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Oncolytic Activity of Bacteria used in Cancerous Disease Gene Therapy

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Abstract

Gene therapy is a therapeutic strategy based on using genes as pharmaceuticals. Gene therapy holds promise for treating a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS. Various types of genetic material are used in gene therapy; double-strained DNA (dsDNA), single strained DNA (ssDNA), plasmid DNA and antisense oligodeoxynucleotides (ASON), adenoviruses, retroviruses, undeveloped/ plasmid DNA and bacteria. The use of bacteria in cancer therapy can be advantageous for various reasons compared to classic chemotherapy or other microorganisms. Bacteria can adhere and invade tumor cells, and they are capable of proliferation and of establishing extracellular colonies. Other than that, their genome length enables them to be recipient to a quantum of exogenous therapeutic genes (for example, enzymes activating precursors and cytokines). The most important thing from the clinical safety view is they can be killed by antibiotics (such as metronidazole) if complications in further treatment arise. For comparison, the capacity of viral vectors is limited and in case of side effects viruses cannot be eliminated by antibiotics.

Index terms— gene therapy, salmonella spp., clostridium spp., therapeutic strategy.

1 I. Introduction

he use of bacteria in cancer therapy can be advantageous for various reasons compared to classic chemotherapy or other microorganisms, such as vectors on the basis of viruses used in gene therapy. Several bacterial species are motile and have the capability of active movement against the diffuse gradient pressure built up in the abnormal environment of a tumor. On the other hand, small molecules of medicaments or viruses are dependent on streaming for them to disseminate in the tumor. For this reason, interstitial pressure in tumors limits their penetration (1). Bacteria can adhere and invade tumor cells, and they are capable of proliferation and of establishing extracellular colonies. Other than that, their genome length enables them to be recipient to a quantum of exogenous therapeutic genes (for example, enzymes activating precursors and cytokines). The most important thing from the clinical safety view is they can be killed by antibiotics (such as metronidazole) if complications in further treatment arise. For comparison, the capacity of viral vectors is limited and in case of side effects viruses cannot be eliminated by antibiotics (2). Clostridia: several studies from half of the 20 th century (3) shown that Gram-positive anaerobic Clostridia can proliferate in hypoxic or necrotic tissues in tumor regions and so oncolytic means for cancer treatment were proved. Clostridia are spore-forming anaerobic bacteria which must be injected to a patient in the form of spores. These spores migrate to the localization of the tumor and are capable of budding only in anoxic environment (note: this type of environment is present in large tumors; 2).

One of the first strains tested as an anti-cancer agent is Clostridium histolyticum. A direct injection of spores to mice sarcomas induced a visible tumor regression and lysis. Simultaneous microscopic examination of these bacteria proliferating inside a tumor revealed a presence of an extremely virulent strain of Clostridium tetania few years later. Despite their ability to diminish tumors, these species invoked high toxicity after injection,

