

Chemosensitization by Antisense Oligonucleotides Targeting MDM2

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Abstract: The MDM2 oncogene is overexpressed in many human cancers, including sarcomas, certain hematologic malignancies, and breast, colon and prostate cancers. The p53-MDM2 interaction pathway has been suggested as a novel target for cancer therapy. To that end, several strategies have been explored, including the use of small polypeptides targeted to the MDM2-p53 binding domain, anti-MDM2 antisense oligonucleotides, and natural agents. Different generations of anti-human-MDM2 oligonucleotides have been tested in *in vitro* and *in vivo* human cancer models, revealing specific inhibition of MDM2 expression and significant antitumor activity. Use of antisense oligos potentiated the effects of growth inhibition, p53 activation and p21 induction by several chemotherapeutic agents. Increased therapeutic effectiveness of chemotherapeutic drugs in human cancer cell lines carrying p53 mutations or deletions have shown the ability of MDM2 inhibitors to act as chemosensitizers in various types of tumors through both p53-dependent and p53-independent mechanisms. Inhibiting MDM2 appears to also have a role in radiation therapy for human cancer, regardless of p53 status, providing a rationale for the development of a new class of radiosensitizers. Moreover, MDM2 antisense oligonucleotides potentiate the effect of epidermal growth factor receptor (EGFR) inhibitors by affecting *in vitro* and *in vivo* proliferation, apoptosis and protein expression in hormone-refractory and hormone-dependent human prostate cancer cells. These data support the development, among other MDM2 inhibitors, of anti-MDM2 antisense oligonucleotides as a novel class of anticancer agents, and suggest a potentially relevant role for the oligonucleotides when integrated with conventional treatments and/or other signaling inhibitors in novel therapeutic strategies.

INTRODUCTION

The phenotype of human cancer cells arises from the derangement of several signaling pathways, resulting in the capacity for sustained proliferation, loss of apoptosis, invasion into surrounding tissue, and increased angiogenesis. Oncogenes and tumor suppressor genes have a crucial role in initiating and sustaining the process of cell transformation. The tumor suppressor gene TP53/p53 product, known as p53, is a transcription factor which regulates the transcription of genes that control progression through the cell cycle, cell cycle arrest, and apoptosis following DNA damage induction [1-5]. The p53-induced cell growth arrest is related to its ability to regulate the expression of genes such as MDM2 [6], GADD45 [7] and p21^{WAF1/CIP1} [8]. Alteration of the p53 gene is one of the most frequent genetic abnormalities in human cancers, and correlates with poor prognosis and a lack of response to conventional anticancer therapies [9-11]. Many chemotherapeutic drugs, as well ionizing radiation, exert their cytotoxic effect through the activation of wild-type (wt) p53, leading to cell cycle arrest or apoptosis [12-15]. Restoration of wt p53 can increase the sensitivity of tumors to DNA-damaging agents and, moreover, may overcome the drug resistance of human tumors associated with p53 loss of function mutations. Human cancer cell lines carrying p53 mutations or deletions usually exhibit less growth inhibition and apoptosis compared to wt p53 lines following treatment

with several anticancer agents, such as cisplatin, 5-fluorouracil, and bleomycin. Cancer cell lines with wt p53 display a better response to both chemotherapy and radiation therapy [16]. These data strongly suggest that p53 is an ideal target for cancer gene therapy or for improving therapeutic effects of conventional chemotherapy. Blocking the expression of mutant p53 has been demonstrated to be an effective therapeutic approach in both *in vivo* and *in vitro* tumor models, alone or in combination with chemotherapeutic agents [17-20]. Moreover, since p53's activities are negatively regulated by the MDM2 oncoprotein, the activation of p53 by DNA damage from cancer chemotherapy or -irradiation may be limited in cancer cells with MDM2 expression, especially if MDM2 is overexpressed. It is possible, then, that MDM2 inhibition may increase the magnitude of wild type p53 activation following DNA damage induced by chemotherapeutic agents. For these reasons, several therapeutic strategies involving p53 have been directed to MDM2 blockade, including p53-derived peptides that inhibit the p53-MDM2 interaction, non-peptidic or natural inhibitors, like chalcone derivatives, and antisense oligonucleotides [18, 21-23].

