Inhibition of BCL-2 in Small Cell Lung Cancer Cell Lines with Oblimersen, an Antisense BCL-2 Oligodeoxynucleotide (ODN): In Vitro and In Vivo Enhancement of Radiation Response

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Abstract. Background: Oblimersen, an ODN targeting BCL-2 RNA, has been shown to be effective in reducing BCL-2 expression in vitro and in vivo models engineered to overexpress BCL-2. The present study evaluated the efficacy of combining BCL-2 ODN and radiation in small-cell lung cancers (SCLC) cell lines. Materials and Methods: The in vitro effect was determined using short term (cell viability) and long term (clonogenic) assays. Apoptosis, BCL-2 expression and intratumoural uptake of the FAM-ODN with or without prior radiation treatment were also evaluated. Combination of ODN and RT was also assessed in vivo. Results: Radiation was shown to increase intracellular and intratumoural penetration of oblimersen, confirming previous results obtained in prostate cancer xenograft models. Oblimersen decreased BCL-2 protein expression in vitro and in vivo. BCL-2 ODN sensitised H69 cells to radiation in vitro and in vivo. Oblimersen increased radiation-induced apoptosis and decreased in vivo tumoural vascularisation. Conclusion: Oblimersen was shown to increase in vitro and in vivo effect of RT on SCLC cell lines. Radiation increases intracellular and intratumoural penetration of ODN. This pre-clinical study argues in favour of clinical development in localised SCLC.

Defects in the ability to appropriately regulate apoptotic processes are among the fundamental occurrences that underlie cancer (1). BCL-2 was the first identified member of a family of apoptotic regulators which have at least one BCL-2 homology domain in common. BCL-2 family members include antiapoptotic (prosurvival) proteins (e.g. BCL-2, BCL-xL and MCL-1), multidomain proapoptotic proteins (e.g. BAX and BAK) and BH3-only proapoptotic proteins (e.g. BIM, BID, NOXA and PUMA) (2). Interactions between and relative ratios of proapoptotic and antiapoptotic BCL-2 family members are key determinants of cellular sensitivity to multiple cell death triggers, including many standard chemotherapeutic agents and ionising radiation (3-4). Overexpression of BCL-2 is known to increase clonogenic survival and inhibit radiation-induced apoptosis (3-4), but there is limited information on the in vivo efficacy of combining radiation with BCL-2 family therapeutics for cancer therapy.

Lung cancer is the leading cause of cancer death for both men and women in western countries (5). Small cell lung cancer (SCLC) accounts for 15% of all lung cancer cases and is distinguished from non-SCLC by its characteristic cellular appearance, rapid proliferation, and early dissemination to metastatic sites (6). The standard treatment strategy for patients with limited stage SCLC and good performance status is a combination of chest radiotherapy and chemotherapy using etoposide and cisplatin, which results in complete response rates of 50-80% and 12-25% 5-year survival rates (7-8). While SCLC tumours are primarily chemo- and radio-sensitive, the development of acquired chemo- and radio-resistance occurs within the first 12 months and the overall 5-year survival rate is about 10% for patients in this setting (9). Increased expression of BCL-2 has been reported in 73% to 90% of SCLC. BCL-2 overexpression, BAX down-regulation, and BCL-2 to BAX ratio >1 are correlated with low values of apoptotic index (10) and are associated with chemotherapeutic resistance in SCLC cell lines (11). Overexpression of BCL-2 can abrogate chemotherapy-induced apoptosis in lung cancer cell lines (11). Apoptosis is one the key mechanism that causes SCLC...
cells to die when given radiotherapy (12, 13). Hence, defects in apoptosis could be correlated with radioresistance in SCLC (12).

