7 Lipid-based Nanocarriers for Cancer Gene Therapy

Rajagopal Ramesh, Manish Shanker, Jiankang Jin, Sarah J. West and Jack A. Roth

7.1 Introduction

Cancer is the second most common cause of death in the US. The American Cancer Society (http://www.acs.org/) estimated that about 1,479,350 new cases of cancer, excluding noninvasive cancer and basal and squamous cell skin cancers, were diagnosed in 2009 [1]. About 562,340 of the patients diagnosed with cancer will die of the disease. Additionally, in the US, the lifetime risk of developing cancer for men is less than one in two, while for women the risk is more than one in three [1]. Although the incidence of cancer is very high, the occurrence of certain types of cancer could be greatly reduced by minimising the exposure to external factors (tobacco, chemicals, radiation, alcohol, infectious organisms). For example, the incidence of alcohol- and tobacco-related cancers could be reduced by discontinuing their use. Behavioural changes could also significantly reduce the development of certain types of cancer, such as cancer of the liver and cervix. Reducing exposure to the sun and to indoor tanning devices could similarly decrease the incidence of skin cancer.

By utilising modern screening methods for early detection and dissemination of cancer awareness, a reduction in the incidence of certain cancers has been observed. For example, stopping the use of cigarettes has reduced the incidence of lung cancer in men. However, in recent years, an increasing trend of cigarette smoking with corresponding higher incidence of lung cancer has been observed in women, indicating the need for the development of more robust methods for preventing lung cancer incidence in women.

Development of new therapies has also contributed to improvements in the 5-year survival rates of cancer patients. The 5-year survival rate for all cancers diagnosed from 1996 to 2004 was 66% compared to the survival rate of 50% for the period 1975–1977 [1]. Despite the advances made in cancer prevention and therapy, the overall 5-year survival rates for certain types of cancer, especially those of epithelial origin, are less than 15% [2]. These results suggest that, in addition to improving cancer screening and diagnostic methods, new and effective treatments are warranted.
A novel and attractive form of cancer treatment is gene therapy, which relies on transferring therapeutic genes to the tumour. Several gene therapy-based clinical trials have been conducted for treatment of numerous human diseases including cancer [3]. The most widely tested gene delivery vehicle has been adenovirus (Ad) due to its ability to transfect both dividing and nondividing cells with high efficiency and with a low probability of nonhomologous recombination with the host genome. Ad-based gene transfer strategies with the \( p53 \) tumour suppressor gene (TSG) have shown clinical promise in phase I/II trials [4–7]. However, the drawbacks of Ad-based gene therapy are the ability of the virus to induce host immune response and toxicity and the potential to produce infectious virus [8–11]. As a result, Ad-based cancer therapy has been limited to locoregional treatment and it is ineffective as systemic delivery vehicle for the treatment of metastatic cancers [10, 11]. Therefore, there is still a need to overcome the inability to treat metastatic disease. An alternative to adenoviral vectors is the nonviral nanocarrier-based gene delivery system, which was shown effective in delivering therapeutic genes to metastatic sites when administered systemically [3, 12]. The advantage of using nonviral nanocarrier systems is that they are easy to manufacture and they help avoid the problems often encountered with Ad [12, 13].

The ideal strategy envisioned for cancer treatment is a nanocarrier that systemically delivers a therapeutic gene to the primary tumour site and to distant metastatic sites without undue toxicity to normal tissues. Development of such effective systemic therapies will have broad applications in cancer treatment.

In the following sections, we will discuss some of the lipid-based nanocarriers that have been developed and tested in preclinical studies followed by a discussion on nanocarriers that are being tested in the clinic for cancer gene therapy.

### 7.2 Preclinical Studies Testing Lipid-based Nanocarriers for Cancer Gene Therapy

Lipid-based nanocarriers are small particles of 5–500 nm in size and can be produced from various lipids or lipid mixtures that can vary in charge (positive, negative and neutral), fluidity and packing geometry. A majority of the nanocarriers currently being developed are used in diagnosis and detection of a disease [14–19]. However, nanocarriers are also being tested to deliver therapeutic genes to treat diseases such as cancer [20–22].

