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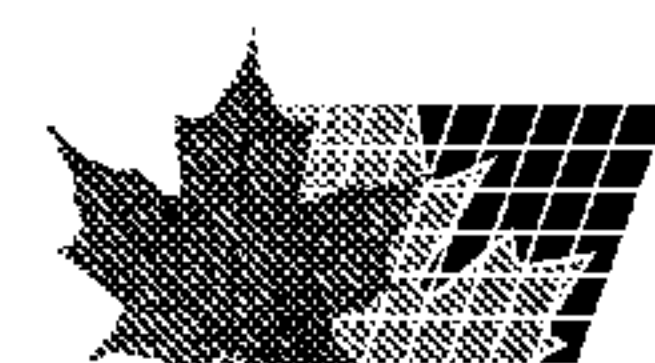
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(57) **Abrégé/Abstract:**

R1 α antisense oligonucleotides and pharmaceutical compositions thereof are disclosed. Methods for treating certain cancers in animals comprising administering to animals an effective amount of an R1 α antisense oligonucleotide, or a pharmaceutical composition thereof, are also disclosed.



ABSTRACT

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RI_α antisense oligonucleotides and pharmaceutical compositions thereof are disclosed. Methods for treating certain cancers in animals comprising administering to animals an effective amount of an RI_α antisense oligonucleotide, or a pharmaceutical composition thereof, are also disclosed.

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ANTISENSE OLIGONUCLEOTIDES FOR TREATMENT OF CANCER

FIELD OF THE INVENTION

10 The invention is in the field of medicinal chemistry. In particular, the invention relates to certain antisense oligonucleotides and the use thereof for the treatment of cancer.

BACKGROUND OF THE INVENTION

15 Control mechanisms for cell growth and differentiation are disrupted in neoplastic cells
20 (Potter, V.R. (1988) Adv. Oncol. 4, 1-8; Strife, A. & Clarkson, B. (1988) Semin. Hematol. 25, 1-19; Sachs, L. (1987) Cancer Res. 47, 1981-1986). cAMP, an intracellular regulatory agent, has been considered to have a role in the control of cell proliferation and
25 differentiation (Pastan, I., Johnson, G.S. & Anderson, W.B. (1975) Ann. Rev. Biochem. 44, 491-522; Prasad, K.N. (1975) Biol. Rev. 50, 129-165; Cho-Chung, Y.S. (1980) J. Cyclic Nucleotide Res. 6, 163-177; Puck, T.T. (1987) Somatic Cell Mot. Genet. 13, 451-457).
30 Either inhibitory or stimulatory effects of cAMP on cell growth have been reported previously in studies in which cAMP analogs such as N⁶-O^{2'}-dibutyryladenosine 3',5'-cyclic monophosphate or agents that raise

intracellular cAMP to abnormal and continuously high levels were used, and available data are interpreted very differently (Chapowski, F.J., Kelly, L.A. & Butcher, R.W. (1975) Adv. Cyclic Nucleotide Protein Phosphorylat. Res. 6, 245-338; Cho-Chung, Y.S. (1979) in Influence of Hormones on Tumor Development, eds. Kellen, J.A. & Hilf, R. (CRC, Boca Raton, FL), pp. 55-93); Prasad, K.N. (1981) in The Transformed Cell, eds. Cameron, L.L. & Poof, T.B. (Academic, New York), pp. 235-266; Boynton, A.L. & Whitfield, J.F. (1983) Adv. Cyclic Nucleotide Res. 15, 193-294).

Recently, site-selective cAMP analogs were discovered which show a preference for binding to purified preparations of type II rather than type I cAMP-dependent protein kinase in vitro (Robinson-Steiner, A.M. & Corbin, J.D. (1983) J. Biol. Chem. 258, 1032-1040; Øgreid, D., Ekanger, R., Suva, R.H., Miller, J.P., Sturm, P., Corbin, J. D. & Døskeland, S.O. (1985) Eur. J. Biochem. 150, 219-227), provoke potent growth inhibition, differentiation, and reverse transformation in a broad spectrum of human and rodent cancer cell lines (Katsaros, D., Tortora, G., Tagliaferri, P., Clair, T., Ally, S., Neckers, L., Robins, R.K. & Cho-Chung, Y.S. (1987) FEBS Lett. 223, 97-103; Tortora, G., Tagliaferri, P., Clair, T., Colamonici, O., Neckers, L.M., Robins, R.K. & Cho-Chung, Y.S. (1988) Blood, 71, 230-233; Tagliaferri, P., Katsaros, D., Clair, T., Robins, R.K. & Cho-Chung, Y.S. (1988) J. Biol. Chem. 263, 409-416). The type I and type II protein kinases are distinguished by their regulatory subunits (RI and RII, respectively) (Corbin, J.D., Keely, S.L. & Park, C.R. (1975) J. Biol. Chem. 250, 218-225; Hofmann, F., Beavo, J.A. & Krebs, E.G. (1975) J. Biol. Chem. 250, 7795-7801). Four different regulatory subunits [RI_α (previously designated RI) (Lee, D.C., Carmichael, D.F., Krebs, E.G. & McKnight, G.S. (1983) Proc. Natl. Acad. Sci.

USA 80, 3608-3612), RI_{β} (Clegg, C.H., Cadd, G.G. & McKnight, G.S. (1988) Proc. Natl. Acad. Sci. USA 85, 3703-3707), RII_{α} (RII_{54}) (Scott, J.D., Glaccum, M.B., Zoller, M.J., Uhler, M.D., Hofmann, D.M., McKnight, G.S. & Krebs, E.G. (1987) Proc. Natl. Acad. Sci. USA 84, 5192-5196) and RII_{β} (RII_{51}) (Jahnsen, T., Hedin, L., Kidd, V.J., Beattie, W.G., Lohmann, S.M., Walter, U., Durica, J., Schulz, T.Z., Schlitz, E., Browner, M., Lawrence, C.B., Goldman, D., Ratoosh, S.L. & Richards, J.S. (1986) J. Biol. Chem. 261, 12352-12361)] have now been identified at the gene/mRNA level. Two different catalytic subunits [C_{α} (Uhler, M.D., Carmichael, D.F., Lee, D.C. Chrivia, J.C., Krebs, E.G. & McKnight, G.S. (1986) Proc. Natl. Acad. Sci. USA 83, 1300-1304) and C_{β} (Uhler, M.D., Chrivia, J.C. & McKnight, G.S. (1986) J. Biol. Chem. 261, 15360-15363; Showers, M.O. & Maurer, R.A. (1986) J. Biol. Chem. 261, 16288-16291)] have also been identified; however, preferential coexpression of either one of these catalytic subunits with either the type I or type II protein kinase regulatory subunit has not been found (Showers, M.O. & Maurer, R.A. (1986) J. Biol. Chem. 261, 16288-16291).

The growth inhibition by site-selective cAMP analogs parallels reduction in RI_{α} with an increase in RII_{β} , resulting in an increase of the RII_{β}/RI_{α} ratio in cancer cells (Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Øgreid, D., Døskeland, S.O., Jahnsen, T. & Cho-Chung, Y.S. (1988) Proc. Natl. Acad. Sci. USA 85, 6319-6322; Cho-Chung, Y.S. (1989) J. Natl. Cancer Inst. 81, 982-987).

Such selection modulation of RI_{α} versus RII_{β} is not mimicked by treatment with N^6, O^2' -dibutyryladenine 3',5'-cyclic monophosphate, a previously studied cAMP analog (Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Øgreid, D., Døskeland, S.O., Jahnsen, T. & Cho-Chung, Y.S. (1988) Proc. Natl.

Acad. Sci. USA 85, 6319-6322). The growth inhibition further correlates with a rapid translocation of RII β to the nucleus and an increase in the transcription of the RII β gene (Ally, S., Tortora, G., Clair, T., Grieco, D., Merlo, G., Katsaros, D., Øgreid, D., Døskeland, S.O., Jahnsen, T. & Cho-Chung, Y.S. (1988) Proc. Natl. Acad. Sci. USA 85, 6319-6322). These results support the hypothesis that RII β plays an important role in the cAMP growth regulatory function (Cho-Chung, Y.S. (1989) J. Natl. Cancer Inst. 81, 982-987).

Antisense RNA sequences have been described as naturally occurring biological inhibitors of gene expression in both prokaryotes (Mizuno, T., Chou, M-Y, and Inouye, M. (1984), Proc. Natl. Acad. Sci. USA 81, (1966-1970)) and eukaryotes (Heywood, S.M. Nucleic Acids Res., 14, 6771-6772 (1986)), and these sequences presumably function by hybridizing to complementary mRNA sequences, resulting in hybridization arrest of translation (Paterson, B.M., Roberts, B.E., and Kuff, E.L., (1977) Proc. Natl. Acad. Sci. USA, 74, 4370-4374. Antisense oligodeoxynucleotides are short synthetic nucleotide sequences formulated to be complementary to a specific gene or RNA message. Through the binding of these oligomers to a target DNA or mRNA sequence, transcription or translation of the gene can be selectively blocked and the disease process generated by that gene can be halted. The cytoplasmic location of mRNA provides a target considered to be readily accessible to antisense oligodeoxynucleotides entering the cell; hence much of the work in the field has focused on RNA as a target. Currently, the use of antisense oligodeoxynucleotides provides a useful tool for exploring regulation of gene expression in vitro and in tissue culture (Rothenberg, M., Johnson, G., Laughlin, C., Green, I.,

Craddock, J., Sarver, N., and Cohen, J.S. (1989) J. Natl. Cancer Inst., 81:1539-1544.

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SUMMARY OF THE INVENTION

5 The invention is related to the discovery that inhibiting the expression of RI_{α} in leukemia cells by contact with an antisense O-oligonucleotides and S-oligonucleotides for RI_{α} results in the inhibition of proliferation and the stimulation of cell differentiation. Accordingly, the invention is directed to RI_{α} antisense oligonucleotides and pharmaceutical compositions thereof for the treatment of cancer.

10 In particular, the invention is related to 15- to 30-mer antisense oligonucleotides which are complementary to a region in the first 100 N-terminal codons of RI_{α} (Seq. ID No:6).

15 The invention is also related to 15- to 30-mer antisense oligonucleotides which are a fragment of antisense DNA complementary to RI_{α} (Seq. ID No: 5).

20 The invention is also related to pharmaceutical compositions comprising at least one 15- to 30-mer antisense oligonucleotide which is complementary to a region in the first 100 N-terminal codons of RI_{α} (Seq. ID No:6); and a pharmaceutically acceptable carrier.

25 The invention is also related to a method for treating cancer by suppressing growth of cancer cells susceptible to growth suppression and for inducing cancer cell differentiation in an animal comprising administering to an animal in need of such treatment a cancer cell growth suppressing amount of an RI_{α} antisense oligonucleotide.

DESCRIPTION OF THE FIGURES

Fig. 1 depicts a graph showing the effect of RI_{α} antisense oligodeoxynucleotide on the basal rate of growth of HL-60 leukemic cells (A) and the growth of these cells when treated with cAMP analogs or TPA (B). A, cells were grown (see the Examples) in the absence (O) or presence (•) of RI_{α} antisense oligodeoxynucleotide (15 μ M). At indicated times, cell counts in duplicate were performed. Data represent the average values $\pm$ SD of four experiments. B, On day 4 of experiment A, cells exposed or unexposed to RI_{α} antisense oligodeoxynucleotide were reseeded (day 0) at 5×10^5 cells/dish, and cells pre-exposed to RI_{α} antisense oligodeoxynucleotide were further treated with the oligomer at day 0 and day 2. cAMP analogs and TPA were added one time at day 0. Cell counts were performed on a Coulter counter on day 4. 8-Cl, 8-Cl-cAMP (10 μ M); 8-Cl + N^6 -B, 8-Cl-cAMP (5 μ M) + N^6 -benzyl-cAMP (5 μ M); TPA (10^{-8} M). The data represent the average values $\pm$ SD of four experiments.

