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Lung Cancer

Prevention, Management, and Emerging Therapies

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Gene-Based Therapies for Lung Cancer

John Nemunaitis and Jack Roth

Abstract Recent advances in genetics, molecular biology, molecular pharmacology, and biomolecular technology have brought targeted therapeutic opportunities to the forefront of clinical development. Physician and patient communities are highly attracted to lung cancer management opportunities that may involve a personalized approach based on utilizing a unique cancer signal with a target-specific therapy. In this chapter, we will review several advanced clinical developments involving gene-based targeted therapies in lung cancer. Discussion will focus on replacement therapies for abnormal p53 function, FUS1 mediated molecular therapy, antisense technologies, and early developments with RNA interference technology.

Keywords Gene • Molecular • Lung • Cancer therapy

Introduction

Non-small cell lung cancer (NSCLC) management over the last 10 years has significantly improved with the successful development of angiogenesis inhibitors and EGFR inhibitors. However, despite these recent additions to our oncology armament, metastatic disease patients receiving frontline treatment with doublet platinum based chemotherapy in combination with angiogenesis inhibition still have a median survival of less than 1 year. Survival of second line patients is approximately 8 months. Survival of small cell lung cancer patients treated with etoposide based chemotherapy regimes is similar to the

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survival of patients with advanced NSCLC. In patients with advanced disease, both histologic types of lung cancer have 5-year-survival rates of 1–2% regardless of treatment.

Understanding of the biomolecular basis of cancer has exploded over the last 10 years. Developments in genetics, molecular biology, molecular pharmacology, and biomolecular technology promise to dramatically alter strategies of cancer treatment. Like other cancers, NSCLC and SCLC are driven by a complex adaptive network of dynamic evolving spatial–temporal biomolecular interactions. Six essential alterations in the neoplastic physiolome collectively dictate malignant growth. These include self sufficiency, insensitivity to growth inhibition (including immune "escape"), independence from programmed cell death, unlimited replicative potential, sustained angiogenesis, and local and metastatic invasiveness (1). Although it appears intuitive that disruption of any one of these global physiologic capabilities would provide a therapeutic opportunity, each cancer is a robust system capable of maintaining its functional characteristics following internal or external perturbation (2, 3). Cancer cells are able to buffer the impact of genetic modification by virtue of having redundant functional pathways in which different structural elements have overlapping functions, termed "degeneracy" (4). Positive and negative feedback controls allow for stochastic robustness by dampening natural noise. Multileveled functional complementation results from self-contained modules at each organizational level (genome → transcriptosome → proteome → metabolome) which interrelate in a functional organizational hierarchy (5). Work is now underway to integrate theoretical and experimental programs to map out and model in quantifiable terms topological and dynamic properties of networks which control the behavior of one cell. Development of high throughput data collection techniques (i.e., microarrays) allows for simultaneous interrogation of the status of a cell's components at any given time. New technology platforms, such as protein chips and yeast two hybrid screens, help define how proteins interact with each other and will enable us to determine various types of interactive networks (protein to protein interaction, metabolic, signaling, and transcription/regulatory networks) (6, 7). Interestingly, the modulation of pathways that produce "robustness" in certain insults are generally associated with enhanced "fragility" of other perturbations (2), thereby exposing an "Achilles heel" of cancer and potentially permitting a reasoned coordinated multitarget lethal attack on the cancer (8, 9). Specifically, technologies have been developed that enable the systematic discovery of the molecular pathways thereby setting the stage for targeted therapeutics which focus on driving reduction in proliferation and tumor growth following transcriptional and translational modulation.

Efforts to improve these statistics recently have centered around a number of innovative approaches involving immune mediated anticancer effect and/or molecular inhibitory approaches. The purpose of this chapter is to summarize key molecular directed approaches in lung cancer, specifically, p53 gene therapy, antisense technology, and RNA interference technology.

Gene Therapy to Replace Genes Including Missing/Defective Tumor Suppressor Genes

Mechanism of p53 Tumor Suppression and Rationale for p53 Gene Therapy

Many studies over the past 20 years have established a genetic basis for lung cancer. Genes that suppress tumors and repair DNA can be damaged by more than 100 carcinogens contained in tobacco smoke (10). Lung cancers show multiple genetic lesions even in histologically normal bronchial mucosa from people with a smoking history. These genetic abnormalities provide an array of targets for therapy. The *p53* tumor suppressor gene appears to play a central role in lung cancer development and was the initial focus of gene therapy approaches to lung cancer.

Two tumor suppressor genes, *Rb* (retinoblastoma gene) and *p53*, which are both regulated at the protein level by oncogenes and other tumor suppressor genes, regulate cell proliferation. The Rb protein regulates the maintenance of, and release from, the G1 phase. The p53 protein monitors cellular stress and DNA damage, either causing growth arrest to facilitate DNA repair or inducing apoptosis if DNA damage is extensive (11). When a cell is stressed by oncogene activation, hypoxia, or DNA damage, an intact p53 pathway may determine whether the cell will receive a signal to arrest at the G1 stage of the cell cycle, whether DNA repair will be attempted, or whether the cell will self-destruct via apoptosis (programmed cell death). Apoptosis plays a key role in numerous normal cellular mechanisms, from embryogenesis to destruction of cells that have sustained irreparable DNA damage due to random mutations, ionizing radiation, or DNA damaging chemicals including chemotherapeutic agents. The observation that expression of a wild-type *p53* gene in a cancer cell triggers apoptosis provided the rationale for gene therapy approaches (12). Previously, it was believed that gene therapy could not replace all the damaged genes in a cancer cell, and thus would not have a significant effect. The fact that restoration of only one of the defective genes is enough to trigger apoptosis suggests that the DNA damage present in a cancer cell may prime it for an apoptotic event that can be provided through a single pathway.

The *p53* gene product is a transcription factor that plays a major role in regulating the apoptosis genes (13). p53 also downregulates the prosurvival (or antiapoptotic) genes, including the antiapoptotic genes *bcl-2* and *bcl-XL*, and upregulates the proapoptotic genes *bax*, *bad*, *bid*, *puma*, and *noxa* (14). Available transcripts of each of the pro and antiapoptotic genes with *bcl2* homology-3 domains interact with one another to form heterodimers, and the relative ratio of proapoptotic to prosurvival proteins in these heterodimers determines the activity of the resulting molecule, thereby determining whether the cell lives or undergoes apoptosis. p53 also targets the death-receptor signaling pathway, including DR5 and Fas/CD95, and the apoptosis machinery, including caspase-6, Apaf-1, and PIDD. It may also directly mediate cytochrome *c* release.

The p53 pathway is regulated at the protein level by other tumor suppressor genes and by several oncogenes (11). For example, mdm2 normally binds to the N-terminal transactivating domain of *p53*, prohibiting p53 activation and leading to its rapid degradation. In normal cells, mdm2 is inhibited by expression of *p14ARF*, a tumor suppressor gene encoded by the same gene locus as *p16INK4a* but expressed as an alternate reading frame (15). Deletion or mutation of the tumor suppressor gene *p14ARF*, which has been noted in some cancers, results in increased levels of mdm2 and subsequent inactivation of p53, resulting in an inappropriate progression through the cell cycle. The expression of *p14ARF* is induced by hyperproliferative signals from oncogenes such as *ras* and *myc*, thus indicating an important role for p53 in protecting cells from oncogene activation. Importantly, p53 also plays a central role in mediating cell cycle arrest. This function is significant, as prolonged tumor stability has often been observed in clinical trials of *p53* gene replacement, suggesting that this effect may be predominant over apoptosis in some tumors. p53 is involved in regulating cell cycle checkpoints, and p53 expression can promote cell senescence through its control of cell cycle effectors such as p21CIP1/WAF1. Loss of function in the p53 pathway is the most common alteration identified in human cancer to date. About 50% of common epithelial cancers have *p53* mutations (16–18). In some cancers, loss of *p53* also appears to be linked to resistance to conventional DNA damaging therapies that require functional cellular apoptosis to accomplish cell death.

Preclinical Studies of *p53* Gene Replacement

The studies described above suggest that expressing a wild-type *p53* gene in cancer cells defective in p53 function could mediate either apoptosis or cell growth arrest, both of which would be of therapeutic benefit to a cancer patient. Initial studies showed that restoration of functional *p53* using a retroviral vector suppressed the growth of some, but not all, human lung cancer cell lines (19). Because of limitations inherent in the use of retroviruses, subsequent studies of *p53* gene replacement in lung cancer made use of an adenoviral vector (*Ad-p53*) (20). The original adenoviral vector was a serotype 5 replication-defective vector with a deleted E1 region, which has been used in all *p53* clinical trials. The first published study of *p53* gene therapy showed suppression of tumor growth in an orthotopic human lung cancer model using a retroviral expression vector (21). This was the first study to show that restoring the function of a single tumor suppressor gene could result in the regression of human cancer cells in vivo.

Ad-p53 also induced apoptosis in cancer cells with nonfunctional *p53* without significantly affecting the proliferation of normal cells (22). Subsequent studies with *Ad-p53* demonstrated inhibition of tumor growth in a mouse model of human orthotopic lung cancer (23) and induction of apoptosis and suppression of proliferation in various other cancer cell lines and in vivo mouse xenograft tumor models (24–26). Bystander killing (killing of nontransduced cells by transduced cells), now known to be an important phenomenon in the success of gene therapy, appears to involve regulation of angiogenesis (27, 28), immune upregulation (29–31), and secretion of soluble proapoptotic proteins (32).