Numerous studies using cationic, neutral and anionic lipids have documented the potential use of lipid-based nanocarriers for gene transfer [23–29]. However, the results
from a majority of these studies have established cationic lipids to be the preferred lipid component for nucleic acid delivery, owing to the high efficiency of nucleic acid transfer associated with these lipid formulations. Often, cationic lipids are mixed with neutral lipids to increase the rigidity and stability of the nanocarrier. However, the usefulness of these cationic lipid-based formulations for in vivo gene delivery has been limited by inflammation-associated toxicity induced by the nanocarrier and their poor stability in vivo [30–34]. The interaction between the lipid component of the nanocarrier and serum proteins has a dramatic negative impact on the stability of the nanocarrier [35, 36].

Cationic lipid-based nanocarriers attract and bind negatively charged serum proteins, leading to their rapid removal from circulation by macrophages and the reticuloendothelial system (RES). As a result, the majority of the in vivo nanocarrier delivery systems use subcutaneous (SC), intradermal, intratumoral (IT) or intraperitoneal (IP) injection to avoid the inflammation-associated toxicity and stability problems associated with cationic nanocarriers in circulation [37]. The interaction between lipid nanocarriers and plasma proteins is responsible for the disparity between the efficiency of in vitro and in vivo gene transfer [38–45].

To improve the in vivo stability of cationic lipid nanocarriers and increase their accumulation in the tumour, PEGylating the outer surface of the nanocarrier using polyethylene glycol (PEG) has been investigated. Studies have shown that PEGylation does improve the stability and half-life of cationic lipid nanocarriers in vivo and evades the host immune system resulting in increased accumulation of the drug in the tumour and enhanced therapeutic efficacy [46, 47]. Another approach taken to minimise the interaction between positively charged lipid nanocarrier and serum proteins is to use neutral or anionic lipids in formulating the nanocarrier rather than cationic lipids [47]. Several reports using neutral and anionic lipid formulations have demonstrated increased gene delivery to the tumour and enhanced therapeutic effect and have attributed these observations to the ability of the nanocarriers to escape serum protein interaction and RES clearance [48–50]. Although neutral and anionic lipids have shown effective gene delivery, these lipid formulations are usually used to deliver siRNA, antisense oligonucleotides and chemotherapeutic drugs for cancer treatment [51–54]. Several chemotherapeutic drugs formulated in these neutral or anionic lipid formulations are routinely used in the clinic. Despite the advances made with neutral and anionic lipid-based nanocarriers, they have not been developed and tested as gene delivery vehicles for clinical cancer gene therapy.

One cationic lipid nanocarrier that has been tested as gene transfer vehicle and shown to be stable in vivo is the N-[1-(2, 3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP):cholesterol (DOTAP:Chol) lipid formulation. Early studies in murine models using DOTAP:Chol nanocarrier at an equimolar ratio demonstrated
Rajagopal Ramesh et al.

that these nanocarriers are efficient in vivo nucleic acid transfer vehicles [24–26]. Furthermore, intravenous (IV) administration of a plasmid DNA carrying the chloramphenicol acetyltransferase (CAT) reporter gene and encapsulated in the DOTAP:Chol nanocarrier (DOTAP:Chol-CAT) resulted in preferential transgene expression in the mouse lung with lower expression levels in a variety of other tissues [24]. Systemic administration of DOTAP:Chol nanocarrier containing the luciferase (Luc) marker gene showed preferential accumulation of the particles in the lung as noted by higher expression of Luc [55]. Luc expression was initially observed in both the lung and liver. However, Luc expression increased in the lung over a period of 24 hours, while it was lost in the liver, demonstrating that retention and expression of transgene continues in the lung over an extended period. Loss of Luc expression in the liver is likely due to rapid RES-mediated clearance of the nanocarrier.

