JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 405–413

Received August 27, 2007
Accepted November 26, 2007
DOI 10.1211/jpp.60.4.0001
ISSN 0022-3573

Review Article

Gene therapy for osteosarcoma: steps towards clinical studies

Crispin R. Dass and Peter F. M. Choong

Abstract

Gene therapy, an applied form of biotechnology, relies on the delivery of foreign DNA into cells. More than 50% of all reported clinical trials for gene therapy are for cancer, though only a scant number for osteosarcoma. Osteosarcoma is a neoplasm afflicting young adults, who in their prime years of life suffer debilitation if not death. The disease is not entirely curable, even with surgery combined with aggressive chemotherapy. Thus, other forms of therapies are being evaluated, including gene therapy. There exist two major forms of gene transfer: viral and non-viral. This review only covers proof-of-principle work carried out in cancer beyond the cell culture stage, in animals. Drawing from the experiences of gene therapy against other cancers, studies for which have already reached the clinical phase, the review discusses potential pitfalls and solutions to enhance gene therapy for osteosarcoma.

Introduction

Osteosarcoma is the second highest cause of cancer-related death in the paediatric age group and the common most primary tumour of the bone in general. Children and adolescents are most commonly affected as the peak incidence coincides with the period of rapid skeletal growth. Thus, incidence coincides with the prime period of the human life cycle. Tumours frequently localize to the distal femur and proximal tibia region (Whelan 1997), and the 5-year survival rate is between 50 and 65% (Stiller et al 2001; Mankin et al 2004).

While modern therapy has significantly improved tumour resectability and the long-term outcome of these patients, 25–50% of patients with initially non-metastatic disease subsequently develop metastases, which usually signals terminal stage disease (Link et al 1986). Approximately one-third of all patients develop lung metastasis despite aggressive multi-regime chemotherapy and surgery.

Gene therapy—quick overview

The field of gene therapy, which has gained rapid momentum in the last one-and-a-half decades as the next major form of therapy for such ailments as cancer, rheumatoid arthritis and cystic fibrosis, has undergone a major overhaul due to a general lack of efficacy in clinical trials. Recently, the fact that viral vector-mediated gene therapy may in fact cause patient death led to significant questioning of further clinical studies being performed using viral vectors (Kaiser 2007). However, viral gene delivery is still showing some rather promising results clinically with novel safer vectors (Favaro & Indraccolo 2007).

The main reasons for the discrepancy between otherwise promising preclinical studies and clinical trials are several fold. Firstly, inappropriate or non-robust disease models were chosen in animal studies and, secondly, the vehicles used for gene transfer in man were lacking in the ability to selectively deliver the therapeutic gene construct to the disease sites at a level within the therapeutic window. Such results were relevant for all the major gene therapeutic approaches, including plasmid DNA, retroviral, adenoviral and other viral vectors.

This is the same for the other therapies that are usually bunched under the gene therapy field, such as nucleic acid constructs like ribozymes, antisense molecules, decoy oligodeoxynucleotides (ODNs) and DNAzymes. The development of small interfering RNA

Department of Orthopaedics, University of Melbourne, St. Vincent's Hospital Melbourne, Australia

Crispin R. Dass, Peter F. M. Choong

Bone and Soft Tissue Sarcoma Service, Peter MacCallum Cancer Institute, Melbourne, Australia

Peter F. M. Choong

Correspondence: C. R. Dass, Department of Orthopaedics, St. Vincent's Hospital Melbourne, P.O. Box 2900, Fitzroy 3065, Australia. E-mail: crispin.dass@svhm.org.au

Funding: This study was generously supported by grants from the Cancer Council of Victoria, the Australian Orthopaedic Association and the Victorian Orthopaedic Research Trust Grant.

(siRNA), a newcomer, has progressed with the benefit of hindsight of previous difficulties encountered during antisense drug development. Ribozymes, DNAzymes and antisense molecules cause degradation of the target RNA either by Watson–Crick-based base-pairing and cleavage due to RNAseH enzymatic activity (antisense) or by directly acting as catalytic molecules (ribozymes and DNAzymes). For antisense at least, immunostimulatory effects in-vivo are dependent on certain chemistries used for manufacture of oligonucleotides. One example of this is the phosphorothioate (PO) backbone used for synthesising enzyme-resistant oligonucleotides (Dass et al 2002a).

siRNA utilises the cell's inherent machinery (called Dicer) to degrade target mRNA. Several delivery vehicles for siRNAs are currently being tested, with the major class being cationic liposomes (Kawakami & Hashida 2007). Due to its RNA-based backbone, the ribozyme is rapidly degraded in-vivo and thus cannot be delivered as a conventional drug molecule, and therefore is delivered as an expressible construct. For cancer, the DNAzyme molecule, composed of a DNA-based backbone and a relatively newcomer to the gene therapy field, is still largely being tested in cell culture (Dass et al 2002a; De Bock et al. 2006), and to date only few studies have looked at the effects of these entities in-vivo (Fahmy et al 2003; Mitchell et al 2004; Zhang et al 2004). Decoy ODNs, double-stranded ODNs, act to prevent a specific transcription factor from activating a certain gene's promoter region.

Transfection of cells with decoy ODNs corresponding to the *cis* sequence results in attenuation of the authentic *cis–trans* interaction, leading to the removal of the *trans* factors from the endogenous *cis* element, with subsequent modulation of gene expression. By this mechanism, the normal transcription factors that drive and regulate gene expression are not permitted to perform their normal task at the required site due to the decoy oligonucleotides binding to and thereby inactivating them. Thus, the decoy approach enables treatment of disease by modulation of endogenous transcriptional regulation.