causing quick death in tumor-bearing mice (4). Scientists decided to change the strain and used non-pathogenic Clostridium butyricum M55, its non-pathogenic character speeding the start of clinical studies (5). In 1967 Carey carried out a small experiment with conclusions variating from: without tumor lysis, with tumor lysis and even death (6). Roughly in the same time, for the benefits of amplifying effectiveness of Clostridium, scientist started to combine bacteria with numerous agents, such as heavy metals and classical chemotherapy (7). Many similar researches were carried out later in the 70‘s (8; 9). Dang et al., examined many species targeted at tumors, of which two showed promising effects (10). An ability of targeting the tumor and disseminating in it was found in Clostridium novyi and Clostridium sordellii. Other than that, these strains were capable of evenly inducing the destruction of surrounding tissue. Despite this, no surprise was the effectiveness of the clostridia led to the death of all animals with tumors. The authors of the experiment had the suspicion, that this toxicity could have been a consequence of toxin secretion. It is commonly known clostridia hold anumber of potentially dangerous genes for toxins. For this reason, Clostridium novyi was selected for the purpose of later studies, and was attenuated by elimination of the gene coding the lethal NT toxin from its genome. This new strain preserved its capability of targeting the tumor and was still capable of destroying live tumor cells in the proximity of their growth. For the amplification of therapeutic effectiveness, Dang used several chemotherapeutic medications in co-operation with Clostridium novyi (10). The association of C. novyi with classical chemotherapy brought extreme tumor regression. This type of therapy was named ”combination bacteriolytic therapy” (COBALT). Later in vivo experiments on a vast scale of tumor cell-lines shown C. novyi potentiates the effect of standard radiation modes (11). It was explained lately, that C. novyi -NT can be uses as a tool for liposome lysis initiation and can help in liposomal distribution of therapeutic substances to tumors (12). In the clinical study Roberts et al (2014) use volunteers with C. novyi-NT.C. novyi-NT has been shown in preclinical settings to have excellent tumor colonizing properties (13). Roberts et al. use non-armed C. novyi-NT bacteria, and it is the specific proteolytic nature of the strain that, once germinated, induces tumor necrosis. Previous studies showed that a single dose of C. novyi-NT spores injected intravenously in syngeneic tumor-bearing animals often led to localized tumor necrosis and oncolysis, leading to cures in up to one-third of treated animals, without excessive toxicity (14,15)].Strains such as C. sporogenes also have inherent anti-tumor effects as a consequence of proteolysis, but to a lesser extent, and significant efficacy improvement can be obtained by arming these bugs with additional therapeutic genes. Most studies with armed clostridia have however been performed with so-called prodrug converting enzymes (PCE). Such PCE can convert a non-toxic prodrug into a chemotherapeutic agent (16). Since the PCE is only expressed within the tumor where clostridia reside, the conversion also only takes place locally within the tumor, thereby avoiding the side effects commonly occurring following systemic therapy. In addition, most of these prodrug/PCE combinations are characterized by a potent bystander effect as the converted prodrug can diffuse from the site of conversion towards non-exposed neighbouring cells within its vicinity. The proof-of-principle of this approach has been shown with PCE expressed from a plasmid (17,18) and more importantly, recently also with a nitroreductase PCE stably integrated into the chromosome (19). Salmonella: Salmonellae are Gram-negative, facultative anaerobes growing in oxygen-rich conditions as well as oxygen-deficient.

When wild type Salmonella typhimurium is injected in mice, Salmonellae disseminate in the organism and reach high concentrations in the liver (20). Although animals eventually died of organ failure, there was an apparent presence of bacteria in tumors. This observation led scientists to studying the use of Salmonella for therapeutic usage against cancer (2).

The modified Salmonella typhimurium strain for the uses of cancer therapy was designed at the turn of the century by Vion Pharmaceuticals, Icn. S. typhimurium (ATCC 14028) was attenuated in sequence leading to the birth of strain YS1646 (commertial designation VNP20009; 21). This strain was deficient in purine synthesis, which forced the bacteria to use an external source of purines for them to survive. Purine deficiency had two consequences. First, the bacteria became partially attenuated, second, as was observed in mice, proliferation in normal tissues was inhibited, while the capability of proliferation in tumors was preserved. After previous atenuation, the gene coding msbB was removed from the bacterial genome (21). The msbB protein catalyzes the addition of the terminal myristoyl group to lipid A. Lipid A is a component of the lipopolysacharides (LPS) found in Gram-negative bacteria, including E. coli and Salmonella. During infection, lipid A stimulates the production of cytokines as TNF-?, leading to inflammation and toxic shock. It was proved even earlier, that mutations in the gene coding msbB limited the capability of Salmonella to invoke disease, but not its ability to target tumors (22). Toxicity trials after VNP20009 application to mice, rats and small monkeys proved their safe character. This conclusion was verified in the first phase of clinical testing on volunteers (23).

Anti-tumor qualities of strain VNP20009 were also found. It was shown this strain is effective against a vast scale of tumors, as well as against some metastatic lesions (24). But the mechanism of tumor suppression induced by Salmonella has still not been explained. One study points to specific genes linked with pathogenicity more than to genes connected with the invasive character of the bacteria (25). However, this theory is in a contrary to evidence of the attenuated Salmonella not being directly toxic to tumor cells (26).

Another study shows to the immune system, which can play a key role in tumor suppression. Local inflammatory reactions in subsequence to a large bacterial count in the localization of the tumor were documented. Histological examinations of tumors in mice with B16 melanoma tumor-bearings shown massive neutrophil infiltration as a result of Salmonella application. The bacteria alone can lead to tumor suppression, as was

proved in tests on mice with neutrophil depletion (27). More, there is evidence supporting that bacteria can induce toxicity by nitric oxide production specifically in the location of the tumor (28). Besides the mentioned, other bacteria-mediated tumor regression mechanisms were found, for example, toxin secretion and direct competition for nutrition with the tumor cells (2).