Clinical Trials of *p53* Gene Replacement

The first clinical trial protocol for *p53* gene-replacement utilized a replication-defective retroviral vector expressing wild-type *p53* driven by a beta-actin promoter (33). The gene/vector construct was injected into tumors of nine patients with unresectable NSCLC that had progressed after conventional therapy. Three of the nine patients showed evidence of tumor regression with no vector-related toxicity, demonstrating the feasibility and safety of *p53* gene therapy.

Subsequent *p53* clinical trials were conducted with the adenovirus *p53* vector described above. A phase I trial enrolled 28 NSCLC patients whose cancers had not responded to conventional treatments. Successful gene transfer was demonstrated in 80% of evaluable patients (34). Expression of *p53* was detected in 46% of patients, apoptosis was seen in all but one of the patients expressing the gene, and, importantly, no significant toxicity was observed. More than a 50% reduction in tumor size was observed in two patients, with one patient remaining free of tumor more than a year after concluding therapy and another experiencing nearly complete regression of a chemotherapy- and radiation-resistant upper lobe endobronchial tumor. Additional studies in patients with head and neck cancer helped to establish *Ad-p53* gene transfer as a clinically feasible strategy resulting in successful gene transfer and gene expression, low toxicity, and strong evidence of tumor regression.

Gene Replacement in Combination with Conventional DNA Damaging Agents in NSCLC

Preclinical studies of *p53* gene therapy combined with cisplatin in cultured NSCLC cells and in human xenografts in nude mice showed that sequential administration of cisplatin and *p53* gene therapy resulted in enhanced expression of the *p53* gene product (35, 36), and similar studies of *Ad-p53* gene transfer combined with radiation therapy indicated that delivery of *Ad-p53* increases the sensitivity of *p53*-deficient tumor cells to external beam radiation (26).

Many tumors are resistant to chemotherapy and radiation therapy and, therefore, fail initial therapeutic interventions. *P53*, often missing or nonfunctional in radiation- and chemotherapy-resistant tumors, is known to play a key role in detecting damage to DNA and either directing repair or inducing apoptosis. Once apoptosis was implicated as a mechanism of cell killing in response to these DNA damaging agents, it followed that a defect in the normal apoptotic pathway might confer resistance to some tumor cells. Due to *Ad-p53*'s low toxicity (less than a 5% incidence of serious adverse events) in initial trials, therapeutic strategies combining *Ad-p53* gene replacement and conventional DNA damaging therapies were logical extensions of earlier studies (37).

Clinical Trials of Tumor Suppressor Gene Replacement Combined with Chemotherapy

Twenty-four NSCLC patients with tumors previously unresponsive to conventional treatment were enrolled in a phase I trial of *p53* in sequence with cisplatin (38). Seventy-five percent of the patients had previously experienced tumor progression on cisplatin- or carboplatin-containing regimens. Up to six monthly courses of intravenous cisplatin, each followed 3 days later by intratumoral injection of *Ad-p53*, resulted in 17 patients remaining stable for at least 2 months, two patients achieving partial responses, four patients continuing to exhibit progressive disease, and one patient unevaluable due to progressive disease. Seventy-nine percent of tumor biopsies showed an increase in the number of apoptotic cells, 7% showed a decrease in apoptosis, and 14% showed no change.

A phase II clinical trial evaluated two comparable metastatic lesions in each NSCLC patient enrolled in the study (39). All patients received chemotherapy, either three cycles of carboplatin plus paclitaxel or three cycles of cisplatin plus vinorelbine, and then *Ad-p53* was injected directly into one lesion. *Ad-p53* treatment resulted in minimal vector-related toxicity and no overall increase in chemotherapy-related adverse events. Detailed statistical analysis of the data indicated that patients receiving carboplatin plus paclitaxel, the combination of drugs that provided the greatest benefit on its own, did not realize additional benefit from *Ad-p53* gene transfer. However, patients treated with the less-successful cisplatin and vinorelbine regimen experienced significantly greater mean local tumor regression, as measured by size, in the *Ad-p53*-injected lesion than in the control lesion.

Clinical Trials of p53 Gene Replacement Combined with Radiation Therapy

Preclinical studies suggesting that *p53* gene replacement might confer radiation sensitivity to some tumors (26, 40–43) led to a phase II clinical trial of *p53* gene transfer in conjunction with radiation therapy (44). Patients with a poor performance status who could not undergo surgery and would be at high risk for combined chemotherapy and radiation received 60 Gy over 6 weeks with *Ad-p53* injected on days 1, 18, and 32. Nineteen patients with localized NSCLC were treated, resulting in a complete response in one patient (5%), partial response in 11 patients (58%), stable disease in three patients (16%), and progressive disease in two patients (11%). Two patients (11%) were not evaluable due to tumor progression or early death. Three months after the completion of therapy, biopsies revealed no viable tumor in 12 patients (63%) and viable tumor in three (16%). Tumors of four patients (21%) were not biopsied because of tumor progression, early death, or weakness. The 1-year progression-free survival rate was 45.5%. Among 13 evaluable patients after 1 year, five (39%) had a complete response and three (23%) had a partial response or disease stabilization. Most treatment failures were caused by metastatic disease without local progression.

In that study, biopsies of the tumor were performed before and after treatment so that detailed studies of gene expression were possible. *Ad-p53* vector-specific DNA was detected in biopsy specimens from 9 of 12 patients with paired biopsies (days 18 and 19). The ratio of copies of *Ad-p53* vector DNA to copies of actin DNA was 0.15 or higher in eight of nine patients (range, 0.05–3.85), with four patients having a ratio >0.5. For 11 patients with adequate samples for both vector DNA and mRNA analysis, eight showed a postinjection increase in mRNA expression associated with detectable vector DNA. Postinjection increases in *p53* mRNA were detected in 11 of 12 paired biopsies obtained 24 h after *Ad-p53* injection, with 10 of 11 increasing threefold or more. Preinjection biopsy specimens that were shown by immunohistochemistry to be negative for p53 protein expression were stained for p53 protein expression after *Ad-p53* injection. Staining results confirmed that the p53 protein was expressed in the posttreatment samples in the nuclei of cancer cells. For *p21* (*CDKN1A*) mRNA, increases of statistical significance were noted 24 h after *Ad-p53* injection and during treatment, as compared with the pretreatment biopsy. *MDM2* mRNA levels were higher during treatment than before treatment. Levels of *FAS* mRNA did not change significantly during treatment. BAK mRNA expression increased significantly 24 h after injection of *Ad-p53* and thus appeared to be the marker most acutely upregulated by *Ad-p53* injection.

The safety profile for intratumoral injection of *Ad-p53* has been excellent. The most frequently reported adverse events related to treatment with *Ad-p53* injection were fever and chills, asthenia, injection site pain, nausea, and vomiting. The vast majority of these events were mild to moderate.

To date, no maximum tolerated dose for *Ad-p53* injection has been established.

Systemic Gene Therapy for Metastases

Local control of cancers is important, but most patients with lung cancer die from systemic metastases. The development of a cancer vaccine to p53 is one approach. Although the p53 protein is expressed by normal cells, it has a short half-life and is thus present at low levels. Mutant p53 is conformationally altered and resists degradation in cancer cells. Thus, it has a prolonged half-life and is expressed at high levels in cancer cells. These differences in expression between normal and cancer cells suggest that p53 could function as a tumor antigen and vaccine target (45–48). Several studies have shown in cultured cells and mouse models induction of anti-p53 cytotoxic lymphocytes that killed cancer cells but not normal cells. A strategy was developed using dendritic cells, which are the most effective antigen-presenting cells, transduced with *Ad-p53* (49).

Patients with extensive-stage small-cell lung cancer (SCLC) were entered into a trial. SCLC patients with extensive stage disease have a median survival of 2–4 months untreated or 6–8 months with chemotherapy. In that trial, the patients' autologous dendritic cells were treated ex vivo with *Ad-p53*, which activates the cells and results in the expression of high levels of p53 protein. Patients were first treated with conventional chemotherapy. Those who achieved at least stable disease

received the vaccine biweekly for a total of three to six injections. If patients progressed, they were treated with chemotherapy. Of the 29 patients treated, one had a partial response, seven had stable disease, and 21 had progression. Patients having progression then received second-line chemotherapy. Clinical follow-up was completed for 21 patients. Complete or partial responses to the second-line chemotherapy were observed in 61.9% of the 21 patients treated. Eleven of the patients were alive 1 year after the first vaccine treatment. These clinical responses were correlated with induction of immune responses to the vaccine. Published objective response rates for second-line chemotherapy in extensive-stage SCLC patients ranges from 5 to 30%.

Gene delivery to distant sites of cancer is essential for successful cancer gene therapy. Recently, the development of nanoscale synthetic particles that can encapsulate plasmid DNA and deliver it to cells after intravenous injection has been reported. This has been studied in mouse xenograft models of disseminated human lung cancer. In addition to p53, other tumor suppressor genes have been delivered using this technique. Multiple 3p21.3 genes show different degrees of tumor suppression activities in various human cancers in vitro and in preclinical animal models. One of the tumor suppressor genes at this locus is FUS1, which is not expressed in most lung cancers. When wild-type FUS1 is expressed in a lung cancer cell, apoptosis occurs. To translate these findings to clinical applications for molecular cancer therapy, we recently developed a systemic treatment strategy by using a novel FUS1-expressing plasmid vector complexed with DOTAP:cholesterol (DOTAP:Chol) liposome, termed FUS1 nanoparticle, for treating lung cancer and lung metastases (50, 51). In a preclinical trial, we showed that intratumoral administration of FUS1 nanoparticles to subcutaneous NSCLC H1299 and A549 tumor xenografts resulted in significant inhibition of tumor growth. Intravenous injections of FUS1 nanoparticles into mice bearing experimental A549 lung metastasis significantly decreased the number of metastatic tumor nodules. Lung tumor-bearing animals treated with FUS1 nanoparticles survived longer (median survival time: 80 days) than control animals. These results demonstrate the potent tumor suppressive activity of the FUS1 gene, making it a promising therapeutic agent for treatment of primary and disseminated human lung cancer (50, 51). Based on these studies, a phase I clinical trial with FUS1-mediated molecular therapy by systemic administration of FUS1 nanoparticles is now under way in stage IV lung cancer patients at The University of Texas M. D. Anderson Cancer Center in Houston, Texas.

