Gene transfer presents a potentially useful approach for the treatment of diseases refractory to conventional therapies. Various preclinical and clinical strategies have been explored for treatment of gynecological diseases. Given the direst need for novel treatments, much of the work has been performed with gynecological cancers and ovarian cancer in particular. Although the safety of many approaches has been demonstrated in early phase clinical trials, efficacy has been mostly limited so far. Major challenges include improving gene transfer vectors for enhanced and selective delivery and achieving effective penetration and spread within advanced and complex tumor masses. This review will focus on current and developmental gene transfer applications for gynecological diseases.

Key Words: gene transfer, gene therapy, ovarian cancer, cervical cancer, gynecological disease
therapy approaches featuring taxanes and platinum, when given following optimal cytoreductive surgery, can increase the survival of patients, treatment of metastatic disease eventually results in drug resistance and disseminated disease cannot be cured. Therefore, novel treatment approaches are needed. Gene therapy, even at its current rather adolescent stage, is an attractive modality for ovarian cancer as this cancer frequently presents with metastases confined to the peritoneal cavity, creating a rationale for locoregional delivery.

**Targeting Vectors to Ovarian Cancer Cells**

The majority of gene therapy approaches for ovarian cancer (Table 1) are based on adenovirus serotype 5 (Ad5), which binds to the coxsackie—adenovirus receptor (CAR). A number of approaches have been tested in phase I clinical trials with impressive safety data. Moreover, successful gene transfer has been demonstrated in most cases in which it has been analyzed. In contrast, only rare examples of efficacy have been published. This is partly influenced by trial design (phase I trials usually have safety as the main endpoint), but nevertheless the lack of response implies that there is a discrepancy between preclinical and clinical efficacy.

One possible reason is that there might be a tendency for researchers to use models that allow effective transduction, and therefore variable CAR expression has been recognized only upon analysis of clinical substrates. Another reason might be the greater complexity of advanced solid tumor masses in comparison to relatively rapidly growing xenografts. By extension, this implies that it is crucial to perform extensive sampling and biopsies in phase I trials to acquire material for correlative studies. Obviously, this is hampered by compliance and cost issues and the fact that traditionally phase I trials have mostly looked at safety.

Heretofore, all published studies have been performed with CAR-binding viruses. Unfortunately, concurrent studies have suggested that expression of CAR is frequently dysregulated in many types of advanced cancers, including ovarian cancer [2]. Various strategies have been evaluated to modify adenovirus tropism to circumvent CAR deficiency, for increased transduction of tumor cells and reduced normal tissue tropism. Transductional targeting can be achieved by utilizing bispecific molecules that block the interaction with CAR and redirect the virus to a novel receptor. Several ligands, including basic fibroblast growth factor [3], anti-TAG-72 [4], and anti-CD40 [5], have been physically linked to an Ad5-fiber-binding moiety for enhanced transduction.

Another strategy involves genetic modifications of the viral capsid. Enhanced infectivity of ovarian cancer cells has been demonstrated by incorporating an integrin-binding RGD-4C motif in the HI loop of the fiber knob [6]. Fiber pseudotyping has also been evaluated. 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 [7,8].

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 elevations [9,10], suggesting that transduction of normal tissue has the potential for toxicity. Also, while very safe in comparison to, e.g., chemotherapy, it is now well known that adenoviruses can cause even fatal immune reactions [11]. Therefore, it has become attractive to restrict expression of viral genes or transgenes to tumor cells by using tumor-specific promoters (TSPs) in a strategy called transcriptional targeting. Several TSPs have been evaluated for ovarian cancer specificity, including L-plastin [12], midkine [13], cyclooxygenase-2 (cox-2) [13], ovarian-specific promoter-1 [14], secretory leukoprotease inhibitor promoter (SLPI) [15,16], and mesothelin [17].

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 toward infected tumor 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. Other approaches for reducing immune responses toward adenovirus are discussed in the last section.

**Replacement of an Altered Tumor Suppressor Gene**

Mutation of the p53 tumor suppressor gene is one of the most frequent genetic changes in cancer and it has been found in nearly 60% of advanced ovarian cancers [18]. Preclinical studies have demonstrated that adenovirus-mediated delivery of wild-type p53 inhibits growth of ovarian cancer cells both in vitro and in vivo [19,20] (Fig. 1A). p53 gene transfer to ovarian cancer cells using cationic nonviral vector has also been reported [21]. Adp53 was evaluated in a phase I/II trial and the treatment was well tolerated [9,22,23]. Gene transfer and biological activity were also demonstrated [24].