Size fractionation studies suggested that nanocarriers in the range of 200–450 nm in size were optimal for nucleic acid transfer. However, more recent studies suggest that nanocarriers that are 10–100 nm are better for IV delivery and they escape the RES, thereby improving gene delivery and transfer [56, 57]. Nanocarriers that are less than 100 nm in size appear to be more efficiently taken up by tumour cells, while nanocarriers that are greater than 400 nm in size are efficiently taken up by the host macrophages and cleared rapidly. Nanocarriers whose size is in the range of 100–400 nm appear to strike a balance between tumour uptake and macrophage clearance. Although the size of the nanocarrier is critical and important for efficient gene delivery, it should be noted that for different cancers and diseases, the particle size will likely need to be modified. Furthermore, studies comparing nanocarriers of different sizes and formulations in a broad range of tumour models are warranted prior to making a conclusion on the size of the nanocarriers needed for efficient gene transfer in humans diagnosed with cancer.

Crook and co-workers [58] reported that DOTAP when mixed with cholesterol increased the in vitro delivery of nucleic acids in the presence of serum. The amount of serum proteins associating with the DOTAP:Chol nanocarrier was determined to be equivalent in DOTAP:Chol and DOTAP nanocarriers. The increase in nucleic acid transfer was attributed to the increased binding of the cholesterol-containing nanocarrier. The presence of cholesterol in the formulation stabilises the nanocarrier against serum protein disruption and supports its use as an in vivo nucleic acid delivery vehicle. Thus, the DOTAP:Chol nanocarrier achieves a balance between toxicity and in vivo nucleic acid transfer efficiencies compared to other cationic nanocarriers [24–26].

Based on these reports, we have tested the use of DOTAP:Chol nanocarrier as a local and systemic gene delivery vector in experimental mouse models. Initial studies were focused on IT treatments with a marker gene to determine the efficiency of gene
transfer. IT administration of β-galactosidase (β-gal) marker gene into SC H1299 lung tumour xenograft showed about 15–20% transduction, as determined by the transgene expression in the tumour tissue (Figure 7.1) [26]. This degree of transgene expression was unexpected as nonviral gene transfer and expression in vivo has been reported to be very low and in the range of 5–10%.

It is unclear how the DOTAP:Chol nanocarrier formulation provides such high-efficiency gene transfer and expression, but nevertheless our findings were encouraging to conduct additional in vivo studies. Subsequent therapeutic studies from our laboratory showed that daily IT treatment of p53 gene encapsulated in the DOTAP:Chol nanocarrier for a total of six doses resulted in suppression of SC lung tumour growth [26]. Tumour suppression was accompanied by tumour cell apoptosis. Additionally, the DOTAP:Chol-p53 nanocarrier treatment was shown to be effective in lung tumours irrespective of the endogenous p53 status (wild type, mutant or null).

To determine whether the IT treatment approach was restricted to p53 therapy, we conducted studies testing additional TSG such as Fhit, Fus1 and the melanoma differentiation-associated gene-7 (mda-7)/interleukin (IL)-24 in lung cancer [26, 59, 60]. In these studies, a significant delay in tumour growth was observed in mice whose tumours were treated with the nanocarrier containing the appropriate therapeutic gene (Figure 7.2).

The effective tumour suppression produced by the individual TSG varied, with complete tumour regression observed in a few mice that were treated with mda-7/IL-24. These results provide support for applying DOTAP:Chol nanocarrier-based gene therapy for treatment of localised tumours that are unresectable and have failed to respond to conventional chemotherapy or radiotherapy.
We next investigated whether DOTAP:Chol nanocarrier was effective in systemically delivering TSG to experimental lung metastasis established in a nude mouse model. Prior to the start of the efficacy studies, we conducted marker gene studies. Human A549 lung tumour-bearing nude mice were injected with DOTAP:Chol nanocarrier containing β-gal gene IV via the tail vein. At 24 hours after injection of the nanocarrier, the mice were euthanised and tumour-bearing lungs were isolated and stained for β-gal expression. As shown in Figure 7.3, tumours in the lung stained for β-gal as evidenced by the blue staining of pulmonary tumour nodules.

