Mini-review

Suicide gene therapy in cancer: Where do we stand now?

Sónia Duarte, Georges Carle, Henrique Faneca, Maria Pedroso de Lima, Valérie Pierrefite-Carle

PII: S0304-3835(12)00329-1
DOI: http://dx.doi.org/10.1016/j.canlet.2012.05.023
Reference: CAN 11033

To appear in: *Cancer Letters*

Received Date: 21 December 2011
Revised Date: 11 April 2012
Accepted Date: 21 May 2012

Please cite this article as: S. Duarte, G. Carle, H. Faneca, M.P. de Lima, V. Pierrefite-Carle, Suicide gene therapy in cancer: Where do we stand now?, *Cancer Letters* (2012), doi: http://dx.doi.org/10.1016/j.canlet.2012.05.023

Suicide gene therapy in cancer: Where do we stand now?

Sónia Duarte[1], Georges Carle[2], Henrique Faneca[1], Maria Pedroso de Lima[1,3,*] and Valérie Pierrefite-Carle[2]

[1]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [2]UMR 6235 CNRS, Université de Nice Sophia Antipolis, Faculté de Médecine, Avenue de Valombrose, 06107 Nice cédex 2, France**;** [3]Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Keywords: suicide gene therapy; cancer; bystander effect, combined strategies

Abbreviations: AMSCs: adipose tissue mesenchymal stromal cells; CD: cytosine deaminase; CSC: cancer stem cells; DOTAP: 1,2-dioleoyl-3-(trimethylammonium) propane; Chol: cholesterol; 5-FC: 5-fluorocytosine; 5-FU: 5-fluorouracil; GCV: ganciclovir; GvHD: graft-versus-host disease; HSV-tk: herpes simplex virus thymidine kinase; MSC: mesenchymal stem cells; NK: natural killer; NSCs: neural stem cells; PSA: prostate-specific antigen; TK: thymidine kinase; UPRT: uracil phosphoribosyltransferase

*Corresponding author:

Maria C. Pedroso de Lima, Professor
Department of Life Sciences, University of Coimbra,
Apartado 3046
3001- 401 Coimbra, Portugal
Phone: +351-239 104 397
Fax.: +351-239 855 789
E-mail: mdelima@ci.uc.pt

Abstract

Suicide gene therapy is based on the introduction into tumor cells of a viral or a bacterial gene, which allows the conversion of a non-toxic compound into a lethal drug. Although suicide gene therapy has been successfully used in a large number of in vitro and in vivo studies, its application to cancer patients has not reached the desirable clinical significance. However, recent reports on pre-clinical cancer models demonstrate the huge potential of this strategy when used in combination with new therapeutic approaches. In this review, we summarize the different suicide gene systems and gene delivery vectors addressed to cancer, with particular emphasis on recently developed systems and associated bystander effects. In addition, we review the different strategies that have been used in combination with suicide gene therapy and provide some insights into the future directions of this approach, particularly towards cancer stem cell eradication.

1. Introduction

Chemotherapy, radiotherapy and surgery constitute the conventional treatments for cancer, and among these, complete surgical resection is still the most effective approach to treat cancer patients. However, these patients often exhibit advanced stage tumors limiting the possibility of surgery or favouring relapses after resection. In addition, the recent discovery in a large number of cancers of a rare population of cells with stem cell-like properties called «cancer stem cells» (CSCs), which fuel tumor growth and exhibit different features compared to differentiated tumor cells, appears as a new challenge for cancer treatment. Therefore, as the majority of cancers remain resistant to the current therapeutic options, the development of more efficient strategies is still urgent [1; 2].

Gene therapy appears as a good alternative and holds a great promise for the treatment of various diseases including cancer, as evidenced by the significant number of recently reported clinical trials [3; 4; 5; 6; 7; 8]. In cancer gene therapy, different approaches can be used such as mutation correction; enhancement of the immune response against tumor cells; RNA interference, targeted lysis of tumor cells using selective replicative viruses; anti-angiogenic and suicide gene therapies [9].

In this review, we will focus on suicide gene therapy, summarizing the different suicide gene systems and gene delivery vectors addressed to cancer, giving particular emphasis to recently developed systems and associated bystander effects. In addition, we will review the different strategies that have been used in combination with suicide gene therapy and provide some insights into the future directions of this approach.

2. The concept of suicide gene therapy and the different suicide systems

Suicide gene therapy is based on the introduction into tumor cells of a viral or a bacterial gene, which allows the conversion of a non-toxic compound into a lethal drug (Figure 1). Among the large number of suicide systems that have been reported (Table I), the herpes simplex virus thymidine kinase gene (HSV-tk) with ganciclovir (GCV) as prodrug and the cytosine deaminase gene (CD) of *E. coli*, which converts the non-toxic antifungal agent 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), are the most extensively studied. These two systems will be described in more detail.

2.1. The TK/GCV suicide system

HSV-tk/GCV is one of the most promising "suicide" gene therapy systems. The expression of the HSV-tk gene leads to the production of viral thymidine kinase that metabolizes GCV to ganciclovir monophosphate. Cellular kinases then convert monophosphorylated GCV into ganciclovir triphosphate. As the latter compound is an analogue of deoxyguanosine

triphosphate, inhibiton of DNA polymerase and/or incorporation into DNA occurs causing chain termination and tumoral cell death [10; 11; 12].

It was demonstrated that erroneous GCV triphosphate incorporation into DNA results in S-phase delay, as well as G2-phase arrest by the activation of 3' exonuclease and postreplicative endonuclease repair mechanisms [13; 14; 15; 16]. As a result of GCV-induced cell cycle arrest, Wei *et al.* found that apoptosis rather than a direct chemical effect was involved in HSV-tk-transduced B16F10 melanoma cell death [17]. Beltinger *et al.*, reported that TK/GCV-induced apoptosis in cultured human neuroblastoma cells involves accumulation of p53, translocation of CD95 to the cell surface mediated by p53 and CD95-L-independent formation of a death-inducing signaling complex containing Fas-associated death domain protein (FADD) and caspase-8 [18]. In contrast, Tomicic *et al.* and Fischer *et al.* showed that, upon application of HSV-tk suicide gene therapy in Chinese hamster ovary and glioma cell lines, GCV-induced apoptosis occurred mainly by activating the mitochondrial damage pathway, in which a decline in Bcl-2 levels was observed [19; 20]. Therefore, caspase activation may be a consequence of different initiation events depending on the cell type. In a recent study, the relevance of the cell cycle control towards the sensitivity of pancreatic tumour cells to the cytotoxicity induced by the HSV-tk/GCV system was demonstrated, since a Chk1 activation was associated with a greater HSV-tk/GCV extent of cell death [21].

In vivo anti-tumor activity of the TK/GCV system has been demonstrated in several carcinoma animal models, including leukemia [22], glioma [23; 24], bladder cancer [25], intrahepatic metastasis of liver cancer [26], colon adenocarcinoma [27], and oral cancer, [28; 29].

The promising results achieved in the pre-clinical studies with the HSV-tk/GCV system led to its application in a number of clinical trials towards different types of cancer [30; 31; 32; 33; 34; 35]. In a prospective phase I/II clinical study, Voges *et al.* treated patients suffering from recurrent glioblastoma multiforme with HSV-1-tk gene-bearing cationic liposomal vector and

systemic ganciclovir and observed a therapeutic benefit in some of the patients. The results of this phase I/II study demonstrated the feasibility and safety of this therapeutic strategy [31]. In a more recent study, Nasu *et al.* conducted a phase I clinical trial addressed to hormone-refractory prostate cancer in 8 patients, which involved the administration of HSV-tk mediated by adenovirus followed by ganciclovir. In 5 patients, a clear decrease of prostate-specific antigen (PSA) values was observed, confirming the safety profile and possibility of clinical response at the surrogate marker level [32].

Given the successful results obtained with the HSV-tk/GCV system in humans, some studies have pursued to phase III clinical trials [30].

Table I-Suicide gene systems

Enzyme	Prodrug	Drug	References
Herpes simplex virus thymidine kinase (HSV-Tk)	Ganciclovir (GCV)	Ganciclovir triphosphate (GCV-3P)	[36; 37]
Varicella-Zoster virus thymidine kinase (VZV-tk)	6-methoxypurine arabinoside (ara-M)	Adenine arabinoside triphosphate (ara-ATP)	[38]
Cytosine deaminase (CD)	5-Fluorocytosine (5-FC)	5- Fluorouracil (5-FU)	[39; 40]
Purine nucleoside phosphorylase (PNP)	6-methylpurine-2-deoxyriboside	6-methylpurine	[41; 42]
Nitroreductase	5-aziridinyl-2,4-dinitrobenzamide (CB1954)	5-(Aziridinyl)-4-hidroxylamine-2-nitrobenzamide	[43; 44]
beta-Galatosidase	N-[4"-(beta- D-galactopyranosyl)-3"-nitrobenzyloxycarbonyl]daunomycin (Daun02)	Daunomycin	[45]
Hepatic cytochrome P450-2B1	Cyclophosphamide (CPA) and Ifosfamide (IFO)	Phosphoramide mustard and acrolein	[46; 47]
Linamarase	Linamarin	Cyanide	[48; 49]
Horseradish peroxidase	Horseradish Indole-3-acetic acid (IAA) and derivatives, paracetamol	Free radicals	[50; 51]
Carboxypeptidase A	Methotrexate (MTX)-α-peptides	MTX	[52]
Carboxypeptidase G2	N,N-[(2-chloroethyl) (2-mesyloxy-ethyl) amino] benzoyl-L-glutamic acid (CMDA)	N,N-[(2-chloroethyl) (2-mesyloxyethyl) amino] benzoic acid (CMBA)	[53; 54]

2.2. The CD/5-FC suicide system

The CD enzyme, found in several bacteria and fungi but not in mammalian cells, catalyses the hydrolytic deamination of cytosine into uracil. It can therefore convert the non-toxic prodrug 5-FC to 5-FU, which is then transformed by cellular enzymes into potent pyrimidine antimetabolites (5-FdUMP, 5-FdUTP, 5-FUTP). Three pathways are involved in the induced cell death: thymidylate synthase inhibition, formation of (5-FU) RNA and of (5-FU) DNA complexes [55].

Similarly to HSV-tk/GCV system, apoptosis is also involved in the mechanism of cytotoxicity induced by the CD/5-FC suicide system [20]. It was reported that in glioma cells, the mitochondrial pathway is involved in the process of cell death induced by both suicide gene systems, while p53 and death receptors are not implicated in such process. Although it was shown that the cytotoxicity induced by the HSV-tk/GCV and CD/5-FC suicide systems follow a mitochondrial pathway, the mechanisms of modulation of Bcl-2 proteins were found to be different [20]). Finally, a study of Negroni *et al*. suggested that the activation of heat shock protein 90-beta by phosphorylation in CD-expressing colon carcinoma cells upon 5-FC treatment, might contribute to tumor regression and tumor immunogenicity [56].

5-FU has been widely used in cancer chemotherapy but high doses are generally required for tumor response. This suicide system results in tumor targeted chemotherapy and allows bypassing the toxic side effects generally associated to systemic 5-FU chemotherapy. The CD gene has been cloned from *Escherichia coli* [57] and has been shown in a number of in vitro studies to confer mammalian cell sensitivity to 5-FC.

The CD/5-FC system has been further improved in several studies by the inclusion of the uracil phosphoribosyltransferase (UPRT) gene allowing the conversion of 5-FU to 5-fluorouridine monophosphate, the first step of its pathway to activation [58]. Importantly, this CD-UPRT/5-FC suicide system was shown to be effective against 5-FU-resistant human primary cancer cells [59].

Finally, a bifunctional chimeric protein designated FCU1, combining the yeast enzymatic activities of CD and uracil phosphoribosyltransferase was shown to highly increase CD activity [60].