ANTISENSE MDM2 AND CHEMOTHERAPY

Except for antisense anti-MDM2 oligonucleotides, none of the mentioned agents have been tested in combination therapy with conventional chemotherapeutic drugs. The first class of specific antisense oligos (AS) against human MDM2 tested in cancer cell lines were phosphorothioate (PS) oligodeoxynucleotides (PS-AS-MDM2). These oligonucleotides were able to strongly inhibit MDM2

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expression in human choriocarcinoma JAR cells and osteosarcoma SJSA cells [24]. Both cell lines harbor wild-type p53, amplified MDM2 genes, and overexpress MDM2 protein. MDM2 inhibition correlated with an elevation in p53 activities in these cell lines, as demonstrated by the dose-dependent induction of p21 (a p53-inducible gene) expression following antisense treatment. MDM2 inhibition also correlated with a strong induction of apoptotic cell death. Neither p21 expression, nor p53-induced apoptosis occurs in the p53-null tumor cell line H1299, suggesting that these effects are due to activation of p53. Inhibition of MDM2 expression also enhances DNA damage-induced activation of p53 and cell death in a breast cancer cell model. In fact, treatment of MCF-7 cells, a breast tumor cell line carrying wt p53 and no MDM2 amplification, with the topoisomerase I inhibitor camptothecin (CPT) results in a strong, synergistic activation of p53 when used in combination with PS-AS-MDM2. Co-incubation with CPT and PS-AS-MDM2 also resulted in significantly increased apoptotic cell death in both MCF-7 and H1299 cell lines, demonstrating that inhibition of MDM2 expression can synergistically cooperate with DNA damaging agents to boost p53 activity. A second generation, mixed-backbone oligonucleotide (MBO) targeting MDM2 (MBO AS-MDM2), containing centrally modified or end-modified nucleosides, also showed significant *in vitro* antitumor activity in SJSA and JAR tumor cell lines, in a time- and dose-dependent manner [25]. The inhibition of MDM2 expression and tumor proliferation also extended to an *in vivo* model: dose-dependent growth inhibition of SJSA tumor xenografts was found following treatment with the MBO AS-MDM2. Moreover, the oligonucleotide significantly increased the therapeutic effects of two DNA damaging agents, adriamycin and 10-hydroxycamptothecin (HCPT), in SJSA xenografts in a dose-dependent manner. The mismatch oligonucleotide, Oligo ASM, showed no effect on the therapeutic effectiveness of HCPT. Co-treatment with HCPT and MBO AS-MDM2 also caused a synergistic inhibition of tumor growth in nude mice bearing JAR xenografts. In fact, while the chemotherapeutic drug alone was unable to modify animal survival compared to the untreated group, and AS-MDM2 alone moderately inhibited tumor growth and increased survival, the combined treatment of MBO AS-MDM2 plus HCPT significantly improved the survival rate with almost complete tumor regression in 50% of the animals. These data demonstrate that the combination of MDM2 inhibition and DNA damaging agents has better therapeutic effects compared to single agent therapy, resulting in improved tumor regression and animal survival.

