

REVIEW ARTICLE



Ewing sarcoma treatment: a gene therapy approach

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Ewing sarcoma (ES) is an aggressive malignant tumor, characterized by non-random chromosomal translocations that produce fusion genes. Fusion genes and fusion protein products are promising targets for gene therapy. Therapeutic approaches and strategies vary based on target molecules (nucleotides, proteins) of interest. We present an extensive literature review of active molecules for gene therapy and methods of gene therapy delivery, both of which are necessary for successful treatment.

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INTRODUCTION

Ewing sarcoma is an aggressive malignant tumor most commonly affecting children and adolescents, characterized by rapid tumor growth and active metastasis [1]. The peak incidence occurs in the second decade of life, and males suffer from ES about one and a half times more often than females [2]. ES most commonly affects bones, particularly the pelvis, femur, or axial skeleton, but it can also involve soft tissues in up to 30% of patients [3–5].

ES refers to Ewing sarcoma family tumors (ESFT), which are characterized by non-random chromosomal translocations that produce fusion genes. At the C-terminus of the transcribed region, a TET-family protein allows RGG repeats (arginine-glycine-glycine) of the fusion protein to bind RNA and participate in transcription, processing, and RNA splicing. The N-terminus of the fusion protein is formed by a protein from the ETS family of transcription factors (FLI1, ERG, FEV, and others). The translocation (t) (11; 22) (q24; q12) is present in 85% of tumors and fuses the genes *EWSR1* and *FLI1*, producing a fusion protein (EWS/FLI). Further, the t (21; 22) (q22; q12), t (7;22) (p22;q12), and other less common translocations represent the remaining 10–15% of cases [6], and these fusion products regulate various cellular signaling and regulatory pathways, including IGF1R and RAS-Rac1 and oncogenesis in general [7].

Despite therapeutic advances in ES over the past decade that have improved the 5-year survival rate from 10% to 55–60%, one quarter of cases are diagnosed with distant metastases, reducing the 5-year survival rate to 15–30% [8]. In general, ES is difficult to treat. Patients have frequent relapses and require complex treatment regimens, including surgery, radiotherapy, and chemotherapy [9]. The complexity of ES therapy and its low efficiency necessitates new treatment modalities. While the need to develop new drugs, active molecules, and combinatorial approaches is obvious, an equally important element of treatment often remains in the shadows—an effective way to deliver active substances to target cells. Effective delivery systems are needed to precisely deliver drugs to avoid side effects associated with non-specific

chemotherapy regimens, and also to increase therapeutic efficacy [10].

Our review focuses on two main aspects of gene therapy: the analysis of existing active agents and the consideration of the advantages and disadvantages of their possible delivery systems.

DELIVERY SYSTEMS-AVAILABILITY AND SELECTION

Viral-based approaches

Remarkable scientific discovery has allowed researchers to harness one of the original enemies of humanity—viruses—for biotechnological and medical purposes. Such vectors have several positive qualities: they often have an affinity for certain tissues, can penetrate target cells, and by their nature, they carry nucleic acids that encode genetic information. The structure of employed viruses is well studied, and the mechanism of interaction with the cell is clear and therefore amenable to control and change. However, many delivery systems are based on retroviruses (mainly gamma-retroviruses and lentiviruses), adenoviruses, and adeno-associated viruses which are currently in use for clinical trials (Table 1).

Retroviral vectors

In the discussion of viral delivery platforms, retroviral vectors are worth considering first. Retroviruses belong to the seventh Baltimore group and are capable of reverse transcription (the production of DNA from RNA using their enzyme). They can be simple or complex viruses with single-stranded RNA and are approximately 100 nm in diameter [11]. In bioengineering and medicine, representatives of only two genera are used from the relatively large and diverse family: gamma-retroviruses and lentiviruses. The vectors obtained from representatives of these viruses are similar in many characteristics. They have a packaging capacity of up to 9 kb [11] and the immunological issues associated with their use are typically minor [12]. However, gamma-retroviruses have a clear disadvantage: they are unable to

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Table 1. Viral and non-viral delivery systems and their application for ES anticancer therapy.