In vivo anti-tumor activity of the CD/5-FC combination has been demonstrated in several animal models, including fibrosarcomas [61], carcinomas [62; 63; 64; 65; 66], gliomas [67] and metastatic formations of different origin [68; 69].

A number of clinical trials have been reported using the CD/5-FC system, although its application in the clinic has been limited [35; 70; 71; 72]. The first clinical trial using the CD/5-FC system was performed in breast cancer patients and involved specific targeting to the erbB-2 oncogene overexpressed in this type of cancer. The authors showed that their approach was safe and selective to erbB-2–positive tumor cells [70].

In another study, Nemunaitis *et al.* performed a pilot trial in refractory cancer patients, which involved intratumoral injection of TAPET-CD, an attenuated Salmonella bacterium expressing the *E. coli* CD gene in 3 patients. The authors reported the absence of adverse effects induced by TAPET-CD and the results demonstrated that Salmonella bacterium can be utilized as a delivery vehicle of the CD gene to malignant tissue, and the delivered gene was found to be functional [71].

A different delivery system, which consisted in the use of an oncolytic adenovirus containing a CD/HSV-1 TK fusion gene, was applied in a phase I clinical trial in 75 patients with newly diagnosed, intermediate- to high-risk prostate cancer [72]. It was found that the transgene expression persisted in the prostate for up to 3 weeks after the adenovirus injection. A combination of this therapeutic system with conventional-dose three-dimensional conformal radiation therapy resulted in significant declines in PSA in all patients, and this combined approach was shown to be safe [72].

3. Delivery systems

3.1. Viral vectors

Because viruses have evolved natural mechanisms to deliver their genomes into cells, they are excellent vectors to deliver foreign DNA. These vectors are designed by replacing non-essential genes involved in viral replication or pathogenic protein production with foreign therapeutic genes. Production of recombinant viral vectors is achieved by providing in *trans* the non-essential genes, either integrated into the genome of a packaging cell line or in a plasmid. The choice of viral vector depends on several parameters such the characteristics of the cancer type and the therapeutic strategy. The commonly used viral vectors for gene therapy derive from adenoviruses, retroviruses, vaccinia virus, poxviruses, adeno-associated viruses, herpes simplex virus and lentiviruses [73]. In the context of cancer stem cells which can remain quiescent for long periods [74], the latter represents a promising newcomer as a gene transfer vector due to its abilities to efficiently and stably transduce non-dividing cells.

Along with these replication-defective viral vectors, replicative-competent viruses, able to replicate selectively in tumor cells with specified oncogenic phenotypes have been used [75]. These oncolytic viruses can be either naturally occurring or modified to obtain tumor specificity and can be adapted as vectors for cancer gene therapy. The "armed" viruses can then directly kill cancer cells as a consequence of the lytic viral cycle, in combination with the effect of the therapeutic gene incorporated in the viral genome.

Despite their high potential for gene delivery, immune recognition for most of them, mutagenic integration (retroviral & lentiviral vectors), and inflammatory toxicity (adenoviral vectors) still appear as limitations for the use of viral vectors. These considerations led to a renewed interest in non-viral methods.

3.2. Non-viral vectors

At present, approximately 70% of the 1714 protocols approved for gene therapy clinical trials involve the use of viral vectors, which is justified by the high gene delivery/expression

efficiency of these systems (http://www.wiley.com/legacy/wileychi/genmed/clinical/, Wiley website). However, the drawbacks associated with the application of viral vectors, especially safety concerns, prompted investigators to develop non-viral gene delivery systems. Non-viral approaches can be divided into three groups: naked DNA, physical approaches, such as the hydrodynamics methods, gene gun and electroporation, and chemical methods, which mainly involve vectors that have cationic components in their composition, such as cationic liposomes and cationic polymers [76; 77].

Naked or plasmid DNA exhibit low cellular uptake and rapid clearance. As a consequence, naked DNA injection is used when the low gene transfer efficiency can be relayed by immune system activation and/or local bystander effects. Naked DNA is now used in clinical trials almost as frequently (19%) as adenoviral (24.2%) or retroviral (20.7%) vectors [Wiley website].

Regarding the physical methods, their potential to be used in clinical applications of gene therapy has already been demonstrated as shown by the approval of 5 clinical trials involving gene gun protocols (Wiley website). Nevertheless, further optimization of these gene delivery protocols will be needed in order to increase their efficiency without inducing a severe toxicity [76; 77].

On the other hand, cationic liposomes and cationic polymers represent the most extensively investigated and commonly used non-viral gene delivery methods [76; 77]. These non-viral vectors, because of their positive charge, can interact with the negatively charged DNA through electrostatic interactions leading to the formation of lipoplexes, in the case of cationic liposomes, or polyplexes, when using cationic polymers. The widespread application of lipoplexes for gene delivery is due to a number of important advantages, including their capacity to transport large amounts of genetic material; their physico-chemical versatility, allowing innumerous modifications; their easy and inexpensive large scale production; and their low immunogenic response [78; 79]. Since cationic liposomes were first described by

Felgner *et al*. [80] for gene delivery, an increasing number of new cationic lipids have been produced and used in transfection protocols of different cell lines, animal models and patients submitted to gene therapy clinical trials [77]. At present, 6.4% (n=109) of the protocols approved for gene therapy trials involve lipoplexes, these being mainly applied in the treatment of cancer and cystic fibrosis (http://www.wiley.com/legacy/wileychi/genmed/clinical/). Both lipoplexes and polyplexes have proven to be promising systems to efficiently transfect a broad range of cell types in tissue culture. However, despite the extensive work in the last years, which resulted in remarkable progress culminating in the use of lipoplexes in clinical trials, the in vivo efficiency of these vectors is still unsatisfactory [76; 77]. Such low in vivo efficiency is due to some limitations that are associated to these systems like their poor levels of transfection, particularly when compared to viral vectors, and the considerable reduction of their biological activity by serum components [78]. Moreover, systemic administration of lipoplexes can result in some toxicity, most probably due to their positive charge and propensity to aggregate, which also limits their clinical application [81]. However, the potential advantages of these systems over viral vectors encouraged investigators to further improve their performance by developing novel formulations. In this regard, much effort has been devoted to the synthesis of numerous novel cationic lipids and polymers, which could improve the stability and efficiency of their complexes with nucleic acids; incorporation of hydrophilic components, such as polyethylene glycol (PEG), which could mask the positive charge of the complexes, thus increasing their circulation time in blood stream and reducing toxicity; and association of proteins, antibodies, peptides or other agents that could enhance the biological activity and specificity to target cells [81; 82; 83; 84]. Therefore, it is expected that in a near future the improvements performed in non-viral gene delivery systems will result in the generation of vectors that fulfill the standard requirements for clinical use in terms of efficiency and specificity, namely for systemic administration.

3.3. Cellular vehicles

Various mammalian cells exhibiting tumor-tropism have been recently considered as vehicles for cancer gene therapy. Mesenchymal stem cells or bone marrow stromal cells (MSCs) are adult stem cells with unique immunologic tolerance allowing their engraftment into a xenogeneic environment, while preserving their ability of homing to the tumor sites where they participate in tumor stroma formation (reviewed in, [85]. These features have led to the use of MSCs as cellular vehicles for gene delivery to multiple tumor sites.

Neural stem cells (NSCs) also possess an inherent tumor tropism that supports their use as a reliable delivery vehicle to target therapeutic gene products to primary brain tumors and metastatic cancers throughout the brain. The NSCs have been successfully used to deliver therapeutic gene products to primary and secondary invasive glioma, medulloblastoma, melanoma brain metastases and neuroblastoma throughout the brain and extracerebral loci (reviewed in, [86]). Tumor growth is dependent on angiogenesis and tumor vasculature represents a common target for cancer treatment. Recent evidence suggests that endothelial progenitor, precursor, and blood outgrowth endothelial cells are attracted to the tumor vasculature and could then be used as delivery vector for cancer gene therapy (reviewed in, [87]).

3.4. "Unconventional" vectors

In this section we summarize newly developed vectors including both non-viral biological agents exhibiting natural properties that can be exploited for specific tumor gene delivery andnanovectors that appear as promising tools due to their size and supramolecular structure. Non-viral biological gene delivery vehicles include bacteria, bacteriophages, virus-like particles, erythrocyte ghosts, and exosomes (reviewed in, [88]). Among them, bacterial vectors have been the most extensively studied for cancer gene therapy. Obligate or

facultative anaerobic bacteria such as strains of Clostridia, Bifidobacteria and Salmonellae are able to selectively colonize the hypoxic areas of tumors and destroy the tumor cells, resulting in a bacterial oncolytic therapy [89]. Bacterial vectors can also be modified to deliver bacterially expressed therapeutic proteins and/or plasmid DNA encoding a therapeutic gene or interfering RNA [90]. The most frequently applied anti-cancer approach using bacterial vectors involves systemic administration of bacteria carrying a suicide gene [91; 92; 93] and seven clinical trials using bacterial vectors to treat cancers have been recently reported (Wiley website).

Due to the unique properties of nano-scale matter, nanoparticle-based delivery systems have also emerged as potential gene carriers [94]. These vectors can enhance tumor accumulation of the carried biologically active agent due to the so-called enhanced permeability and retention effect (EPR effect). This effect results from the combination of an increased permeability of tumor blood vessels and a decreased rate of clearance within the tumor [95]. As a consequence, nanocarriers passively accumulate in solid tumors after their intravenous administration. This type of vectors is presently used more often for siRNA than for plasmid DNA delivery.

3.5. Tracing vector biodistribution and transgene expression by non-invasive molecular imaging

Whatever the vector used, monitoring vector biodistribution and transgene expression remains a critical issue in gene therapy protocols. One of the advantages of suicide gene therapy is that the therapeutic gene can also be used as reporter gene for non-invasive imaging to determine the distribution, magnitude and kinetics of vector-mediated gene expression. The most commonly used reporter gene for small-animal molecular imaging studies using radiolabelled probes and positron-emission tomography is HSV-TK [96; 97]. Indeed, like GCV,

radiolabelled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (FHBG) or 2'-fluoro-nucleoside analogues of thymidine such as 1-(2-deoxy-2-fluoro-D-arabinofuranosyl)-5-iodouracil (FIAU) can be used as HSV-TK substrates [98; 99]. Using these approaches, it was demonstrated that radiotracer accumulation, which corresponds to the extent of TK expression, can predict response to therapy [99; 100].

The CD-mediated conversion of 5-FC to 5-FU can also be quantified *in vivo* using magnetic resonance spectroscopy [101]. The same method was used to demonstrate that the therapeutic efficiency is enhanced by combining CD and UPRT, which improves the conversion of 5-FC to toxic metabolites [102]. Altogether, research in this field should lead to a better understanding of suicide gene therapy at the molecular level, allowing improving the efficacy and safety of current clinical protocols.

4. Bystander effects and mechanisms

Suicide gene therapy is associated with two distinct bystander effects. The so-called local bystander effect is known to induce tumor regression although only a fraction of tumor cells express the suicide gene [62; 103]. The second effect, named distant bystander effect, is observed *in vivo* and consists in the regression of WT tumors distant from those expressing the suicide gene [104; 105]. Several hypotheses have been proposed to explain killing of neighbouring untransfected tumor cells (Figure 2): (i) passive diffusion of the drug; (ii) passage of the drug through gap junctions; (iii) endocytosis of apoptotic vesicles; (iv) release of soluble factors; (v) stimulation of the immune sytem *in vivo*. The two bystander effects are described below together with some typical results illustrating their relevance in promoting an antitumoral effect.