The effect of MDM2 inhibition through antisense oligos in combination with cytotoxic drugs has been investigated in several human cancer models, including breast, prostate and colon cancer cells lines. MBO AS-MDM2 treatment caused *in vitro* dose-dependent growth inhibition of GEO human colon cancer cells [26], while an MBO with a scrambled sequence at the same doses showed only a modest inhibitory effect. AS Oligo treatment had an effect on the expression of the target gene product MDM2, as well as on p53, and the expression of the p53-inducible p21/WAF1 gene product. Treatment with MDM2 antisense oligo decreased the expression of MDM2 in a dose-dependent

manner, and increased expression of both p53 and p21/WAF1 in GEO cells, a wt p53 cell line with a moderate overexpression of MDM2. Conversely, the scramble MBO was unable to modify expression of these proteins, even at the highest dose. The AS-MDM2 in these cells is able to cooperate with a variety of antitumor drugs because it acts by a different mechanism. A clear cooperative effect is observed when AS-MDM2 is used in combination with the platinum-derived cisplatin and carboplatin, with the topoisomerase I-selective drug topotecan, the topoisomerase II-selective drug doxorubicin, and with the two taxanes, paclitaxel and docetaxel. The cooperative effect is more evident with lower doses of cytotoxic drug, since an already maximal effect is achieved by increasing the single-agent dose. The scramble MBO is unable to increase the inhibitory effect of any cytotoxic drug tested. When AS-MDM2 is used in GEO cells in combination with different doses of 5-fluorouracil, methotrexate or etoposide, an additive growth-inhibitory effect is observed on soft agar colony formation. The proliferation arrest caused by combination of AS-MDM2 and different chemotherapeutic agents is the result of strong induction of apoptotic cell death in GEO cancer cells. Treatment with AS-MDM2 alone, but not with scramble MBO, leads to a moderate increase in apoptotic index (about 1.5 fold) compared to untreated control cells. A cooperative effect toward apoptosis was observed when AS-MDM2 was used in combination with different cytotoxic drugs. For instance, while topotecan alone induces a moderate increase in apoptosis (about 1.5 fold), similar to that induced by antisense alone, the combination of the two agents increases apoptosis over 4.5-fold, as compared to control untreated GEO cells. A super-additive effect on the induction of apoptosis is also obtained with AS-MDM2 in combination with cisplatin and docetaxel. Marked anti-tumor activity is obtained in nude mice bearing GEO cell xenografts upon sequential treatment with AS-MDM2 in combination with chemotherapeutic drugs. Compared with untreated animals, a variable and significant reduction of tumor growth is observed in mice treated with AS-MDM2, cisplatin or topotecan. Combination of AS-MDM2 with either cisplatin or topotecan results in a potent and statistically significant inhibition of tumor growth when compared with the tumor progression observed in untreated mice, mice treated with the scramble MBO, or mice treated with either agent alone. Moreover, mice treated with AS-MDM2 in combination with either cisplatin or topotecan experienced a significantly prolonged delay in tumor growth and a significantly increased life span compared to those treated with single agents. Interestingly, MDM2 protein expression in GEO tumor specimens is down-regulated to a greater extent with the combination of AS-MDM2 plus topotecan, as compared to AS-MDM2 alone [26].

The role of the MBO AS-MDM2 as a chemosensitizer has also been investigated in human breast cancer models [27], using the MCF-7 cell line containing wt p53 and the MDA-MB-468 cell line containing mutant p53. AS-MDM2 treatment in MCF-7 cells correlates, *in vitro*, with the inhibition of MDM2 protein expression in a sequence-specific and dose-dependent manner, leading to elevated levels of p53 and p21, as expected, and with inhibition of tumor cell growth and proliferation. *In vitro* MCF-7 cells exposed to the cytotoxic drugs adriamycin, HCPT and 5-