In comparison, antisense molecules (such as those against the oncogene c-myc; Figure 1) have proceeded from being tested in cell culture, and after numerous preclinical and clinical trials the first antisense drug was released into the market in 1999. This was Formivirsen (ISIS 2922), sold for the control of cytomegalovirus-mediated retinitis (Perry & Balfour 1999). It is anticipated that a few more antisense drugs will be released into the market within the next five years of research and development. The vested interests of major drug companies in these biotech-pharmaceutical drugs has paved the way for research centres and universities to develop and test antisense-based drugs at a steady but cautious pace. Other antisense test agents with potential against cancer include Eye001 (Eyetech Study Group 2002; target VEGF), aprinocarsen (Paz-Ares et al 2006; Ritch et al 2006; target protein kinase C-α), OGX-011 (Chi et al 2005; target

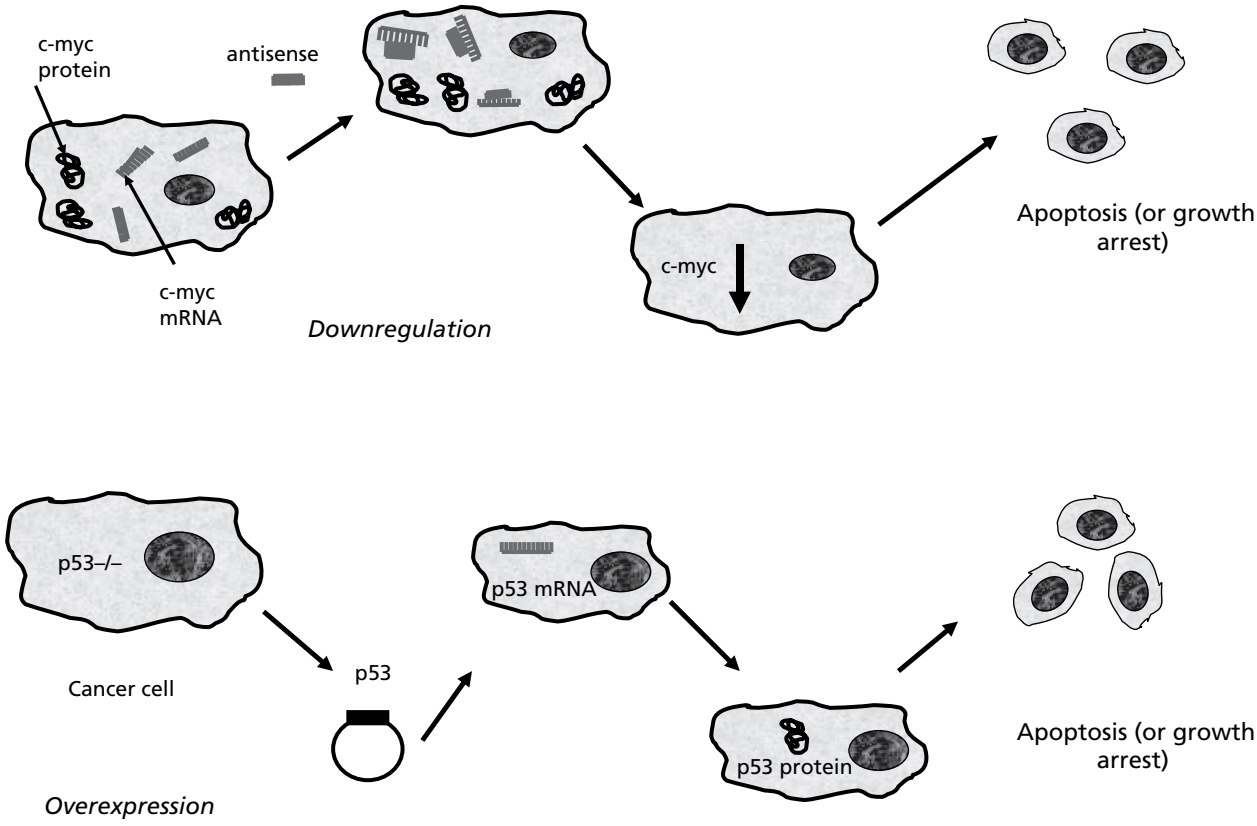

Figure 1 The two most common modes of gene therapy. Conventional gene therapy consists of either new expression of a gene in the target cell or forced downregulation of a target gene in affected cells.

clusterin), and even immunostimulatory ODNs being developed by several companies.

In contrast, overexpression of such genes as tumour suppressors (for example p53; Figure 1) using plasmid DNA has been the traditional approach for cancer gene therapy (Dass 1997). The major two forms of non-viral carriers are cationic liposomes and poly-ethyleneimine (PEI), both of which are discussed in depth below in the context of usage in osteosarcoma gene therapy. The early lack of transfectability with these vectors then led to further development of viral vectors that possessed intercellular transfer and hence transfection, although at a cost.

This review does not cover studies using cells transfected ex-vivo, and then transplanted into the animal. Only strict in-vivo gene therapy evaluations where the administration of the genotherapeutic construct was performed in a live animal are mostly covered herein.

Viral versus non-viral gene therapy

The field of clinical gene therapy is now nearly 15 years old, with the original gene therapy clinical trial being performed for adenosine deaminase (ADA) deficiency (Culver et al 1991). Since then, a plethora of gene vectors have been synthesised and trialled, including a range of viral ones, as well as the most efficient of the plasmid vectors. Viral vectors have had some major successes in genotherapy (Ko et al 2005), but there are still issues, mainly targeting, which plague their widespread use (Baker et al 2005). A further problem is the inability to repeatedly dose patients with these vectors due to immunorecognition of the viral capsid proteins (Lee et al 2005). Uncertainties and hesitation in using a vector that has the inherent potential to recombine with other viruses present in cells has also marred their rapid adoption by gene therapists.

Also, safety concerns during preparation and use preclude ease-of-use. More regulation is due to occur arising from biosafety issues, especially in light of the unfortunate recent clinical problems discussed below. These restrictions need to be carefully assessed and weighed against potential benefits of using viral vectors for gene transfer, as years of effort and substantial amounts of resources may lead to useless technology.

For instance, a report from 2003 (Hacein-Bey et al 2003) highlights two cases in children where retroviral genotherapy for severe combined immunodeficiency caused de-novo T-cell leukaemia 3 years after commencement of therapy. Retrovirus vector integration occurred in proximity to the LMO2 proto-oncogene promoter, leading to aberrant transcription and expression of LMO2. A major blow to viral gene therapy came with the death of an 18-year-old patient (Jesse Gelsinger) in September 1999 receiving adenoviral vectors (Raper et al 2002). A report in 2005 (Check 2005) states that a third child may have acquired de-novo cancer as a result of gene therapy.

