REVIEW ARTICLE OPEN

AAV for ovarian cancer gene therapy

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Recent advancements in ovarian cancer treatment, particularly with PARP inhibitors, have markedly enhanced the recurrence-free interval, shifting the treatment paradigm and increasing treatment success in patients with BRCA mutations or HRD (homologous recombination deficiency). However, a significant proportion of cases experience relapse, resulting in poorer long-term survival rates when compared to other female cancers, such as breast cancer. This review explores the potential of adeno-associated virus (AAV) vectors for gene therapy in ovarian cancer and examines rational gene therapy strategies by categorizing them based on target cells and target genes to determine the most effective approach for ovarian cancer treatment. Specifically, it examines strategies such as anti-angiogenesis and immune modulation, highlighting the strategy of gene supplementation to hinder ovarian cancer progression. Innovations in AAV capsid design now allow for targeted delivery, focusing on ovarian cancer stem cells (CSCs) identified by specific markers. Additionally, leveraging DNA sequencing technologies enhances the identification and incorporation of therapeutic genes into AAV vectors, promising new avenues for ovarian cancer gene therapy.

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INTRODUCTION

Globally, ovarian cancer is the eighth most common cancer in women, accounting for an estimated 3.7% of cases and 4.7% of cancer deaths in 2020 [1]. It is the leading cause of gynecological cancer-related deaths and the second most common gynecologic cancer [2]. Over 90% of ovarian cancers are epithelial ovarian cancers (EOC), with high-grade serous ovarian cancer (HGSOC) being the predominant subtype [3] (Hereafter, these will be referred to as ovarian cancer). Most ovarian cancers (75%) are diagnosed at an advanced stage (III or IV) and initially respond well to standard treatment (platinum-based chemotherapy and cytoreductive surgery) with a response rate of over 80% [4]. However, the recurrence rate is nearly 80% of advanced ovarian cancers, with progressively shorter progression-free intervals and repeated chemotherapy cycles [5]. Consequently, the 5-year survival rate is 26% for stage III and 14% for stage IV [6] (Fig. 1a). Treatment following recurrence is primarily ineffective, with a median survival time of only 2 years, making this disease essentially lethal [7].

Gene therapy encompasses the delivery of genes (DNA or RNA) to patients, aiming to inhibit the expression of oncogenes (gene silencing), restore mutated-tumor suppressor genes with normal genes (gene restoration), provide therapeutic genes to target cells (gene supplementation) [8]. Initially, research primarily concentrated on treating hereditary diseases with well-defined causes. However, current investigations have expanded to include a variety of diseases, such as neurodegenerative diseases [9], rheumatoid arthritis [10], cardiovascular diseases [11], infectious diseases [12], and aging-related diseases [13]. Moreover, the expanded understanding of genes implicated in cancer formation, growth, and metastasis has stimulated research [14] and clinical investigations [15] into cancer gene therapy, leading to notable advancements in the field.

For cancer gene therapy, two distinct groups of delivery vehicles exist viral and non-viral vectors. While both vectors have their advantages and limitations, the central challenge of vectors for cancer gene therapy is achieving efficient and safe gene delivery. Non-viral vectors include carriers such as lipid nanoparticles (LNPs), cationic liposomes, peptides, and cationic polymers like polyethyleneimine (PEI) [16]. Although non-viral vectors often face limitations in tumor-targeting specificity due to their relatively simple structures [17], several studies have demonstrated tissue-specific delivery [18]. Furthermore, similar to viral vectors, non-viral vectors can be functionalized with biomolecules (e.g., peptides, antibodies, or aptamers) to enhance their targeting ability and therapeutic efficacy. These advantages, however, are often outweighed by viral vectors' inherent strengths. Compared to non-viral systems, viral vectors typically offer significantly longer-lasting transgene expression [19, 20] and intrinsic mechanisms for efficient cellular entry and genome integration or persistence [21]. These features make viral vectors especially effective for in vivo gene delivery [22], explaining why they remain the primary focus in a substantial portion of cancer gene therapy research [23]. Notably, viral vectors, such as retroviruses and adenoviruses, can potentially achieve cancer cell-specific delivery by modifying the proteins present in the viral envelope or, in the case of AAV (Adeno-associated virus), altering the structure of the viral capsid [14, 15].

AAV has a protein structure known as the viral capsid, allowing for AAV capsid engineering through systematic gene modifications [24]. This characteristic facilitates the development of AAV variants with specific tissue tropism for research purposes. As a

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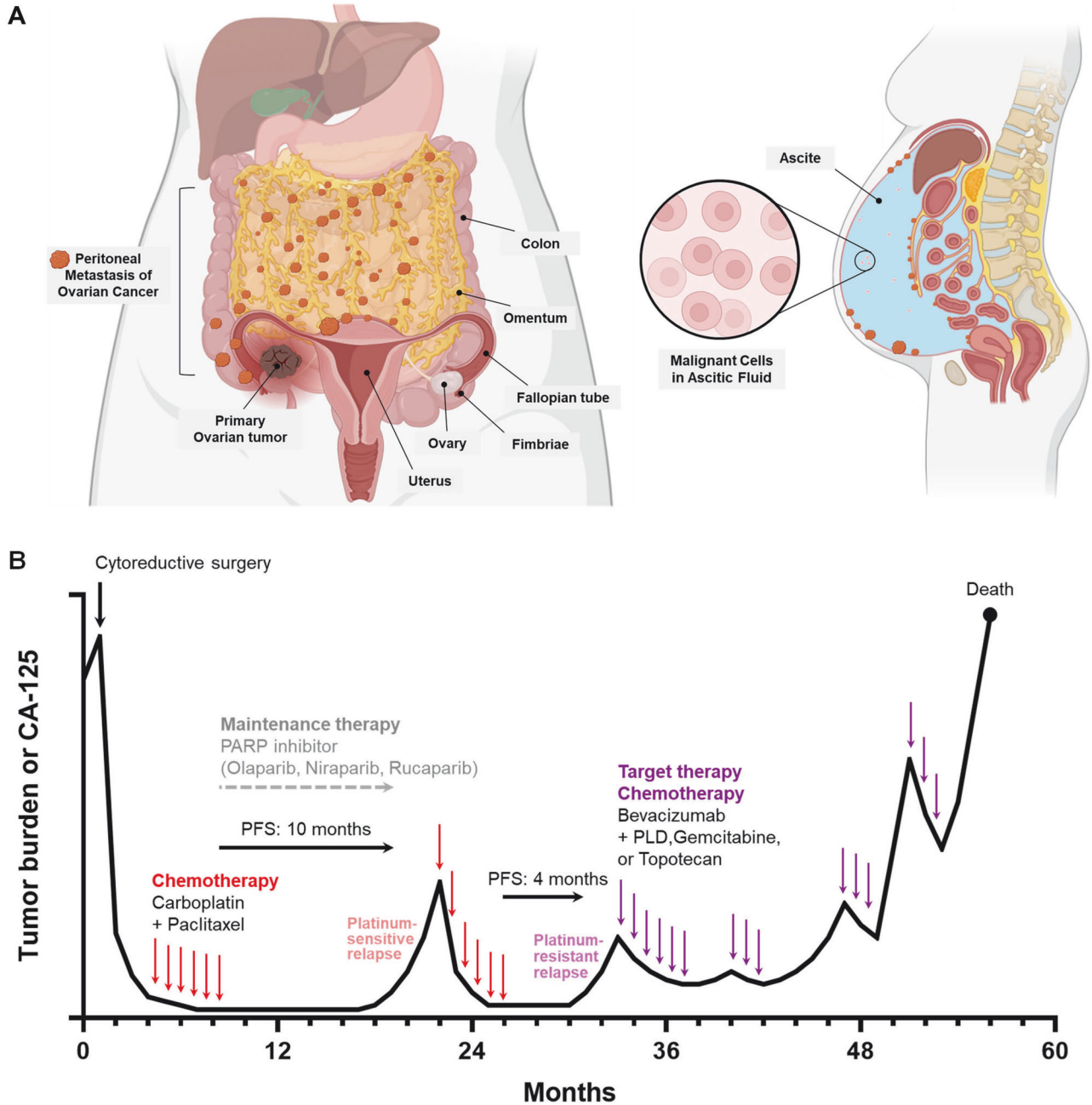