fluorouracil produce elevated levels of p21 and p53 in a dose-dependent manner. Combination of MBO AS-MDM2 and any of the three chemotherapeutic agents results in a significant elevation in both p53 and p21 levels compared to the single-agent treatment. The effect of combined treatment on *in vivo* tumor proliferation has been evaluated in an MCF-7 xenograft model using antisense MDM2 plus 5-fluorouracil, paclitaxel or irinotecan. Following the combination of the oligonucleotide with 5-fluorouracil or paclitaxel, a significant additive effect on tumor growth inhibition was observed; a similar significant synergistic effect was shown when the AS-MDM2 was combined with irinotecan. Interestingly, at least in this experimental model and conditions, the mismatch control oligonucleotide also showed a similar effect when combined with irinotecan, probably due to the effects of the mismatch oligo on the pharmacokinetics and metabolism of this topoisomerase I inhibitor. The role of negative modulation of MDM2 in tumor growth inhibition appears to be relevant regardless of p53 status [27]. For instance, in the MDM-MB-468 cell line, which contains mutant p53, the use of AS-MDM2 resulted in a strong, dose-dependent reduction in MDM2 protein expression with no significant changes in the levels of mutant p53. The p21 levels were elevated following AS-MDM2 treatment, which is independent of p53. When combined with the cytotoxic agent adriamycin, the AS-MDM2 is able to increase the magnitude of p21 induction. Similar to MCF-7 tumors, MDM-MB-468 xenografts experienced significant synergistic effects on tumor growth inhibition following combined treatment with AS-MDM2 and 5-fluorouracil, paclitaxel or irinotecan. Therefore, the combination treatment of AS-MDM2 and cytotoxic drugs in both breast cancer cell lines induced tumor inhibition, demonstrating the effect of AS-MDM2 as a sensitizer for chemotherapy, through both p53-dependent and p53-independent mechanisms.

Human prostate cancer models have been extensively used to investigate the cellular responses to MDM2 inhibition and its role in tumor sensitization to cytotoxic agents. In a study conducted in a cohort of prostate cancer patients, MDM2 expression was associated with features of more advanced disease, suggesting that its overexpression inactivates p53 and favours prostate cancer progression [28].

The use of a MBO AS-MDM2 [29] in LNCaP (containing wt p53), DU145 (p53 mutant) and PC3 (p53 null) human prostate cancer cell lines, in which MDM2 is not overexpressed, results in the inhibition of proliferation, and induction of apoptosis in a dose-dependent manner in all three cell lines, regardless of p53 status. At the same time, pretreatment with AS sensitizes all the cell lines to the cancer chemotherapeutic drugs 10-hydroxycamptothecin (HCPT) and paclitaxel. MDM2 inhibition by MBO AS produced significant antitumor activity in a dose-dependent manner, and increased the therapeutic effectiveness of paclitaxel in severe combined immunodeficient (SCID) mice bearing LNCaP xenografts. Moreover, the *in vivo* PC3 model, which does not express p53, demonstrated strong sensitization to paclitaxel by the AS-MDM2, providing direct evidence for the p53-independent activity of MDM2. Interestingly, after AS-MDM2 treatment in both LNCaP and PC3 cell lines, induction of expression of p21 and Bax is observed *in vitro* and *in vivo*, regardless of p53 status. Bax

elevation is accompanied by Bcl-2 reduction in LNCaP cells and xenografts. Since Bcl-2 up-regulation is associated with androgen-independence and resistance to chemotherapy in prostate cancer, these results provide an explanation, at least in part, for the significant antitumor and chemosensitization effects of antisense therapy. AS-MDM2 combination treatment with cytotoxic agents such as HCPT, adriamycin, 5-fluorouracil and paclitaxel [30] results in inhibition of MDM2 expression, dose-dependent activation of p53 and p21, and induction of apoptotic cell death in LNCaP cells. Pretreatment with AS-MDM2 also increased the levels of p21 and apoptosis induction arising from cytotoxic DNA-damage in p53-mutated DU145 cell line, with no changes in p53 levels. Moreover, AS-MDM2 has *in vivo* anti-tumor activity in DU145 and PC3 models in combination with irinotecan, in a sequence-independent manner, and in a synergistic fashion. AS-MDM2 in combination with the chemotherapeutic agents camptothecin and irinotecan in CD-1 mice did not alter the host toxicity of either cytotoxic drug, regardless of the administration schedule.