However, non-viral vehicles also suffer due to problems in their own right. These include heightened immunostimulation when complexed with nucleic acids. Side-effects as a result of sometimes harsh chemistry come to light when these vectors are taken from cell culture testing to in-vivo usage and evaluation. It is important that researchers quickly highlight deficiencies in their gene delivery technology, specifically those that may actually harm the patient. In the Jesse Gelsinger case, it was found out by the US Food and Drug Administration (FDA) that information was withheld from patients, including the fact that monkeys had died earlier when tested, and that human volunteers earlier in the study had side-effects that should have been mentioned.

Viral-vector-mediated gene therapy

Viral vectors are genetically modified viruses, which are able to transfer their genetic material into a host cell. The types of viral vector that have been used to date for osteosarcoma gene therapy include integrating vectors based on retroviruses and adeno-associated viruses, as well as non-integrating vectors based on adenoviruses, lentiviruses and herpes viruses.

Mutations in p53 are quite frequently noted in osteosarcoma (Miller et al 1990, 1996; Florenes et al 1994), which makes the restoration of p53 function in these neoplastic cells an attractive target. Phelan et al (1998) demonstrated that the herpes protein VP22 used to deliver functional p53 was capable of inducing apoptosis in p53–/– osteosarcoma cell lines. However, p53 mutations are not present in all cancers, as is the case for osteosarcoma where it is estimated that 50% of tumours harbour a defective p53 cellular status (Ragland et al 2002). Thus, the scope of this approach is limited, and when combined with the lack of clinical efficacy to date (Ternovoi et al 2006), the prospects are not too bright. Even with cells not expressing p53, overexpression of p53 does not lead to an expected cell death or growth arrest in various osteosarcoma lines tested in-vitro (Hellwinkel et al 2005).

The introduction of viral vectors expressing IL-12 into osteosarcoma cells inhibits the ability of these cells to form metastases in mice (Worth et al 2000). Viral particles (2.5×10^8 pfu) were instilled into each nostril in a drop-wise manner. Treatment was given twice a week for 5 weeks, resulting in a significant decrease in the number of lung metastases in mice bearing intravenously injected SaOS-LM6 osteosarcoma cells.

Tsuji et al (2002) demonstrated that an adenovirus-vector-mediated B7-1 (stimulatory molecule, which when expressed on tumour cells leads to better recognition of neoplastic cells by immune cells of the body) gene transfer into rats induced immunity against pre-established primary osteosarcoma as well as systemic immunity against pre-established secondary MSK-8G tumours in rat lungs. While one intratumoral (5×10^8 pfu) injection was sufficient to give the results attained, these tumours were established intravenously, and are thus not truly orthotopic in nature. More irrelevant was the subcutaneous injection of MSK-4E cells, which were also administered with one intratumoral injection of 5×10^8 viral units. Lung metastases were significantly reduced in the treatment group.

The herpes simplex thymidine kinase (HSV-TK) system was used (Charissoux et al 1999) for ex-vivo transduction of tumours cells that were then implanted in the hind limbs of rats. Both primary tumours and secondary pulmonary tumours were significantly inhibited using this technology. The HSV-TK gene enhances tumour cell sensitivity to nucleoside analogues such as aciclovir and ganciclovir. The

enzyme converts the nucleosides to their non-phosphorylated structure, and further metabolism of these molecules interferes with DNA replication and transcription. Such interference is fatal to cells. The question to ask is whether this approach would be applicable to gene therapy proper, where the constructs will be administered post-establishment of tumours, as will the case be for real patients.

An alternative suicide gene therapy approach was taken by Ramnaraine et al (2003) who used the cytosine deaminase (CD) gene to direct cancer cell death in bone sarcomas in 9 out of 10 mice. However, the cells were transfected ex-vivo before being transplanted back into the mice. CD converts the relatively inert compound 5-fluorocytosine to 5-fluorouracil, which is highly cytotoxic. These authors then went on (Ramnaraine et al 2006) to demonstrate that in cell culture, osteoclasts transduced with the suicide gene were capable of a bystander killing of osteosarcoma cells with the CD fusion gene. However, the effect in-vivo remains to be seen, especially when the osteoclasts themselves would undergo destruction. However, in case the stem cell precursors could be continuously stimulated to divide ex-vivo, then this gives therapists a continuous arsenal to fend off osteosarcoma cells via repeat cell transplantations. It would be interesting to find out whether these cells could be made to seek out bone lesions when administered intravenously.

An adenoviral vector expressing the secreted form of carboxylesterase (sCE) was demonstrated to significantly delay the growth of MG-63 osteosarcoma xenografts in mice when combined with the prodrug CPT-11 (Oosterhoff et al 2003). CPT-11, a derivative of camptothecin belonging to the class of topoisomerase I inhibitors, is converted by CE to its cytotoxic derivative SN-38. Injections of both drug (daily over 7 days) and viral vector units (once, 1×10^9 pfu) were intratumoral.

One promising approach to limit gene transfer to osteosarcoma tumours in-vivo has been exploiting the osteosarcoma cell selectivity of the osteocalcin (OC) promoter. In one study (Cheon et al 1997), 80% of mice with subcutaneous osteosarcoma tumours treated with an adenovirus coding the HSV-TK gene under the control of the OC promoter (Ad-OC-Tk) survived. This survival was further elevated when the adenoviral gene transfer was combined with methotrexate treatment. Both the human MG63 and rat ROS osteosarcoma tumours were intratumorally administered thrice with 5×10^8 pfu of viral units. However, both tumour models were established with subcutaneous injections of cells.

In a separate study (Shirakawa et al 1998), this virus Ad-OC-Tk, when co-administered with aciclovir, was also effective in eradicating ROS 17/2.8 pulmonary growths in mice, with a concomitant increased lifespan. Tumours were smaller in diameter and more necrotic in co-treatment groups. Cells were injected intravenously and 5×10^8 pfu of viral vector units were administered intravenously, with aciclovir given intraperitoneally. The authors used the OC promoter to limit expression of the transgene to the osteosarcoma growths, although no attempt was made to look at expression in other organs, or even look at destruction of distal bone growth or other organ sites.

