Antisense therapy in malignant diseases: status quo and quo vadis?

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ABSTRACT
Preclinical and clinical studies indicate a role for AS ODNs (antisense oligonucleotides) as therapeutics for malignant diseases. The principle of antisense technology is the sequence-specific binding of an AS ODN to the target mRNA, resulting in a translational arrest. The specificity of hybridization makes antisense strategy attractive to selectively modulate the expression of genes involved in the pathogenesis of malignant diseases. One antisense drug has been approved for local therapy of CMV (cytomegalovirus) retinitis, and a number of AS ODNs are currently being tested in clinical trials, including AS ODN targeting Bcl-2, XIAP (X-linked inhibitor of apoptosis protein) and TGF-β2 (transforming growth factor β-2). AS ODNs are well tolerated and may have therapeutic activity. In particular, an AS ODN to Bcl-2 has been tested in phase III clinical trials in chronic lymphocytic leukaemia, multiple myeloma and malignant melanoma. In this review, therapeutic concepts, clinical studies and new promising molecular targets to treat malignancies with AS ODNs are summarized.

INTRODUCTION
AS ODNs (antisense oligonucleotides) are typically 18–25 bp in length, consisting of sequences that are complementary to the target RNA. Once delivered into cells, an AS ODN binds to its RNA counterpart and suppresses expression of the protein encoded by target RNA. The specificity of this approach is based on the probability that any sequence longer than a minimal number of nucleotides – 13 for RNA and 17 for DNA – occurs only once within the human genome [1]. Paterson et al. [2] were the first to report that gene expression can be modified through the use of exogenous nucleic acids by using single-stranded DNA to inhibit translation of a complementary RNA in a cell-free system. In 1978, Zamecnik and Stephenson [3] added a 13-mer synthetic AS ODN, complementary to the 3′-end of the Rous sarcoma virus, to the medium of chicken fibroblasts in tissue culture, along with Rous sarcoma virus itself. The antisense construct inhibited the formation of new virus and prevented transformation of chicken fibroblasts into sarcoma cells. In a cell-free system, translation of the Rous

Key words: antisense oligonucleotide (As ODN), Bcl-2, cancer, malignant disease, targeted therapy, transforming growth factor-β2 (TGF-β2), X-linked inhibitor of apoptosis protein (XIAP).
Abbreviations: AML, acute myelogenous leukaemia; AS ODN, antisense oligonucleotide; CLL, chronic lymphocytic leukaemia; CML, chronic myelogenous leukaemia; CR, complete remission; CRp, CR by all criteria except recovery of a normal platelet count; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FDA, Food and Drug Administration; IAP, inhibitor of apoptosis; BIR, baculovirus IAP repeat; cIAP, cellular IAP; IFN α, interferon α; MALT, mucosa-associated lymphoid tissue; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; 2′MOE, 2′-O-methoxyethyl; MTD, maximum-tolerated dose; NHL, non-Hodgkin’s lymphoma; nPR, nodular partial remission; PBMC, peripheral blood mononuclear cell; PKA, protein kinase A; PKCα, protein kinase Ca; PNA, peptide nucleic acid; PSA, prostate specific antigen; R-CHOP, rituximab and cyclophosphamide, adriamycin, oncovin and prednisolone; SCLC, small cell lung cancer; NSCLC, non-SCLC; STAT-3, signal transducer and activator of transcription-3; XIAP, X-linked IAP.
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FROM UNSPECIFIC KILLING TO TARGETED THERAPY

The classical treatment options in haematology and oncology include surgery, chemotherapy and irradiation. Over the last few years, attention has been focused on more molecularly orientated therapeutic approaches. These include the development of monoclonal antibodies to specifically target cancer cells and small-molecule inhibitors of cell signalling pathways that have been linked to oncogenesis [7].

Another interesting molecular targeting strategy is the introduction of AS ODNs into cancer cells aiming at specific molecules involved in cell proliferation and cell death [8]. In general, the critical steps in the rational drug design process are the identification of an appropriate target responsible for a certain disease and the development of a therapeutic agent with specific recognition and affinity to this target. For the majority of drugs in use to date the mechanism of action is not well defined. In contrast, the specificity of Watson–Crick hybridization is the basis for ‘rational drug design’ of AS ODNs, leading to a new class of selective protein synthesis inhibitors. At the same time, the elucidation of the pathogenetic role of individual target proteins for certain diseases is progressing rapidly, most notably in the field of cancer research.

Several approaches are available to specifically manipulate gene expression at the DNA or RNA level. Gene therapy uses integration of new genetic material into the genome. This approach can replace defective genes or block the effects of unwanted genes by the introduction of a counteracting gene. An alternative strategy is to use single-stranded AS ODNs to modify gene expression at the translational level [9]. AS ODNs are designed to specifically hybridize to the corresponding RNA by Watson–Crick binding. They inhibit mRNA function by several mechanisms, including modulation of splicing and inhibition of protein translation by disrupting ribosome assembly. However, the most important mechanism appears to be the utilization of endogenous RNase H enzymes by AS ODNs. RNase H recognizes the mRNA–oligonucleotide duplex and cleaves the mRNA strand leaving the AS ODN intact. The released AS ODN can bind to another target RNA [9–11]. The specificity of this mechanism has led to a new class of drugs with a wide range of potential clinical applications. One approved antisense drug and a number of clinical antisense trials demonstrate the feasibility of this approach, with some evidence for clinical efficiency [6]. In addition, other non-antisense-mediated biological effects of oligonucleotides are known. The immunostimulatory activity of unmodified phosphodiester oligonucleotides is strongly dependent on the presence of unmethylated CG dinucleotides in certain base contexts, so-called CpG motifs. CpG-dependent immune stimulation of a DNA molecule represents a highly evolved immune defence mechanism whose actual goal is the detection of microbial nucleic acids. In contrast with vertebrate DNA, in which CpG dinucleotides are suppressed and highly methylated, microbial genomes do not generally feature CpG suppression or methylation. Immune effector cells, such as B-cells and dendritic cells, have evolved pattern recognition receptors that, by binding the microbe-restricted structure of CpG motifs, trigger protective immune responses. CpG oligonucleotides, which are designed to provide optimum immune stimulation, are promising anticancer drugs. They are being tested in several clinical trials.

Since AS ODNs inhibit gene expression in a sequence-specific manner, it is possible to selectively alter the expression of genes with closely related sequences. The antisense strategy allows the detailed analysis of signal transduction pathways, which often comprise families of highly homologous proteins. Furthermore, it may lead to the identification of new therapeutic targets and provide the corresponding drug at the same time. Since most tumour cells have a different pattern of gene expression compared with normal cells, AS ODNs can theoretically be used to specifically target tumour-associated genes or mutated genes without altering gene expression of normal cells [12]. In addition, the selective inhibition of a single member of a protein family that plays a role in cancer progression allows other members of the family to perform their normal cellular function. Also, most of the proteins involved in the pathogenesis of cancer operate inside the cell and thus are not accessible to protein-based drugs. Targeting those genes by AS ODNs might be more effective in vivo.

