Chapter 20
Oligonucleotide Therapeutics

Cy A. Stein, Britta Hoehn, and John Rossi

20.1 Introduction

The idea of sequence-specific gene silencing by synthetic oligonucleotides targeting mRNA is at least 40 years old, but it was only in the mid-1980s when technical advances made the chemical synthesis of oligonucleotides possible that practical steps could be taken toward its implementation. The result was a deluge of experimental data in a variety of systems [1], most of which employed the phosphorothioate (PS) backbone modification, and much of which was ultimately, and unfortunately, uninterpretable.

The reason for uninterpretability is somewhat complicated. A PS oligonucleotide contains a sulfur atom that has been substituted for a nonbridging oxygen atom at each phosphorus in the oligonucleotide chain. These molecules were produced [2] because phosphodiester oligonucleotides (containing linkages identical to what is found in normal DNA) could not be used to silence gene expression either in tissue cultures or in vivo because they were very sensitive to nuclease digestion, especially to 3'-exonucleases [3] and were also rapidly cleared from the plasma through the kidneys. In contrast, phosphorothioates are degraded relatively slowly by nuclease [4] and are also cleared by the kidneys relatively slowly because of their low-affinity binding to plasma proteins (predominantly albumin) [5–7]. Further, because sulfur is immediately beneath oxygen in the periodic table, the PS linkage retains the same negative charge as the PO linkage, thus bestowing the property of extreme aqueous solubility. Importantly, the biophysical behavior of PS and PO oligonucleotides in solution are governed by their backbone charge and not by their sequence. In addition, the PS linkage retains the property of being a substrate for the RNase H, a ubiquitous, predominately nuclear enzyme that cleaves the mRNA strand of an mRNA–DNA duplex [8] and apparently functions naturally to eliminate Okazaki fragments. This ostensibly permits gene silencing to occur via

C.A. Stein (*)  
Department of Oncology, Albert Einstein-Montefiore Cancer Center, Montefiore Medical Center, 111 E. 210 St, Bronx, NY 10467, USA  
e-mail: cstein@montefiore.org
a “pseudocatalytic mechanism” requiring only submicromolar concentrations of PS oligonucleotides for efficacy, at least when they are transfected into cells by employing carrier lipids or other vehicles. For all practical purposes, only PO and PS oligonucleotides elicit RNase H activity. (Interestingly, whether the RNase H mechanism of gene silencing is correct or not, there is mounting evidence that it may be responsible for only a small percentage of total gene silencing. Silencing may actually occur predominately due to the activity of Ago2, the same enzyme with slicer function that cleaves the mRNA strand of an siRNA–mRNA duplex in the RISC complex) [9].

However, despite these important properties of PS oligonucleotides, which have led to their being featured in several important clinical cancer trials, the down side to this chemical modification is that PS oligonucleotides are biochemically fundamentally different from PO oligonucleotides. These differences are most significant in their ability to hybridize to a complementary mRNA strand, which is greatly diminished with respect to an isosequential PO oligonucleotide, and with respect to off-target effects, which are greatly enhanced. Diminished hybridization, reflected in a lower melting temperature ($T_m$) of the PS-oligo-mRNA duplex [4], directly correlates with diminished antisense activity and may be part of the reason why some clinical trials with PS oligonucleotides have not been successful, as will be discussed, although other clinically important aspects of study design and disease characteristics may also explain this observation. Fortunately, the more recent development of sugar-modified locked nucleic acids (LNAs) as antisense oligonucleotides appears to have solved the diminished hybridization problem of the PS oligonucleotide. Each LNA incorporated into a PS oligonucleotide (four are commonly employed, two at each molecular terminus) may raise the $T_m$ of the LNA–mRNA duplex by 4–8°C [10, 11], and hence potentiate gene silencing [12–15]. Clinical trials with an anti-Bcl-2 LNA oligonucleotide in chronic lymphocytic leukemia (CLL) are currently underway, but data are not yet available.

The off-target effects of PS oligonucleotides fall into two basic categories: their ability to bind to heparin-binding proteins and their immunostimulatory properties, which can induce splenomegaly, B-lymphocyte proliferation, and cytokine production. Rodents appear to be particularly sensitive to this phosphorothioate class effect [16]. Neither category of off-target effect will be dealt with extensively in this review, but if these effects are not rigorously controlled for, particularly in experiments in immunosuppressed mice, it may be difficult, if not impossible, to differentiate sequence-specific effects from off-target effects. This has led to a great difficulty in interpreting experimental data [17], as previously noted. The problem is further compounded by the fact that the extent of immune-stimulation by PS oligonucleotides (with or without CpG motifs) in a mouse is very different from that in a human. Unfortunately, this means that preclinical animal models employing PS oligonucleotides may have had little or no predictive value for response in clinical trial patients. Nevertheless, despite these problems, some extremely interesting data have emerged from recent large, randomized, multicenter phase III trials with antisense oligonucleotides, particularly in melanoma and CLL. These trials,
in addition to a few provocative phase II–III trials in other cancers, form the basis for the majority of this review. Phase I trials and later-phase trials performed in populations other than cancer patients will not be discussed.

20.2  Clinical Trials of Oblimersen

Oblimersen (G3139 or Genasense®) is an 18-mer PS oligonucleotide that is complementary to codons 1–6 of the Bcl-2 mRNA [18] and was first synthesized approximately 15 years ago [19]. Relative to all other PS oligonucleotides of similar length studied, it is a molecule with unusual and entirely unexplained properties – its measured $T_m$, when in a duplex with its complementary mRNA, is substantially higher than predicted.

The molecule has been studied extensively in phase I and phase II trials. A compilation of these studies has recently been published [20]. Indeed, in the opinion of the author, sufficient data to support the antisense activity of oblimersen as the sole mechanism of action in any clinical trial is presently lacking, although the drug has demonstrated evidence of clinical activity, as described below.

Webb et al. [21] began a phase I trial with oblimersen in nine patients with lymphoma who had relapsed after at least two chemotherapy interventions and showed overexpression of Bcl-2 protein based on lymph node biopsies. Oblimersen was administered as a continuous subcutaneous infusion for 2 weeks at doses ranging from 4.6 to 73.6 mg/m²/day. The maximum-tolerated dose (MTD) was not reached. One patient had a complete response (CR) and three patients had stable disease. Enrollment continued and a total of 21 patients were treated with oblimersen at doses up to 195.8 mg/m²/day [22]. The MTD was 147.2 mg/m²/day. A CR was attained in one patient, a minor response in two patients, and stable disease in nine patients. In 7 of 16 evaluable patients, Bcl-2 protein was decreased in cells from lymph nodes (two patients) and in samples of peripheral blood or bone marrow (five patients).

20.2.1  Phase III Trial of Oblimersen in Chronic Lymphocytic Leukemia

There is substantial evidence to indicate that in CLL cells, Bcl-2 silencing may lead to significant cellular apoptosis. Therefore, a randomized phase III trial of fludarabine (F) plus cyclophosphamide (C) with or without oblimersen was initiated in patients with relapsed or refractory CLL [23]. A total of 241 patients were stratified and randomized according to three criteria: responsive vs. refractory to prior fludarabine therapy, number of prior regimens (1–2 vs. ≥3), and duration of response to last therapy (>6 months vs. ≤6 months). All patients were required to have received treatment with at least one prior chemotherapy regimen that included at least two cycles of fludarabine. Using standard definitions, patients were
considered relapsed after prior treatment with fludarabine if they achieved at least a PR lasting more than 6 months; patients who did not achieve at least a PR lasting more than 6 months after their last fludarabine treatment were considered to be refractory.