2 Volume XVI Issue III Version I

3 Bacteria as gene transport systems

One of the problems connected to the use of bacteria as anti-cancer tools is the toxicity of bacteria in therapeutic dosage. This applies in individual application or in combination with radiation or chemotherapy (10). Reduction of the dosage significantly reduces the toxicity as well as their effect. Some bacteria, such as probiotic bifidobacteria or nonpathogenic bacteria, for example E.coli Dh5a can effectively colonize tumors, but they do not have any therapeutic effect due to their non-pathogenic character (2).

The process overcoming both of these limiting factors is to "arm" bacteria with protein coding genes, which can induce cytotoxicity. This provides the therapeutic potential to harmless strains and amplifies effectiveness in more toxic strains. The advantage of this is that in clinical practice a lower and therefore a safer dose of bacteria can be administer do the patient, lowering the systemic toxicity, but maintaining the therapeutic effectiveness in the tumor location (2). A progress in development of Clostridia and Salmonella strains as non-modified and autonomous anti-cancer pharmaceuticals is expected. In the meantime, many other bacterial strains were developed as tumor interfering agents (29).Some of them are attenuated and some are naturally harmless, as non-pathogenic anaerobic Gram-positive bifidobacteria, belonging to a group of bacteria commonly introduced as lactic acid bacteria or probiotic bacteria, which live in symbiosis in lower parts of the small intestine in humans and other mammals (2).

4 Bacteria-directed enzyme/prodrug therapy

Bacteria-directed enzyme/prodrug therapy (BDEPT) is found on a process of amplifying effectiveness of bacterial vectors and it reduces therapeutic doses. This procedure uses bacteria for the delivery of the enzyme to the tumor bearings, and involves "arming" bacteria with genes coding an enzyme for transforming the prodrug (that does not have a human homologue and/or has a better enzyme kinetics as a similar human enzyme). BDEPT is a two step therapy. In the first step, the "armed" vector is administered to the patient and it targets specifically in the tumor location, where the enzyme is expressed. In the second step, as soon as the level of enzyme expression is optimal, the predrug is administered and converted by the expressed enzymes to a cytotoxic medicament directly in the tumor location. This leads to a tumor-selective cytotoxicity (2).

There are numbers of homologous therapeutic strategies similar to BDEPT. Antibody-directed enzyme/ prodrug therapy (ADEPT) was designed for the first time more than 20 years ago (30,31). It is based on extracellular targeting of tumor antigens by monoclonal antibodies, chemically connected to a purified predrug-converting enzyme. Many ADEPT systems are being studied; some of them underwent clinical studies (32). Virus-directed enzyme/prodrug therapy (VDEPT) has shown itself as a promising therapeutic method in preclinical and clinical testing (33). Another similar therapy is Polymer-directed enzyme/prodrug therapy (PDEPT; 34), Ligand-directed enzyme/prodrug therapy (35), Melanocyte-directed enzyme/prodrug therapy (MDEPT; 36), and precursor monotherapy (37). The broad term Gene directed enzyme/prodrug therapy (GDEPT) includes all strategies on the principle of gene expression of the precursor-converting enzymes in tumor cells (38). One of the most widely described GDEPT systems became the combination of a Herpes Simplex Virus-tymidine-kinase (HSV-tk) nucleoside analog and it dates to the 1980‘s (39). The distribution of genes coding HSV-tkin vivo was achieved with the use of many vectors, for example: retroviruses, adenoviruses and liposomes (40). In BDEPT method and other precursor-converting methods, the medicament is created in situ as a consequence of intervention with the tumor. This grants many advantages with comparison with conventional procedures. High tumor selectivity is achieved, because the precursor is converted only inside the tumor, which reduces side effects in other organs. An amplifying effect is created as a result of the capability of one therapeutic molecule enzyme to activate many prodrug molecules. This leads to high concentrations of active medicament in the location of the tumor. A "bystander effect" is occurring, defined as a capability of bacterial cells to express enzymes stimulating the killing of cells in the proximity of tumor cells not expressing the enzyme. For this reason, bacteria can group to colonies in the stroma of the tumor and they do not need to attack cancer cells for the successful eradication/regression of the tumor (38).