The rapidly growing number of clinical antisense trials represents the growing interest in this technology (Table 1) [9]. In general, systemic AS ODN treatment is well tolerated and side-effects are dose-dependent. Dose-limiting toxicities include thrombocytopenia, hypotension, fever and asthenia [13,14]. Furthermore, elevation of the liver enzymes aspartate aminotransferase and alanine aminotransferase, as well as complement activation and a prolonged PTT (partial thromboplastin time) have been reported [15].

MOLECULAR TARGETING OF GENES BY AS ODNs

Several proteins involved in cell proliferation, angiogenesis, apoptosis and metastasis have been characterized as
Table 1  AS ODNs in clinical trials in haematology and oncology

<table>
<thead>
<tr>
<th>Compound</th>
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<th>Protein target</th>
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<td>Vitravene™ (Fomivirsen)</td>
<td>Isis Pharmaceuticals (<a href="http://www.isispharm.com">http://www.isispharm.com</a>) and Giba Vision (<a href="http://www.cibavision.com">http://www.cibavision.com</a>)</td>
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<td>CMV retinitis</td>
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<td>Genta (<a href="http://www.genta.com">http://www.genta.com</a>)</td>
<td>Bcl-2</td>
<td>Malignant melanoma, B-cell CLL and multiple myeloma</td>
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<td>AML and NSCLC</td>
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<td>SCLC and prostate, colon and breast, liver, renal and pancreatic cancers</td>
<td>Phase I/II</td>
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<td>Isis Pharmaceuticals and Eli Lilly Pharmaceuticals (<a href="http://www.lilly.com">http://www.lilly.com</a>)</td>
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<td>Raf kinase</td>
<td>Solid cancers: ovarian and others</td>
<td>Phase II</td>
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<td>PKA</td>
<td>Solid cancers</td>
<td>Phase I, II</td>
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<td>Head and neck and metastatic renal cancers</td>
<td>Phase II</td>
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<td>R2 component of ribonucleotide reductase</td>
<td>Renal cancer</td>
<td>Phase II</td>
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<td>Solid cancers</td>
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<td>STAT-3</td>
<td>Solid tumour cell lines</td>
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<td>IGFBP-2 and IGFBP-5</td>
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<td>LY 2275796</td>
<td>Eli-Lilly Pharmaceuticals and Isis Pharmaceuticals</td>
<td>elf-4E</td>
<td>Solid tumour cell lines</td>
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Molecular targets for antisense therapy in vitro and are now being tested clinically for haematological and solid malignancies in combination with chemotherapeutic drugs. The first generation of antisense agents contains backbone modifications such as replacement of the oxygen atom of the phosphate linkage by sulphur (phosphorothioates), methyl group (methylphosphonates) or amines (phosphoroamidates) [1]. Of these, the phosphorothioates have been most widely used for gene silencing because of their sufficient resistance to nucleases and ability to induce RNase H functions. In contrast, binding to the target sequence is less satisfactory. Most AS ODNs currently in clinical testing are first-generation antisense, however, continuous or frequent intravenous or subcutaneous infusions are required to administer first generation phosphorothioates because of their short tissue lives, which remains a major technical problem [16].

The second generation of antisense modifications was aimed at improving these properties, among which substitutions of position 2′ of ribose with an alkoxyl group (e.g. methyl or methoxyethyl groups) were most successful. 2′-O-Methyl and 2′MOE (2′-O-methoxyethyl) derivates can be combined further with a phosphorothioate linkage [17]. 2′MOE AS ODNs form duplexes with RNA with a significantly higher affinity relative to unmodified phosphorothioate AS ODNs. This increased affinity has been shown to result in improved antisense potency in vitro and in vivo. In addition, 2′MOE AS ODNs display significantly improved resistance against nuclease-mediated metabolism relative to first-generation phosphorothioate AS ODNs. This property results in an improved tissue half-life in vivo, which produces a longer duration of action [1,16,18,19]. This could allow for a more relaxed dosing regimen, e.g. more
convenient subcutaneous bolus administration instead of the continuous infusions used in most clinical trials to date. The third generation of AS ODNs contains structural elements, such as zwitterionic oligonucleotides (possessing both positive and negative charges in the molecule), locked nucleic acids/bridged nucleic acids, morpholino [20], PNA (peptide nucleic acids) [21] and, more recently, hexitol nucleic acids [22]. All of the modifications enhanced AS ODNs in terms of nuclease resistance, specific binding and, with agents such as PNA and morpholino, cellular uptake. However, the ability of AS ODNs to induce RNase H cleavage was abolished by these alterations. Therefore chimaeric AS ODNs with an unmodified RNase H-susceptible core flanked by modified nuclease-resistant nucleotides have recently been proposed to address this issue [1,23].

AS ODNs are taken up primarily by cells via endocytosis. Only a portion of AS ODNs is able to escape the endosome/lysosome, enter the nucleus and bind to its RNA complement. Because of the hydrophilic and macromolecular nature of the cell membrane, permeation of AS ODNs across this is relatively difficult. Even after two decades of research, safe and efficient delivery of AS ODNs in vivo still remains a major barrier to the clinical success of AS ODN therapies. Cationic liposomes and electroporation are commonly used carriers in vitro. More recently, nanoparticles and AS ODN conjugates have shown improved cellular uptake, biodistribution and targeted delivery, especially in cancer treatment [24]. Inhalation and topical applications of AS ODNs in patients have shown satisfactory profiles of uptake and distribution [25]. However, so far, most AS ODNs that are therapeutically valuable in animal models and in patients have been administered in the form of naked compounds, despite the progress in AS ODN delivery [1].

Cancer is the major target of ongoing clinical trials using antisense therapies, followed by viral diseases such as AIDS and hepatitis. The targets of AS ODNs for cancer treatment include genes involved in cell growth, apoptosis, angiogenesis and metastasis. A limitation for AS ODNs as a therapy for cancer may be the single-target approach. Even if the target is successfully inhibited by AS ODNs, other targets may be activated and compensate for AS ODN inhibition [1].

**TARGETING Bcl-2 USING AS ODNs**

Anti-apoptotic molecules are exciting targets for antisense therapy, for example Bcl-2 and the IAP (inhibitor of apoptosis protein) family. The apoptosis-suppressing Bcl-2 gene was discovered as a proto-oncogene found at the breakpoints of t(14;18) chromosomal translocations. The oncogenic impetus of Bcl-2 overexpression, found in most follicular lymphomas and some cases of diffuse large cell lymphomas and B-cell CLL (chronic lymphocytic leukemia), was verified in Bcl-2 transgenic mice. These mice all accumulated excess non-cycling mature B-lymphocytes. In parallel, B-cell CLL is a classical example of a human malignancy in which the neoplastic cell expansion can be attributed primarily to failed apoptosis rather than rapid cell division [26].