In the FC group, patients received fludarabine 25 mg/m²/day intravenously (IV) followed by cyclophosphamide 250 mg/m²/day IV on days 1–3. In the oblimersen/FC group, patients were given oblimersen 3 mg/kg/day by continuous IV infusion on days 1–7, with FC administered at the above doses on days 5–7. Cycles were 28 days in duration, and up to six cycles were administered. The primary end point of the study was the between-treatment difference in the proportion of patients who achieved CR+nodular partial response (nPR, which is the clinical equivalent of CR; heretofore CR+nPR will be referred to as CR).

Demographic characteristics between the two groups were well balanced. The median number of prior treatment regimens in both groups was three, and previous therapy was balanced between-treatment groups [24].

Twenty patients (17%) in the oblimersen/FC group achieved a CR, as opposed to 8 (7%; *P*= 0.025) in the FC group. Moreover, these CRs were significantly more durable in the oblimersen/FC group. At 36 months of follow-up, the duration of complete remission in FC-treated patients was 22 months, whereas the median had not been reached but was estimated to exceed 36 months for patients in the oblimersen/FC group (*P*= 0.031). With 54 months of follow-up, 12/20 (60%) of oblimersen/FC patients with CR remained alive, including five who remained in complete remission [25]. Of the eight FC-treated patients with CR, only three were alive at 5 years, and all three had relapsed. Maximum benefit was seen in fludarabine-sensitive patients, e.g., those who had a PR or better for more than 6 months after prior fludarabine treatment. In this population, there was a fourfold increase in the CR rate in the oblimersen/FC group as compared to the FC control (25% vs. 6%; *P*= 0.016). With 5 years of follow-up, among all patients who achieved a response, including both complete and partial responses (*N*= 103), there was an 18-month median survival benefit for patients in the oblimersen/FC group (HR = 0.60; *P*= 0.038) [24]. In nonresponding patients, there was no difference in survival outcome between groups.

With respect to nonhematologic toxicities, nausea, pyrexia, and fatigue (primarily grade 1–2) were the most commonly occurring and affected more patients in the oblimersen/FC group than in the FC group [23]. Grade 3–4 occurrences of nausea, pyrexia, and fatigue were limited (8% vs. 2% of patients; 3% of patients in both groups; and 6% vs. 4% of patients, respectively). Importantly, in a population in which infection and immunosuppression are the most common cause of death, the incidence of grade 4 neutropenia (7% vs. 11%) was not increased with the addition of oblimersen to the FC regimen. Grade 4 thrombocytopenia was more frequent in the oblimersen/FC group, but was not associated with an increased incidence of grade 3–4 bleeding events (4% vs. 2%). In approximately 3% of patients, oblimersen administration was associated with first-cycle reactions (cytokine release with or without tumor lysis).
These data demonstrate the importance of achieving a CR for long-term survival in CLL, a point that was initially contested by the FDA. Although oblimersen has, to date, not yet been approved in this indication, the FDA is currently reconsidering that decision on the basis of the recently reported 5-year survival data.

### 20.2.2 Bcl-2 Silencing and Chemosensitization

While there is no doubt that oblimersen can silence Bcl-2 expression in tissue cultures, its ability to do this in vivo, and most importantly, the extent to which Bcl-2 silencing actually chemosensitizes malignant cells, as it is predicted to do, are matters of great debate. Bcl-2 is far from the only antiapoptotic protein present in the vast majority of malignant cells. Even in follicular lymphomas bearing the t14:18 chromosomal translocation, which produces a fused Bcl-2/immunoglobulin mRNA in about 65–70% of cases [26], it is not clear whether the Bcl-2 protein is necessary and sufficient for the maintenance of the neoplastic phenotype. In some tumors, elevated expression of Bcl-2 protein in tumor cells may merely be an epiphénomemonon, despite documented clinical correlations between the expression (or “overexpression”) of Bcl-2 protein and a poor prognosis in cancer patients with tumors [27–30]. Blagosklonny [31] when addressing this question, noted that Bcl-2 expression in colorectal, breast, and lung carcinomas was associated with an “increased apoptotic index, lower risk of distant metastases, and improved prognosis.” Furthermore, concordant with much experimental data, cell lines may “…become resistant due to a strong selection during establishment of cells in culture, overexpression of Bcl-2 simply cannot further increase resistance and [the] effects of Bcl-2 are undetectable.” Tumor cells may also become resistant to cytotoxic therapeutics by downregulating proteins in the apoptotic cascade. An example in melanoma is Apaf-1, which is downstream of Bcl-2, and in whose absence, the level of Bcl-2 protein expression would appear to be irrelevant [32].

Further complicating any potential value of Bcl-2 silencing is the role of this protein in melanoma. Its role in the pathogenesis and prognosis of clinical melanoma is controversial [33] because the protein can be found in normal melanocytes, benign nevi, and primary melanomas, in addition to melanoma metastases [34]. Interestingly, in some studies, Bcl-2 expression was decreased in melanoma cells vs. normal melanocytes [35–38]. However, this finding has not been confirmed in other studies [34, 39, 40], in which minimal differences in the expression of Bcl-2 have been observed. To add to the confusion, in advanced melanoma, about one-third of the data suggest an increase in Bcl-2 expression, while one-third suggest a decrease [33], although the function that the Bcl-2 protein actually serves, rather than just the amount of Bcl-2 protein present, would probably be more important. However, one study that was insufficiently powered has demonstrated that if lymph node deposits express Bcl-2, advanced melanoma patients have a poorer prognosis than those who do not [33]. In toto, conflicting data render the role of Bcl-2 in advanced melanoma unclear.
20.2.3 Clinical Trials in Advanced Melanoma

Dacarbazine (DTIC), the only approved chemotherapy drug for advanced melanoma, was combined with oblimersen in a phase I/II trial (N=14) in patients with advanced disease [41]. Oblimersen was administered via continuous IV infusion for 14 days each month. The initial dose was 0.6 mg/kg/day, increasing to a dose maximum of 6.5 mg/kg/day. Dacarbazine 200 mg/m² was given IV on days 5–9. Six patients also received the same total daily dose of oblimersen administered as twice-daily subcutaneous injections on days 1–7 and dacarbazine 800 mg/m² IV on day 5. The maximum decrease in Bcl-2 expression in patients’ biopsy specimens was highly variable, and no conclusions could be drawn due to insufficient sampling. Responses included one CR, two PRs, and three minor responses, including two in patients whose disease stabilized for a period of at least 1 year.

These data led to the initiation of the largest phase III trial (GM301) in advanced melanoma to date. Between July 2000 and February 2003, 771 chemotherapy-naïve patients with advanced melanoma were randomly assigned to receive treatment with dacarbazine alone 1,000 mg/m²/day IV for 60 min or oblimersen 7 mg/kg/day by continuous IV infusion for 5 days followed by the same dose of dacarbazine [42].