Summary and Conclusions

Current therapy such as radiation and chemotherapy controls less than 50% of lung cancers, and overall 5-year survival is only 15%. Combining existing treatments has reached a plateau of efficacy, and the addition of conventional cytotoxic agents is limited because of toxicity. The clinical trials summarized in this article clearly demonstrate that, contrary to initial predictions that gene therapy would not be suitable for cancer, gene replacement therapy targeted to a tumor suppressor gene can cause cancer regression by activation of known pathways with minimal toxicity.

Gene expression has been documented and occurs even in the presence of an antiadenovirus immune response, clinical trials have demonstrated that direct intratumoral injection can cause tumor regression or prolonged stabilization of local disease, and the low toxicity associated with gene transfer indicates that tumor suppressor gene replacement can be readily combined with existing and future treatments. Initial concerns that the wide diversity of genetic lesions in cancer cells would prevent the application of gene therapy to cancer appear unfounded; on the contrary, correction of a single genetic lesion has resulted in significant tumor regression.

Studies using the transfer of tumor suppressor genes in combination with conventional DNA damaging treatments indicate that correction of a defect in apoptosis induction can restore sensitivity to radiation and chemotherapy in some resistant tumors, and indications that sensitivity to killing might be enhanced in already sensitive tumors may eventually lead to reduced toxicity from chemotherapy and radiation therapy. The most recent laboratory data demonstrating damage to tumor suppressor genes in normal tissue and premalignant lesions suggests that these genes could someday be useful in early intervention, diagnosis, and even prevention of cancer. Preclinical studies have shown that systemic delivery for treatment of metastases can be achieved. The ready availability of gene libraries, the ability to administer genes without the extensive reformulation required of small molecules, and their specificity make this an attractive therapeutic approach. Despite the obvious promise evident in the results of these studies, though, it is critical to recognize that there are still gaps in knowledge and technology to address. The major issues for the future development of gene therapy include:

1. Development of more efficient and less toxic gene delivery vectors for systemic gene delivery.
2. Identification of the optimal genes for various tumor types.
3. Optimizing combination therapy.
4. Monitoring gene uptake and expression by cancer cells.
5. Overcoming resistance pathways.

However, given the rapid progress in the field, it is likely that many of these technological problems will be solved in the near future.

Antisense Technology in NSCLC

Antisense oligonucleotides (AS ODNs) are unmodified or chemically modified single-stranded DNA molecules of 13–25 nucleotides in length that are designed to specifically hybridize to corresponding RNA by Watson–Crick binding. They inhibit mRNA function by several mechanisms, one, through inhibition of protein translation by disrupting ribosome assembly, and two, through utilization of endogenous RNase H enzymes that cleave the mRNA strand (52–56). The specificity of

hybridization of an AS ODN to the target mRNA makes the AS strategy attractive for selective modulation of expression of genes involved in the pathogenesis of malignant disease. One AS ODN has been approved for local therapy of cytomegalovirus (CMV) retinitis, and a number of AS ODN's are currently being tested in clinical trials. These include ODN's that target C-Raf kinase, C-Raf 1, H ras, protein kinase A-Type I, protein kinase C-alpha, bcl-2, survivin, and DNA methyltransferase (57, 58).

Most oligonucleotides being clinically tested have a phosphorothioate backbone in which one of the oxygens on the phosphate moiety is replaced with a sulfur. Phosphorothioate oligonucleotides enable mRNA degradation through enzymatic cleavage via activation of RNase H, and they carry a negative charge which has been shown to bind plasma proteins in a manner similar to heparin (59, 60). This characteristic protects them from filtration thereby prolonging product half-life. However, the negative charge has also been correlated with side effects, including thrombocytopenia and activation of the complement cascade (61). Phosphorothioates accumulate predominantly in the liver but also in the kidneys (62–64).

Protein Kinase C-α: ISIS 3521

Protein Kinase C (PKC) is a family of phospholipid-dependent cytoplasmic serine threonine kinases that comprises distinct isoenzymes which differ in their biochemical properties, tissue-specific expression, and intracellular localization (65, 66). PKC isoenzymes provide signals that lead to proliferation or differentiation (66, 67) PKC-α activity specifically appears to be involved in signaling (68) malignant transformation and proliferation. Overexpression of the PKC-α gene in breast cancer cells results in increased proliferation, anchorage-independent growth, and enhanced tumorigenicity (69). PKC-α expression is also elevated in human breast cancers (70). Inhibition of PKC-α limits growth of hepatoma (71) and medulloblastoma (72). ISIS 3521 (also designated ISI 641A) is a 20-mer phosphorothioate oligodeoxynucleotide that hybridizes to the 3′-untranslated region of the human PKC-α mRNA, resulting in a site amenable to degradation by RNase H (73). Phase I testing demonstrated acceptable safety and evidence of clinical activity (two patients with lymphoma had complete response to ISIS 3521) (74).

Early evaluation of ISIS 3521 in NSCLC involved combination with carboplatin and paclitaxel. Forty-eight evaluable patients with advanced stage IIIB or IV NSCLC were entered into trial (75). Minimal toxicity consisting of neutropenia and thrombocytopenia lead to treatment delays in less than 15% of patients. Patients received a median of six cycles and achieved a response rate of 48%, with 2% (one patient) obtaining complete response and 46% (22 patients) partial response. The median time to progression and the median survival were 6.3 and 15.9 months, respectively. A second phase II trial tested ISIS 3521 in combination with cisplatin and gemcitabine. Forty-four chemotherapy-naïve patients with advanced NSCLC were entered into trial. Toxicity was moderate but included thrombocytopenia, neutropenia, anemia, fatigue, dehydration, sepsis and neutropenic fever (76). In the updated analysis of the trial, the response rate was 37%, including one complete remission and 11 partial remissions.

Based on these phase II data, two large randomized phase III trials were initiated as first-line treatment in patients with NSCLC. The first enrolled 600 patients with stage IV NSCLC using ISIS 3521 in combination with carboplatin and paclitaxel. Results were disappointing. No difference was observed in time to progression or overall survival between treatment and control groups. There were, however, indications of antitumor activity, as a subset of patients who completed the prescribed course of ISIS 3521 (six cycles) had a median survival of 17.4 months when compared with 14.3 months in patients who did not ($p=0.048$). Negative results were also obtained in the second phase III trial involving advanced NSCLC patients testing ISIS 3521 in combination with gemcitabine and cisplatin. Therapy was well tolerated, but median survival was roughly 10 months in both groups.

Clusterin: OGX-011

Overexpression of clusterin prolongs cell survival and leads to enhanced metastatic potential of cancer cells in vitro (77). AS against clusterin significantly enhanced chemosensitivity in prostate and renal carcinoma cells in vitro (78). A phase I trial using OGX-011 for patients with localized prostate cancer has been published (79). The most frequently reported side effects were mild (grade 1 or 2) and included fevers, rigors, fatigue, and transient elevations of aspartate aminotransferase and alanine aminotransferase. A second phase I study was designed to determine the recommended dose of OGX-011 in combination with docetaxel (Taxotere™) in various solid tumors (80, 81). OGX-011 is currently in phase II development for patients with prostate, breast, and lung cancer.

Combination of OGX-011 and docetaxel in 38 patients with different solid tumors reveals a linear dose-dependent pharmacokinetics of OGX-011, with no apparent interaction with docetaxel. Similar results with OGX-011 were found in combination with cisplatin and gemcitabine. A dose-dependent increase in OGX-011 C_{max} and AUC was noted, with no apparent interaction with either chemotherapeutic (82). In another trial, OGX-011 was administered in combination with docetaxel (80). The study enrolled 38 patients with a variety of solid tumors (including NSCLC, and prostate, ovarian, renal cell, and breast cancer). A significant decrease in serum clusterin levels was observed in relation to dose of OGX-011. Of 24 patients with measurable disease, there was one patient with a partial response (PR) and eight patients with stable disease (SD). In a subsequent clinical trial involving ten chemotherapy-naïve patients with advanced NSCLC OGX-011 was administered in combination with cisplatin and gemcitabine. Two of nine patients with stable disease to prior therapy achieved a PR to OGX-011, cisplatin and gemcitabine. Toxicity primarily occurred within the first week of therapy and diminished with continued dosing. Hematological adverse effects included grade 1

leukopenia, thrombocytopenia, and anemia. Other self-limiting common adverse events were fever, fatigue and rigors occurring several hours after infusion, and grade 1 and 2 elevations in hepatic transaminase levels. No apparent dose-dependent induction of serum complement was observed (79).

H-ras: ISIS 2503

ISIS 2503 is a 20-base phosphorothioate AS ODN that binds to the translation initiation region of human H-ras mRNA (ISIS 2503) and that selectively reduced the expression of H-ras mRNA and protein in cell culture. In a phase I trial, ISIS 2503 administration was not associated with any dose-limiting toxicity. Out of 23 patients, four had stabilization of their disease for six to ten cycles of therapy. No consistent decreases in H-ras mRNA levels were observed in peripheral blood lymphocytes (83). A subsequent multicenter phase II trial analyzed ISIS 2503 in stage IIIB/IV NSCLC. Out of 20 evaluable patients, 7 achieved SD and 13 progressed within the first three cycles. There were no partial or complete responses (84). Given that limited activity was seen and most relevant mutations involving ras oncogene in NSCLC0 are K-ras rather than H-ras, further development if ISIS 2503 in NSCLC has not been done.