BCL-2-targeted antisense oligodeoxynucleotides are members of the therapeutic family of pro-apoptotic agents. Anti-BCL-2 oligonucleotide therapy was first introduced by Reed et al. (14) and has been further evaluated in the form of G3139 (oblimersen sodium, Genasense; Genta, Inc., Berkeley Heights, NJ, USA). G3139 is a phosphorothioate-modified antisense sequence complementary to the first six codons of human BCL-2 mRNA. Although clinical trials have shown that G3139 has a favourable safety profile, clinical efficacy is limited when given as a single agent (15). Oblimersen has been previously shown to increase radiation response in in vivo prostate cancer models engineered to overexpress BCL-2, especially by inducing apoptosis and reduction of angiogenesis (16).

This study investigated the role of phosphorothioate-modified BCL-2 oligodeoxynucleotide antisense (BCL-2 ASO) therapy (oblimersen), in combination with radiation for small-cell lung cancer. It intended to evaluate the relationship between pharmacodynamic parameters (oblimersen uptake, BCL-2 inhibition) in a naturally BCL-2 overexpressing model and the potential of oblimersen as an enhancer of radiation response.

Materials and Methods

Cells and culture conditions. Human small-cell lung cancer cell lines H69 (characterised by marked BCL-2 overexpression, and p53 mutated) were purchased from ATCC (Manassas, VA, USA). These cells were maintained in RPMI-1640 supplemented with 2 mM L-glutamine, 10% fetal bovine serum (Gibco, Inc., France), 100 units/ml of penicillin and 100 μg/ml of streptomycin. All cells were incubated at 37°C in a humidified atmosphere of 5% CO2 in air.

Antisense oligonucleotides. All oligodeoxynucleotides were twice high-performance liquid chromatography-purified phosphorothioates obtained from Genta, Inc; Berkeley Heights, NJ, USA. BCL-2 antisense oligodeoxynucleotide (ODN) G3139 (oblimersen sodium) is a fully phosphorothioated 18mer ODN directed against the first six codons of the human BCL-2 mRNA translation initiation site (5’-TCTCCCAGCGTGCCGACAT-3’). G3622 is a fully phosphorothioated 18mer ODN of reversed polarity G3139 sequence (5’-TACCGGTGACCCCTCT-3’) and was used as control ODN in all experiments. FITC-ASO oligonucleotides consisted of a FITC molecule labeled on the 5’ end of an ASO oligonucleotide.

For in vitro experiments, lyophilised antisense oligonucleotides were diluted in PBS (PBS without Ca2+ and Mg2+ was used throughout this study). For in vivo experiments, injections of ODNs were given i.p. at a dose of 10 mg/kg in a volume of 10 μl PBS.

X-ray irradiation. For in vitro experiments, cells were irradiated at room temperature using a 200-kV X-ray irradiator at a dose rate of 0.85 Gy/min. For in vivo irradiation, radiation was given using mouse jigs designed to expose only the tumour bed to radiation at a dose rate of 1.1 Gy/min.

Antisense uptake assays. H69 cells were seeded in T-25 flasks (5×105 per flask). First, H69 cells were incubated with ODN-FAM with or without lipofectamine (Invitrogen, France) according to the manufacturer’s recommendation and cellular uptake was determined 24 hours later. In a second experiment, 24 hours after cell seeding, experimental and control cells were given radiation at a dose of 6 Gy either 3 hours before, after or concomitantly with the administration of ODN-FAM. Twenty-four hours after antisense administration, the cells were examined under the Axiovert S 100 microscope (Zeiss, Gottigen, Germany) at 488 nm. Subsequently, the cells were pelleted and resuspended in PBS and analysed using CellQuest software (Becton Dickinson, Mountain View, CA, USA) with a FACSCalibur flow cytometer (Becton Dickinson). For in vivo experiments, xenograft tumour-bearing mice were injected with FAM-ASO (10 mg/kg for 3 days, 100 μl, i.p.) with or without radiation administered at different times. Tumours were harvested 2 hours following the last injection. Tumours were imaged using a Zeiss Axiosvert 200M (Zeiss) for widefield microscopy (FITC: excitation, 480 nm; beam splitter, 505 nm; emission, 535 nm; Hoechst 33342: excitation, 360 nm; beam splitter, 395 nm; emission, 460 nm).