Since the discovery of Bcl-2 as an anti-apoptotic protein in 1988, several theories concerning its mechanism of action have been presented, which have been reviewed extensively elsewhere [27,28]. The Bcl-2 family has been implicated not only in the pathogenesis of cancer, but also in resistance to therapy. Anticancer drugs and radiation ultimately kill cancer cells by inducing the apoptotic cell suicide pathway. Abundant evidence has been amassed indicating that Bcl-2 represents a multidrug-resistance protein, which prevents apoptosis induction by radiation and essentially all chemotherapeutic agents currently in clinical use [29]. Conversely, several in vitro studies have demonstrated that reduction in Bcl-2 achieved by antisense methods increase the susceptibility of cancer cells to apoptosis induction by multiple chemotherapeutic drugs [30]. For example, after engraftment of DoHH2 lymphoma cell line cells into SCID (severe combined immunodeficient) mice, studies showed that in vivo subcutaneous infusion of 5 mg·kg⁻¹ of body weight·day⁻¹ for 21 days of an 18-mer AS ODN complementary to the first six codons of the Bcl-2 open reading frame resulted in complete eradication of this tumour [31].

In various phase I studies, pharmacokinetics, toxicity and therapeutic activity of this AS ODN (oblimersen; Table 1) have been evaluated [14,32]. For example, 21 patients with Bcl-2-positive relapsed NHL (non-Hodgkin’s lymphoma) received a 14-day subcutaneous infusion of oblimersen. Eight cohorts of patients received doses between 4.6 and 195.8 mg·m⁻²·day⁻¹. No significant systemic toxicity was seen at doses up to 110.4 mg·m⁻²·day⁻¹. All patients displayed skin inflammation at standard criteria, there was one complete response, two minor responses, nine cases ofstable disease and nine cases of progressive disease. Bcl-2 protein was reduced in seven of 16 assessable patients as determined by FACS (fluorescence-activated cell sorting). In two of these seven patients, reduced levels of Bcl-2 was detected in tumour cells derived from lymph nodes and in five patients in PBMC (peripheral blood mononuclear cell) or peripheral bone marrow mononuclear cell populations. Expression of HLA, which was used as a control protein, was not affected by antisense therapy. From these results, the authors [14] concluded that: (i) Bcl-2 AS ODN therapy is feasible, (ii) it shows potential for antitumour activity in NHL, and
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(iii) down-regulation of Bcl-2 protein, but not HLA, suggests a specific antisense mechanism [14]. However, it is important to note that Bcl-2 was diminished in less than half of the treated patients. The mean inhibition of Bcl-2 expression was moderate (24%) and the biological significance of this relatively small decline for response is uncertain [33].

Although the maximum tolerated dose of oblimersen was 12 mg·kg⁻¹ of body weight·day⁻¹ in melanoma, 2 mg·kg⁻¹ of body weight·day⁻¹ was noted as a biologically active dose that could effectively down-regulate Bcl-2 protein in 3–5 days of continuous infusion [34–36]. The oblimersen dose explored in ongoing clinical trials ranges from 3–7 mg·kg⁻¹ of body weight·day⁻¹ and varies with the disease under investigation. For example, in diseases with high tumour burden such as CLL, a lower dose of oblimersen is used to avoid tumour lysis syndrome. Ongoing clinical trials will determine the clinical effectiveness and tolerability of these doses in patients with malignancies [36]. Clinical outcome may well be affected by the various doses and modes of administration employed. The pharmacokinetics of oblimersen have been studied in various tumour types. It is highly protein bound in animal models, suggesting the kidneys as the primary route of elimination [37]. In the phase I study reported by Waters et al. [14], mean plasma half-life for elimination of oblimersen was 7.46 h with continuous subcutaneous infusion. No difference in half-life was noted among the various dose levels. At a dose of 2–3 mg·kg⁻¹ of body weight·day⁻¹, a steady-state concentration of >1 µg/ml was consistently reached, a concentration noted to be biologically active in animal studies [36,38].

Based on the results of this and other phase I studies [37,39–42], phase II and III trials have been completed using oblimersen for various malignant diseases.

CLL

Over 75% of patients with CLL overexpress Bcl-2 protein. Previous studies have shown that reduction of Bcl-2 expression by antisense therapy sensitizes CLL cells to chemotherapy-induced apoptosis. In vitro, oblimersen enhances the apoptotic response in CLL cells to fludarabine, corticosteroids, alemtuzumab and rituximab [43,44].

Based on these in vitro data, a phase I trial in patients with fludarabine refractory/relapsed CLL was initiated to determine the MTD, efficacy, safety and pharmacokinetics of oblimersen. Oblimersen was administered at doses ranging from 3–7 mg·kg⁻¹ of body weight·day⁻¹ as a 5-day continuous intravenous infusion in cycle 1 and as a 7-day continuous intravenous infusion in subsequent cycles every 3 weeks in stable or responding patients. Forty patients were enrolled and treated. Dose-limiting reactions included hypotension and fever, and the MTD was established at 3 mg·kg⁻¹ of body weight·day⁻¹. Two of the 26 assessable patients achieved a partial response. Other evidence of antitumour activity included reduction in splenomegaly, hepatomegaly and lymphadenopathy or reduction in circulating lymphocyte counts. Adverse events included transient hypotension, fever, fatigue, diarrhoea, nausea, vomiting, hypokalaemia and cough. Plasma concentration of oblimersen and its metabolites were variable. Altogether, dosing with oblimersen in patients with CLL was limited by development of a cytokine release syndrome that is characterized by fever, hypotension and back pain. Oblimersen showed modest single-agent activity in these heavily pretreated patients with advanced CLL [42].

Subsequently, the updated results from a randomized phase III clinical trial of oblimersen injection in patients with relapsed or refractory CLL showed that the addition of oblimersen significantly increased the proportion of patients who achieved a major response, which was the primary end-point of this trial [45]. Patients were eligible for this trial if they had failed standard treatment for CLL that had included fludarabine. Patients (n = 241) were randomized to receive chemotherapy with fludarabine and cyclophosphamide with or without oblimersen (continuous intravenous infusion of 3 mg·kg⁻¹ of body weight·day⁻¹; days 1–7). Thus, in this trial, the dose of oblimersen was used as determined in the CLL phase I trial, which is lower than the dose employed in trials of oblimersen for solid tumours. The primary objective of the study was to evaluate whether the addition of oblimersen would increase the proportion of patients who achieved CR (complete remission) or nPR (nodular partial remission).

In this trial, 120 patients were randomized to receive oblimersen plus chemotherapy and 121 patients were randomized to receive chemotherapy alone. Approximately half of the patients in each group had failed three or more treatment regimens. All patients had received fludarabine previously, and most had received either cyclophosphamide or another alkylating agent [45,46]. The addition of genasense to fludarabine plus cyclophosphamide significantly increased the proportion of patients who achieved a CR/nPR compared with patients treated with chemotherapy alone (17 compared with 7% respectively; P = 0.025). The median duration of CR/nPR was 21 months in the chemotherapy-alone group, whereas the median had not been reached in the oblimersen group. Although too early to formally compare, median overall survival was 28 months for patients treated with chemotherapy and the median had not been reached in the oblimersen group. The incidence of grade 3 or 4 serious adverse events was higher in the oblimersen group. Specific events that were significantly higher in the oblimersen group included thrombocytopenia, nausea, fever, fatigue, back pain, tumour lysis syndrome, weight loss, dehydration and intravenous
catheter complications [45]. It will be important to see whether a significantly better overall survival can be achieved with oblimersen in follow-up analyses. Based on these phase III data, as well as phase I and II studies of oblimersen in patients with relapsed or refractory CLL and safety data obtained in more than 1000 patients who received oblimersen in clinical trials in other indications, Genta (www.genta.com) initiated submission of a new drug application with the FDA (Food and Drug Administration) seeking marketing approval of oblimersen.

