expression of early genes such as E1A, which is essential for viral replication. Promoters that have shown preclinical utility for ovarian cancer include IAI.3B [82], cox-2 [80] and SLPI [83]. However, none of these viruses has entered a clinical trial yet. Of note, the combination of both replication control strategies has shown promise in murine ovarian cancer models. The cox-2 promoter increased the specificity of a Δ24-based virus containing the Ad5/3 chimeric fibres, whereas in vivo efficacy was not compromised [84].

Wild-type measles is a negative-strand RNA virus causing rash, fever, cough and conjunctivitis [85]. The virus strain used for cancer therapy is derived from an attenuated vaccination strain (MV-Edm). Tumour cell killing is a consequence of cell-to-cell fusion and subsequent syncytial formation. A Phase I/II dose-escalation trial of an intraperitoneally administered carcinoembryonic antigen (CEA)-expressing measles virus (MV-CEA) in patients with recurrent ovarian cancer is ongoing and preliminary data suggests good safety and some evidence of efficacy has also already been seen in the form of CA-125 decreases [86]. The virus produces CEA, which allows monitoring of the degree and longevity of virus replication. A similar approach has been used preclinically in the context of adenovirus [87,88].

3. Gene therapy for other gynaecological cancers

Although ovarian cancer is the most problematic gynaecological cancer in developed societies, cervical cancer remains the leading cause of mortality worldwide [2]. Unfortunately, improvements in surgery, chemotherapy and radiotherapy have not decreased mortality much, and patients with advanced, recurrent or metastatic disease still have a poor chance of being cured. The pathogenesis of cervical cancer follows a natural history characterised by human papilloma virus (HPV) infection, a long latency period, and progression in a fraction of patients through dysplasia and carcinoma in situ to invasive cancer and metastatic disease [89]. Only a few viral strains are specifically responsible for cervical neoplasms, and HPV16 accounts for > 50% of reported cases.

Carson et al. reported a strategy for killing of papilloma virus-infected cells by conditional expression of the HSV-TK gene delivered with AAV followed by GCV treatment [90]. TK expression was specifically induced as a result of homologous recombination between the TK gene cassette and endogenous virus genome in infected cells. Also, adenoviruses have been studied as gene transfer vectors for cervical cancer cells. A serotype 5/35 chimeric vector enhanced the infectivity of primary cancer cell cultures derived from cervix biopsies. When expression of the relevant receptor was analysed on Pap smears, all samples from patients with invasive cervical cancer stained positive for CD46 (the Ad35 receptor) and negative for CAR [91]. In another recent study, Ad5-Δ24RGD demonstrated effective oncolysis of cervical...
cancer cells and therapeutic efficacy was demonstrated in a mouse model of cervical cancer with both intratumoural and intravenous application [92]. Importantly, the virus caused no toxicity to human peripheral blood mononuclear cells. Another interesting approach, which takes advantage of similarities between gene products of DNA viruses, is complementation of an E1-deleted adenovirus by HPV E6/E7 genes [93]. This virus was further armed with a p53 variant resistant to E6-mediated degradation. The in vitro oncolytic potency was enhanced although selectivity was reported retained [94].

An alternative approach is based on the observation that tumour suppressor functions of p53 are downregulated in most cervical cancer cells. The product of HPV oncogene E6 binds to and inactivates p53 by promoting its degradation. p73 is similar to p53 in structure and function but not degraded by E6. Das et al. demonstrated growth inhibition of E6-positive cell lines in vitro following infection with Ad-p73 [95]. Adenovirus vectors have also been used for the delivery of HPV16 E6/E7 antisense RNA into cervical cancer cells in vitro, ex vivo and in vivo. p53 and Rb protein expression increased after infection, and the infected cells underwent apoptosis [96].

HPV-induced premalignant and malignant diseases have been treated with an attenuated vaccinia virus expressing E6 and E7 oncoproteins and IL-2. Encouraging results have been observed with this immunotherapy approach in clinical trials [97].

In the first published adenovirus virotherapy trial, various wild-type strains were injected intratumourally, intravenously and intraarterially to cervical cancer patients, with or without concomitant immune suppression [98]. No severe toxicities were reported. Transient responses were seen but eventually all patients had progressive disease. Unfortunately, the approach was discontinued for nearly 50 years.

Endometriosis, the growth of ectopic endometrial tissue, is an estrogen-dependent disease that causes pain and infertility. Moreover, an association between untreated endometriosis and development of ovarian cancer has been proposed [105]. Typically, endometriosis is treated with surgical removal of lesions and medical therapy aiming at a hypoestrogenic state [102]. A central feature of active endometriosis is pronounced vascularisation and, therefore, antiangiogenic gene therapy has been evaluated [106]. In a murine model, intraperitoneal delivery of an adenovirus encoding angiostatin caused a decrease in the number, size and density of blood vessels. More importantly, established endometriosis was eradicated in all treated mice within 18 days. For evaluation of HSV-TK/GCV in the context of endometriosis, human endometrial fragments were infected ex vivo and injected subcutaneously into nude mice. GCV induced a significant regression of endometrial implants [107].