4.1. Local bystander effect

One of the main advantages of the CD/5-FC system is the strong local bystander effect that

does not require cell-to-cell contact, since 5-FU can diffuse in and out of cells by non-facilitated diffusion [106]. Experiments conducted in vitro by exposing mixed wild-type (WT) and CD-expressing cells to 5-FC showed that 1-30% of suicide cells could generate sufficient 5-FU to inhibit the growth of the untransfected neighbouring cells [62; 66; 67].

Significant tumor cell killing through the bystander effect has also been observed in vivo in pre-clinical studies. Treatment with 5-FC of nude mice bearing tumor xenografts generated by CD-positive and negative human WiDr colorectal carcinoma cells caused tumour regression even when only 4% of the tumor cells expressed the enzyme-encoding gene [62; 107].

Local bystander effect has also been observed by using cellular vehicles expressing a suicide gene. Mouse embryonic endothelial progenitor cells, which home preferentially to hypoxic lung metastases when administered intravenously, were shown to exert a local bystander effect on lung tumor cells in vitro and in vivo upon CD gene expression followed by 5-FC treatment [108]. Human adipose tissue–derived mesenchymal stem cells (ATMSC) were also used as a vehicle for the CD gene and were able of tumor targeting and growth inhibition after systemic administration and 5-FC treatment [39; 40]. Similarly, multiple transplantations of CD-expressing MSCs in established C6 brain tumors, followed by 5-FC treatment, were able to successfully repress tumor growth [109]. Neural stem cells, which exhibit an extensive tumor tropism, were also shown to be an efficient vehicle for the CD gene in a glioma animal model [110]. Finally, mutant forms of bacterial CD were recently described to significantly improve 5-FC cell sensitization and bystander effect compared with wild-type CD [111].

A significant anti-tumor effect was also induced by GCV in tumors containing 50% HSV TK-negative and positive cells [107]. However, in contrast with FU, GCV triphosphate cannot passively diffuse to neighboring cells and local bystander effect was shown to be mediated by different mechanisms. It was first demonstrated that the bystander effect requires cell-to-cell contact, suggesting the passage of toxic GCV metabolites from TK-expressing cells to unmodified tumor cell [112]. The requirement of gap junctions was then evidenced by

comparing the bystander effect in tumor cell lines transfected or not with connexin genes [113], which was also revealed in human primary malignant glioma cell cultures [114]. In parallel, using flow cytometric and electron microscopic analysis, Freeman *et al*. demonstrated that the bystander effect can also be mediated via apoptotic bodies generated from dying TK-expressing cells and phagocytosed by unmodified neighboring cells [103]. Finally, some studies reported a gap junction-independent local bystander effect mediated by soluble factors, likely corresponding to phosphorylated GCV metabolites [115; 116]. More recently, the expression of E-cadherin, which is involved in the formation and function of gap junctions, was shown to correlate strongly with the TK/GCV bystander effect. The co-expression of TK and E-cadherin genes mediated by an adenoviral vector improved TK/GCV cytotoxicity and triggered a potent anti-tumor effect, superior to the one generated by an adenoviral vector expressing only TK. Moreover, the increased expression of E-cadherin was found to be associated to a decrease in the bcl-2 content, which suggests that a high E-cadherin content improved TK/GCV therapy by both enhancing the bystander effect and facilitating apoptosis induction [117].

Several types of TK-expressing cellular vehicles have also been used to target tumor cells in vivo. Human adipose tissue mesenchymal stromal cells (AMSCs) expressing renilla luciferase and TK were used as cellular autologous delivery vehicles for GCV-mediated bystander killing of firefly luciferase-expressing tumors [118]. By using a non-invasive bioluminescence imaging to continuously monitor both tumor cells and AMSCs, the authors showed a significant bystander killing in mice bearing prostate tumors after treatment with therapeutic AMSCs and GCV [118].

As AMSCs, neural stem cells (NSCs) were also used as a cellular therapeutic delivery system to assess the anti-tumor effect of TK gene in medulloblastoma. In vivo co-implantation of TK-transduced neural stem cells and human medulloblastoma cells (1:8, transduced cells:

non-transduced cells) resulted in tumor growth inhibition and significant prolonged survival of mice, indicating the occurrence of the bystander phenomenon [119].

Co-expression of TK and connexin 43 (Cx43) in bone marrow-derived stem cells (BMSCs), which exhibit tumor tropic properties, enhanced the TK bystander effect and resulted in tumor growth inhibition and increased survival in the rat C6 glioma model [120].

Using bone marrow–derived tumor-infltrating cells (BM-TIcs) expressing TK, Miletic *et al.* demonstrated the induction of a high bystander cell killing mediated by gap junction formation between BM-TIcs and brain tumor cells, which resulted in a strong anti-tumor effect in a rat malignant glioma [121]. In another study, the same authors demonstrated that TK suicide gene transfer using pseudotyped lentiviral vectors was very effective in the treatment of rat glioma. An interesting finding was that the normal brain cells surrounding the tumor, transduced with these vectors survived GCV treatment, due to lower division rate and contributed significantly to the bystander killing of tumor cells. These authors highlighted the fact that high selectivity of gene transfer to tumor cells may not always be required and normal cells, such as brain cells, might contribute to the therapy by long-term expression of therapeutic genes [122].

In a clinical trial, 27 patients undergoing malignant glioma resection were injected with murine retroviral vector producer cells (VPCs) for HSV-TK suicide gene therapy. Local inflammation and devascularisation were the mechanisms responsible for the observed bystander effect in this type of tumor [123].

Finally, it is of interest to note that Wilson *et al.* developed a physiologically relevant tissue culture model for quantifying local bystander effect in vitro by using three-dimensional (3D) multilayer co-cultures of transduced and non-transduced cells. They used nitroreductase (NTR) gene-dependent enzyme-prodrug therapy to show that the local bystander effect was underestimated in 2D-culture and the 3D-culture system appears as a valuable tool to quantify and optimize bystander effects [43].

4.2. Distant bystander effect

The other effect associated to suicide gene therapy, named distant bystander effect, consists in the regression of WT tumors distant from those expressing the suicide gene. Regression of untransduced tumors growing at a distance from transduced tumors is well documented in the TK/GCV system [104; 124; 125; 126; 127]. Several hypotheses have been proposed to explain the TK-related distant anti-tumor effect. The involvement of the immune system was proposed in most studies, based on the presence of T lymphocyte infiltrates within the tumors [104; 125; 126; 127]. However, a distant bystander effect was also detected in immunodeficient SCID mice, which suggests that alternative mechanisms may be operating, such as the release of a soluble factor that might contribute to the observed distant anti-tumor effect [124].

Regarding the CD/5-FC suicide system, we and others provided evidence for the existence of a distant bystander effect, which was observed after injection of CD-expressing tumor cells [68; 105; 128; 129]; or after CD gene delivery via naked DNA injection [130]. Such an immune effect acting on pre-established wild-type tumors was dependent on both CD4+ and CD8+ lymphocytes as well as on natural killer cells [68; 105; 131].

5. **Suicide gene therapy in combination with other therapeutic strategies**

The combination of CD/5-FC and HSV-TK/GCV suicide systems has resulted in enhanced anti-tumor activity *in vitro* [132; 133] and *in vivo* [133]. In this regard, it was shown that sequential prodrug treatment was more efficient than their simultaneous addition as CD/5-FC-mediated reduction of dTTP results in a concurrent decrease of dGTP, which is the endogenous competitor of GCV triphosphate [133].

Suicide gene therapy was also shown to synergistically act with classical anti-cancer treatments such as radiotherapy [134; 135; 136]. This effect was further enhanced by the use

of replication-competent vectors [137; 138; 139] and several clinical trials have demonstrated the potential therapeutic benefit and the safety of this approach [35; 72; 140].

Oncolytic viral vectors armed with a suicide gene were also successfully used in several pre-clinical models [141; 142; 143; 144; 145].

The combination of suicide gene therapy with conventional chemotherapy was also shown to result in a great antitumoral activity, both *in vitro* [146; 147; 148; 149] and *in vivo* [146; 147; 148]. The additional combination with RNAi-based gene therapy was found to be more efficient when compared to each therapy application alone [150]. These authors developed a novel delivery system combining HSV-tk gene with MDR1 shRNA cassette and were able to show enhanced cell sensitivity to anti-cancer drugs [150].

As suicide gene therapy is able to trigger an immune response, various combined strategies have involved the cotransfer of a suicide gene and an immune-stimulatory gene such as cytokine/chemokine genes [28; 151; 152; 153; 154; 155; 156]. As shown in Ambade *et al.*, addition of IL-2 gene delivery in combination with HSV-TK resulted in an improvement of tumor cell apoptosis compared to each strategy *per se* [28]. Another recently developed combined strategy consisted of the association of suicide gene therapy with the targeting of tumor angiogenesis [157; 158; 159; 160; 161]. Chen *et al.* recently showed that delivery of a fusion gene between endostatin and CD followed by 5-FC treatment was more efficient and less toxic than the combination of an antibody against VEGF-A (Bevacizumab) and 5-FU in human breast and colorectal orthotopic animal models [160]**.** Suicide gene therapy is also frequently used in adoptive transfer strategies. This approach, which consists in the transfer of gene-modified T cells, is able to mediate tumor regression in patients with metastatic cancer [162]. However, the adoptive cell therapy may lead to severe autoimmune reaction or to the occurrence of graft-versus-host disease (GvHD), limiting the clinical application of this strategy. In the case of severe GvHD, transfer of a suicide gene into infused T cells appears as a safety switch preserving the antitumoral effect and enabling the destruction of donor T cells.

In this regard, several suicide systems have been used such as HSV-TK/GCV, either alone [163] or associated with other transgenes stimulating alloreactivity [22], CD20/anti-CD20 antibody rituximab [164], and an inducible caspase 9 that is activated using a specific chemical inducer of dimerization [165; 166].

Finally, it is important to mention that nanocarriers have emerged as ideal platforms for achieving multi-functionalization and appear as optimal vectors for application of combined strategies [94]. This is examplified in a study of Li *et al.* describing the use of a nanoplex carrying magnetic resonance imaging reporters for *in vivo* detection and optical reporters for microscopy, to image the delivery of siRNA and a functional prodrug enzyme into breast tumors towards an image-guided molecular targeted cancer therapy [167].

6. Future directions

Recent literature about suicide gene therapy for cancer treatment clearly shows that this field is still under intense investigation. The use of new delivery vectors together with the therapeutic strategies described above have significantly improved the efficiency of suicide gene therapy.

The majority of cancers remain resistant to the current therapeutic options and it recently appeared that CSCs could be one of the key determinants of treatment failure [2; 74]. Therefore, CSCs are now considered as a new promising target for therapeutic approaches aiming at improving the clinical cancer therapy field. CSCs express various molecules protecting them from cytotoxic agents such as ATP-binding cassette (ABC) transporters which actively efflux drugs from cells [168]. In addition, these cells can exhibit a relative quiescence, possess an active DNA-repair capacity and resistance to apoptosis which make them resistant to classical drug-based therapies [169]. However, we can hypothesize that an *in situ* continuous lethal drug production by CSCs themselves should be more effective than the systemic exposure to the drug. Indeed, in a quiescent state, CSCs should behave as drug-producing cells which, within a tumor, should eliminate their differentiated

progeny. Therefore, it is expected that upon division, suicide gene-expressing CSCs will die. Indeed, in a glioma nude rat model generated by CSC spheroids derived from patient biopsies, Huszthy *et al.* recently showed that the TK suicide gene delivered by lentiviral pseudotyped vectors mediated a complete tumor remission [170]. However, Hu *et al.* demonstrated in this same type of cancer that TK suicide gene therapy was less efficient in cancer stem-like cells than in differentiated tumor cells due to the elimination of GCV by ABCG2-mediated efflux [171]. Combined approaches associating suicide gene therapy and the inhibition of CSC-specific properties thus appear as a new challenge for the efficient treatment of cancer.