ANTISENSE MDM2 IN COMBINATION WITH CYTOTOXIC AGENTS

The most common and successful treatment for advanced prostate cancer is androgen deprivation. However, although this therapy causes dramatic regression of prostate tumors, the vast majority of patients experience only a temporary response, and then progress to an androgen-independent condition. Androgen ablation shrinks tumors by inducing apoptosis and reducing cell proliferation. Since abnormal or suppressed p53 expression has been associated with resistance to androgen deprivation, it has been proposed that MDM2 may contribute to the modulation of the apoptotic response following androgen ablation [31]. In fact, the use of a specific AS-MDM2 oligonucleotide strongly increases growth inhibition of LNCaP prostate cancer cells when cultured in the absence of androgen hormones in a dose-dependant manner. In addition, the use of the synthetic androgen R1881 partially restores the growth of LNCaP cells treated with the combination of AS-MDM2 plus androgen deprivation. The inhibitory effect on cell proliferation is related to the induction of apoptotic cell death: the AS-MDM2 treatment sensitizes LNCaP cells to androgen deprivation by significantly increasing apoptosis, an effect reversed by R1881. The growth inhibitory and cell death effects of the combined treatment seem to be related to MDM2 expression; in fact, AS-MDM2 is able to inhibit MDM2 expression and induce apoptosis to a greater extent in LNCaP cells, as compared to LNCaP-MST, a stable transfected cell line that overexpresses MDM2. Androgen deprivation alone causes a reduction in MDM2, p53 and p21 protein levels in LNCaP cells; but combination with AS-MDM2 results in a further reduction of MDM2 levels, while the expression of p53 and p21 remain normal [31]. These results demonstrate that the apoptotic response of prostate cancer to androgen deprivation is strongly influenced by MDM2 expression, and that the AS-MDM2 MBO has broad potential as a therapeutic agent to sensitize prostate cancer cells to androgen ablation therapy by enhancing apoptotic cell death.

The progression of prostate cancer from androgen-dependent to hormone-refractory disease is a complex multi-

step process involving multiple factors, such as oncogenes, tumor suppressor genes, growth factor receptors, signal transduction molecules and molecules involved in angiogenesis. Among them, a key role is played by the activated EGFR, a major transducer of mitogenic signals, and an inducer of angiogenic growth factors and neoangiogenesis, which are involved in pathogenesis and progression of several human cancers [32]. It has been demonstrated that EGFR expression increases during the natural history of prostate cancer, and the progression from hormone-dependence to hormone-refractory disease [33, 34], and it has a potent independent prognostic effect on disease-free survival, when evaluated by a Cox multivariate analysis [35]. On this basis, EGFR-targeted drugs could be of therapeutic relevance in prostate cancer. Gefitinib (ZD1839, Iressa) is an orally active EGFR tyrosine kinase inhibitor anticancer drug that has shown antitumor activity in a variety of human cancer types, including prostate cancer, alone and in combination with other agents [34, 36-38]. Moreover, a relevant direct functional link has been demonstrated between MDM2 and the ras-raf-MAPK signalling pathway [39, 40]. For these reasons, combined inhibition of EGFR and MDM2 has been employed to investigate their roles in the development of hormone-independent prostate cancer and the control of several key signal transducers, using hormone refractory PC3 and DU145, and hormone sensitive LNCaP human prostate cancer cells [41]. The effects of drugs, alone and in combination, demonstrate that AS-MDM2 and gefitinib have dose-dependent anti-proliferative effects in all the three cell lines tested. The LNCaP cells were the most sensitive to the AS-MDM2. While a positive cooperation is observed with AS-MDM2 in combination with gefitinib, the mismatch control antisense oligonucleotide is unable to significantly modify the inhibitory effect of gefitinib. A combination analysis demonstrated a strong synergism between the action of AS-MDM2 and gefitinib in hormone-independent PC3 cells and in DU145 cells, while the effect is only additive in hormone-dependent LNCaP cells. Gefitinib or the control oligo Mm-ON, even at high doses, do not have an effect on MDM2 expression, while a suboptimal dose of AS-MDM2 caused inhibition of target protein expression. The combination of AS-MDM2 and gefitinib further decreased MDM2 protein levels. Interestingly, unlike total Akt and MAPK expression, which are only slightly affected by each agent used alone or in combination, the levels of activated pMAPK and pAkt are inhibited by gefitinib and, although to a lesser degree, also by AS-MDM2. The combination of the two agents almost completely suppressed both activated proteins.

