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Review

# Making sense of antisense

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**Abstract**

The specific and rational targeting of key genes, identified to be vital to driving cancer growth, has recently led to the successful development of several small molecule and antibody therapeutics. However, despite considerable efforts, antisense oligonucleotides (ASO) have yet to prove their worth as targeted therapies. However, many important genes cannot be readily targeted by antibodies or small molecules, and could be blocked by ASOs. Moreover, the latest generation of ASOs is safe, well tolerated and able to modulate target protein expression both in surrogate and tumour tissue in the clinic. This review will describe the experience acquired with these agents to date and will raise critical issues relevant to the further optimal development of these agents. Future clinical studies need to evaluate combinations of several different ASO targeting multiple key targets, including strategies that reverse functional redundancy of the key target (*e.g.*, targeting several *Bcl* family members including *Bcl-2* and *Bcl-x*). Approaches to maximise the duration of target blockade yet avert the need for prolonged intravenous infusions, with the consequent risk of line infection and thrombosis, are also needed. These may include slow-release depot subcutaneous formulations. Short interfering (Si) RNA therapeutics, which are now being evaluated in early clinical trials, are also envisioned to impact the future utility of this class of therapeutics. The high manufacture cost of these agents, when compared with small chemical molecules, could however, limit their success unless cost-effective manufacturing processes are developed.


*Keywords:* Antisense; Therapy; Cancer

## 1. Introduction

Antisense therapeutics have evolved significantly since they were first demonstrated in 1977, to specifically inhibit transcription and translation in a cell-free system by hybridisation of a mRNA sequence to its complementary DNA. Further studies reported the successful inhibition of replication of the Rous sarcoma virus by small oligonucleotides of antisense DNA (the non-coding complementary sequence that acts as an RNA template) [1]. The importance of antisense technology became more apparent when both prokaryotes and eukaryotes were reported to employ an antisense protein cascade as a means of controlling gene expression to eradicate foreign viral DNA or RNA as part of the innate host defence system. These observations fuelled interest in hijacking this defence mechanism for the purpose of targeting particular genes or proteins known to play a key role in cancer progression. Further studies led to the successful administration *in vivo* of antisense DNA to the gene *N-myc*. When administered as a continuous subcutaneous infusion this down-regulated *N-myc* gene expression and induced xenograft tumour regression. This was followed by encouraging results in other animal models and cell lines, using antisense against genes such as *Raf* [2], *Survivin* [3] and *MDM-2* [4]. However, early studies of ASOs were limited by a number of factors, resulting in much controversy about

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their utility. These first generation ASOs, which usually comprised of deoxyribonucleotides, were vulnerable to rapid degradation by endo- and exonucleases; poorly soluble; and had limited intracellular uptake. Interest in the field of antisense has, however, been reignited in recent years. Following the sequencing of the human genome many new key gene targets have been identified, several of which remain difficult to target by small molecules or antibodies but are more applicable for targetting by ASOs. Moreover, a new generation of ASOs has improved pharmacological properties and efficacy. This has been possible through the use of computing software that can help predict the accessibility of RNA binding sites and minimise the targeting of inaccessible sites [5]. Furthermore, better assays have been designed to demonstrate "knock-down" of the particular gene or protein, allowing improved assessment of antisense efficacy. Additionally, the discovery of RNA interference (RNAi) and the widespread use of short interfering (Si) RNA have substantially enhanced the credibility of antisense biology.

ASOs are now being widely evaluated for the treatment of many diseases including genetic disorders, hypertension, cardiovascular disease and viral infections. Significantly, an ASO therapeutic has also now received regulatory approval for the treatment of CMV retinitis (vitravene™; fomivirsen sodium). In cancer medicine, the modern, post-genomic goal for anticancer drug development is the targeting of individual proteins involved in carcinogenesis and the development of ASOs remains an attractive strategy since many critical targets driving cancer growth are difficult to target by small molecules or antibodies. These agents can reduce target protein expression and have now demonstrated *in vitro* and *in vivo* antitumour activity in many preclinical models. However, overall clinical trials of many ASOs have yet to demonstrate convincing evidence that they merit incorporation into the routine care of cancer patients. Nonetheless, this class of agents has shown considerable promise for anticancer therapy and it is envisioned that further manipulation of their mechanisms of action will make them effective anticancer therapeutics in the future.