Acknowledgements

This work was supported by CNRS and PESSOA-EGIDE Program (2010-2011), Portuguese Foundation for Science and Technology and France Cancer

Conflict of interest statement

The authors declare no conflict of interest.

References

[1]L.J. Goldstein, I. Pastan, M.M. Gottesman, Multidrug resistance in human cancer. Crit Rev Oncol Hematol 12 (1992) 243-253.

[2]M.P. Shekhar, Drug resistance: challenges to effective therapy. Curr Cancer Drug Targets 11 (2011) 613-623.

[3]Y.S. Guan, Y. Liu, Q. He, X. Li, L. Yang, Y. Hu, Z. La, p53 gene therapy in combination with transcatheter arterial chemoembolization for HCC: one-year follow-up. World J Gastroenterol 17 (2011) 2143-2149.

[4]B. Sangro, G. Mazzolini, M. Ruiz, J. Ruiz, J. Quiroga, I. Herrero, C. Qian, A. Benito, J. Larrache, C. Olague, J. Boan, I. Penuelas, B. Sadaba, J. Prieto, A phase I clinical trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma. Cancer Gene Ther 17 (2010) 837-843.

[5]H.L. Kaufman, S.D. Bines, OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. Future Oncol 6 (2010) 941-949.

[6]P. Nokisalmi, S. Pesonen, S. Escutenaire, M. Sarkioja, M. Raki, V. Cerullo, L. Laasonen, R. Alemany, J. Rojas, M. Cascallo, K. Guse, M. Rajecki, L. Kangasniemi, E. Haavisto, A. Karioja-Kallio, P. Hannuksela, M. Oksanen, A. Kanerva, T. Joensuu, L. Ahtiainen, A. Hemminki, Oncolytic adenovirus ICOVIR-7 in patients with advanced and refractory solid tumors. Clin Cancer Res 16 (2010) 3035-3043.

[7]D.H. Sterman, A. Recio, A.R. Haas, A. Vachani, S.I. Katz, C.T. Gillespie, G. Cheng, J. Sun, E. Moon, L. Pereira, X. Wang, D.F. Heitjan, L. Litzky, C.H. June, R.H. Vonderheide, R.G. Carroll, S.M. Albelda, A phase I trial of repeated intrapleural adenoviral-mediated interferon-beta gene transfer for mesothelioma and metastatic pleural effusions. Mol Ther 18 (2010) 852-860.

[8]K. Anwer, M.N. Barnes, J. Fewell, D.H. Lewis, R.D. Alvarez, Phase-I clinical trial of IL-12 plasmid/lipopolymer complexes for the treatment of recurrent ovarian cancer. Gene Ther 17 (2010) 360-369.

[9]G. Vassaux, P. Martin-Duque, Use of suicide genes for cancer gene therapy: study of the different approaches. Expert Opin Biol Ther 4 (2004) 519-530.

[10]H.H. Balfour, Jr., Antiviral drugs. N Engl J Med 340 (1999) 1255-1268.

[11]J. Wang, X.X. Lu, D.Z. Chen, S.F. Li, L.S. Zhang, Herpes simplex virus thymidine kinase and ganciclovir suicide gene therapy for human pancreatic cancer. World J Gastroenterol 10 (2004) 400-403.

[12]A. Ketola, A.M. Maatta, T. Pasanen, K. Tulimaki, J. Wahlfors, Osteosarcoma and chondrosarcoma as targets for virus vectors and herpes simplex virus thymidine kinase/ganciclovir gene therapy. Int J Mol Med 13 (2004) 705-710.

[13]C.P. Selby, A. Sancar, Molecular mechanism of transcription-repair coupling. Science 260 (1993) 53-58.

[14]A. Sancar, Mechanisms of DNA excision repair. Science 266 (1994) 1954-1956.

[15]A. Kazantsev, A. Sancar, Does the p53 up-regulated Gadd45 protein have a role in excision repair? Science 270 (1995) 1003-1004; author reply 1005-1006.

[16]P.J. Halloran, R.G. Fenton, Irreversible G2-M arrest and cytoskeletal reorganization induced by cytotoxic nucleoside analogues. Cancer Res 58 (1998) 3855-3865.

[17]S.J. Wei, Y. Chao, Y.M. Hung, W.C. Lin, D.M. Yang, Y.L. Shih, L.Y. Ch'ang, J. Whang-Peng, W.K. Yang, S- and G2-phase cell cycle arrests and apoptosis induced by ganciclovir in murine melanoma cells transduced with herpes simplex virus thymidine kinase. Exp Cell Res 241 (1998) 66-75.

[18]C. Beltinger, S. Fulda, T. Kammertoens, E. Meyer, W. Uckert, K.M. Debatin, Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. Proc Natl Acad Sci U S A 96 (1999) 8699-8704.

[19]M.T. Tomicic, R. Thust, B. Kaina, Ganciclovir-induced apoptosis in HSV-1 thymidine kinase expressing cells: critical role of DNA breaks, Bcl-2 decline and caspase-9 activation. Oncogene 21 (2002) 2141-2153.

[20]U. Fischer, S. Steffens, S. Frank, N.G. Rainov, K. Schulze-Osthoff, C.M. Kramm, Mechanisms of thymidine kinase/ganciclovir and cytosine deaminase/ 5-fluorocytosine suicide gene therapy-induced cell death in glioma cells. Oncogene 24 (2005) 1231-1243.

[21]D. Abate-Daga, L. Garcia-Rodriguez, L. Sumoy, C. Fillat, Cell cycle control pathways act as conditioning factors for TK/GCV sensitivity in pancreatic cancer cells. Biochim Biophys Acta 1803 (2010) 1175-1185.

[22]A. Bondanza, L. Hambach, Z. Aghai, B. Nijmeijer, S. Kaneko, S. Mastaglio, M. Radrizzani, K. Fleischhauer, F. Ciceri, C. Bordignon, C. Bonini, E. Goulmy, IL-7 receptor expression identifies suicide gene-modified allospecific CD8+ T cells capable of self-renewal and differentiation into antileukemia effectors. Blood 117 (2011) 6469-6478.

[23]E.J. Ribot, S. Miraux, J.P. Konsman, V. Bouchaud, L. Pourtau, M.H. Delville, J.M. Franconi, E. Thiaudiere, P.J. Voisin, In vivo MR tracking of therapeutic microglia to a human glioma model. NMR Biomed 24 (2011) 1361-1368.

[24]F.I. Staquicini, M.G. Ozawa, C.A. Moya, W.H. Driessen, E.M. Barbu, H. Nishimori, S. Soghomonyan, L.G. Flores, 2nd, X. Liang, V. Paolillo, M.M. Alauddin, J.P. Basilion, F.B. Furnari, O. Bogler, F.F. Lang, K.D. Aldape, G.N. Fuller, M. Hook, J.G. Gelovani, R.L. Sidman, W.K. Cavenee, R. Pasqualini, W. Arap, Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. J Clin Invest 121 (2011) 161-173.

[25]W. Tang, Y. He, S. Zhou, Y. Ma, G. Liu, A novel Bifidobacterium infantis-mediated TK/GCV suicide gene therapy system exhibits antitumor activity in a rat model of bladder cancer. J Exp Clin Cancer Res 28 (2009) 155.

[26]K. Kakinoki, Y. Nakamoto, T. Kagaya, T. Tsuchiyama, Y. Sakai, T. Nakahama, N. Mukaida, S. Kaneko, Prevention of intrahepatic metastasis of liver cancer by suicide gene therapy and chemokine ligand 2/monocyte chemoattractant protein-1 delivery in mice. J Gene Med 12 (2010) 1002-1013.

[27]L.S. Chen, M. Wang, W.C. Ou, C.Y. Fung, P.L. Chen, C.F. Chang, W.S. Huang, J.Y. Wang, P.Y. Lin, D. Chang, Efficient gene transfer using the human JC virus-like particle that inhibits human colon adenocarcinoma growth in a nude mouse model. Gene Ther 17 (2010) 1033-1041.

[28]A.V. Ambade, G.V. Joshi, R. Mulherkar, Effect of suicide gene therapy in combination with immunotherapy on antitumour immune response & tumour regression in a xenograft mouse model for head & neck squamous cell carcinoma. Indian J Med Res 132 (2010) 415-422.

[29]K. Greish, J. Frandsen, S. Scharff, J. Gustafson, J. Cappello, D. Li, B.W. O'Malley, Jr., H. Ghandehari, Silk-elastinlike protein polymers improve the efficacy of adenovirus thymidine kinase enzyme prodrug therapy of head and neck tumors. J Gene Med 12 (2010) 572-579.

[30]N.G. Rainov, A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 11 (2000) 2389-2401.

[31]J. Voges, R. Reszka, A. Gossmann, C. Dittmar, R. Richter, G. Garlip, L. Kracht, H.H. Coenen, V. Sturm, K. Wienhard, W.D. Heiss, A.H. Jacobs, Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. Ann Neurol 54 (2003) 479-487.

[32]Y. Nasu, T. Saika, S. Ebara, N. Kusaka, H. Kaku, F. Abarzua, D. Manabe, T.C. Thompson, H. Kumon, Suicide gene therapy with adenoviral delivery of HSV-tK gene for patients with local recurrence of prostate cancer after hormonal therapy. Mol Ther 15 (2007) 834-840.

[33]F. Xu, S. Li, X.L. Li, Y. Guo, B.Y. Zou, R. Xu, H. Liao, H.Y. Zhao, Y. Zhang, Z.Z. Guan, L. Zhang, Phase I and biodistribution study of recombinant adenovirus vector-mediated herpes simplex virus thymidine kinase gene and ganciclovir administration in patients with head and neck cancer and other malignant tumors. Cancer Gene Ther 16 (2009) 723-730.

[34]N. Li, J. Zhou, D. Weng, C. Zhang, L. Li, B. Wang, Y. Song, Q. He, D. Lin, D. Chen, G. Chen, Q. Gao, S. Wang, G. Xu, L. Meng, Y. Lu, D. Ma, Adjuvant adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of liver transplantation in patients with advanced hepatocellular carcinoma. Clin Cancer Res 13 (2007) 5847-5854.

[35]S.O. Freytag, B. Movsas, I. Aref, H. Stricker, J. Peabody, J. Pegg, Y. Zhang, K.N. Barton, S.L. Brown, M. Lu, A. Savera, J.H. Kim, Phase I trial of replication-competent adenovirus-mediated suicide gene therapy combined with IMRT for prostate cancer. Mol Ther 15 (2007) 1016-1023.

[36]C. Fillat, M. Carrio, A. Cascante, B. Sangro, Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: fifteen years of application. Curr Gene Ther 3 (2003) 13-26.

[37]F.L. Moolten, J.M. Wells, R.A. Heyman, R.M. Evans, Lymphoma regression induced by ganciclovir in mice bearing a herpes thymidine kinase transgene. Hum Gene Ther 1 (1990) 125-134.

[38]B.E. Huber, C.A. Richards, T.A. Krenitsky, Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. Proc Natl Acad Sci U S A 88 (1991) 8039-8043.

[39]L. Kucerova, V. Altanerova, M. Matuskova, S. Tyciakova, C. Altaner, Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res 67 (2007) 6304-6313.

[40]V. Altanerova, M. Cihova, M. Babic, B. Rychly, K. Ondicova, B. Mravec, C. Altaner, Human adipose tissue-derived mesenchymal stem cells expressing yeast cytosinedeaminase::uracil phosphoribosyltransferase inhibit intracerebral rat glioblastoma. Int J Cancer 130 (2012) 2455-2463.

[41]J.H. Zhou, B. Tang, X.L. Liu, D.W. He, D.T. Yang, hTERT-targeted E. coli purine nucleoside phosphorylase gene/6-methylpurine deoxyribose therapy for pancreatic cancer. Chin Med J (Engl) 120 (2007) 1348-1352.