C-Raf-1: ISIS 5132

Raf kinases are serine/threonine kinases that regulate mitotic signaling pathways, most notably those involving the mitogen-activated protein kinase pathway signals from ras. This regulation of ras-dependent pathways by raf is potentially important since the ras oncogene is dysregulated or mutated more frequently than any other oncogene studied in human cancer (85, 86). In several tumors, including breast and NSCLC, the presence of a ras mutation is a negative prognostic factor (87). C-raf has also been reported to bind to Bcl-2 and to be involved in the regulation of apoptosis. An AS ODN directed to the 3′ untranslated region of the c-raf mRNA (ISIS 5132) inhibits growth of human tumor cell lines in vitro and in vivo in association with specific downregulation of target message expression. In a phase I trial, changes in c-raf-1 mRNA expression were analyzed in peripheral blood mononuclear cells (PBMC) collected from patients with advanced cancers treated with ISIS 1532. Significant reductions of c-raf-1 expression from baseline were detected in 13 of 14 patients. Clinical toxicities included fever and fatigue, neither of which were dose limiting. Two patients experienced prolonged disease stabilization for more than 7 months. In both of these cases, this was associated with reduction in c-raf-1 expression in PBMC. Initial results of a phase I trial testing continuous IV infusion of ISIS 5132 in 34 patients with a variety of solid tumors refractory to standard therapy reported one patient at high dose with fever as a dose-limiting toxicity

(88), three patients had grade 3 or 4 thrombocytopenia, and one patient had grade 3 leukopenia. One patient with refractory ovarian cancer had a dramatic reduction in her CA-125 level (97%), and two other patients had prolonged disease stabilization for 9 and 10 months, respectively. No objective responses were seen in a phase II trial for 22 patients with progressive lung cancer (18 NSCLC, 4 SCLC) (89). Hematological toxicity did not exceed grade 2. Nonhematological toxicity was mild to moderate. More recently, a different Raf-1 AS ODN has been developed in a new formulation called LErafAON (NeoPharm, Lake Forest, IL) (90). To avoid the need to chemically protect the oligonucleotide from degradation and to improve intracellular delivery, LErafAON has been encapsulated in a cationic liposome (90). LErafAON is undergoing phase I testing in patients with advanced solid tumors (91).

Bcl-2: Oblimersen

Oblimersen is an AS ODN which downregulates Bcl-2 protein expression. Animal studies validated mechanism, safety, and clinical opportunity (92–98). Phase I, II, and III studies have been and are being performed testing oblimersen in patients with multiple advanced cancers including lymphoma, melanoma, breast cancer, hormone-refractory prostate cancer, and a small number of lung cancer patients (99, 100).

Phase I and II trial investigation in non Hodgkin's lymphoma (101, 102) demonstrated dose-related safety. Two patients achieved complete remissions. Reduction in Bcl-2 protein as predicted was able to be demonstrated in a subset of patients (102). Fever and transient grade 3 increases in hepatic enzymes were observed. Phase III investigation involving melanoma (103) did demonstrate limited efficacy; however, it was not sufficient for the Food and Drug Administration (FDA) approval. In melanoma, the overall response rate of the combination of dacarbazine and oblimersen was 12.4% vs. 6.8% for dacarbazine alone ($p=0.0007$) (103). The median progression-free survival was 2.4 months vs. 1.6 months ($p=0.0003$), but there was only a trend toward improvement in overall median survival (9.0 months vs. 7.8 months, $p=0.077$).

Oblimersen has been tested in combination with paclitaxel and in combination with carboplatin and etoposide in advanced small cell lung cancer patients, but limited efficacy has been demonstrated (100).

Survivin: LY2181308

Survivin is a member of the IAP gene family, and has an important role in both cell division and apoptosis inhibition (104–106). Survivin is expressed at a high level in a wide range of human cancer types, including lung, colon, pancreas, breast and prostate cancers (105, 107). However, survivin is generally not expressed in normal tissue. Survivin expression levels correlate with lower apoptotic index in tumor

cells and poor prognosis in cancer patients, and serial analysis of gene expression studies have indicated that survivin is the fourth most common gene that is uniformly expressed in cancer cells but not in normal tissues (108). A novel 2′-MOE ASO (called LY2181308) has been constructed. It specifically downregulates survivin expression in a broad range of human cancer cells and has produced potent antitumor activity in human tumor xenograft models (109, 110). Antitumor activity displayed by LY2181308 in these models is oligonucleotide-sequence specific, and is associated with reduced survivin levels in tumor tissue. Clinical development of LY2181308 is moving forward.

RNA Interference

RNA interference (RNAi) is an evolutionarily conserved gene-silencing mechanism which functions during vertebrate embryonic development and is incorporated as an additional layer in the immune defense mechanism (111) whereby small sequences of intrinsic antisense RNA or extrinsic dsRNA (i.e., viral) trigger translational suppression. In cells that endogenously express a gene, introduction of siRNA molecules that target the gene triggers mRNA degradation. The degradation process occurs following interaction of siRNA with ATP dependent helicase and with the ATP dependent RNase enzyme Dicer through the formation of a "RNA interfering silencing complex" (RISC) (112). Endonucleolytic cleavage of the target mRNA occurs at a single site at the center of the target mRNA-siRNA antisense strand duplex (113) and is mediated by Slicer (Ago2) (114).

The use of synthetic siRNA molecules has gained wide acceptance as a laboratory tool for target validation, but clinical trials in oncology patients have not yet commenced. Nevertheless RNAi has gained greater acceptance in 2 years than traditional antisense oligonucleotides (ASO) and ribozymes (RBZ) achieved in 20 years (115). Unlike single-stranded RNA, duplex RNA is quite stable and does not require chemical modifications to achieve a satisfactory half-life in cell-culture media (116, 117). While antisense oligonucleotides have been tested clinically (74, 83, 88), the backbone modifications required for oligonucleotide stability increased the risk of toxicity thereby limiting administration at dose levels sufficient to induce significant tumor response, and siRNA methods could potentially avoid this problem.

Progress in the development of RNAi technology benefited from previous research aimed at optimizing traditional ASO and RBZ nucleotides. For example, cellular uptake was a major obstacle for efficient gene inhibition inside cells and lessons learned from difficulties in transfecting cells with ASO and RBZ's were applied to RNAi (118). Wide varieties of efficient delivery systems for nucleic acids have now been developed and are commercially available. In addition, researchers using traditional ASOs had already described potential pitfalls and developed criteria for the essential control experiments needed to validate preliminary data (119).

Finally, biodistribution studies of single-stranded ASOs had been performed, providing suggestions about potential target requirements for siRNA (120–122).

Antitumor Effects of RNAi

The exquisite specificity of RNAi has been utilized in multiple studies to exploit phenotypic differences between cancer cells and normal cells. The early work of Martinez and coworkers (123) demonstrated that the *p53* mutant-specific RNAi molecule can knockdown the mutant message that differed from wild type by only a single nucleotide, and restored wild type *p53* function in heterozygous tumor cells. Similarly, mutant *ras* silencing by RNAi produced an antitumor effect that nullified the oncogenic phenotype (124). Kawasaki showed that mutant *ras* decreased by 90% through RNA silencing without altering wild type messages in vitro and in vivo (125, 126). Mutant *K-ras* knockdown also produced ~70% reduction of cancer cell growth in the human colon carcinoma cell line SW480. Retroviral delivery of an RNAi molecule specifically inhibited the mutant *K-ras*$^{v\text{-}12}$ allele in the human pancreatic carcinoma cell line CAPAN-1 without affecting wild type K-ras level, and collaterally led to a loss of anchor independent growth and tumorigenicity. Similar success has also been attained by targeting the mutant H-ras oncogene (126–128). The targeting of p53 and ras reflect widely different requirements for siRNA reagent design. Insofar as p53 point mutations are located throughout the 11 exon sequences, custom reagents have to be designed for each mutation. By contrast, ras mutations are primarily limited to "hotspots," thereby allowing a limited number of reagents to cover the most mutated messages. In vitro cancer growth inhibition has been achieved by targeting unique cancer oncogenetic messages that are derived from novel gene fusions (e.g., *bcr-abl* in myelogenous leukemia) (129), virally-expressed genes (HPV *E6/E7* in cervical cancer cells) (130), or overexpressed messages (including *HER-2/neu* in human breast and ovarian cancer cells (131, 132), *protein kinase A* in pancreatic cancer cells (133), multidrug resistance genes (134), telomerase (135), and the antiapoptotic bcl-2 gene (136)). In vivo studies have also led to favorable outcomes by RNAi targeting of critical components for tumor cell growth (124, 137–140), metastasis (141–143), angiogenesis (144, 145), and chemoresistance (146, 147).

As with ASOs and RBZ's, efficacy of siRNA depends on the cell type as well as the level of expression of the targeted gene (148). Nonetheless, RNAi has repeatedly proven to be more robust in terms of consistency of transcript knockdowns at threshold concentrations that are several orders of magnitude below typically-used ASOs (113, 148–151). Theoretically, approximately 1–3 molecules of duplexed RNA per cell are effective at knocking down gene expression (112), although most studies in mammalian cells require an intracellular concentration at the nanomolar range. At these concentrations, more prolonged knockdown activity has been observed in vivo as compared with ASO and RBZ (152).