These findings led to a randomized phase II/III trial in which Adp53 was given intraperitoneally in combination with chemotherapy. Although complete results have unfortunately not been published, the first interim analysis suggested a lack of therapeutic effect but increased toxicity and the study was closed [25]. In parallel with most trials with this approach, trans-
<table>
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<td>Measles virus</td>
<td>Virotherapy</td>
<td>In vitro and in vivo</td>
<td>Killing of ovarian cancer cells, expression of soluble marker peptide [107]</td>
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<td>Ad-MN/Ca9-E1a</td>
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<td>HSV-TK suicide gene therapy</td>
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<td>Inhibition of endometrial cancer cell growth, induction of apoptosis [71]</td>
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<td>pNFn-B-TK</td>
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TABLE 1 (continued)

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<td>\textit{In vitro}</td>
<td>High transgene expression in teratocarcinoma cells</td>
<td>[112]</td>
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Inhibition of Growth Factor Receptors

Growth factor receptors such as erbB1–erbB4 of the epidermal growth factor receptor family can be targeted for replacement or inactivation. Deshane \textit{et al.} constructed a gene that encodes an intracellular single-chain antibody (intrabody) against erbB2/HER-2/neu [28]. This receptor is highly expressed in 10–15% of ovarian cancers with correlation with poor prognosis [29]. Adenovirus (Ad21)-mediated transfer of the intrabody to ovarian tumors resulted in induction of apoptosis and cytotoxicity \textit{in vitro} and enhanced efficacy and survival in animal models of ovarian cancer [30–32]. The strategy was subsequently evaluated in a phase I trial [33]. Intraperitoneal treatment was well tolerated without dose-limiting toxicity, and gene transfer was demonstrated but no responses were detected.

Adenoviral E1A has been shown to downregulate erbB2 expression with concomitant growth inhibition [34]. Hortobagyi \textit{et al.} evaluated cationic liposome-mediated E1A transfer in a phase I trial with breast and ovarian cancer patients [35]. Expression of E1A and downregulation of erbB2 expression were demonstrated in peritoneal samples. Following dose escalation, abdominal pain eventually identified the maximum tolerated dose, but stable disease was detected in only 17% of patients, a rather low figure perhaps reflecting the effectiveness of plasmid-based transduction in the context of advanced disease. A similar strategy was used in another phase I trial [36].

Molecular Chemotherapy

Molecular chemotherapy (a.k.a. suicide gene therapy) is a strategy based on delivery of genes encoding a prodrug-activating enzyme (Fig. 1B). The most popular approach in the context of ovarian cancer has been herpes simplex virus thymidine kinase (HSV-TK), which converts the prodrug ganciclovir (GCV) into a toxic metabolite. The HSV-TK/GCV system is associated with a “bystander effect,” i.e., killing of uninfected neighboring cells. Based on promising preclinical results [37,38] Alvarez \textit{et al.} utilized intraperitoneal delivery of a replication-deficient adenovirus (AdHSV-TK) followed by intravenous GCV [39]. No dose-liming side effects were seen and 38% of patients had stable disease for the duration of the study. Transgene expression could be detected from ascites samples of patients. Another phase I study combined intraperitoneal AdHSV-TK with intravenous acyclovir and topotecan [10]. Again, no dose-limiting adverse effects were seen, and the most common side effect was myelosuppression most likely related to chemotherapy. The median survival of these patients was 18.5 months [40]. As an example of bench-to-bedside-and-back translational work, when clinical specimens revealed variable expression of CAR, the efficacy of the HSV-TK/GCV approach was subsequently enhanced \textit{in vitro} and \textit{in vivo} by incorporating an integrin-binding RGD-4C motif into the adenoviral fiber [41,42], and a trial is forthcoming.