Recently, chemo-immunotherapeutic approaches using a combination of fludarabine with rituximab has shown improved clinical results in patients with CLL, suggesting a trend towards a survival benefit in patients with CLL [47]. To improve upon the quality of the induction of remission by this therapeutic approach, a multicentre study of oblimersen in combination with fludarabine and rituximab in patients with previously treated and untreated CLL has been started [36]. Oblimersen is given as a continuous intravenous infusion at a dose of 3 mg·kg\(^{-1}\) of body weight·day\(^{-1}\) for 7 days. In cycle 1, rituximab will be given on a dose-escalating scheme on days 4 (125 mg/m\(^2\)) and 6 (250 mg/m\(^2\)) while fludarabine (25 mg/m\(^2\)) will be given for 3 days starting on day 6 of a 28 day cycle. In subsequent cycles, rituximab (375 mg/m\(^2\)) will be given on day 5 and fludarabine (25 mg/m\(^2\)) will be given on days 5–7 of a 28-day cycle. These studies will help identify the role of oblimersen in the treatment of CLL [36].

NHL

Previous studies have shown preclinical synergy of oblimersen with rituximab in NHL [48]. In another study for malignant lymphoma, oblimersen was administered daily for 7 days on weeks 1, 3 and 5, and rituximab was administered weekly for 6 weeks. Thirty-five patients who had failed a median of two prior chemotherapy regimens with or without rituximab were entered into this ongoing trial. Six patients achieved a complete response and nine other patients achieved a partial response for an overall response rate of 42 %. Patients with the specific subset of follicular lymphoma showed a response rate of 56 %. Twelve other patients had stable disease. Side-effects of the combination appeared qualitatively similar to that for rituximab alone, including neutropenia, fever, infection, anaemia and fatigue (http://www.genta.com).

In addition, a phase II study of oblimersen in patients with mantle cell lymphoma has been initiated. Thirty-seven patients received oblimersen (3 mg·kg\(^{-1}\) of body weight·day\(^{-1}\)) for 7 days over a 21-day cycle [49]. At the time of progression, newly diagnosed patients were switched to a combination of oblimersen and chemotherapy [R-CHOP (rituximab and cyclophosphamide, adriamycin, oncovin and prednisolone)] and patients treated previously were taken off study. Of the 25 evaluable patients, nine were newly diagnosed and 16 were relapsed. Of the patients treated previously, there was one CR with single agent oblimersen and six patients with stable disease. Oblimersen was well tolerated in this population. As a single agent, oblimersen achieved 40 % stable disease rate, the drug could be safely added to R-CHOP chemotherapy. These preliminary data are intriguing and further studies of oblimersen in this difficult to treat lymphoma are eagerly awaited.

AML (acute myelogenous leukaemia)

Pharmacological down-regulation of Bcl-2 increases chemosensitivity in AML. The feasibility of this approach was tested in untreated elderly AML patients by administering oblimersen during induction and consolidation treatments [50]. Untreated patients with primary or secondary AML, who were 60 years of age, received induction with oblimersen, cytarabine and daunorubicin at one of two different dose levels (45 and 60 mg/m\(^2\)) and, on achievement of CR, consolidation with oblimersen and high-dose cytarabine. An ELISA-based assay was used to measure plasma and intracellular concentrations of oblimersen. Bcl-2 mRNA and protein levels were quantified by real-time RT (reverse transcriptase)-PCR and ELISA respectively, in bone marrow samples collected before induction treatment and after 72 h of oblimersen infusion prior to initiation of chemotherapy. Of the 29 treated patients, 14 achieved CR. With a median follow-up of 12.6 months, seven patients had relapsed. Side-effects of this combination were similar to those expected with chemotherapy alone and were not dose limiting at both dose levels. After infusion of oblimersen for 72 h, BCL-2/ABL mRNA copies were decreased compared with baseline (P = 0.03) in CR patients and increased in non-responders (P = 0.05). Changes in Bcl-2 protein showed a similar trend. Although plasma pharmacokinetics did not correlate with disease response, the median IC of the antisense was higher in the CR patients compared with non-responders. The authors [50] conclude that oblimersen can be administered safely in combination with intensive chemotherapy, and the degree of Bcl-2 down-modulation may correlate with response to therapy.

A phase II multicentre study of oblimersen plus gemtuzumab (Mylotarg\textsuperscript{TM}), a cytotoxic antibody directed against CD33-positive cells, in relapsed patients of 60 years of age and older has been reported [51]. The protocol called for two 7-day infusions of oblimersen, plus two infusions of gemtuzumab, over a 21-day treatment period. The primary end point of the trial was a determination of the proportion of patients who achieved a CR or CRp (CR by all criteria except recovery of a normal platelet count). Forty-eight patients were enrolled in the study, which comprised the ‘intent-to-treat’
population: 39 patients (79%) who actually received the prescribed 21 days of treatment comprised the ‘per-protocol’ population. Twelve patients achieved CR ($n = 5$) or CRp ($n = 7$), for an intent-to-treat remission rate of 25% and a per-protocol rate of 31%. The median time to remission was 52 days. With limited follow-up, ten of the 12 complete responders survived longer than 6 months. Adverse events were qualitatively similar to those that have been reported previously for gemtuzumab used alone, including fever, neutropenia and thrombocytopenia. A portion (21%) of patients discontinued treatment due to adverse events [51]. Results of a randomized trial will be required to evaluate whether the addition of oblimersen improves the response to gemtuzumab in this patient population.

In another paper [52], the investigators reported the development of a new highly sensitive assay that can detect very low concentrations of oblimersen within a cancer cell. They applied this technique to the evaluation of oblimersen uptake in a human leukaemic cell line and also in blood cells and bone marrow taken from patients with AML who were being treated with oblimersen. Results with the cell line showed that the assay successfully detected intracellular oblimersen concentrations that were sufficient to down-regulate protein levels of Bcl-2. Subsequent studies were carried out using AML cells taken directly from patients undergoing oblimersen treatment. These data showed that intravenous therapy with oblimersen achieved intracellular concentrations, which were sufficient to down-regulate Bcl-2.

Findings from these reports have been extended into a randomized Phase III trial of approx. 400 patients with previously untreated AML who received daunorubicin plus cytosine arabinoside with or without oblimersen ([http://www.genta.com](http://www.genta.com)).

**Multiple myeloma**

Not all phase II data translate into a significant clinical benefit when studied in phase III. Based on promising phase I and II data, a randomized phase III clinical trial of oblimersen in patients with relapsed or refractory multiple myeloma was started. The trial did not meet its primary end point, which was the demonstration of a statistically significant increase in time-to-disease progression [36].