RNAi Delivery

Building on the premise that RNAi molecules may have a higher therapeutic index than ASO and RBZ's, a markedly lower intracellular concentration of RNAi is needed for the desired effect of targeted gene knockdown. Hence the success of RNAi therapy, which requires effective and global delivery of RNAi to target cells, is more likely to be attained. This is particularly applicable to cancer therapy, where multiorgan metastatic foci are largely responsible for the morbidity and mortality of advanced cancer. Murine studies show that RNAi can be administered "hydrodynamically" (153) by rapidly injecting duplex RNAi molecules through the tail vein. This strategy is not feasible clinically and produces severe cardiovascular side effects (152). RNAi molecule delivery by lipid-based technologies (cationic liposomes, liposome-protamine/DNA) (154–156) or viral vectors (157, 158) have also been explored in animal models.

Liposomal-based technologies allow for up to 90% transfection efficiency in vitro, but they are costly, difficult to generate and associated with induction of clinically toxic cytokines (IL6, TNFα) (154). Cell-specific immunoliposomes have been used successfully to deliver chemotherapy drugs to target cells and may serve as a viable alternative for cationic-based RNAi delivery. In a recent study by Zhang et al. (159), weekly intravenous injection of pegylated immunoliposomes effectively delivered epidermal growth factor receptor siRNA to xenografts of intracranial gliomas, resulting in 95% suppression of EGFR function and an 88% increase in survival time.

A number of studies have documented stable transduction of siRNA-expressing constructs with viral vectors. Retroviral delivered siRNA effectively targeted p53 in both cell lines and primary fibroblasts (160). Lentiviral vectors were similarly effective, with a lasting effect of >25 days (112). Lentiviral delivery of antiviral siRNA inhibited HIV production from primary human T cells and macrophages (161, 162) in vitro, and silenced target genes in vivo in transgenic mice (163). However, concern over the risk of insertional mutagenesis with retroviruses precludes their clinical use for cancer therapy at this time. Theoretically, bacterial vectors could also be utilized (164), but to date, most cancer gene transfer trials, whether intratumoral, intravenous or intra-arterial, involve adenoviral delivery vehicles.

Potential Hurdles for siRNA Cancer Therapeutics

siRNA faces unique hurdles as a cancer gene therapeutic in addition to common concerns that it shares with ASO- and RBZ-based therapies. There are concerns regarding the specificity of siRNA gene silencing with respect to interferon (IFN) induction and "off-target" activity. Contrary to the initial observations of Elbashir et al. (113), Sledz et al. identified JAK–STAT pathway activation and global upregulation of IFN-stimulated genes following PKR activation by a 21-bp siRNA molecule (165). However, nonspecific IFN induction or toxicity was not observed

in various in vivo studies (112, 166). Other contributing elements of IFN activation include the plasmid vector used for siRNA delivery, which may cause formation of long hairpin RNA duplexes, or chemical modifications (e.g., 3′ triphosphates on the duplex) at the 3′ end of siRNA (167). The liposomal transfecting agent may also contribute to nonspecific toxicity (168). Hence, each siRNA construct and its delivery system should be carefully scrutinized with respect to its likelihood of soliciting a nonspecific IFN response that would negatively impact therapeutic outcome.

In a recent gene expression profile analysis, Jackson and coworkers suggested that siRNAs may exhibit silencing activities on unintended target sequences having less than 18-nucleotide homology with the intended target sequence (169). This apparent lack of fidelity may be explained by an inadequacy of transcriptome search (170). A more extensive evaluation of siRNA's that had been designed for specific targets revealed multiple examples of other nucleotide sequence homology in addition to the intended target sequence (170). In fact, Snove and Holen identified unintended target sequences with three or fewer mismatches in 75% of 359 published siRNA sequences (170), highlighting the potential risk of siRNA design based on limited sequence analysis.

The recent discovery of endogenous microRNAs (miRNAs) furthered misgivings regarding the off-target activity of siRNA. miRNAs are single-stranded RNAs of 21–25 nucleotides found in all multicellular organisms (171–173). In humans, 200–255 genes in the human genome encode miRNAs (172). miRNAs are generated from genome hairpin RNAs through processing by Drosher, and are believed to serve a regulatory function. miRNA inhibits the translation of mRNAs into proteins through imperfect base pairing with the target mRNA, but does not impede transcription or destroy mRNAs. It appears, however, that siRNAs "acting as miRNA" contribute minimally to off-target activity, as synergism between multiple, partially complementarity-bound miRNAs are needed for effective translational silencing (171). Furthermore, unique, target sequence-independent signatures of individual siRNAs remain a laboratory manifestation defined by gene array analyses. The impact of such off-target activity has not been evident in animal studies (152). Preliminary evidence suggests that in vitro off-target activity may be reduced further through chemical modifications, such as nucleotide selection in key positions, and the intentional introduction of mismatches at defined positions between the siRNA sense and antisense strands (174).

Development of siRNA technology is moving forward. Initial delivery vehicles will include nonviral strategies (i.e., cationic liposomes). Some of the initial targets will likely include similar genes identified for ASO development.

Concluding Remarks

In conclusion, a broad array of targeted gene-based therapies are under active clinical development in NSCLC. Common attributes of these therapies include remarkable safety with virtually no evidence of clinically significant off target effect

outside of the target specificity. Tolerable toxicity, however, is observed in relation to delivery components. Further development is ongoing to reduce toxicity attributed to delivery of gene-based targeted therapeutics. Evidence of clinical activity has been demonstrated and further phase II and a phase III investigation is moving forward. At the same time quantitative proteomic and genomic technology is becoming more accessible, thereby enabling personalized attempts to match a particular targeted therapy with a unique cancer specific molecular signal.

References

1. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
2. Carlson JM, Doyle J (2002) Complexity and robustness. Proc Natl Acad Sci U S A 99(Suppl 1):2538–2545
3. Stelling J, Sauer U, Szallasi Z, Doyle FJ III, Doyle J (2004) Robustness of cellular functions. Cell 118(6):675–685
4. Edelman GM, Gally JA (2001) Degeneracy and complexity in biological systems. Proc Natl Acad Sci U S A 98(24):13763–13768
5. Laub MT, McAdams HH, Feldblyum T, Fraser CM, Shapiro L (2000) Global analysis of the genetic network controlling a bacterial cell cycle. Science 290(5499):2144–2148
6. Barabasi AL, Oltvai ZN (2004) Network biology: understanding the cell's functional organization. Nat Rev 5(2):101–113
7. Papin JA, Hunter T, Palsson BO, Subramaniam S (2005) Reconstruction of cellular signalling networks and analysis of their properties. Nat Rev Mol Cell Biol 6(2):99–111
8. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH (1997) Integrating genetic approaches into the discovery of anticancer drugs. Science 278(5340):1064–1068
9. Jeong H, Mason SP, Barabasi AL, Oltvai ZN (2001) Lethality and centrality in protein networks. Nature 411(6833):41–42
10. Denissenko MF, Pao A, Tang M, Pfeifer GP (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science 274(5286):430–432
11. Burns TF, El-Deiry WS (1999) The p53 pathway and apoptosis. J Cell Physiol 181(2):231–239
12. Fujiwara T, Grimm EA, Mukhopadhyay T, Cai DW, Owen-Schaub LB, Roth JA (1993) A retroviral wild-type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis. Cancer Res 53(18):4129–4133
13. Raycroft L, Wu HY, Lozano G (1990) Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science 249(4972):1049–1051
14. Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281(5381):1322–1326
15. Kamijo T, Zindy F, Roussel MF et al (1997) Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell 91(5):649–659
16. Isobe T, Hiyama K, Yoshida Y, Fujiwara Y, Yamakido M (1994) Prognostic significance of p53 and ras gene abnormalities in lung adenocarcinoma patients with stage I disease after curative resection. Jpn J Cancer Res 85(12):1240–1246
17. Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F (1992) p53 Expression and prognosis in gastric carcinoma. Int J Cancer 50(6):859–862
18. Quinlan DC, Davidson AG, Summers CL, Warden HE, Doshi HM (1992) Accumulation of p53 protein correlates with a poor prognosis in human lung cancer. Cancer Res 52(17):4828–4831