Measurement of cell growth. Cells were seeded in 96-well plates, 24 h before transfection with various concentrations of oligonucleotides for 72 h. The number of viable cells was determined by using WST-1 assay according to the manufacturer’s instructions (Roche, France). The absorbance was measured at 450 nm with a 96-well plate reader (Biorad, France). Absorbance values were normalised to the values obtained from untreated cells to determine survival rates. Each assay was performed in triplicate.

Clonogenic survival assays. To investigate the effect of G3139 cell response to radiation, a standard clonogenic assay was performed. Survival following radiation exposure was defined as the ability of the cells to maintain clonogenic capacity and form colonies. Briefly, after exposure with G3139 or G3622 at doses ranging from 50 nmol/l to 500 nmol/l for 3 days, cells were exposed to radiation or harvest at radiation doses ranging from 2 Gy to 6 Gy using 200 kV X-rays; they were then separated by gently pipeting, counted and seeded for colony formation in 35 mm dishes at 500 to 2,000 cells/dish. Upon incubation intervals of 21 days, colonies were stained with crystal violet and manually counted. All colonies of 50 cells or more were then counted. The survival fraction (SF) was estimated according to the formula: SF = number of colonies formed/number of cells seeded then separated by gently pipeting, counted and seeded for colony formation in 35 mm dishes at 500 to 2,000 cells/dish. Upon incubation intervals of 21 days, colonies were stained with crystal violet and manually counted. All colonies of 50 cells or more were then counted. The survival fraction (SF) was estimated according to the formula: SF = number of colonies formed/number of cells seeded plating efficiency of the control group. Experiments were performed in triplicate.

Western blot analysis. Cells were seeded (3×106 per T-75 flask), incubated with G3139 or G3622 (500 nmol/l) and harvested at different times. Cells extracts were prepared using a lysis buffer, and protein concentration was determined using the Bio-Rad Detergent-compatible protein assay. Immunoblotting was performed as previously described (17). Briefly 45 μg of protein were loaded into Tris-glycine (4-12%) protein gels (Invitrogen) for electrophoresis; semidry transfer was done using nitrocellulose membranes and a Trans-Blot SD semidy transfer Cell (Bio-Rad) and blocking was
H209 cells were harvested in exponential phase growth and 5×10⁶ from Janvier CERT (Le Genest St. Isle, France) were used. H69 and Agriculture. Female athymic nude mice (6-8 weeks old) obtained the Animal Care license n˚C94-076-11 (French Ministry of experiments were carried out at the Institut Gustave Roussy under Netherland’s was performed after xylene treatment and methyl green, and then mounted. 

Cell cycle analysis. Cells were harvested after 72 hours exposure to either G3139 or G3622, or 24 hours after exposure to 6 Gy radiation, or the combination of G3139 or G3622 and irradiation. Cells were harvested by trypsinisation, washed with PBS, fixed and stored at 4˚C before DNA analysis. After removal of ethanol by centrifugation, cells were incubated with phosphate-citric acid buffer at room temperature for 45 minutes. After centrifugation, cells were then stained with a propidium iodide solution for 24 hours. Stained nuclei were analysed for DNA-propidium iodide fluorescence using a Becton Dickinson FACSscan flow cytometer. Resulting DNA distributions were analysed by Modfit (Verity Software House, Inc., Topsham, ME, USA) for the proportion of cells in sub-G0, G1, S, and G2-M phases of the cell cycle.