This study showed successful gene transfer and transgene expression in the lung tumours after systemic administration of DOTAP:Chol nanocarrier.

Subsequent studies focused on delivering therapeutic TSG for treatment of experimental lung metastasis. IV administration of DOTAP:Chol-p53 nanocarrier to nude mice bearing experimental human lung tumour metastasis resulted in marked reduction in the number of tumour nodules in the lung and increased animal survival [26]. The therapeutic effect on lung tumour suppression was also observed when other TSG such as the Fhit gene were used (Figure 7.4), indicating that DOTAP:Chol nanocarrier can be used for delivering several therapeutic genes.
**Figure 7.3** Lung tumour nodules from mice treated IV with DOTAP:Chol nanocarrier containing the β-gal gene stain positive for β-gal

**Figure 7.4** Gross and histopathological photographs showing that IV delivery of DOTAP:Chol nanocarrier containing Fhit TSG suppresses lung tumour growth as evidenced by the reduction in the number of tumour nodules compared to the tumour nodules in the lungs of mice that were untreated or treated with a nanocarrier containing control plasmid DNA
Furthermore, repeated treatments in mice showed an increase in the transgene expression level suggesting additive effects. One argument frequently made is that when repeated treatments are spanned across less than a 3-day interval, transgene expression is reduced or shut off due to induction of host inflammatory response [61]. The findings from these studies are in contrast to our findings, and the discrepancy in the study results was argued to be due to the use of immunodeficient mice in our studies, which likely produced reduced inflammatory response against the nanocarrier, and as a consequence transgene expression was unaffected after repeated treatments. The majority of the studies conducted by other laboratories used immunocompetent mice to demonstrate the inhibitory effects of successive repeated treatments on transgene expression.

To investigate if difference in mouse strain contributed to different outcomes regarding the effect of repeated treatments on transgene expression, we conducted studies in an immunocompetent C3H mouse model bearing syngeneic tumours. Experimental lung tumours were established in C3H mice by injecting UV2237 fibrosarcoma. Treatment of these tumour-bearing mice showed a dose-dependent increase in p53 transgene expression with further increases in transgene expression after each treatment. These results showed that repeated treatments with a therapeutic gene contained in DOTAP:Chol nanocarrier are feasible, and the transgene expression additively increases with multiple treatments irrespective of the mouse strain [26]. However, one major observation was that in all of our studies we have used mice bearing experimental lung metastasis while studies from other laboratories often used non-tumour-bearing mice. This led us to speculate that the host pathology could contribute in regulating the inflammatory response.

We hypothesised that the immune status of mice bearing in situ tumours is likely altered and less functional than the immune status of mice that do not have tumours. We also hypothesised that in tumour-bearing mice inflammatory response is reduced compared to mice that do not have tumours. To test our hypothesis, we conducted studies comparing tumour-bearing (TB) and non-tumour-bearing (NTB) immunocompetent mice and measured the inflammatory response to the DOTAP:Chol nanocarrier carrying a plasmid DNA. The inflammatory response as determined by the cytokine profile and alveolar macrophage activity was markedly reduced in TB mice compared to NTB mice [62]. Furthermore, the plasmid DNA dose required to induce inflammatory response in TB mice equivalent to the level of inflammatory response produced in NTB mice was much higher. For the first time, our results demonstrated that the host pathology status plays a role in regulating the inflammatory response induced by lipid-based nanocarriers.