Patients were stratified according to ECOG performance status (0 vs. 1–2), presence or absence of liver metastasis, and disease site/serum LDH level. This latter category included two groups, patients with nonvisceral disease (skin, subcutaneous tissue, or lymph node disease) and normal LDH, and patients with visceral disease (excluding liver) or elevated LDH [baseline serum level more than 1.1 times the upper limit of normal (ULN)] [42]. The primary end point of the study was an intent-to-treat (ITT) comparison of overall survival between the two treatment groups. Secondary end points included progression-free survival, overall and durable response (i.e., response ≥6 months in duration), and duration of response.

The baseline characteristics of the groups were well balanced. With a minimum follow-up of 24 months, the median overall survival in the oblimersen/DTIC group was 9 months, compared with 7.8 months in the DTIC-alone group (HR=0.87; 95% CI 0.75–1.01; P=0.077) [42]. Overall response rates (CRs + PRs) were 13.5% for patients treated with oblimersen/DTIC and 7.5% for patients receiving DTIC alone (P=0.007). Durable responses were also increased in the oblimersen/DTIC group (7.3% vs. 3.6%; P=0.03). Eleven patients (2.8%) in the oblimersen/DTIC group achieved a CR in comparison to three patients (0.8%) in the DTIC-alone group. Median progression-free survival was also significantly longer among patients who received oblimersen/DTIC than among those treated with DTIC (2.6 months vs. 1.6 months, HR=0.75; P<0.001).

Outcome data were subsequently analyzed according to the LDH stratification category. Serum LDH has long been recognized as an important independent biomarker of poor prognosis in malignant melanoma [43] and, in the GM301 study, an interaction between treatment and baseline serum LDH was observed. Patients with LDH values ≤1.1 × ULN who received oblimersen/DTIC (approximately two-thirds [508] of the 771 subjects) were observed to have significantly better treatment
outcomes for all efficacy end points. These included overall survival (median, 11.4 months vs. 9.7 months; \(P=0.02\)), progression-free survival (median, 3.1 months vs. 1.6 months, \(P<0.001\)), overall response (17.2\% vs. 9.3\%; \(P=0.009\)), complete response (3.4\% vs. 0.8\%), and durable response (9.6\% vs. 4.0\%; \(P=0.01\)) [42]. On the other hand, significant differences between-treatment groups were not observed for patients with elevated baseline LDH (LDH > 1.1 × ULN). Recent data demonstrate that the extent to which pretreatment LDH level is increased, even within the “normal” range, is predictive of prognosis in advanced melanoma [44]. For example, a retrospective examination of data obtained from EORTC study 18951 (\(N=330\)) demonstrates a monotonic progression to improved prognosis in advanced melanoma patients as the value of LDH decreases, similar to what has been observed in the GM301 trial. For patients with baseline LDH ≤ 0.8 × ULN in study GM301 (\(N=274\)), the median survival at 24 months in the oblimersen/DTIC group vs. the DTIC group was 12.3 and 9.9 months, respectively (HR = 0.64, \(P<0.001\)). A confirmatory trial (AGENDA, GM307) of 300 patients, similar in design to study GM301 but with a double-blind design and limited to patients with baseline LDH ≤ 0.8 × ULN, is currently ongoing, with recruitment expected to be completed in early 2009. The results from this study should provide important prospective confirmatory data for the previously discussed observations in the GM301 trial.

But why should overall prognosis in advanced melanoma depend on pretreatment levels of serum LDH? LDH is a ubiquitous enzyme, but its expression is frequently elevated in neoplastic cells because of their shift to glycolysis secondary to relatively poor vascularization and diminished oxygen delivery. Cells dying via the process of necrosis will release LDH, but LDH is not commonly released after apoptosis. Tumor cell survival and the rate of necrosis of tumor cells may often depend on the balance between their rate of proliferation vs. the rate of vascularization of the growing tumor. Therefore, it is possible that high LDH levels in patients may reflect disease that is still growing, but is, at least in part, poorly vascularized. These types of tumors are frequently highly resistant to chemotherapy due to poor oxygen delivery, as well as possibly poor drug delivery (hence the lack of response to treatment).

Hypoxia can ultimately be an important survival factor for some tumor cells. For example, hypoxia can induce genetic instability that can select for tumor cells with increased metastatic potential [45–47] and, via c-met protooncogene activation, lead to cells that are more aggressive and invasive [48, 49]. Diminished blood flow and low pH can also compromise the functions of tumor-infiltrating immune effector cells and cytokines. Clinical studies [50] have demonstrated that the presence of hypoxic regions within tumors correlates with poor prognosis and increased metastatic risk regardless of treatment – viz., what is observed in advanced melanoma. Thus, tumor hypoxia leads to necrosis (and thus spillage of LDH) and also ultimately to more aggressive tumors and a poorer prognosis. These ideas predict that the size of the tumor is not the critical factor in either serum LDH levels or prognosis (which it was not in the GM301 trial), but rather that the balance between oxygen supply to the tumor and its intrinsic growth rate is critical.
In the GM301 trial, neutropenia and thrombocytopenia were the most significant adverse effects, but were not associated with an increase in serious infections or bleeding. In the oblimersen/DTIC group, the incidence of grade 3–4 neutropenia with infection was 4.3% for the combination vs. 2.8% for DTIC alone. Grade 1–2 bleeding events (primarily epistaxis or hematuria) were also increased in the combination-treatment group to 13.7% from 9.2% observed for the DTIC group, but more grade 3–4 bleeding events (mostly gastrointestinal) occurred with DTIC (3.1% vs. 2.2%). These rates are substantially lower than those associated with other drugs and drug regimens used for the treatment of advanced melanoma [51–53]. An increased rate of catheter-related events (venous thrombosis, infection, occlusion) was observed in the oblimersen/DTIC group (19.1% vs. 8.6%). Lower rates of adverse events resulting in treatment discontinuation or death and serious adverse events were observed in patients without elevated baseline LDH values [42].

### 20.2.4 Other Trials of Oblimersen

Oblimersen was added to a regimen of etoposide and carboplatin in a randomized (3:1) trial in 56 assessable patients with small-cell lung cancer [54]. In each 21-day cycle, patients in one group received oblimersen 7 mg/kg/day on days 1–8, carboplatin on day 6, and etoposide on days 6–8. Patients in the control group received the same carboplatin and etoposide regimen beginning on day 1 of each cycle. Treatment groups were balanced with respect to baseline characteristics. Response rates were nearly identical in the two treatment groups, and survival at 1 year was actually worse with oblimersen (24%, 95% CI 12–40%) than without oblimersen (47%, 95% CI=21–73%). The incidence of grade 3–4 hematologic toxicity was also somewhat increased with the addition of oblimersen (88% vs. 60%, $P=0.05$). The authors offer several possible explanations for the lack of improved efficacy with the oblimersen-containing regimen, one plausible explanation being that oblimersen does not adequately suppress Bcl-2 levels in patients with small-cell lung cancer, as demonstrated in the phase I study undertaken to determine the regimen for this phase II study.