5 II. Conclusion

In BDEPT the aiming of bacteria to the targeted structures is based on the physical rather than biochemical characteristics of the tumor; nonpathogenic bacteria not toxic for the host can be used; there is a large number of molecular biology techniques using bacteria and they have relatively few obstacles in bacterial gene expression; it is possible to avoid every potential trangene toxicity (which could occur for reasons of striking outside of targeted structures), because genes are enclosed in the bacteria; serum components can‘t inhibit enzymes protected by bacterial membranes and cell wall; there is a collection of cofactors as NADH and NADPH which can be used

by therapeutic enzymes needing reductive environment; bacteria can be, in difference to viruses, relatively easily reduced in size or modified for clinical uses.

One important difference between BDEPT and other bacterial therapies is, BDEPT uses constitutively toxic genes (for example Salmonella), in BDEPT expressing the apoptotic cytokine Fas ligand the toxicity is controlled and induced after prodrug administration, while in other types of bacterial therapy can be toxic subsequent to injecting to the patient. Systemic toxicity can be induced mostly in the case of bacteria secreting the therapeutic protein. Beside this, bacteria carrying therapeutic genes under the control of eukaryotic promoters can cause problems if the vector targets healthy cells, outcomming as "non-target toxicity". In ideal cases, BDEPT could be combined with imaging technique, so workers in clinical practice could correctly evaluate the aiming to target structures and decide ahead the application of the prodrug.

6 Volume XVI Issue III Version I

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[Strahlentherapie ()] , Strahlentherapie . 1977. 153 p. .

[Org. Biomol. Chem ()] , *Org. Biomol. Chem* 2005. 3 (21) p. .

[Spooner et al. ()] 'A novel vascular endothelial growth factor-directed therapy that selectively activates cytotoxic prodrugs'. R A Spooner , F Friedlos , K Maycroft , S M Stribbling , J Roussel , J Brueggen , B Stolz , T O'reilly , J Wood , A Matter , R Marais , C J Springer . *Br. J. Cancer* 2003. 88 (10) p. .

[Bagshawe ()] 'Antibody directed enzymes revive anti-cancer prodrugs concept'. K D Bagshawe . *Br. J. Cancer* 1987. 56 (5) p. .

[Bagshawe ()] 'Antibody-directed enzyme/prodrug therapy (ADEPT)'. K D Bagshawe . *Biochem. Soc. Trans* 1990. 18 (5) p. . (Review)

[Carey et al. ()] 'Association of cancer of the breast and acute myelocytic leukemia'. R W Carey , J F Holland , P R Sheehe , S Graham . *Cancer* 1967. 20 (7) p. .

[Lehouritis et al. ()] 'Bacterialdirected enzyme prodrug therapy'. P Lehouritis , C Springer , M Tangney . *J Control. Release* 2013. 170 (1) p. .

[Avogadri F 1 et al. ()] 'Cancer immuno-therapy based on killing of Salmonella-infected tumor cells'. Avogadri F 1 , C Martinoli , L Petrovska , C Chiodoni , P Transidico , V Bronte , R Longhi , M P Colombo , G Dougan , M Rescigno . *Cancer Res* 2005. 65 (9) p. .

[Minton et al. ()] 'Chemotherapeutic tumour targeting using clostridial spores'. N P Minton , M L Mauchline , M J Lemmon , J K Brehm , M Fox , N P Michael , A Giaccia , J M Brown . *FEMS Microbiol. Rev* 1995. 17 p. .

[Theys and Lambin ()] 'Clostridium to treat cancer: dream or reality? Ann'. J Theys , P Lambin . *Transl. Med* 2015. (1) p. S21. (Suppl)

[Dang et al. ()] 'Combination bacteriolytic therapy for the treatment of experimental tumors'. L H Dang , C Bettegowda , D L Huso , K W Kinzler , B Vogelstein . *Proc. Natl. Acad. Sci. USA* 2001. (26) p. .

[Low et al. ()] 'Construction of VNP20009: a novel, genetically stable antibioticsensitive strain of tumor-targeting Salmonella for parenteral administration in humans'. K B Low , M Ittensohn , X Luo , L M Zheng , I King , J M Pawelek , D Bermudes . *Methods Mol. Med* 2004. 90 p. .