Assay for tumour growth in athymic nude mice. The in vivo experiments were carried out at the Institut Gustave Roussy under the Animal Care license n°C94-076-11 (French Ministry of Agriculture). Female athymic nude mice (6-8 weeks old) obtained from Janvier CERT (Le Genest St. Isle, France) were used. H69 and H209 cells were harvested in exponential phase growth and 5×10⁶ cells were injected subcutaneously into the flank area of 6- to 8-week-old female athymic nude mice on day 0. When tumours reached appropriate size, mice were randomised into 6 mice per group and treated with G3139 or RC or saline solution 10 mg/kg i.p. q.d ×6, ×1 week, or irradiation with 6 Gy or the combination of G 3139 or RC, and irradiation (administered on day 3). Mice were weighed, and the tumour size was measured twice a week with an electronic caliper. Individual mouse follow-up was performed over the 30 days following the beginning of the treatment. The tumour volume was estimated from two-dimensional tumour measurements by the formula:

Tumour volume= length (mm) x width² (mm²)/2

In each group (six mice per group), the relative tumour volume was expressed as the Vt/Vo ratio (Vt is the mean tumour volume on a given day during the treatment and Vo is the mean tumour volume at the beginning of the treatment).

Immunohistochemistry. Animals were euthanised 24 hours after the 6-day treatment with ASO. Tumours were excised and fixed in PFA (Milestone, Italy) to prepare paraffin sections (4 μm thick). For terminal deoxynucleotidyl transferase–mediated nick end labeling (TUNEL) assays (R&D Systems, Minneapolis, MN, USA), frozen tissue sections (10 Amol/l thick) were treated with 1,500 proteinase K solution (20 μg/ml) for 30 min, and endogenous peroxidase was blocked by using 3% hydrogen peroxide for 5 min. The samples were incubated for 1 h at 37˚C with terminal deoxynucleotidyl transferase buffer and for 1 h at 37˚C with antitumorstatoyoxyuridine. After counterstaining with peroxidase-conjugated streptavidin, the slides were incubated with diaminozobenzidine, counterstained with methyl green, and then mounted.

For CD34 staining, immunohistochemistry anti-CD34-positive murine endothelial cells (HyCult biotechnology b.v., The Netherlands) was performed after xylene treatment and rehydration. Heat-induced epitope retrieval was achieved with pH8 Tris-EDTA at 98˚C for 40 min. Endogenous peroxidase activity was quenched by 3% H2O2 for 10 min. The sections were placed in coverplates (Shandon, USA) and incubated with blocking serum Biogenex wash buffer 1:10 (San Ramon, CA, USA) for 10 min. This step was followed by incubation with anti-mouse CD34 1:20, diluted in blocking serum 1:10 during 1 hr. Slides were then incubated with a rabbit anti-rat 1:400 (Southern Biotech., AL, USA), diluted in blocking serum 1:10. The revelation step used a Rabbit PowerVision kit (ImmuNoVision Technologies, CA) 20 min and DAB 10 min. Slides were counterstained with Mayer’s haematoxylin and mounted (Pertex). Tumour necrosis was assessed by light microscopy.

Statistical analysis. Results are expressed as mean±standard deviation (S.D.). All statistical analyses were made with a two-sided Student’s t-test, where p<0.05 was considered statistically significant. SPSS software version 17.0 (Bois-Colombes, France) was used for all statistical analysis.

Results

H69 cell uptake of antisense oligonucleotides. To determine the efficiency of antisense oligonucleotide uptake in vitro, H69 cells were incubated with 500 nmol/l FAM-G3139 for 24 hours. Using flow cytometry, H69 cells were found to have high levels of uptake, with 98% of cells having a fluorescent signal of >100.0% of untreated cells (Figures 1a and 1b). When 6 Gy of irradiation was combined with ASO (3 hours before, during or after FAM-G3139 administration), antisense uptake varied greatly (Figure 1c). ASO followed by 6 Gy irradiation was the most efficient combination for improving uptake of antisense oligonucleotides.

BCL-2 ASO effectively decreases BCL-2 expression in SCLC. The effect of BCL-2 ASO on H69 cell BCL-2 expression was determined in vitro using Western blotting. Relative to RC-exposed cells, treatment with 500 nmol/l BCL-2 ASO for 3 days decreased BCL-2 protein levels (Figure 2a).