Fig. 2 depicts a graph showing the effect of RI_{α} antisense oligodeoxynucleotide on the morphologic transformation of HL-60 cells. Cells either exposed or unexposed to RI_{α} antisense oligodeoxynucleotide were treated with cAMP analogs or TPA as described in Fig. 1B. On day 4 (see Fig. 1B), cells were washed twice in Dulbecco's phosphate-buffered saline and were pelleted onto a glass slide by cytocentrifuge. The resulting cytopreparations were fixed and stained by Wright's stain. x 180.

Fig. 3 depicts a Northern blot showing decreased RI_{α} mRNA expression in HL-60 leukemic cells exposed to RI_{α} antisense oligodeoxynucleotide. Cells were either exposed or unexposed to RI_{α} antisense oligodeoxynucleotide (15 μ M) for 8 hr. Isolation of total RNA and Northern blot analysis followed the

methods described in the Examples. A, ethidium bromide staining of RNA; M, markers of ribosomal RNAs; lanes 1, 2, cells unexposed or exposed to RI $_{\alpha}$ antisense oligomer. B, Northern blot analysis; the same nitrocellulose filter was hybridized to both RI $_{\alpha}$ and actin probes in sequential manner. Lanes 1, 2, cells unexposed or exposed to RI $_{\alpha}$ antisense oligomer.

Fig. 4 depicts an SDS-PAGE showing the effect of RI $_{\alpha}$ antisense oligodeoxynucleotide on the basal and induced levels of RI $_{\alpha}$ and RII $_{\beta}$ CAMP receptor proteins in HL-60 leukemic cells. Cells were either exposed to RI $_{\alpha}$ antisense oligodeoxynucleotide (15 μ M) or treated with CAMP analogs as described in Fig. 1. Preparation of cell extracts, the photoactivated incorporation of 8-N $_3$ -[32 P]CAMP and immunoprecipitation using the anti-RI $_{\alpha}$ or anti-RII $_{\beta}$ antiserum and protein A Sepharose, and SDS-PAGE of solubilized antigen-antibody complex followed the methods described in the Examples. Pre-immune serum controls were carried out simultaneously and detected no immunoprecipitated band. M, 14 C-labeled marker proteins of known molecular weight; RI $_{\alpha}$, the 48,000 molecular weight RI (Sigma); RII $_{\alpha}$, the 56,000 molecular weight RII (Sigma). Lanes RI $_{\alpha}$ and RII $_{\beta}$ are from photoaffinity labeling with 8-N $_3$ -[32 P]CAMP only; lanes 1 to 3, photoaffinity labeling with 8-N $_3$ -[32 P]CAMP followed by immunoprecipitation with anti-RI $_{\alpha}$ or anti-RII $_{\beta}$ antiserum. 8-Cl, 8-Cl-cAMP (5 μ M); N 6 -benzyl, N 6 -benzyl-cAMP (5 μ M). The data in the table represent quantification by densitometric scanning of the autoradiograms. The data are expressed relative to the levels in control cells unexposed to RI $_{\alpha}$ antisense oligomer and untreated with CAMP analog, which are set equal to 1 arbitrary unit. The data represent an average $\pm$ SD of three experiments. A and B, immunoprecipitation with anti-RI $_{\alpha}$ and anti-RII $_{\beta}$ antisera, respectively.

Fig. 5 depicts graphs showing the growth inhibition of human cancer cell lines by RI_α antisense oligodeoxynucleotide having SEQ ID No: 1 (O-oligo and S-oligo derivatives), compared to controls. Cell lines: SK-N-SH, neuroblastoma; LS-174T, colon carcinoma; MCF-7, breast carcinoma; TMK-1, gastric carcinoma. E₂, estradiol-17β.

Fig. 6 depicts the change in morphology of SK-N-SH human neuroblastoma cells exposed to RI_α antisense oligodeoxynucleotide having SEQ ID No: 1.

Fig. 7 depicts a graph showing that RI_α antisense oligodeoxynucleotide and its phosphorothioate analog inhibit the in vivo growth of LS-174T human colon carcinoma in athymic mice. Figure 7A shows the oligodeoxynucleotide concentration-dependent inhibition of tumor growth. O-oligo, RI_α antisense oligodeoxynucleotide; S-oligo, phosphorothioate analog of RI_α antisense oligomer. The cholesterol pellets (total weight 20 mg) containing the indicated doses of O-oligo or S-oligo were implanted s.c. one time, at zero time, and tumor sizes were measured. Tumor volume (see Materials and Methods, Example 3) represents an average ± S.D. of 7 tumors. Figure 7B shows the temporal effect of antisense oligodeoxynucleotide phosphorothioate analogs on tumor growth. S-oligos as indicated at 0.3 mg dose in cholesterol pellets (total weight 20 mg) were implanted s.c. 2x/week, and tumor volume (see Materials and Methods, Example 3) represents an average ± S.D. of 7 tumors.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Antisense therapy is the administration of exogenous oligonucleotides which bind to a target polynucleotide located within the cells. The term "antisense" refers to the fact that such oligonucleotides are complementary to their intracellular targets, e.g. RI α . See for example, Jack Cohen, OLIGODEOXYNUCLEOTIDES, Antisense Inhibitors of Gene Expression, CRC Press, 1989; and Synthesis 1:1-5 (1988). The RI α antisense oligonucleotides of the present invention include derivatives such as S-oligonucleotides (phosphorothioate derivatives or S-oligos, see, Jack Cohen, supra) which exhibit enhanced cancer cell growth inhibitory action (see Figures 5 and 7A).

S-oligos (nucleoside phosphorothioates) are isoelectronic analogs of an oligonucleotide (O-oligo) in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom. The S-oligos of the present invention may be prepared by treatment of the corresponding O-oligos with 3H-1,2-benzodithiol-3-one-1,1-dioxide which is a sulfur transfer reagent. See Iyer, R.P. et al., J. Org. Chem. 55: 4693-4698 (1990); and Iyer, R.P. et al., J. Am. Chem. Soc. 112:1253-1254 (1990).

The RI α antisense oligonucleotides of the present invention may be RNA or DNA which is complementary to and stably hybridizes with the first 100 N-terminal codons of the RI α genome or the corresponding mRNA. Use of an oligonucleotide complementary to this region allows for the selective hybridization to RI α mRNA and not to mRNA specifying other regulatory subunits of protein kinase. Preferably, the RI α antisense oligonucleotides of the present invention are a 15 to 30-mer fragment of the antisense DNA molecule having

SEQ ID NO:5 which hybridizes to Ri_{α} mRNA. Alternatively, RI_{α} antisense oligonucleotide is a 15- to 30-mer oligonucleotide which is complementary to a region
5 in the first 100 N-terminal codons of RI_{α} (Seq. ID No:6). Most preferably, the RI_{α} antisense oligonucleotide has SEQ ID No: 1, SEQ ID No: 2, SEQ ID No: 3, or SEQ ID No: 4.

Included as well in the present invention are pharmaceutical compositions comprising an effective amount of at least one of the RI_{α} antisense oligonucleotides
10 of the invention in combination with a pharmaceutically acceptable carrier. In one embodiment, a single RI_{α} antisense oligonucleotide is utilized. In another embodiment, two RI_{α} antisense oligonucleotides are utilized which are complementary to adjacent regions of the RI_{α} genome. Administration of two RI_{α} antisense oligonucleotides which are complementary to adjacent regions of the Ri_{α}
15 genome or corresponding mRNA may allow for more efficient inhibition of RI_{α} genomic transcription or mRNA translation, resulting in more effective inhibition of cancer cell growth.

Preferably, the RI_{α} antisense oligonucleotides is coadministered with an agent which enhances the uptake of the antisense molecule by the cells. For
20 example, the RI_{α} antisense oligonucleotide may be combined with a lipophilic cationic compound which may be in the form of liposomes. The use of liposomes to introduce nucleotides into cells is taught, for example, in U.S. Patent Nos. 4,897,355 and 4,394,448. See also U.S. Patent Nos. 4,235,871, 4,231,877, 4,224,179, 4,753,788, 4,673,567, 4,247,411, 4,814,270 for general methods of
25 preparing liposomes comprising biological materials.

Alternatively, the RI_{α} antisense oligonucleotide may be combined with a lipophilic carrier such as any

one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol.

5 In addition, the RI_{α} antisense oligonucleotide may be conjugated to a peptide that is ingested by cells. Examples of useful peptides include peptide hormones, antigens or antibodies, and peptide toxins. By choosing a peptide that is selectively taken up by the neoplastic cells, specific delivery of the
10 antisense agent may be effected. The RI_{α} antisense oligonucleotide may be covalently bound via the 5'OH group by formation of an activated aminoalkyl derivative. The peptide of choice may then be covalently attached to the activated RI_{α} antisense
15 oligonucleotide via an amino and sulfhydryl reactive hetero bifunctional reagent. The latter is bound to a cysteine residue present in the peptide. Upon exposure of cells to the RI_{α} antisense oligonucleotide bound to the peptide, the peptidyl antisense agent is
20 endocytosed and the RI_{α} antisense oligonucleotide binds to the target RI_{α} mRNA to inhibit translation. See PCT Application Publication No. PCT/US89/02363.

25 As antineoplastic agents, the RI_{α} antisense oligonucleotides of the present invention are useful in treating a variety of cancers, including, but not limited to, gastric, pancreatic, lung, breast, anal, colorectal, head and neck neoplasms, neuroblastomas, melanoma and various leukemias.

30 The RI_{α} antisense oligonucleotides of the invention may also be active against the following tumor systems: F9 teratocarcinoma, SK-N-SH neuroblastoma, TMK-1 gastric carcinoma, HL-60 promyelocytic Leukemia, Leukemia L-1210, Leukemia P388, P1534 leukemia, Friend Virus Leukemia, Leukemia
35 L4946, Mecca lymphosarcoma, Gardner lymphosarcoma, Ridgway Osteogenic sarcoma, Sarcoma 180 (ascites), Wagner osteogenic sarcoma, Sarcoma T241, Lewis lung

carcinoma, Carcinoma 755, CD8F, MCF-7 breast carcinoma, Colon 38, LS-174T colon carcinoma, Carcinoma 1025, Ehrlich carcinoma (ascites & solid), Krubs 2 carcinoma (ascites), Bashford carcinoma 63, Adenocarcinoma E 0771, B16 Melanoma, Hardin-Passey melanoma, Giloma 26, Miyona adenocarcinoma, Walker carcinosarcoma 256, Flexner-Jobling carcinoma, Jensen sarcoma, Iglesias sarcoma, Iglesias ovarian tumor, Murphy-Sturn lymphosarcoma, Yoshida sarcoma, Dunning leukemia, Rous chicken sarcoma, and Crabb hamster sarcoma.

The RI_α antisense oligonucleotides and the pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions wherein the RI_α antisense oligonucleotide is contained in an amount which is effective to achieve inhibition of proliferation and/or stimulate differentiation of the subject cancer cells. While individual needs vary, determination of optimal ranges of effective amounts of each component is with the skill of the art. Typically, the RI_α antisense oligonucleotide may be administered to mammals, e.g. humans, at a dose of 0.005 to 1 mg/kg/day, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated.

In addition to administering the RI_α antisense oligonucleotides as a raw chemical in solution, the RI_α antisense oligonucleotides may be administered as

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part of a pharmaceutical preparation containing suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the RI_n antisense oligonucleotide into preparations which can be used pharmaceutically.

Suitable formulations for parenteral administration include aqueous solutions of the RI_n antisense oligonucleotides in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The antisense oligonucleotides of the present invention may be prepared according to any of the methods that are well known to those of ordinary skill in the art. Preferably, the antisense oligonucleotides are prepared by solid phase synthesis. See, Goodchild, J., Bioconjugate Chemistry, 1:165-167 (1990), for a review of the chemical synthesis of oligonucleotides. Alternatively, the antisense oligonucleotides can be obtained from a number of companies which specialize in the custom synthesis of oligonucleotides.

Having now generally described this invention, the same will be understood by reference to an example which is provided herein for purposes of illustration only and is not intending to be limited unless otherwise specified.