Placental disorders and dysfunction can cause significant fetal and maternal morbidity, including fetal growth retardation, pre-eclampsia, eclampsia and even death. Initially, there is defective development of the early placenta and its maternal blood supply. The clinical syndrome arises from subsequent generalised maternal endothelial dysfunction [102]. Pathologically, a hypoxic and dysfunctional placenta releases factors such as sFlt-1, which binds VEGF and placental growth factor [108]. Increased understanding of these mechanisms can facilitate the development of gene therapeutic strategies for treatment of pre-eclampsia, which might help prolong the pregnancy. One promising approach is delivery of gene-modified placental cells for secretion of proteins throughout gestation without deleterious effects reported [109]. Further, plasmid DNA and adenoviruses have been guided with angiography to rabbit uterine arteries for transfection of trophoblast cells. Transfection efficiency was as high as 34% with adenovirus, whereas plasmid complexes achieved much lower rates [110]. Insulin-like growth factors (IGFs) I and II are critical in fetal growth because of their role in placental development and function, and reduced levels have been reported in intrauterine growth retardation. Adenoviruses encoding IGF-I or IGF-II were used for in vitro gene transfer to fresh, human primary placental fibroblasts.

4. Gene therapy for benign obstetric and gynaecological disorders

Leiomyomas are benign estrogen-dependent uterine tumours, which only become clinically relevant when they enlarge enough to elicit symptoms such as pain or bleeding. Further, they can cause infertility and miscarriages. At present, treatment is usually hysterectomy or myomectomy [102]. However, because the disease is localised to the uterus, it is an ideal target for local gene therapy via ultrasound-guided injections, laparoscopy or hysteroscopy (Table 2). A plasmid-based strategy with HSV-TK/GCV was assessed in vitro both in human clinical samples and a rat leiomyoma cell line. A bystander effect was demonstrated, and interestingly, it increased with estradiol treatment [103]. In a murine leiomyoma xenograft model, adenoviral expression of a dominant negative estrogen receptor inhibited subcutaneous tumour growth and cell proliferation, and increased apoptosis was found [104].

Endometriosis, the growth of ectopic endometrial tissue, is an estrogen-dependent disease that causes pain and infertility. Moreover, an association between untreated endometriosis and development of ovarian cancer has been proposed [105]. Typically, endometriosis is treated with surgical removal of lesions and medical therapy aiming at a hypoestrogenic state [102]. A central feature of active endometriosis is pronounced vascularisation and, therefore, antiangiogenic gene therapy has been evaluated [106]. In a murine model, intraperitoneal delivery of an adenovirus encoding angiostatin caused a decrease in the number, size and density of blood vessels. More importantly, established endometriosis was eradicated in all treated mice within 18 days. For evaluation of HSV-TK/GCV in the context of endometriosis, human endometrial fragments were infected ex vivo and injected subcutaneously into nude mice. GCV induced a significant regression of endometrial implants [107].
IGFs exerted both autocrine and paracrine effects on cell proliferation, migration and survival \[113\].

Molecular defects have been implicated in embryo implantation disorder making it a possible target for gene therapy. Homeobox (HOX) genes are transcription factors necessary for embryonic development. Unlike in most adult tissues, HOXA10 and HOXA11 expression persists in the endometrium, and they are essential for endometrial development and regulation by sex steroids. Interestingly, it has been shown that mice with disruption of HOXA10 are infertile because of implantation failure \[112\]. More importantly, defects in endometrial HOX gene expression in infertile women have been demonstrated \[112\]. Thus, augmenting HOX expression for improving implantation becomes attractive and has already been achieved in mice by intratuerine administration of a HOXA10 plasmid/liposome complex \[113\].

In general, non-malignant gynaecological diseases are less severe and more treatable with current therapies than gynaecological cancers. Therefore, clinical translation of gene therapy strategies probably requires even more stringent safety information. Moreover, given the immunogenic nature of adenoviruses, other vectors such as lentiviruses and AAV may be more attractive for this group of diseases.

### 5. Conclusion

Recent evidence suggests that relatively conventional gene therapy approaches, when applied following maximal cyto-reduction, can increase the survival of cancer patients \[114\]. Nevertheless, only a few pioneering studies have managed to fully harness the power of correlative analysis in Phase I trials and these studies have implied that traditional delivery systems usually result in insufficient gene transfer when faced with advanced tumour masses \[115\]. To improve the quality and quantity of correlative data in early phase trials, it is important to increase the capacity for evaluation of the persistence and magnitude of virus replication. Because obtaining serial biopsies is difficult due to safety, cost and compliance issues, noninvasive strategies are most attractive. Some promising approaches include functional imaging of transgenes, incorporation of secretable marker proteins, and detection of fluorescent proteins incorporated into virus capsids \[53,87,88,116,117\].