Patients were eligible for this trial if they had failed standard treatment for myeloma. Patients ($n = 224$) were randomized to receive standard therapy using high-dose dexamethasone with or without oblimersen. The primary objective of the study was to evaluate whether the addition of oblimersen would significantly increase the time-to-progression. In the trial, 110 patients were randomized to receive oblimersen plus dexamethasone, and 114 patients were randomized to receive dexamethasone alone. Prior to entering the study, patients in both groups had received extensive prior treatment with corticosteroids such as dexamethasone. The median time-to-progression was 3.1 months for patients treated with oblimersen plus dexamethasone and 3.5 months for patients treated with dexamethasone alone, which was not significantly different ($P = 0.26$). Sixteen patients (15%) who were treated with oblimersen plus dexamethasone achieved a major clinical response (defined as a partial response or a response with $\geq 75\%$ reduction of myeloma protein) compared with 20 patients (18%) who were treated with dexamethasone alone ($P = 0.6$) ([http://www.genta.com](http://www.genta.com)).

Specific adverse events that were significantly higher in the oblimersen/dexamethasone group included nausea, fever, constipation, diarrhoea and intravenous catheter complications. Serious adverse events that resulted in discontinuation of therapy were equal between the treatment arms (16% for each group). The incidence of grade 3–4 neutropenia (4%) and anaemia (12%) was identical in the two treatment groups. The incidence of grade 3–4 thrombocytopenia in the oblimersen/dexamethasone group was 14% and 5% in the dexamethasone group. Renal failure, which is a common complication in patients with advanced myeloma, occurred with equal frequency: three patients in the oblimersen/dexamethasone group and four patients in the dexamethasone group. Mortality within 30 days from the last dose of treatment (irrespective of the study drugs) occurred in 13 patients treated with oblimersen/dexamethasone and ten patients treated with dexamethasone ($P$ value was not significant). The excess mortality was due to fatal progression of disease (six patients in the oblimersen/dexamethasone group and one patient in the dexamethasone group) ([http://www.genta.com](http://www.genta.com)). Thus there is currently no clear indication for oblimersen in multiple myeloma.

Altogether, oblimersen is the best studied AS ODN in patients so far; however, issues regarding best target disease, dosing and regimen persist.

**Oblimersen in solid cancers**

**SCLC (small cell lung cancer)**

Preliminary results have been reported from a randomized phase II trial in patients with extensive-stage SCLC who received carboplatin plus etoposide with or without oblimersen. The primary end point of the study was to evaluate landmark survival at 1 year. Using a 3:1 randomization ratio, 63 patients were enrolled into the trial, 41 of whom received oblimersen. The major adverse event was neutropenia; however, the overall incidence of this reaction was similar between the two treatment arms. Survival data are still immature and no benefit in survival is yet apparent ([53]; [http://www.genta.com](http://www.genta.com)).
NSCLC (non-SCLC)

The usefulness of the combination of oblimersen with another non-chemotherapeutic drug has been shown at the preclinical level for oblimersen and gefitinib (Iressa™) in NSCLC. Gefitinib has recently been approved for clinical treatment of patients with advanced NSCLC [54]. Gefitinib is an inhibitor of the EGFR (epidermal growth factor receptor) tyrosine kinase. Recent studies have suggested that clinical response to gefitinib is highly dependent upon the presence of specific mutations in the EGFR gene, which are found in only a small subset of patients with NSCLC. Using experimental xenograft models of human NSCLC cells, oblimersen used alone showed somewhat greater inhibition of tumour growth in mice compared with single-agent gefitinib. However, the combination of both drugs yielded enhanced antitumour activity. Separate studies have shown that these cell lines do not carry the EGFR mutations that are known to be associated with gefitinib sensitivity [54]. Thus the data suggest that the addition of oblimersen may overcome naturally occurring resistance to gefitinib in vivo. Finally, preliminary data using pemetrexed (Alimta™), an antifol that was recently approved for pleuramesothelioma and NSCLC, have revealed similar results in combination with oblimersen in these models. These studies extend the synergistic potential of oblimersen into a new class of anticancer compounds, the EGFR tyrosine kinase inhibitors. A previous study using another tyrosine kinase inhibitor has shown that oblimersen could enhance the antileukaemic activity of imatinib (Gleevec™) in a model of CML (chronic myelogenous leukaemia) ([55]; http://www.genta.com).

Prostate cancer

Data from a study in 20 patients with hormone-refractory prostate cancer, who were treated with oblimersen plus docetaxel (Taxotere™), have been reported [41]. In this phase I study, seven out of 12 patients who had not received a taxane previously had a decrease in PSA (prostate specific antigen), a marker of prostate cancer activity. The median PSA decrease in responding patients was 78% (range, 52–99%). Bcl-2 was also measured in blood cells taken from four patients, and decreases ranging from 92–98% of baseline levels were observed after oblimersen treatment. Lastly, one patient who underwent serial biopsies of metastatic prostate cancer had a 40% decrease in Bcl-2 in tumour tissue on day 6 of oblimersen therapy [41]. These data suggest a down-regulation of the antisense target in vivo.

Final data from a phase II trial of oblimersen (7 mg · kg⁻¹ of body weight · day⁻¹ for 7 days) plus docetaxel (Taxotere™; 75 mg/m² on day 6, repeated every 21 days) in patients with hormone-refractory prostate cancer have been published [56]. Twenty-seven patients were treated with the drug combination and 14 (52%) achieved a major reduction in PSA. Median survival for all patients irrespective of response was 20 months. Side-effects of the combination were qualitatively similar to the use of docetaxel alone and included neutropenia, fever, hair loss, nausea and diarrhoea [56]. The EORTC (European Organization for the Research and Treatment of Cancer) is evaluating docetaxel with or without oblimersen in a randomized phase II trial of approx. 100 patients with hormone-refractory prostate cancer who have not received chemotherapy previously (http://www.genta.com).

Malignant melanoma

Patients with stage IV metastatic melanoma or stage III disease that was not surgically resectable were studied in a phase III trial. Patients were randomly assigned to receive dacarbazine (the standard chemotherapy drug for stage IV disease at 1000 mg/m² after a 5-day pre-treatment regimen of 7 mg · kg⁻¹ of body weight · day⁻¹ oblimersen administered by continuous infusion) alone or in combination with oblimersen. The primary end point of the trial was to compare overall survival between the two treatment arms using an intent-to-treat analysis. Secondary end points included comparative analyses of tumour response, progression-free survival and safety. A total of 771 patients were enrolled at 139 sites in nine countries. Stratification achieved a good balance across the various risk factors. The median age of patients who enrolled in the trial was 60 years.