In acute myelogenous leukemia (AML), Bcl-2 expression may contribute to a lower CR rate and shorter patient survival [55, 56]. In a phase I study, Marcucci et al. enrolled 29 untreated patients with AML [55]. All patients were over 60 years of age, had either intermediate or adverse cytogenetics, and initially received induction therapy with oblimersen 7 mg/kg/day by continuous IV infusion on days 1–10 + cytarabine by continuous IV infusion on days 4–10 + daunorubicin IV at one of two doses on days 4–6. CR was achieved in 14 patients (48%), and an incomplete remission was achieved in three patients (10%). Levels of normalized Bcl-2 mRNA expression in bone marrow mononuclear cells were found to be decreased from baseline ($P=0.03$) in patients with CR, but increased from baseline ($P=0.05$) in nonresponding patients. Expression of Bcl-2 protein in bone marrow mononuclear cells after 72 h of oblimersen demonstrated a small (about 20%), but statistically significant decrease ($P=0.004$) in patients
with CR vs. nonresponding patients. However, given recent data that the gymnastic (i.e., naked) delivery of oligonucleotides to cells is a very slow process requiring 6 days or more to produce antisense effects, it is possible that 72 h was an insufficiently long time point for meaningful measurement of the Bcl-2 protein. All patients developed pancytopenia. Toxicities were independent of the daunorubicin dose, as well as reversible and/or “not directly attributable” to oblimersen.

A phase II trial of oblimersen + gemtuzumab ozogamicin (Mylotarg; a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin) was performed in patients ≥60 years of age with AML at first relapse [57]. Oblimersen 7 mg/kg/day was administered as a continuous IV infusion on days 1–7 and 15–21, with gemtuzumab given IV on days 4 and 18. A total of 48 patients were enrolled at 18 centers, but the study was eventually terminated due to slow accrual. Based on an ITT analysis, five patients (10%) achieved a CR and seven patients (15%) achieved a CR without platelet recovery (CRp), for an overall ITT response rate of 25%. (These findings are similar to those previously reported for single-agent Mylotarg in a more favorable patient population.) [58] For the CR + CRp patients, median relapse-free survival was 3.75 months (95% CI 3.3–6.3 months), and median survival was not reached at 6 months. The probability of surviving at 6 months was 0.80, 0.86, and 0.17 for the CR, CRp, and nonresponding patients, respectively. A total of 13 patients (27%) withdrew before completing therapy, the most common reason being toxicity (6 of 13 patients). Of 16 patients who died within 30 days of last dose of study medication, five did so from treatment toxicity. Nausea was the most common nonhematologic event (79% of patients) and febrile neutropenia the most common hematologic event (50% of patients).

A Phase III trial (CALGB 10201) in which 503 untreated older patients with AML were randomized to induction treatment with cytosine arabinoside + daunorubicin followed by high-dose cytarabine consolidation therapy, with or without oblimersen 7 mg/kg/day (days 1–10 for induction, days 1–8 for consolidation) showed no differences in CR rates, overall survival, disease-free survival, or toxicity [59]. Further trials of oblimersen in AML are not planned.

Another hematologic malignancy in which oblimersen was not successful in phase III was multiple myeloma. In a phase II trial [60], 33 patients relapsing after prior chemotherapy or transplantation received oblimersen 5–7 mg/kg/day for 7 days by continuous IV infusion. On day 4, patients received dexamethasone 40 mg orally for 4 days and thalidomide 200 mg/day increasing to 400 mg/day, if tolerated, for the study duration. Responding and stable patients received maintenance dosing for up to 2 years, and the cycles were repeated every 35 days. A total of 24 of 33 patients (73% [50% historically for the combination of dexamethasone + thalidomide] [20]) had responses, including two CRs, four near CRs, 12 PRs, and six minor responses. The median duration of response was 13 months and the median overall survival was 17.4 months. A rise in polyclonal IgM (from a median of 35.5 to 94 mg/dL) was found to be predictive of response and was suggested to be due to immunostimulation by the oligonucleotide. Of seven assessable patients, three demonstrated a decrease in Bcl-2 protein in malignant cells, but there was no correlation between Bcl-2 protein levels and expression and response in this limited number of patients. The most
common grade 3 toxicities were neutropenia \((n=8)\), thrombocytopenia \((n=5)\), infection \((n=5)\), and hypocalcemia \((n=6)\). Grade 4 events were limited to neutropenia in four patients and increased serum creatinine in one patient.

The dosing scheme in the phase II trial was not pursued. Instead a randomized, multinational phase III trial of dexamethasone 40 mg/day orally for 4 days during weeks 1–3 (Cycle 1) or during week 1 (all other cycles) ± oblimersen 7 mg/kg/day by continuous intravenous infusion beginning 3 days before dexamethasone treatment in weeks 1 and 3 (Cycle 1) and in week 1 (all other cycles) was conducted in a total of 224 patients with relapsed or refractory disease [61]. The primary end point was a comparison of time to disease progression between the two groups.

At baseline, an imbalance was observed between the treatment groups in several important prognostic factors [62]. ECOG Performance Status at baseline was significantly worse in the oblimersen/dexamethasone group \((P=0.03)\). In addition, more patients in the oblimersen/dexamethasone group were categorized as having Durie–Salmon stage III, IIIa, or IIIb disease than in the dexamethasone group (70% vs. 61%, respectively). Imbalances between the two groups in baseline laboratory parameters also suggested that patients in the oblimersen/dexamethasone group were more seriously impaired than those in the dexamethasone group (ANC <1,000/mm\(^3\): 5 and 2%, respectively; platelet count <50,000/mm\(^3\): 7 and 3%, respectively; creatinine >2.0 mg/dL: 5 and 0%, respectively; and lactate dehydrogenase >ULN: 27 and 14%, respectively).

There was no statistically significant difference between the groups in time to tumor progression [62]. The oblimersen/dexamethasone regimen was generally well tolerated, with fatigue, fever, and nausea as the most commonly observed adverse events. Failure to show an advantage over standard treatment (dexamethasone) may be attributable to significant differences between-treatment groups at baseline that favored the dexamethasone group and/or the fact that many patients in this heavily pretreated population were refractory to dexamethasone.

### 20.3 OGX-011

This oligonucleotide is targeted to the mRNA of clusterin, an antiapoptotic protein that apparently promotes chemo- and radioresistance through inhibition of the function of the pro-apoptotic bax protein [63]. The compound has a phosphorothioate backbone and is further modified by the presence of 2'-methoxyethyl (MOE) substituents on the four 3' and 5' terminal ribose sugar moieties. The MOE modification appears to dramatically increase the tissue half-life of this oligomer, in part, by increasing its stability vs. nucleases. There is also some evidence that MOE “gap-mers” may have fewer off-target effects, in addition to diminished immunostimulatory properties. In a phase I study in combination with docetaxel [63], serum clusterin levels in the 640 mg dosing group declined approximately 35% after Cycle 1. However, declines in clusterin expression in peripheral blood mononuclear cells
could not be assessed because of the wide variability in pretreatment expression. This trial was followed by a randomized phase II trial of OGX-11 plus docetaxel vs. docetaxel plus prednisone in chemotherapy-naive patients with metastatic hormone-refractory prostate cancer [64]. Eighty-two patients at 12 centers were randomized to each arm. The docetaxel dose was 75 mg/m², and the OGX-11 dose was 640 mg. In the initial 56 patients, the toxicity due to OGX-011 included grade 1–2 fever and rigors in 37 and 67% of the patients, respectively. Based on a recent press report by Oncogenex, the median survival for patients in the OGX-011 arm was 27.5 months, but only 16.9 months in the control arm. This is certainly an encouraging signal with respect to proceeding to a large, phase III randomized trial in this indication.