EXAMPLESExample 1

Oligodeoxynucleotides. The 21-mer oligodeoxynucleotides used in the present studies were synthesized at Midland Certified Reagent Co. (Midland, TX) and had the following sequences: human RI_α (Sandberg, M., Tasken, K., Oyen, O., Hansson, V. & Jahnsen, T. (1987) Biochem. Biophys. Res. Commun. 149, 939-945) antisense, 5'-GGC-GGT-ACT-GCC-AGA-CTC-CAT-3' (SEQ ID NO:1); human RII_β (Levy, F.O., Oyen, O., Sandberg, M., Tasken, K., Eskild, W., Hansson, V. & Jahnsen, T. (1988) Mol. Endocrinol., 2, 1364-1373) antisense 5'-CGC-CGG-GAT-CTC-GAT-GCT-CAT-3' (SEQ ID NO:8); human RII_α Oyen, O., Mykelbust, F., Scott, J.D., Hansson, V. & Jahnsen, T. (1989) FEBS Lett. 246, 57-64) antisense 5'-CGG-GAT-CTG-GAT-GTG-GCT-CAT-3' (SEQ ID NO:9); and the random sequence oligodeoxynucleotide was made of a mixture of all four nucleotides at every position.

Cell Growth Experiment. Cells grown in suspension culture in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (50 U/ml), streptomycin (500 µg/ml), and 1 mM glutamine (Gibco, Grand Island, NY) were seeded at 5 x 10⁵ cells per dish. Oligodeoxynucleotides were added after seeding and every 48 hr thereafter. Cell counts were performed on a Coulter counter. Cells unexposed or exposed to oligodeoxynucleotides for 4 days were reseeded (day 0) at 5 x 10⁵ cells/dish, and cells pre-exposed to the oligodeoxynucleotide were further treated with the oligomer at day 0 and day 2. cAMP analogs (kindly provided by Dr. R.K. Robins, Nucleic Acid Research Institute, Costa Mesa, CA) or 12-O-tetradecanoylphorbol-13-acetate (TPA) were added one time at day 0. Cell counts were performed on day 4.

Immunoprecipitation of RI α and RII β cAMP Receptor
Proteins after Photoaffinity Labeling with 8-N $_3$ -
[32 P]cAMP. Cell extracts were prepared at 0-4°C. The
cell pellets (2 x 10 6 cells), after two washes with
PBS, were suspended in 0.5 ml buffer Ten (0.1 M NaCl,
5 mM MgCl $_2$, 1% Nonidet P-40, 0.5% Na deoxycholate, 2
KIU/ml bovine aprotinin, and 20 mM Tris-HCl, pH 7.4)
containing proteolysis inhibitors (Tortora, G., Clair,
T. & Cho-Chung, Y. S. (1990) Proc. Natl. Acad. Sci.
USA 87, 705-708), vortex-mixed, passed through a 22-
gauge needle 10 times, allowed to stand for 30 min at
4°C, and centrifuged at 750 x g for 20 min; the
resulting supernatants were used as cell lysates. The
photoactivated incorporation of 8-N $_3$ -[32 P]cAMP (60.0
Ci/mmol), and the immunoprecipitation using the anti-
RI α or anti-RII β antiserum (kindly provided by Dr. S.O.
Døskeland, University of Bergen, Bergen, Norway) and
protein A Sepharose $^{\text{TM}}$ and SDS-PAGE of solubilized
antigen-antibody complex followed the method
previously described (Tortora, G., Clair, T. & Cho-
Chung, Y. S. (1990) Proc. Natl. Acad. Sci. USA 87,
705-708; Ekanger, R., Sand, T. E., OGREID, D.,
Christoffersen, T. & Døskeland, S.O. (1985) J. Biol.
Chem. 260, 3393-3401).

cAMP-Dependent Protein Kinase Assays. After two
washes with Dulbecco's phosphate-buffered saline, cell
pellets (2 x 10 6 cells) were lysed in 0.5 ml of 20 mM
Tris (pH 7.5), 0.1 mM sodium EDTA, 1 mM
dithiothreitol, 0.1 mM pepstatin, 0.1 mM antipain, 0.1
mM chymostatin, 0.2 mM leupeptin, 0.4 mg/ml aprotinin,
and 0.5 mg/ml soybean trypsin inhibitor, using 100
strokes of a Dounce homogenizer. After centrifugation
(Eppendorf 5412) for 5 min, the supernatants were
adjusted to 0.7 mg protein/ml and assayed (Uhler, M.
D. & McKnight, G. S. (1987) J. Biol. Chem. 262, 15202-
15207) immediately. Assays (40 μ l total volume) were

performed for 10 min at 30°C and contained 200 μ M ATP, 2.7 x 10⁶ cpm γ [³²P]ATP, 20 mM MgCl₂, 100 μ M Kemptide (Sigma K-1127) (Kemp, B. E., Graves, D. J., Benjamin, E. & Krebs, E. G. (1977) J. Biol. Chem. 252, 4888-4894), 40 mM Tris (pH 7.5), $\pm$ 100 μ M protein kinase inhibitor (Sigma P-3294) (Cheng, H.-C., Van Patten, S. M., Smith, A. J. & Walsh, D. A. (1985) Biochem. J. 231, 655-661), $\pm$ 8 μ M cAMP and 7 μ g of cell extract. The phosphorylation of Kemptide was determined by spotting 20 μ l of incubation mixture on phosphocellulose filters (Whatman, P81) and washing in phosphoric acid as described (Roskoski, R. (1983) Methods Enzymol. 99, 3-6). Radioactivity was measured by liquid scintillation using Econofluor-2 (NEN Research Products NEF-969).

Isolation of Total RNA and Northern Blot Analysis. The cells (10⁸ washed twice with phosphate-buffered saline) were lysed in 4.2 M guanidine isothiocyanate containing 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl (N-lauroylsarcosine Na⁺), and 0.1 M β -mercaptoethanol, and the lysates were homogenized, and total cellular RNA was sedimented through a CsCl cushion (5.7 M CsCl, 10 mM EDTA) as described by Chirgwin et al. (Chirgwin, J. M., Przybyla, A. E., MacDonald, R. Y. & Rutter, W. J. (1977) Biochemistry 18, 5284-5288). Total cellular RNA containing 20 mM 3-[N-morpholine]propane-sulfonic acid (pH 7.0), 50% formamide, and 6% formaldehyde was denatured at 65°C for 10 min and electrophoresed through a denaturing 1.2% agarose-2.2 M formaldehyde gel. The gels were then transferred to BiotransTM nylon membranes (ICN Biomedicals) by the method of Thomas (Thomas, P. S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205) and hybridized to the following two ³²P-labeled nick-translated cDNA probes: 1.5 kilobase (kb) cDNA clone containing the entire coding region for the human

cAMP-dependent protein kinase type I regulatory subunit, RI $_{\alpha}$ (Sandberg, M., Tasken, K., Oyen, O., Hansson, V. & Jahnsen, T. (1987) Biochem. Biophys. Res. Commun. 149, 939-945) (kindly provided by Dr. T. Jahnsen, Institute of Pathology, Rikshospitalet, Oslo, Norway), and human β actin (Oncor p7000 β actin).

RESULTS

The RI $_{\alpha}$ antisense oligodeoxynucleotide at 15 μ M concentration had immediate effects on the rate of proliferation of HL-60 cells. By 4-5 days in culture, while cells unexposed to RI $_{\alpha}$ antisense oligomer demonstrated an exponential rate of growth, cells exposed to the antisense oligomer exhibited a reduced growth rate and eventually stopped replicating (Fig. 1A). This inhibitory effect on cell proliferation persisted throughout the culture period. The growth inhibition was not due to cell killing; cells were over 90% viable after exposure to RI $_{\alpha}$ antisense oligomer (15 μ M) for 7 days as assessed by flow cytometry using forward and side scatter. RI $_{\alpha}$ sense, RII $_{\alpha}$, or RII $_{\beta}$ antisense, or a random sequence oligodeoxynucleotide had no such growth inhibitory effect.

Cells unexposed or exposed to RI $_{\alpha}$ antisense oligodeoxynucleotide for 4 days in culture were reseeded and examined for their response to treatment with cAMP analogs or TPA. In cells unexposed to RI $_{\alpha}$ antisense oligodeoxynucleotide, 8-Cl-cAMP (10 μ M) produced 60% growth inhibition, and 80% growth inhibition was achieved by 8-Cl-cAMP (5 μ M) plus N^6 -benzyl-cAMP (5 μ M) (Fig. 1B) (Tortora, G., Tagliaferri, P., Clair, T., Colamonici, O. Neckers, L. M., Robins, R. K. & Cho-Chung, Y. S. (1988) Blood 71, 230-233), and TPA (10^{-8} M) exhibited 60% growth inhibition (Fig. 1B). In contrast, cells exposed to

antisense oligodeoxynucleotide exhibited retarded growth (25% the rate of growth of cells unexposed to the antisense oligomer) and neither cAMP analogs nor TPA brought about further retardation of growth (Fig. 1B).

HL-60 cells undergo a monocytic differentiation upon treatment with site-selective cAMP analogs. Cells either unexposed or exposed to RI_{α} antisense oligodeoxynucleotide were examined for their morphology before and after treatment with cAMP analogs. As shown in Fig. 2, in cells unexposed to RI_{α} antisense oligomer, 8-Cl-cAMP plus N^6 -benzyl-cAMP induced a monocytic morphologic change characterized by a decrease in nuclear-to-cytoplasm ratio, abundant ruffled and vacuolated cytoplasm, and loss of nucleoli. Strikingly, the same morphologic change was induced when cells were exposed to RI_{α} antisense oligodeoxynucleotide (Fig. 2). Moreover, the morphologic changes induced by antisense oligomer were indistinguishable from that induced by TPA (Fig. 2).

To provide more evidence that the growth inhibition and monocytic differentiation induced in HL-60 cells exposed to the RI_{α} antisense oligodeoxynucleotide were due to an intracellular effect of the oligomer, the RI_{α} mRNA level was determined. As shown in Fig. 3, 3.0 kb RI_{α} mRNA (Sandberg, M., Tasken, K., Oyen, O., Hansson, V. & Jahnsen, T. (1987) Biochem. Biophys. Res. Commun. 149, 939-945) was virtually undetectable in cells exposed for 8 hr to RI_{α} antisense oligodeoxynucleotide (Fig. 3B, lane 2), and the decrease in RI_{α} mRNA was not due to a lower amount of total RNA as shown by the ethidium bromide staining (compare lane 2 with lane 1 of Fig. 3A). Conversely, an enhanced level of actin mRNA was detected in cells exposed to RI_{α} antisense oligomer (Fig. 3B). Whether the increase in actin

mRNA level represents changes in cytoskeletal structure is not known.

5 The levels of cAMP receptor proteins in these cells was then determined by immunoprecipitation using anti-RI α and anti-RII β antisera (Tortora, G., Clair, T. & Cho-Chung, Y. S. (1990) Proc. Natl. Acad. Sci. USA 87, 705-708; Ekanger, R., Sand, T. E., OGREID, D., Christoffersen, T. & DØSKELAND, S.O. (1985) J. Biol. Chem. 260, 3393-3401) after photoaffinity labeling of
10 these receptor proteins with 8-N₃-[³²P]cAMP. In control cells, treatment with 8-Cl-cAMP plus N⁶-benzyl-cAMP brought about a 70% reduction in RI α with a 3-fold increase in RII β , resulting in a 10-fold increase in the ratio of RII β /RI α (Fig. 4) (Cho-Chung, Y. S. (1989) J. Natl. Cancer Inst. 81, 982-987).
15 Exposure of these cells to RI α antisense oligodeoxynucleotide for 4 days brought about marked changes in both RI α and RII β levels; an 80% reduction in RI α with a 5-fold increase in RII β resulted in a 25-fold increase in the ratio of RII β /RI α compared with that in control cells (Fig. 4). Since
20 growth inhibition and differentiation were appreciable after 3-4 days of exposure to RI α antisense oligomer, the changing levels of RI α and RII β proteins appears to be an early event necessary for commitment to differentiation.