On the basis of our findings, the therapeutic effects and efficacy of systemic delivery of DOTAP:Chol nanocarrier carrying TSG (p53, Fhit, Fus1, mda-7/IL-24) were
investigated in the laboratory [26, 59, 60]. IV administration of DOTAP:Chol nanocarrier containing \( p53 \) into experimental lung tumour-bearing mice for a total of six doses resulted in effective gene delivery to the tumours and in a therapeutic effect as determined by a marked reduction in the number of lung tumour nodules and increased animal survival [26]. We showed that the nanocarrier was selectively and increasingly taken up by the tumours when compared to the surrounding normal lung tissues, resulting in increased transgene expression in the tumours and enhanced therapeutic effect [63]. The tumour size in the lung dictated the amount of nanocarrier uptake, with larger tumours showing increased uptake of the nanocarrier compared to smaller tumours. We also showed that these DNA-containing nanocarriers induced no significant toxicity in TB mice at the plasmid DNA doses used. Our results demonstrated that, in addition to size, shape and charge of the nanocarrier, other factors such as host immune status and pathology, and tumour size also contribute to overall uptake of the nanocarrier, transgene expression and therapeutic efficacy.

Based on our preclinical findings demonstrating the systemic use of DOTAP:Chol nanocarrier-based gene delivery for cancer therapy, we at the M.D. Anderson Cancer Center have recently initiated a phase I clinical trial for the systemic treatment of non-small cell lung cancer (NSCLC), which is discussed in Section 7.3.

Although studies from our laboratory have shown that DOTAP:Chol nanocarrier is useful for cancer gene therapy and is safe with no major toxicity at the plasmid DNA doses tested, DNA concentrations above 50 \( \mu \)g showed inflammation-associated toxicity \textit{in vivo} (unpublished data). A dose-dependent increase in toxicity with acute toxicity and death of mice occurring at 100 \( \mu \)g DNA concentration was observed. Our findings concurred with reports from other laboratories [31, 32]. The molecular phenomenon underlying lipid nanocarrier-induced toxicity has been attributed to the presence of CpG motifs in the plasmid backbone [64–66]. Studies have shown that reducing the CpG motifs or modifying the CpG motifs with synthetic sequences reduced the inflammatory response [67, 68]. However, despite the modifications made to the CpG sequences and the plasmid backbone, the inflammatory response is not completely abolished [69]. There are reports that lipid nanocarrier-mediated induction of acute inflammatory response can occur in the absence of any CpG motif, suggesting that CpG is not the sole contributor for inducing inflammation [70, 71]. To date, the underlying molecular mechanism by which lipid nanocarrier containing plasmid DNA elicits inflammatory response remains unclear and is an active area of research in several laboratories.

While laboratories developing nanocarrier-based gene therapy for cancer are studying the role of CpG in inflammation, studies in our laboratory have focused on alternative methods to overcome nanocarrier-induced inflammatory response, even in the presence of CpG sequences in the plasmid DNA. The rationale is that by developing
new approaches to overcome CpG-induced inflammatory response one may be able to translate and apply the lipid nanocarrier-based gene delivery in the clinic. Treatment of normal lung fibroblast cells with DOTAP:Chol nanocarrier containing a plasmid DNA induced inflammation-associated signalling pathways resulting in the release of proinflammatory cytokines. Induction of the inflammatory response occurred as early as 2 hours after treatment. However, in the presence of anti-inflammatory agents, such as naproxen, the nanocarrier-mediated inflammatory response was abrogated [30]. Similar observations were made when additional anti-inflammatory agents such as ibuprofen, sulindac sulfate and celecoxib were used to determine their inhibitory effects on nanocarrier-induced inflammation (unpublished data). To further test if naproxen could suppress the inflammatory response \textit{in vivo}, immunocompetent C3H mice were either pretreated or not pretreated with naproxen followed by IV administration of DOTAP:Chol nanocarrier containing a lethal dose (100 μg) of plasmid DNA. The group of mice not pretreated with naproxen was susceptible to the acute inflammatory response and showed signs of morbidity and eventually mortality, which often occurred within 24 hours of nanocarrier injection. However, the group of mice that was pretreated with naproxen tolerated the lethal dose with no signs of toxicity even after 2 weeks (unpublished data). These results demonstrated that use of anti-inflammatory agents could overcome lipid nanocarrier-mediated inflammatory response. By using plasmid DNA with reduced CpG motifs and combining with the anti-inflammatory agent, it is expected that the inflammatory response will be completely abrogated and the therapy will be safer for clinical use.