	Nonviral delivery systems		
	Viral vectors	Liposome-like particles	Virus-like particles
Based mechanisms	Retroviruses Vector integrates into the DNA of the target cell. Gamma-retroviruses can only infect dividing cells.	Liposome-like particles Vector fuses with the membrane of the target cell due to the positive charge of the complex.	Virus-like particles Vector is taken up by endocytosis by the target cell. The active agent is released into the cell.
Packaging capacity	up to 9 kb	>30 kb	Up to 30 kb
Security concerns	Insertional mutagenesis	High particle concentration for greater efficiency, but safety has not been fully studied.	High particle concentration for greater efficiency, but safety has not been fully studied.
Immune response	Low	Low	Low
Experiments with ES	In vitro: [17–21, 24], in vivo: [23]	In vitro & ex vivo: [31]; in vivo: [36]	In vivo: [37, 38]

ES Ewing sarcoma, DNA deoxyribonucleic acid, AAV adeno-associated virus.

infect non-dividing cells, which is explained by their inability to penetrate the nuclear membrane. Moreover, gamma retroviruses have a proclivity to disrupt normal gene synthesis due to their preferential insertion into the proximity of transcription start sites in the genome and this is one of the main reasons why vectors based on lentiviruses are commonly used in gene therapy [13, 14] including ES.

The main disadvantage of lentiviral vectors pertains to safety. Most commercial plasmids are based on HIV, which carries inherent danger. Compliance with the safety rules may not guarantee protection. As such, several generations of plasmids have been produced to create safer vectors. In addition, several viral genes were removed and/or were moved to separate plasmids within special cell cultures (with the removal of the vector) to prevent the assembly of full-fledged infectious particles [15, 16]. Lentiviruses have been used as therapeutic agents in several clinical trials for a variety of indications including HIV (NCT02054286, completed phase 2), COVID-19 (NCT04299724, phase 1), hereditary diseases such as β -thalassaemia (NCT02906202, completed phase 3) and various cancers (NCT04571892, NCT00569985, NCT02135406, NCT02976857).

The use of lentiviral vectors is also promising for gene therapy in the treatment of Ewing sarcoma. In particular, lentiviral vectors are used to deliver small hairpin RNA (shRNA). Of special interest in this area is the work of Schaefer et al. [17] in which extensive RNA interference screening for ES (authors screened for targets specific to the A673 cell line) was performed. As part of this work, lentiviral shRNA libraries were created to identify potential therapeutic targets for the therapy of Ewing sarcoma. Transduction efficiency was measured by the level of green-fluorescent protein (GFP) expression from GIPZ shRNA plasmids. Crompton et al. [18] investigated the potential of focal adhesion kinase (FAK) as a candidate therapeutic target for Ewing sarcoma treatment. In this study, various shRNAs targeting different regions of the FAK transcript were used and resulted in five unique shRNA sequences that robustly downregulated FAK protein levels. HEK-293T cells were used for lentivirus packaging and production and were transfected with pLKO.1 lentiviral vector and packaging plasmids (pCMV8.9 and pCMV-VSVG, which donate a strong promoter) according to the FuGENE 6 (Roche) protocol. The resulting vectors were used to successfully transduce ES cells.

shRNAs are used for therapy and to study individual molecules and their associated signaling pathways and cascades. For example, Hatano et al. [19] used shRNA to study the role of the adhesion molecule Cadherin-11 in ES cells and its role in metastatic seeding to the bone. Cells were successfully transduced with three lentiviral vectors: lentivirus containing Cad-11 shRNA (from Sigma-Aldrich, TRCN0000054334) or control shRNA and lentiviral luciferase vector (transfer vectors pCSII-EF-MCS-IRES2-Venus, pLP1, pLP2, and pLP/VSVG (Invitrogen). A rather complex manual assembly of the vector containing the shRNA for BMI-1 was carried out by Levetzow et al. [20]. The pCLS lentiviral backbone was modified. Firstly, the rtTA IRES Puro expression cassette was amplified from pTRIPZ (Open Biosystems, Huntsville, AL) and ligated into pCLS. Secondly, two additional restriction enzyme sites (AgeI and NheI) were inserted. Finally, the expression cassette (TRE/CMV minimal promoter, tRFP, and the shRNA cassette) was amplified from pTRIPZ and ligated into AgeI and NheI sites in the modified pCLS. Then, to selectively target the resulting construct on BMI-1, the authors added a miR-30-based shRNA expression cassette. Thus, lentiviral vectors can be widely used to deliver shRNA to ES cells.

