Gene Therapy (2006) 13, 101–105

www.nature.com/gt

*REVIEW*

# *Bacteria in gene therapy: bactofection* versus *alternative gene therapy*

R Pálffy[1,2], R Gardlík[1,2], J Hodosy[1,3,4], M Behuliak[1,2], P Reško[1,2], J Radvánský[1,2] and P Celec[1,2,3]
[1]*BiomeD Research and Publishing Group, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic;* [2]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic;* [3]*Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic and* [4]*Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*

*Recent advances in gene therapy can be attributed to improvements of gene delivery vectors. New viral and nonviral transport vehicles that considerably increase the efficiency of transfection have been prepared. However, these vectors still have many disadvantages that are difficult to overcome, thus, a new approach is needed. The approach of bacterial delivery could in the future be important for gene therapy applications. In this article we try to summarize the most important modifications that are used for the preparation of applied strains, difficulties that are related with bacterial gene delivery and the current use of bactofection in animal experiments and clinical trials. Important differences to the alternative gene therapy (AGT) are discussed. AGT resembles bacteria-mediated protein delivery, as the therapeutical proteins are produced not by host cells but by the bacteria* in situ *and the expression can be regulated exogenously. Although the procedure of bacterial gene delivery is far from being definitely solved, bactofection remains a promising technique for transfection in human gene therapy.*
Gene Therapy (2006) **13,** 101–105. doi:10.1038/sj.gt.3302635; published online 15 September 2005

**Keywords:** *bactofection; alternative gene therapy; gene delivery; transfection*

## Introduction

Current gene therapy belongs to the most perspective therapeutical approach not only against monogenic diseases, but also cancer, ischemic heart disease and other polygenic diseases.[1] Despite the recent drawback in enthusiasm caused by the side effects in first clinical applications, gene therapy has the potential to cure and save the lives of many patients.[2] The basic principle of gene therapy lies in the delivery of a nucleic acid (a functional gene copy or an oligonucleotide) affecting the expression of a target gene in the desired location of the patient body. To achieve this goal, delivery vectors for the gene transfer are needed. Viral vectors derived from retroviruses, adenoviruses, poxviruses, parvoviruses and herpesviruses belong to the most frequently used.[3] However, naked plasmid DNA or its combination with compounds increasing the efficiency of cell membrane penetration (cationic lipids, lipoplexes etc.) can be also used, especially for short-term applications.[4] Another option is the use of bacterial delivery systems called bactofection.[5] Every vector system has its own strengths and weaknesses that are summarized in Table 1.

## What is bactofection?

The technique using bacteria for the direct gene transfer into the target organism, organ or tissue is called bactofection (Figure 1; Table 2). The basic idea is nowadays celebrating its 25th birthday.[6] Transformed bacterial strains deliver the genes localized on plasmids into the cells, where these genes can be expressed as a therapeutical gene product. The delivery process might involve intracellular localization of the bacteria, but gene delivery from extracellularly localized bacteria was also reported via conjugational apparatus of *Agrobacterium tumefaciens*.[7] Bacteria used for bactofection should not be pathogenic. Plasmids contain sequences that are needed for the transcription and translation of the transferred genes. Bacteria-mediated transfer of plasmid DNA into mammalian cells (bactofection) is a potent approach to express plasmid-encoded heterologous proteins (protein antigens, hormones, toxins or enzymes) in a large set of different cell types including phagocytic and nonphagocytic mammalian cells.

The main problem of bactofection is the possibility of unwanted side effects related to the host–bacteria interactions. The response of the immune system might cause rapid clearance of bacteria or even autoimmune reactions. On contrary, the bacterial strains can acquire the virulence factors back and might cause serious infections. Therefore, to reduce the risk of clinically symptomatic infections to minimum, the bacteria are genetically modified. Moreover, most of the used strains contain a suicide gene that eases bacterial destruction. During the destruction of vectors, plasmids are released and delivered into the nucleus. Of course, the products of the lysis are released from the cells and they can induce immune system activation. Thus, the bacteria are attenuated not to produce superantigens. However, the use and application of complex systems, for example, bacterial cultures into a mammal, are

Correspondence: Dr R Pálffy, Malé Dvorníky 162, Dunajská Streda 929 01, Slovak Republic.
E-Mail: palffyroli@yahoo.co.uk
Received 24 March 2005; revised 7 May 2005; accepted 1 August 2005; published online 15 September 2005

always accompanied with unpredictable side effects that can be studied seriously only in large randomized clinical trials.