7.3 Clinical Studies Testing Lipid-based Nanocarriers for Cancer Gene Therapy

Numerous targeted and untargeted lipid-based nanocarriers, as described in Section 7.2, have been developed and tested as gene delivery vehicles and have been shown to effectively deliver therapeutic genes and produce anti-cancer effects [26–30, 72–74]. However, of the many tested and promising nanocarriers in preclinical studies, only a few have successfully been translated to the clinic. The inability to test many of the nanocarriers in the clinic could be attributed to numerous reasons, among which the prime factors are toxicity, stability and inability to be produced in large scale for clinical testing. Additionally, despite the development of methods such as PEGylation of the vector to reduce toxicity and increase \textit{in vivo} stability, complete elimination of toxicity has not been achieved and has hampered the testing of these nonviral vectors in the clinic.

All of the nanocarriers that have entered phase I clinical trial to date are cationic lipid-based formulations and have been tested against solid tumours and via IP or
IV routes. The first lipid-based nanocarrier to be tested in the clinic was formulated with cationic 3\([N-(N’N’-dimethylaminoethane)-carbamoyl]cholesterol\) (DC-Chol) in combination with dioleoylphosphatidylethanolamine (DOPE) for delivering \(E1A\) gene for the treatment of human ovarian and breast cancers [75, 76]. In this phase I clinical trial, \(E1A\) gene encapsulated in DC-Chol nanocarrier (DCC-\(E1A\)) was administered to patients diagnosed with ovarian cancer \((n = 12)\) or breast cancer \((n = 6)\) whose tumours either overexpressed HER-2/neu or expressed low HER-2/neu and had failed to respond to conventional therapies. DCC-\(E1A\) was administered to ovarian and breast cancer patients once a week via IP and intrapleural routes, respectively. The objective of this trial was to determine the maximum tolerated dose (MTD) of DCC-\(E1A\) and the ability to deliver \(E1A\) gene and observe any \(E1A\)-mediated HER-2/neu repression in tumour cells. Patients were treated in cohorts with initial starting DNA dose at 1.8 mg/m\(^2\) and subsequent doses increasing 100% to 3.6 and 7.2 mg/m\(^2\). The results from this clinical trial showed the MTD to be 3.6 mg/m\(^2\). The most common treatment-related toxicities observed with the highest DNA dose was nausea, fever, vomiting and discomfort at the injection site. Molecular analysis demonstrated that DCC-\(E1A\) treatment reduced HER-2/neu expression in tumour cells and induced tumour cell apoptosis [76].

Successful completion of the DCC-\(E1A\) phase I trial was followed by a multicentre phase I clinical trial testing DCC-\(E1A\) in patients with recurrent epithelial ovarian cancer that overexpressed HER-2/neu [77]. In this trial, 15 patients were recruited and treated with increasing doses (1.8, 3.6 and 7.2 mg DNA/m\(^2\)). The MTD was 3.6 mg DNA/m\(^2\) and concurred with the report by Hortobagyi and co-workers [76]. However, in this trial, no correlation between dose and biological activity was observed. The authors concluded that DCC-\(E1A\) therapy is safe and feasible but for DCC-\(E1A\) therapy to be effective it has to be combined with other conventional or novel therapies [77].