A study by Chen et al (1997) treated MNNG/HOS cell subcutaneous tumours with an intratumorally administered antisense cyclin G1 expressing retroviral vector. Histologic sections from the antisense cyclin G1 expression vector-treated tumours showed decreased cell division and increased stroma formation within the residual tumours. A decrease in the number of cells in S and G2/M phases of the cell cycle and an accumulation of cells in the G1 phase were noted. This study demonstrated the in-vivo efficacy of a high-titre antisense cyclin G1 retroviral vector, though in a clinically irrelevant model of osteosarcoma.

However, doubts have been thrown on such selective gene therapy for osteosarcoma, as Pollmann et al (2004) demonstrated quite (somewhat surprisingly) limited specificity of promoters for restricting gene transfer to osteosarcoma in-vivo. Suffice to say that use of tissue-specific promoters has not been taken any further despite promising results in cell culture in various types of cancers.

Way forward for viral gene therapy of osteosarcoma

As mentioned above, viral vectors have received a considerable blow due to the death of one patient and incidences of de-novo tumorigenesis in at least three others. Their ability to stimulate a patient's immune system is also a rather frustrating drawback.

Since application is mainly in adolescents, it is important that before viral vectors are used, proper studies for genotoxicity are performed. In a growing body, insertional mutagenesis of the viral construct may lead to relatively more deleterious consequences. While the past decade has been devoted to making viruses target cancerous tissues better, not much progress has been made, though several intricate mechanisms for viral generation have been developed.

Although the use of stronger promoters to drive anti-osteosarcoma genes may aid in therapeutic viral gene transfer in certain osteosarcoma cell lines (de Wilt et al 2001), these vectors are still able to proliferate within cells or insert their genome into that of the mammalian target cell. Thus, absolute guarantee that they are harmless yet tranduce the transgenes efficiently is still, in the authors' opinion, years away.

Non-viral-vector-mediated gene therapy

Against a backdrop of safety concerns with viral vectors, non-viral vectors are increasingly being employed for gene therapy of osteosarcoma. This is usually with cationic liposomes and other carriers such as PEI. Major advantages include safety, ease of preparation and cost-effectiveness, and the major disadvantage is dose-limiting toxicity (Dass et al 2002b; Dass 2004a). When cationic liposomes are complexed to plasmids (or in fact to nucleic acids in general), these complexes are called lipoplexes.

In an orthotopic model of osteosarcoma, when mice were administered with aerosolised plasmid p53-expressing DNA complexed to PEI, a significant reduction of tumour nodules and tumour size was noted (Densmore et al 2001). It is important to note that the cells used for the in-vivo model, SaOS-LM6, are p53 –/–. These cells were injected intravenously to form lung metastases.

Aerosolised PEI:pDNA complexes were effectively delivered to lung metastases in mice (Jia et al 2002). Complexes were instilled twice weekly for two weeks. Significant expression of IL-12 (the transgene) was noted in lungs. The PEI vehicle was superior to bis-guanidinium-tren-cholesterol:DOPE liposomes. The PEI procedure is capable of significant reduction of tumour growth in-vivo as well as reducing metastases to the lungs. SaOS-LM6 tumours were established with an intravenous injection of cells. Importantly, expression of IL-12 in the liver was not seen, and this is one study that looked at such a side-effect.

Administering lipoplexes with a plasmid encoding endostatin (potent inhibitor of angiogenesis) to rats with an orthotopic model of osteosarcoma (1547), Dutour et al (2005) demonstrated that not only were tumours inhibited at the primary site, but also development of lung metastases were stunted. Of note is that in this study, administrations were intravenous, thrice a week after tumours were detectable via tomoscintigraphy. Thus, there is a real potential for this sort of approach. As demonstrated elsewhere and in this study, the lipoplexes were able to target the tumour vasculature, resulting in a reduced microvessel density in the endostatin treatment group. However, tumour blocks were implanted in the paratibial position, but could form metastases.

We have recently demonstrated the anti-osteosarcoma activity of pigment epithelium-derived factor (PEDF) in orthotopic human SaOS-2 (Dass et al 2006) and rat UMR 106-01 (Fisher et al 2001) models. When PEDF plasmids were administered in a naked form and co-mixed with cells before injection, they were able to reduce both growth of tumour cells at the primary intratibial site and significantly decrease the development of metastasis in the lungs of immunocompromised mice (Ek et al 2007a). These data were corroborated with findings in-vitro where decreased cellular proliferation and invasion were noted, and increased adhesion and bone formation (signalling increased cellular differentiation) resulted. We have also demonstrated the ability of this recombinant protein and its short peptides to have anti-osteosarcoma activity as well, both in-vitro and in-vivo.

We have more recently shown that when the PEDF plasmid is encapsulated into chitosan nanoparticles, the activity persists both in-vitro and in-vivo (Dass et al 2007). Adhesion was increased and invasion decreased when encapsulated PEDF plasmids were incubated with SaOS-2 cells in-vitro. In-vivo, a dramatic decrease in primary tumour size and the number of macrometastases in the lungs of mice were noted. We are currently developing this technology further and evaluating whether pre-established tumours can be controlled.

It should be noted that not all studies report positive findings. For instance, while lipoplexes containing a troponin I-encoding plasmid, which expectedly blocks endothelial cell growth in culture, were effective against endothelial cells in-vitro, they were not able to reduce tumour progression in-vivo in a rat osteosarcoma model (Dutour et al 2004). This is not surprising given that occasionally, anti-angiogenic approaches tend to fail in-vivo despite promising in-vitro results. It is important that such findings are reported in the literature, albeit in small journals, as these may save other researchers valuable time and resources, and hasten the progress in the fight to find better alternatives for osteosarcoma management.

Way forward for non-viral gene therapy of osteosarcoma

Other vehicles, such as solid nanoparticles (Dass 2004a) or cyclodextrins (Dass 2004b), need to be tried for gene therapy of osteosarcoma. Dendrimers for example (Fahmy et al 2003; Zhang et al 2004) have been shown to transfect cancer cells via intratumoral administration. New vehicles are surely needed and, as well as not enhancing the natural progression of the neoplasm, these have to be compatible with both the carried therapeutic nucleic acid and the host environment. Slow-release carriers such as microspheres or the use of implants, which deliver the genotherapeutic construct at a sustained rate, may prove worthwhile.