Several strategies are being explored to improve transduction of target cells and effective penetration of solid tumours. For example, gene transfer by viral vectors can be enhanced by using modified agents that are retargeted to receptors highly expressed on target cells \[118\]. Nonetheless, viral spread in the tumour can be limited by physical barriers such as stromal cells and matrix, necrotic, hypoxic or hyperbaric regions. For overcoming these obstacles, selectively oncolytic viruses may be useful and transductional targeting of oncolytic viruses to tumour cells is a logical sequel to work done with replication-deficient viruses. RGD-4C-modified oncolytic adenovirus, Ad5-Δ24RGD, which was able to replicate in ovarian cancer primary cell spheroids and resulted in significantly prolonged survival in an aggressive orthotopic ovarian cancer model, is entering a multi-centre Phase I clinical trial for treatment of ovarian cancer \[3,69\]. For further potentiation, replication competent viruses can be armed with therapeutic transgenes such as cytokines, suicide genes, fusogenic, proteolytic or antiangiogenic moieties \[119\]. A powerful approach recently validated in clinical trials is utilisation of gene transfer in combination with conventional anticancer therapies in a multimodal antitumour approach \[72,120\]. Gene therapy differs from traditional modalities with regard to mechanism and side effects, providing a possibility for additive or synergistic interactions \[122\].
The aforementioned intratumoural complexities also hinder conventional antitumour approaches such as chemotherapy, and it is known that effective treatments usually require multiple rounds of administration. Thus, clinical gene transfer might also benefit from readministration of the virus, whose efficacy may be inhibited by pre-existing neutralising antibodies (NAb). Strategies for facilitating retreatment include alternating related viruses with different capsids (sero-switch) [122], co-treatment with immunosuppressive drugs such as cyclophosphamide for temporary abrogation of NAb induction [123] or physical removal of NAb by using immunoapheresis or an adenovirus capsid protein column [124].

6. Expert opinion

Translation of preclinical advances into clinical trials remains the bottleneck that rate-limits development of the field. Only in patients can we find out which approaches work and why. Laboratory work can provide preliminary data and allow for testing of hypotheses but definite answers can only be obtained in humans. Comprehensive correlative analysis of specimens obtained from treated patients would allow the translational process to cycle rapidly back to the lab for development of next generation agents. Unfortunately, despite an obvious unmet clinical need on one hand and a plethora of promising agents on the other, clinical translation is progressively becoming more difficult. In the European Union, current rules are severely limiting and have made noncommercial trials nearly impossible [125]. Also, the increasingly complex nature of treatment agents complicates intellectual property aspects and reduces the interest of the pharmaceutical industry in the field. Technical challenges include improving gene delivery and potency to levels compatible with more regular clinical responses. In particular, dealing with intratumoural complexity and immunological issues remains challenging. Also, given the recent success of monoclonal antibodies and small-molecular inhibitors as effective and relatively nontoxic antitumour agents, gene therapy and oncolytic viruses need to deliver emphatic clinical results to attract resources compatible with transformation of promising approaches to clinically successful strategies. Fortunately, recent watershed clinical trials [70,114,126-128] have demonstrated that the theoretical hypotheses behind gene delivery for therapeutic effect can be reproduced in humans, and the technology remains a viable and potent approach for treatment of diseases resistant to available modalities. In particular, the tremendously active Chinese biotechnology environment may help bring gene therapeutics forward from the fringe [71]. Our opinion is that all that is needed to release the gene therapy avalanche is one or two unequivocal successes. Our view is that the technology is already potent enough for many purposes and safety has been consistently excellent. Therefore, we are optimistic that during the next decade, the first gene therapy agents will be globally approved and become part of clinical routine.

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Gene therapy of gynaecological diseases

• First report of both transductional and transcriptional vector targeting in the context of gynecological cancers.
• The only report on an unpublished, randomized, Phase III ovarian cancer gene therapy trial.
• Interesting Phase I ovarian cancer trial.
• Interesting Phase I trial.
• Interesting Phase I ovarian cancer trial.
• New generation adenovirus allows prodruk conversion and imaging.
growth factor.


Gene therapy of gynaecological diseases


106. IMMONEN A, VAPALAHTI M, TYYNELA K et al.: AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant

** Landmark randomised study demonstrating a survival advantage in glioma patients treated with adenoviral gene therapy.


- Interesting approach for overcoming neutralizing antibodies.


- The capsid column for removing anti-adenovirus antibodies is of particular interest in this study.


** One of the first examples of successful gene therapy of humans.


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