[42]V.K. Gadi, S.D. Alexander, J.E. Kudlow, P. Allan, W.B. Parker, E.J. Sorscher, In vivo sensitization of ovarian tumors to chemotherapy by expression of E. coli purine nucleoside phosphorylase in a small fraction of cells. Gene Ther 7 (2000) 1738-1743.

[43]W.R. Wilson, S.M. Pullen, A. Hogg, N.A. Helsby, K.O. Hicks, W.A. Denny, Quantitation of bystander effects in nitroreductase suicide gene therapy using three-dimensional cell cultures. Cancer Res 62 (2002) 1425-1432.

[44]P. Patel, J.G. Young, V. Mautner, D. Ashdown, S. Bonney, R.G. Pineda, S.I. Collins, P.F. Searle, D. Hull, E. Peers, J. Chester, D.M. Wallace, A. Doherty, H. Leung, L.S. Young, N.D. James, A phase I/II clinical trial in localized prostate cancer of an adenovirus expressing nitroreductase with CB1954 [correction of CB1984]. Mol Ther 17 (2009) 1292-1299.

[45]D. Farquhar, B.F. Pan, M. Sakurai, A. Ghosh, C.A. Mullen, J.A. Nelson, Suicide gene therapy using E. coli beta-galactosidase. Cancer Chemother Pharmacol 50 (2002) 65-70.

[46]P. Karle, M. Renner, B. Salmons, W.H. Gunzburg, Necrotic, rather than apoptotic, cell death caused by cytochrome P450-activated ifosfamide. Cancer Gene Ther 8 (2001) 220-230.

[47]B.P. Murray, V.G. Zgoda, M.A. Correia, Native CYP2C11: heterologous expression in Saccharomyces cerevisiae reveals a role for vacuolar proteases rather than the proteasome system in the degradation of this endoplasmic reticulum protein. Mol Pharmacol 61 (2002) 1146-1153.

[48]W. Girald, A. Collin, M. Izquierdo, Toxicity and delivery methods for the linamarase/linamarin/glucose oxidase system, when used against human glioma tumors implanted in the brain of nude rats. Cancer Lett 313 (2011) 99-107.

[49]J. Li, H. Li, L. Zhu, W. Song, R. Li, D. Wang, K. Dou, The adenovirus-mediated linamarase/linamarin suicide system: a potential strategy for the treatment of hepatocellular carcinoma. Cancer Lett 289 (2010) 217-227.

[50]M. Dai, J. Liu, D.E. Chen, Y. Rao, Z.J. Tang, W.Z. Ho, C.Y. Dong, Tumor-targeted gene therapy using Adv-AFP-HRPC/IAA prodrug system suppresses growth of hepatoma xenografted in mice. Cancer Gene Ther 19 (2012) 77-83.

[51]Y. Xu, Z. Liu, H. Kong, W. Sun, Z. Liao, F. Zhou, C. Xie, Y. Zhou, Co-expression of interleukin 12 enhances antitumor effects of a novel chimeric promoter-mediated suicide gene therapy in an immunocompetent mouse model. Biochem Biophys Res Commun 412 (2011) 763-768.

[52]D.A. Hamstra, M. Page, J. Maybaum, A. Rehemtulla, Expression of endogenously activated secreted or cell surface carboxypeptidase A sensitizes tumor cells to methotrexate-alpha-peptide prodrugs. Cancer Res 60 (2000) 657-665.

[53]D. Hedley, L. Ogilvie, C. Springer, Carboxypeptidase-G2-based gene-directed enzyme-prodrug therapy: a new weapon in the GDEPT armoury. Nat Rev Cancer 7 (2007) 870-879.

[54]S. Schepelmann, R. Spooner, F. Friedlos, R. Marais, Methods to improve efficacy in suicide gene therapy approaches: targeting prodrug-activating enzymes carboxypeptidase G2 and nitroreductase to different subcellular compartments. Methods Mol Med 90 (2004) 279-301.

[55]C.J. Springer, N.-D. I, Gene-directed enzyme prodrug therapy (GDEPT): choice of prodrugs Advanced Drug Delivery Reviews 22 (1996) 351-364.

[56]L. Negroni, M. Samson, J.M. Guigonis, B. Rossi, V. Pierrefite-Carle, C. Baudoin, Treatment of colon cancer cells using the cytosine deaminase/5-fluorocytosine suicide system induces apoptosis, modulation of the proteome, and Hsp90beta phosphorylation. Mol Cancer Ther 6 (2007) 2747-2756.

[57]E.A. Austin, B.E. Huber, A first step in the development of gene therapy for colorectal carcinoma: cloning, sequencing, and expression of Escherichia coli cytosine deaminase. Mol Pharmacol 43 (1993) 380-387.

[58]F. Kanai, T. Kawakami, H. Hamada, A. Sadata, Y. Yoshida, T. Tanaka, M. Ohashi, K. Tateishi, Y. Shiratori, M. Omata, Adenovirus-mediated transduction of Escherichia coli uracil phosphoribosyltransferase gene sensitizes cancer cells to low concentrations of 5-fluorouracil. Cancer Res 58 (1998) 1946-1951.

[59]C. Richard, W. Duivenvoorden, D. Bourbeau, B. Massie, W. Roa, J. Yau, J. Th'ng, Sensitivity of 5-fluorouracil-resistant cancer cells to adenovirus suicide gene therapy. Cancer Gene Ther 14 (2007) 57-65.

[60]P. Erbs, E. Regulier, J. Kintz, P. Leroy, Y. Poitevin, F. Exinger, R. Jund, M. Mehtali, In vivo cancer gene therapy by adenovirus-mediated transfer of a bifunctional yeast

cytosine deaminase/uracil phosphoribosyltransferase fusion gene. Cancer Res 60 (2000) 3813-3822.

[61]C.A. Mullen, M.M. Coale, R. Lowe, R.M. Blaese, Tumors expressing the cytosine deaminase suicide gene can be eliminated in vivo with 5-fluorocytosine and induce protective immunity to wild type tumor. Cancer Res 54 (1994) 1503-1506.

[62]B.E. Huber, E.A. Austin, C.A. Richards, S.T. Davis, S.S. Good, Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. Proc Natl Acad Sci U S A 91 (1994) 8302-8306.

[63]B.E. Huber, E.A. Austin, S.S. Good, V.C. Knick, S. Tibbels, C.A. Richards, In vivo antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically modified to express cytosine deaminase. Cancer Res 53 (1993) 4619-4626.

[64]A. Ohwada, E.A. Hirschowitz, R.G. Crystal, Regional delivery of an adenovirus vector containing the Escherichia coli cytosine deaminase gene to provide local activation of 5-fluorocytosine to suppress the growth of colon carcinoma metastatic to liver. Hum Gene Ther 7 (1996) 1567-1576.

[65]F. Kanai, K.H. Lan, Y. Shiratori, T. Tanaka, M. Ohashi, T. Okudaira, Y. Yoshida, H. Wakimoto, H. Hamada, H. Nakabayashi, T. Tamaoki, M. Omata, In vivo gene therapy for alpha-fetoprotein-producing hepatocellular carcinoma by adenovirus-mediated transfer of cytosine deaminase gene. Cancer Res 57 (1997) 461-465.

[66]M. Bentires-Alj, A.C. Hellin, C. Lechanteur, F. Princen, M. Lopez, G. Fillet, J. Gielen, M.P. Merville, V. Bours, Cytosine deaminase suicide gene therapy for peritoneal carcinomatosis. Cancer Gene Ther 7 (2000) 20-26.

[67]T. Ichikawa, T. Tamiya, Y. Adachi, Y. Ono, K. Matsumoto, T. Furuta, Y. Yoshida, H. Hamada, T. Ohmoto, In vivo efficacy and toxicity of 5-fluorocytosine/cytosine deaminase gene therapy for malignant gliomas mediated by adenovirus. Cancer Gene Ther 7 (2000) 74-82.

[68]M. Consalvo, C.A. Mullen, A. Modesti, P. Musiani, A. Allione, F. Cavallo, M. Giovarelli, G. Forni, 5-Fluorocytosine-induced eradication of murine adenocarcinomas engineered to express the cytosine deaminase suicide gene requires host immune competence and leaves an efficient memory. J Immunol 154 (1995) 5302-5312.

[69]N. Topf, S. Worgall, N.R. Hackett, R.G. Crystal, Regional 'pro-drug' gene therapy: intravenous administration of an adenoviral vector expressing the E. coli cytosine deaminase gene and systemic administration of 5-fluorocytosine suppresses growth of hepatic metastasis of colon carcinoma. Gene Ther 5 (1998) 507-513.

[70]H.S. Pandha, L.A. Martin, A. Rigg, H.C. Hurst, G.W. Stamp, K. Sikora, N.R. Lemoine, Genetic prodrug activation therapy for breast cancer: A phase I clinical trial of erbB-2-directed suicide gene expression. J Clin Oncol 17 (1999) 2180-2189.

[71]J. Nemunaitis, C. Cunningham, N. Senzer, J. Kuhn, J. Cramm, C. Litz, R. Cavagnolo, A. Cahill, C. Clairmont, M. Sznol, Pilot trial of genetically modified, attenuated Salmonella expressing the E. coli cytosine deaminase gene in refractory cancer patients. Cancer Gene Ther 10 (2003) 737-744.

[72]S.O. Freytag, H. Stricker, J. Pegg, D. Paielli, D.G. Pradhan, J. Peabody, M. DePeralta-Venturina, X. Xia, S. Brown, M. Lu, J.H. Kim, Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer. Cancer Res 63 (2003) 7497-7506.

[73]Y. Lu, C.O. Madu, Viral-based gene delivery and regulated gene expression for targeted cancer therapy. Expert Opin Drug Deliv 7 (2010) 19-35.