20.4 AP 12009

AP 12009 is an antisense PS oligonucleotide targeted to the TGF-β2 mRNA. The justification for targeting TGF-β2 as an important anticancer target has been previously made by Hau et al. [65]. In brief, TGF-β2 is widely overexpressed in human tumors and is negatively correlated with prognosis. The protein blocks the proliferation and cytotoxic activity of T- and NK cells and is a potent immunosuppressant, while at the same time acting, in gliomas, as a growth and angiogenic factor. However, similar to the oblimersen story, it is unclear to what extent the in vivo mechanism of action of AP 12009 is related to these observations.

In early phase I/II trials, the drug was delivered by convection-enhanced delivery directly into the tumors of patients with grade 3 (anaplastic astrocytoma) or grade 4 (glioblastoma multiforme) disease via an implanted catheter either for four or seven days continuously. In another trial, multiple cycles of drug were administered. Twenty-four patients were enrolled, receiving a total of 48 cycles. Many of the patients had been pretreated with temozolamide. Seven showed stable disease after 28 days. One patient had a CR after one cycle of AP 12009 without further therapy; a second patient (who received a total of 12 cycles) also had a CR and was still in remission after 4.5 years. No treatment-related deaths, grade 4 events, or catheter-related infections were observed. Two adverse events were grade 3, and the MTD was not reached after more than a 100-fold dose escalation. Plasma levels of AP 12009 after intracerebral infusion were below the limit of detection, and no laboratory abnormalities were observed. One serious event (brain edema) was considered possibly drug related. All told, the drug appeared to be extremely well tolerated [66]. A phase IIb international, open-label trial in 134 patients with high-grade (3 or 4) glioma was designed to compare (1:1:1) low (10 μM) and high (80 μM) doses of AP 12009 vs. standard chemotherapy (temozolamide + procarbazine + lomustine + vincristine) [67]. The test drug was administered weekly via convection-enhanced delivery for 6 months. Six serious adverse events possibly related to the study drug, and 37 procedure-related serious adverse events (92% grade 1 or 2) were reported. “Several long-term tumor responses were observed by local MRI reading;” response rates by central reading have not yet been presented, to our knowledge.
20.5 Affinitak

This molecule is a 20-mer PS oligonucleotide targeted to the 3′ untranslated region of the PKC-α mRNA, whose translation product was believed to be a very important signal transduction protein. The compound was evaluated in several phase I and phase II trials [68–72]. A total of 55 patients with non-small-cell lung cancer received 80 mg/m² cisplatin and either gemcitabine 1,000 or 1,250 mg/m² + Affinitak 2 mg/kg/day for 14 days via continuous IV infusion, repeated every 3 weeks. Sixteen of 48 (33%) evaluable patients achieved a response (1 CR, 15 PRs). The median overall duration of response was 7 months (95% CI 4.2–7.8 months), and the median duration of stable disease was 4 months (95% CI 3–5.5 months). Based on these data, a large, multicenter, randomized phase III trial in non-small-cell lung cancer was performed. The details of this trial have apparently not been published, but it is understood that Affinitak did not add anything to the gemcitabine + cisplatin combination, and it is no longer being clinically pursued.

20.6 Conclusions

The results of phase III studies of oblimersen in melanoma, CLL, and multiple myeloma suggest that oblimersen may not be as active in patients who have advanced disease and have received multiple prior chemotherapy regimens. Despite the evidence of clinical benefit at this point, our understanding of the mechanism of action of oblimersen, to date the only clinically active anticancer antisense oligonucleotide, is far from complete. While this is of little consequence to the advanced cancer patient, it is far from an optimal situation for those who view antisense as a platform technology. Does this mean that oblimersen is a one-off, a clinical oddity not to be repeated? Will increasing the $T_m$ of the oligonucleotide–mRNA duplex by inclusion of LNA lead to improved clinical efficacy? Will recent advances in our understanding of the uptake of oligonucleotides by cancer cells suggest improved dosing schedules? Are siRNAs too “clean” to be active anticancer agents, and how can they be distributed efficiently to targeted cells? There are a large number of questions that need to be answered, but we believe that additional significant clinical advances can only be achieved rationally by a more complete understanding of the fundamental properties of these highly pleiotropic, biologically active compounds.

20.7 RNAi and siRNAs

The field of oligonucleotide-based therapy experienced a revival with the discovery of RNA interference (RNAi) in 1998 [73]. RNAi is a conserved endogenous mechanism, which is triggered by double-stranded (ds) RNAs leading to target-specific
inhibition of gene expression by promoting mRNA degradation or translational repression. There are two RNAi pathways that are guided either by small-interfering RNAs (siRNAs), which are perfectly complementary to the mRNA or by microRNAs (miRNAs), which bind imperfectly to their target mRNA [74]. A breakthrough in the field of siRNA therapeutic agents was achieved by Elbashir et al. [75], who demonstrated that synthetic, exogenously applied dsRNAs of 21 nucleotides in length can induce silencing in mammalian cells. In addition to the siRNA design of 21-mer duplex with 3’-overhangs at both sides, Dicer-substrate formats such as 27-mers or short hairpin (sh) RNAs have been developed that elicit a more potent gene-silencing effect at lower concentrations as compared to conventional 21-mer siRNAs [76–78].

It is remarkable how quickly after its discovery RNAi has been established as the method of choice for targeted inhibition of gene expression in mammalian systems. Because RNAi uses a natural pathway for gene silencing, it generally results in a greater potency of knockdown than antisense oligonucleotides or ribozymes. Preclinical results have confirmed the effectiveness of RNAi and have generated serious optimism about the potential for siRNA drugs. As with the other oligonucleotide-based approaches, the applications of siRNAs as a therapeutic agent face most of the above mentioned challenges. Some of these challenges, however, have already been addressed in the course of antisense oligonucleotide and ribozyme development.

Many of the standard stabilizing oligonucleotide modifications that have been already explored for antisense strategies were employed in siRNA designs. SiRNA properties can be beneficially improved by the introduction of certain chemical modifications at distinct positions in the sequence, including thermal stability of the duplex, resistance against degradation, specificity for the target mRNA, reduction of off-target effects, biodistribution, and cellular uptake [79]. In a systematic study, Jackson and coworkers reported that many individual nucleotides in the antisense strand may be modified with 2’-O-Me groups without loss of the silencing potential. A similar study has been performed with 2’-fluoro (2’-F) and 2’-O-MOE [80]. An additional advantage of using 2’-O-Me nucleotides is a reduction in off-target effects [81], as well as avoidance of the interferon responses [82]. The strategic placement of these modifications is crucial. Modifications at the 5’-end of the guide strand can inhibit the silencing effect [83], while modifications at the 5’-end of the passenger strand can improve stability as well as guide strand selection and targeting specificity [84, 85]. Incorporation of 3’-S-phosphorothiolate [86], boranophosphates [87], 4’-thioriboses [88], and LNAs [89, 90], were also reported to enhance target-binding affinity and increase silencing potency.