[Westphal et al. ()] 'Containment of tumor-colonizing bacteria by host neutrophils'. K Westphal , S Leschner , J Jablonska , H Loessner , S Weiss . *Cancer Res* 2008. (8) p. .

[Tietze et al. ()] 'Duocarmycin-based prodrugs for cancer prodrug monotherapy'. L F Tietze , H J Schuster , K Schmuck , I Schuberth , F Alves . *Bioorg. Med. Chem* 2008. (12) p. .

[Gericke et al. ()] 'Further progress with oncolysis due to apathogenic clostridia'. D Gericke , F Dietzel , W König , I Rüster , L Schumacher . *Zentralbl Bakteriol Orig A* 1979. 243 (1) p. .

[Niculescu-Duvaz et al. ()] 'Gene-directed enzyme prodrug therapy'. I Niculescu-Duvaz , R Spooner , R Marais , C J Springer . *Bioconjug. Chem* 1998. 9 (1) p. . (Review)

[Finzi et al. ()] 'Improved retroviral suicide gene transfer in colon cancer celll ines after cell synchronization with methotrexate'. L Finzi , A Kraemer , C Capron , S Noullet , D Goere , C Penna , B Nordlinger , J Legagneux , J F Emile , R Malafosse . *J. Exp. Clin. Cancer. Res* 2011. p. 92.

[Dietzel and Gericke] *Intensification of the oncolysis by clostridia by means of radio-frequency hyperthermy in experiments on animals–dependence on dosage and on intervals*, F Dietzel , D Gericke .

[Roberts et al. (20146)] 'Intratumoral injection of Clostridium novyi-NT spores induces antitumor responses'. N J Roberts , L Zhang , F Janku , A Collins , R Y Bai , V Staedtke , A W Rusk , D Tung , M Miller , J Roix , K V Khanna , R Murthy , R S Benjamin , T Helgason , A D Szvalb , J E Bird , S Roy-Chowdhuri , H H Zhang , Y Qiao , B Karim , J Mcdaniel , A Elpiner , A Sahora , J Lachowicz , B Phillips , A Turner , M K Klein , G Post , L A DiazJr , G J Riggins , N Papadopoulos , K W Kinzler , B Vogelstein , C Bettegowda , D L Huso , M Varterasian , S Saha , S Zhou . *Sci. Transl. Med* 20146. p. .

[Low et al. ()] 'Lipid A mutant Salmonella with suppressed virulence and TNFalpha induction retain tumor-targeting in vivo'. K B Low , M Ittensohn , T Le , J Platt , S Sodi , M Amoss , O Ash , E Carmichael , A Chakraborty , J Fischer , S L Lin , X Luo , S I Miller , L Zheng , I King , J M Pawelek , D Bermudes . *Nat. Biotechnol* 1999. 17 (1) p. .

[Malmgren and Flanigan ()] 'Localization of the vegetative form of Clostridium tetani in mouse tumors following intravenous spore administration'. R A Malmgren , C C Flanigan . *Cancer Res* 1955. (7) p. .

[Jain and Baxter ()] 'Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure'. R K Jain , L T Baxter . *Cancer Res* 1988. 48 p. .

[Knaggs et al.] *New prodrugs derived from 6-aminodopamine and 4-aminophenol as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT)*, S Knaggs , H Malkin , H M Osborn , N A Williams , P Yaqoob .

[Gericke and Engelbart ()] 'Oncolysis by clostridia. Ii. Experiments on a tumor spectrum with a variety of clostridia in combination with heavy metal'. D Gericke , K Engelbart . *Cancer Res* 1964. 24 p. .

[Engelbart and Gericke ()] 'Oncolysis by clostridia. V. Transplanted tumors of the hamster'. K Engelbart , D Gericke . *Cancer res* 1964. 24 p. .

[Liu et al. ()] 'Optimized clostridium-directed enzyme prodrug therapy improves the antitumor activity of the novel DNA cross-linking agent PR-104'. S C Liu , G O Ahn , M Kioi . *Cancer Res* 2008. 68 p. .

[Bettegowda et al. ()] 'Overcoming the hypoxic barrier to radiation therapy with anaerobic bacteria'. C Bettegowda , L H Dang , R Abrams , D L Huso , L Dillehay , I Cheong , N Agrawal , S Borzillary , J M Mccaffery , E L Watson , K S Lin , F Bunz , K Baidoo , M G Pomper , K W Kinzler , B Vogelstein , S Zhou . *Proc. Natl. Acad. Sci. USA* 2003. 100 (25) p. .