Regardless of the vehicle or device used, they have to be biocompatible, preferably biodegradable and easy to prepare and use. The route of administration may have a huge bearing on the efficiency of gene therapy, whether it is based on a viral or non-viral delivery mode. Most nucleic acid constructs degrade rapidly in blood, and the loss due to binding to cells and other macromolecules in the circulation cannot be underestimated. The solution, as past research with cancer in general demonstrates, lies not in increasing dosage or frequency of administration, but in the ability to target or selectively deliver the therapeutic gene or down-regulation agent close to the primary osteosarcoma or its secondary growths.

Future directions and opportunities for gene therapy of osteosarcoma

There is a definite need for better models that closely mimic the clinical progression of the disease. Existence of syngeneic and human xenograft orthotopic models, such as UMR 106-01 (Fisher et al 2001) and SaOS-2 (Dass et al 2006), should aid in the search for better therapeutics. Other cell lines such as 143B (Luu et al 2005) also exist, but these are genetically altered (transformed) cells. While other studies show efficiency of various gene therapy approaches, they suffer from the fact that only xenograft tumours (subcutaneous: Chen et al 1998; Wang et al 1999; Bougeret et al 2000; Majumdar et al 2001) or tumours induced via an intravenous injection of cells (mostly SaOS-LM7: Duan et al 2006; Li et al 2006) were used to evaluate their therapeutic constructs. The effect of the bone milieu on growth of osteosarcoma tumours at the primary site and subsequent spread to the lungs as the major secondary site cannot be overlooked.

It is logical that any gene therapeutic approach be tested in combination with the current frontline chemotherapy. For osteosarcoma, such a conventional chemotherapeutic regimen consists of methotrexate, cisplatin and doxorubicin (Ek & Choong 2006). If complete cure with gene therapy is not possible for osteosarcoma, being that it is a complex and varied disease, then the goal may be shifted to prolonging and improving the quality of life of sufferers.

Of all gene therapy clinical trials performed to date, at least 50% are for cancer. Thus, while osteosarcoma has not been extensively challenged in clinical trials with gene therapy, it is just a matter of time before that becomes a reality. Also of note is the fact that a clear majority (> 90%) of all studies look at overexpressing a gene, with no evaluation of gene downregulation as yet, as discussed below.

For osteosarcoma, one study, which was an in-vivo study, looked at the downregulation of the urokinase plasminogen activator receptor (uPAR) in an orthotopic syngeneic model. The uPA/uPAR system is widely implicated in cancer cell migration and invasion, and hence tumour metastasis, so presents itself as a rational target. In this study (Dass et al 2005), rat osteosarcoma UMR106-01 cells were transfected with either antisense-uPAR or vector control plasmids, and an antisense clone downregulated uPAR and demonstrated decreased adhesion, migration and invasion in cell-based assays in-vitro. However, cell proliferation was not perturbed by uPAR downregulation. A significant reduction of 80% in tibial tumour volumes and total inhibition of pulmonary metastases were observed in mice injected with the antisense-transfected cells. This seminal study paves the way for future analysis of downregulation of tangible gene targets in osteosarcoma. Furthermore, given the fact that we now have siRNA as a potent tool for downregulation, why not look at this mode of tumour inhibition?

The major challenge is the need for vehicles to selectively target tumours in-vivo. Delivery to non-target tissues should be minimised. The ideal carrier may need to have several attributes (Figure 2), borrowed from other vehicles used currently as well as the culmination of novel ideas, to both protect and deliver the construct to the lesion at therapeutically relevant doses. For osteosarcoma, since the field is relatively new, much can be learnt from the attempts to perform gene therapy for other cancers such as brain, lung, prostate and breast. Thus, while osteosarcoma is a rare cancer, translation

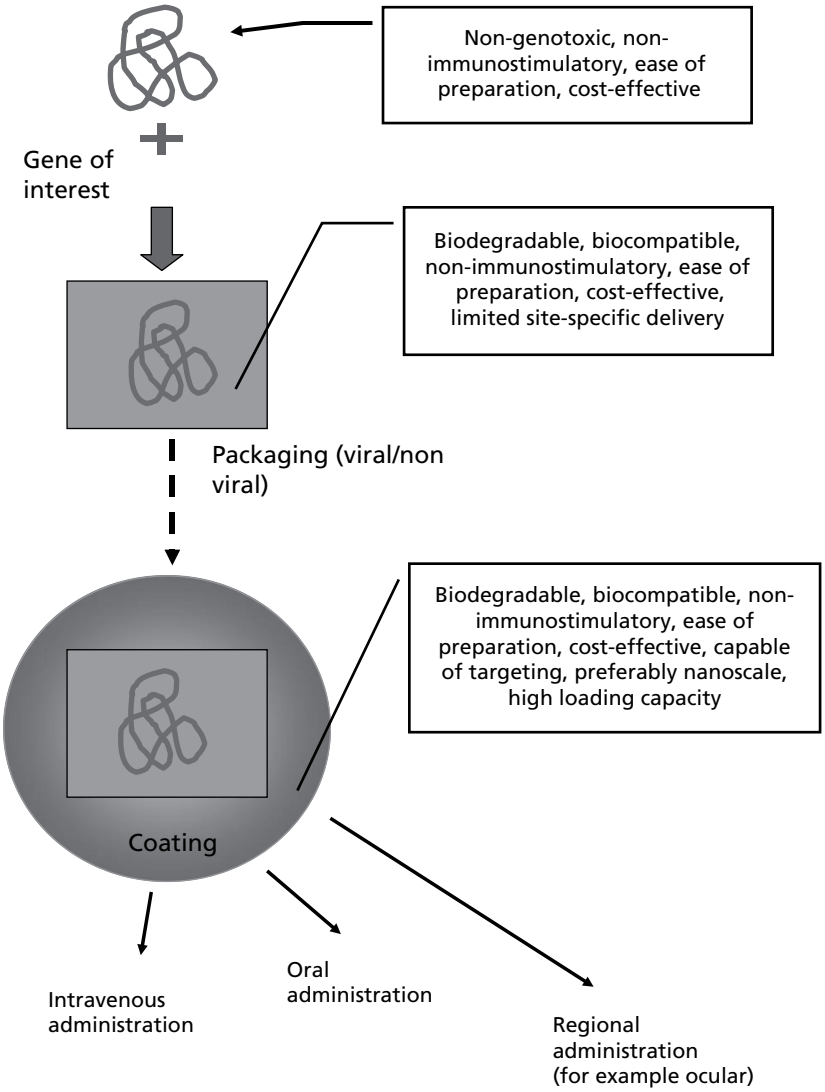

Figure 2 A generic view of essential features of vectors for selective tumour gene therapy. The features that may aid in better delivery of genotherapeutic constructs for osteosarcoma therapy.

of murine pre-clinical findings into dogs with osteosarcoma (more clinically-relevant model), then into humans, may provide a relevant and safe pathway of clinical development.