Preclinical studies have demonstrated the safe use and the potential for therapeutic benefit of RNAi-mediated gene silencing [91, 92]. SiRNAs are in early-stage clinical trials for the treatment of viral infections, cancer, and ocular diseases. Phase I studies are planned for numerous other diseases, including neurodegenerative diseases, asthma/allergies, and inflammatory diseases [93]. The most advanced-stage testing for a siRNA-based drug is for the treatment of viral infection and was developed by Alnylam Pharmaceuticals (Cambridge, MA, USA). The siRNA
ALN-RSV01 was designed against the respiratory syncytial virus (RSV), which causes severe respiratory illness, primarily in infants [94]. The unmodified siRNAs, administered by inhalation, showed significant viral reduction in experimentally infected adult volunteers compared to the placebo group in a phase II GEMINI study and is now being tested in patients with naturally acquired RSV infection. Other examples of antiviral applications have been proposed for severe acute respiratory syndrome (SARS) [95], herpes simplex virus 2 [96], and HIV-1 [97, 98]. Serious concerns about the rapid development of drug-resistant HIV variants make the use of multiple-drug combinations inevitable. Recently, a pilot study of safety and feasibility of stem cell therapy for lymphoma patients with AIDS was initiated using a lentivirus vector encoding three anti-HIV RNAs [99]. The combinatorial approach involves a shRNA targeting tat/rev, an RNA TAR decoy, and an anti-chemokine receptor 5 (CCR5) ribozyme.

The lead product of Intradigm (Palo Alto, CA, USA) targets angiogenesis (http://www.intradigm.com) by an RNAi nanoplex particle ICS-283 comprised of a nanoparticle and two siRNAs, one against vascular endothelial growth factor (VEGF) and the other against the VEGF’s main receptor (VEGFR2). The product is in preclinical development for a variety of cancer indications, and the company expects to initiate clinical evaluation in 2009. Two ongoing clinical trials also aim at angiogenesis in age-related macular deficiency (AMD). Bevasiranib (previously known as Cand5) was developed against VEGF and AGN 211745 (previously known as Sirna-027) against its receptor (VEGFR1). Early clinical studies showed that the therapeutic reagents were well tolerated and could prevent neovascularization in the eye after intravitreal injection. AGN 211745 is being investigated in a phase II study in combination with ranibizumab, and patients are currently being enrolled in a phase III study to evaluate the safety and effectiveness of bevasiranib. Controversially, a report was recently published suggesting that the suppression of neovascularization in two animal models is a generic property of siRNAs through TLR3 activation, independent of the sequence [100]. This example clearly demonstrates that preclinical studies need to be carefully conducted to prove safety and a specific siRNA-mediated silencing effect. Encouraged by earlier achievements of oligonucleotide-based therapeutics, some RNAi strategies may have been rushed into clinical trials. It is crucial to understand the basic mechanism of RNAi and its diverse related effectors to avoid toxic side effects and to develop rationally designed biopharmaceuticals.

References

1. Stein CA, Cheng YC: Antisense oligonucleotides as therapeutic agents: is the bullet really magical? Science 261:1004–1012, 1993
2. Stec WJ, Zon G, Egan W, et al: Automated solid-phase synthesis, separation and stereochemistry of phosphorothioate analogs of oligodeoxyribonucleotides. J Am Chem Soc 106:6077–6079, 1984
3. Eder PS, DeVine RJ, Dagle JM, et al: Substrate specificity and kinetics of degradation of antisense oligonucleotides by a 3’ exonuclease in plasma. Antisense Res Dev 1:141–151, 1991
4. Stein CA, Subasinghe C, Shinozuka K, et al: Physicochemical properties of phosphorothioate oligodeoxynucleotides. Nucleic Acids Res 16:3209–3221, 1988
5. Watanabe TA, Geary RS, Levin AA: Plasma protein binding of an antisense oligonucleotide targeting human ICAM-1 (ISIS 2302). Oligonucleotides 16:169–180, 2006
6. Geary RS, Watanabe TA, Truong L, et al: Pharmacokinetic properties of 2’-O-(2-methoxyethyl)-modified oligonucleotide analogs in rats. J Pharmacol Exp Ther 296:890–897, 2001
7. Geary RS, Yu RZ, Watanabe T, et al: Pharmacokinetics of a tumor necrosis factor-alpha phosphorothioate 2’-O-(2-methoxyethyl) modified antisense oligonucleotide: comparison across species. Drug Metab Dispos 31:1419–1428, 2003
8. Walder RY, Walder JA: Role of RNase H in hybrid-arrested translation by antisense oligonucleotides. Proc Natl Acad Sci USA 85:5011–5015, 1988
9. Stein CA, Hansen B, Lai J, et al: Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. Nucl. Acids Res. 2009, doi: 10.1093/nar/gkp841
10. Koshkin AA, Singh SK, Nielsen P, et al: LNA (locked nucleic acids): synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation and unprecedented nucleic acid recognition. Tetrahedron 54:3607–3630, 1998
11. Singh SK, Nielsen P, Koshkin AA, et al: LNA (locked nucleic acids): synthesis and high-affinity nucleic acid recognition. Chem Commun (Camb) 4:455–456, 1998
12. Grünweller A, Wyszko E, Bieber B, et al: Comparison of different antisense strategies in mammalian cells using locked nucleic acids, 2’-O-methyl RNA, phosphorothioates and small interfering RNA. Nucleic Acids Res 31:3185–3193, 2003
13. Fluiter K, Frieden M, Vreijling J, et al: On the in vitro and in vivo properties of four locked nucleic acid nucleotides incorporated into an anti-H-Ras antisense oligonucleotide. Chembiochem 6:1104–1109, 2005
14. Elayadi AN, Braasch DA, Corey DR: Implications of high-affinity hybridization by locked nucleic acid oligomers for inhibition of human telomerase. Biochemistry 41:9973–9981, 2002
15. Braasch DA, Liu Y, Corey DR: Antisense inhibition of gene expression in cells by oligonucleotides incorporating locked nucleic acids: effect of mRNA target sequence and chimera design. Nucleic Acids Res 30:5160–5167, 2002
16. Monteith DK, Henry SP, Howard RB, et al: Immune stimulation – a class effect of phosphorothioate oligodeoxynucleotides in rodents. Anticancer Drug Des 12:421–432, 1997
17. Gekeler V, Gimmiich P, Hofmann HP, et al: G3139 and other CpG-containing immunostimulatory phosphorothioate oligodeoxynucleotides are potent suppressors of the growth of human tumor xenografts in nude mice. Oligonucleotides 16:83–93, 2006
18. Klasa RJ, Gillum AM, Klem RE, et al: Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. Antisense Nucleic Acid Drug Dev 12:193–213, 2002
19. Kitada S, Takayama S, De Riel K, et al: Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of bcl-2 gene expression. Antisense Res Dev 4:71–79, 1994
20. Gjertsen BT, Bredholt T, Anensen N, et al: Bcl-2 antisense in the treatment of human malignancies: a delusion in targeted therapy. Curr Pharm Biotechnol 8:373–381, 2007
21. Webb A, Cunningham D, Cotter F, et al: BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet 349:1137–1141, 1997
22. Waters JS, Webb A, Cunningham D, et al: Phase I clinical and pharmacokinetic study of bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin’s lymphoma. J Clin Oncol 18:1812–1823, 2000
23. O’Brien S, Moore JO, Boyd TE, et al: Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. J Clin Oncol 25:1114–1120, 2007
24. O'Brien S, Moore JO, Boyd TE, et al: 5-year survival in patients with relapsed or refractory CLL in randomized Phase III trial of fludarabine plus cyclophosphamide with or without oblimersen: the Oblimersen CLL Study Group. J Clin Oncol. 27:5208–5212, 2009