## 2. Mechanisms of action

ASOs are single strands of short deoxynucleotide sequences (18–21 oligomers) that cause targeted transcript destruction by binding to the mRNA of a particular gene by Watson–Crick hybridisation. ASOs are designed to interact with their complementary target RNA in a sequence-specific manner and block gene transcription. This process can activate endogenous nucleases, such as RNase H, which are deployed to cleave the RNA and release the specific oligonucleotide (Fig. 1). Following the destruction of its mRNA, transcription of the corresponding protein cannot occur, resulting in cells becoming depleted of that protein. Other non-antisense mechanisms are, however, also thought to play a role in targeted mRNA destruction and to contribute to the biological effects of ASO-mediated antisense.

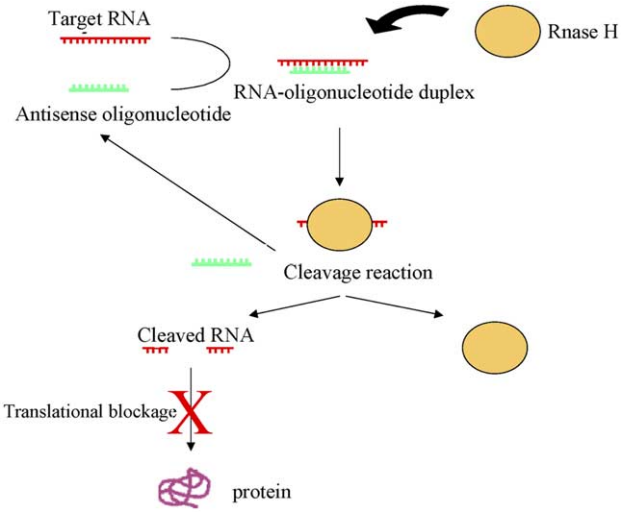


Fig. 1. Antisense-specific mechanism of action of ASOs can involve the activation of RNase H, which cleaves the targeted RNA and releases the ASO.

RNase H is a family of endogenous enzymes that cleave the RNA strand of a heteroduplex RNA–DNA complex and activate RNA degradation. Although the activation of RNase H is the principal mechanism of action of many ASOs, some such as RNA interference, do not exhibit this property although both can possess similar potency, duration of activity, selectivity and efficacy [6]. In addition, some ASOs exhibit non-catalytic antisense effects, such as the occupancy of target RNA causing translational arrest or the modulation of RNA splicing [7].

It has been reported that some ASOs, in particular earlier generation ASOs, also mediate non-specific, less selective effects on a variety of proteins. These interactions raise concerns about the pharmacological properties and toxicities of these agents. These effects appear to be less common with subsequent generations of ASOs. Some oligonucleotides also possess specific sequences that confer non-antisense activity, such as the sequences CpG and GGGG. These induce immune stimulation and have been postulated to contribute to some of the less specific effects of these agents. Although some of these ASOs have been tested in clinical trials, the use of CG dinucleotides remains controversial as they may be associated with undesirable side effects secondary to immune activation [8].

Unlike ASO, SiRNA comprises double stranded (ds) RNA complementary to the gene of interest that can, after introduction into cells, generate a "knock-out"

phenotype. Following transfection, SiRNA is cut into smaller lengths by the cytoplasmic RNase III enzyme Dicer and is then targeted by a protein complex called the RNA-induced silencing complex (RISC). This unwinds the two strands of RNA and releases the antisense strand to guide RISC to its homologous target mRNA for endonucleolytic cleavage. The RISC complex continues to degrade homologous mRNAs, amplifying the silencing effect [9,10]. SiRNA has been widely and very successfully evaluated in the laboratory to study gene function in cultured cells. However, non-specific effects of SiRNAs have been described [11]. Expression profiling studies have revealed SiRNA-specific, rather than target-specific, signatures. Direct silencing of non-targeted genes has also been reported by certain SiRNAs, including genes with as few as eleven contiguous nucleotides of similar identity to the SiRNA, raising concerns that SiRNA may cross-react with targets of limited sequence similarity. Moreover, other studies indicate that certain SiRNAs can also activate components of the interferon pathway in a non-sequence-specific manner through the activation of endogenous cell-signalling pathways important to defence against dsRNA viral infection [12,13]. Nonetheless, although some questions remain about the specificity of SiRNA, their efficacy is unquestioned and the first clinical trials of SiRNA agents have recently commenced. A detailed understanding of the pharmacological properties of these antisense molecules will be required to ensure their future success in the clinic.