BCL-2 ASO does not reduce cell viability in combination with radiation. The cytotoxic effects of BCL-2 ASO and radiation were assessed using the WST-1 inner salt assay and compared with the effects of RC or no treatment (Figure 2b). A slight reduction in cell viability was observed when BCL-2 ASO was given alone. The fraction of viable cells decreased to 0.7 (500 nmol/l BCL-2 ASO) after 3 days of incubation. In contrast, no dose-dependent and time-dependent reduction in cell viability 24 hours or 48 hours after increasing the dose of radiation was observed when BCL-2 ASO was given in combination (data not shown).

BCL-2 ASO decreases clonogenic survival in combination with radiation. H69 cells were incubated with mock, RC or BCL-2 ASO for 3 days and then were given radiation at

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increasing doses (0, 2, 4 or 6 Gy). At 4 Gy, SF fraction decreased from 0.3 with mock or MM to 0.2 with ASO (p<0.05) and at 6 Gy, SF fraction decreased from 0.1 with mock to 0.05 with ASO (p<0.05) (Figure 2c).

**BCL-2 ASO slightly increase apoptosis.** The presence of a sub-G1 DNA peak/population detected by flow cytometry suggested apoptosis-related cell death. BCL-2 ASO increased the apoptotic fraction over untreated or RC cells (p<0.05) (Figure 3).

**BCL-2 ASO and radiation inhibit in vivo tumour growth.** To determine the efficacy of the combination of BCL-2 ASO and radiation in SCLC, H69 xenograft tumours were established in nude mice and then were given radiation (6 Gy on day 3) with or without ASO (10 mg/kg, one injection daily for 6 days). Combination of radiation with G3139 in sequential schedule decreased tumour growth. RTV5 was obtained on day 10 for untreated mice and day 11 for mice RC-treated. In contrast, BCL-2 ASO extended RTV5 until day 17 and radiation combined with RC alone extended RTV5 until day 16. BCL-2 ASO plus radiation extended survival until day 21 (p<0.05) (Figure 4a). Western blot analysis on tumour specimens excised on day 7 showed BCL-2 down-regulation in mice treated with BCL-2 ASO compared to mice treated with RC or mock (Figure 4b).

**BCL-2 oligodeoxynucleotides increases in vivo radiation-induced apoptosis.** To determine whether BCL-2 oligodeoxynucleotide could improve in vivo radiation-induced apoptosis, nude mice bearing H69 xenografts were given mock, RC or G3139 for 6 days alone or combined with radiation (RT) on day 3. Tumours were excised on day
7 and analysed for TUNEL assay (Figure 5a). G3139 alone increased TUNEL-positive H69 cells (from 2.6% with mock plus RT to 12.4 % with ASO plus RT, \( p<0.05 \)) suggesting activation of apoptosis. When combined with radiation, the fraction of apoptotic cells increased significantly (Figure 5b).

**BCL-2 oligodeoxunucleotides decreases in vivo tumoural vascularisation.** As BCL-2 exhibits pro-angiogenic properties, this study evaluated whether G3139 targeting BCL-2 mRNA could induce antiangiogenic effect via staining of endothelial cells using the CD34 expression assay. As shown in Figure 5c, G3139 decreased vascularisation and this effect was more pronounced in the combination of G3139 with radiation.

**Antisense oligonucleotides display systemic tumour penetration effects.** Some systemically administered molecules have limited penetration into tumours. This study evaluated whether radiation could improve intratumoural penetration of ASO. As shown previously in vitro, mice treated with ASO and then radiation displayed better intratumoural penetration of ASO compared with other combinations or ASO alone (Figure 6). Hence, the antisense was observed to distribute throughout the tumour, with at least some fluorescence evident throughout all areas of the tumour, whereas a lower apparent distribution was observed in mice treated with ASO alone or other combinations of radiation and ASO.