The main advantages of bactofection are the simplicity of application[8–11] and the selectivity of gene transfer, which can be improved in the future by modulating, for example, organ-specific activity.[12]

## What is alternative gene therapy?

Another interesting approach that might be used in gene therapy is the so-called alternative gene therapy (AGT).[13] Bacteria are not used for the gene transfer but should persist in the target tissues. Persisting bacteria produce the therapeutical polypeptide *in situ*, thus, this technique resembles bacterial protein delivery (Figure 2; Table 3). In comparison to classic gene therapy using gene transfer into the mammalian cells AGT offers the extremely important possibility of gene expression regulation using low molecular weight inductors of expression dependent on the used expression system. If needed, the therapy can always be stopped. Bacteria can be eliminated using antibiotics as their resistance spectrum is defined. This negative regulation cannot be performed in classic gene therapy or bactofection. The use of antibiotics in bactofection or suicide genes (e.g. tymidine kinase) in viral vectors can increase the safety of delivery, but it does not affect the expression of therapeutic genes itself. AGT can be improved using experience from bactofection experiments since the transport of bacteria in the organism does not differ significantly among these two methods. Although bactofection and AGT share some characteristics such as their side effects and other similarities, the key difference lies in the expression of the desired gene. In bactofection, the transgene is expressed in the eukaryotic host cell; in AGT bacteria are the producer of the therapeutical peptide.

**Table 1** The comparison of main vector types in selected parameters[3]

| | *Viral vectors* | *Nonviral vectors* | *Bacterial vectors* |
|---|---|---|---|
| Safety | + | +++ | + |
| Efficiency | +++ | + | + |
| Low production costs | + | ++ | +++ |
| Simple production | + | ++ | +++ |
| Simple delivery | ++ | + | +++ |
| Amount of delivered DNA | ++ | + | +++ |

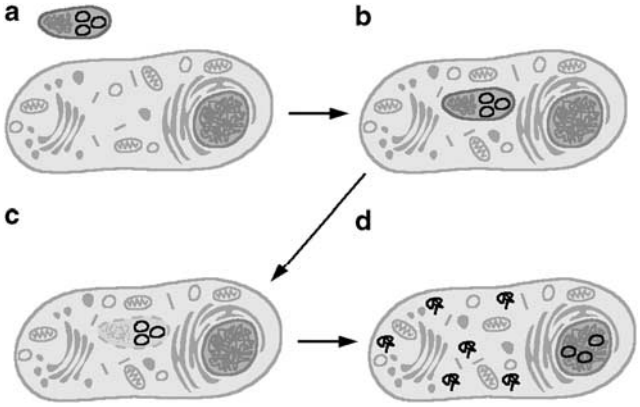


**Figure 1** Bactofection: bacteria are used as a vehicle/vector to transport the genetic information into the eukaryotic cell. (**a**) Transformed bacteria that contain plasmids carrying the transgene are applicated into the target tissue. (**b**) Genetically engineered bacteria penetrate into the cells. (**c**) Vectors are destructed or undergo lysis induced by their presence in the cytoplasm. (**d**) The released plasmids get into the nucleus and the therapeutical transgene is expressed by the eukaryotic transcription and translation machinery.