Data in Fig. 4 showed that suppression of RI α by the antisense oligodeoxynucleotide brought about a compensatory increase in RII β level. Such coordinated
30 expression of RI and RII without changes in the amount of C subunit has been shown previously (Hofman, F., Bechtel, P. J. & Krebs, E. G. (1977) J. Biol. Chem. 252, 1441-1447; Otten, A. D. & Mcknight, G. S. (1989) J. Biol. Chem. 264, 20255-20260). The increase in
35 RII β may be responsible for the differentiation induced in these cells after exposure to RI α antisense oligodeoxynucleotide. The increase in RII β mRNA or

RII β protein level has been correlated with ~~cAMP~~ analog-induced differentiation in K-562 chronic myelocytic leukemic cells (Tortora, G., Clair, T., Katsaros, D., Ally, S., Colamonici, O., Neckers, L. M., Tagliaferri, P., Jahnsen, T., Robins, R. K. & Cho-Chung, Y. S. (1989) Proc. Natl. Acad. Sci. USA **86**, 2849-2852) and in erythroid differentiation of Friend erythrocytic leukemia cells (Schwartz, D. A. & Rubin, C. S. (1985) J. Biol. Chem. **260**, 6296-6303). In a recent report (Tortora, G., Clair, T. & Cho-Chung, Y. S. (1990) Proc. Natl. Acad. Sci. USA **87**, 705-708), we have provided direct evidence that RII β is essential for the cAMP-induced differentiation in HL-60 cells. HL-60 cells that were exposed to RII β antisense oligodeoxynucleotide became refractory to treatment with cAMP analogs and continued to grow.

The essential role of RII β in differentiation of HL-60 cells was further demonstrated when these cells were exposed to both RI α and RII β antisense oligodeoxynucleotides simultaneously. As shown in Table 1, RI α antisense oligodeoxynucleotide induced a marked increase in the expression of monocytic surface antigens [Leu 15 (Landay, A., Gartland, L. & Clement, L. T. (1983) J. Immunol. **131**, 2757-2761) and Leu M3 (Dimitriu-Bona, A., Burmester, G. R., Waters, S. J. & Winchester, R. J. (1983) J. Immunol. **130**, 145-152)] along with a decrease in markers related to the immature myelogenous cells [My9 (Talle, M. A., Rao, P. E., Westberg, E., Allegar, N., Makowski, M., Mittler, R. S. & Goldstein, G. (1983) Cell. Immunol. **78**, 83.; Todd, R. F. III, Griffin, J. D., Ritz, J., Nadler, L. M. Abrams, T. & Schlossman, S. F. (1981) Leuk. Res. **5**, 491)]. These changes in surface marker expression were abolished when cells were exposed simultaneously to both RI α and RII β antisense oligodeoxynucleotides (Table 1). RII α cAMP receptor was not detected in HL-60 cells (Cho-Chung, Y. S.,

Clair, T., Tagliaferri, P., Ally, S., Katsaros, D., Tortora, G., Neckers, L., Avery, T. L., Crabtree, G. W. & Robins, R. K. (1989) Cancer Invest. 7(2), 161-177), and RII $_{\alpha}$ antisense oligodeoxynucleotide showed no interference with the effects of RI $_{\alpha}$ antisense oligomer (Table 1).

Cells exposed to both RI $_{\alpha}$ and RII $_{\beta}$ antisense oligodeoxynucleotides were neither growth inhibited nor differentiated regardless of cAMP analog treatment. We interpret these results to reflect the blockage of cAMP-dependent growth regulatory pathway. Cells under these conditions are no longer cAMP-dependent but survive and proliferate probably through an alternate pathway. Thus, suppression of both RI $_{\alpha}$ and RII $_{\beta}$ gene expression led to an abnormal cellular growth regulation similar to that in mutant cell lines (Gottesman, M. M. (1980) Cell 22, 329-330), those that contain either deficient or defective regulatory subunits of cAMP-dependent protein kinase and are no longer sensitive to cAMP stimulus.

Our results demonstrated that cAMP transduces signals for dual controls, either positive or negative, on cell proliferation, depending on the availability of RI $_{\alpha}$ or RII $_{\beta}$ receptor proteins. The RI $_{\alpha}$ antisense oligodeoxynucleotide which brought about suppression of RI $_{\alpha}$ along with enhancement of RII $_{\beta}$ expression led to terminal differentiation of HL-60 leukemia with no sign of cytotoxicity.

It is unlikely that free C subunit increase in cells exposed to RI $_{\alpha}$ antisense oligodeoxynucleotide was responsible for the differentiation, because cells exposed to RII $_{\beta}$ antisense or both RI $_{\alpha}$ and RII $_{\beta}$ antisense oligodeoxynucleotides, conditions which also would produce free C subunit, continued to grow and became refractory to cAMP stimulus. In order to directly verify this we measured phosphotransferase activity in cells that are exposed or unexposed to the

antisense oligodeoxynucleotides using kemptide (Kemp, B. E., Graves, D. J., Benjamin, E. & Krebs, E. G. (1977) J. Biol. Chem. 252, 4888-4894) as a substrate in the presence and absence of a saturating concentration of cAMP and in the presence and absence of the heat-stable protein kinase inhibitor (Cheng, H.-C., Van Patten, S. M., Smith, A. J. & Walsh, D. A. (1985) Biochem. J., 231, 655-661). This method of assay gives accurate determination of the relative levels of dissociated C and total C activity. Cell extracts from untreated HL-60 cells exhibited a very low level of dissociated C and were stimulated 36-fold by cAMP (Table 2). This cAMP-stimulated activity was almost completely inhibited by the heat-stable protein kinase inhibitor (Table 2), indicating that the total C activity measured was cAMP-dependent protein kinase. In cells exposed to RI_{α} antisense, RII_{β} antisense, or RI_{α} and RII_{β} antisense oligodeoxynucleotide, the free C activity was not increased as compared to unexposed control cells, although there was a small difference in the total cAMP-stimulated activity (Table 2). These results provide direct evidence that free catalytic subunit is not responsible for the differentiation observed in HL-60 cells.

Over expression of RI_{α} cAMP receptor protein has also been found in the majority of human breast and colon primary carcinomas examined (Bradbury, A. W., Miller, W. R., Clair, T., Yokozaki, H. & Cho-Chung, Y. S. (1990) Proc. Am. Assoc. Cancer Res. 31, 172), suggesting an important *in vivo* role of cAMP receptor in tumor growth as well. However, the precise role of RI_{α} in cell proliferation is not known at present. RI_{α} may suppress RII_{β} production by titrating out C subunit, or it may be a transducer of mitogenic signals leading to cell proliferation. Our results demonstrate that RI_{α} antisense oligodeoxynucleotide provides a useful genetic tool for studies on the role

of cAMP receptor proteins in cell proliferation and differentiation, and contribute to a new approach in the control of malignancy.

Table 1. Modulation of differentiation markers in HL-60 cells by RI $_{\alpha}$ antisense oligodeoxynucleotide

Surface Makers			
Treatment	Leu15	LeuM3	My9
Control	10	2	100
RI $_{\alpha}$ antisense	80	98	80
RI $_{\alpha}$ antisense + RII $_{\beta}$ antisense	11	2	100
RII $_{\beta}$ antisense	13	3	100
RI $_{\alpha}$ antisense + RII $_{\alpha}$ antisense	85	100	80

Surface antigen analysis was performed by flow cytometry using monoclonal antibodies reactive with either monocytic or myeloid cells. The monoclonal antibodies used were Leu 15, Leu M3, and My9. 2×10^4 cells were analyzed for each sample, and cell gating was performed using forward and side scatter. The numbers represent % positive and represent the average values of three experiments.

Table 2. Protein kinase activity in HL-60 cells

Treatment	Activity -cAMP	Relative to control	Activity +cAMP	Relative to control	Stimulation (fold)
- PKI					
Control	23.0 ± 6.6	1.0	837 ± 87	1.0	36
RI _α antisense	22.9 ± 5.4	1.0	944 ± 18	1.1	41
RII _β antisense	22.8 ± 8.4	1.0	1,028 ± 154	1.2	45
RI _α and RII _β antisense	24.3 ± 7.0	1.1	802 ± 36	1.0	33
+ PKI					
Control	17.5 ± 8.7	1.0	37.0 ± 8.4	1.0	2.1
RI _α antisense	25.0 ± 8.8	1.4	22.6 ± 8.8	0.6	0.9
RII _β antisense	24.0 ± 2.6	1.4	24.8 ± 3.9	0.7	1.0
RI _α and RII _β antisense	19.0 ± 5.9	1.1	19.1 ± 8.2	0.5	1.0

Cells were exposed to each of 15 μM concentrations of RI_α, RII_β, or RI_α and RII_β antisense oligodeoxynucleotide for 4 days as shown in Fig. 1A. The data represent an average ± SD of duplicate determinations of three identical experiments.

*Picomoles phosphate transferred to Kemptide per min/mg protein.

Example 2

Next, the RI_α antisense oligonucleotide having SEQ ID NO:1 was administered to mice having an experimental tumor. A pellet of RI_α antisense oligonucleotide (25 mg/Kg) and cholesterol (1000 mg/Kg) was implanted s.c. in the left flank of athymic mice which had been injected in the right flank with LS-174T human colon cancer cells (2x10⁶ cells) suspended in phosphate-buffered saline. Tumor measurements and mouse weights were recorded on the initial day of treatment (staging day), and at the end of treatment (staging day +5). The mean tumor weight

change (Δ), was based on length and width measurements in millimeters. After a few days, the tumor growth was inhibited when compared to control cells (see Table 3). No change in body weight was noted in the control and treated animals.

Table 3. Effect of RI $_{\alpha}$ antisense oligodeoxynucleotide s.c. pellet on the growth of LS-174T human colon carcinoma in athymic mice			
	Initial mean ^c tumor wt (mg)	Final mean ^d tumor wt (mg)	% $\Delta T/\Delta C^e$
Treatment ^a s.c. pellet implanted			
Control	25	450	--
RI $_{\alpha}$ antisense (0.5 mg)	25	230	48.
8-Cl cAMP (1 mg) ^b + N ⁶ benzyl cAMP (1 mg)	34	250	51
a. 20 mg pellet lyophilized consisting of indicated doses of RI$_{\alpha}$ antisense or cAMP analogs plus supplement doses of cholesterol. b. The growth inhibitory effect of these cAMP analogs correlate with decrease in RI$_{\alpha}$ (Natl. Cancer Inst. 81 982 (1989)) and is shown here for comparison. c. Mean tumor weight per group (4 mice) on staging day. d. Mean tumor weight per group on staging day +5. e. % of change in test tumor weight (ΔT)/change in control tumor weight (ΔC).			

In other in vitro experiments, the RI $_{\alpha}$ antisense oligonucleotide having SEQ ID NO: 1 was added to dishes containing neuroblastoma, colon carcinoma, breast carcinoma and gastric carcinoma cells. As shown in Figure 5, the RI $_{\alpha}$ antisense oligonucleotide having SEQ ID No: 1 inhibited proliferation of all

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cancer cell types when compared to control cells. Moreover, the RI_α antisense oligonucleotide having SEQ ID No: 1 caused differentiation of the human neuroblastoma cells (see Figure 6).

Example 3

Next, the effect of O-oligo and S-oligo RI_α antisense oligonucleotides on the growth of LS-174T human colon carcinoma in athymic mice was compared.

Materials and Methods. We synthesized [Milligen Biosearch 8700 DNA synthesizer (Bedford, MA)] the 21-mer antisense oligodeoxynucleotides and their phosphorothioate analogs complementary to the human RI_α, human RII_β mRNA transcripts starting from the first codon, and mismatched sequence (random) oligomers of identical size. The oligomers had the following sequences: RI_α antisense, 5'-GCC-GGT-ACT-GCC-AGA-CTC-CAT-3' (SEQ ID No: 1); RII_β antisense, 5'-CGC-CGG-GAT-CTC-GAT-GCT-CAT-3' (SEQ ID No: 8); and random oligo, 5'-CGA-TCG-ATC-GAT-CGA-TCG-TAC-3' (SEQ ID No: 7).