Moreover, lentiviral vectors are used not only to deliver shRNA to target cells but also to introduce DNA sequences of functional proteins into cancer cells. For example, Joo et al. [21] used lentiviruses to achieve two alternative means of inhibiting glioma-associated oncogene (GLI1) which is an upregulated target of EWS/ETS and is the principal transcriptional effector of the

Hedgehog-GLI (HH-GLI) signaling pathway. Researchers inhibited GLI1 by the introduction of shRNA and by the transduction of the DNA sequence suppressor of EWS/FLI1 fusion, which acts as an endogenous inhibitor of the HH-GLI1 pathway. It has previously been shown that this fusion protein is synthesized in cancer cells and that it disrupts the HH-GLI signaling pathway [22].

Another example of successful lentiviral vectors use to introduce functional proteins into Ewing sarcoma cells as part of therapy is the work of Rademacher et al. [23]. While systemic administration of interleukin-12 (IL-12) has detrimental toxicities, its introduction with a lentiviral vector has shown promising results for cancer immunotherapy. Lentiviral constructs contained a fused form of human IL-12 α chain (p35), β chain (p40) and enhanced green-fluorescent protein or firefly luciferase within a HIV-1-based backbone. The article details the vector synthesis route, using the lentiviral backbone pDY.cPPT-EF1 α .WPRE, previously synthesized in the same laboratory [24]. The successful LV transduction was verified by the specific production of IL-12 in the supernatant of transduced cell spheroids. Thus, the variety of lentiviral vectors used for efficient transfection demonstrates the potential for this tool in ES gene therapy.

Adenoviral and adeno-associated viral vectors

Adenoviruses are double-stranded DNA viruses with 80–100 nm in diameter. Since basic human adenovirus type 2/5 vector recognizes Coxsackievirus and adenovirus receptor (CAR) present on the cellular membrane of ES cells [25, 26], adenoviral vectors represent a promising system of therapy for Ewing sarcoma. To increase the specificity of adenovirus replication to tumor cells, genetic engineering can enhance their ability to target cellular signaling. For example, the oncolytic adenovirus XVir-N-31 is being studied in combination with a CDK4/6 inhibitor (abemaciclib), which selectively infects cells expressing YB-1. Improvements in viral replication, viral particle formation, and oncolysis were demonstrated. Engineering of oncolysis with ablation of RB binding site (Rb-independent) creates oncolytic virus that uses free E2F to drive viral replication in cancer cells. The effect of adenovirus is enhanced by stopping the cell cycle with an inhibitor in the G1 phase, accompanied by a decrease in the level of proteins Rb and E2F1, which are participants of the CDK4/6-RB/E2F pathway that is dysregulated in tumors [27].

In another study, researchers improved the susceptibility of Ewing sarcoma cells (TC71) to agents targeting type IIA topoisomerase (topo IIA), including VP-16 and doxorubicin, by transferring the E1A gene using adenovirus Ad-E1A. The vector is based on adenovirus serotype 5, and contains wild-type E1A, but deleted E1B and E3 regions [28]. With the same Ewing sarcoma cell line, experiments were conducted in vivo on a mouse model where MSC cultures were established from mouse bone marrow and used as a viral delivery system. A bicistronic adenovector containing protein subunits of IL-12 (p35 and p40). After 30 days of treatment, the tumor size decreased 3-fold compared to the control group [29].

Clinical studies using adenovectors as methods of treatment have gained interest in recent years. For example, the Shanwen Zhang group used the rAd-p53 adenovector (Gendicine) based on the adenovirus 5 serotype, defective in replication, where the E1 region was replaced by a gene cassette containing the wild-type p53 protein gene. The study involved patients diagnosed with inoperable soft tissue sarcoma, including Ewing sarcoma. For 34 out of 36 patients, injections of viral particles at tumor sites were performed with a concentration of 1×10^{12} virus particles/ml in combination with radiotherapy and hyperthermia. For the treatment vs. control group, the progression-free survival was 12 vs. 5 months, and overall survival was 36.5 vs. 16 months. These results demonstrate that gene therapy can synergize with traditional treatment modalities to significantly improve therapeutic efficacy and survival [30].