[74]J.E. Visvader, G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 8 (2008) 755-768.
[75]R.M. Eager, J. Nemunaitis, Clinical development directions in oncolytic viral therapy. Cancer Gene Ther 18 (2011) 305-317.
[76]S. Kawakami, Y. Higuchi, M. Hashida, Nonviral approaches for targeted delivery of plasmid DNA and oligonucleotide. J Pharm Sci 97 (2008) 726-745.
[77]M.S. Al-Dosari, X. Gao, Nonviral gene delivery: principle, limitations, and recent progress. AAPS J 11 (2009) 671-681.
[78]S. Simoes, A. Filipe, H. Faneca, M. Mano, N. Penacho, N. Duzgunes, M.P. de Lima, Cationic liposomes for gene delivery. Expert Opin Drug Deliv 2 (2005) 237-254.
[79]M.C. Pedroso de Lima, S. Simoes, P. Pires, H. Faneca, N. Duzgunes, Cationic lipid-DNA complexes in gene delivery: from biophysics to biological applications. Adv Drug Deliv Rev 47 (2001) 277-294.
[80]P.L. Felgner, T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, M. Danielsen, Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A 84 (1987) 7413-7417.
[81]M. Morille, C. Passirani, A. Vonarbourg, A. Clavreul, J.P. Benoit, Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. Biomaterials 29 (2008) 3477-3496.
[82]H. Faneca, S. Simoes, M.C. Pedroso de Lima, Association of albumin or protamine to lipoplexes: enhancement of transfection and resistance to serum. J Gene Med 6 (2004) 681-692.
[83]K.S. Kim, Y.K. Lee, J.S. Kim, K.H. Koo, H.J. Hong, Y.S. Park, Targeted gene therapy of LS174 T human colon carcinoma by anti-TAG-72 immunoliposomes. Cancer Gene Ther 15 (2008) 331-340.
[84]S. Neves, H. Faneca, S. Bertin, K. Konopka, N. Duzgunes, V. Pierrefite-Carle, S. Simoes, M.C. Pedroso de Lima, Transferrin lipoplex-mediated suicide gene therapy of oral squamous cell carcinoma in an immunocompetent murine model and mechanisms involved in the antitumoral response. Cancer Gene Ther 16 (2009) 91-101.
[85]Y.L. Hu, Y.H. Fu, Y. Tabata, J.Q. Gao, Mesenchymal stem cells: a promising targeted-delivery vehicle in cancer gene therapy. J Control Release 147 (2010) 154-162.
[86]S.U. Kim, Neural stem cell-based gene therapy for brain tumors. Stem Cell Rev 7 (2011) 130-140.
[87]A.Z. Dudek, Endothelial lineage cell as a vehicle for systemic delivery of cancer gene therapy. Transl Res 156 (2010) 136-146.
[88]Y. Seow, M.J. Wood, Biological gene delivery vehicles: beyond viral vectors. Mol Ther 17 (2009) 767-777.
[89]M.Q. Wei, K.A. Ellem, P. Dunn, M.J. West, C.X. Bai, B. Vogelstein, Facultative or obligate anaerobic bacteria have the potential for multimodality therapy of solid tumours. Eur J Cancer 43 (2007) 490-496.
[90]R. Gardlik, M. Behuliak, R. Palffy, P. Celec, C.J. Li, Gene therapy for cancer: bacteria-mediated anti-angiogenesis therapy. Gene Ther 18 (2011) 425-431.
[91]I. King, D. Bermudes, S. Lin, M. Belcourt, J. Pike, K. Troy, T. Le, M. Ittensohn, J. Mao, W. Lang, J.D. Runyan, X. Luo, Z. Li, L.M. Zheng, Tumor-targeted Salmonella expressing cytosine deaminase as an anticancer agent. Hum Gene Ther 13 (2002) 1225-1233.
[92]F. Friedlos, P. Lehouritis, L. Ogilvie, D. Hedley, L. Davies, D. Bermudes, I. King, J. Martin, R. Marais, C.J. Springer, Attenuated Salmonella targets prodrug activating enzyme carboxypeptidase G2 to mouse melanoma and human breast and colon carcinomas for effective suicide gene therapy. Clin Cancer Res 14 (2008) 4259-4266.

[93]C.M. Cheng, Y.L. Lu, K.H. Chuang, W.C. Hung, J. Shiea, Y.C. Su, C.H. Kao, B.M. Chen, S. Roffler, T.L. Cheng, Tumor-targeting prodrug-activating bacteria for cancer therapy. Cancer Gene Ther 15 (2008) 393-401.
[94]L. Jabr-Milane, L. van Vlerken, H. Devalapally, D. Shenoy, S. Komareddy, M. Bhavsar, M. Amiji, Multi-functional nanocarriers for targeted delivery of drugs and genes. J Control Release 130 (2008) 121-128.
[95]A.K. Iyer, G. Khaled, J. Fang, H. Maeda, Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today 11 (2006) 812-818.
[96]Y. Waerzeggers, P. Monfared, T. Viel, A. Winkeler, J. Voges, A.H. Jacobs, Methods to monitor gene therapy with molecular imaging. Methods 48 (2009) 146-160.
[97]D. Abate-Daga, N. Andreu, J. Camacho-Sanchez, R. Alemany, R. Herance, O. Millan, C. Fillat, Oncolytic adenoviruses armed with thymidine kinase can be traced by PET imaging and show potent antitumoural effects by ganciclovir dosing. PLoS One 6 (2011) e26142.
[98]A. Jacobs, J. Voges, R. Reszka, M. Lercher, A. Gossmann, L. Kracht, C. Kaestle, R. Wagner, K. Wienhard, W.D. Heiss, Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas. Lancet 358 (2001) 727-729.
[99]I. Penuelas, G. Mazzolini, J.F. Boan, B. Sangro, J. Marti-Climent, M. Ruiz, J. Ruiz, N. Satyamurthy, C. Qian, J.R. Barrio, M.E. Phelps, J.A. Richter, S.S. Gambhir, J. Prieto, Positron emission tomography imaging of adenoviral-mediated transgene expression in liver cancer patients. Gastroenterology 128 (2005) 1787-1795.
[100]S.S. Yaghoubi, J.R. Barrio, M. Namavari, N. Satyamurthy, M.E. Phelps, H.R. Herschman, S.S. Gambhir, Imaging progress of herpes simplex virus type 1 thymidine kinase suicide gene therapy in living subjects with positron emission tomography. Cancer Gene Ther 12 (2005) 329-339.
[101]L.D. Stegman, A. Rehemtulla, B. Beattie, E. Kievit, T.S. Lawrence, R.G. Blasberg, J.G. Tjuvajev, B.D. Ross, Noninvasive quantitation of cytosine deaminase transgene expression in human tumor xenografts with in vivo magnetic resonance spectroscopy. Proc Natl Acad Sci U S A 96 (1999) 9821-9826.
[102]D.A. Hamstra, K.C. Lee, J.M. Tychewicz, V.D. Schepkin, B.A. Moffat, M. Chen, K.J. Dornfeld, T.S. Lawrence, T.L. Chenevert, B.D. Ross, J.T. Gelovani, A. Rehemtulla, The use of 19F spectroscopy and diffusion-weighted MRI to evaluate differences in gene-dependent enzyme prodrug therapies. Mol Ther 10 (2004) 916-928.
[103]S.M. Freeman, C.N. Abboud, K.A. Whartenby, C.H. Packman, D.S. Koeplin, F.L. Moolten, G.N. Abraham, The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 53 (1993) 5274-5283.
[104]W. Bi, Y.G. Kim, E.S. Feliciano, L. Pavelic, K.M. Wilson, Z.P. Pavelic, P.J. Stambrook, An HSVtk-mediated local and distant antitumor bystander effect in tumors of head and neck origin in athymic mice. Cancer Gene Ther 4 (1997) 246-252.
[105]V. Pierrefite-Carle, P. Baque, A. Gavelli, M. Mala, M. Chazal, J. Gugenheim, A. Bourgeon, G. Milano, P. Staccini, B. Rossi, Cytosine deaminase/5-fluorocytosine-based vaccination against liver tumors: evidence of distant bystander effect. J Natl Cancer Inst 91 (1999) 2014-2019.
[106]B.A. Domin, W.B. Mahony, T.P. Zimmerman, Transport of 5-fluorouracil and uracil into human erythrocytes. Biochem Pharmacol 46 (1993) 503-510.
[107]Q.T. Trinh, E.A. Austin, D.M. Murray, V.C. Knick, B.E. Huber, Enzyme/prodrug gene therapy: comparison of cytosine deaminase/5-fluorocytosine versus thymidine kinase/ganciclovir enzyme/prodrug systems in a human colorectal carcinoma cell line. Cancer Res 55 (1995) 4808-4812.
[108]J. Wei, S. Blum, M. Unger, G. Jarmy, M. Lamparter, A. Geishauser, G.A. Vlastos, G. Chan, K.D. Fischer, D. Rattat, K.M. Debatin, A.K. Hatzopoulos, C. Beltinger,

Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery. Cancer Cell 5 (2004) 477-488.

[109]D.Y. Chang, S.W. Yoo, Y. Hong, S. Kim, S.J. Kim, S.H. Yoon, K.G. Cho, S.H. Paek, Y.D. Lee, S.S. Kim, H. Suh-Kim, The growth of brain tumors can be suppressed by multiple transplantation of mesenchymal stem cells expressing cytosine deaminase. Int J Cancer 127 (2010) 1975-1983.

[110]K.S. Aboody, A. Brown, N.G. Rainov, K.A. Bower, S. Liu, W. Yang, J.E. Small, U. Herrlinger, V. Ourednik, P.M. Black, X.O. Breakefield, E.Y. Snyder, Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 97 (2000) 12846-12851.

[111]M. Fuchita, A. Ardiani, L. Zhao, K. Serve, B.L. Stoddard, M.E. Black, Bacterial cytosine deaminase mutants created by molecular engineering show improved 5-fluorocytosine-mediated cell killing in vitro and in vivo. Cancer Res 69 (2009) 4791-4799.

[112]W.L. Bi, L.M. Parysek, R. Warnick, P.J. Stambrook, In vitro evidence that metabolic cooperation is responsible for the bystander effect observed with HSV tk retroviral gene therapy. Hum Gene Ther 4 (1993) 725-731.

[113]A.A. Elshami, A. Saavedra, H. Zhang, J.C. Kucharczuk, D.C. Spray, G.I. Fishman, K.M. Amin, L.R. Kaiser, S.M. Albelda, Gap junctions play a role in the 'bystander effect' of the herpes simplex virus thymidine kinase/ganciclovir system in vitro. Gene Ther 3 (1996) 85-92.

[114]T. Asklund, I.B. Appelskog, O. Ammerpohl, I.A. Langmoen, M.S. Dilber, A. Aints, T.J. Ekstrom, P.M. Almqvist, Gap junction-mediated bystander effect in primary cultures of human malignant gliomas with recombinant expression of the HSVtk gene. Exp Cell Res 284 (2003) 185-195.

[115]F. Princen, P. Robe, C. Lechanteur, M. Mesnil, J.M. Rigo, J. Gielen, M.P. Merville, V. Bours, A cell type-specific and gap junction-independent mechanism for the herpes simplex virus-1 thymidine kinase gene/ganciclovir-mediated bystander effect. Clin Cancer Res 5 (1999) 3639-3644.

[116]R.R. Drake, K. Pitlyk, R.A. McMasters, K.E. Mercer, H. Young, M.P. Moyer, Connexin-independent ganciclovir-mediated killing conferred on bystander effect-resistant cell lines by a herpes simplex virus-thymidine kinase-expressing colon cell line. Mol Ther 2 (2000) 515-523.

[117]L. Garcia-Rodriguez, D. Abate-Daga, A. Rojas, J.R. Gonzalez, C. Fillat, E-cadherin contributes to the bystander effect of TK/GCV suicide therapy and enhances its antitumoral activity in pancreatic cancer models. Gene Ther 18 (2011) 73-81.

[118]M. Vilalta, I.R. Degano, J. Bago, E. Aguilar, S.S. Gambhir, N. Rubio, J. Blanco, Human adipose tissue-derived mesenchymal stromal cells as vehicles for tumor bystander effect: a model based on bioluminescence imaging. Gene Ther 16 (2009) 547-557.

[119]K. Pu, S.Y. Li, Y. Gao, L. Ma, W. Ma, Y. Liu, Bystander effect in suicide gene therapy using immortalized neural stem cells transduced with herpes simplex virus thymidine kinase gene on medulloblastoma regression. Brain Res 1369 (2011) 245-252.

[120]Q. Huang, X.Z. Liu, C.S. Kang, G.X. Wang, Y. Zhong, P.Y. Pu, The anti-glioma effect of suicide gene therapy using BMSC expressing HSV/TK combined with overexpression of Cx43 in glioma cells. Cancer Gene Ther 17 (2010) 192-202.

[121]H. Miletic, Y. Fischer, S. Litwak, T. Giroglou, Y. Waerzeggers, A. Winkeler, H. Li, U. Himmelreich, C. Lange, W. Stenzel, M. Deckert, H. Neumann, A.H. Jacobs, D. von Laer, Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. Mol Ther 15 (2007) 1373-1381.

[122]H. Miletic, Y.H. Fischer, T. Giroglou, M.A. Rueger, A. Winkeler, H. Li, U. Himmelreich, W. Stenzel, A.H. Jacobs, D. von Laer, Normal brain cells contribute to

the bystander effect in suicide gene therapy of malignant glioma. Clin Cancer Res 13 (2007) 6761-6768.