Summary

Osteosarcoma is a cancer with logistically complex and toxic standard therapy. While current surgical and chemotherapeutic intervention has improved the life of numerous sufferers, recurrence of the disease, especially at secondary sites, is a major problem. Novel ways of management are required, and slowly gene therapy has been included in this expanding list of newcomers. However, for osteosarcoma, relatively little has been done by way of gene therapy, and much more remains to be performed. The importance of assessing this new technology in clinically relevant animal models cannot be over-emphasised. The lessons learnt from gene therapy in other major cancers are to be used as guidance. Finally, the ability of gene therapy on its own may not be that great, but may need complementation with currently used chemotherapeutics for enhanced tumour death and patient survival. With additional research, particularly if applied to dogs before people, gene therapy of osteosarcoma has the potential to become a reality.

References

Baker, A. H., Kritz, A., Work, L. M., Nicklin, S. A. (2005) Cell-selective viral gene delivery vectors for the vasculature. *Exp. Physiol*. **90**: 27–31

Bougeret, C., Virone-Oddos, A., Adeline, E., Lacroix, F., Lefranc, C., Ferrero, L., Huet, T. (2000) Cancer gene therapy mediated by CTS1, a p53 derivative: advantage over wild-type p53 in growth inhibition of human tumors overexpressing MDM2. *Cancer Gene Ther*. **7**: 789–798

Charissoux, J. L., Grossin, L., Leboutet, M. J., Rigaud, M. (1999) Treatment of experimental osteosarcoma tumors in rat by herpes simplex thymidine kinase gene transfer and ganciclovir. *Anticancer Res*. **19**: 77–80

Check, E. (2005) Gene therapy put on hold as third child develops cancer. *Nature* **433**: 561

Chen, D. S., Zhu, N. L., Hung, G., Skotzko, M. J., Hinton, D. R., Tolo, V., Hall, F. L., Anderson, W. F., Gordon, E. M. (1997) Retroviral vector-mediated transfer of an antisense cyclin G1 construct inhibits osteosarcoma tumor growth in nude mice. *Hum. Gene Ther*. **8**: 1667–1674

Chen, X., Li, Y., Xiong, K., Aizicovici, S., Xie, Y., Zhu, Q., Sturtz, F., Shulok, J., Snodgrass, R., Wagner, T. E., Platika, D. (1998) Cancer gene therapy by direct tumor injections of a nonviral T7 vector encoding a thymidine kinase gene. *Hum. Gene Ther*. **9**: 729–736

Cheon, J., Ko, S. C., Gardner, T. A., Shirakawa, T., Gotoh, A., Kao, C., Chung, L. W. (1997) Chemogene therapy: osteocalcin promoter-based suicide gene therapy in combination with methotrexate in a murine osteosarcoma model. *Cancer Gene Ther*. **4**: 359–365

Chi, K. N., Eisenhauer, E., Fazli, L., Jones, E. C., Goldenberg, S. L., Powers, J., Tu, D., Gleave, M. E. (2005) A phase I pharmacokinetic and pharmacodynamic study of OGX-011, a 2'-methoxyethyl antisense oligonucleotide to clusterin, in patients with localized prostate cancer. *J. Natl Cancer Inst*. **97**: 1287–1296

Culver, K. W., Anderson, W. F., Blaese, R. M. (1991) Lymphocyte gene therapy. *Hum. Gene Ther*. **2**: 107–109

Dass, C. R. (1997) Cationic liposomes and gene therapy for solid tumors. *Drug Deliv*. **4**: 151–165

Dass, C. R. (2004a) Oligonucleotide delivery to tumours using macromolecular carriers. *Biotechnol. Appl. Biochem*. **40**: 113–122

Dass, C. R. (2004b) Cyclodextrins and oligonucleotide delivery to solid tumours. *J. Drug Target*. **12**: 1–9

Dass, C. R., Saravolac, E. G., Li, Y., Sun, L. Q. (2002a) Cellular uptake, distribution, and stability of 10–23 deoxyribozymes. *Antisense Nucleic Acid Drug Dev*. **12**: 289–299

Dass, C. R., Walker, T. L., Burton, M. A. (2002b) Liposomes containing cationic dimethyl dioctadecyl ammonium bromide: formulation, quality control, and lipofection efficiency. *Drug Deliv*. **9**: 11–18

Dass, C. R., Nadesapillai, A. P., Robin, D., Howard, M. L., Fisher, J. L., Zhou, H., Choong, P. F. (2005) Downregulation of uPAR confirms link in growth and metastasis of osteosarcoma. *Clin. Exp. Metastasis* **22**: 643–652

Dass, C. R., Ek, E. T., Contreras, K. G., Choong, P. F. (2006) A novel orthotopic murine model provides insights into cellular and molecular characteristics contributing to human osteosarcoma. *Clin. Exp. Metastasis* **23**: 367–380

Dass, C. R., Contreras, K. G., Dunstan, D. E., Choong, P. F. (2007) Chitosan microparticles encapsulating PEDF plasmid demonstrate efficacy in an orthotopic metastatic model of osteosarcoma. *Biomaterials* **28**: 3026–3033

de Bock, C. E., Lin, Z., Itoh, T., Morris, D., Murrell, G., Wang, Y. (2005) Inhibition of urokinase receptor gene expression and cell invasion by anti-uPAR DNAzymes in osteosarcoma cells. *FEBS J*. **272**: 3572–3582

de Wilt, J. H., Bout, A., Eggermont, A. M., van Tiel, S. T., de Vries, M. W., ten Hagen, T. L., de Roos, W. K., Valerio, D., van der Kaaden, M. E. (2001) Adenovirus-mediated interleukin 3 beta gene transfer by isolated limb perfusion inhibits growth of limb sarcoma in rats. *Hum. Gene Ther*. **12**: 489–502