**Discussion**

The current treatment strategy for localised SCLC combines radiation and chemotherapy either sequentially or concurrently. However, only 10% of patients with localised small-cell lung cancer are disease free at 5 years. Therefore, new radiotherapeutic techniques or new radiation-sensitising strategies must be developed if the outcome of patients treated with radiation therapy is to improve (18). Failure of cells to undergo apoptosis could lead to resistance to treatment of SCLC (19). Reducing the anti-apoptotic BCL-2 protein may induce an increase of apoptotic response to anti-tumoural therapies (11). This study demonstrated a sensitisation of SCLC to RT by down-regulating BCL-2 expression with antisens oligonucleotides. This down-regulation resulted in a slight inhibition of cell viability but in a marked inhibition of clonogenic survival with an increase of RT-induced apoptosis. Previous reports have shown that clonogenic survival is more sensitive than viability assay such as WST-1 tetrazolium assay that relies on metabolic active cells only (20). Cells that have lost their reproductive capacity immediately but are still viable after the combination of radiation and ASO, have been scored in WST-1 assay but not in clonogenic assay (21). In accordance
with these results, combination of ASO targeting BCL-2 mRNA with radiation significantly induced tumour growth delay in nude mice.

BCL-2 proteins, such as BCL-2, BCL-xL, and MCL-1, have been found to be essential in the resistance to radiotherapy in many cancers including prostate cancer (22-23), lymphomas (24) and head and neck cancers (25). BCL-2 proteins have been found to be involved in the development and progression of SCLC. Indeed, some studies have underscored the role of altered apoptotic balance in the pathogenesis of SCLC with the involvement of the amplification of anti-apoptotic genes including \(BCL2\) gene and a deletion of proapoptotic genes such as \(MAPK10\) and \(TNFRSF6\), leading the apoptotic balance towards survival (26). Up-regulation of BCL-2 has been shown to be involved in the mediation of chemotherapy resistance in human small cell lung cancer cell lines (11).

Figure 3. Bcl-2 ASO induces apoptosis in H9 cells. H69 cells were incubated with RC or BCL-2 ASO for 3 days before the administration of 6 Gy radiation (where indicated). The cells were harvested for sub-G1 flow cytometry 1 day later. Representative experiment from two independent experiments. % Apoptotic cells: sub-G1 fraction, G0/G1 fraction, S fraction and G2-M fraction.

Figure 4. a: BCL-2 ASO and local radiation produce an in vivo therapeutic benefit. H69 xenografts were established in nude mice and randomised into the following groups: no treatment, RC, G3139, radiation (RT), RC plus RT or G3139 plus RT. Six injections of antisense (10 mg/kg) from day 1 through day 6 with or without one dose of radiation (6 Gy on day 3) were given. Two independent experiments were conducted, with 5 mice per group in each experiment. Bars, standard deviation. b: BCL-2 ASO decreases in vivo BCL-2 expression. Mice bearing H69 xenograft were treated as described in (a). Tumours were excised on day 7 and BCL-2 expression was analysed by Western blot. Representative blot of two experiments.
Figure 5. a: H69 xenografts were established in nude mice and randomised into the following groups: no treatment, RC, BCL-2 ASO, radiation (RT), RC plus RT or G3139 plus RT. Six injections of antisense (10 mg/kg) from day 1 to day 6 with or without one dose of radiation (6 Gy on day 3) were given. Tumours were then excised on day 7 and stained for TUNEL assay. b: Quantification of apoptotic staining. At least 1,000 cells were counted for each sample, with two mice in each group. Columns, means; bars, standard deviation; *Statistically significant difference (p<0.05). c: Quantification of microvessel density. Mice bearing H69 xenografts were treated with G3139 (10 mg/kg for 6 days) with or without RT (6 Gy on day 3). Tumours were excised on day 7 and tissue sections were stained for CD34 (as described in the Materials and Methods).
One mechanism explaining this positive association consists of an induction of apoptosis, which occurred with both the BCL-2 ASO alone and when combined with radiation, as indicated in vivo by terminal deoxynucleotidyl transferase-mediated nick-end labelling staining (Figure 4a and 4b). The efficacy of oblimersen seems to be more potent in vivo than in vitro, suggesting that others mechanisms may be involved in this interaction. Others studies assessing the potential value of the combination of oblimersen and radiation reported an increase of radiation-induced apoptosis by oblimersen (27). Moreover, some studies have shown that oblimersen exhibits an antiangiogenic effect provided that BCL-2 has been shown to play role in angiogenesis (28). In this study oblimersen decreased CD34 cells alone and in combination with radiation contributing at least partially to the antitumoural effect (Figure 4C). Other studies have reported similar data. Anai et al. reported that antisense BCL-2 ODN and irradiation decreases VEGF expression and MVD in PC-3-BCL-2 xenografts (16). They showed that in PC-3-BCL-2 xenografts, the proportion of CD31-expressing endothelial cells undergoing apoptosis was highest in tumours treated with the combination of antisense BCL-2 ODN and irradiation resulting in a reduction of microvessel density, CD 31 and VEGF expression.