Extended follow-up for a minimum of 24 months in all patients showed that the addition of oblimersen to dacarbazine improved median overall survival to 9.0 months compared with 7.8 months for patients treated with dacarbazine alone; however, the difference did not reach statistical significance (P = 0.077). Using RECIST (Response Evaluation Criteria in Solid Tumors) criteria, the overall response rate (complete plus partial responses) was 13.5% for patients treated with oblimersen + dacarbazine compared with 7.5% for patients treated with chemotherapy alone (P = 0.007). The complete response rates were 2.8 and 0.8% respectively (P = 0.03). The number of patients who achieved durable responses exceeding 6 months in duration was 7.3 and 3.6% respectively (P = 0.02). Patients treated with oblimersen + dacarbazine had a significant increase in median progression-free survival to 2.6 months compared with 1.6 months for patients treated with dacarbazine alone (P = 0.0007). Adverse events that were significantly greater in the oblimersen-treatment group included nausea, vomiting, neutropenia, thrombocytopenia, fever and catheter-related complications (http://www.genta.com).

In May 2004, a New Drug Application based on 6-months of minimum follow-up data from this trial failed to receive an affirmative vote for approval by an Advisory Committee to the FDA, because overall survival of the intent-to-treat population was not
Antisense therapy in malignant diseases

There are two classic pathways of caspase activation, one involving death receptor ligation, followed by recruitment of adaptor molecules and activation of upstream caspases, the other involving release of cytochrome c from mitochondria, binding of APAF-1 and activation of caspase 9. Once activated, caspase 8 or 9 can, in turn, activate the downstream caspases 3, 6 and 7, which execute the apoptotic programme. Bcl-2 blocks apoptosis at the mitochondrial level, and IAPs by binding and inhibiting specific caspases. CARD, caspase recruitment domain; DD, death domain; DED, death effector domain; FADD, FAS adaptor death domain; Flips, FLICE/caspase 8-inhibitory proteins; tBID, truncated BID; TNFR1, TNFR type 1; TRADD, TNFR-associated DD.

IAP: BLOCKING THE BLOCKER

The IAP family constitute a group of apoptosis suppressors [XIAP (X-linked IAP), cIAP (cellular IAP) 1 and 2, NAIP (neuronal apoptosis inhibitory protein), survivin and apollon] which function as direct inhibitors of certain caspases (Figure 1) [58,59]. So far, only caspases 3, 7 and 9 have been shown to be bound and inhibited by IAPs. Caspases 1, 6, 8 and 10 are not. Thus the caspases inhibited by IAPs operate mostly in the distal portion of apoptotic proteolytic cascades (i.e. caspases 3 and 7), functioning as ultimate effectors of apoptosis by cleaving various proteins essential for life. The ability of cIAP1 and cIAP2 to associate with TRAFs [(tumour necrosis factor)-receptor-associated factors] suggests that they may inhibit the proteolytic processing of effector caspases already at the receptor complex [60]. Because caspases are central for most apoptotic pathways, it is not surprising that IAPs protect cells from several anticancer drugs as well as other inducers of apoptosis [27].
Survivin is overexpressed in a large proportion of human cancers, providing evidence that altered expression of these proteins can occur during tumorigenesis [61,62]. In colorectal, gastric, breast, bladder and lung cancers, survivin expression is associated with shorter survival and, in neuroblastoma, survivin expression correlates with higher stage of disease [63–68]. Interestingly, survivin is expressed in a cell-cycle-dependent manner with highest levels in G2/M and rapid down-regulation following cell-cycle arrest [69]. At the beginning of mitosis, survivin associates with the mitotic spindle and disruption of this interaction results in a loss of its anti-apoptotic function. The overexpression of survivin in cancer may thus overcome this apoptosis-related cell-cycle checkpoint and favour aberrant progression of transformed cells through mitosis. A previous study [70] underlines the role of survivin as a cell-cycle regulator, as survivin was shown to bind to cdk4 (cyclin-dependent kinase 4). Thus survivin bridges apoptosis and the cell cycle. Mutation of a conserved cysteine in the survivin BIR (baculovirus IAP repeat) domain abolished the cytoprotective abilities of survivin. However, the BIR domain mutant retained the ability to associate with microtubules similar to wild-type survivin and interfered with the function of endogenous survivin by competing for microtubule binding. Thus, in contrast with p53 which links DNA replication in the S phase of the cell cycle to apoptosis, survivin appears to couple the cell-suicide response to the checkpoint machinery involved in later cell-cycle steps (G2/M) [71,72].

cIAP2 at 11q21, and a novel gene, MLT, at 18q21, are involved in t(11;18)(q21;q21) associated with MALT (mucosa-associated lymphoid tissue) lymphoma [73]. Although the functional significance of this is not clear, the finding suggests a role for cIAP2 in the pathogenesis of MALT lymphoma, as this rearrangement is found in approx. 50 % of low-grade MALT lymphoma [27,74].

One therapeutic strategy to inhibit IAPs uses ASODNs to decrease the target IAP mRNA. XIAP AS ODNs can reduce XIAP mRNA and protein. XIAP antisense molecules can directly induce apoptosis as well as sensitize cells to chemotherapy and irradiation [75–77]. In a lung carcinoma xenograft, XIAP antisense therapy combined with vinorelbine reduced tumour establishment in mice [78]. Clinically, XIAP AS ODNs are being developed as therapeutic agents by Aegera Therapeutics (Table 1). Their antisense XIAP molecule, AEG 35156, a 19-mer that incorporates 2’-O-methyl chemistry with a phosphorothioate backbone, is currently in phase I clinical trials as a 7-day continuous intravenous infusion in patients with advanced cancers [79].

The efficacy of survivin AS ODNs has been demonstrated both in vitro and in vivo. In vitro, survivin antisense molecules directly induce apoptosis in lung cancer and mesothelioma cell lines overexpressing this IAP. However, cell lines and normal cells, such as PBMCs that do not express survivin, are unaffected by the AS ODN [80,81]. In vivo, survivin AS ODNs administered by transfecting tumour cells with plasmids encoding survivin antisense before tumour implantation reduced tumour growth in xenograft models of gastric carcinoma [82] and thymic lymphoma [83]. Although not strictly an antisense strategy, adenoviral delivery of a dominant-negative survivin suppressed de novo tumour formation in a breast cancer xenograft and shrunk existing tumours by almost half without significant toxicity to the mouse [84]. LY 2181308/ISIS 23722 (Table 1), a second-generation antisense drug currently under development by Isis Pharmaceuticals (http://www.isispharm.com) and Eli Lilly Pharmaceuticals (www.lilly.com), has entered phase I clinical trials for cancer patients [79].

TARGETING Raf KINASE

Another interesting molecular target of AS ODNs in oncology is the MAPK (mitogen-activated protein kinase) pathway, which includes the kinases Raf, MEK [MAPK/ERK (extracellular-signal-regulated kinase) kinase] 1/2 and ERK1/2. A variety of agents have been discovered to interfere with Raf kinase, including AS ODNs [85].