19. Cai DW, Mukhopadhyay T, Roth J (1993) A novel ribozyme for modification of mutated p53 pre-mRNA in non-small cell lung cancer cell lines. In: 3rd antisense workshop, 13 Nov 1993
20. Zhang WW, Fang X, Mazur W, French BA, Georges RN, Roth JA (1994) High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus. Cancer Gene Ther 1(1):5–13
21. Fujiwara T, Cai DW, Georges RN, Mukhopadhyay T, Grimm EA, Roth JA (1994) Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. J Natl Cancer Inst 86(19):1458–1462
22. Wang J, Bucana CD, Roth JA, Zhang WW (1995) Apoptosis induced in human osteosarcoma cells is one of the mechanisms for the cytocidal effect of Ad5CMV-p53. Cancer Gene Ther 2(1):9–17
23. Georges RN, Mukhopadhyay T, Zhang Y, Yen N, Roth JA (1993) Prevention of orthotopic human lung cancer growth by intratracheal instillation of a retroviral antisense K-ras construct. Cancer Res 53(8):1743–1746
24. Bouvet M, Fang B, Ekmekcioglu S et al (1998) Suppression of the immune response to an adenovirus vector and enhancement of intratumoral transgene expression by low-dose etoposide. Gene Ther 5(2):189–195
25. Nielsen LL, Dell J, Maxwell E, Armstrong L, Maneval D, Catino JJ (1997) Efficacy of p53 adenovirus-mediated gene therapy against human breast cancer xenografts. Cancer Gene Ther 4(2):129–138
26. Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA (1996) Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation. Clin Cancer Res 2(10):1665–1671
27. Dameron KM, Volpert OV, Tainsky MA, Bouck N (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 265(5178):1582–1584
28. Miyashita T, Reed JC (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80(2):293–299
29. Carroll JL, Nielsen LL, Pruett SB, Mathis JM (2001) The role of natural killer cells in adenovirus-mediated p53 gene therapy. Mol Cancer Ther 1(1):49–60
30. Molinier-Frenkel V, Le Boulaire C, Le Gal FA et al (2000) Longitudinal follow-up of cellular and humoral immunity induced by recombinant adenovirus-mediated gene therapy in cancer patients. Hum Gene Ther 11(13):1911–1920
31. Yen N, Ioannides CG, Xu K et al (2000) Cellular and humoral immune responses to adenovirus and p53 protein antigens in patients following intratumoral injection of an adenovirus vector expressing wild-type. P53 (Ad-p53). Cancer Gene Ther 7(4):530–536
32. Owen-Schaub LB, Zhang W, Cusack JC et al (1995) Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol Cell Biol 15(6):3032–3040
33. Roth JA, Nguyen D, Lawrence DD et al (1996) Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. Nat Med 2(9):985–991
34. Swisher SG, Roth JA, Nemunaitis J et al (1999) Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. J Natl Cancer Inst 91(9):763–771
35. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang WW, Owen-Schaub LB, Roth JA (1994) Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res 54(9):2287–2291
36. Nguyen DM, Spitz FR, Yen N, Cristiano RJ, Roth JA (1996) Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. J Thorac Cardiovasc Surg 112(5):1372–1376 discussion 6–7
37. Yver A, Dreiling LK, Mohanty S et al (1999) Tolerance and safety of RPR/INGN 201, an adeno-viral vector containing a p53 gene, administered intratumorally in 309 patients with advanced cancer enrolled in phase I and II studies world-wide. Proc Am Soc Clin Oncol 19:460a

38. Nemunaitis J, Swisher SG, Timmons T et al (2000) Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. J Clin Oncol 18(3):609–622
39. Schuler M, Herrmann R, De Greve JL et al (2001) Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: results of a multicenter phase II study. J Clin Oncol 19(6):1750–1758
40. Broaddus WC, Liu Y, Steele LL et al (1999) Enhanced radiosensitivity of malignant glioma cells after adenoviral p53 transduction. J Neurosurg 91(6):997–1004
41. Feinmesser M, Halpern M, Fenig E et al (1999) Expression of the apoptosis-related oncogenes bcl-2, bax, and p53 in Merkel cell carcinoma: can they predict treatment response and clinical outcome? Hum Pathol 30(11):1367–1372
42. Jasty R, Lu J, Irwin T, Suchard S, Clarke MF, Castle VP (1998) Role of p53 in the regulation of irradiation-induced apoptosis in neuroblastoma cells. Mol Genet Metab 65(2):155–164
43. Sakakura C, Sweeney EA, Shirahama T et al (1996) Overexpression of bax sensitizes human breast cancer MCF-7 cells to radiation-induced apoptosis. Int J Cancer 67(1):101–105
44. Swisher S, Roth JA, Komaki R et al (2000) A phase II trial of adenoviral mediated p53 gene transfer (RPR/INGN 201) in conjunction with radiation therapy in patients with localized non-small cell lung cancer (NSCLC). Am Soc Clin Oncol 19:461a
45. Chada S, Mhashilkar A, Roth JA, Gabrilovich D (2003) Development of vaccines against self-antigens: the p53 paradigm. Curr Opin Drug Discov Devel 6(2):169–173
46. Ishida T, Chada S, Stipanov M et al (1999) Dendritic cells transduced with wild-type p53 gene elicit potent anti-tumour immune responses. Clin Exp Immunol 117(2):244–251
47. Mayordomo JI, Loftus DJ, Sakamoto H et al (1996) Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. J Exp Med 183(4):1357–1365
48. Nikitina EY, Clark JI, Van Beynen J et al (2001) Dendritic cells transduced with full-length wild-type p53 generate antitumor cytotoxic T lymphocytes from peripheral blood of cancer patients. Clin Cancer Res 7(1):127–135
49. Antonia SJ, Mirza N, Fricke I et al (2006) Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clin Cancer Res 12(3 Pt 1):878–887
50. Ito I, Ji L, Tanaka F et al (2004) Liposomal vector mediated delivery of the 3p FUS1 gene demonstrates potent antitumor activity against human lung cancer in vivo. Cancer Gene Ther 11(11):733–739
51. Uno F, Sasaki J, Nishizaki M et al (2004) Myristoylation of the fus1 protein is required for tumor suppression in human lung cancer cells. Cancer Res 64(9):2969–2976
52. Clark RE (2000) Antisense therapeutics in chronic myeloid leukaemia: the promise, the progress and the problems. Leukemia 14(3):347–355
53. Baker BF, Monia BP (1999) Novel mechanisms for antisense-mediated regulation of gene expression. Biochim Biophys Acta 1489(1):3–18
54. Crooke ST (1999) Molecular mechanisms of action of antisense drugs. Biochim Biophys Acta 1489(1):31–44
55. Gewirtz AM (2000) Oligonucleotide therapeutics: a step forward. J Clin Oncol 18(9):1809–1811
56. Koller E, Gaarde WA, Monia BP (2000) Elucidating cell signaling mechanisms using antisense technology. Trends Pharmacol Sci 21(4):142–148
57. Coppelli FM, Grandis JR (2005) Oligonucleotides as anticancer agents: from the benchside to the clinic and beyond. Curr Pharm Des 11(22):2825–2840
58. Tamm I, Wagner M (2006) Antisense therapy in clinical oncology: preclinical and clinical experiences. Mol Biotechnol 33(3):221–238
59. Kurreck J (2003) Antisense technologies. Improvement through novel chemical modifications. Eur J Biochem/FEBS 270(8):1628–1644
60. Brown DA, Kang SH, Gryaznov SM et al (1994) Effect of phosphorothioate modification of oligodeoxynucleotides on specific protein binding. J Biol Chem 269(43):26801–26805

61. Levin AA (1999) A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochim Biophys Acta 1489(1):69–84
62. Zhang R, Iyer RP, Yu D et al (1996) Pharmacokinetics and tissue disposition of a chimeric oligodeoxynucleoside phosphorothioate in rats after intravenous administration. J Pharmacol Exp Ther 278(2):971–979
63. Zhang R, Lu Z, Zhang X et al (1995) In vivo stability and disposition of a self-stabilized oligodeoxynucleotide phosphorothioate in rats. Clin Chem 41(6 Pt 1):836–843
64. Zhang R, Diasio RB, Lu Z et al (1995) Pharmacokinetics and tissue distribution in rats of an oligodeoxynucleotide phosphorothioate (GEM 91) developed as a therapeutic agent for human immunodeficiency virus type-1. Biochem Pharmacol 49(7):929–939
65. Nishizuka Y (1988) The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334(6184):661–665
66. Basu A (1993) The potential of protein kinase C as a target for anticancer treatment. Pharmacol Ther 59(3):257–280
67. Blobe GC, Obeid LM, Hannun YA (1994) Regulation of protein kinase C and role in cancer biology. Cancer Metastasis Rev 13(3–4):411–431
68. Yuspa SH (1994) The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis – thirty-third G. H. A. Clowes Memorial Award Lecture. Cancer Res 54(5):1178–1189
69. Ways DK, Kukoly CA, de Vente J et al (1995) MCF-7 breast cancer cells transfected with protein kinase C-alpha exhibit altered expression of other protein kinase C isoforms and display a more aggressive neoplastic phenotype. J Clin Invest 95(4):1906–1915
70. O'Brian C, Vogel VG, Singletary SE, Ward NE (1989) Elevated protein kinase C expression in human breast tumor biopsies relative to normal breast tissue. Cancer Res 49(12):3215–3217
71. Perletti GP, Smeraldi C, Porro D, Piccinini F (1994) Involvement of the alpha isoenzyme of protein kinase C in the growth inhibition induced by phorbol esters in MH1C1 hepatoma cells. Biochem Biophys Res Commun 205(3):1589–1594
72. Adesina AM, Dooley N, Yong VW, Nalbantoglu J (1998) Differential role for protein kinase C-mediated signaling in the proliferation of medulloblastoma cell lines. Int J Oncol 12(4):759–768
73. Dean N, McKay R, Miraglia L et al (1996) Inhibition of growth of human tumor cell lines in nude mice by an antisense of oligonucleotide inhibitor of protein kinase C-alpha expression. Cancer Res 56(15):3499–3507
74. Nemunaitis J, Holmlund JT, Kraynak M et al (1999) Phase I evaluation of ISIS 3521, an antisense oligodeoxynucleotide to protein kinase C-alpha, in patients with advanced cancer. J Clin Oncol 17(11):3586–3595
75. Yuen AR, Halsey J, Fisher GA et al (1999) Phase I study of an antisense oligonucleotide to protein kinase C-alpha (ISIS 3521/CGP 64128A) in patients with cancer. Clin Cancer Res 5(11):3357–3363
76. Villalona-Calero MA, Ritch P, Figueroa JA et al (2004) A phase I/II study of LY900003, an antisense inhibitor of protein kinase C-alpha, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. Clin Cancer Res 10(18 Pt 1):6086–6093
77. Miyake H, Chi KN, Gleave ME (2000) Antisense TRPM-2 oligodeoxynucleotides chemosensitize human androgen-independent PC-3 prostate cancer cells both in vitro and in vivo. Clin Cancer Res 6(5):1655–1663
78. Zellweger T, Miyake H, July LV, Akbari M, Kiyama S, Gleave ME (2001) Chemosensitization of human renal cell cancer using antisense oligonucleotides targeting the antiapoptotic gene clusterin. Neoplasia 3(4):360–367
79. Chi KN, Eisenhauer E, Fazli L et al (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(17):1287–1296