### 2.1. Pharmacological properties

Phosphodiester oligonucleotides were the first ASOs described. Although these bind and activate RNase H, their therapeutic efficacy was limited by an inability to selectively hybridise to their target RNA sequence; a susceptibility to degradation by endogenous endonucleases; and low intracellular uptake. Modifications of the base, sugar or phosphodiester structure of these oligonucleotides have overcome these limitations and have improved both RNase activation and pharmacokinetic properties. This was successfully demonstrated by the development of the first and subsequently, second generation ASOs that have successively improved on the properties of previous ASOs (Fig. 2).

The best known ASOs are the phosphorothioate class, first synthesised in 1969. These first generation ASOs are formed by the substitution of the oxygen atoms in the phosphate group with a sulphur atom, resulting in ASOs that are negatively charged, highly soluble and more resistant to endonucleases with a greater hybridisation affinity for target RNA. However, these modifications also result in an affinity for non-specific binding to proteins such as serum albumin; platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF); and clotting factors that can influence the pharmacology and toxicity of these agents. In addition, these first generation ASOs could also exert non-sequence-specific binding, including the inhibition of non-target DNA or RNA polymerases; the enhancement of NK cell

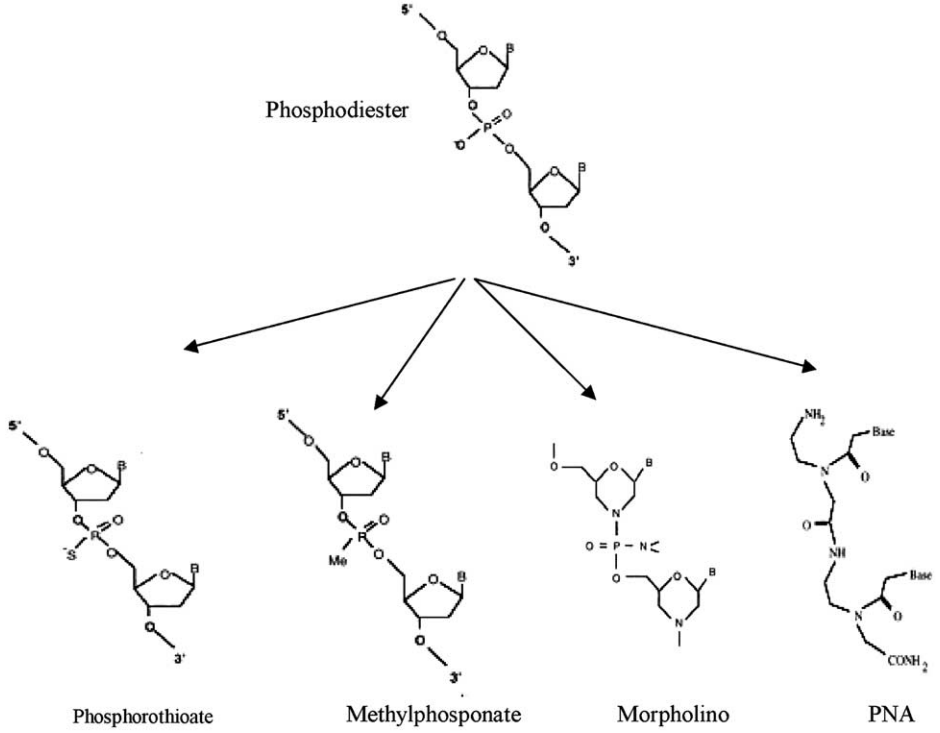


Fig. 2. Modifications of the chemical backbone of ASO have been pursued in order to optimise the pharmacological properties of these agents.

activity; or the activation of transcription factors [14,15]. The polyanionic properties of these ASOs also inhibited their passive entry into target cells resulting in a dependence on active transport mechanisms. Strategies to improve the pharmacological properties of these drugs, such as conjugation to polycationics, coupling to cholesterol or the use of liposome formulations or vectors have been explored [16].