LS-174T human colon carcinoma cells (2×10^6) were injected s.c. in athymic mice, and the antisense oligodeoxynucleotides in the form of either a cholesterol pellet or 50% sesame oil emulsion were administered s.c. 1 week later when mean tumor sizes usually were 25-50 mg. Tumor volume was based on length and width measurements and calculated by the formula $\frac{4}{3} \pi r^3$, where $r = (\text{length} + \text{width})/4$.

Results and Discussion. Fig. 7 shows the dose- and time-dependent effect of an RI_α antisense oligodeoxynucleotide (O-oligo) at 0.2 and 0.5 mg doses in cholesterol pellets administered s.c. one time (at zero time); it brought about 20 and 46% growth inhibition, respectively, in 7 days, when compared with control (untreated) tumors (Fig. 7A). Strikingly, the RI_α antisense phosphorothioate analog (S-oligo) at a 0.2 mg dose (cholesterol pellet, s.c.) gave a 60%

growth inhibition at day 7, exhibiting a 3-fold greater potency than the O-oligo antisense (Fig. 7A). The growth inhibitory effect of RI $_{\alpha}$ antisense S-oligo was even greater when animals were treated for a longer period. The RI $_{\alpha}$ antisense S-oligo at a 0.3 mg dose in a cholesterol pellet, 2 times/week s.c. implantation for 3 weeks, resulted in a 80% growth inhibition; the tumor growth almost stopped after 2 weeks of treatment (Fig. 7B). RI $_{\alpha}$ antisense O-oligo or S-oligo administered s.c. as 50% sesame oil emulsion gave similar results. RI $_{\alpha}$ antisense S-oligo brought about no apparent toxicity in animals; no body weight loss or other toxic symptoms were observed during the 3 weeks of treatment.

The growth inhibitory effect brought about by RI $_{\alpha}$ antisense S-oligo was the specific effect of the oligomer: RII $_{\beta}$ antisense or random (mismatched sequence) S-oligos of the identical size as the RI $_{\alpha}$ antisense oligomer had no effect on the tumor growth (Fig. 7B).

To provide more evidence that the growth inhibition observed in colon carcinomas in athymic mice treated with RI $_{\alpha}$ antisense oligodeoxynucleotide was due to an intracellular effect of the oligomer, the levels of RI $_{\alpha}$ and RII $_{\beta}$ cAMP receptor proteins in these tumors were determined. RI $_{\alpha}$ levels were determined by immunoblotting (Ally, S., Proc. Natl. Acad. Sci. USA 85:6319-6322 (1988)) using monoclonal antibody against human RI $_{\alpha}$ (kindly provided by Drs. T. Lea, University of Oslo, Oslo, Norway, and S.O. Døskeland, University of Bergen, Bergen, Norway), and RII $_{\beta}$ was measured by immunoprecipitation (Tortora, G., et al., Proc. Natl. Acad. Sci. USA 87:705-708 (1990)) with anti-RII $_{\beta}$ antiserum (kindly provided by Dr. S.O. Døskeland) after photoaffinity labeling of RII $_{\beta}$ with [32 P] 8-N $_3$ -cAMP. As shown in Table 4, RI $_{\alpha}$ antisense S-oligomer treatment brought about a marked

reduction (80% decrease) of RI_{α} level in tumors as compared with that in untreated control tumors. This suppression of RI_{α} expression by RI_{α} antisense S-oligomer brought about a 2-fold increase in RII_{β} level (Table 4). Such coordinated expression of RI_{α} and RII_{β} without changes in the amount of catalytic subunit of protein kinase has been shown in HL-60 leukemia cells that demonstrated growth inhibition and differentiation upon exposure to RI_{α} antisense oligodeoxynucleotide. On the other hand, a 50% increase in RI_{α} level along with 80% suppression in RII_{β} level was observed in tumors after treatment with RII_{β} antisense S-oligomer (Table 4) which had no effect on tumor growth (Fig. 7). Random (mismatched sequence) S-oligomer which had no effect on tumor growth (Fig. 7) also showed no effect on RI_{α} levels (Table 4). Thus, reduction in RI_{α} expression appears to trigger a decrease or halt in tumor growth upon treatment with RI_{α} antisense oligomer. Our results demonstrated that cAMP transduces signals for dual control, either positive or negative, on cell proliferation, depending on the availability of RI_{α} or RII_{β} receptor proteins. The RI_{α} antisense oligodeoxynucleotide, which suppressed RI_{α} and enhanced RII_{β} expression, led to inhibition of in vivo growth of solid colon carcinoma in athymic mice with no symptoms of toxicity in animals. The phosphorothioate analog (S-oligo) of RI_{α} antisense oligomer exhibited a greater potency than the antisense of unmodified oligodeoxynucleotide (O-oligo). It has been shown that S-oligos, as compared with O-oligos, more readily enter cells, are more resistant to endonucleases, and yet exhibit high efficacy in hybridization with target mRNAs or DNAs (Stein, C.A., et al., In: J.S. Cohen (ed.), Oligodeoxynucleotides: Antisense Inhibitors of Gene

Expression, pp. 97-117. Boca Raton, FL, CRC Press, Inc. (1989)).

These results demonstrate here for the first time the striking in vivo effect of antisense oligodeoxynucleotide in the suppression of malignancy. The depletion of RI_{α} , the type I regulatory subunit of cAMP-dependent protein kinase, by means of an antisense oligodeoxynucleotide, especially with its phosphorothioate analog, leads to a successful halt of tumor growth in vivo with no symptoms of toxicity, suggesting great potential of this antisense oligodeoxynucleotide for clinical application.

Table 4

Suppression of RI_{α} cAMP Receptor Expression by RI_{α} Antisense Oligodeoxynucleotide (S-oligo) Results in Compensatory Increase in RII_{α} Receptor

Treatment	Relative Levels	
	RI_{α}	RII_{β}
None	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
RI_{α} antisense S-oligo	0.2 $\pm$ 0.03	2.0 $\pm$ 0.2
RII_{β} antisense S-oligo	1.5 $\pm$ 0.2	0.2 $\pm$ 0.02
Random S-oligo	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1

Treatment with S-oligos as indicated were the same as that in Fig. 7B. At the end of the experiment (3 weeks), tumor extracts were prepared as previously described (Ally, S. et al., Cancer Res. 49:5650-5655 (1980)) and immunoblotting and immunoprecipitation of RI_{α} and RII_{β} , respectively, were performed as previously described by Ally, S., et al., Proc. Natl. Acad. Sci. USA 85:6319-6322 (1988) and Tortora, G., et al., Proc. Natl. Acad. Sci. USA 87:705-708 (1990). Data are from quantification by densitometric scanning of autoradiograms. Data are expressed relative to levels in control tumors (no treatment), which are set to equal to one as an arbitrary unit.

Data represent an average $\pm$ S.D. of 7 tumors.

In the following sequence listing, Seq ID No: 1 represents an antisense sequence corresponding to the first 7 N-terminal codons for RI_{α} . Seq ID No: 2 represents an antisense sequence corresponding to the 8th-13th codon for RI_{α} . Seq ID No: 3 represents an antisense sequence corresponding to the 14th-20th codon for RI_{α} . Seq ID No: 4 represents an antisense sequence corresponding to the 94th-100th codon for RI_{α} . Seq ID No: 5 represents an antisense sequence corresponding to the 1st-100th codon for RI_{α} . Seq ID No: 6 represents the sense sequence corresponding to the 1st-100th codon for RI_{α} .

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Yoon S. Cho-Chung
- (ii) TITLE OF INVENTION: Antisense Oligonucleotides for Treatment of Cancer
- (iii) NUMBER OF SEQUENCES: 9
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: MBM & CO.
 - (B) STREET: P.O. BOX 809, STATION B
 - (C) CITY: OTTAWA
 - (D) PROVINCE: ONTARIO
 - (E) COUNTRY: CANADA
 - (F) POSTAL CODE: K1P 5P9
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Ver. 2.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: CA 2,054,325
 - (B) FILING DATE: 1991-10-28
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 607,113
 - (B) FILING DATE: 1990-11-02
 - (C) CLASSIFICATION:
 - (A) APPLICATION NUMBER: US 680,198
 - (B) FILING DATE: 1991-04-05
 - (C) CLASSIFICATION:
 - (A) APPLICATION NUMBER: US 702,163
 - (B) FILING DATE: 1991-05-20
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: SWAIN, Margaret
 - (B) REGISTRATION NUMBER: 10926
 - (C) REFERENCE/DOCKET NUMBER: 127-115
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 613/567-0762
 - (B) TELEFAX: 613/563-7671

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: DNA

-32-

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCGGTACTG CCAGACTCCA T

21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCGTGCCTCC TCACTGGC

18

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

-33-

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAGCTCACAT TCTCGAAGGC T

21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: Antisense oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATAGCACCT CGTCGCCTCC T

21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 300
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: Antisense DNA complementary to Protein Kinase subunit RI α mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATAGCACCT CGTCGCCTCC TACCTTTAAC CACTGGGTTG GGTGGAGGAG GAGAAATCTC 60

-34-

ATCCTCCCTT GAGTCTGTAC GAGTGCCTGC TTTCTGCAGA TTGTGAATCT GTTTTGCCTC 120
 CTCCTTCTCC AACCTCTCAA AGTATTCCCT GAGGAATGCC ATGGGACTCT CAGGTCGAGC 180
 AGTGCACAAC TGCACAATAG AATCTTTGAG CAGTGCTTGA ATGTTATGCT TCTGGACGTA 240
 GAGCTCACAT TCTCGAAGGC TCGTGCCTC CTCCTGGCG GCGGTACTGC CAGACTCCAT 300

INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 300
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: DNA
- (iv) ANTISENSE: No
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Sequence: Partial sequence of Protein Kinase Regulatory Subunit RI α
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGGAGTCTG GCAGTACCGC CGCCAGTGAG GAGGCACGCA GCCTTCGAGA ATGTGAGCTC 60
 TACGTCCAGA AGCATAACAT TCAAGCACTG CTCAAAGATT CTATTGTGCA GTTGTGCACT 120
 GCTCGACCTG AGAGACCCAT GGCATTCTC AGGGAATACT TTGAGAGGTT GGAGAAGGAG 180
 GAGGCAAAAC AGATTCAGAA TCTGCAGAAA GCAGGCACTC GTACAGACTC AAGGGAGGAT 240
 GAGATTTCTC CTCCTCCACC CAACCCAGTG GTTAAAGGTA GGAGGCGACG AGGTGCTATC 300

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: DNA
- (iv) ANTISENSE: Yes
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Artificial Sequence
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:

-35-

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense
oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGATCGATCG ATCGATCGTA C

21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense
oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCCGGGATC TCGATGCTCA T

21

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(iv) ANTISENSE: Yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: Antisense
oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGGATCTGG ATGTGGCTCA T

21

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An antisense oligonucleotide, or derivative thereof, which is 15 to 30 nucleotides in length and complementary to a region of the sequence of nucleotides of the first 100 N-terminal codons of RI_{α} (SEQ ID NO:6), wherein said oligonucleotide is other than a 21-mer O-oligonucleotide having SEQ ID NO:1.
2. The antisense oligonucleotide, or derivative thereof, of claim 1, which is DNA, RNA or a combination thereof.
3. The antisense oligonucleotide, or derivative thereof, of claim 1, which comprises the sequence of SEQ ID NO:2.
4. The antisense oligonucleotide, or derivative thereof, of claim 1, which comprises the sequence of SEQ ID NO:3.
5. The antisense oligonucleotide, or derivative thereof, of claim 1, which comprises the sequence of SEQ ID NO:4.
6. The antisense oligonucleotide, or derivative thereof, of any one of claims 1 – 5, in which the internucleotide linkages are phosphodiester.
7. The antisense oligonucleotide, or derivative thereof, of any one of claims 1 – 5, in which the internucleotide linkages are phosphorothioate phosphodiester.
8. A pharmaceutical composition comprising one or more antisense oligonucleotides, or derivatives thereof, of any one of claims 1 – 7 and a pharmaceutically acceptable carrier.