[123]F.W. Floeth, N. Shand, H. Bojar, H.B. Prisack, J. Felsberg, E. Neuen-Jacob, A. Aulich, K.J. Burger, W.J. Bock, F. Weber, Local inflammation and devascularization--in vivo mechanisms of the "bystander effect" in VPC-mediated HSV-Tk/GCV gene therapy for human malignant glioma. Cancer Gene Ther 8 (2001) 843-851.

[124]M.S. Dilber, M.R. Abedi, B. Bjorkstrand, B. Christensson, G. Gahrton, K.G. Xanthopoulos, C.I. Smith, Suicide gene therapy for plasma cell tumors. Blood 88 (1996) 2192-2200.

[125]A.R. Kianmanesh, H. Perrin, Y. Panis, M. Fabre, H.J. Nagy, D. Houssin, D. Klatzmann, A "distant" bystander effect of suicide gene therapy: regression of nontransduced tumors together with a distant transduced tumor. Hum Gene Ther 8 (1997) 1807-1814.

[126]K.M. Wilson, P.J. Stambrook, W.L. Bi, Z.P. Pavelic, L. Pavelic, J.L. Gluckman, HSV-tk gene therapy in head and neck squamous cell carcinoma. Enhancement by the local and distant bystander effect. Arch Otolaryngol Head Neck Surg 122 (1996) 746-749.

[127]M.X. Wei, P. Bougnoux, B. Sacre-Salem, M.B. Peyrat, C. Lhuillery, J.L. Salzmann, D. Klatzmann, Suicide gene therapy of chemically induced mammary tumor in rat: efficacy and distant bystander effect. Cancer Res 58 (1998) 3529-3532.

[128]V. Pierrefite-Carle, A. Gavelli, N. Brossette, P. Baque, D. Benchimol, A. Bourgeon, P. Staccini, B. Rossi, Re: Cytosine deaminase/5-fluorocytosine-based vaccination against liver tumors: evidence of distant bystander effect. J Natl Cancer Inst 92 (2000) 494-495.

[129]A. Khatri, B. Zhang, E. Doherty, J. Chapman, K. Ow, H. Pwint, R. Martiniello-Wilks, P.J. Russell, Combination of cytosine deaminase with uracil phosphoribosyl transferase leads to local and distant bystander effects against RM1 prostate cancer in mice. J Gene Med 8 (2006) 1086-1096.

[130]P. Baque, V. Pierrefite-Carle, A. Gavelli, N. Brossette, D. Benchimol, A. Bourgeon, P. Staccini, M.C. Saint-Paul, B. Rossi, Naked DNA injection for liver metastases treatment in rats. Hepatology 35 (2002) 1144-1152.

[131]S. Bertin, S. Neves, A. Gavelli, P. Baque, N. Brossette, S. Simoes, M.C. Pedroso de Lima, V. Pierrefite-Carle, Cellular and molecular events associated with the antitumor response induced by the cytosine deaminase/5-fluorocytosine suicide gene therapy system in a rat liver metastasis model. Cancer Gene Ther 14 (2007) 858-866.

[132]X.R. Luo, J.S. Li, Y. Niu, L. Miao, Targeted killing effects of double CD and TK suicide genes controlled by survivin promoter on gastric cancer cell. Mol Biol Rep 38 (2010) 1201-1207.

[133]P.D. Boucher, M.M. Im, S.O. Freytag, D.S. Shewach, A novel mechanism of synergistic cytotoxicity with 5-fluorocytosine and ganciclovir in double suicide gene therapy. Cancer Res 66 (2006) 3230-3237.

[134]J. Xia, K. Xia, Y. Feng, A. Tang, Y. Tang, L. Wu, D. Liang, F. Cai, Q. Pan, Z. Long, H. Dai, Y. Zhang, S. Zhao, Z. Chen, The combination of suicide gene therapy and radiation enhances the killing of nasopharyngeal carcinoma xenographs. J Radiat Res (Tokyo) 45 (2004) 281-289.

[135]K.R. Rogulski, J.H. Kim, S.H. Kim, S.O. Freytag, Glioma cells transduced with an Escherichia coli CD/HSV-1 TK fusion gene exhibit enhanced metabolic suicide and radiosensitivity. Hum Gene Ther 8 (1997) 73-85.

[136]J. Liu, H. Harada, M. Ogura, T. Shibata, M. Hiraoka, Adenovirus-mediated hypoxia-targeting cytosine deaminase gene therapy enhances radiotherapy in tumour xenografts. Br J Cancer 96 (2007) 1871-1878.

[137]K.R. Rogulski, M.S. Wing, D.L. Paielli, J.D. Gilbert, J.H. Kim, S.O. Freytag, Double suicide gene therapy augments the antitumor activity of a replication-competent lytic

adenovirus through enhanced cytotoxicity and radiosensitization. Hum Gene Ther 11 (2000) 67-76.
[138]S.O. Freytag, K.R. Rogulski, D.L. Paielli, J.D. Gilbert, J.H. Kim, A novel three-pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene, and radiotherapy. Hum Gene Ther 9 (1998) 1323-1333.
[139]S.O. Freytag, K.N. Barton, S.L. Brown, V. Narra, Y. Zhang, D. Tyson, C. Nall, M. Lu, M. Ajlouni, B. Movsas, J.H. Kim, Replication-competent adenovirus-mediated suicide gene therapy with radiation in a preclinical model of pancreatic cancer. Mol Ther 15 (2007) 1600-1606.
[140]S.O. Freytag, H. Stricker, J. Peabody, J. Pegg, D. Paielli, B. Movsas, K.N. Barton, S.L. Brown, M. Lu, J.H. Kim, Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer. Mol Ther 15 (2007) 636-642.
[141]S. Leveille, S. Samuel, M.L. Goulet, J. Hiscott, Enhancing VSV oncolytic activity with an improved cytosine deaminase suicide gene strategy. Cancer Gene Ther 18 (2011) 435-443.
[142]J.D. Dias, I. Liikanen, K. Guse, J. Foloppe, M. Sloniecka, I. Diaconu, V. Rantanen, M. Eriksson, T. Hakkarainen, M. Lusky, P. Erbs, S. Escutenaire, A. Kanerva, S. Pesonen, V. Cerullo, A. Hemminki, Targeted chemotherapy for head and neck cancer with a chimeric oncolytic adenovirus coding for bifunctional suicide protein FCU1. Clin Cancer Res 16 (2010) 2540-2549.
[143]S. Chalikonda, M.H. Kivlen, M.E. O'Malley, X.D. Eric Dong, J.A. McCart, M.C. Gorry, X.Y. Yin, C.K. Brown, H.J. Zeh, 3rd, Z.S. Guo, D.L. Bartlett, Oncolytic virotherapy for ovarian carcinomatosis using a replication-selective vaccinia virus armed with a yeast cytosine deaminase gene. Cancer Gene Ther 15 (2008) 115-125.
[144]K. Hiraoka, T. Kimura, C.R. Logg, C.K. Tai, K. Haga, G.W. Lawson, N. Kasahara, Therapeutic efficacy of replication-competent retrovirus vector-mediated suicide gene therapy in a multifocal colorectal cancer metastasis model. Cancer Res 67 (2007) 5345-5353.
[145]M. Ahn, S.J. Lee, X. Li, J.A. Jimenez, Y.P. Zhang, K.H. Bae, Y. Mohammadi, C. Kao, T.A. Gardner, Enhanced combined tumor-specific oncolysis and suicide gene therapy for prostate cancer using M6 promoter. Cancer Gene Ther 16 (2009) 73-82.
[146]W.X. Hu, Z.J. Zeng, S.Q. Luo, Q. Chen, Suicide gene therapy of human breast cancer in SCID mice model by the regulation of Tet-On. Chin Med J (Engl) 117 (2004) 434-439.
[147]J. Martinez-Quintanilla, M. Cascallo, A. Gros, C. Fillat, R. Alemany, Positive selection of gene-modified cells increases the efficacy of pancreatic cancer suicide gene therapy. Mol Cancer Ther 8 (2009) 3098-3107.
[148]K. Hayashi, J.B. Lee, Y. Maitani, N. Toyooka, H. Nemoto, T. Hayashi, The role of a HSV thymidine kinase stimulating substance, scopadulciol, in improving the efficacy of cancer gene therapy. J Gene Med 8 (2006) 1056-1067.
[149]H. Faneca, A. Faustino, M.C. Pedroso de Lima, Synergistic antitumoral effect of vinblastine and HSV-Tk/GCV gene therapy mediated by albumin-associated cationic liposomes. J Control Release 126 (2008) 175-184.
[150]S.Y. Park, W. Lee, J. Lee, I.S. Kim, Combination gene therapy using multidrug resistance (MDR1) gene shRNA and herpes simplex virus-thymidine kinase. Cancer Lett 261 (2008) 205-214.
[151]M. Toda, R.L. Martuza, S.D. Rabkin, Combination suicide/cytokine gene therapy as adjuvants to a defective herpes simplex virus-based cancer vaccine. Gene Ther 8 (2001) 332-339.
[152]A. Khatri, Y. Husaini, K. Ow, J. Chapman, P.J. Russell, Cytosine deaminase-uracil phosphoribosyltransferase and interleukin (IL)-12 and IL-18: a multimodal anticancer

interface marked by specific modulation in serum cytokines. Clin Cancer Res 15 (2009) 2323-2334.
[153]S.H. Chen, X.H. Chen, Y. Wang, K. Kosai, M.J. Finegold, S.S. Rich, S.L. Woo, Combination gene therapy for liver metastasis of colon carcinoma in vivo. Proc Natl Acad Sci U S A 92 (1995) 2577-2581.
[154]X. Cao, X. Huang, D.W. Ju, W. Zhang, H. Hamada, J. Wang, Enhanced antitumoral effect of adenovirus-mediated cytosine deaminase gene therapy by induction of antigen-presenting cells through stem cell factor/granulocyte-macrophage colony-stimulating factor gene transfer. Cancer Gene Ther 7 (2000) 177-186.
[155]J.H. Zhang, M.X. Wan, B.R. Pan, B. Yu, Cytotoxicity of HSVtk and hrTNF-alpha fusion genes with IRES in treatment of gastric cancer. Cancer Biol Ther 3 (2004) 1075-1080.
[156]A.K. Ghulam Muhammad, M. Candolfi, G.D. King, K. Yagiz, D. Foulad, Y. Mineharu, K.M. Kroeger, K.A. Treuer, W.S. Nichols, N.S. Sanderson, J. Yang, M. Khayznikov, N. Van Rooijen, P.R. Lowenstein, M.G. Castro, Antiglioma immunological memory in response to conditional cytotoxic/immune-stimulatory gene therapy: humoral and cellular immunity lead to tumor regression. Clin Cancer Res 15 (2009) 6113-6127.
[157]T.J. Harvey, I.M. Hennig, S.D. Shnyder, P.A. Cooper, N. Ingram, G.D. Hall, P.J. Selby, J.D. Chester, Adenovirus-mediated hypoxia-targeted gene therapy using HSV thymidine kinase and bacterial nitroreductase prodrug-activating genes in vitro and in vivo. Cancer Gene Ther 18 (2011) 773-784.
[158]A. Leng, J. Yang, T. Liu, J. Cui, X.H. Li, Y. Zhu, T. Xiong, Y. Chen, Nanoparticle-delivered VEGF-silencing cassette and suicide gene expression cassettes inhibit colon carcinoma growth in vitro and in vivo. Tumour Biol 32 (2011) 1103-1111.
[159]D. Sha, Y.J. He, W.B. Wang, J.Q. Han, [Anti-nasopharyngeal carcinoma effect in vivo and in vitro of Avastin and adenovirus-thymidine kinase suicide gene]. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 42 (2007) 526-532.
[160]C.T. Chen, H. Yamaguchi, H.J. Lee, Y. Du, H.H. Lee, W. Xia, W.H. Yu, J.L. Hsu, C.J. Yen, H.L. Sun, Y. Wang, E.T. Yeh, G.N. Hortobagyi, M.C. Hung, Dual targeting of tumor angiogenesis and chemotherapy by endostatin-Cytosine deaminase-uracil phosphoribosyltransferase. Mol Cancer Ther 10 (2011) 1327-1336.
[161]T. Liu, L. Ye, Y. He, X. Chen, J. Peng, X. Zhang, H. Yi, F. Peng, A. Leng, Combination gene therapy using VEGF-shRNA and fusion suicide gene yCDglyTK inhibits gastric carcinoma growth. Exp Mol Pathol 91 (2011) 745-752.
[162]N.P. Restifo, M.E. Dudley, S.A. Rosenberg, Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12 (2012) 269-281.
[163]C. Traversari, S. Marktel, Z. Magnani, P. Mangia, V. Russo, F. Ciceri, C. Bonini, C. Bordignon, The potential immunogenicity of the TK suicide gene does not prevent full clinical benefit associated with the use of TK-transduced donor lymphocytes in HSCT for hematologic malignancies. Blood 109 (2007) 4708-4715.
[164]M. Griffioen, E.H. van Egmond, M.G. Kester, R. Willemze, J.H. Falkenburg, M.H. Heemskerk, Retroviral transfer of human CD20 as a suicide gene for adoptive T-cell therapy. Haematologica 94 (2009) 1316-1320.
[165]A. Di Stasi, S.K. Tey, G. Dotti, Y. Fujita, A. Kennedy-Nasser, C. Martinez, K. Straathof, E. Liu, A.G. Durett, B. Grilley, H. Liu, C.R. Cruz, B. Savoldo, A.P. Gee, J. Schindler, R.A. Krance, H.E. Heslop, D.M. Spencer, C.M. Rooney, M.K. Brenner, Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med 365 (2011) 1673-1683.
[166]V. Hoyos, B. Savoldo, C. Quintarelli, A. Mahendravada, M. Zhang, J. Vera, H.E. Heslop, C.M. Rooney, M.K. Brenner, G. Dotti, Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia 24 (2010) 1160-1170.