Densmore, C. L., Kleinerman, E. S., Gautam, A., Jia, S. F., Xu, B., Worth, L. L., Waldrep, J. C., Fung, Y. K., T'Ang, A., Knight, V. (2001) Growth suppression of established human osteosarcoma lung metastases in mice by aerosol gene therapy with PEI-p53 complexes. *Cancer Gene Ther*. **8**: 619–627

Duan, X., Jia, S. F., Koshkina, N., Kleinerman, E. S. (2006) Intranasal interleukin-12 gene therapy enhanced the activity of ifosfamide against osteosarcoma lung metastases. *Cancer* **106**: 1382–1388

Dutour, A., Rabinovich-Chable, H., Kaletta, C., Michaelis, U., Fiorenza, F., Sturtz, F., Rigaud, M. (2004) Is troponin I gene therapy effective for osteosarcoma treatment? Study on a human-like orthotopic rat model. *Anticancer Res*. **24**: 3977–3982

Dutour, A., Monteil, J., Paraf, F., Charissoux, J. L., Kaletta, C., Sauer, B., Naujoks, K., Rigaud, M. (2005) Endostatin cDNA/cationic liposome complexes as a promising therapy to prevent lung metastases in osteosarcoma: study in a human-like rat orthotopic tumor. *Mol. Ther*. **11**: 311–319

Ek, E. T., Choong, P. F. (2006) The role of high-dose therapy and autologous stem cell transplantation for pediatric bone and soft tissue sarcomas. *Exp. Rev. Anticancer Ther*. **6**: 225–237

Ek, E. T., Dass, C. R., Contreras, K. G., Choong, P. F. (2007) Pigment epithelium-derived factor overexpression inhibits orthotopic osteosarcoma growth, angiogenesis and metastasis. *Cancer Gene Ther*. May 4 [Epub ahead of print]

Eyetech Study Group. (2002) Preclinical and phase 1A clinical evaluation of an anti-VEGF pegylated aptamer (EYE001) for the treatment of exudative age-related macular degeneration. *Retina* **22**: 143–152

Fahmy, R. G., Dass, C. R., Sun, L. Q., Chesterman, C. N., Khachigian, L. M. (2003) Transcription factor Egr-1 supports FGF-dependent angiogenesis during neovascularization and tumor growth. *Nat. Med*. **9**: 1026–1032

Favaro, E., Indraccolo, S. (2007) Gene therapy of cancer in the clinic: good news in sight from Asia? *Curr. Opin. Mol. Ther*. **9**: 477–482

Fisher, J. L., Mackie, P. S., Howard, M. L., Zhou, H., Choong, P. F. (2001) The expression of the urokinase plasminogen activator system in metastatic murine osteosarcoma: an in vivo mouse model. *Clin. Cancer Res*. **7**: 1654–1660

Florenes, V. A., Maelandsmo, G. M., Forus, A., Andreassen, A., Myklebost, O., Fodstad, O. (1994) MDM2 gene amplification and transcript levels in human sarcomas: relationship to TP53 gene status. *J. Natl Cancer Inst*. **86**: 1297–1302

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Le Deist, F., Fischer, A., Cavazzana-Calvo, M. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* **302**: 415–419

Hellwinkel, O. J., Muller, J., Pollmann, A., Kabisch, H. (2005) Osteosarcoma cell lines display variable individual reactions on wildtype p53 and Rb tumour-suppressor transgenes. *J. Gene Med*. **7**: 407–419

Jia, S. F., Worth, L. L., Densmore, C. L., Xu, B., Zhou, Z., Kleinerman, E. S. (2002) Eradication of osteosarcoma lung metastases following intranasal interleukin-12 gene therapy using a nonviral polyethylenimine vector. *Cancer Gene Ther*. **9**: 260–206

Kaiser, J. (2007) Clinical research. Death prompts a review of gene therapy vector. *Science* **317**: 580

Kawakami, S., Hashida, M. (2007) Targeted delivery systems of small interfering RNA by systemic administration. *Drug Metab. Pharmacokinet*. **22**: 142–151

Ko, D., Hawkins, L., Yu, D. C. (2005) Development of transcriptionally regulated oncolytic adenoviruses. *Oncogene* **24**: 7763–7774

Lee, T. W. R., Matthews, D. A., Blair, G. E. (2005) Novel molecular approaches to cystic fibrosis gene therapy. *Biochem. J*. **387**: 1–15

Li, X., Jung, C., Liu, Y. H., Bae, K. H., Zhang, Y. P., Zhang, H. J., Vanderputten, D., Jeng, M. H., Gardner, T. A., Kao, C. (2006) Anti-tumor efficacy of a transcriptional replication-competent adenovirus, Ad-OC-E1a, for osteosarcoma pulmonary metastasis. *J. Gene Med*. **8**: 679–689

Link, M. P., Goorin, A. M., Miser, A. W., Green, A. A., Pratt, C. B., Belasco, J. B., Pritchard, J., Malpas, J. S., Baker, A. R., Kirkpatrick, J. A. (1986) The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N. Engl. J. Med*. **314**: 1600–1606

Luu, H. H., Kang, Q., Park, J. K., Si, W., Luo, Q., Jiang, W., Yin, H., Montag, A. G., Simon, M. A., Peabody, T. D., Haydon, R. C., Rinker-Schaeffer, C. W., He, T. C. (2005) An orthotopic model of human osteosarcoma growth and spontaneous pulmonary metastasis. *Clin. Exp. Metastasis* **22**: 319–329

Majumdar, A. S., Hughes, D. E., Lichtsteiner, S. P., Wang, Z., Lebkowski, J. S., Vasserot, A. P. (2001) The telomerase reverse transcriptase promoter drives efficacious tumor suicide gene therapy while preventing hepatotoxicity encountered with constitutive promoters. *Gene Ther*. **8**: 568–578