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9. A pharmaceutical composition comprising one or more antisense oligonucleotides, or derivatives thereof, of claim 1, and a pharmaceutically acceptable carrier.
10. The pharmaceutical composition of claim 9, wherein said one or more antisense oligonucleotides, or derivatives thereof, comprise sequences selected from the group of SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.
11. The pharmaceutical composition of claim 9 or 10, wherein said one or more antisense oligonucleotides, or derivatives thereof, are DNA, RNA or a combination thereof.
12. The pharmaceutical composition of any one of claims 9 – 11, wherein said one or more antisense oligonucleotides, or derivatives thereof, are 5' to 3'-oligodeoxynucleotides.
13. The pharmaceutical composition of any one of claims 9 – 12, wherein said one or more antisense oligonucleotides, or derivatives thereof, comprise internucleotide linkages that are selected from the group of phosphodiesters or phosphorothioate phosphodiesters.
14. The pharmaceutical composition of any one of claims 8 – 13, comprising two antisense oligonucleotides, or derivatives thereof, which are complementary to adjacent regions in the first 100 N-terminal codons of RI_{α} (SEQ ID NO:6).
15. The pharmaceutical composition of any one of claims 8 – 14, wherein said pharmaceutically acceptable carrier is a sterol.
16. The pharmaceutical composition of any one of claims 8 – 14, wherein said pharmaceutically acceptable carrier is a liposome.
17. Use of one or more antisense oligonucleotides, or derivatives thereof, said antisense oligonucleotides, or derivatives thereof, being 15 to 30 nucleotides in length and complementary to a region of the sequence of nucleotides of the first 100 N-terminal codons of RI_{α} (SEQ ID NO:6), wherein said oligonucleotide is other than a 21-mer O-

-38-

oligonucleotide having SEQ ID NO:1, in the preparation of a pharmaceutical composition for the treatment of cancers.

18. The use according to claim 17, wherein said cancer is selected from the group of gastric, pancreatic, lung, breast, anal, colorectal, head and neck neoplasms, neuroblastomas melanoma, sarcoma and carcinoma.
19. The use according to claim 17, wherein said cancer is selected from the group of F9 teratocarcinoma, SK-N-SH neuroblastoma, TMK-1 gastric carcinoma, Mecca lymphosarcoma, Gardner lymphosarcoma, Ridgway Osteogenic sarcoma, Sarcoma 180 (ascites), Wagner osteogenic sarcoma, Sarcoma T241, Lewis lung carcinoma, Carcinoma 755, CD8F, MCF-7 breast carcinoma, Colon 38, LS-174T colon carcinoma, Carcinoma 1025, Erhlich carcinoma (ascites & solid), Krubs 2 carcinoma (ascites), Bashford carcinoma 63, Adenocarcinoma E 0771, B16 Melanoma, Hardin-Passey melanoma, Giloma 26, Miyona adenocarcinoma, Walker carcinosarcoma 256, Flexner-Jobling carcinoma, Jensen sarcoma, Iglesias sarcoma, Iglesias ovarian tumor, Murphy-Sturm lymphosarcoma, Yoshida sarcoma, Dunning leukemia, Rous chicken sarcoma, and Crabb hamster sarcoma.
20. The use according to any one of claims 17 – 19, wherein said one or more antisense oligonucleotides, or derivatives thereof, are DNA, RNA or a combination thereof.
21. The use according to any one of claims 17 – 19, wherein said one or more antisense oligonucleotides, or derivatives thereof, are 5' to 3'-oligodeoxynucleotides.
22. The use according to any one of claims 17 – 21, wherein said one or more antisense oligonucleotides, or derivatives thereof, have internucleotide linkages that are selected from the group of phosphodiester or phosphorothioate phosphodiester.
23. The use according to any one of claims 17 – 22, wherein said one or more antisense oligonucleotides, or derivatives thereof, comprise two antisense oligonucleotides, or

derivatives thereof, which are complementary to adjacent regions of the nucleotides of the first 100 N-terminal codons of RI α (SEQ ID NO:6).

24. The use according to any one of claims 17 – 23, wherein said pharmaceutical composition comprises a pharmaceutically acceptable carrier.
25. The use according to claim 24, wherein said pharmaceutically acceptable carrier is a sterol.
26. The use according to claim 24, wherein said pharmaceutically acceptable carrier is a liposome.
27. The use according to any one of claims 17 – 26, wherein the one or more antisense oligonucleotides, or derivatives thereof, are combined with an agent which enhances uptake of the antisense oligonucleotides, or derivatives thereof, by cells.
28. The use according to claim 27, wherein the agent is a selected from the group of a lipophilic cationic compound, a lipophilic carrier or a peptide, that is ingested by cells.
29. Use of a therapeutically effective amount of one or more antisense oligonucleotides, or derivatives thereof, said antisense oligonucleotides, or derivatives thereof, being 15 to 30 nucleotides in length and complementary to a region of the sequence of nucleotides encoding the first 100 N-terminal codons of RI α (SEQ ID NO:6) wherein said oligonucleotide is other than a 21-mer O-oligonucleotide having SEQ ID NO:1, in the treatment of cancer.
30. The use according to claim 29, wherein the cancer is selected from the group of gastric, pancreatic, lung, breast, anal, colorectal, head and neck neoplasms, neuroblastomas, melanoma, sarcoma and carcinoma.
31. The use according to claim 29, wherein said cancer is selected from the group of F9 teratocarcinoma, SK-N-SH neuroblastoma, TMK-1 gastric carcinoma, Mecca

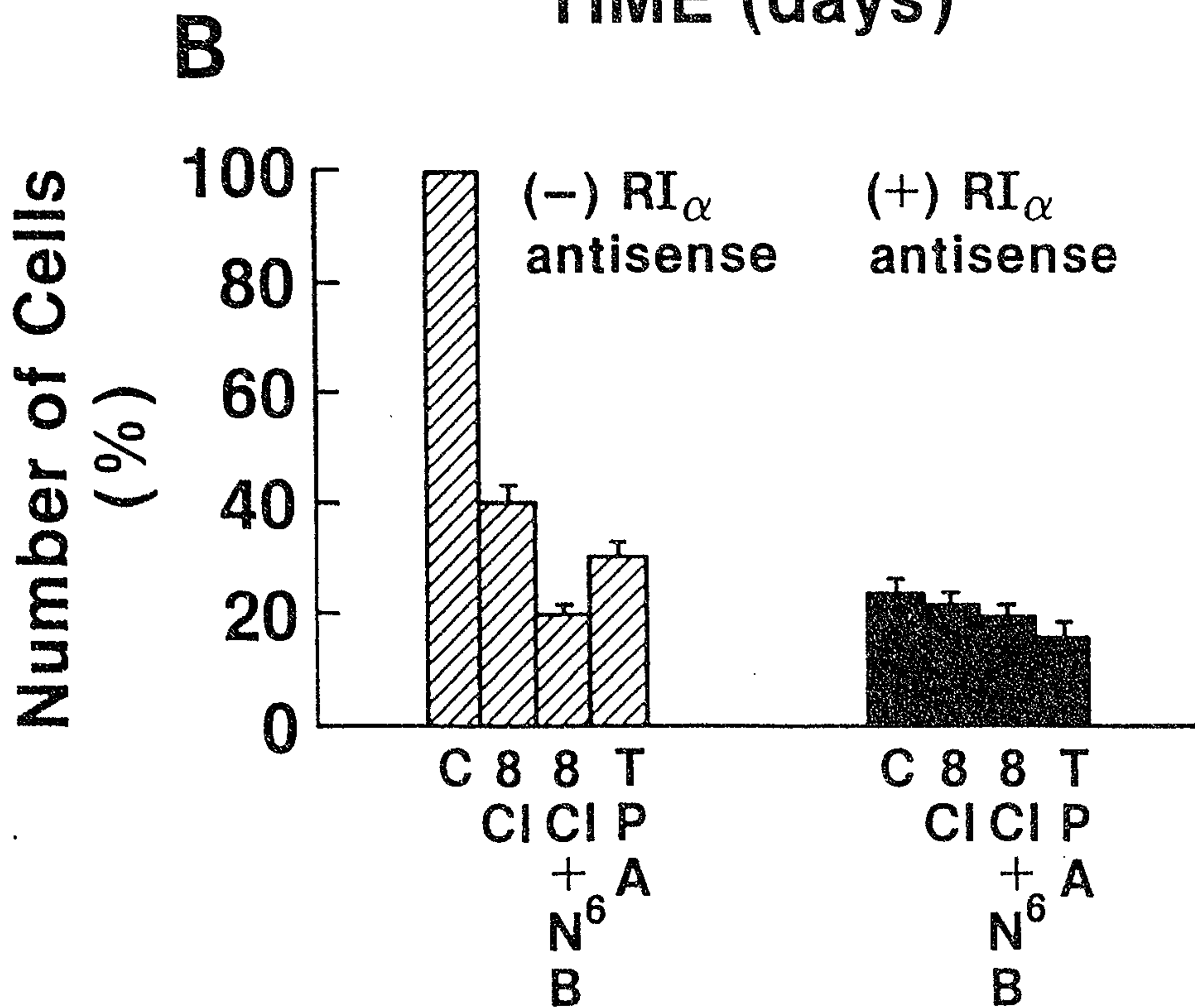
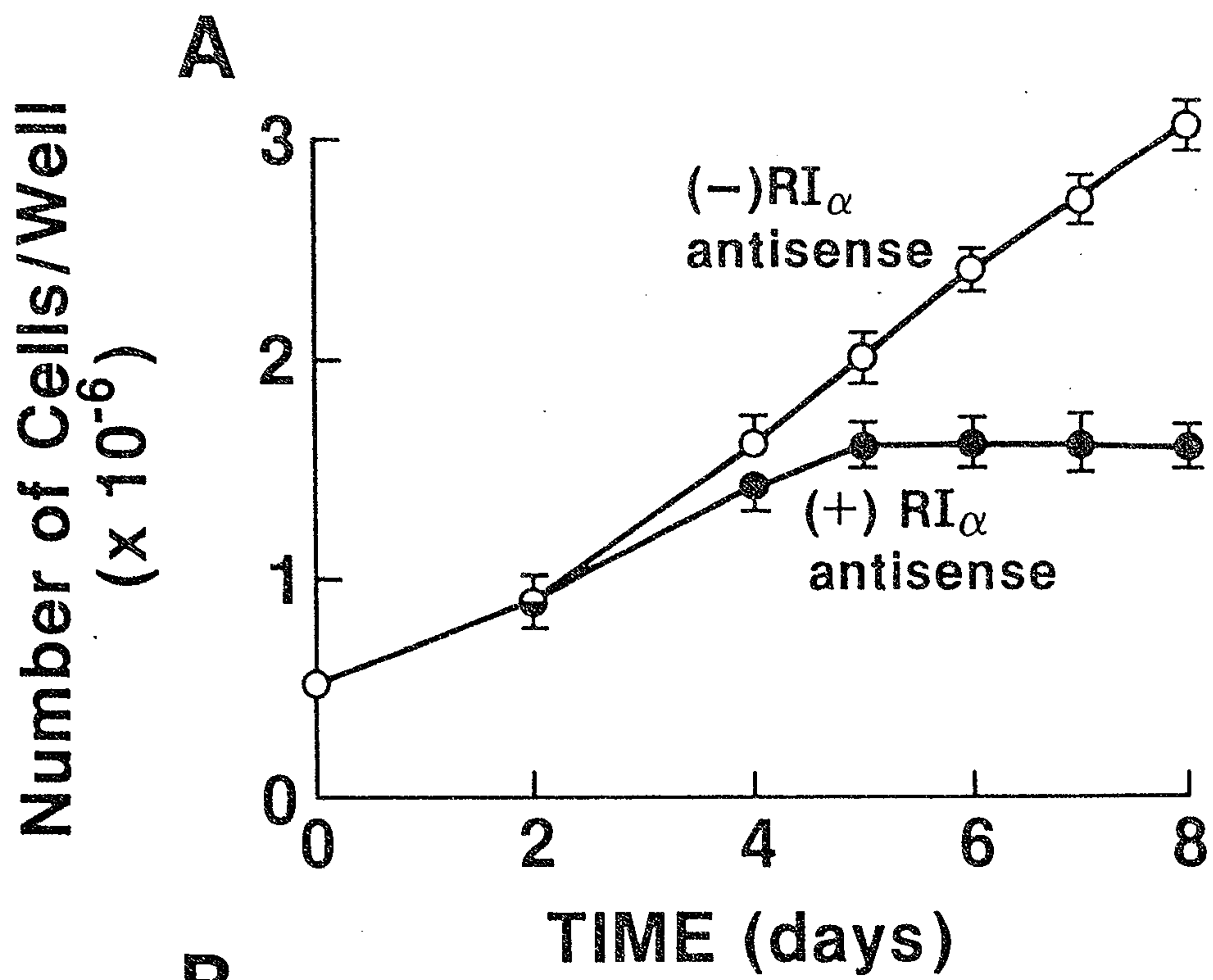
lymphosarcoma, Gardner lymphosarcoma, Ridgway Osteogenic sarcoma, Sarcoma 180 (ascites), Wagner osteogenic sarcoma, Sarcoma T241, Lewis lung carcinoma, Carcinoma 755, CD8F, MCF-7 breast carcinoma, Colon 38, LS-174T colon carcinoma, Carcinoma 1025, Erhlich carcinoma (ascites & solid), Krubs 2 carcinoma (ascites), Bashford carcinoma 63, Adenocarcinoma E 0771, B16 Melanoma, Hardin-Passey melanoma, Giloma 26, Miyona adenocarcinoma, Walker carcinosarcoma 256, Flexner-Jobling carcinoma, Jensen sarcoma, Iglesias sarcoma, Iglesias ovarian tumor, Murphy-Sturm lymphosarcoma, Yoshida sarcoma, Dunning leukemia, Rous chicken sarcoma, and Crabb hamster sarcoma.