DCC nanocarrier-based \(E1A\) therapy has also been investigated as a treatment for recurrent head and neck cancer [78]. In this trial, nine patients were treated with DCC-\(E1A\) via IT administration. The DNA dose tested in this trial was 15, 30, 60 and 120 \(\mu\)g DNA/cm of tumour. Although treatment was well tolerated, most of the patients reported pain and bleeding at the injection site. No MTD was achieved in this trial. In some patients, minor tumour response and/or stabilisation of disease was observed. In a follow-up phase II trial, DCC-\(E1A\) nanotherapy was conducted in 24 patients with recurrent, unresectable head and neck cancer [79]. Patients were treated with DCC-\(E1A\) (30 \(\mu\)g/cm\(^3\)) by IT injection for a total of 10 doses over a period of 8 weeks. The results showed that DCC-\(E1A\) therapy was safe and well tolerated. Additionally, DCC-\(E1A\) therapy produced clinical responses in a few patients, which ranged from complete response \((n = 1)\) to partial response \((n = 2)\) to disease stabilisation \((n = 7)\). Although some degree of therapeutic benefits was observed, combination
of DCC-E1A therapy with other treatment modalities was warranted to produce an enhanced tumour response.

Despite the encouraging results obtained from the initial phase I/II clinical trials, there were no advances in the testing of DCC-E1A in phase II/III trials and in making DCC-E1A a clinical product.

Using the cationic 1,2-dimyristoylpropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE):DOPE formulation, a phase I clinical trial for IT delivery of IL-2 gene was performed for advanced head and neck cancer [80]. In this dose-escalating phase I clinical trial, 11 patients diagnosed with head and neck cancer were treated with the cationic lipid-based nanocarrier containing a plasmid DNA expressing IL-2 from 0.06 to 0.6 mg of plasmid DNA. The results from this trial showed that the IL-2 nanocarrier treatment was safe and well tolerated with no adverse toxicity observed. However, clinical responses were observed in only one patient. These studies have not continued or progressed to phase II clinical trial for further testing.

Another cationic lipid-based nanocarrier that is currently being tested in a phase I clinical trial is the DOTAP:Chol formulation [81]. In this clinical trial, DOTAP:Chol nanocarrier is being tested as systemic gene delivery vehicle for systemic treatment of NSCLC. This is the first systemic lipid nanocarrier-based gene therapy clinical trial in the world. The objective of this trial is to IV deliver the Fus1 TSG encapsulated in the DOTAP:Chol nanocarrier and determine the MTD. To date, patients diagnosed with metastatic stage IV NSCLC who have failed chemotherapy have been recruited in the trial and have received DOTAP:Chol-Fus1 treatment. Preliminary results show that treatment is well tolerated with no signs of treatment-related toxicity [81]. When completed, the results from this clinical trial will identify the MTD and allow conducting subsequent phase II trials with a defined Fus1 DNA dose and focus on both toxicity and therapeutic efficacy.

Based on the preclinical studies and the phase I trial testing systemic DOTAP:Chol nanocarrier delivery of Fus1 TSG in NSCLC, two additional phase I clinical trials for the treatment of pancreatic cancer, ovarian cancer and breast cancer have been approved by the Food and Drug Administration (FDA) and initiated in our institution. For treatment of pancreatic cancer, a plasmid DNA containing a mutant form of Bcl2-interacting killer (Bik) gene called BikDD that is under the control of cholecystokinin type A receptor promoter and regulated by VP16-GAL4-WPRE integrated systemic amplifier (VISA) will be used. The CVISA-BikDD plasmid encapsulated in DOTAP:Chol nanocarrier will be administered IV to determine the MTD and optimal biologically active dose and compared with the clinical response. This trial is currently open and recruiting patients. The results of this trial are expected to be known in 2012.
For the breast cancer clinical trial, patients will be treated IV with DOTAP:Chol nanocarrier containing the E1A plasmid DNA. Expression of E1A is controlled by the widely used cytomegalovirus (CMV) promoter. Use of CMV promoter entails constitutive and high gene expression levels.