Changes in the ASO sugar moiety can increase affinity for RNA and confer nuclease resistance. These modifications have been utilised in the generation of second generation ASOs. The most promising modifications are the insertion in position 2 of either 2′0-methyl, 2′0-methoxy-ethyl, or a 2′0-alkyl group. This chemical modification, however, also blocks the ability of ASOs to activate RNase. Other modifications evaluated include mixed-backbone oligonucleotides (MBOs), which involve the addition of segments of 2′-O-methyl oligoribonucleotide phosphates at both the 3′ and 5′ ends. This structure provides a more stable phosphate with a higher duplex affinity; increased nuclease resistance; increased tissue uptake; a longer half-life *in vivo*; and less toxicity [17]. Many other modifications of the ASO backbone or phosphate linkages are currently being evaluated. Reports indicate that the absence of charged moieties avoids non-specific interactions with proteins. Peptic nucleic acid (PNA) oligonucleotides, synthesised by completely replacing the sugar–phosphate backbone with a peptide-based backbone have been evaluated but have an unfavourable pharmacological profile with poor water solubility and low intracellular uptake. Morpholino-oligonucleotides are another class of next generation ASOs with a phosphorodiamidate backbone and a morpholino moiety. These confer nuclease stability; improved solubility and uptake by cells; and low non-specific activity [18]. Further clinical study of these next generation ASOs is now required. Concerns remain that some of these ASOs may still have non-specific, non-antisense, biological effects that can lead to unexpected toxicity in the clinic.

#### 2.1.1. ASO toxicology and safety profiles

Overall, despite concerns about non-specific effects, ASOs have very acceptable toxicity profiles. These can broadly be defined as being sequence-specific or non-specific. Toxicity from the most commonly studied ASOs, the phosphorothioate ASOs, is mainly due to the sulphur backbone modification, which results in non-specific protein interactions. In animal toxicology studies, inter-species variation in toxicity has been reported. In rodents, toxicity appears related to cytokine release whilst in primates it is secondary to complement activation and the transient inhibition of the clotting cascade. Effects that are secondary to immune stimulation may be related to specific sequences that induce lymphocyte activation and the production of anti-DNA antibodies. The clotting alterations may be related to binding to plasma clotting factors, that are characterised by a prolongation of activated partial thromboplastin times (aPTT) and are related to peak plasma concentrations [19]. A large number of clinical trials have reported that ASOs are safe at doses required for antisense activity. The dose-limiting toxicities reported include thrombocytopaenia, hypotension, fever, asthenia, transaminitis, complement activation and prolonged partial thromboplastin time. Many of these toxicities reported in the clinic are related to peak ASO plasma concentrations and are similar for different agents. This similarity in toxicity profiles of different ASOs is not surprising and is related to the fact that the majority of the compounds tested to date have been phosphorothioates. Chemical modifications in next generation ASOs are reported to result in less stimulation of the immune system and consequently, fewer non-antisense effects. However, these chemical modifications designed to improve ASO delivery and efficacy could result in side effects not seen with the phosphorothioates.

### 2.2. ASO delivery and target modulation

ASO sequence has little effect on the physicochemical properties of these agents, resulting in remarkably similar drug delivery profiles. Independent of sequence, preclinical mass-balance studies of $^{14}C$ radiolabelled phosphorothioate ASOs indicate that up to 50% of the $^{14}C$ is exhaled as $^{14}CO_2$; in keeping with ASO degradation by nucleases with approximately 15–25% being excreted in urine and faeces; and 20–25% remaining in tissues 10-days after a single administration. After intravenous administration, phosphorothioate ASOs are rapidly cleared from blood, with plasma drug concentrations falling below the limits of quantitation within 5–6 h of the end of infusion. This supports the study of continuous intravenous infusion as the preferred means of administration. ASO pharmacokinetics appears saturable in both preclinical and clinical studies, resulting in non-dose proportional kinetics. This has been attributed to saturation of distribution. This may explain the slower clearance observed with decreasing duration of dose administration. ASO clearance from tissues is much slower than plasma clearance, suggesting a relatively long residual time with tissue half-lives of 1–5 days. This supports more favourable administration schedules in the clinic. ASOs are also well absorbed following intradermal, subcutaneous and pulmonary delivery but are not orally bioavailable. Finally, as ASOs readily bind plasma proteins, especially albumin and alpha-2 macroglubulin there is a potential for pharmacokinetic interactions with concomitantly administered drugs due to competition for protein binding.