32. The use according to any one of claims 29 – 31, wherein said one or more antisense oligonucleotides, or derivatives thereof, are DNA, RNA or a combination thereof.
33. The use according to any one of claims 29 – 32, wherein said one or more antisense oligonucleotides, or derivatives thereof, are 5' to 3'-oligodeoxynucleotides.
34. The use according to any one of claims 29 – 33, wherein said one or more antisense oligonucleotides, or derivatives thereof, have internucleotide linkages that are selected from the group of phosphodiester or phosphorothioate phosphodiester.
35. The use according to any one of claims 32 – 34, wherein said one or more antisense oligonucleotides, or derivatives thereof, comprise two antisense oligonucleotides, or derivatives thereof, which are complementary to adjacent regions of the nucleotides of the first 100 N-terminal codons of RI_α (SEQ ID NO:6).
36. The use according to any one of claims 29 – 35, wherein said one or more antisense oligonucleotides, or derivatives thereof, are contained in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.
37. The use according to claim 36, wherein said pharmaceutically acceptable carrier is a sterol.

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38. The use according to claim 36, wherein said pharmaceutically acceptable carrier is a liposome.
39. The use according to any one of claims 29 – 38, wherein the one or more antisense oligonucleotides, or derivatives thereof, are combined with an agent which enhances uptake of the antisense oligonucleotides, or derivatives thereof, by cells.
40. The use according to claim 39, wherein the agent is selected from the group of a lipophilic cationic compound, a lipophilic carrier or a peptide, that is ingested by cells.
41. Use of a 15-30-mer oligonucleotide complementary to a sequence of the nucleotides encoding the first 100 N-terminal amino acids of RI α , for the preparation of a pharmaceutical composition for the treatment of a cancer selected from the group consisting of gastric, pancreatic, lung, breast, anal, colorectal, head and neck neoplasms, neuroblastomas and melanoma.
42. Use of a 15-30-mer oligonucleotide complementary to a sequence of the nucleotides encoding the first 100 N-terminal amino acids of RI α , for administration to a patient having cancer selected from the group consisting of gastric, pancreatic, lung, breast, anal, colorectal, head and neck neoplasms, neuroblastomas and melanoma.