Mankin, H. J., Hornicek, F. J., Rosenberg, A. E. Harmon, D. C., Gebhardt, M. C. (2004) Survival data for 648 patients with osteosarcoma treated at one institution. *Clin. Orthop*. **429**: 286–291

Miller, C. W., Aslo, A., Tsay, C., Slamon, D., Ishizaki, K., Toguchida, J., Yamamuro, T., Lampkin, B., Koeffler, H. P. (1990) Frequency and structure of p53 rearrangements in human osteosarcoma. *Cancer Res*. **50**: 7950–7954

Miller, C. W., Aslo, A., Won, A., Tan, M., Lampkin, B., Koeffler, H. P. (1996) Alterations of the p53, Rb and MDM2 genes in osteosarcoma. *J. Cancer Res. Clin. Oncol*. **122**: 559–565

Mitchell, A., Dass, C. R., Sun, L. Q., Khachigian, L. M. (2004) Inhibition of human breast carcinoma proliferation, migration, chemoinvasion and solid tumour growth by DNAzymes targeting the zinc finger transcription factor EGR-1. *Nucleic Acids Res*. **32**: 3065–3069

Oosterhoff, D., Witlox, M. A., van Beusechem, V. W., Haisma, H. J., Schaap, G. R., Bras, J., Kruyt, F. A., Molenaar, B., Boven, E., Wuisman, P. I., Pinedo, H. M., Gerritsen, W. R. (2003) Gene-directed enzyme prodrug therapy for osteosarcoma: sensitization to CPT-11 in vitro and in vivo by adenoviral delivery of a gene encoding secreted carboxylesterase-2. *Mol. Cancer Ther*. **2**: 765–771

Paz-Ares, L., Douillard, J. Y., Koralewski, P., Manegold, C., Smit, E. F., Reyes, J. M., Chang, G. C., John, W. J., Peterson, P. M., Obasaju, C. K., Lahn, M., Gandara, D. R. (2006) Phase III study of gemcitabine and cisplatin with or without aprinocarsen, a protein kinase C-alpha antisense oligonucleotide, in patients with advanced-stage non-small-cell lung cancer. *J. Clin. Oncol*. **24**: 1428–1434

Perry, C. M., Balfour, J. A. (1999) Fomivirsen. *Drugs* **57**: 375–380

Phelan, A., Elliott, G., O'Hare, P. (1998) Intercellular delivery of functional p53 by the herpesvirus protein VP22. *Nat. Biotechnol*. **16**: 440–443

Pollmann, A., Kabisch, H., Block, A., Muller, J., Hellwinkel, O. J. (2004) Limited specificity of promoter constructs for gene therapy in osteosarcoma. *Int. J. Mol. Med*. **14**: 737–742

Ragland, B. D., Bell, W. C., Lopez, R. R., Siegal, G. P. (2002) Cytogenetics and molecular biology of osteosarcoma. *Lab. Invest*. **82**: 365–373

Ramnaraine, M., Pan, W., Goblirsch, M., Lynch, C., Lewis, V., Orchard, P., Mantyh, P., Clohisy, D. R. (2003) Direct and bystander killing of sarcomas by novel cytosine deaminase fusion gene. *Cancer Res*. **63**: 6847–6854

Ramnaraine, M., Pan, W., Clohisy, D. R. (2006) Osteoclasts direct bystander killing of cancer cells in vitro. *Bone* **38**: 4–12

Raper, S. E., Yudkoff, M., Chirmule, N., Gao, G. P., Nunes, F., Haskal, Z. J., Furth, E. E., Propert, K. J., Robinson, M. B., Magosin, S., Simoes, H., Speicher, L., Hughes, J., Tazelaar, J., Wivel, N. A., Wilson, J. M., Batshaw, M. L. (2002) A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. *Hum. Gene Ther*. **13**: 163–175

Ritch, P., Rudin, C. M., Bitran, J. D., Edelman, M. J., Makalinao, A., Irwin, D., Lilenbaum, R., Peterson, P., John, W. J. (2006) Phase II study of PKC-alpha antisense oligonucleotide aprinocarsen in combination with gemcitabine and carboplatin in patients with advanced non-small cell lung cancer. *Lung Cancer* **52**: 173–180

Shirakawa, T., Ko, S. C., Gardner, T. A., Cheon, J., Miyamoto, T., Gotoh, A., Chung, L. W., Kao, C. (1998) In vivo suppression of osteosarcoma pulmonary metastasis with intravenous osteocalcin promoter-based toxic gene therapy. *Cancer Gene Ther*. **5**: 274–280

Stiller, C. A., Craft, A. W., Corazziari, I. (2001) Survival of children with bone sarcoma in Europe since 1978: results from the EUROCARE study. *Eur. J. Cancer* **37**: 760–766

Ternovoi, V. V., Curiel, D. T., Smith, B. F., Siegal, G. P. (2006) Adenovirus-mediated p53 tumor suppressor gene therapy of osteosarcoma. *Lab. Invest*. **86**: 748–766

Tsuji, H., Kawaguchi, S., Wada, T., Nagoya, S., Inobe, M., Yamashita, T., Ishii, S., Uede, T. (2002) Adenovirus-mediated in vivo B7-1 gene transfer induces anti-tumor immunity against pre-established primary tumor and pulmonary metastasis of rat osteosarcoma. *Cancer Gene Ther*. **9**: 747–755

Wang, H., Zeng, X., Oliver, P., Le, L. P., Chen, J., Chen, L., Zhou, W., Agrawal, S., Zhang, R. (1999) MDM2 oncogene as a

target for cancer therapy: an antisense approach. *Int. J. Oncol*. **15**: 653–660

Whelan, J. S. (1997) Osteosarcoma. *Eur. J. Cancer* **33**: 1611–1618

Worth, L. L., Jia, S. F., Zhou, Z., Chen, L., Kleinerman, E. S. (2000) Intranasal therapy with an adenoviral vector containing the murine interleukin-12 gene eradicates osteosarcoma lung metastases. *Clin. Cancer Res*. **6**: 3713–3718

Zhang, G., Dass, C. R., Sumithran, E., Di Girolamo, N., Sun, L. Q., Khachigian, L. M. (2004) Effect of deoxyribozymes targeting c-Jun on solid tumor growth and angiogenesis in rodents. *J. Natl Cancer Inst*. **96**: 683–696