[Satchi-Fainaro et al. ()] 'PDEPT: polymer-directed enzyme prodrug therapy. 2. HPMA copolymer-betalactamase and HPMA copolymer-C-Dox as a model combination'. R Satchi-Fainaro , H Hailu , J W Davies , C Summerford , R Duncan . *Bioconjug Chem* 2003. 14 (4) p. .

[Diaz et al. ()] *Pharmacologic and toxicologic evaluation of C. novyi-NT spores. Toxicological sciences: an official journal of the Society of Toxicology*, L A Diaz , Jr , I Cheong , C A Foss , X Zhang , B A Peters , N Agrawal , C Bettegowda , B Karim , G Liu , K Khan , X Huang , M Kohli , L H Dang . 2005. 88 p. .

[Toso et al. ()] 'Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma'. J F Toso , V J Gill , P Hwu , F M Marincola , N P Restifo , D J Schwartzentruber , R M Sherry , S L Topalian , J C Yang , F Stock , L J Freezer , K E Morton , C Seipp , L Haworth , S Mavroukakis , D White , S Macdonald , J Mao , M Sznol , S A Rosenberg . *J. Clin. Oncol* 2002. 20 (1) p. .

[Tietze and Schmuck ()] 'Prodrugs for targeted tumor therapies: recent developments in ADEPT, GDEPT and PMT'. L F Tietze , K Schmuck . *Curr. Pharm. Des* 2011. 17 (32) p. . (Review)

[Lukashev et al. ()] 'Recombination in circulating Human enterovirus B: independent evolution of structural and non-structural genome regions'. A N Lukashev , V A Lashkevich , O E Ivanova , G A Koroleva , A E Hinkkanen , J Ilonen . *J. Gen. Virol* 2005. 86 p. .

[Theys et al. ()] 'Repeated cycles of Clostridium-directed enzyme prodrug therapy resultin sustained antitumour effects in vivo'. J Theys , O Pennington , L Dubois . *Br. J. Cancer* 2006. 95 p. .

[Barak et al. ()] 'Role of nitric oxide in Salmonella typhimurium-mediated cancer cell killing'. Y Barak , F Schreiber , S H Thorne , C H Contag , D Debeer , A Matin . *BMC Cancer* 2010. p. 146.

[Pawelek et al. ()] 'Salmonella pathogenicity island-2 and anticancer activity in mice'. J M Pawelek , S Sodi , A K Chakraborty , J T Platt , S Miller , D W Holden , M Hensel , K B Low . *Cancer Gene Ther* 2002. 9 (10) p. .

[Heap et al. ()] 'Spores of Clostridium engineered for clinical efficacy and safety cause regression and cure of tumors in vivo'. J T Heap , J Theys , M Ehsaan . *Oncotarget* 2014. 5 p. .

[Dang et al. ()] 'Targeting vascular and avascular compartments of tumors with C. novyi-NT and antimicrotubule agents'. L H Dang , C Bettegowda , N Agrawal , I Cheong , D Huso , P Frost , F Loganzo , L Greenberger , J Barkoczy , G R Pettit , Smith , H Gurulingappa , S Khan . *Cancer biology & therapy* 2004. 3 (3) p. .

[Zheng et al. ()] 'Tumor amplified protein expression therapy: Salmonella as a tumor-selective protein delivery vector'. L M Zheng , X Luo , M Feng , Z Li , T Le , M Ittensohn , M Trailsmith , D Bermudes , S L Lin , I C King . *Oncol. Res* 2000. 12 (3) p. .

[Moolten ()] 'Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy'. F L Moolten . *Cancer Res* 1986. 46 (10) p. .

[Cheong and Zhou ()] 'Tumor-specific liposomal drug release mediated by liposomase'. I Cheong , S Zhou . *Methods. Enzymol* 2009. 465 p. .

[Bermudes et al. ()] 'Tumor-targeted Salmonella. Highly selective delivery vectors'. D Bermudes , B Low , J Pawelek . *Adv. Exp. Med. Biol* 2000. 465 p. .

[Morrissey et al. ()] 'Tumour targeting with systemically administered bacteria'. D Morrissey , G C O'sullivan , M Tangney . *Curr. Gene. Ther* 2010. 10 (1) p. . (Review)