Antiangiogenic Gene Therapy

Antiangiogenic gene transfer inhibits formation of neo-vascularature required for tumor growth and may also act by collapsing immature tumor-associated vascular structures (Fig. 1C). Ovarian cancer cells have been shown to express proangiogenic growth factors such as vascular endothelial growth factor (VEGF) [43]. Effects of VEGF are mediated through the endothelium-specific VEGF receptors such as Flt-1 [44]. Soluble FMS-like tyrosine kinase receptor 1 (sFlt-1) is a splice variant of Flt-1 and binds to VEGF, inhibiting its angiogenic actions and may also prevent dimerization of wild-type Flt-1. Mahasreshi \textit{et al.} evaluated the effect of adenovirus-mediated sFlt-1 transfer against ovarian carcinoma [45,46]. Intraperitoneal delivery of an integrin-targeted virus encoding sFlt-1 inhibited ovarian tumor 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 [47]. Also other antiangiogenic genes such as mutant
endothelin have been packaged into AAV for in vivo efficacy [48]. Lentiviruses have not been widely used for ovarian cancer therapy, but transfer of interferon-α has been evaluated in a murine model [49]. Antitumor effects were associated with a decrease in the formation of hemorrhagic ascites and a reduction in microvessel density.

**Virotherapy**

Utilization of the oncolytic potential of viruses for killing of tumor cells predates the concept of gene therapy by more than half a decade [50]. Nevertheless, due to safety concerns, most modern 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 trials with these agents is that the utility of replication-deficient viruses may be limited when faced with advanced and bulky disease. Thus, intratumoral diffusion of nanosize carriers such as viruses may be a limiting step. While tumor targeting and infectivity enhancement have improved transduction rates of replication-deficient viruses preclinically, to our knowledge no trials have been initiated yet, although a number are in preparation (Table 1). A specific obstacle with regard to analysis of oncolytic viruses on clinical specimens is the limited viability of the latter in vitro. This can be partly overcome by maintaining clinical samples as multicellular tumor clusters or spheroids [51]. This technology has been applied to analysis of transductionally targeted oncolytic adenoviruses [52–55], but correlation to clinical responsiveness is not yet available.

**FIG. 1.** Gene therapy approaches. (A) Replacement of a mutated tumor suppressor gene. Delivery and expression of a wild-type gene results in apoptosis and cancer cell death. (B) Molecular chemotherapy. Delivery and expression of a suicide gene results in conversion of a nontoxic prodrug into a cytotoxic metabolite. (C) Antiangiogenic gene therapy. Delivery of a soluble VEGF receptor results in sequestration of VEGF and subsequent inhibition of neovascularization. (D) Virotherapy. Viral infection of cancer cells results in replication, oncolysis, and release of virions to surrounding cells.
To improve tumor penetration, various naturally occurring, inherently tumor-selective or engineered oncolytic viruses have been utilized, including adenovirus, HSV, Newcastle disease virus, vaccinia, reovirus, measles virus, and vesicular stomatitis virus [56]. Conditionally replicating adenoviruses (CRAds) are the most widely studied members of this group (Fig. 1D), and more than 500 cancer patients have been treated with CRAds [2,57].

In type I CRAds, tumor-specific replication is achieved by engineering deletions in genes critical for efficient viral replication in normal but not in tumor cells [58]. The most widely studied CRAd, ONYX-015 (dl1520), carries deletions in E1B, exhibits reduced binding of p53, and replicates selectively in tumor cells [59]. ONYX-015 has been evaluated in a phase I ovarian cancer trial [60]. Treatment resulted in grade 3 abdominal pain and diarrhea in one patient but the maximum tolerated dose was not reached, and the “maximum affordable dose” was $10^{11}$ viral particles. However, there were no clinical or radiological responses in any patients.

In addition to ONYX-015, many type I CRAds have been evaluated preclinically. Integrin-targeted Ad5-Δ24RGD and serotype 3 receptor-targeted Ad5/3-Δ24 contain a 24-bp deletion in the retinoblastoma (Rb) binding site of EIA. Therefore, these viruses replicate selectively in cancer cells deficient in the Rb/p16 pathway. Recent studies have demonstrated that both agents deliver a powerful antitumor effect to ovarian cancer cells in vitro, to clinical ovarian cancers, and in orthotopic models of ovarian cancer, and both viruses are now proceeding toward clinical testing [52,53,61].

Type II CRAds are designed to achieve replicative specificity based on heterologous promoters placed into the adenovirus genome to control the expression of the early genes such as EIA, which is essential for viral replication. The utility of these agents is subservient to the identification of promoters that induce the appropriate inductivity vs specificity profile [62]. Promoters that have shown utility for ovarian cancer include IAI.3B, cox-2, and SLPI [55,63,64].