Recently, the antisense field has experienced some difficulties due to oblimersen not receiving approval from the U.S. Food and Drug Administration after disappointing results from phase III clinical trials in advanced melanoma, multiple myeloma and diffuse SCLC (29-30). The main reason explaining these failures was the inability of oligonucleotides to decrease in vivo BCL-2 protein levels, resulting in early degradation, poor intratumoural penetration and poor intracellular uptake (31-32). The currently used phosphorothioate antisense molecules, such as G3139, represent the first generation of chemical oligonucleotide modifications. Newer 2′-O-methyl and locked nucleic acid modifications, as well as the use of chimeric antisense molecules, provide increased target binding affinity and improved pharmacokinetics (33). Another mechanism involved in this positive interaction between radiation and BCL-2 ODN may be a better tumoural penetration of ODN following irradiation. Drug distribution in tumours is often a challenge for systemic therapies, whether using small molecules, chemotherapies or antibodies (34). Fluorescent ODN was slightly visible at the tumour when administered alone and these data underscore impaired pharmacokinetics with antisense explaining partially the moderate antitumoural effects. However, the present study showed the potential effect of radiation to increase oligonucleotide uptake both in vitro and in vivo. These results are not consistent with others reporting that there is not enhancement of ODN uptake by radiation in nasopharyngeal cancer cell line C666-1 (27). However, Anai et al. have recently reported that the uptake of fluorescent ODN was increased in prostate cancer cells exposed to low doses of irradiation both in vitro and in vivo (16). Irradiation before fluorescent ODN treatment resulted in increased fluorescent signal intensity in xenograft tumours compared with those irradiated after fluorescent ODN treatment. This phenomenon was seen in other cell lines (bladder, kidney and fibroblast). Low-dose irradiation may perturb the cellular membrane enough to allow increased cellular uptake of ODN (16). More stable drugs such as oral inhibitors may contribute to better BCL-2 family protein inhibition leading to improved anti-tumoural efficacy. Recently, several studies have reported the antitumoural efficacy of ABT 737, a BCL-2 and BCL-XL inhibitor in lymphoma, myeloma and SCLC (35-37). Increasing the antitumoural efficacy of radiation by BCL-2 protein down-regulation with ODN represents proof-of-concept of combining BCL-2 family protein and radiation in SCLC, warranting confirmatory studies with these more potent compounds.
In summary, this preclinical study showed that antisense BCL-2 enhances both in vitro and in vivo the antitumour effect of radiation SCLC which overexpresses BCL-2. The improved response to this sensitisation strategy seems to be the result of enhanced induction of apoptosis and antiangiogenic effects. These moderate effects were explained in part by an impaired oligonucleotide uptake in tumour. However, a strong uptake was observed when oligonucleotides were combined with radiation. This study represents a proof-of-concept of combining radiation and BCL-2 protein inhibitors with more potent drugs such as BCL-2/BCL-XL inhibitors.

References


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