However, successful ASOs delivery has been shown to decrease expression of protein targets in both surrogate

and tumour tissue. Oblimersen (Genasense™, G3139), an 18-base phosphorothioate oligonucleotide to the first 6 codons of *bcl-2* mRNA, has been shown to cause depletion of bcl-2 protein expression in peripheral blood mononuclear cells and tumour cells at safe and feasible dosing schedules in the clinic. This has been associated with clinical evidence of antitumour activity (objective responses) in patients with follicular low-grade non-Hodgkin's lymphoma, a disease driven by a translocation involving bcl-2 that results in its increased expression [20].

Studies with several other antisense molecules have also demonstrated evidence of target modulation in the clinic (Table 1). These include trials of an ASO to DNA methyltransferase 1 (*DNMT-1*) known as MG-98 [21] and an antisense to *c-Raf* (ISIS 5132) [22]. However, most of these trials only report data confirming modulation of target expression in surrogate tissue, such as peripheral blood mononuclear cells. A particularly important clinical study is therefore the translational phase I study of the 2′methoxyethyl oligonucleotide OGX-011, targeting clusterin, administered prior to radical prostatectomy intravenously on days 1 and 5 and then weekly as a 2-h infusion. Clusterin, also known as testosterone-repressed prostate message-2 (TRPM-2), is an anti-apoptotic protein that is up-regulated in prostate cancer. Its over-expression confers resistance to androgen ablation, radiation therapy or chemotherapy [23]. Preclinical data demonstrated that clusterin ASOs reduce mRNA and protein levels of clusterin and enhance apoptotic tumour regression after chemotherapy. OGX-011 is the first modified ASO targeting clusterin to be tested in clinical trials [24]. These translational clinical studies confirmed a dose-dependent reduction in target mRNA and protein expression in microdissected prostate cancer cells [25]. At the 640 mg dose-level, clusterin mRNA was reduced to a mean of 8% (SD 4%) compared with lower dose levels and historical controls as assessed by RT-PCR on laser captured microdissected cancer cells. Immunohistochemical studies confirmed these findings, with the mean number of cancer cells expressing no clusterin being increased to

Table 1
Clinical studies of antisense oligonucleotides in cancer patients

| Drug | Protein target | Chemistry properties | Drug delivery | Dose-limiting toxicities | Recommended dose | Evidence for target downregulation (site) |
|---|---|---|---|---|---|---|
| Oblimersen (Genasense, G3139) | BCL-2 | PS | Continuous intravenous infusion for 14 and 21 days | Fatigue, thrombocytopenia and reversible transaminitis | 7 mg/kg/day (shorter infusions) | Yes (PBMCs) surrogate tissue only |
| | | | 14-day subcutaneous infusion in NHL | Thrombocytopenia, hypotension, fever, and asthenia | 110.4 mg/m$^2$/day | Yes (PBMCs and lymph nodes) surrogate and tumour tissue |
| ISIS 3521 (Affinitak) | PKC-α | PS | Continuous intravenous infusion for 3 out of 4 weeks | Thromobytopenia and fatigue | 2.0 mg/kg/day | Not available |
| | | | 2-h intraveneous infusion three times a week for 3 weeks | None | 6.0 mg/kg/day | Not available |
| ISIS 2503 | H-Ras | PS | 14-day continuous intravenous infusion every 3 weeks | None | 10.0 mg/kg/day | Not available |
| ISIS 5132 | Raf-1 | PS | 21-day continuous intravenous infusion every 3 weeks | None | 4 mg/kg/day | Not described |
| | | | 2-h intraveneous infusion three times a week for 3 weeks. | None | 6.0 mg/kg/day | Yes (PBMCs) Surrogate tissue only |
| | | | Weekly 24-h intraveneous infusion | Acute hemolytic anemia, acute renal failure | 24.0 mg/kg/week | Not described |
| GEM 231 (HYBO-165) | PKA-I | MBOs | 5-day continuous intraveneous infusion weekly | Transaminitis | 120.0 mg/m$^2$/day | Yes (serum) |
| OGX-011 (Isis 112989) | Clusterin | MBOs | 2-h continuous intraveneous infusion pre prostatectomy | None | | Yes (prostate tumor) Tumour tissue |
| MG 98 | DNMT 1 | MBOs | Continuous 21-day intravenous infusion every 4 weeks | Reversible transaminitis and fatigue | 80.0 mg/m$^2$/day | Not available |
| | | | 2-h intraveneous infusion twice weekly three weeks out of every four. | Fever, chills and fatigue | 360.0 mg/m$^2$ | Not available |
| | | | 7-day intraveneous continous infusion every 14 days | Thrombocytopenia and transaminitis | 200.0 mg/m$^2$ | Yes (PBMCs) surrogate tissue only |

PBMC, peripheral blood mononuclear cells; NHL, non-Hodgkin's lymphoma; PS, phosphorothioate; MBO, mixed-back bone oligonucleotides.