25. Rai KR, Moore J, Wu J, et al: Effect of the addition of oblimersen (Bcl-2 antisense) to fludarabine/cyclophosphamide for relapsed/refractory chronic lymphocytic leukemia (CLL) on survival in patients who achieve CR/nPR: five-year follow-up from a randomized phase III study. J Clin Oncol 26:374s, 2008 (suppl; abstr 7008)

26. Weiss LM, Warnke RA, Sklar J, et al: Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. N Engl J Med 317:1185–1189, 1987

27. Reed JC, Kitada S, Takayama S, et al: Regulation of chemoresistance by the bcl-2 oncoprotein in non-Hodgkin’s lymphoma and lymphocytic leukemia cell lines. Ann Oncol 5:61–65, 1994

28. Schmitt CA, Rosenthal CT, Lowe SW: Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med 6:1029–1035, 2000

29. Gazitt Y, Hu WX: Fas (APO-1/CD95)-mediated apoptosis is independent of bcl-2: a study with cell lines overexpressing bcl-2 and with bcl-2 transfected cell lines. Int J Oncol 12:211–220, 1998

30. Gleave ME, Miyake H, Goldie J, et al: Targeting bcl-2 gene to delay androgen-independent progression and enhance chemosensitivity in prostate cancer using antisense bcl-2 oligodeoxynucleotides. Urology 54:36–46, 1999

31. Blagosklonny MV: Paradox of Bcl-2 (and p53): why may apoptosis-regulating proteins be irrelevant to cell death? Bioessays 23:947–953, 2001

32. Soengas MS, Capodieci P, Polsky D, et al: Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature 409:207–211, 2001

33. Bush JA, Li G: The role of Bcl-2 family members in the progression of cutaneous melanoma. Clin Exp Metastasis 20:531–539, 2003

34. Leiter U, Schmid RM, Kaskel P, et al: Antiapoptotic bcl-2 and bcl-xL in advanced malignant melanoma. Arch Dermatol Res 292:225–232, 2000

35. Tang L, Tron VA, Reed JC, et al: Expression of apoptosis regulators in cutaneous malignant melanoma. Clin Cancer Res 4:1865–1871, 1998

36. Ramsay JA, From L, Kahn HJ: bcl-2 protein expression in melanocytic neoplasms of the skin. Mod Pathol 8:150–154, 1995

37. Saenz-Santamaria MC, Reed JA, et al: Immunohistochemical expression of BCL-2 in melanomas and intradermal nevi. J Cutan Pathol 21:393–397, 1994

38. Tron VA, Krajewski S, Klein-Parker H, et al: Immunohistochemical analysis of Bcl-2 protein regulation in cutaneous melanoma. Am J Pathol 146:643–650, 1995

39. Plettenberg A, Ballaun C, Pammer J, et al: Human melanocytes and melanoma cells constitutively express the Bcl-2 proto-oncogene in situ and in cell culture. Am J Pathol 146:651–659, 1995

40. Cerroni L, Soyer HP, Kerl H: bcl-2 protein expression in cutaneous malignant melanoma and benign melanocytic nevi. Am J Dermatopathol 17:7–11, 1995

41. Jansen B, Wacheck V, Heere-Ress E, et al: Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet 356:1728–1733, 2000

42. Bedikian AY, Millward M, Pehamberger H, et al: Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. J Clin Oncol 24:4738–4745, 2006

43. Manola J, Atkins M, Ibrahim J, et al: Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials. J Clin Oncol 18:3782–3793, 2000

44. Agarwala S, Gilles E, Wu J, et al: LDH correlation with survival in advanced melanoma from two large, randomized trials: Oblimersen (GM 301) and EORTC 18951. Eur J. Cancer 45:1807–1814, 2009

45. Cairns RA, Kalliomaki T, Hill RP: Acute (cyclic) hypoxia enhances spontaneous metastasis of KHT murine tumors. Cancer Res 61:8903–8908, 2001

46. Postovit LM, Adams MA, Lash GE, et al: Oxygen-mediated regulation of tumor cell invasiveness. Involvement of a nitric oxide signaling pathway. J Biol Chem 277:35730–35737, 2002
58. Rofstad EK, Rasmussen H, Galappathi K, et al: Hypoxia promotes lymph node metastasis in human melanoma xenografts by up-regulating the urokinase-type plasminogen activator receptor. Cancer Res 62:1847–1853, 2002
59. Bottaro DP, Liotta LA: Out of air is not out of action. Nature 423:593–595, 2003
60. Pennacchietti S, Michieli P, Galluzzo M, et al: Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 3:347–361, 2003
61. Höckel M, Vaupel P: Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93:266–276, 2001
62. Avril MF, Aamdal S, Grob JJ, et al: Fotemustine compared with dacarbazine in patients with disseminated malignant melanoma: a phase III study. J Clin Oncol 22:1118–1125, 2004
63. Chapman PB, Einhorn LH, Meyers ML, et al: Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol 17:2745–2751, 1999
64. Eton O, Legha SS, Bedikian AY, et al: Sequential biochemotherapy versus chemotherapy for metastatic melanoma: results from a phase III randomized trial. J Clin Oncol 20:2045–2052, 2002
65. Rudin CM, Salgia R, Wang X, et al: Randomized phase II study of carboplatin and etoposide with or without the bcl-2 antisense oligonucleotide oblimersen for extensive-stage small-cell lung cancer: CALGB 30103. J Clin Oncol 26:870–876, 2008
66. Marcucci G, Stock W, Dai G, et al: Phase I study of oblimersen sodium, an antisense to Bcl-2, in untreated older patients with acute myeloid leukemia: pharmacokinetics, pharmacodynamics, and clinical activity. J Clin Oncol 23:3404–3411, 2005
67. Banker DE, Radich J, Becker A, et al: The t(8:21) translocation is not consistently associated with high Bcl-2 expression in de novo acute myeloid leukemias of adults. Clin Cancer Res 4:3051–3062, 1998
68. Moore J, Seiter K, Kolitz J, et al: A phase II study of Bcl-2 antisense (oblimersen sodium) combined with gemtuzumab ozogamicin in older patients with acute myeloid leukemia in first relapse. Leuk Res 30:777–783, 2006
69. Larson RA, Boogaerts M, Estey E, et al: Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). Leukemia 16:1627–1636, 2002
70. Marcucci G, Moser B, Blum W, et al: A phase III randomized trial of intensive induction and consolidation chemotherapy ± antisense oligonucleotide in untreated acute myeloid leukemia patients >60 years old. J Clin Oncol 25:360s, 2007 (suppl; abstr 7012)
71. Badros AZ, Goloubeva O, Rapoport AP, et al: Phase II study of G3139, a Bcl-2 antisense oligonucleotide, in combination with dexamethasone and thalidomide in relapsed multiple myeloma patients. J Clin Oncol 23:4089–4099, 2005
72. Chanen-Chan AA, Niesvizky R, Hohlf RJ, et al: Randomized multicenter phase 3 trial of high-dose dexamethasone (dex) with or without oblimersen sodium (G3139; Bcl-2 antisense; Genasense) for patients with advanced multiple myeloma (MM). Blood 104:413a, 2004 (abstr 1477)
73. Data on file. Genta Incorporated. Berkeley Heights, NJ
74. Chi K, Siu L, Hirte H, et al: 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 14:833–839, 2007
75. Chi K, Hotte S, Yu E, et al: A randomized phase II study of OGX-011 in combination with docetaxel and prednisone or docetaxel and prednisone alone in patients with metastatic hormone refractory prostate cancer (HRPC). J Clin Oncol 25:252s, 2007 (suppl; abstr 5069)
76. Hau P, Jachimeczak P, Schlingensiepen R, et al: Inhibition of TGF-β2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. Oligonucleotides 17:201–212, 2007
77. Schlingensiepen KH, Fischer-Blass B, Schmaus S, et al: Antisense therapeutics for tumor treatment: the TGF-beta2 inhibitor AP 12009 in clinical development against malignant tumors. Recent Results Cancer Res 177:137–150, 2008
67. Bogdahn U, Oliushine VE, Parfenov VE, et al: Results of G004, a phase IIb study in recurrent glioblastoma patients with the TGF-β2 targeted compound AP 12009. J Clin Oncol 24:71s, 2006 (suppl; abstr 1553)