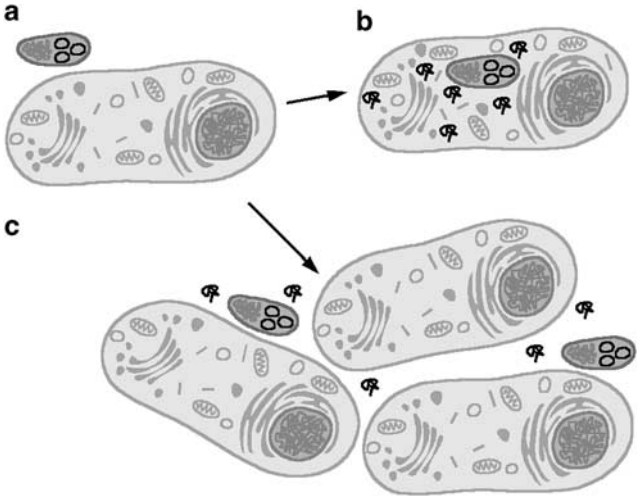


**Figure 2** Alternative gene therapy: transformed bacteria produce the therapeutical polypeptide in situ in the cells or in the intercellular space. (**a**) Transformed bacteria that contain plasmids carrying the transgene are applicated into the target tissue and either enter the cells or stay in the intercellular matrix. (**b**) The transgene is expressed after penetrating into the cell in the cytoplasm by the prokaryotic transcription and translation machinery (bactochondrion). (**c**) Bacteria do not enter the eukaryotic cell, but express the therapeutical transgene in the intercellular space.

**Table 2** Studies using bactofection in various disease models

| *Vector* | *Target gene* | *Disease* | *Model* | *Result* | *Reference* |
|---|---|---|---|---|---|
| *L. monocytogenes* | IL-12 | *L. major*-infection | Mus musculus | Positive | Shen *et al.*, [12] |
| *S. typhimurium* | CD40L | B-cell lymphoma | Mus musculus | Positive | Urashima *et al.*,[9] |
| *S. typhimurium* | VEGFR-2 (FLK-1) | Various carcinomas | Mus musculus | Positive | Niethammer *et al.*,[29] |
| *L. monocytogenes* | CFTR | Cystic fibrosis | CHO-K1 cells | Positive | Krusch *et al.*,[31] |
| *S. typhimurium* | IFNγ | Immunodeficiency | Mus musculus | Positive | Paglia *et al.*,[38] |
| *S. choleraesuis* | Thrombospondin-1 | Melanoma | Mus musculus | Positive | Lee *et al.*,[30] |

**Table 3** Studies using alternative gene therapy in various disease models

| *Vector* | *Target gene* | *Disease* | *Model* | *Result* | *Reference* |
|---|---|---|---|---|---|
| *B. longum* | Endostatin | Liver cancer | Mus musculus | Positive | Fu *et al.*,[8] |
| *S. typhimurium* | Cytosine deaminase | Refractory cancer | Homo sapiens | Positive | Nemunaitis *et al.*,[20] |
| *B. adolescentis* | Endostatin | Liver cancer | Mus musculus | Positive | Li *et al.*,[39] |
| *C. beijerinckii* | Cytosine deaminase | Mammary carcinoma | EMT6 murine carcinoma cells | Positive | Fox *et al.*,[25] |
| *S. typhimurium* | IL-2 | Adenocarcinoma | Mus musculus | Positive | Saltzman *et al.*,[40] |
| *E. coli* | VEGF | Ischemia | Mus musculus | Positive | Celec *et al.*,[13] |
| *C. sporogenes* | Cytosine deaminase | Solid tumor | Mus musculus | Positive | Liu *et al.*,[27] |

## Bacterial strains

Many bacterial strains are used in gene therapy and they can be divided according to where they reside inside the host. They can be localized primarily in cytoplasm (*Listeria*, *Shigella)*, in vacuoles (*Salmonella*, *Yersinia)* or in extracellular space (*Agrobacterium*).[7] However, this classification is far from being strict, as genetic modification can alter these phenotypic characteristics in most used strains.