Proliferation, differentiation, survival and apoptosis of all eukaryotic cells is controlled by a highly interactive network of protein kinases and other signal messengers. Many receptor tyrosine kinases and cytokine receptors in association with G-proteins are known to activate intracellular protein serine/threonine kinases termed MAPKs [85,86]. The basic arrangement of this signal cascade includes the G-protein Ras working upstream of a core module consisting of three kinases: Raf that phosphorylates and thus activates MEK1/2, which in turn culminates in the activation of ERK1/2. Raf-1 is a serine/threonine kinase that was initially discovered as a transforming oncogene in retroviral animal models [87]. Subsequently, two human homologues were discovered and named A-Raf and B-Raf [88]. Experiments with an interfering mutant of Raf-1 placed it in a pathway upstream of the core module consisting of three kinases: Raf that phosphorylates and thus activates MEK1/2, which in turn culminates in the activation of ERK1/2. Raf-1 is a serine/threonine kinase that was initially discovered as a transforming oncogene in retroviral animal models [87]. Subsequently, two human homologues were discovered and named A-Raf and B-Raf [88]. Experiments with an interfering mutant of Raf-1 placed it in a pathway downstream of the Ras oncoproteins, which are activated in many human tumours. Key experiments then showed that Raf binds directly to Ras, and that binding to Ras results in membrane-translocation and activation of Raf kinase activity [89]. Activated Raf phosphorylates the kinase MEK on two serine residues, resulting in MEK activation. In turn, activated MEK phosphorylates the kinase ERK on a threonine and a tyrosine residue, resulting in translocation of ERK to the nucleus. Numerous solid tumours are known to express constitutive levels of phosphorylated ERK1/2. ERK1/2 in turn phosphorylate and activate a variety of transcription factors [85]. Raf is activated in tumour cells containing enhanced growth factor signalling pathways, such as those induced by mutant or constitutively expressed...
EGFR family members. Hence Raf pathway inhibitors possess therapeutic potential for multiple tumours that demonstrate elevated levels of growth factor signalling. Recently, the discovery of activating BRAF mutations in many human cancers has further validated B-Raf as a therapeutic target in additional cancers [90]. Therefore the collective evidence suggests that Raf is a viable anticancer drug target [85].

Two different AS ODNs have been developed: ISIS 5132 (by Isis Pharmaceuticals) and LErafAON (by NeoPharma [http://www.neophrm.com]). ISIS 5132 is a phosphorothioate oligodeoxynucleotide antisense compound that targets the 3′-untranslated region of the human RAF-1 gene. ISIS 5132 appears to mediate degradation of Raf-1 mRNA and thereby suppresses protein expression. Reduction of Raf-1 mRNA was shown to occur in tumour-bearing mice treated with relatively low doses. Importantly, preclinical efficacy and toxicology studies suggested a large therapeutic window for ISIS 5132. Three phase I trials have been initiated using ISIS 5132 in patients with a range of solid tumours [91–93]. This approach was generally well tolerated, producing only mild side-effects. In one of the clinical trials, the level of Raf-1 mRNA was found to be suppressed in PBMCs of treated patients [94]. This demonstrates that ISIS 5132 not only targets Raf-1 in tumour cells, but also in normal tissues. Furthermore, phase II data with single-agent ISIS 5132 in patients with hormone-refractory prostate cancer [95], with locally advanced or metastatic colorectal cancer [96] and in patients with SCLC or NSCLC [97] have been published. However, in the doses and schedules studied, ISIS 5132 failed to induce objective responses in these patients [85].

More recently, a different Raf-1 AS ODN has been developed in a new formulation, called LErafAON [98]. To avoid the need to chemically protect the oligonucleotide from degradation and to improve intracellular delivery, LErafAON has been encapsulated in a cationic liposome [98]. Preclinical studies have shown a >50% inhibition of Raf-1 expression in tumour xenografts associated with tumour growth inhibition and sensitization to radiation. Phase I studies have shown that intravenous delivery of LErafAON was well tolerated in combination with radiation [99]. Another phase I dose-escalation trial has shown dose-independent hypersensitivity reactions and dose-dependent thrombocytopenia, limiting tolerance of LErafAON in a weekly bolus regimen in patients with advanced solid tumours [100]. Another phase I study is recruiting patients (http://clinicaltrials.gov).

**TARGETING c-myb**

Genta obtained rights to G4460 (Table 1), formerly known as LRX3001, from Temple University in December 2004. The drug has been tested in two phase I clinical trials in patients with myeloid leukaemias. Genta plans to pursue further clinical development of G4460 in patients with both haematological malignancies and solid tumours. G4460 targets an oncogene known as c-myb, which is a protein that directly binds to cellular DNA. c-myb is believed to regulate the expression of other genes that are involved in the growth and differentiation of primitive cells, including Bcl-2, Bcl-XL, c-myc, cyclin A1, cyclin D1, cdc2 and COX-2 (cyclo-oxygenase-2). Overexpression of c-myb blocks differentiation, promotes proliferation and decreases apoptosis. Potential clinical targets for G4460 include CML, malignant melanoma, neuroblastoma and cancers of the breast, pancreas and colon (http://www.genta.com).

**TARGETING DNA METHYLTRANSFERASE**

MG98 [developed by MethylGene (http://www.methylgene.com); Table 1] has been investigated in four monotherapy phase I dose- and schedule-optimization trials in patients with solid tumours or haematological malignancies [102]. MG98 was reasonably well tolerated in these studies and, in general, adverse effects were reversible (http://www.methylgene.com). One phase II monotherapy trial was conducted in advanced metastatic renal cell cancer patients as some responses were seen during phase I. The primary objective of this study was to evaluate the preliminary efficacy and safety of MG98 when given as a 2-h infusion twice weekly in 3 out of every 4 weeks in patients with advanced metastatic renal cell cancer. No overall response was observed (http://www.methylgene.com).

The second exploratory phase II monotherapy trial was conducted in recurrent or metastatic head and neck squamous cell cancer in Canada and the United States (http://www.methylgene.com). The effect of MG98 at the molecular level was assessed by measuring DNA methylation levels in serial tumour biopsies taken from patients before and after MG98 treatment. An analysis of DNA from tumour biopsies indicated that systematic demethylation (activation) of two important methylated tumour suppressor genes and the methylation (deactivation) of a cancer-causing oncogene had occurred post-MG98 treatment. Thus some patients appear to respond at the molecular level to MG98 treatment. One patient experienced stable disease on this trial (http://www.methylgene.com).

A randomized two-step phase II trial of MG98 in combination with IFNα (interferon α) in metastatic renal cell cancer is underway. The first step of this trial will involve approx. 30–50 metastatic renal cell cancer patients who have not received chemotherapeutic treatment previously. Patients will be randomly assigned to two dosing schedules which will combine MG98 with IFNα. This first step of the trial will evaluate the safety,
t tolerability, pharmacokinetics, optimal dosing regimen and activity of MG98 combined with IFNα. The second step of the trial will enrol approx. 200 patients at up to 35 sites in North America and Europe. Patients will be randomized to treatment with either a combination of MG98 and IFNα or IFNα alone. The MG98 dosing schedule for the second step will be selected based on the best results obtained in step one. The primary end point of this trial will be median progression-free survival. Secondary end points will be tolerability of the combination therapy, 1 year survival, tumour response and overall survival (http://www.methylgene.com).