[167]C. Li, M.F. Penet, F. Wildes, T. Takagi, Z. Chen, P.T. Winnard, D. Artemov, Z.M. Bhujwalla, Nanoplex delivery of siRNA and prodrug enzyme for multimodality image-guided molecular pathway targeted cancer therapy. ACS Nano 4 (2010) 6707-6716.

[168]M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance. Nat Rev Cancer 5 (2005) 275-284.

[169]C.Y. Park, D. Tseng, I.L. Weissman, Cancer stem cell-directed therapies: recent data from the laboratory and clinic. Mol Ther 17 (2009) 219-230.

[170]P.C. Huszthy, T. Giroglou, O. Tsinkalovsky, P. Euskirchen, K.O. Skaftnesmo, R. Bjerkvig, D. von Laer, H. Miletic, Remission of invasive, cancer stem-like glioblastoma xenografts using lentiviral vector-mediated suicide gene therapy. PLoS One 4 (2009) e6314.

[171]W. Hu, W. Liu, Side populations of glioblastoma cells are less sensitive to HSV-TK/GCV suicide gene therapy system than the non-side population. In Vitro Cell Dev Biol Anim 46 (2010) 497-501.

Legends to figures

Figure 1. Suicide gene therapy. The introduction into tumor cells of a viral or a bacterial gene leads to the expression of an enzyme able to convert a non-toxic prodrug into a lethal drug.

Figure 2. Different mechanisms of local bystander effect. Several hypotheses have been proposed to explain killing of neighbouring untransfected tumor cells, including passive diffusion or passage of the drug through gap junctions, endocytosis of apoptotic vesicles, release of soluble factors and stimulation of the immune system *in vivo*.

Figure 1

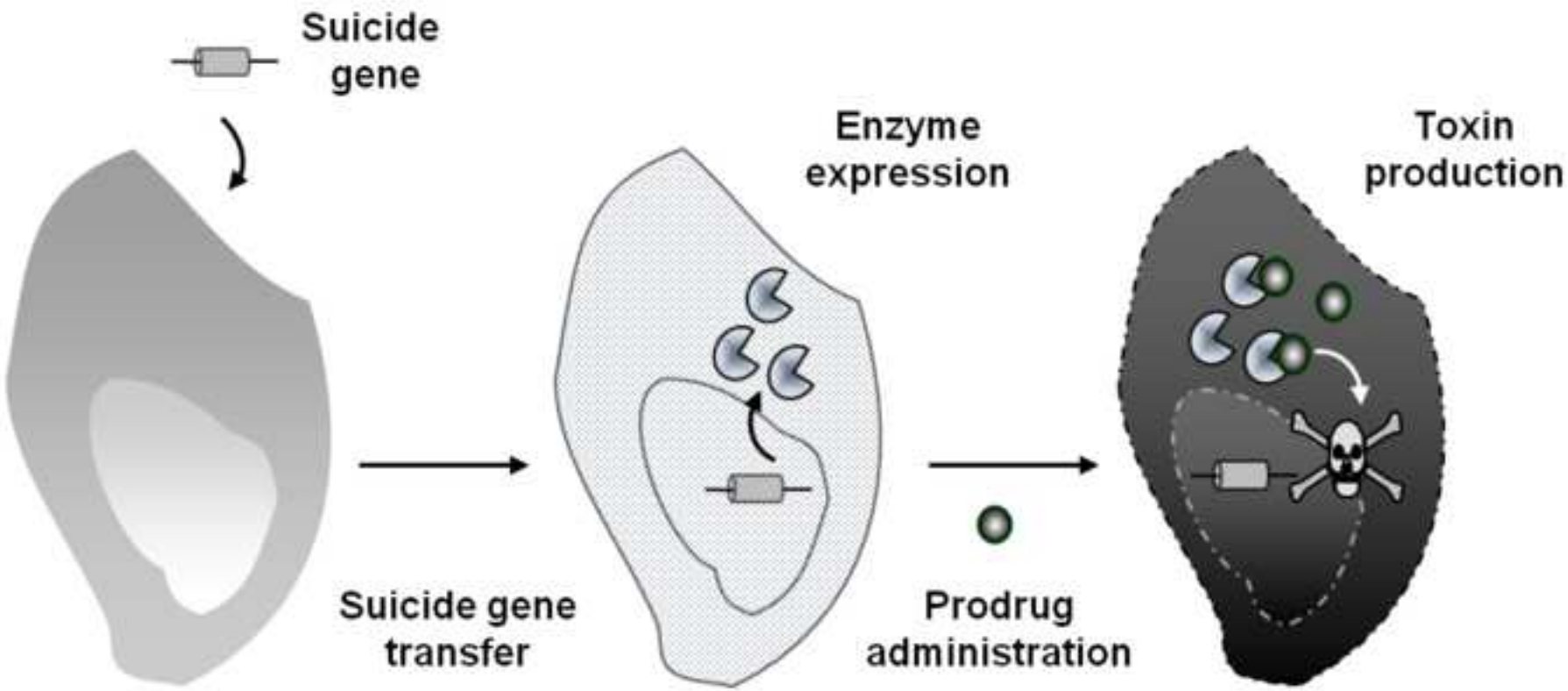
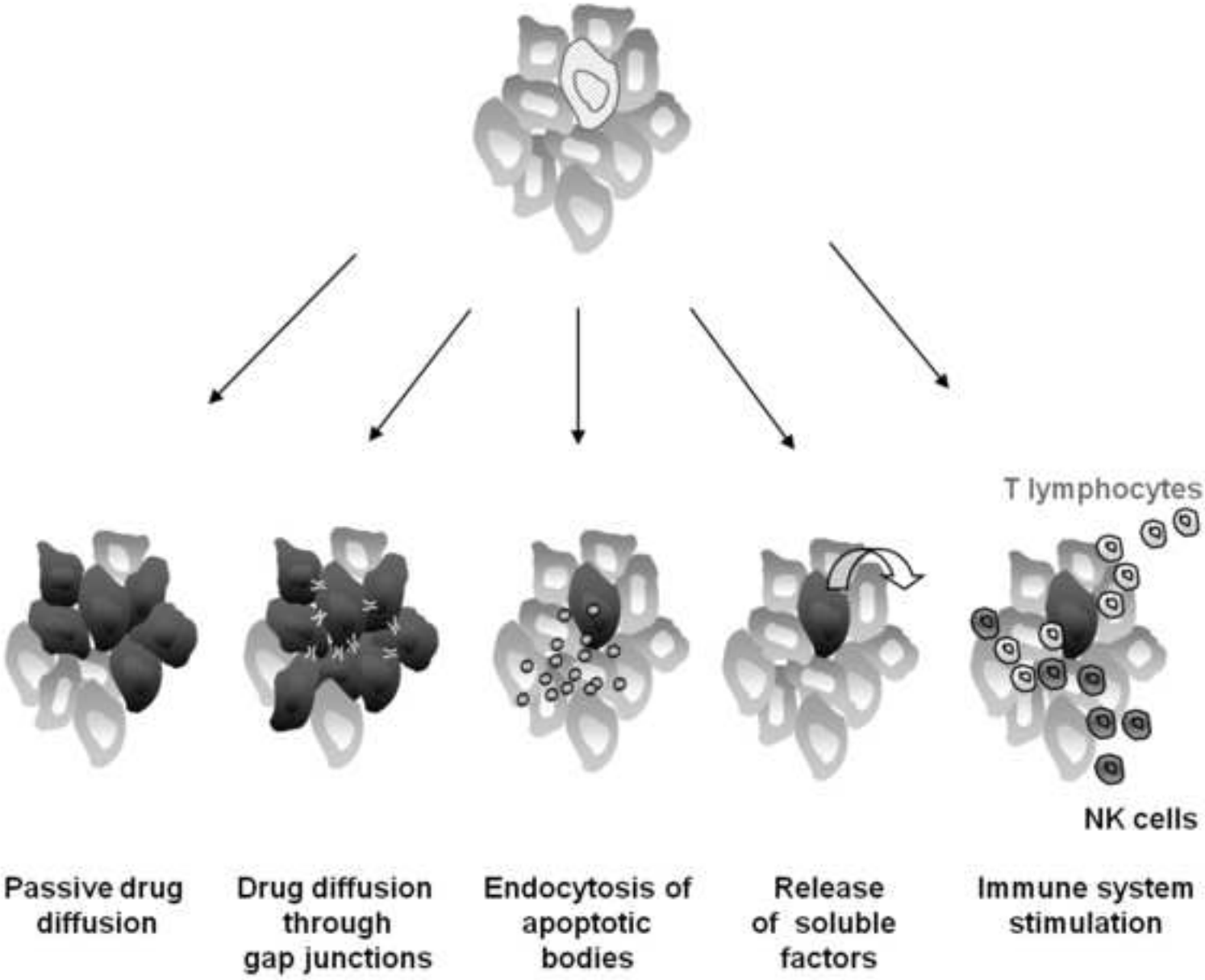


Figure 2



Abstract

Suicide gene therapy is based on the introduction into tumor cells of a viral or a bacterial gene, which allows the conversion of a non-toxic compound into a lethal drug. Although suicide gene therapy has been successfully used in a large number of in vitro and in vivo studies, its application to cancer patients has not reached the desirable clinical significance. However, recent reports on pre-clinical cancer models demonstrate the huge potential of this strategy when used in combination with new therapeutic approaches. In this review, we summarize the different suicide gene systems and gene delivery vectors addressed to cancer, with particular emphasis on recently developed systems and associated bystander effects. In addition, we review the different strategies that have been used in combination with suicide gene therapy and provide some insights into the future directions of this approach, particularly towards cancer stem cell eradication.