Since MDM2 also interferes with Rb function [42], the expression of Rb protein was analysed. AS-MDM2 inhibits the expression of the phosphorylated form of Rb more efficiently than gefitinib, but the combination of the two agents almost completely inhibited phosphorylated Rb. Additionally, VEGF is inhibited by single agent AS-MDM2 or gefitinib, and is almost completely suppressed when the two agents are combined.

The antitumor activity of gefitinib plus AS-MDM2 has been investigated in *in vivo* models in nude mice bearing hormone-independent PC3 or DU145 prostate cancer xenografts [41]. Treatment with gefitinib or AS-MDM2

alone is able to cause, respectively, about 30-40% inhibition of tumor growth. When gefitinib and AS-MDM2 are given in combination, tumor growth inhibition of about 80-90% in PC3 and about 70% in DU145 cells is observed, with no signs of acute or delayed toxicity. Similar results are obtained when the AS-MDM2 is administered intraperitoneally or orally. Preliminary short-term experiments in nude mice bearing LNCaP tumors show an increased inhibitory effect when AS-MDM2 and gefitinib are used in combination, although to a lesser degree than hormone-independent PC3 and DU145. Western blot analysis of PC3 tumors removed at the end of treatment demonstrated an inhibition of MDM2 protein by the specific antisense oligonucleotide, and a marked inhibition by AS-MDM2 and gefitinib combination. As observed *in vitro*, both pMAPK and pAkt are inhibited by each single agent, and the inhibition is increased when the two agents are used in combination. Analysis of VEGF and bFGF expression shows an inhibitory activity by gefitinib, and only a moderate reduction with AS-MDM2, while combination of the two agents resulted in a marked reduction of VEGF and a suppression of bFGF expression [41]. These results demonstrate that the targeted inhibition of EGFR and MDM2 in hormone-independent prostate cancer cells is not only able to restore the expression and function of a variety of critical signalling inhibitors, but also affects the angiogenesis of these tumors.

Taken together, these results support a potentially relevant role for the AS-MDM2 MBO in hormone refractory prostate cancer and a rationale for a further development of its combination with gefitinib in this specific patient setting.

CONCLUSION

In the development of cancer therapeutic strategies, combining agents with different activity and toxicity profiles has been demonstrated to be successful in several human cancer models. Combination of drugs results, in fact, in interference with separate intracellular targets, or in stronger inhibition of a single pathway that is crucial for tumor cell survival and progression, resistance to apoptosis, or cell death. Evidence from several experiments published in the last few years demonstrates that one of the major applications of MDM2 inhibition may be to improve the therapeutic effectiveness of DNA damaging drugs, such as conventional chemotherapeutic agents. DNA damage activates p53-dependent cell cycle arrest and apoptosis, mainly by increasing the levels of intracellular p53 protein through stabilization. One of the mechanisms by which the MDM2 antisense oligonucleotide acts as a chemosensitizer could be activation of p53 by reducing the level of MDM2-p53 complex, thereby enhancing the stimulatory effect of DNA damage on p53. It is possible, therefore, that the ability of traditional chemotherapy to stimulate p53 may be significantly enhanced by simultaneous inhibition of MDM2 functions. Alterations of the p53 gene are the most frequent genetic abnormalities in human cancers, with different frequencies of mutations in different types of malignancies. Several types of tumors, including lymphomas, gliomas, osteosarcomas, breast and prostate cancers, often overexpress the MDM2 oncogene; however, studies analyzing MDM2 amplification and p53 mutations indicate that mutations in