Fig. 1 Anatomy and treatment course in advanced ovarian cancer. A Typical anatomical presentation of FIGO (International Federation of Gynecology and Obstetrics) stage IIIC ovarian cancer. The 5-year survival rate for patients initially diagnosed with stage III ovarian cancer is ~26%. **B** Schematic representation of CA-125 levels and ovarian tumor burden in stage III high-grade serous ovarian cancer (HGSOC). The standard treatment regimen for ovarian cancer typically involves optimal cytoreductive surgery followed by six cycles of chemotherapy using carboplatin and paclitaxel. Post-chemotherapy, PARP inhibitors may be used as maintenance therapy to prolong progression-free survival (PFS). If the patient experiences a relapse more than 6 months after completing the initial chemotherapy, it is classified as a platinum-sensitive relapse, allowing for the same platinum- and taxane-based chemotherapy regimen to be reused. Conversely, if the relapse occurs within six months, it is considered a platinum-resistant relapse. In these cases, targeted therapies such as bevacizumab, combined with agents like pegylated liposomal doxorubicin (PLD), gemcitabine, or topotecan, are often utilized. Typically, PFS decreases with each successive relapse of ovarian cancer, eventually leading to a stage where no effective treatments remain, resulting in patient mortality. Therefore, extending the PFS following the initial chemotherapy is crucial for improving outcomes in ovarian cancer treatment. Promising gene therapy has the potential to significantly increase this initial PFS by targeting specific pathways involved in tumor progression.

result, AAVs have been developed that can cross the human blood-brain barrier (BBB) for CNS transduction [25], target human hepatocytes [26, 27], and transduce human cancer cells more efficiently than wild-type AAV [28]. As a viral vector capable of cell-type-specific or tissue tropism, AAV is relatively safe [29] and provides long-term expression [30], enhancing its potential for FDA approval in gene therapy compared to previously developed viral vectors. Over 200 completed and ongoing clinical trials use AAV as the gene transfer vector. The outstanding virtues of AAV—its relative safety and the ability to provide prolonged transgene expression—suggest that it will play a critical role as a strategic tool in cancer gene therapy aimed at controlling and treating

cancer as a manageable disease. This review examines studies on AAV-mediated cancer gene therapy for the treatment of ovarian cancer and proposes future directions for this therapeutic approach.

UNDERSTANDING OVARIAN CANCER: DIAGNOSIS, GENETICS, INITIAL SURGERY, AND CHEMOTHERAPY

Diagnosis

Ovarian cancer often presents insidiously, making early diagnosis challenging. Most women are diagnosed at stage III or IV, exhibiting symptoms such as abdominal pain or discomfort, menstrual irregularities, dyspepsia, other gastrointestinal disturbances, and urinary frequency or retention. In advanced stages, respiratory symptoms may occur due to ascites or pleural effusion, and bowel obstruction can also be present [31]. The late diagnosis is attributed to subtle onset and vague symptoms of the disease, which patients may mistake for ordinary changes related to childbearing, menopause, or aging. Additionally, these symptoms are often nonspecific and can mimic conditions like irritable bowel syndrome. This difficulty in recognizing ovarian cancer symptoms results in a prolonged and convoluted diagnostic process [32].

Genetics

Hereditary factors account for ~20% of ovarian cancers [33]. Most are due to pathogenic mutations in the BRCA1 or BRCA2 genes, which repair DNA double-stranded breaks via homologous recombination. Inherited mutations in these genes are major risk factors, with germline BRCA1 mutations increasing ovarian cancer risk by 20%–50% and BRCA2 mutations by 10%–20% [34]. These cancers typically occur at a younger age, especially in BRCA1 mutation carriers, with a median diagnosis age in the mid-40s [34]. For this reason, women with suspected hereditary cancer syndromes, such as BRCA1 or BRCA2 mutations, or those with a family history, young age at diagnosis, or high-grade ovarian cancer, should receive genetic testing and counseling. If a mutation is identified, risk-reducing bilateral salpingo-oophorectomy is considered an effective preventive strategy to reduce the risk of ovarian cancer [34]. While BRCA1 and BRCA2 are well-documented as key genetic factors in hereditary ovarian cancer, including mutations in other DNA repair genes, only around 20% of ovarian cancer cases are attributed to genetic causes [33]. The remaining 80% of cases remain unexplained by known genetic mutations.

Initial surgery and chemotherapy

The prognosis for ovarian cancer is largely determined by the maximum diameter of residual disease after cytoreductive surgery [35, 36]. The standard treatment protocol for ovarian cancer includes optimal cytoreductive surgery followed by a chemotherapy regimen of six cycles of carboplatin and paclitaxel, or docetaxel if paclitaxel is not tolerated [37] (Fig. 1b). In advanced-stage cases, the volume of residual disease post-surgery is the most important prognostic indicator [35, 36], necessitating a comprehensive surgical approach that includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and maximal cytoreduction [38]. Additional procedures involve peritoneal washings, multiple peritoneal biopsies, appendectomy in mucinous histology, and resection of bulky para-aortic and pelvic lymph nodes [38]. This standard treatment may also be employed in cases of primary suboptimal cytoreduction. For advanced-stage patients (IIIC or IV) with unresectable tumors, 2–3 cycles of neoadjuvant chemotherapy followed by surgical cytoreduction and further chemotherapy are required [39].

The catalytic activity of PARP1 (poly ADP-ribose polymerase 1) is crucial for mediating various DNA damage repair pathways, including stabilizing DNA replication forks [40, 41]. Additionally, its role in chromatin remodeling is closely linked to its function in DNA repair [42]. Consequently, inhibiting PARP1 is an effective strategy for treating cancers with deficiencies in the homologous recombination repair of DNA double-strand breaks. Studies have shown that BRCA mutations in ovarian cancers disrupt the homologous recombination pathway, leading to increased sensitivity to PARP inhibitors [43, 44]. Indeed, several clinical studies demonstrated the promising efficacy of PARP inhibitors in ovarian cancer patients [45–47]. There is growing evidence supporting that the use of maintenance therapy with PARP inhibitors after a response to platinum-based chemotherapy, in both first-line and second-line settings, has significantly extended the interval between response and disease relapse [48, 49] (Fig. 1b).

Although PARP inhibitors have increased progression-free survival and overall survival compared to control ovarian cancer treatments, patients ultimately experience disease relapse and develop resistance to PARP inhibitors, leading to mortality (Fig. 1b). The most presumptive resistance mechanism of PARP inhibitors is the restoration of BRCA1 or BRCA2 protein functionality through secondary mutations [50]. This mechanism is also shared in resistance to platinum-based treatments in cancer cells [51]. In PARP inhibitor-resistant human pancreatic cancer cell lines, new BRCA2 isoforms were made by an intragenic deletion of the frameshift mutation. This deletion restored the open reading frame (ORF) of the BRCA2 gene, enabling the cells to repair drug-induced DNA double-strand breaks via homologous recombination [52].