54% (SD 24%), compared with 2–15% for lower dose levels and historical controls. Moreover a dose-dependent increase in the number of cancer cells undergoing apoptosis was reported confirming that ASO therapeutics can have the desired effect on cancer cells, inducing the desired downstream tumour cell kill at well-tolerated doses (only grade 1 or 2 toxicity was reported at this dose-level: transaminitis, fatigue, fever).

### 2.3. Anticancer activity in clinical trials

Overall however, while many ASOs have been reported to be safe and tolerable and able to successfully modulate the expression of their target protein at non-toxic doses, efficacy trials with these agents have been disappointing to date. Nonetheless, objective tumour responses have been reported with Oblimersen in follicular low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. However, randomised phase III studies in patients with metastatic melanoma comparing dacarbazine (DTIC) in combination with Oblimersen with single-agent DTIC have failed to reveal clinically meaningful patient benefit [26]. Several reasons could explain this lack of antitumour activity including poor target inhibition, or target inhibition of insufficient duration for clinically demonstrable antitumour effect. Also, as these agents are very target-specific, resistance could occur through activation of redundant signalling pathways or co-existing, unrelated, genetic alterations. Combinations of ASOs against multiple key targets implicated in driving tumour growth, including redundant proteins, need to be evaluated to improve antitumour efficacy.

## 3. Future challenges

Clinical trials to date have shown that ASOs can be safely delivered to patients with tolerable toxicity. Moreover, translational studies have demonstrated that these agents can modulate target protein expression in patients' surrogate and tumour tissue in a dose-dependent manner. These promising results have confirmed proof of principle in the clinic for the biological activity of these agents and have laid the groundwork for future clinical research with these agents. Many future challenges, however, remain. These include:

1. The further optimisation of the ASO chemical backbone to improve stability.
2. The study of improved formulations to facilitate delivery.
3. The development of biology-based combinations (based on target and disease biology) of ASOs.
4. The clinical evaluation and optimisation of short interfering (Si) RNA.

Facilitating the ease of ASO administration in the clinic remains an important goal. Currently, optimal target blockade usually requires continuous intravenous infusion, which mandates the insertion of a central venous catheter or peripheral long line with the inherent risks of central venous thrombosis or line infection. More stable ASO chemical backbones limiting ASO degradation by nucleases, and improved depot ASO formulations allowing protracted target blockade following subcutaneous or intradermal administration are needed.

Finally, although little evidence of clinical anticancer activity or patient benefit has been reported when these highly selective targeted agents are administered as single agents, the translational data from clinical samples confirming successful blockade of target protein expression should encourage further study of ASO as cancer therapeutics. These data confirm that future clinical trials should evaluate biology-based combinations of ASOs targeting several targets simultaneously, in order to maximise antitumour activity. The first-in-humans study of multiple ASOs of the same class, administered in combination, needs to be considered but does cause regulatory challenges. Nonetheless, this could safely expedite the successful future clinical development of ASOs.

## Conflict of interest statement

None declared.

## References

1. Zamecnik PC, Stephenson ML. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc Natl Acad Sci USA* 1978, **75**, 280–284.
2. Cioffi CL, Garay M, Johnston JF, *et al*. Selective inhibition of A-Raf and C-Raf mRNA expression by antisense oligodeoxynucleotides in rat vascular smooth muscle cells: role of A-Raf and C-Raf in serum-induced proliferation. *Mol Pharmacol* 1997, **51**, 383–389.
3. Li F, Altieri DC. The cancer antiapoptosis mouse survivin gene: characterization of locus and transcriptional requirements of basal and cell cycle-dependent expression. *Cancer Res* 1999, **59**, 3143–3151.
4. Ru K, Schmitt S, James WI, *et al*. Growth inhibition and antimetastatic effect of antisense poly-DNP-RNA on human breast cancer cells. *Oncol Res* 1999, **11**(11–12), 505–512.
5. Zuker M. On finding all suboptimal folding of an RNA molecule. *Science* 1989, **244**, 48–52.
6. Vickers TA, Koo S, Bennett CF, *et al*. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis Dean NM, Baker BF. *J Biol Chem* 2003, **278**, 7108–7118.
7. Crooke T. Molecular mechanisms of action of antisense drugs. *Biochim Biophys Acta* 1999, **1489**, 31–44.
8. Gewirth AM. Oligonucleotide therapeutics: a step forward. *J Clin Oncol* 2000, **18**, 1809–1811.