Adeno-associated viruses (AAV) are another viable option that are actively used in clinical and preclinical trials to target ES cells. And although we have not been able to identify any clinical trials of AAV for the treatment of Ewing sarcoma, model studies are underway. In a study by Veldwijk et al. two vectors (rAAV-2-CMV-TK/eGFP, rAAV-2-EF1 α -TK/eGFP) based on AAV2 with cytomegalovirus or elongation-factor 1- α promoters were investigated. The Herpes simplex-derived thymidine kinase (TK) gene was used as a suicide gene. The effect of the vectors was tested on four different sarcoma cell lines (HS-1 epitheloid sarcoma, HT-1080 fibrosarcoma, RD-ES Ewing sarcoma, SK-N-MC Askin tumor). The combined effect of rAAV-2-EF1 α -TK/eGFP at an MOI of 200 and ganciclovir at a concentration of 2.5 μ g/ml on sarcoma cells led to 100% mortality on the 14th day of the study, while the action of the agents individually did not lead to a significant decrease in the number of living cells. In the same study, an in vivo experiment was conducted in which ex vivo treated sarcoma cells (HS-1 cells with rAAV-2-TK/eGFP and GCV) were transplanted into mice. The period of tumor-free survival was more than 5 months for treated mice, yet only 30 days for untreated mice [31].

Schwarzbach et al. investigated the possibility of inducing the sensitization of sarcoma cells to doxorubicin using adeno-associated virus type 2. HS729, RD, A204 (rhabdomyosarcoma), A673 (Ewing sarcoma), U20S, KHOS, SAOS2, MNNG (osteosarcoma), SW872, 195591 (liposarcoma), SW982 (synovial sarcoma), HT1080 (fibrosarcoma) cells were used as model cells. To study proliferation, the cells were infected with a virus (MOI of 10^5 VP/cell) and then incubated with doxorubicin for 24 h. It was shown that AAV2 infection potentiated doxorubicin in all cell types, except for the lines SW872, 195591, and SW982. Although, the molecular mechanisms that caused tumor cell sensitization in this article have not been investigated [32] we speculate that doxorubicin can modulate AAV trafficking to target cells [33]. On the molecular level, AAV2 infection can develop apoptosis [34], which favors doxorubicin cytotoxicity and opposes cytoprotective autophagy [35].

Using a system with elements from AAV and phage (AAVP), Amin Hajitou et al. created a vector controlled by a CMV promoter, which triggered the expression of transgenes containing RGD-4C peptide that binds to tumor TK and tumor integrins. To study the effect of the vector on tumor cells and to visualize the distribution of the vector in the body, an in vivo model using nude rats and a soft tissue sarcoma cell line SKLMS1 was used. Using this vector, researchers were able to visualize the transgene containing a specific radiometric tag using positron emission tomography. The synergistic effect of the vector has been demonstrated using ganciclovir therapy [36].

Thus, the advantages of AAV include the absence or low pathogenicity within the doses necessary for transduction. AAVs, like adenoviral vectors, have a high cell tropism, but unlike an adenoviral vector, AAVs can be used for long-term expression. One of the major disadvantages is the small size of transgenes that can be used in these vectors, but this limitation can be overcome by dividing the transgene of interest among multiple separate vectors.

NONVIRAL DELIVERY SYSTEMS

As we described above, one of the classes of drugs for the treatment of oncological diseases, in particular Ewing sarcoma (ES), uses viral replication to deliver therapeutic payload to the target cells and express it via part of viral genome. The viral delivery systems we have described have their limitations and disadvantages, which are briefly described in Table 1. Non-viral delivery systems are an alternative, and such systems are relatively easy to modify. It is now possible to regulate their specificity both against healthy/cancer cells and different types of cancer cells. Also, the duration of the therapeutic effect can be well controlled

so that nonviral delivery systems are attractive for further study as anticancer agents. Lipids and nanoparticles or their combination have been widely used for the therapy of sarcoma (Table 1). One of them is the cationic lipid-bound complex with DNA composed with liposomes. These are positively charged complexes, which allows them to bind to the membranes of target cells, and the lipids at their base also allow them to then to fuse with the bound membrane. For example, researchers delivered the TNF-dependent apoptosis-inducing ligand gene into cells in a lipophosphoramidate-based transfection system, resulting in an antitumor effect that prevented bone destruction and inhibited tumor growth, which led to an increase in the life expectancy of patients [37]. Others developed a newer method of ES therapy by blocking the ligand of the activator receptor NF- κ B [38]. To do this, the authors injected osteoprotegerin (a protein that inhibits osteoclasts and osteolysis) into ES cells using non-viral gene transfer. The latter was carried out using amphiphilic polymers made of polyethylene and polypropylene oxides [38].