**Gene Therapy for Other Gynecological Cancers**

While ovarian cancer is the most problematic gynecological cancer in developed societies, cervical cancer remains the leading cause of mortality worldwide [1]. Unfortunately, neither improvements in surgery nor radiotherapy has significantly decreased mortality [65], 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 characterized by human papillomavirus (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. Only a few viral strains are specifically responsible for cervical neoplasms, of which HPV16 accounts for more than one-half of reported cases. Carson et al. demonstrated a novel gene-based strategy to prevent virus replication in HPV-infected cells through the conditional expression of the HSV-TK gene [66]. Delivery of HSV-TK with AAV followed by GCV treatment resulted in efficient cell killing of HPV-positive cells.

CRAds represent another promising treatment alternative. In a recent study, Ad5-Δ24RGD demonstrated effective oncolysis in cervical cancer cells [67]. Moreover, therapeutic efficacy could be demonstrated in a mouse model of cervical cancer with both intratumoral and intravenous application. Importantly, no toxicity was seen with human peripheral blood mononuclear cells. Another interesting approach, which takes advantage of similarities between gene products of DNA viruses, is complementation of adenovirus mutants by HPV genes [68].

An alternative approach to inhibiting the growth of cervical cancer cells is based on the observation that tumor suppressor p53 functions 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 the HPV E6 gene product. Das et al. demonstrated growth inhibition of E6-positive cell lines in vitro following infection with Ad-p73 [69].

Endometrial carcinoma is the most common neoplasm of the female reproductive tract and it accounts for nearly one-half of all gynecologic malignancies. Although usually curable with surgery, sometimes aggressive tumors such as uterine papillary serous carcinomas (UPSC) are seen. Immunohistochemical studies suggest that p53 is aberrant in 50–90% of UPSC tumors in comparison to 10–30% in typical endometrioid adenocarcinomas [70]. In a recent study, adenoviral delivery of p53 or p21 resulted in growth suppression and induction of apoptosis in a UPSC cell line [71].

Another interesting gene therapy approach for endometrial cancer is based on the observation that the gonadotropin-releasing hormone receptor (GnRH-R) is expressed by the majority of ovarian and endometrial cancers. GnRH-R is a promising tumor-specific target due to limited normal tissue expression. Grundker et al. demonstrated the efficacy of HSV-TK/GCV controlled by GnRH-R-specific elements in intraperitoneal and subcutaneous mouse models of endometrial and ovarian cancer [72].

**Gene Therapy for Other Gynecological Disorders**

Leiomyomas are benign, proliferating, estrogen-dependent uterine tumors, which become clinically relevant only when they enlarge enough to elicit symptoms such
as abnormal bleeding [73]. Further, they can cause infertility and miscarriages. Current treatment is usually hysterectomy or myomectomy. However, the disease is localized to the uterus, which makes it 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 in a rat leiomyoma cell line. A bystander effect was demonstrated, and interestingly, it was increased with estradiol treatment [74]. In a murine leiomyoma xenograft model adenovirus-mediated expression of a dominant negative estrogen receptor inhibited subcutaneous tumor growth and cell proliferation, while increased apoptosis was found [75].

Endometriosis, the growth of ectopic endometrial tissue, is an estrogen-dependent disease that causes pain and infertility. Moreover, there is an association between untreated endometriosis and development of ovarian cancer. Typically, it is treated with surgical removal of the lesions and medical therapy aiming at a hypoestrogenic state [73]. An important feature of active endometriosis is pronounced vascularization, and therefore antiangiogenic gene therapy has been evaluated [76]. In a murine model, intraperitoneal delivery of an adenovirus encoding the angiogenesis inhibitor 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 [76]. Fortin et al. evaluated the efficacy of HSV-TK/GCV for treatment of endometriosis. Human endometrial fragments were infected ex vivo with an adenovirus containing HSV-TK and injected subcutaneously into nude mice. GCV treatment induced a significant regression in endometrial implants [77].