9. Fjose A, Ellingsen S, Wargelius A, *et al*. RNA interference: mechanisms and applications. *Biotechnol Annu Rev* 2001, **7**, 31–57.
10. Hannon GJ. RNA interference. *Nature* 2002, **418**, 244–251.
11. Jackson AL, Bartz SR, Schelter J, *et al*. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol* 2003, **21**, 635–637.
12. Moss EG, Taylor JM. Small-interfering RNAs in the radar of the interferon system. *Nat Cell Biol* 2003, **5**, 771–772.
13. Sledz CA, Holko M, de Veer MJ, *et al*. Activation of the interferon system by short-interfering RNAs. *Nat Cell Biol* 2003, **5**, 834–839.
14. Perez JR, Li Y, Stein CA, *et al*. Sequence-independent induction of Sp1 transcription factor activity by phosphorothioate oligodeoxynucleotides. *Proc Natl Acad Sci USA* 1994, **91**, 5957–5961.
15. Boggs RT, McGraw K, Condon T, *et al*. Characterization and modulation of immune stimulation by modified oligonucleotides. *Antisense Nucl Acid Drug Dev* 1997, **7**, 461–471.
16. Biroccio M, Leonetti C, Zuppi G. The future of antisense therapy: combination with anticancer treatments. *Oncogene* 2003, **22**, 6579–6588.
17. Agrawal S, Jiang Z, Zhao Q, *et al*. Mixed-backbone oligonucleotides as second generation antisense oligonulcleotides. *In vitro* and *in vivo* studies. *Proc Natl Acad Sci USA* 1997, **94**, 2620–2625.
18. Summerton J, Weller D. Morpholino antisense oligomers: design, preparation and properties. *Antisense Nucl Acid Drug Dev* 1997, **7**, 187–195.
19. Sheehan JP, Phan TM. Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex by an allosteric mechanism. *Biochemistry* 2001, **40**, 4980–4989.
20. Webb A, Cunningham D, Cotter F, *et al*. BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. *Lancet* 1997, **349**, 1137–1141.
21. Leslie M, Coulthardt SA, Plummer ER, *et al*. Decreased expression of DNMT 1 at the mRNA level following 7 day infusion of the antisense compound MG98 in a phase I study. *EJC* 2004, **2**, 133.
22. Stevenson JP, Yao KS, Gallagher M, *et al*. Phase I clinical/pharmacokinetic and pharmacodynamic trial of the c-raf-1 antisense oligonucleotide ISIS5132 (CGP 69846A). *J Clin Oncol* 1999, **17**, 2227–2236.
23. Miyake H, Chi KN, Gleave ME. Antisense TRPM-2 oligodeoxynucleotides chemosensitize human androgen-independent PC-3 prostate cancer cells both *in vitro* and *in vivo*. *Clin Cancer Res* 2000, **6**, 1655–1663.
24. Zellweger T, Miyake H, Cooper S, *et al*. Ethyl chemistry. Antitumour activity of antisense clusterin oligonucleotides is improved *in vitro* and *in vivo* by incorporation of 2′-O-(2-methoxy) ethyl chemistry. *J Pharmacol Exp Ther* 2001, **298**, 934–940.
25. Chi KN, Eisenhauer E, Fazli L, *et al*. A Phase I pharmacokinetic and pharmacodynamic study of OGX-011, a 2′ methoxyethyl phosphorothioate antisense to clusterin, in patients with prostate cancer prior to radical prostatectomy. *Proc Am Soc Clin Oncol* 2004, **23**, 203.
26. Kirkwood JM, Bedikian AY, Millwars MJ, *et al*. Long-term survival results of a randomized multinational phase 3 of dacarbazine (DTIC) with or without bcl-2 antisense (oblimersen sodium)in patients with advanced malignant melanoma. *Proc Am Soc Clin Oncol* 2005, **23**, 711.