Another DOTAP:Chol nanocarrier-based cancer gene therapy trial that is currently being discussed is for the treatment of metastatic melanoma. Although the phase I trial has yet to receive approval from the FDA, plans are under way to deliver the tumour suppressor/cytokine gene, IL-24, encapsulated in the DOTAP:Chol nanocarrier for systemic treatment of metastatic melanoma. This phase I trial, when approved by the FDA, will also be conducted at the M.D. Anderson Cancer Center, Houston, TX, USA.

More recently, a phase I clinical trial for systemic treatment of solid tumours with a tumour-targeted cationic lipid nanocarrier carrying the p53 TSG (SGT-53) has been initiated. This nanocarrier is formulated using DOTAP:DOPE to carry the p53 TSG and coated on the outside with a targeting moiety to achieve tumour-targeted gene delivery. Tumour-specific p53 gene delivery will be achieved via the anti-transferrin receptor single-chain antibody fragment, which is attached to the outside of the nanocarrier and targets the transferrin receptor which is overexpressed in cancer cells. The aim of this dose-escalating study trial is to determine the safety and the MTD. Patients recruited in this trial will be treated with plasmid DNA concentrations from 0.6 mg DNA per IV infusion escalating to 7.2 mg DNA per infusion. According to the treatment schedule planned patients will receive two treatments per week for a total of 5 weeks (10 treatments). Additionally, measurement of dose-related tumour response and toxicity is envisaged (http://www.clinicaltrials.gov/).

### 7.4 Conclusions

Cancer gene therapy has come a long way since the inception of gene therapy to treat genetic diseases two decades ago. During this period, our understanding of the biology of viral vectors has improved, and both their application and limitation in cancer therapy have led to the development and testing of lipid-based nanocarriers for cancer gene therapy. Although nanocarriers have several advantages over viral vectors, they also have limitations, including nanocarrier-induced inflammatory response [82]. Despite these limitations, it is exciting that a few of the lipid-based nanocarriers are currently being tested as gene delivery vehicles in clinical trials for treatment of cancer. It is also evident that cationic lipid-based nanocarriers are still the preferred vector of choice for systemic gene delivery, as evidenced by the number of cancer gene therapy clinical trials that are currently testing these nanocarriers.
When completed, the results from the phase I clinical trials will provide information leading to improvements in nanocarrier formulation, and strategies to overcome potential toxicity will be developed and their subsequent testing in phase II/III clinical trials will be undertaken. Given the rapid growth and interest in the field of nanotechnology and nanomaterials, it is anticipated that in the next few years, new and novel nanocarrier gene delivery systems will be developed and made available for testing in the clinic for cancer treatment. Some of the nanocarriers will be multifunctional in their properties and will be used for diagnosis, molecular imaging and therapy of cancer. Studies testing the utility of lipid-based nanocarriers for magnetic resonance imaging and molecular imaging of tumours have been reported [83, 84]. The use of gold nanoparticles coated on the outside with DNA and encapsulated in a cationic lipid was shown to be efficient in gene transduction and expression [85]. Using a similar concept, it is possible to develop hybrid gold nanoparticles containing an inner magnetic iron oxide core and coated on the outside with DNA, which, in turn, are encapsulated within a lipid nanocarrier that is decorated on the outside with a tumour-targeted ligand, making them multifunctional in their properties. Additionally, in the next few years, significant advances would have been made in the field of nanotechnology and nanomedicine and novel methods devised to regulate and control cationic lipid nanocarrier-mediated toxicity, thereby making more lipid-based multifunctional nanocarriers available for clinical cancer gene therapy.

Acknowledgements

Part of the studies described in this chapter were conducted in the author’s laboratory and supported by grant RO1 CA113450 from the National Institutes of Health (NIH)/National Cancer Institute (NCI).

References


Lipid-based Nanocarriers for Cancer Gene Therapy


Lipid-based Nanocarriers for Cancer Gene Therapy