*Escherichia coli* as a classic model and best-described laboratory tool in molecular biology has been used also in gene therapy, although only after several defined modifications. One of these is the mutation *ΔdapAΩcat* in *E. coli*. This mutation causes an accelerated disintegration of the bacterial cell in media without diaminopimelic acid similarly to the environment in mammalian lysosomes (decreased virulence). This modification considerably decreases the pathogenic potential of the laboratory strains. On contrary, *E. coli* strains used for bactofection are genetically engineered for better penetration through the cellular membrane (increased virulence) and for the facilitation of the release of the carried plasmid into the cytoplasm of the target cell. These features are ensured by genes like *inv* from *Yersinia pseudotuberculosis* that encodes invasin and *hly* from *Listeria monocytogenes* that encodes listeriolysin O (LLO). Invasin enables *E. coli* to penetrate cells expressing β1-integrins on their surface (e.g. airway epithelial cells). LLO causes the breakdown of lysosomal membranes after phagocytosis.[14,15]

*L. monocytogenes*, which is capable of surviving inside a mammalian cell, can also be used for bactofection, but its pathological effects have to be eliminated.[16] Gene *ply118* encoding a phage endopeptidase (or lysin) is responsible for bacterial lysis and subsequently for the plasmid release. Regulatory sequences of *ply118* contain the Pact promoter, which is preferentially activated after *Listeria* enters the host cytoplasm.[17] Thus, the bacterial lysis induced by *ply118* is restricted to the cytoplasm of the mammalian cell.[12,18]

*Bifidobacterium longum* is a facultative anaerobic microbe. After systemic application, its growth is limited to hypoxic areas such as the tissue of solid tumors.[8] Similar metabolism, and thus, similar applications have *Salmonella enterica* serovar *typhimurium* and *S. choleraesuis* in bacteria-mediated gene delivery.[19] The attenuation is achieved by deletion of not only *aroA*, *aroC* (both involved in synthesis of aromatic amino acids), but also *ssaV* (causes defects in the secretion apparatus encoded by a region of *Salmonella* pathogenicity island 2 – SPI2) or *sifA* (mutants cannot preserve the enveloping endosomal vacuoles).[5,11] Increased specificity of the growth in the tumor tissue is achieved by the deletion of *purI*[20,21] or by creating Leu/Arg-dependent auxotrophic *Salmonella* mutants.[22] These bacterial mutants are dependent on exogenous adenine sources. Tumor tissues represent such an adenine source due to the increased cell turnover. The TNFα production as a marker of inflammation is reduced by inhibiting the production of lipid A or lipopolysaccharide,[20] which can be achieved also by the deletion of *msbB* gene.[21,23] Other approaches to limit the pathogenity of *Salmonella* strains include affecting the synthesis of adenylate cyclase, adenine methylase, various receptor proteins or the global regulatory system phoP/phoQ.[24]

Other prokaryotes including *Yersinia enterocolytica*,[24] *Shigella flexneri*,[18] *Clostridium beijerinckii*,[25] *Toxoplasma gondii*,[26] *Clostridium sporogenes*[27] and other *Clostridium* strains[21,28] are rarely used. Nevertheless, some of these bacterial strains can efficiently invade intestinal epithelia and as the factors of virulence are studied in detail, future studies on gene therapy using these potentially valuable vectors can be awaited.

## Experimental and clinical studies

### *Bactofection*

Bactofection has shown to be a useful tool in the therapy of several tumors in mice. Melanoma, colon carcinoma and lung carcinoma in mice can be used as an example. DNA vaccination using *S. enterica* serovar *typhimurium* transformed with plasmid-carrying vascular endothelial growth factor receptor 2 (VEGFR-2 or FLK-1) gene has been described. This approach reduced growth of established metastases of these tumors in mice.[29] Other *Salmonella* strain *Salmonella choleraesuis* allowed targeted gene delivery into the tumor tissue. In this study, bacteria were used as a vector for the delivery of thrombospondin-1 gene to murine melanoma. This approach significantly inhibited the tumor growth and prolonged survival in the murine melanoma model.[30] The possibility of treatment of not only solid tumors was described in study using oral delivery system with *S. typhimurium* carrying CD40 ligand gene against B-cell lymphoma in mice.[9]

Invasive strains of *E. coli* are capable of transferring a functional gene copy into target cells (airway epithelium) in various models of monogenic diseases like cystic fibrosis (CF).[14] A major problem of this study was the unsatisfying selectivity of gene transfer. The treatment of CF was also a goal of a study using *Listeria monocytogenes*. These bacteria delivered CF transmembrane conductance regulator to mammal cells indicating the

possibilities of bactofection usage in the treatment of inherited disorders.[31] A completely different problem was solved in a similar fashion using *L. monocytogenes*. The bacteria delivered interleukin 10 and 12 genes into mammalian cells, while this production affected the progress of Leishmania infection. The study was conducted *in vitro* and *in vivo* in mice, but despite promising results this method has not been tested clinically yet.[12] Also, protection against lethal doses of *L. monocytogenes* infection in mice after multiple application of *S. typhimurium*-carrying plasmid-encoding LLO from *L. monocytogenes* as a antigen was described.[32]