Placental disorders and dysfunction cause significant fetal and maternal morbidity, including fetal growth retardation, preeclampsia or eclampsia, and mortality. Initially, there is defective development of the early placenta and its maternal blood supply. The clinical syndrome arises from subsequent generalized maternal endothelial dysfunction [73]. Pathologically, a hypoxic and dysfunctional placenta releases factors such as sFlt-1, which binds VEGF and placental growth factor [78]. Increased understanding of these mechanisms facilitates development of gene therapeutic strategies for treatment of preeclampsia and prolonging the pregnancy. Senut et al. delivered gene-modified placental cells to the rodent placenta in vivo and demonstrated that gene products were secreted throughout gestation without deleterious effects [79]. Plasmid DNA and adenoviruses have been guided with angiography to uterine arteries in rabbits for transfection of trophoblast cells. Transfection efficiency was as high as 34% with adenovirus, while plasmid complexes led to much lower rates [80]. 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 utilized for in vitro gene transfer to fresh, human primary placental fibroblasts. IGFs exerted both autocrine and paracrine effects on cell proliferation, migration, and survival [81].

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 receptivity in response to sex steroids. Interestingly, it has been shown that mice with disruption of the HOXA10 gene are infertile because of implantation failure [82]. More importantly, defects in endometrial HOX gene expression in infertile women have been demonstrated [82]. Thus, augmenting HOX gene expression with gene therapy to improve implantation becomes attractive and has already been achieved.

<table>
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<th>TABLE 2: Gene therapy approaches for noncancer gynecological diseases</th>
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<td>HOXA10 cDNA</td>
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with intrauterine administration of \textit{HOXA10} plasmid/liposome complex to mice \cite{83}.

In general, nonmalignant gynecological diseases are less severe and more treatable than gynecological 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.

\textbf{FUTURE DIRECTIONS}

Recent evidence suggests that relatively conventional gene therapy approaches, when applied following maximal cytoreduction, can increase the survival of cancer patients \cite{84}. Nevertheless, only a few pioneering studies have managed to harness fully 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 tumor masses \cite{85}. To improve the quality and quantity of correlative data in early phase trials, it is important to increase our capacity for detection 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 \cite{42,86,87}.

Several strategies are currently being explored to improve transduction of target cells and effective penetration of solid tumors. For example, gene transfer by viral vectors can be enhanced by using modified agents that are retargeted to receptors highly expressed on target cells \cite{88}. Nonetheless, viral spread in the tumor can be limited by physical barriers such as stromal cells and matrix and necrotic, hypoxic, or hyperbaric regions. For overcoming these obstacles, selectively oncolytic viruses may be useful and targeting oncolytic viruses to tumor cells is a logical sequel \cite{52,61}. For further potentiation, replication-competent viruses can be armed with therapeutic transgenes such as cytokines, suicide genes, and fusogenic, proteolytic, or antiangiogenic moieties \cite{89}.

A powerful approach for increasing efficacy is utilization of gene transfer in combination with conventional anticancer therapies in a multimodal antitumor approach \cite{90}, which has recently been validated in randomized trials \cite{57,91,92}. Gene therapy differs from traditional modalities with regard to mechanism and side effects, providing a possibility for additive or synergistic interactions \cite{93,94}.

The aforementioned intratumoral complexities hinder also conventional antitumor approaches such as chemotherapeutic and, it is known that effective treatments usually require multiple rounds of administration; solid tumors can usually be reduced only layer by layer. Thus, clinical gene transfer might benefit from readministration of virus, whose efficacy may be inhibited by neutralizing antibodies (NAb). Strategies for facilitating re-treatment include alternating related viruses with different capsids (sero-switch) \cite{95}, cotreatment with immunosuppressive drugs for temporary abrogation of NAb induction \cite{96}, or physical removal of NABs by using immunopheresis or an adenovirus capsid protein column \cite{97}.

Most importantly, it remains crucial to translate preclinical advances quickly into clinical trials, because only in patients can we find out which approaches work and which do not. Comprehensive correlative analysis of specimens obtained in these trials allows the translational process to cycle rapidly back to the lab for development of next generation agents. It may be that the biggest obstacle cancer gene therapy faces is the continually increasing difficulty in rapidly setting up phase I trials in an ever-tightening regulatory environment. Other challenges include improving gene delivery and potency to levels compatible with clinical responses. Also, given the recent success of monoclonal antibodies and small molecular inhibitors as effective and relatively nontoxic antitumor agents, gene therapy needs to deliver emphatic clinical results to attract resources compatible with transformation of a promising approach to a clinically successful strategy. Fortunately, recent watershed clinical trials \cite{57,84,92,98–100} have demonstrated that the theoretical considerations behind gene delivery for therapeutic effect are sound, and the technology remains a viable and potent approach for treatment of diseases resistant to available modalities.

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