both genes do not generally occur within the same tumor [43]. It has been demonstrated that MDM2 gene amplification in tumor cells results in inactivation of p53, and that the MDM2 pathway is the major limiting factor in p53 activation upon DNA-induced damage. The inhibitory effect of MDM2 during the DNA damage response is independent from MDM2 gene amplification: it is possible that in human cancers with MDM2 overexpression, treatment with MDM2 antisense oligonucleotides could activate p53 to a level sufficient to induce apoptotic cell death. In tumors with functional p53, but without MDM2 overexpression, p53 is more effectively activated by combination treatment with chemotherapeutic agents and MDM2 antisense oligos. Because MDM2 can be stabilized and accumulated by mutated p53 [44], it may also play a role in human tumors harboring mutant p53, regardless of its amplification status. However, the functional relationship between the p53-MDM2 interaction and sensitivity to chemotherapeutic agents remains to be elucidated. In at least some tumor cell models, there is evidence that exogenous wild-type expression can sensitize mutant p53-expressing cells to the cytotoxic effects of some DNA-damaging agents, but wt p53 expression has no effect on the chemosensitivity of cells that overexpress MDM2 [45].

The role of MDM2 inhibition appears to be relevant to anti-cancer strategies, especially considering that MDM2 has p53-independent activities, such as inactivation of the retinoblastoma tumor suppressor (pRb) [42], modulation of the activity, stability and apoptotic function of E2F1/DP1 transcription factors [46, 47], induction of p21 proteasome-mediated degradation by direct binding to p21 protein [48], and inhibition of interactions with the tumor suppressor protein p19^{ARF} [46, 49]. Because the p53-independent activities of MDM2 may play a role in MDM2-related tumorigenicity, and because almost 50% of human cancers have mutant forms of p53, the inhibition of MDM2 appears to be a relevant anti-tumor approach. Therefore, combining MDM2 antisense oligonucleotides and chemotherapeutic drugs can result in strong inhibition of tumor cell proliferation through interference with the multiple and independent pathways involved in cell growth and apoptosis. Furthermore, the combined treatment approach may increase the therapeutic effects of the cytotoxic drugs without increasing their toxicity profile. All these data provide the rationale for further development of MDM2 antisense in combination with conventional anticancer agents in a clinical setting.

MDM2 expression and activity appear to strongly influence the apoptotic response of prostate cancer to androgen deprivation, and its inhibition through antisense approaches results in sensitization of prostate cancer cells to anti-androgen therapy by enhancing apoptotic cell death. At the same time, MDM2 may be important in the development of androgen-independence of prostate cancer, a condition frequent in advanced disease which is often related to alterations of p53, drug resistance, and overexpression of intracellular signalling molecules like EGFR. In fact, combined blockade of EGFR and MDM2 by the selective and non-cytotoxic inhibitors gefitinib and MDM2 antisense oligonucleotides causes the down-regulation of critical proteins involved in cell growth and angiogenesis, resulting in a potent antitumor effect with a low incidence of side

effects. Moreover, since both agents are active by oral administration, this strategy may be worthy of investigation in a clinical setting either to treat androgen-independent prostate cancer, or to prevent the tumor progression from hormone-dependent to hormone-independent condition.

ABBREVIATIONS

CPT	=	Camptothecin
EGFR	=	Epidermal growth factor receptor
HCPT	=	10-Hydroxycamptothecin
MDM2	=	Murine Double Minute 2
MBO AS -MDM2	=	Antisense mixed-backbone oligodeoxynucleotides targeting MDM2
Oligo	=	Oligonucleotide
PS-AS -MDM2	=	Antisense phosphorothioate oligodeoxynucleotides targeting MDM2
SCID	=	Severe combined immunodeficient

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