Bactofection can also be used for DNA vaccination against numerous microbial agents including viruses, parasitical protozoa, fungi and even other bacteria.[5,10,11,24] Plasmids in bacterial vectors carry genes encoding antigens that are expressed in the eukaryotic cells after transfection. Attenuated bacteria can also express the antigens themselves without a transfer of DNA into the eukaryotic cells and in addition they act as adjuvans.[33] Currently, RNA interference identified in numerous organisms is currently being used for the post-transcriptional gene silencing. Genetic information for specific dsRNA production can be delivered into the target cells also via bactofection.[34,35]

### *Alternative gene therapy*

A promising and very sophisticated approach is the clinical use of transformed *S. typhimurium* producing cytosine deaminase (CD) in the therapy of colorectal cancer as one of the civilization diseases – a tumor with very high incidence and prevalence in Caucasian population. Although the study was a phase II clinical trial with a low number of participating patients, the results are very motivating and point towards the potential of this procedure.[20] As the gene was not transferred into the host cells but expressed in the bacteria, this approach cannot be classified as bactofection, on contrary, it resembles the principle of AGT. The produced protein has not a direct therapeutical effect. This enzyme catalyzes the conversion of an exogenously applied harmless molecule into an agent used often in chemotherapy with detrimental effects on tumor tissue. Thus, the usage of tissue-specific growth and expression limits the side effects of this kind of chemotherapy. Similar studies using *S. typhimurium* were published focusing on the treatment of spleen cancer and various lymphatic tumors.[11]

The same mechanism of delivering CD into solid tumor using *C. sporogenes*[27] was described. *Clostridium* strains as a tool of AGT in cancer therapy were well described in review article from Minton.[28] In another study, the authors described *C. acetobutylicum* expressing murine-TNFα as a possible tool for cancer therapy.[36]

*B. longum* transformed with plasmid containing the gene for human endostatin has been used for the treatment of liver tumors in BALB/c mice. As a potent inhibitor of angiogenesis, endostatin inhibited the progress of the tumors, as the growth of solid tumors is dependent on oxygen and nutrient supply via new vessels.[8] Main advantages of this approach are inexpensiveness, target tissue specificity, easy and safe delivery in comparison to other gene therapy vectors or direct protein administration. In this very interesting study, another possibility of using bacteria therapeutically has been shown. Prokaryotes precultivated in selenite-rich medium have been used for the transport of this anorganic salt – sodium selenite into the target tumor tissue. Genetically modified bacteria are also experimentally used as recombinant probiotics for the therapy of gastrointestinal disorders, but their clinical usage is dependent on a wide acceptance in the society, what is currently not the case.

Although most studies using bacteria for various gene therapy-related procedures are focusing on the treatment of cancer, expectations are put into the usage of prokaryotes in other clinical entities like cystic fibrosis[14,37] or ischemic diseases.[13]

## Conclusion

In summary, bactofection seems to be a valuable tool equivalent to other methods using nonbacterial vectors for gene delivery. Many details must be explored, important problems solved in the near future. Although the studies using bactofection and AGT are still relatively rare, considerable advantages of prokaryotic vectors in some indications make this method a perspective approach that after future extensive experimental investigations might also have a heavy impact on the clinical therapy of various diseases.

## Acknowledgements

We are greatful to L'ubomír Tomáška for the motivation to write this review. The authors are supported by Grant of the Comenius University 116/2004 and 117/2004 and the Grant Agency for Science and Technology APVT-20-003104.