Although current treatment approaches are optimum, most women with advanced-stage ovarian cancer will relapse eventually due to resistance to platinum-based drugs, PARP inhibitors, or because of refractory cancer (Fig. 1b). This challenge has driven significant interest in developing new, more targeted strategies for treatment, with gene therapy presenting a potential new option.

ADENO-ASSOCIATED VIRUS AS A DELIVERY VECTOR: FROM BASICS TO THERAPEUTIC APPLICATIONS

Adeno-associated virus (AAV) belongs to human Parvovirus with a single-stranded DNA genome and is one of the smallest known viruses (~25 nm). Although it can transduce human cells, it has not been identified as a causative agent of any specific disease [53, 54]. AAV does not replicate well within host cells unless co-infected with adenovirus [55]. Unlike adenovirus, AAV exhibits low immunogenicity and does not strongly elicit a host immune response [56].

Wild-type AAV contains a single-stranded DNA viral genome flanked by inverted terminal repeat (ITR) sequences that form hairpin structures [57] (Fig. 2a). Between these ITR sequences, the AAV genome encodes the Rep gene, which is involved in the rescue and replication of the viral genome, and the Cap gene, which encodes the icosahedral capsid proteins responsible for packaging the ssDNA viral genome [58]. In contrast to wild-type AAV, recombinant AAV (rAAV) replaces the viral genes between the ITR sequences with a therapeutic transgene (Fig. 2b). To produce rAAV, host cells such as 293T cells are co-transfected with plasmids encoding the Rep and Cap genes, as well as adenovirus helper genes E4, E2a, and VA, to facilitate the assembly of AAV particles containing the therapeutic gene [59].

A major limitation of using adeno-associated virus (AAV) for cancer gene therapy is its relatively small viral genome size compared to other viral vectors such as lentivirus and adenovirus [60]. The ideal size for a therapeutic gene delivered by AAV is generally <5 kb [61]. Although the wild-type AAV genome itself is ~4.7 kb (4675 bp) [57] (Fig. 1a), this size includes essential regulatory elements like promoters and poly-A regions, which leaves limited space for the therapeutic gene. Consequently, for genetic disorders such as cystic fibrosis (CFTR, 4443 bp) or

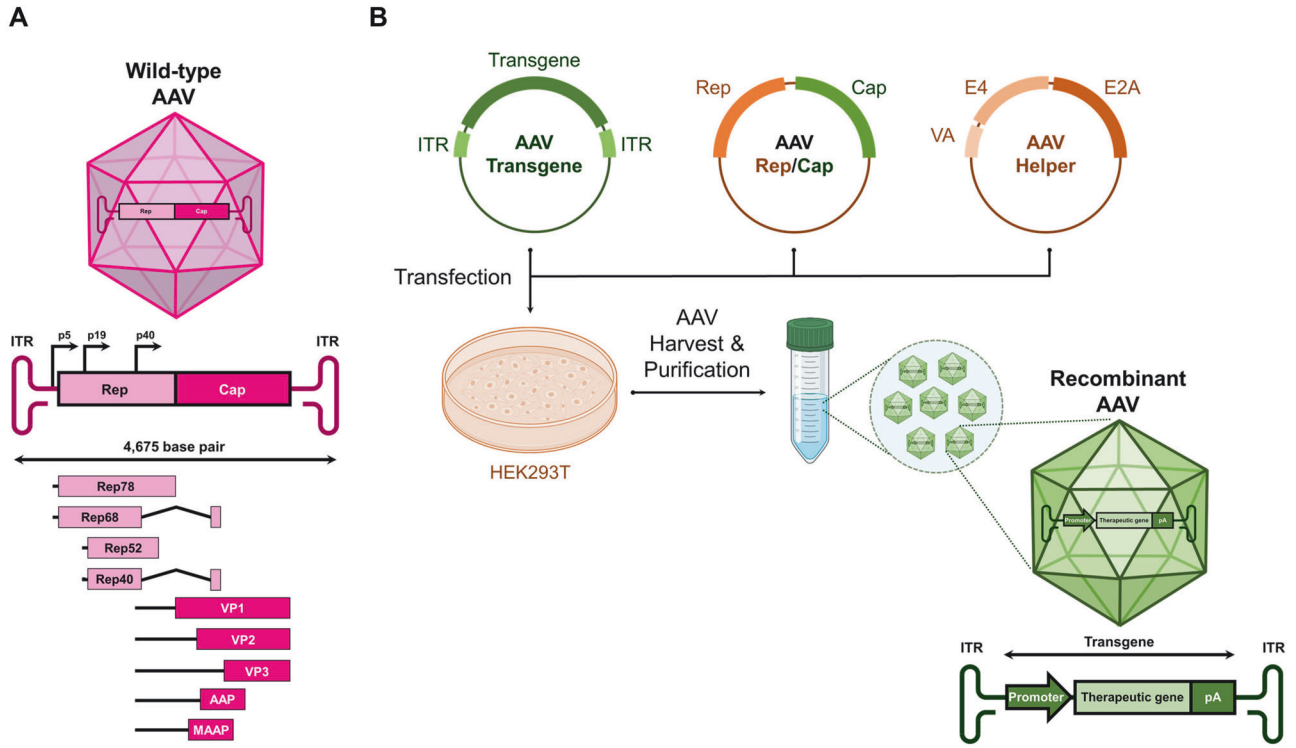

Fig. 2 Comparison of wild-type AAV and recombinant AAV. A Schematic representation of wild-type AAV, featuring its single-stranded viral genome. The wild-type AAV genome comprises single-stranded DNA with two inverted terminal repeats (ITRs) at either end and includes the essential genes Rep and Cap for viral replication. This genome produces at least three different transcript variants, each originating from distinct starting points. The p5 and p19 promoters are responsible for transcribing mRNA for the Rep78, Rep68, Rep52, and Rep40 proteins, while the p40 promoter transcribes the genes for the viral capsid proteins VP1, VP2, and VP3, as well as the non-structural proteins AAP and MAAP, which are crucial for virus production. **B** Production of recombinant AAV and its representative structure. To produce AAV, a eukaryotic cell, such as a HEK293T cell, is transfected with an AAV transgene plasmid containing the therapeutic gene intended for gene therapy, a Rep/Cap plasmid derived from wild-type AAV, and a helper plasmid derived from adenovirus that contains genes promoting efficient AAV production. After allowing sufficient time for the cells to produce AAV particles, AAV is harvested and purified from the cell lysate and supernatant. This process results in the generation of functional recombinant AAV capable of therapeutic gene expression in the host. Unlike wild-type AAV, recombinant AAV cannot replicate or propagate after transducing the host cell due to the absence of Rep/Cap genes.

Duchenne muscular dystrophy (DMD, 11,034 bp) where the size of the therapeutic gene exceeds 4 kb, AAV-based gene therapy may become either unfeasible or exceptionally challenging.

In addition to the limitation imposed by the AAV packaging capacity, a significant challenge in systemic AAV-mediated gene delivery—particularly in the context of repeated dosing—is the host immune system's recognition of the viral capsid [62]. Pre-existing neutralizing antibodies (NAbs), which develop from natural exposure to wild-type AAV, can bind to the recombinant AAV vectors and prevent it from reaching target cells [56]. These antibodies block viral entry by interfering with receptor interactions and promote rapid clearance through the reticuloendothelial system [63]. Notably, seroprevalence studies indicate that around half of the population develops AAV-specific NAbs by the age of two, and once formed, these antibodies typically persist throughout life [64–66]. Given this high prevalence, efforts to develop effective AAV-based gene therapies for widespread applications, such as anti-tumor treatments, must also address the challenge posed by pre-existing NAbs. In the context of cancer, where broad patient applicability is essential, strategies to evade or overcome AAV neutralization—through capsid engineering, immune modulation, or alternative serotype selection—are likely to be critical for clinical success.