68. Nemunaitis J, Holmlund JT, Kraynak M, et al: Phase I evaluation of ISIS 3521, an antisense oligodeoxynucleotide to protein kinase C-alpha, in patients with advanced cancer. J Clin Oncol 17:3586–3595, 1999

69. Yuen AR, Halsey J, Fisher GA, et al: Phase I study of an antisense oligonucleotide to protein kinase C-alpha (ISIS 3521/CGP 64128A) in patients with cancer. Clin Cancer Res 5:3357–3363, 1999

70. Cripps MC, Figueredo AT, Oza AM, et al: Phase II randomized study of ISIS 3521 and ISIS 5132 in patients with locally advanced or metastatic colorectal cancer: a National Cancer Institute of Canada clinical trials group study. Clin Cancer Res 8:2188–2192, 2002

71. Tolcher AW, Reyno L, Venner PM, et al: A randomized phase II and pharmacokinetic study of the antisense oligonucleotides ISIS 3521 and ISIS 5132 in patients with hormone-refractory prostate cancer. Clin Cancer Res 8:2530–2535, 2002

72. Villalona-Calero MA, Ritch P, Figueroa JA, et al: A phase I/II study of LY900003, an anti-sense inhibitor of protein kinase C-α, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. Clin Cancer Res 10:6086–6093, 2004

73. Fire A, Xu S, Montgomery MK, et al: Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806–811, 1998

74. Rana TM: Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol 8:23–36, 2007

75. Elbashir SM, Harborth J, Lendeckel W, et al: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494–498, 2001

76. Rose SD, Kim DH, Amarzguioui M, et al: Functional polarity is introduced by Dicer processing of short substrate RNAs. Nucleic Acids Res 33:4140–4156, 2005

77. Kim DH, Behlke MA, Rose SD, et al: Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. Nat Biotechnol 23:222–226, 2005

78. Siolas D, Lerner C, Burchard J, et al: Synthetic shRNAs as potent RNAi triggers. Nat Biotechnol 23:227–231, 2005

79. Corey DR: Chemical modification: the key to clinical application of RNA interference? J Clin Invest 117:3615–3622, 2007

80. Prakash TP, Allerson CR, Dande P, et al: Positional effect of chemical modifications on short interference RNA activity in mammalian cells. J Med Chem 48:4247–4253, 2005

81. Fedorov Y, Anderson EM, Birmingham A, et al: Off-target effects by siRNA can induce toxic phenotype. RNA 12:1188–1196, 2006

82. Morrissey DV, Lockridge JA, Shaw L, et al: Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat Biotechnol 23:1002–1007, 2005

83. Chiu YL, Rana TM: siRNA function in RNAi: a chemical modification analysis. RNA 9:1034–1048, 2003

84. Chen PY, Weinmann L, Gaidatzis D, et al: Strand-specific 5′-O-methylation of siRNA duplexes controls guide strand selection and targeting specificity. RNA 14:263–274, 2008

85. Kubo T, Zhelev Z, Ohba H, et al: Modified 27-nt dsRNAs with dramatically enhanced stability in serum and long-term RNAi activity. Oligonucleotides 17:445–464, 2007

86. Gaynor JW, Brazier J, Cosstick R: Synthesis of 3′-S-phosphorothioate oligonucleotides for their potential use in RNA interference. Nucleosides Nucleotides Nucleic Acids 26:709–712, 2007

87. Hall AH, Wan J, Shaughnessy EE, et al: RNA interference using boranophosphate siRNAs: structure-activity relationships. Nucleic Acids Res 32:5991–6000, 2004

88. Hoshika S, Minakawa N, Matsuda A: RNA interference induced by siRNAs modified with 4′-thioribonucleosides. Nucleic Acids Symp Ser (Oxf) 49:77–78, 2005

89. Mook OR, Baas F, de Wissel MB, et al: Evaluation of locked nucleic acid-modified small interfering RNA in vitro and in vivo. Mol Cancer Ther 6:833–843, 2007
90. Elmén J, Thonberg H, Ljungberg K, et al: Locked nucleic acid (LNA) mediated improvements in siRNA stability and functionality. Nucleic Acids Res 33:439–447, 2005
91. de Fougerolles A, Vornlocher HP, Maraganore J, et al: Interferring with disease: a progress report on siRNA-based therapeutics. Nat Rev Drug Discov 6:443–453, 2007
92. Behlke MA: Progress towards in vivo use of siRNAs. Mol Ther 13:644–670, 2006
93. Kim DH, Rossi JJ: Strategies for silencing human disease using RNA interference. Nat Rev Genet 8:173–184, 2007
94. Bitko V, Musiyenko A, Shulyayeva O, et al: Inhibition of respiratory viruses by nasally administered siRNA. Nat Med 11:50–55, 2005
95. Li BJ, Tang Q, Cheng D, et al: Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat Med 11:944–951, 2005
96. Palliser D, Chowdhury D, Wang QY, et al: An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. Nature 439:89–94, 2006
97. Jacque JM, Triques K, Stevenson M: Modulation of HIV-1 replication by RNA interference. Nature 418:435–438, 2002
98. Coburn GA, Cullen BR: Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference. J Virol 76:9225–9231, 2002
99. Rossi JJ, June CH, Kohn DB: Genetic therapies against HIV. Nat Biotechnol 25:1444–1454, 2007
100. Kleinman ME, Yamada K, Takeda A, et al: Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature 452:591–597, 2008