The second class of delivery systems simulates viruses. Virus-like particles are multi-subunit self-assembling protein structures that have an overall structure similar to the corresponding biological virus. One example is complex with a polycation, such as a polylysine, along with a ligand that provides specificity for binding to the cell and permitting endocytosis, coupled to an endosomal agent that releases DNA into the target cell cytoplasm. Replication-defective adenovirus particles have been used for this purpose and animal adenoviruses are most often used since they are unable to replicate in human cells. The use of virus-like particles seems promising, but unfortunately there have been no studies for the treatment of Ewing sarcoma.

Active agents to deliver of DNA and RNA to target cells

The gene therapy agents for Ewing sarcoma can be divided into several groups of drugs including those designed to transfer DNA into cells, drugs designed to suppress gene expression using RNA interference, and drugs used for direct gene editing. Next, we will summarize and analyze the insights gained from researchers who have investigated active agents for gene therapy.

Targeting tumor DNA to modulate gene expression

DNA was one of the first to be widely used as a therapeutic agent [38, 39]. Using cleverly designed systems, DNA molecules have been delivered by both viral and non-viral delivery methods. For example, researchers used a retroviral vector expressing a hybrid of FLI1-ERF—the product of the fusion of FLI1 and ERF genes—mitogen-activated and protein kinase-regulated protein that stops RAS-induced cell transformation and arrests the cell cycle at the G0/G1 stage [40]. The authors showed that the retroviral vector with the chimeric protein ERF reduces the oncogenicity of ES cells and the cancer phenotype. Nanoparticles or liposomes are used as non-viral DNA delivery methods, as suggested by Picarda et al. [38]. The authors wanted to develop a new method of ES therapy by blocking the ligand of the activator receptor NF- κ B. To do this, the osteoprotegerin gene was introduced using amphiphilic polymers made of polyethylene and polypropylene oxides, which inhibited tumor growth.

Targeting RNA of tumor

Interfering RNA is a class of drugs used in the treatment of malignant and benign tumors since they specifically inhibit gene expression and do not rely upon active cell division. The process of RNA interference suppresses mRNA maturation with the help of small RNA molecules. RNA, like DNA, is a carrier of genetic information, which makes it possible to use it as a therapeutic agent for various pathologies, including ES. In preclinical trials of a cyclodextrin polymer-based nanoparticle, there is a method of introducing mRNA as part of a cyclodextrin polymer-based nanoparticle, specifically silencing the translocation of EWS-FLI1 [41].

Another method of ES gene therapy used plasmid transfection of a plasmid containing siRNA to the Lyn gene, a tyrosine kinase that controls cell proliferation, adhesion, mobility, and invasiveness [42]. The researchers were able to reduce the growth of the tumor and inhibited metastasis. In addition, the authors noted the suppression of tumor growth and a decrease in its lytic phenotype. In a more recent study, researchers delivered mRNA to ES cells using exosomes [43]. By transferring specific mRNAs, the authors were able to reduce the malignancy of sarcoma cells. These results show that the development of new specific drugs for ES therapy based on nucleic acids can improve patient outcomes.

The discovery of genome editing technologies has allowed the creation of cancer cell models and the identification of possible targets for therapy. Clustered regularly interspaced short palindromic repeats called CRISPR sequences were first detected in *E. coli*. CRISPR is an important component of the prokaryotic immune system. In 2013, CRISPR/Cas9 gene editing technology was used for the first time to edit the genomes of mammalian cells by Mali [44] and Cong [45] and coll. Overall, CRISPR/Cas9 exceeds many aspects of Zinc Finger Nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) methods that were used to edit genomes previously. In the case of Ewing sarcoma, ZFN and TALEN have been used to create de novo oncogenes [46]. Due to the advantages of the CRISPR/Cas9 system, gene knockouts or genome editing of Ewing sarcoma cells using ZFN or TALEN is not currently used. CRISPR/Cas9 has several advantages: CRISPR/Cas9 is cheaper and the same Cas9 can be used for editing, while only needing to replace the sgRNA (single guide RNA) sequence [47, 48]. With the help of this system [49] it is possible to edit several genes simultaneously with fantastic accuracy. This technology has been widely adopted in biomedical research and is being investigated for the treatment of human diseases.