A notable advancement in AAV technology is capsid engineering, which allows modification of the virus's infection efficiency and tissue tropism [67]. One of the most significant achievements in this area is the development of AAV capsids capable of crossing the blood-brain barrier (BBB) to achieve central nervous system (CNS) transduction in several animal models, including mouse and NHP (non-human primate) [25, 68, 69]. Subsequently, a breakthrough study translated animal model research into a human context by engineering an AAV capsid that specifically targets the human transferrin receptor on the blood-brain barrier [70]. When tested in transgenic mice expressing this receptor, the engineered AAV was shown to effectively traverse the barrier and deliver a therapeutically relevant gene to the CNS [70]. These efforts hold great potential for treating CNS disorders.

The US FDA approved the most successful AAV-based gene therapy in 2019 for treating spinal muscular atrophy, marketed as Zolgensma (onasemnogene abeparvovec) [71, 72]. Subsequently, multiple other AAV-based gene therapies have also gained approval, solidifying AAV's position as a promising viral vector in the gene therapy market [58].

CHALLENGES OF GENE THERAPY IN OVARIAN CANCER

Gene therapy represents a promising strategy for treating ovarian cancer, as it enables the introduction of various genes that regulate molecular processes. This approach can inhibit tumor growth, angiogenesis, invasion, and metastasis, and modulate immune response [73]. Nonetheless, at least two significant challenges must be addressed to ensure the success of these therapeutic strategies.

Firstly, current knowledge of the molecular mechanisms driving tumorigenesis and cancer progression remains incomplete. For example, High-grade serous ovarian cancer (HGSOC), which

constitutes over two-thirds of ovarian cancers, is characterized by high intra-tumoral heterogeneity [74]. This genomic instability leads to the emergence of tumor subclones with competitive advantages, such as faster growth rates or resistance to chemotherapy. These advantageous subclones ultimately dominate the tumor through a process known as "clonal expansion", and an elevated extent of clonal expansion has a detrimental effect on a patient's overall survival [75]. This observation of drug resistance driven by clonal expansion through intra-tumoral heterogeneity underscores the current limitations in our understanding of the specific stages of ovarian cancer progression and the identification of optimal cellular (e.g., cancer stem cells) or genetic targets for effective gene therapy.

Second, the erratic blood supply in ovarian cancer presents substantial challenges for the direct delivery of therapeutic genes to ovarian cancer cells in vivo. Uncontrolled tumor growth contributes to the development of abnormal vasculature through rapid and uncoordinated vascular expansion [76]. As tumors grow uncontrollably, the demand for oxygen and nutrients in hypoxic status increases, leading to the formation of haphazardly arranged blood vessels that lack proper structure and function. The aggressive expansion of tumor mass often outpaces the capacity of the newly formed blood vessels to provide sufficient blood supply, resulting in a chaotic and ineffective vascular network [77]. In addition, dysregulated angiogenesis signaling pathways in ovarian cancer lead to the secretion of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), angiopoietin (ANGPT), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) [78]. These factors facilitate the formation of new yet structurally disorganized blood vessels, characterized by increased permeability and suboptimal vessel maturation, ultimately undermining the effectiveness of therapeutic gene delivery to ovarian cancer cells via systemic administration.

Thus, for the successful implementation of gene therapy in ovarian cancer, selecting the appropriate target cells and therapeutic genes tailored to the stages of ovarian cancer progression is necessary. Furthermore, taking advantage of the unique anatomical location of ovarian cancer within the abdominal cavity (Fig. 1a), it is essential to assess the efficacy of intraperitoneal gene therapy when combined with traditional systemic chemotherapy.

STRATEGIES FOR TARGETING OVARIAN CANCER CELLS IN GENE THERAPY

The theoretical strategies for targeting ovarian cancer cells in gene therapy can be broadly categorized based on two main criteria: the target cell types for therapeutic gene expression and the therapeutic gene types used to suppress cancer cell growth (Fig. 3).

The first criterion focuses on the types of cells within the tumor microenvironment and other organs or systemic compartments that are targeted to receive the therapeutic gene expression. Three main strategies fall under this criterion.

The first strategy focuses on selectively targeting cancer cells within the tumor mass, with the goal of directing gene therapy predominantly to malignant cells while limiting effects on surrounding non-cancerous components. While not exclusively specific, this approach enhances targeting toward cancer cells, potentially limiting unintended effects on surrounding normal cells or non-malignant cell types within the tumor microenvironment. For example, intratumoral injection of an AAV vector encoding SaCas9-KKH and sgRNA has been reported to reduce tumor growth and moderately improve overall survival in an orthotopic glioblastoma mouse model [79]. In addition, AAV capsid engineering has been shown to enhance tumor cell-specific transduction. One study developed AAV6 vectors incorporating RGD peptides and capsid mutations (Y705-731F, T492V, K531E), which significantly increased transduction efficiency in integrin-overexpressing cancer cells and improved tumor specificity in vivo [28]. Another study engineered AAV2 capsids to display a plectin-1 targeting peptide (PTP), resulting in a 37-fold preference for pancreatic ductal adenocarcinoma (PDAC) tumors over liver tissue, thus demonstrating the potential of engineered capsids for tumor-targeted gene delivery [80].

The second strategy broadens the scope to include the cancer cells, the tumor microenvironment, and other organs or systemic compartments. This approach leverages the supportive roles that these cells play in tumor growth, aiming to disrupt the tumor's supportive microenvironment. By simultaneously targeting cancer cells and their surrounding support systems, this strategy potentially enhances therapeutic efficacy and contributes to a more hostile environment for tumor survival. For example, systemic administration of AAV8 vectors encoding soluble VEGF receptors (sVEGFR2 and sVEGFR3) led to transgene expression in both ovarian tumor cells and their surrounding microenvironment. This approach effectively reduced intratumoral angiogenesis and, when combined with chemotherapy, suppressed tumor growth and ascites formation, ultimately improving overall survival in an ovarian cancer model [81, 82]. Additionally, AAV capsid engineering has produced RGD-modified vectors with enhanced muscle-specific transduction after systemic delivery [83]. These capsids outperformed natural serotypes in mouse models of genetic muscle disease and showed conserved efficacy in non-human primates, highlighting their therapeutic potential. This approach enables a strategy to maximize anti-tumor effects by increasing specificity toward cancer cells or their surrounding microenvironment.

The third strategy targets the surrounding tissue cells within the tumor microenvironment, as well as cells in other organs or systemic compartments, deliberately excluding the cancer cells. Here, the therapeutic genes are introduced into the surrounding supportive cells in the tumor microenvironment. By modifying these supportive cells, this strategy can indirectly impair cancer growth by weakening the resources and support that the tumor relies on. A representative example of this approach is an AAV-based vaccine that, despite being delivered intramuscularly to normal tissue, induced strong and durable antigen-specific T and B cell responses. This immune activation led to effective tumor suppression in mouse models, including the poorly immunogenic B16/F10-Ova melanoma, highlighting the potential of targeting the tumor microenvironment rather than cancer cells directly [84, 85].

The second criterion for categorizing gene therapy strategies in ovarian cancer pertains to the type of therapeutic genes used. These genes fall into three main types: gene silencing, gene restoration, and gene supplementation. Gene silencing aims to deactivate oncogenes or genes that play a role in cancer progression, such as mutant KRAS [86], CLDN3 [87], and EGFR [88]. By silencing these cancer-promoting genes, this approach effectively reduces the proliferative and invasive potential of cancer cells.