80. Chi KN, Siu LL, Hirte H et al (2008) A phase I study of OGX-011, a 2′-methoxyethyl phosphorothioate antisense to clusterin, in combination with docetaxel in patients with advanced cancer. Clin Cancer Res 14(3):833–839
81. Gleave M, Miyake H (2005) Use of antisense oligonucleotides targeting the cytoprotective gene, clusterin, to enhance androgen- and chemo-sensitivity in prostate cancer. World J Urol 23(1):38–46
82. Laskin J, Chi KN, Melosky B et al (2006) Phase I study of OGX-011, a second generation antisense oligonucleotide (ASO) to clusterin, combined with cisplatin and gemcitabine as first-line treatment for patients with stage IIB/IV non-small cell lung cancer (NSCLC). J Clin Oncol 24(18S):17078
83. Cunningham CC, Holmlund JT, Geary RS et al (2001) A Phase I trial of H-ras antisense oligonucleotide ISIS 2503 administered as a continuous intravenous infusion in patients with advanced carcinoma. Cancer 92(5):1265–1271
84. Dang T, Johnson DH, Kelly K, Rizvi N, Holmlund J, Dorr A (2001) Multicenter phase II trial of an antisense inhibitor of H-ras (ISIS-2503) in advanced non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 20:1325a
85. Bollag G, McCormick F (1991) Regulators and effectors of ras proteins. Annu Rev Cell Biol 7:601–632
86. Bos JL (1989) ras Oncogenes in human cancer: a review. Cancer Res 49(17):4682–4689
87. Eckhardt SG, Rizzo J, Sweeney KR et al (1999) Phase I and pharmacologic study of the tyrosine kinase inhibitor SU101 in patients with advanced solid tumors. J Clin Oncol 17(4):1095–1104
88. Cunningham CC, Holmlund JT, Schiller JH et al (2000) A phase I trial of c-Raf kinase antisense oligonucleotide ISIS 5132 administered as a continuous intravenous infusion in patients with advanced cancer. Clin Cancer Res 6(5):1626–1631
89. Coudert B, Anthoney A, Fiedler W et al (2001) Phase II trial with ISIS 5132 in patients with small-cell (SCLC) and non-small cell (NSCLC) lung cancer. A European Organization for Research and Treatment of Cancer (EORTC) Early Clinical Studies Group report. Eur J Cancer 37(17):2194–2198
90. Gokhale PC, Zhang C, Newsome JT et al (2002) Pharmacokinetics, toxicity, and efficacy of ends-modified raf antisense oligodeoxyribonucleotide encapsulated in a novel cationic liposome. Clin Cancer Res 8(11):3611–3621
91. Rudin CM, Marshall JL, Huang CH et al (2004) Delivery of a liposomal c-raf-1 antisense oligonucleotide by weekly bolus dosing in patients with advanced solid tumors: a phase I study. Clin Cancer Res 10(21):7244–7251
92. Reed JC, Stein C, Subasinghe C et al (1990) Antisense-mediated inhibition of BCL2 protooncogene expression and leukemic cell growth and survival: comparisons of phosphodiester and phosphorothioate oligodeoxynucleotides. Cancer Res 50(20):6565–6570
93. Kitada S, Miyashita T, Tanaka S, Reed JC (1993) Investigations of antisense oligonucleotides targeted against bcl-2 RNAs. Antisense Res Dev 3(2):157–169
94. Kitada S, Takayama S, De Riel K, Tanaka S, Reed JC (1994) Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of bcl-2 gene expression. Antisense Res Dev 4(2):71–79
95. Cotter FE, Johnson P, Hall P et al (1994) Antisense oligonucleotides suppress B-cell lymphoma growth in a SCID-hu mouse model. Oncogene 9(10):3049–3055
96. Gleave ME, Miayake H, Goldie J, Nelson C, Tolcher A (1999) Targeting bcl-2 gene to delay androgen-independent progression and enhance chemosensitivity in prostate cancer using antisense bcl-2 oligodeoxynucleotides. Urology 54(6A Suppl):36–46
97. Miyake H, Tolcher A, Gleave ME (1999) Antisense Bcl-2 oligodeoxynucleotides inhibit progression to androgen-independence after castration in the Shionogi tumor model. Cancer Res 59(16):4030–4034
98. Cotter FE, Corbo M, Raynaud F et al (1996) Bcl-2 antisense therapy in lymphoma: in vitro and in vivo mechanisms, efficacy, pharmacokinetics and toxicity studies. Ann Oncol 7:32

99. Tolcher AW, Chi K, Kuhn J et al (2005) A phase II, pharmacokinetic, and biological correlative study of oblimersen sodium and docetaxel in patients with hormone-refractory prostate cancer. Clin Cancer Res 11(10):3854–3861
100. Rudin CM, Otterson GA, Mauer AM et al (2002) A pilot trial of G3139, a bcl-2 antisense oligonucleotide, and paclitaxel in patients with chemorefractory small-cell lung cancer. Ann Oncol 13(4):539–545
101. Webb A, Cunningham D, Cotter F et al (1997) BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet 349(9059):1137–1141
102. Waters JS, Webb A, Cunningham D et al (2000) Phase I clinical and pharmacokinetic study of bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma. J Clin Oncol 18(9):1812–1823
103. Bedikian AY, Millward M, Pehamberger H et al (2006) Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol 24(29):4738–4745
104. Li F, Ambrosini G, Chu EY et al (1998) Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 396(6711):580–584
105. Ambrosini G, Adida C, Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3(8):917–921
106. Tamm I, Wang Y, Sausville E et al (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 58(23):5315–5320
107. Lu CD, Altieri DC, Tanigawa N (1998) Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. Cancer Res 58(9):1808–1812
108. Lal A, Lash AE, Altschul SF et al (1999) A public database for gene expression in human cancers. Cancer Res 59(21):5403–5407
109. Li F, Ackermann EJ, Bennett CF et al (1999) Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. Nat Cell Biol 1(8):461–466
110. Chen J, Wu W, Tahir SK et al (2000) Down-regulation of survivin by antisense oligonucleotides increases apoptosis, inhibits cytokinesis and anchorage-independent growth. Neoplasia 2(3):235–241
111. Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
112. Ichim TE, Li M, Qian H et al (2004) RNA interference: a potent tool for gene-specific therapeutics. Am J Transplant 4(8):1227–1236
113. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411(6836):494–498
114. Liu J, Carmell MA, Rivas FV et al (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305(5689):1437–1441
115. Paroo Z, Corey DR (2004) Challenges for RNAi in vivo. Trends Biotechnol 22(8):390–394
116. Braasch DA, Jensen S, Liu Y et al (2003) RNA interference in mammalian cells by chemically-modified RNA. Biochemistry 42(26):7967–7975
117. Hough SR, Wiederholt KA, Burrier AC, Woolf TM, Taylor MF (2003) Why RNAi makes sense. Nat Biotechnol 21(7):731–732
118. Hogrefe RI (1999) An antisense oligonucleotide primer. Antisense Nucleic Acid Drug Dev 9(4):351–357
119. Crooke ST (2000) Evaluating the mechanism of action of antiproliferative antisense drugs. Antisense Nucleic Acid Drug Dev 10(2):123–126 discussion 7
120. Sands H, Gorey-Feret LJ, Cocuzza AJ, Hobbs FW, Chidester D, Trainor GL (1994) Biodistribution and metabolism of internally 3H-labeled oligonucleotides. I. Comparison of a phosphodiester and a phosphorothioate. Mol Pharmacol 45(5):932–943