## References

1 Mountain A. Gene therapy: the first decade. *Trends Biotechnol* 2000; **18**: 119–128.
2 Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P *et al*. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**: 415–419.
3 Gardlik R, Palffy R, Hodosy J, Lukacs J, Turna J, Celec P. Vectors and delivery systems in gene therapy. *Med Sci Monit* 2005; **11**: RA110–RA121.
4 Page DT, Cudmore S. Innovations in oral gene delivery: challenges and potentials. *Drug Discov Today* 2001; **6**: 92–101.
5 Loessner H, Weiss S. Bacteria-mediated DNA transfer in gene therapy and vaccination. *Expert Opin Biol Ther* 2004; **4**: 157–168.
6 Schaffner W. Direct transfer of cloned genes from bacteria to mammalian cells. *Proc Natl Acad Sci USA* 1980; **77**: 2163–2167.
7 Weiss S, Chakraborty T. Transfer of eukaryotic expression plasmids to mammalian host cells by bacterial carriers. *Curr Opin Biotechnol* 2001; **12**: 467–472.
8 Fu GF, Li X, Hou YY, Fan YR, Liu WH, Xu GX. Bifidobacterium longum as an oral delivery system of endostatin for gene therapy on solid liver cancer. *Cancer Gene Ther* 2005; **12**: 133–140.
9 Urashima M, Suzuki H, Yuza Y, Akiyama M, Ohno N, Eto Y. An oral CD40 ligand gene therapy against lymphoma using attenuated Salmonella typhimurium. *Blood* 2000; **95**: 1258–1263.
10 Darji A, zur Lage S, Garbe AI, Chakraborty T, Weiss S. Oral delivery of DNA vaccines using attenuated Salmonella