Gene restoration is another therapeutic approach that involves reintroducing or restoring the function of tumor suppressor genes, such as TP53 [89, 90] and PTEN [91, 92], which are often mutated or inactivated in cancer. Restoring these genes can re-enable cellular mechanisms that prevent uncontrolled cell division and tumor growth, contributing to cancer suppression.

Finally, gene supplementation involves the addition of therapeutic genes that aid in cancer treatment, including genes encoding cytokines [93–95], anti-angiogenic proteins [96, 97], and immune checkpoint inhibitors such as bevacizumab [98] and pembrolizumab [99]. This supplementation strategy introduces additional therapeutic agents to the tumor environment, aiming to improve the immune response against cancer cells, inhibit blood vessel formation needed for tumor growth, and enhance overall therapeutic outcomes.

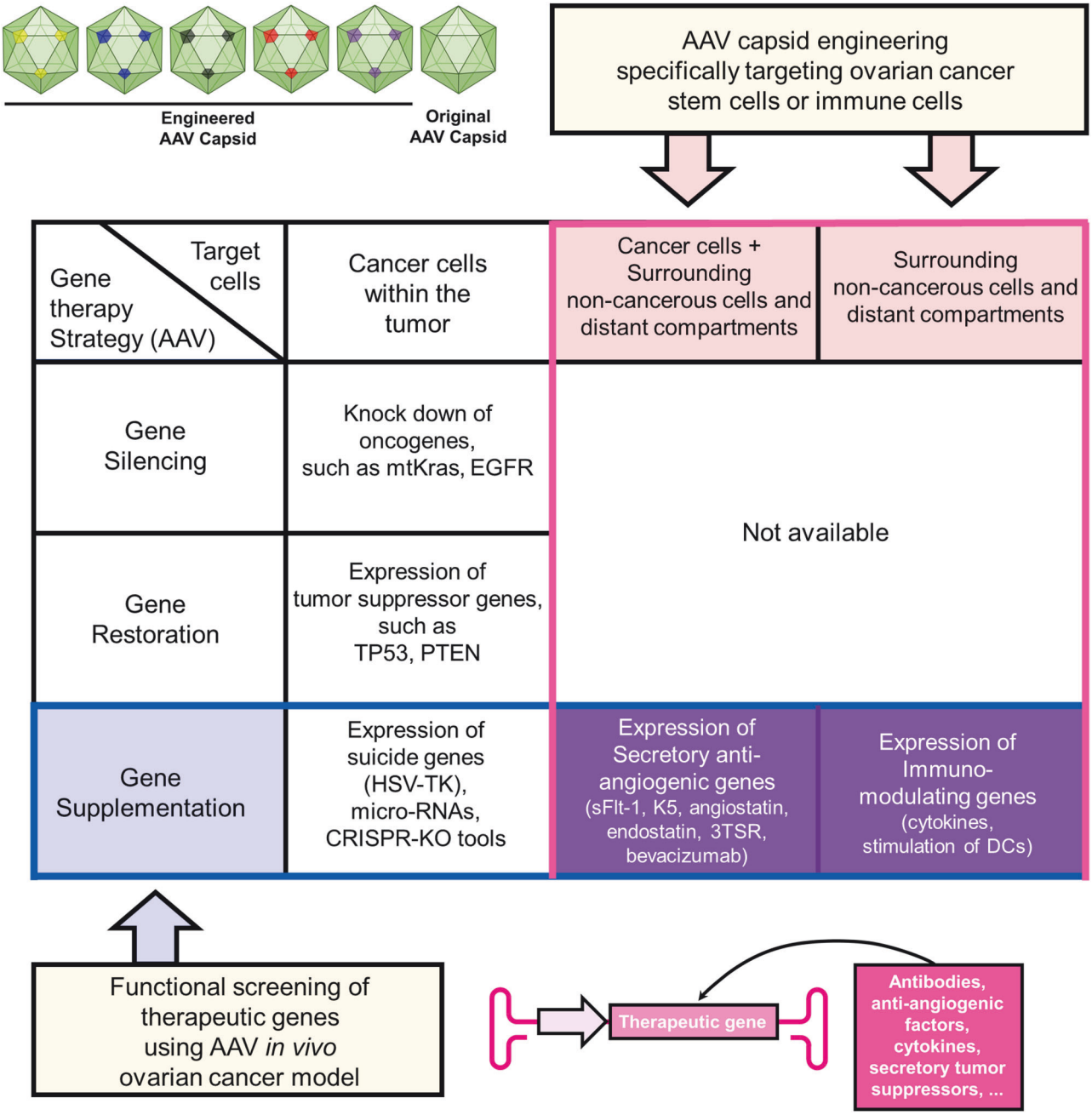

Fig. 3 AAV gene therapy strategies for targeting ovarian cancer cells: focus on gene supplementation. Schematic representation of gene therapy strategies targeting ovarian cancer cells using AAV. The strategies are categorized based on the target cells and the specific gene therapy approaches employed. The diagram highlights the engineering of AAV capsids to selectively target ovarian cancer stem cells or immune cells. Additionally, functional screening of therapeutic genes could be conducted using an in vivo ovarian cancer model with AAV to identify potential therapeutic genes, including antibodies, anti-angiogenic factors, cytokines, and secretory tumor suppressors.

LIMITATIONS OF GENE SILENCING AND GENE RESTORATION STRATEGIES

The necessity of categorizing effective anticancer gene therapy strategies based on target cells and therapeutic genes arises from the inherent limitation that gene delivery vectors cannot realistically transduce 100% of ovarian cancer cells. As previously mentioned, it is practically challenging to achieve complete transduction of the ovarian cancer mass, which can grow to several centimeters in size [100], with poorly formed blood vessels leading to hypoxic necrotic regions [101]. In particular, when employing gene silencing strategies, the specificity and efficiency of vector-mediated transduction of cancer cells become critically important. In this context, the cancer cell transduction rate of the delivery vector becomes a crucial determinant for the success of gene therapy.

Even if not all ovarian cancer cells are transduced, effectively inhibiting and killing the transduced cells to extend progression-free survival by several months can still be considered beneficial. However, given the high production and distribution costs of gene therapies, these treatments must demonstrate substantial efficacy, akin to the significant improvements in overall survival seen with PARP1 inhibitors in ovarian cancer treatment [46, 47].

From this perspective, although gene supplementation strategies are employed, approaches such as expressing suicide genes

specifically in cancer cells [102] (Fig. 3), using the CRISPR-Cas9 system to knock out overexpressed oncogenes [103], or inducing the expression of microRNAs to inhibit oncogene translation [104] within cancer cells may fail due to the realistic limitation of not being able to transduce all cancer cells.

The gene restoration strategy, which involves delivering intact tumor suppressor genes, similarly faces the intrinsic limitation that the transduction efficiency of the delivery vector cannot reach 100%. Even if the theoretical inhibition efficiency of genes delivered for gene silencing and gene restoration is 100%, if not all cancer cells are eradicated, the clonal expansion of untransduced cancer cells may prevent patients from experiencing the full therapeutic benefits [105, 106]. Therefore, the issue of delivery efficiency significantly undermines the gene silencing and gene restoration strategies, which rely on specifically transducing cancer cells (Fig. 3).