CRISPR/Cas9 has also been used to construct a model of Ewing sarcoma cells with EWSR1-FLI1 translocation mutations in the HEK293 cell line and human mesenchymal stem cells (hMSCs), after which the expression of the EWSR1-FLI1 fusion protein was analyzed. Six genes targeted by the EWSR1-FLI1 protein were also activated, demonstrating the potential impact of this method [50]. Torres-Ruiz et al. [51] developed a method for connecting ssODN-RNP CRISPR/Cas9 for a more efficient generation of translocations t(11, 22) in hMSC and hiPSC (human-induced pluripotent stem cells). In the same year, Spraggon et al. developed a new method that combines CRISPR/Cas9 with HDR to develop and modulate the expression of chromosomal translocation products. This method also allows timely monitoring of the expression of the *EWSR1-FLI1* fusion gene, which effectively solves the problem of constant generation of the *EWSR1-FLI1* fusion gene mediated by changes in the expression of its intracellular target genes in a short time. Povedano et al. used CRISPR/Cas9 technology to knock out the *MSH2* gene in A673 cell lines. This increased the frequency of mutations, which can be used to identify new multi-compound targets for therapy. In a recent study, the TRIM8 gene was knocked out using CRISPR/Cas9, which expressed a ligase of the same name involved in the degradation of the EWS/FLI-1 fusion protein, the expression of which was significantly increased in modified cells [52].

Stolte et al. used CRISPR/Cas9 technology to knock out *MDM2*, *MDM4*, *PPM1D*, and *USP7* genes in mutant TP53 cells and wild-type TP53 cells, after which the survival rate of wild-type cells decreased. It has been demonstrated that the knockout of the *MDM2/MDM4* gene pair leads to an antitumor effect [53]. It was also shown that editing and subsequent knockout of *PHF19* significantly reduced cell proliferation, colony formation, and the ability to invade, and increased sensitivity of SK-N-MC cell lines to the inhibitor of the protein BET-bromodomain JQ1, in addition to reducing proliferation and stimulating apoptosis of Ewing sarcoma [54]. He et al. found that TNC knockout in A673

and SKNMC cell lines reduced tumor cell proliferation, migration, and angiogenesis, and when these cells were injected into nude mice, cells showed less motility and a decreased ability to colonize *in vivo* compared to control samples [55]. One recent study of the efficacy of *EWS/FLI1* knockout by editing exon 9 using CRISPR/Cas9 found that editing *EWS/FLI1* in the A673 cell line almost completely stops proliferation and works more efficiently than *EWS/FLI1* silencing [56]. Another study found an association between *RRM2* knockout in Ewing sarcoma cells and reduced tumor growth, and induction of apoptosis *in vitro* and *in vivo* models. The sensitivity of ES cells to *RRM2* knockout is partially related to the overexpression of the DNA restriction factor SLFN11, which is a direct transcriptional target of *EWS-FLI1* [57]. Schmidt et al. discovered that HDAC1 and HDAC2 knockouts inhibited tumor growth and invasiveness in xenografts [58], implying that histone acetylation and cell cycle division are hallmarks of ES pathogenesis.

The presented studies show that CRISPR technology as a gene editing tool is an excellent technology that promises new possibilities in the treatment of genetically determined diseases including Ewing sarcoma. Knockout of disease-related genes or their replacement shows positive experimental results. However, this strategy has its problems including off-target effects, a low editing efficiency, or unintended consequences from gene editing [59]. In addition, the Cas9 complex may cut in an undesirable region and potentially cause a catastrophic obstacle, however research aimed at mitigating such challenges of the CRISPR/Cas9 system is underway [60].

CONCLUSIONS

This article presents the most recent gene therapy research for Ewing sarcoma and illustrates how this therapy is a promising approach for patients with this disease. Its main advantage over classical methods of treatment is greater selectivity of the therapeutic agent. Unlike radiation therapy and chemotherapy, which are non-specific in comparison leading to adverse side effects that reduce patient quality of life [61]. The development of future drugs used for gene therapy must consider the need for the high specificity, as well as low toxicity to non-target cells and tissues. In addition, the optimal therapy should include a combination of an effective delivery system and the possibility of long-term and short-term controllable activity.

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AUTHOR CONTRIBUTIONS

ST, DF, PT, and IU designed the review; ST, DF, PT, IK, and IU performed the literature search and analyzed data; ST, DF, PT, OK, BAS, and IU wrote the review, PT, provided administrative and lab support; all authors edited the paper. The author(s) read and approved the final paper.

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The authors declare no competing interests.

ADDITIONAL INFORMATION

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