121. Geary RS, Watanabe TA, Truong L et al (2001) Pharmacokinetic properties of 2'-O-(2-methoxyethyl)-modified oligonucleotide analogs in rats. J Pharmacol Exp Ther 296(3):890–897
122. Geary RS, Yu RZ, Levin AA (2001) Pharmacokinetics of phosphorothioate antisense oligodeoxynucleotides. Curr Opin Investig Drugs 2(4):562–573
123. Martinez LA, Naguibneva I, Lehrmann H et al (2002) Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. Proc Natl Acad Sci U S A 99(23):14849–14854
124. Brummelkamp TR, Bernards R, Agami R (2002) Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer cell 2(3):243–247
125. Kawasaki H, Suyama E, Iyo M, Taira K (2003) siRNAs generated by recombinant human Dicer induce specific and significant but target site-independent gene silencing in human cells. Nucleic Acids Res 31(3):981–987
126. Kawasaki H, Taira K (2003) Short hairpin type of dsRNAs that are controlled by tRNA(Val) promoter significantly induce RNAi-mediated gene silencing in the cytoplasm of human cells. Nucleic Acids Res 31(2):700–707
127. Yang G, Thompson JA, Fang B, Liu J (2003) Silencing of H-ras gene expression by retrovirus-mediated siRNA decreases transformation efficiency and tumorgrowth in a model of human ovarian cancer. Oncogene 22(36):5694–5701
128. Yin JQ, Gao J, Shao R, Tian WN, Wang J, Wan Y (2003) siRNA agents inhibit oncogene expression and attenuate human tumor cell growth. J Exp Ther Oncol 3(4):194–204
129. Scherr M, Battmer K, Winkler T, Heidenreich O, Ganser A, Eder M (2003) Specific inhibition of bcr-abl gene expression by small interfering RNA. Blood 101(4):1566–1569
130. Yoshinouchi M, Yamada T, Kizaki M et al (2003) In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by E6 siRNA. Mol Ther 8(5):762–768
131. Choudhury A, Charo J, Parapuram SK et al (2004) Small interfering RNA (siRNA) inhibits the expression of the Her2/neu gene, upregulates HLA class I and induces apoptosis of Her2/neu positive tumor cell lines. Int J Cancer 108(1):71–77
132. Yang G, Cai KQ, Thompson-Lanza JA, Bast RC Jr, Liu J (2004) Inhibition of breast and ovarian tumor growth through multiple signaling pathways by using retrovirus-mediated small interfering RNA against Her-2/neu gene expression. J Biol Chem 279(6):4339–4345
133. Farrow B, Rychahou P, Murillo C, O'Connor KL, Iwamura T, Evers BM (2003) Inhibition of pancreatic cancer cell growth and induction of apoptosis with novel therapies directed against protein kinase A. Surgery 134(2):197–205
134. Yague E, Higgins CF, Raguz S (2004) Complete reversal of multidrug resistance by stable expression of small interfering RNAs targeting MDR1. Gene Ther 11(14):1170–1174
135. Kosciolek BA, Kalantidis K, Tabler M, Rowley PT (2003) Inhibition of telomerase activity in human cancer cells by RNA interference. Mol Cancer Ther 2(3):209–216
136. Cioca DP, Aoki Y, Kiyosawa K (2003) RNA interference is a functional pathway with therapeutic potential in human myeloid leukemia cell lines. Cancer Gene Ther 10(2):125–133
137. Aharinejad S, Paulus P, Sioud M et al (2004) Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice. Cancer Res 64(15):5378–5384
138. Li K, Lin SY, Brunicardi FC, Seu P (2003) Use of RNA interference to target cyclin E-overexpressing hepatocellular carcinoma. Cancer Res 63(13):3593–3597
139. Uchida H, Tanaka T, Sasaki K et al (2004) Adenovirus-mediated transfer of siRNA against survivin induced apoptosis and attenuated tumor cell growth in vitro and in vivo. Mol Ther 10(1):162–171
140. Verma UN, Surabhi RM, Schmaltieg A, Becerra C, Gaynor RB (2003) Small interfering RNAs directed against beta-catenin inhibit the in vitro and in vivo growth of colon cancer cells. Clin Cancer Res 9(4):1291–1300

141. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE (2004) CEACAM6 gene silencing impairs anoikis resistance and in vivo metastatic ability of pancreatic adenocarcinoma cells. Oncogene 23(2):465–473
142. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE (2004) RNA interference targeting the M2 subunit of ribonucleotide reductase enhances pancreatic adenocarcinoma chemosensitivity to gemcitabine. Oncogene 23(8):1539–1548
143. Salisbury AJ, Macaulay VM (2003) Development of molecular agents for IGF receptor targeting. Horm Metab Res 35(11–12):843–849
144. Filleur S, Courtin A, Ait-Si-Ali S et al (2003) SiRNA-mediated inhibition of vascular endothelial growth factor severely limits tumor resistance to antiangiogenic thrombospondin-1 and slows tumor vascularization and growth. Cancer Res 63(14):3919–3922
145. Takei Y, Kadomatsu K, Yuzawa Y, Matsuo S, Muramatsu T (2004) A small interfering RNA targeting vascular endothelial growth factor as cancer therapeutics. Cancer Res 64(10):3365–3370
146. Chen J, Wall NR, Kocher K et al (2004) Stable expression of small interfering RNA sensitizes TEL-PDGFbetaR to inhibition with imatinib or rapamycin. J Clin Invest 113(12):1784–1791
147. Lakka SS, Gondi CS, Yanamandra N et al (2004) Inhibition of cathepsin B and MMP-9 gene expression in glioblastoma cell line via RNA interference reduces tumor cell invasion, tumor growth and angiogenesis. Oncogene 23(27):4681–4689
148. Bass BL (2001) RNA interference. The short answer. Nature 411(6836):428–429
149. Aoki Y, Cioca DP, Oidaira H, Kamiya J, Kiyosawa K (2003) RNA interference may be more potent than antisense RNA in human cancer cell lines. Clin Exp Pharmacol Physiol 30(1–2):96–102
150. Coma S, Noe V, Lavarino C et al (2004) Use of siRNAs and antisense oligonucleotides against survivin RNA to inhibit steps leading to tumor angiogenesis. Oligonucleotides 14(2):100–113
151. Miyagishi M, Hayashi M, Taira K (2003) Comparison of the suppressive effects of antisense oligonucleotides and siRNAs directed against the same targets in mammalian cells. Antisense Nucleic Acid Drug Dev 13(1):1–7
152. Lieberman J, Song E, Lee SK, Shankar P (2003) Interfering with disease: opportunities and roadblocks to harnessing RNA interference. Trends Mol Med 9(9):397–403
153. McCaffrey AP, Meuse L, Pham TT, Conklin DS, Hannon GJ, Kay MA (2002) RNA interference in adult mice. Nature 418(6893):38–39
154. Sioud M, Sorensen DR (2003) Cationic liposome-mediated delivery of siRNAs in adult mice. Biochem Biophys Res Commun 312(4):1220–1225
155. Sorensen DR, Leirdal M, Sioud M (2003) Gene silencing by systemic delivery of synthetic siRNAs in adult mice. J Mol Biol 327(4):761–766
156. Tan Y, Zhang JS, Huang L (2002) Codelivery of NF-kappaB decoy-related oligodeoxynucleotide improves LPD-mediated systemic gene transfer. Mol Ther 6(6):804–812
157. Cao X, Daniel J, Ozvaran M et al (2004) Bcl-XL silencing in thoracic malignancies using short interfering RNA (siRNA) (Abstract). Cancer Gene Ther (in press)
158. Xia H, Mao Q, Paulson HL, Davidson BL (2002) siRNA-mediated gene silencing in vitro and in vivo. Nat Biotechnol 20(10):1006–1010
159. Zhang Y, Zhang YF, Bryant J, Charles A, Boado RJ, Pardridge WM (2004) Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. Clin Cancer Res 10(11):3667–3677
160. Barton GM, Medzhitov R (2002) Retroviral delivery of small interfering RNA into primary cells. Proc Natl Acad Sci U S A 99(23):14943–14945
161. Lee MT, Coburn GA, McClure MO, Cullen BR (2003) Inhibition of human immunodeficiency virus type 1 replication in primary macrophages by using Tat- or CCR5-specific small interfering RNAs expressed from a lentivirus vector. J Virol 77(22):11964–11972

162. Qin XF, An DS, Chen IS, Baltimore D (2003) Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5. Proc Natl Acad Sci U S A 100(1):183–188
163. Tiscornia G, Singer O, Ikawa M, Verma IM (2003) A general method for gene knockdown in mice by using lentiviral vectors expressing small interfering RNA. Proc Natl Acad Sci U S A 100(4):1844–1848
164. Lin J, Lin E, Nemunaitis J (2004) Bacteria in the treatment of cancer. Curr Opin Mol Ther 6(6):629–639
165. Sledz CA, Holko M, de Veer MJ, Silverman RH, Williams BR (2003) Activation of the interferon system by short-interfering RNAs. Nat Cell Biol 5(9):834–839
166. Hill JA, Ichim TE, Kusznieruk KP et al (2003) Immune modulation by silencing IL-12 production in dendritic cells using small interfering RNA. J Immunol 171(2):691–696
167. Kim DH, Longo M, Han Y, Lundberg P, Cantin E, Rossi JJ (2004) Interferon induction by siRNAs and ssRNAs synthesized by phage polymerase. Nat Biotechnol 22(3):321–325
168. Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. Cell 115(2):209–216
169. Jackson AL, Bartz SR, Schelter J et al (2003) Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol 21(6):635–637
170. Snove O Jr, Holen T (2004) Many commonly used siRNAs risk off-target activity. Biochem Biophys Res Commun 319(1):256–263
171. Novina CD, Sharp PA (2004) The RNAi revolution. Nature 430(6996):161–164
172. Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP (2003) Vertebrate microRNA genes. Science 299(5612):1540
173. Moss EG (2003) Silencing unhealthy alleles naturally. Trends Biotechnol 21(5):185–187
174. Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A (2004) Rational siRNA design for RNA interference. Nat Biotechnol 22(3):326–330

Lung Cancer Resistance to Chemotherapy

David J. Stewart

Abstract Metastatic lung cancer remains incurable by chemotherapy. Several factors contribute to resistance to chemotherapy, including many factors that are adaptations of systems that evolved to protect normal cells from a hostile environment. Tumor cell characteristics, tumor cell interactions with extracellular matrix and stromal cells, and tumor physical characteristics all contribute to resistance. Resistance may arise from gene upregulation or downregulation as a downstream consequence of the oncogene mutations or tumor suppressor gene deletions that underlie tumorigenesis or may also arise due to tumor hypoxia or due to exposure to therapy. Host gene polymorphisms may alter resistance by determining the half-life or enzymatic activity of upregulated resistance factors. Resistance may arise from decreased drug delivery to tumor, impact of extracellular pH on drug uptake, altered drug uptake transporters or cell membrane characteristics, increased drug efflux or detoxification, decreased drug binding, altered drug targets, increased DNA repair, decreased proapoptotic factors, increased antiapoptotic factors, altered cell cycling or mitotic checkpoints, or altered transcription factors. This diversity of resistance mechanisms magnifies the challenges facing us in predicting patient prognosis and in overcoming resistance.

Keywords Lung cancer • Chemotherapy • Resistance

Lung Cancer and Resistance

As outlined elsewhere in this text, despite 20–50% of patients with advanced non-small cell lung cancer (NSCLC) and 60–80% of patients with extensive small cell lung cancer (SCLC) initially responding to chemotherapy, widely metastatic

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