TP53 gene therapy as a representative example of gene restoration therapy

TP53 mutations are nearly ubiquitous in ovarian cancer, with a prevalence of 96% [107]. Initial gene therapy efforts focused on delivering normal TP53 to cancer cells via adenovirus, aiming to restore its function and induce growth arrest and apoptosis [108]. While these approaches were effective in vitro and in vivo, they did not translate successfully to clinical trials [109]. Two main reasons for this failure are the limited understanding of TP53's biological functions and the inadequacy of delivery vectors and routes. The assumption that delivering normal TP53 would restore its function overlooked the dominant-negative effect of mutant TP53. In cancer cells, endogenous mutant p53 exerts a dominant-negative effect on wild-type (WT) p53, thereby inhibiting the tumor-suppressing activities of WT p53 [110]. TP53 functions as a tetramer [111], and in the presence of mutant TP53 or without a significantly higher amount of normal TP53, functional restoration is insufficient. Moreover, adenovirus vectors, used in clinical trials for TP53 delivery, induce only transient expression of the therapeutic gene [112], which is inadequate for sustained anticancer effects in ovarian cancer. Additionally, adenoviruses elicit strong immune responses, limiting their delivery efficiency to cancer cells when administered via peritoneal injection [89].

Two independent studies have investigated the restoration of wild-type TP53 using AAV vectors. One study demonstrated that AAV-mediated expression of WT TP53 inhibited cancer cell proliferation in vitro [113]. Another study showed tumor growth suppression after cancer cells, transduced with AAV expressing WT TP53, were injected subcutaneously into immunodeficient mice [114]. However, these experimental designs do not provide sufficient evidence to evaluate the in vivo transduction efficiency of AAV in tumor cells. Therefore, based on these findings, the strategy of TP53 gene restoration may need to be reconsidered for its applicability in in vivo gene therapy.

PROPOSED OVARIAN CANCER GENE THERAPY: GENE SUPPLEMENTATION

Anti-angiogenic gene supplementation using AAV in ovarian cancer

Considering the failures of adenovirus-mediated TP53 gene therapy in ovarian cancer, gene supplementation emerges as a promising alternative (Fig. 3). AAV vectors have been employed in ovarian cancer gene therapy to induce the expression of anti-angiogenesis factors or VEGFR-inhibiting antibodies [54]. Gene supplementation aligns with the mechanisms of modern immunotherapies [115] and CAR-T therapy [116], which strengthen host immune responses against cancer cells rather than targeting cancer cells directly. These therapies fall under the category of targeting cells within the tumor microenvironment, excluding cancer cells (Fig. 3). From this perspective, studies that have demonstrated the efficacy of ovarian cancer gene therapy using AAV by expressing secretory proteins as gene supplements are as follows.

AAV encoding the human soluble FMS-like tyrosine kinase receptor 1 (sFlt-1), which functions by both sequestering vascular endothelial growth factor (VEGF) and forming inactive heterodimers with other membrane-spanning VEGF receptors, leads to stable expression and significantly inhibits the growth of angiogenesis-dependent human ovarian cancer cells in a mouse xenograft model [96, 97] Compared to the control group, treatment with AAV-sFlt-1 resulted in an 80% reduction in tumor size and protected 83% of mice from death [96]. Kringle 5 (K5), a fragment of plasminogen, serves as an effective endogenous inhibitor of angiogenesis by both inhibiting endothelial cell proliferation and migration and inducing endothelial cell apoptosis [117, 118]. Administration of AAV-K5 led to a significant reduction in both subcutaneous and intraperitoneal growth of human ovarian cancer cells [119]. Compared to the control group, treatment with AAV encoding K5 resulted in a 60% reduction in tumor size and a 30% decrease in tumor weight [119]. A single intramuscular administration of AAV encoding angiostatin and endostatin inhibits intraperitoneal ovarian cancer growth in a preclinical mouse model [120]. Compared to the control mice, those treated with AAV encoding sFlt-1 exhibited significant tumor-free survival, reduced ascites volume, and lower levels of vascular endothelial growth factor (VEGF) in the ascites. Specifically, AAV-sFlt-1 treatment led to a 20% reduction in tumor-induced ascites volume, a 50% decrease in tumor weight, and protected 30% of the mice from tumor-related death [120]. Similarly, when AAV-encoding endostatin and angiostatin were administered intraperitoneally, it also showed sustained antitumor effects on the growth and dissemination of epithelial ovarian cancer in a mouse model [121]. Surprisingly, while AAV-mediated expression of endostatin and angiostatin alone extended mouse survival by ~25 days, the combination of AAV-mediated endostatin and angiostatin expression with paclitaxel treatment provided 90% protection from ovarian cancer–related death over a 240-day observation period [121]. Likewise, using AAV to express a point-mutated endostatin (P125A-endostatin) with enhanced endothelial cell binding and antiangiogenic activity resulted in sustained expression for 9 weeks from a single intramuscular administration and significantly inhibited the growth of human ovarian cancer cells in athymic nude mice, leading to an 80% reduction in tumor volume [122]. Treatment with AAV-P125A-endostatin in combination with carboplatin resulted in 60% of the animals remaining tumor-free for over 200 days, which was significantly better than treatment with AAV-vehicle and/or carboplatin alone [123].

Thrombospondin-1 (TSP-1) exhibits potent antiangiogenic activity due to its three type 1 repeat (3TSR) domain binding to CD36 on endothelial cells [124, 125]. Additionally, the KRFK motif in the second of these repeats is involved in activating the TGF-β pathway and demonstrates antitumor activity [126]. Pretreatment with 3TSR combined with chemotherapy significantly induced ovarian tumor regression, normalized tumor vasculature, and improved drug uptake, resulting in a reduction in ovarian tumor size compared to PBS controls and enhanced survival in advanced-stage ovarian cancer [127]. This combination also reduced tumor hypoxia by 50% and decreased tumor weight by 70%. In addition, the AAV-mediated delivery of 3TSR effectively decreases the formation of primary and secondary lesions and significantly prolongs survival in a murine model of orthotopic epithelial ovarian cancer at 90 days post-tumor implantation [128]. Furthermore, AAV-mediated delivery of 3TSR and the Fc domain-modified Fc3TSR, which is designed to prolong transgene expression, significantly enhances survival in a mouse model of epithelial ovarian cancer, although it did not outperform AAV-Bevacizumab in terms of survival benefit [129].

Bevacizumab, a humanized monoclonal antibody that targets and neutralizes VEGF, effectively inhibits tumor angiogenesis and exhibits significant anticancer activity [130]. The use of AAV vectors for delivering bevacizumab has shown significant results in cancer therapy. AAV-mediated delivery to the pleura effectively inhibits metastatic lung tumors [131], and persistent AAV-mediated bevacizumab therapy demonstrates notable tumor growth suppression in ovarian cancer models, reducing tumor weight by more than 80% and enabling the treated mice to survive twice as long as the control mice [132]. Furthermore, the use of AAV-mediated sVEGFR (soluble VEGFR) decoy gene therapy effectively inhibits intra-tumoral angiogenesis and tumor growth in an ovarian cancer model by targeting the VEGF/VEGFR signaling pathway, resulting in a twofold increase in survival rate. This approach demonstrates the potential of antiangiogenic gene therapy as a viable treatment for ovarian cancer [81].

AAV gene supplementation for enhancing immune responses against ovarian cancer

Utilizing AAV for the expression of cytokines represents a promising approach for the development of immune-based cancer immunotherapies [93–95]. IL-12 is a cytokine known for its potent immune-stimulating activity and plays a critical role in initiating and augmenting cell-mediated immunity [133]. IL-12 is primarily synthesized by immune cells such as dendritic cells and macrophages, and it plays a crucial role in driving Th1 cell differentiation, stimulating activation of T, B, and NK cells, and inducing the reprogramming of immunosuppressive cells [134]. Indeed, AAV-mediated delivery of IL-12 and tumor-associated cell-based vaccines represents an innovative approach in ovarian cancer immunotherapy [135].