NEWER MOLECULAR TARGETS

Clusterin

Also known as TRPM-2 (testosterone-repressed prostate message-2), clusterin is associated with a wide variety of physiological and pathological processes [16]. High levels of clusterin are associated with numerous tumours, including prostate, lung and breast cancer, lymphoma and renal cell carcinoma. In prostate cancer, experimental and clinical studies support the hypothesis that clusterin expression is associated with androgen-independent progression and has a protective role against apoptotic cell death. Increased expression of clusterin in prostate cancer is closely correlated with higher Gleason score [104] and cancer prognosis [105]. Clusterin levels also increase in prostate and other cancer cells after chemotherapy and radiation [106]. Thus clusterin seems to act as a survival protein. Inhibiting clusterin might enhance the effect of traditional therapies in cancer treatment [16].

To identify the most potent antisense sequence to move into human trials, the clusterin gene was ‘walked’ with a series of 80 AS ODN sequences [16]. This gene walk identified a 21-mer targeting the AUG translational initiation site, the sequence used in all preclinical human xenografts, as the most potent AS ODN sequence. This 21-mer antisense was incorporated into 2′MOE-gapmer backbone and synthesized for human trials as OGX-011 [developed by Onco Genex Technology (http://www.oncogenex.ca)]. A phase I trial using OGX-011 for patients with localized prostate cancer has been published. OGX-011 was administered prior to radical prostatectomy and thus a pharmacodynamic end point (i.e. inhibition of clusterin expression) could be evaluated for each patient and dose level [107]. OGX-011 was given as a 2-h intravenous infusion over 2 h on days 1, 3, 5, 8, 15, 22 and 29 with radical prostatectomy carried out within 7 days of the last dose. Relevant concentrations of OGX-011 were achieved that inhibited expression of clusterin in human cancer tissue in a dose-dependent fashion. Concentrations of OGX-011 associated with preclinical effect were achieved in tumour tissue and a biologically effective dose, based on clusterin target suppression by up to 90%, was identified. Furthermore, a well-tolerated phase II dose was established based on biological effectiveness, rather than the traditional phase I end point of maximum tolerated dose, which may not be relevant for targeted therapeutics. OGX-011 was well tolerated at the doses studied. 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. No dose-limiting toxicities were observed in the trial. A second phase I study was designed to determine recommended dose of OGX-011 in combination with docetaxel (TAXOTERE™) in various solid tumours [16,108]. OGX-011 is currently in phase II development for patients with prostate, breast and lung cancers.

TGF (transforming growth factor)-β2

High-grade (malignant) glioma are highly aggressive tumours showing marked overexpression of TGF-β2. TGF-β plays a key role in malignant progression by inducing proliferation, invasion and metastasis, angiogenesis and immunosuppression and is responsible for the immunodeficient state of malignant glioma patients. AP 12009, a phosphorothioate AS ODN specific for the human TGF-β2 mRNA, has been developed by Antisense Pharma (http://www.antisense-pharma.com; Table 1) as a targeted antitumour therapy. AP 12009 has already proven safety and shown antitumour activity in phase I/II clinical studies as therapy for recurrent high-grade glioma after intratumoural infusion. A phase IIb multinational study in adult patients with recurrent high-grade glioma is currently ongoing. Patients are randomized into three treatment groups to receive either one of two doses of AP 12009 or standard chemotherapy, i.e. temozolomide, or the combination PCV (procarbazine/lomustine/vincristine). The primary objectives of this study are response rate, progression-free survival and overall survival. AP 12009 is administered intratumourally as continuous high-flow microperfusion for 7 days every other week for up to 11 cycles. Both efficacy and safety will be used as criteria for evaluation. In the previous phase I/II studies, the median overall survival time was longer than that of historic controls on standard chemotherapy. Data on antitumour activity in phase I/II studies with 24 patients included several patients with stabilizations and two patients with complete tumour remissions, both of them long-lasting without recurrence. In the current phase IIb study more than 120 patients have been enrolled [109].

Another phase I trial tested this AS ODN in pancreatic cancer [110]. AP 12009 was tested for its antitumour activity in various preclinical studies in pancreatic carcinoma. TGF-β2 levels in pancreatic carcinoma patients were more than 3-fold higher compared with controls. AP 12009 inhibited TGF-β2 secretion in several
human pancreatic carcinoma cell lines. In functional assays, AP 12009 reduced pancreatic tumour cell proliferation in a dose-dependent manner by up to 76%. Migration of tumour cells was completely blocked compared with untreated controls in a spheroid migration model in contrast with an anti-(TGF-β2) antibody, which had no effect. After AP 12009 treatment, cytotoxicity from IL-2 (interleukin-2)-activated PBMCs co-incubated with pancreatic carcinoma cells increased by up to 401% of the untreated control. Systemic and local tolerability studies in rodents, rabbits and monkeys showed a favourable toxicity profile. Based on these clinical data and the preclinical efficacy in pancreatic carcinoma, a multisite dose-escalation trial with AP 12009 in pancreatic carcinoma patients has been started to assess the maximum tolerated dose [110].

Other AS ODNs tested clinically are shown in Table 1. Affinitak™ targets PKCa (protein kinase Ca); however, Affinitak™ recently failed in a phase III trial for NSCLC. GEM 231 targets PKA (protein kinase A) and is currently in phase I/II clinical testing. GTI 2501 targets different components of ribonucleotide reductase and is currently in phase I/II clinical testing.

**PRECLINICAL TARGETS**

Various molecules are being targeted using AS ODNs in vitro. For example, LY2275796 (Table 1) is a second-generation antisense drug targeting eIF-4E (eukaryotic initiation factor-4E), a protein that is up-regulated in a variety of cancers, including breast, head and neck, prostate, lung, bladder, colon and thyroid cancers and non-Hodgkin’s lymphomas. The molecule facilitates the synthesis of tumour angiogenic factors, growth factors and survival factors by selectively enhancing their translation (http://wwwisispharm.com).

ISIS 345794 (Table 1) is a second-generation antisense drug targeting STAT-3 (signal transducer and activator of transcription-3), a protein that regulates cell division and growth and prevents cell death. In preclinical studies, antisense inhibition of STAT-3 significantly delayed tumour growth and increased the rate of cancer cell death in multiple cell and animal models of cancer. STAT-3 is a member of a multigene family called signal transducers and activators of transcription, which is involved in the regulation of cell growth. STAT-3 appears to play an important role in cell development and death, and is active in a wide range of cancers, including both solid and haematological cancers. Activated STAT-3 is present in numerous malignancies, including head and neck, prostate, breast and lung cancers and in multiple myeloma, anaplastic lymphoma, CML and melanoma. The control of both the activation and inactivation of STAT-3 is equally important to maintain normal cell growth (http://wwwisispharm.com).

**CONCLUSIONS**

There have been a lot of ups and downs in the last decade of development of AS ODNs as therapeutics for human diseases. The biggest up was the approval of formivirs as a drug, the first AS ODN drug ever to enter the market. In addition, oblimersen has been filed for approval as a new drug for CLL and melanoma. On the other hand, the recent lack of success regarding overall survival in large randomized phase III trials testing AS ODNs against Bel-2 for multiple myeloma and PKCa for NSCLC dampened enthusiasm for AS ODN therapeutics. Improved chemistry and better design of molecular therapeutics and clinical trials offer significant advantages for increased response rates.

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