typhimurium as carrier. *FEMS Immunol Med Microbiol* 2000; **27**: 341–349.
11 Michael A, Stratford R, Khan S, Dalgleish A, Pandha H. Novel strains of Salmonella typhimurium as potential vectors for gene delivery. *FEMS Microbiol Letts* 2004; **238**: 345–351.
12 Shen H, Kanoh M, Liu F, Maruyama S, Asano Y. Modulation of the immune system by Listeria monocytogenes-mediated gene transfer into mammalian cells. *Microbiol Immunol* 2004; **48**: 329–337.
13 Celec P, Gardlik R, Palffy R, Hodosy J, Stuchlik S, Drahovska H *et al*. The use of transformed *Escherichia coli* for experimental angiogenesis induced by regulated *in situ* production of vascular endothelial growth factor - an alternative gene therapy. *Med Hypotheses* 2005; **64**: 505–511.
14 Fajac I, Grosse S, Collombet JM, Thevenot G, Goussard S, Danel C *et al*. Recombinant Escherichia coli as a gene delivery vector into airway epithelial cells. *J Control Release* 2004; **97**: 371–381.
15 Grillot-Courvalin C, Goussard S, Huetz F, Ojcius DM, Courvalin P. Functional gene transfer from intracellular bacteria to mammalian cells. *Nat Biotechnol* 1998; **16**: 862–866.
16 Pilgrim S, Stritzker J, Schoen C, Kolb-Maurer A, Geginat G, Loessner MJ *et al*. Bactofection of mammalian cells by Listeria monocytogenes: improvement and mechanism of DNA delivery. *Gene Ther* 2003; **10**: 2036–2045.
17 Dietrich G, Bubert A, Gentschev I, Sokolovic Z, Simm A, Catic A *et al*. Delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide Listeria monocytogenes. *Nat Biotechnol* 1998; **16**: 181–185.
18 Grillot-Courvalin C, Goussard S, Courvalin P. Bacteria as gene delivery vectors for mammalian cells. *Curr Opin Biotechnol* 1999; **10**: 477–481.
19 Weiss S. Transfer of eukaryotic expression plasmids to mammalian hosts by attenuated Salmonella spp. *Int J Med Microbiol* 2003; **293**: 95–106.
20 Nemunaitis J, Cunningham C, Senzer N, Kuhn J, Cramm J, Litz C *et al*. Pilot trial of genetically modified, attenuated Salmonella expressing the *E. coli* cytosine deaminase gene in refractory cancer patients. *Cancer Gene Ther* 2003; **10**: 737–744.
21 Sznol M, Lin SL, Bermudes D, Zheng LM, King I. Use of preferentially replicating bacteria for the treatment of cancer. *J Clin Invest* 2000; **105**: 1027–1030.
22 Zhao M, Yang M, Li XM, Jiang P, Baranov E, Li S *et al*. Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing Salmonella typhimurium. *Proc Natl Acad Sci USA* 2005; **102**: 755–760.
23 Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ *et al*. Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma. *J Clin Oncol* 2002; **20**: 142–152.
24 Medina E, Guzman CA. Use of live bacterial vaccine vectors for antigen delivery: potential and limitations. *Vaccine* 2001; **19**: 1573–1580.
25 Fox ME, Lemmon MJ, Mauchline ML, Davis TO, Giaccia AJ, Minton NP *et al*. Anaerobic bacteria as a delivery system for cancer gene therapy: *in vitro* activation of 5-fluorocytosine by genetically engineered clostridia. *Gene Therapy* 1996; **3**: 173–178.
26 Chakrabarty AM. Microorganisms and cancer: quest for a therapy. *J Bacteriol* 2003; **185**: 2683–2686.
27 Liu SC, Minton NP, Giaccia AJ, Brown JM. Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia/necrosis. *Gene Therapy* 2002; **9**: 291–296.
28 Minton NP. Clostridia in cancer therapy. *Nat Rev Microbiol* 2003; **1**: 237–242.
29 Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G *et al*. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med* 2002; **8**: 1369–1375.
30 Lee CH, Wu CL, Shiau AL. Systemic administration of attenuated Salmonella choleraesuis carrying thrombospondin-1 gene leads to tumor-specific transgene expression, delayed tumor growth and prolonged survival in the murine melanoma model. *Cancer Gene Ther* 2005; **12**: 175–184.
31 Krusch S, Domann E, Frings M, Zelmer A, Diener M, Chakraborty T *et al*. Listeria monocytogenes mediated CFTR transgene transfer to mammalian cells. *J Gene Med* 2002; **4**: 655–667.
32 Darji A, Guzman CA, Gerstel B, Wachholz P, Timmis KN, Wehland J *et al*. Oral somatic transgene vaccination using attenuated S. *typhimurium*. *Cell* 1997; **91**: 765–775.
33 Dietrich G, Spreng S, Favre D, Viret JF, Guzman CA. Live attenuated bacteria as vectors to deliver plasmid DNA vaccines. *Curr Opin Mol Ther* 2003; **5**: 10–19.
34 Timmons L, Fire A. Specific interference by ingested dsRNA. *Nature* 1998; **395**: 854.
35 Timmons L, Court DL, Fire A. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. *Gene* 2001; **263**: 103–112.
36 Theys J, Nuyts S, Landuyt W, Van Mellaert L, Dillen C, Bohringer M *et al*. Stable Escherichia coli-Clostridium acetobutylicum shuttle vector for secretion of murine tumor necrosis factor alpha. *Appl Environ Microbiol* 1999; **65**: 4295–4300.
37 Klink D, Schindelhauer D, Laner A, Tucker T, Bebok Z, Schwiebert EM *et al*. Gene delivery systems–gene therapy vectors for cystic fibrosis. *J Cyst Fibros* 2004; **3** (Suppl 2): 203–212.
38 Paglia P, Terrazzini N, Schulze K, Guzman CA, Colombo MP. *In vivo* correction of genetic defects of monocyte/macrophages using attenuated Salmonella as oral vectors for targeted gene delivery. *Gene Therapy* 2000; **7**: 1725–1730.
39 Li X, Fu GF, Fan YR, Liu WH, Liu XJ, Wang JJ *et al*. Bifidobacterium adolescentis as a delivery system of endostatin for cancer gene therapy: selective inhibitor of angiogenesis and hypoxic tumor growth. *Cancer Gene Ther* 2003; **10**: 105–111.
40 Saltzman DA, Heise CP, Hasz DE, Zebede M, Kelly SM, Curtiss III R *et al*. Attenuated Salmonella typhimurium containing interleukin-2 decreases MC-38 hepatic metastases: a novel anti-tumor agent. *Cancer Biother Radiopharm* 1996; **11**: 145–153.