While preclinical studies have demonstrated strong anti-tumor activity of AAV vectors carrying IL-12 [136–139], systemic administration of recombinant IL-12 in mice and humans has been associated with severe adverse effects [140, 141]. To reduce the toxicity associated with IL-12 and achieve controlled transgene expression, the Tet-On system has been employed for regulatable AAV-mediated IL-12 expression [142].

AAV-Tet-On-IL-12 has demonstrated high efficacy in preventing the establishment of metastasis and inducing a robust T-cell memory response against tumor cells. Additionally, a tetracycline-dependent riboswitch has been developed, allowing potent regulation of AAV-based transgene expression via a tetracycline aptamer [143]. The Tet-On system enables inducible gene expression in the presence of doxycycline or tetracycline, allowing temporal control over transgene activation. This system is designed to enable protein production from mRNA only in the presence of tetracycline, offering reversibility and repeatable induction capabilities for treating hepatocellular cancer in mice [144]. Considering the long-term expression characteristic of AAV in cancer gene therapy, a system that enables AAV transgene expression only when treatment is needed could be a valuable application for future AAV-mediated therapeutics.

AAV-based antigen loading of dendritic cells generates efficient cytotoxic T-cell responses in vitro [145, 146]. A quantitative transcriptomic-based investigation demonstrated that AAV particles are efficiently internalized without inducing detectable transcriptomic changes in monocyte-derived dendritic cells, in contrast to adenoviral infection, which upregulates anti-viral pathways. This report indicates that AAV has an immunologically favorable profile for the activation of dendritic cells [147]. AAV-mediated Her-2/neu expression in dendritic cells robustly stimulated cytotoxic T cells targeting ovarian cancer cells [148]. Dendritic cells transduced with an AAV-expressing Sperm protein 17 (Sp17) have been shown to generate a robust antigen-specific cytotoxic T-cell response against ovarian cancer cells. Additionally, these transduced dendritic cells significantly enhance the differentiation of cytotoxic T-cells, demonstrating their potential efficacy in immunotherapeutic strategies against ovarian cancer [149].

ADDITIONAL CONSIDERATIONS FOR SUCCESSFUL OVARIAN CANCER GENE THERAPY USING AAV

Significant advancements in AAV biotechnology, particularly in AAV capsid engineering and transgene expression, can be applied to ovarian cancer gene therapy (Fig. 3). This integration can enhance ovarian cancer gene therapy from the following two perspectives:

Capsid engineering for ovarian cancer stem cell-specific transduction

Recent studies have demonstrated that AAV capsid engineering can enhance cancer cell-specific transduction efficiency [28, 80, 150–152], thereby increasing antitumor effects while reducing cytotoxicity to normal cells [153]. Although these studies have shown promising antitumor effects in both in vitro and in vivo models, the practical challenges of achieving comprehensive transduction of all cancer cells, particularly due to the clonal expansion of cancer cells and drug resistance associated with cancer stem cells and intra-tumoral heterogeneity, highlight the limitations of strategies targeting the entire tumor mass in ovarian cancer gene therapy.

One of the major challenges in treating ovarian cancer is overcoming chemoresistance, which is often caused by intra-tumor heterogeneity [154, 155]. A significant concern is cancer stem cells, which are considered to be a source of tumor heterogeneity [156, 157]. These cells can survive initial chemotherapy and lead to relapse through clonal expansion [156, 157]. Cancer organoids, derived from cells with stemness, are valuable for their ability to accurately replicate the complexities and heterogeneity of primary tumors. They retain the genetic and molecular characteristics of the original tumor, including mutations, gene expression profiles, and drug resistance [158]. Thus, ovarian cancer stem cell-derived organoids serve as valuable models for screening therapeutic transgenes to overcome chemoresistance [159], as it is known that organoids form from ovarian cancer stem cells [160–162]. These ovarian cancer organoids are already used for drug screening in personalized cancer therapy [163–165]; however, the limited availability of existing anticancer drugs poses a significant challenge to organoid-assisted precision personalized therapy. In this situation, gene therapy could offer a broader range of therapeutic genes, potentially fulfilling the concept of personalized cancer treatment more effectively.

AAV vectors can be engineered to transduce specific cell types, due to their protein-based capsid structure. By modifying the AAV capsid genes, vectors can be designed to target ovarian cancer stem cells specifically, addressing chemoresistance and preventing ovarian cancer relapse. Thus, research focused on engineering AAV capsids for specific transduction of ovarian cancer stem cells represents a promising direction (Fig. 3). This approach holds promise as it may enable targeted therapy against ovarian cancer stem cells, which are critical in tumor relapse and drug resistance. Considering that organoid-based drug screening using patient-derived ovarian cancer stem cells is currently being pursued [163–165], developing AAV capsids with the capability to specifically transduce ovarian cancer stem cells represents a feasible and strategic research direction. Similarly, a study reported that combining capsid engineering with microRNA-dependent gene editing enhances tumor specificity. Specifically, a mosaic-capsid AAV vector—composed of AAV2 and AAV.CAP-B22, the latter known for its ability to cross the mouse blood-brain barrier—was used to target glioblastoma-initiating cells (GICs). This approach selectively eliminated GICs, reduced tumor growth, and extended survival in mice. Compared to the control group, which had a median survival of 32 days, treated mice survived for 55 days—an increase of ~72%. In addition, tumor volume was reduced by about 60%, highlighting the potential of this strategy for precision cancer therapy. These findings suggest that capsid

engineering can be effectively utilized to target cancer stem cell populations, providing a viable strategy for overcoming therapeutic resistance. This supports the feasibility of applying similar approaches to ovarian cancer stem cells, reinforcing the promise of AAV capsid engineering as a precision tool for targeting the root of tumor relapse and progression [166].

Furthermore, evaluating therapeutic transgenes utilizing such AAVs to target ovarian cancer stem cells is crucial, as overcoming

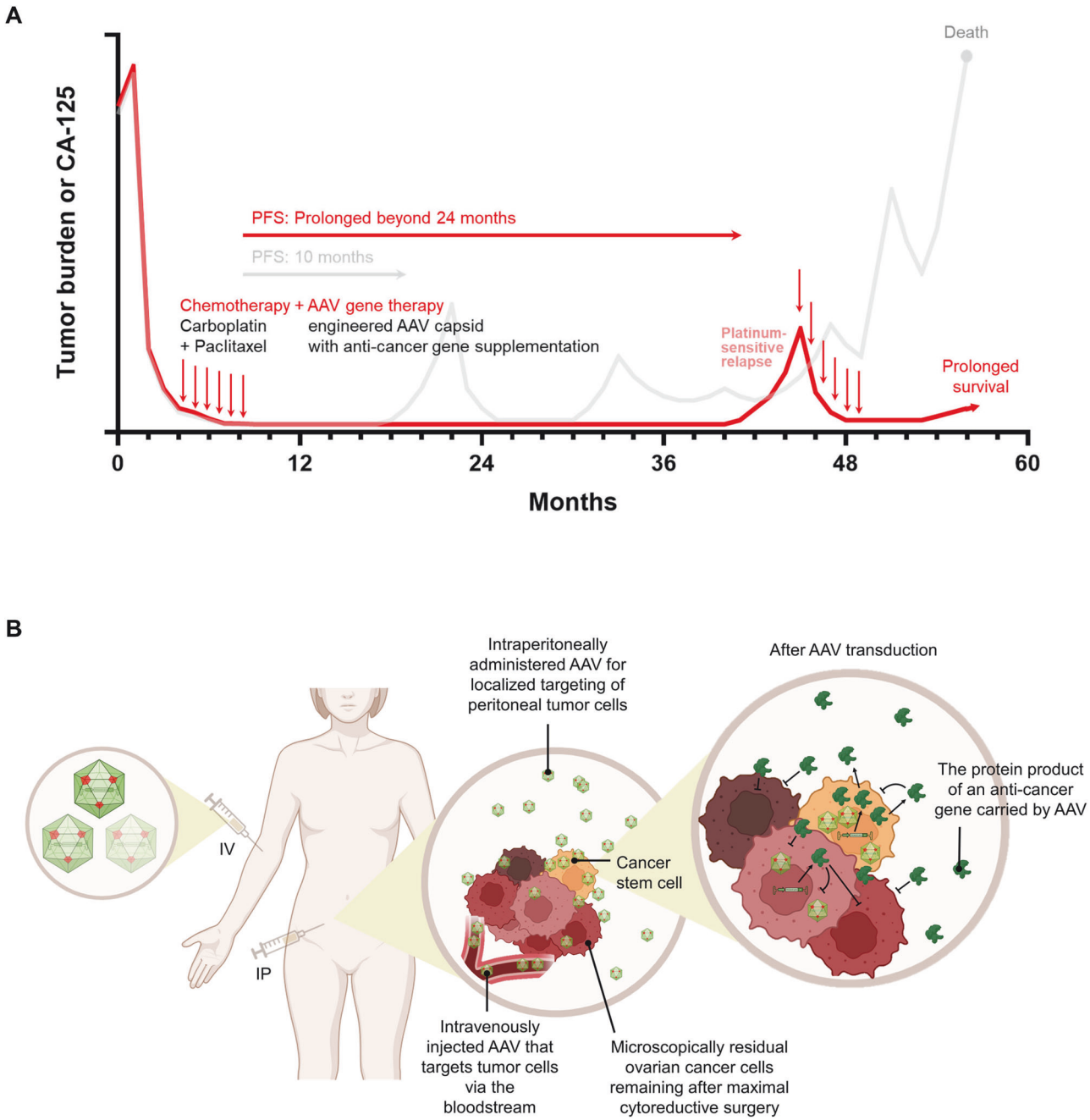

Fig. 4 Schematic overview of therapeutic application and mechanism of AAV gene therapy targeting ovarian cancer. A This schematic illustrates a conceptual framework for incorporating AAV-based gene therapy into the current standard treatment regimen for ovarian cancer. Following maximal cytoreductive surgery and adjuvant chemotherapy with paclitaxel and carboplatin, AAV gene therapy—targeting angiogenesis or immune modulation pathways—is administered either in parallel with or sequentially after chemotherapy. The addition of gene therapy aims to enhance the durability of treatment response and significantly extend the duration of PFS beyond what is typically achieved with chemotherapy alone. This strategy addresses the critical need for therapeutic interventions that can delay relapse and improve long-term outcomes in ovarian cancer patients. **B** This illustration depicts the proposed molecular mechanism of AAV-mediated gene therapy targeting ovarian cancer. AAV vectors can be administered either intravenously (IV) or intraperitoneally (IP). Although the evaluation of administration routes lies beyond the scope of this review, it remains a critical consideration due to the anatomical characteristics of ovarian cancer, which predominantly resides within the peritoneal cavity. Determining the more effective route for AAV delivery—IV or IP—may significantly influence therapeutic efficacy. Engineered AAV capsids capable of efficiently infecting residual, microscopic ovarian cancer cells following maximal cytoreductive surgery can facilitate the delivery and expression of therapeutic genes within both ovarian tumor cells and cancer stem cells. Ideally, the anti-cancer proteins encoded by these genes should exert not only cell-autonomous anti-tumor effects in transduced cells, but also paracrine effects on neighboring malignant cells, thereby enhancing the overall therapeutic response.

chemotherapy resistance remains a significant challenge in the effective treatment of ovarian cancer.

Screening for the most effective therapeutic genes deliverable by AAV

Screening for the most effective anticancer genes to be delivered via AAV is challenging. Traditionally, research approaches in cancer gene therapy have focused on delivering genes with well-defined mechanisms of action, such as Inhibition of angiogenic pathways, or immune activation [153]. The advantage of these studies lies in the ease of hypothesis formulation and the straightforward interpretation of results. However, the major drawback is the difficulty in adopting an unbiased approach, making it impossible to discover entirely new therapeutic methods unpredictably.

Recently, research utilizing the CRISPR-Cas9 system for genetic screening has been actively conducted to elucidate the mechanisms underlying drug resistance in cancer and to identify related genes [167, 168]. These studies employed lentivirus as a delivery vector, which allows for easy control of the MOI (multiplicity of infection), and utilized barcodes and UMIs (unique molecular identifier) to track delivered sgRNAs (single guide RNA), with data analysis facilitated by NGS (next-generation sequencing) techniques. Similarly, constructing AAVs loaded with various potential anticancer genes as transgenes and conducting screenings in vivo or in vitro could establish a critical strategic screening platform for discovering novel anticancer genes previously unknown [169, 170].

CONCLUSION AND FUTURE DIRECTION

Ovarian cancer, similar to pancreatic cancer, often lacks clear symptoms in its early stages, making early detection very difficult [32]. As a result, it is usually diagnosed at an advanced stage. Due to the ovary's anatomical structure, which is directly exposed to the peritoneal cavity, and the nature of cancers originating from ovarian epithelium, ovarian cancer is often found with peritoneal metastasis, frequently accompanied by significant ascites. Despite this dismal situation, the initial response rate to treatment for ovarian cancer is relatively high. This achievement is due to a well-established standard protocol that includes maximal cytoreductive surgery combined with platinum-based chemotherapy, as well as PARP inhibitor maintenance therapy. While complete remission is sometimes observed, over 80% of patients with advanced ovarian cancer experience recurrence and eventually develop resistance to all administered chemotherapies, leading to a high mortality rate [5].

To address the therapeutic limitations associated with ovarian cancer, a range of innovative strategies has been investigated. This review focuses on the potential of AAV as a gene delivery vector for ovarian cancer therapy, with particular emphasis on approaches involving anti-angiogenic and immune-modulating genes. Specifically, AAV-mediated gene therapy for ovarian cancer should be considered for administration either in combination with, or sequentially following, standard chemotherapy regimens involving paclitaxel and carboplatin after maximal cytoreductive surgery (Fig. 4a). The primary objective of this therapeutic strategy would ideally be to significantly prolong progression-free survival beyond two years post-surgery and, ultimately, to achieve complete prevention of disease recurrence.

This approach underscores the practical applicability of gene supplementation strategies in the context of ovarian cancer suppression. For optimal efficacy, the therapeutic gene encoded within the AAV vector should demonstrate preferential transduction of ovarian tumor cells and cancer stem cells over normal tissue. Moreover, genes capable of exerting anti-tumor effects not only within directly transduced cells but also on neighboring malignant cells would represent ideal candidates for inclusion in such gene therapy platforms (Fig. 4b).

Recent advances in capsid engineering have shown that AAV can target specific tissues or cells. By taking advantage of this property, developing AAV that targets cancer stem cells using markers known to identify ovarian cancer stem cells (CD24, CD44, CD133, CD117, ALDH1, SOX2, and OCT4) [171] presents an effective and promising gene therapy strategy, given the role of cancer stem cells in drug resistance and tumor heterogeneity. Additionally, with the rapid advancements in DNA sequencing technologies, research aimed at identifying therapeutic genes for ovarian cancer gene therapy through functional screening and incorporating them into AAV vectors could lead to new directions for ovarian cancer gene therapy.

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AUTHOR CONTRIBUTIONS

HCY Conceived, drafted, and wrote the manuscript; SL and JYP reviewed the manuscript for essential intellectual content; EL supported the draft design and provided revisions and contributed to the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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