FIG.1



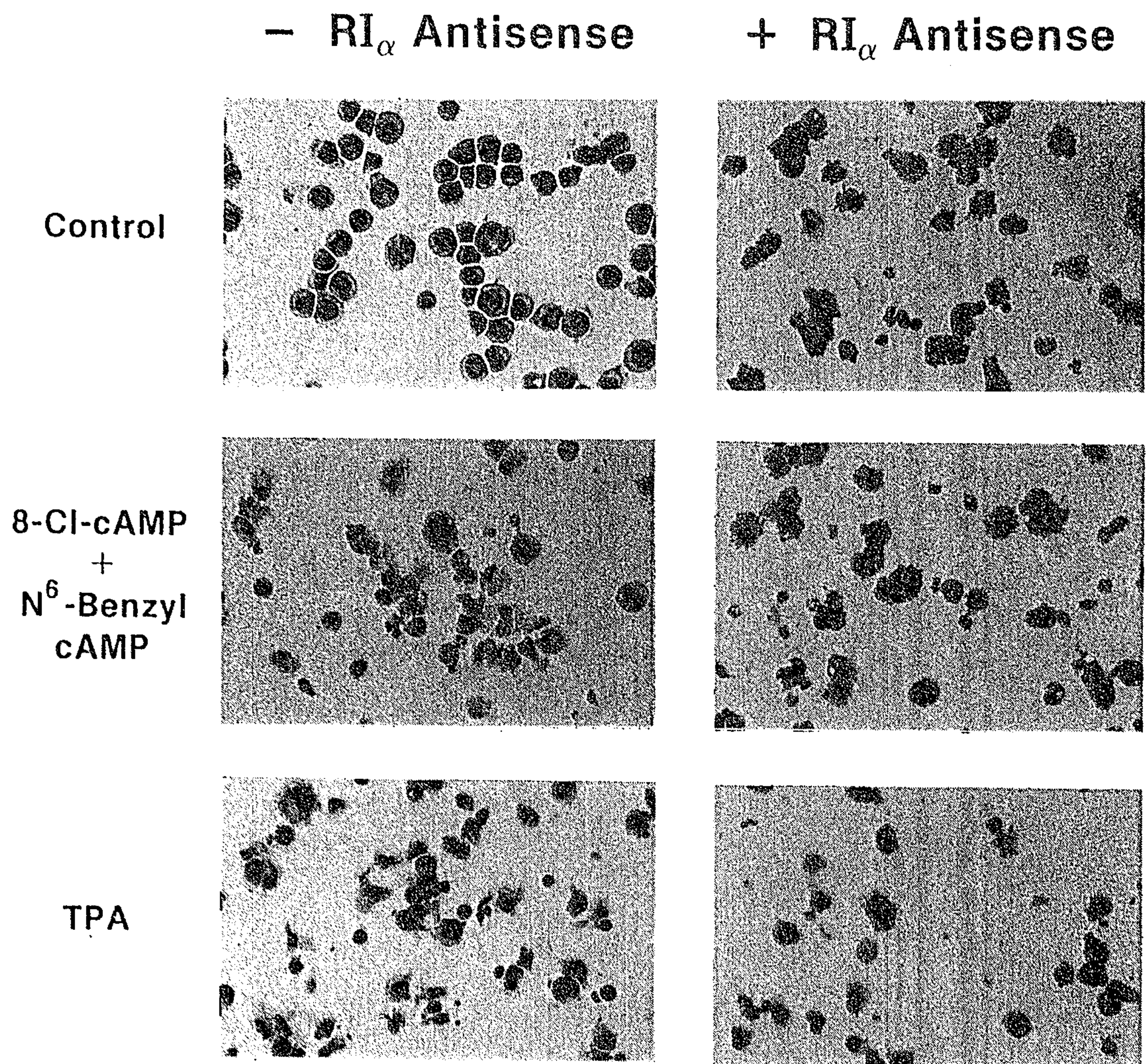


FIG. 2

*Kirby, Eades, Gale,
Baker & Potvin*

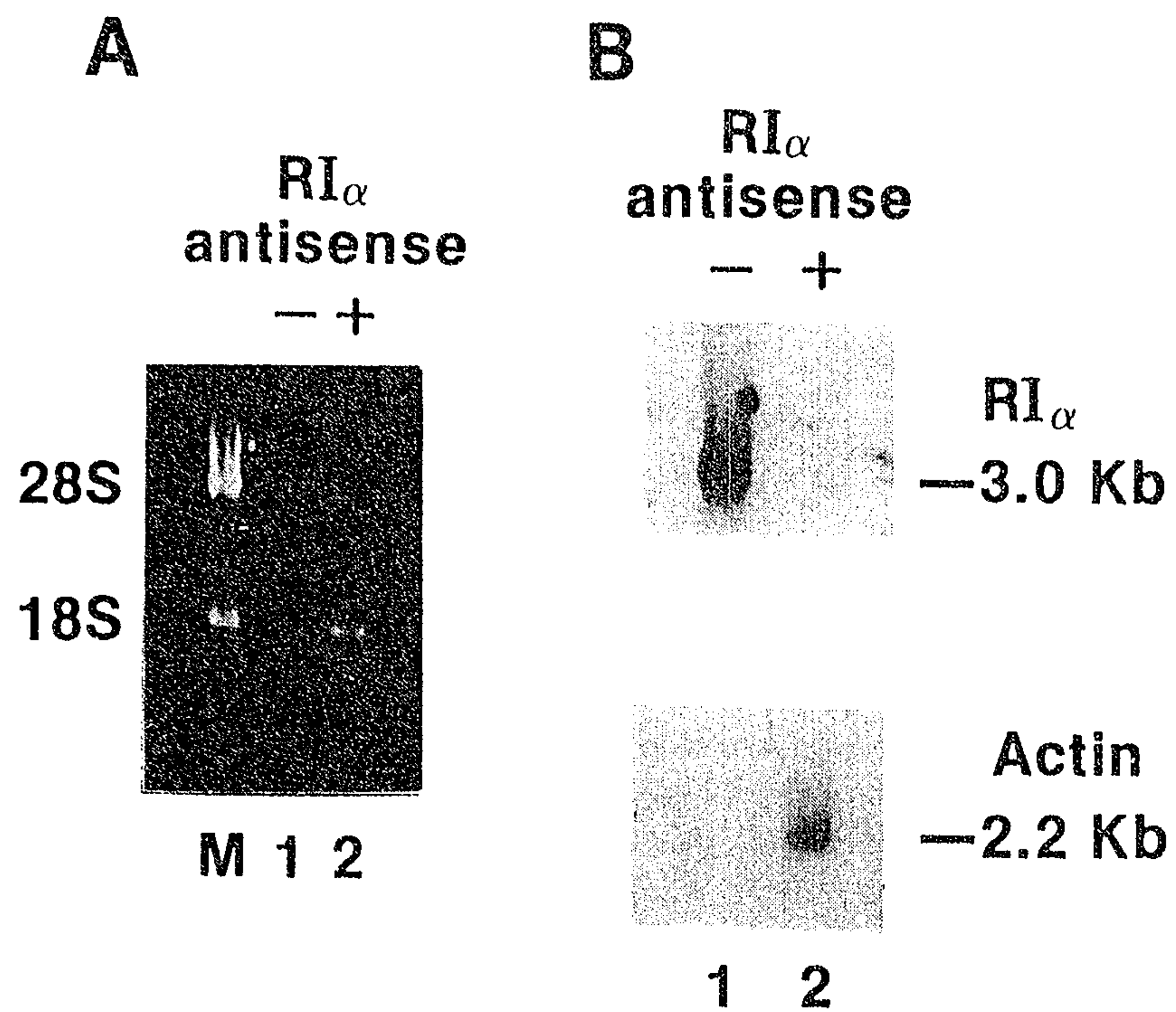
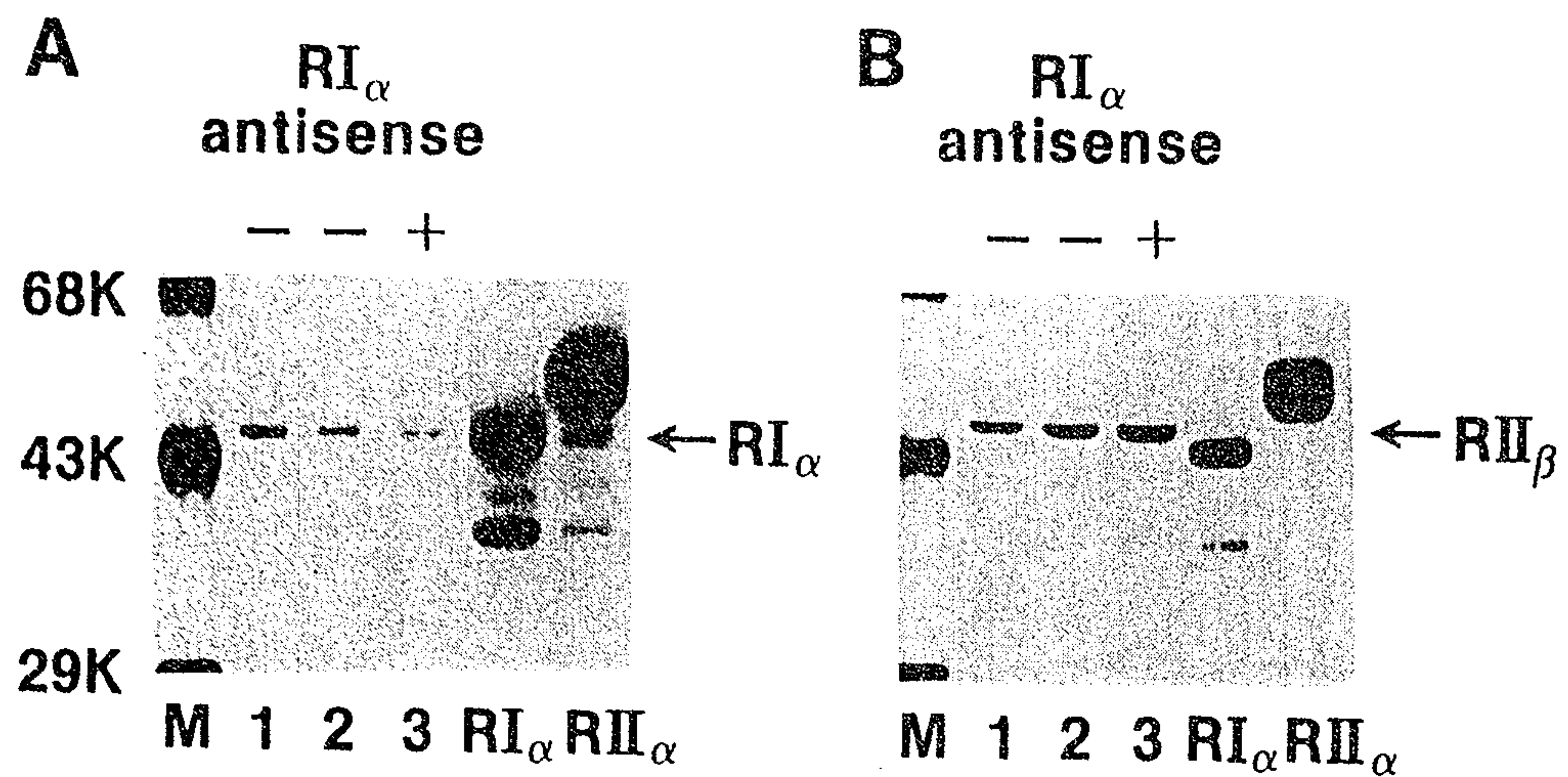


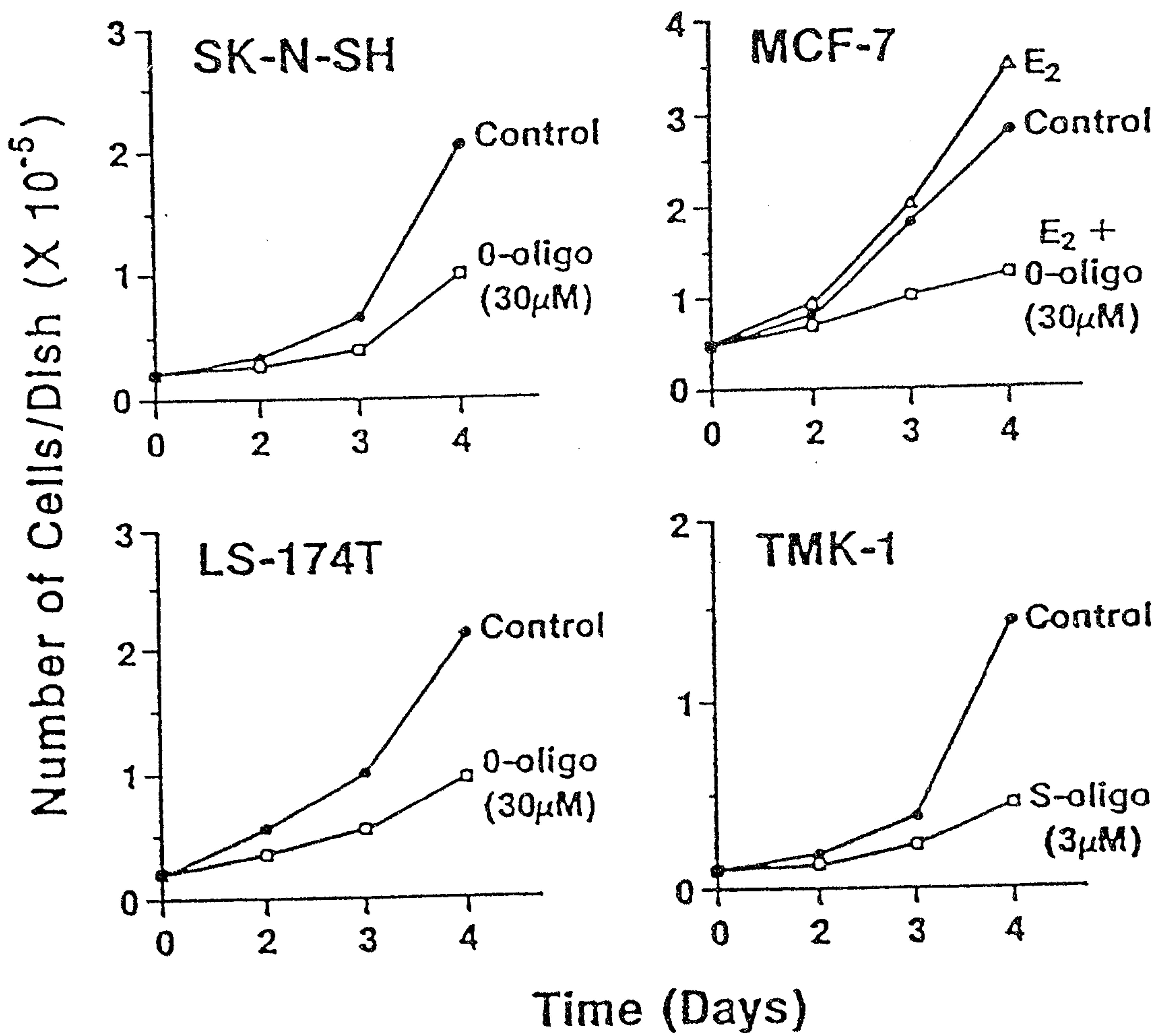
FIG.3



Lane	Treatment	Relative Levels	
		RI_{α}	RII_{β}
1	Control	1.0 ± 0.1	1.0 ± 0.1
2	8-Cl + N ⁶ -Benzyl	0.3 ± 0.04	3.0 ± 0.3
3	RI_{α} Antisense	0.2 ± 0.03	5.0 ± 0.4

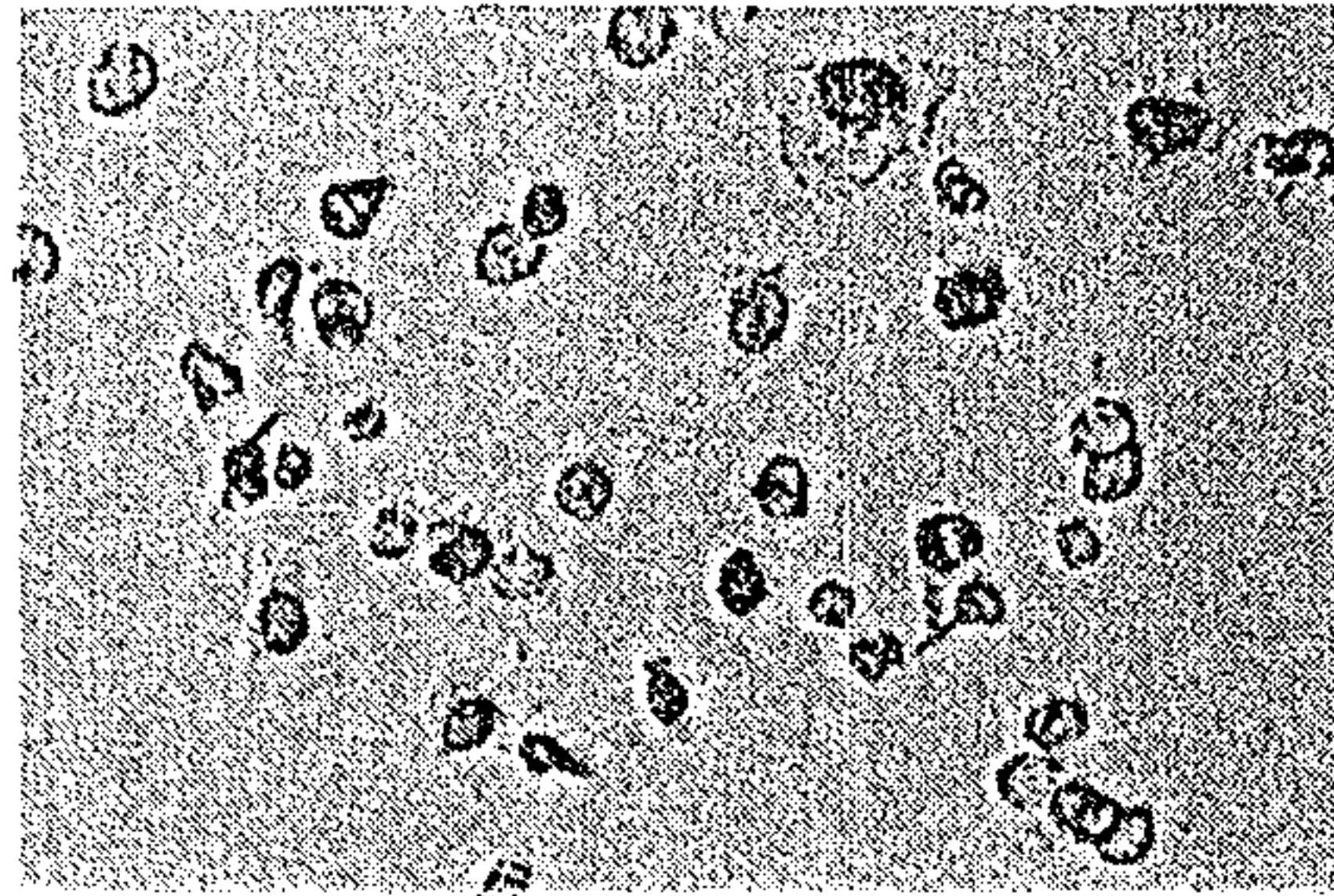
FIG.4

FIG. 5

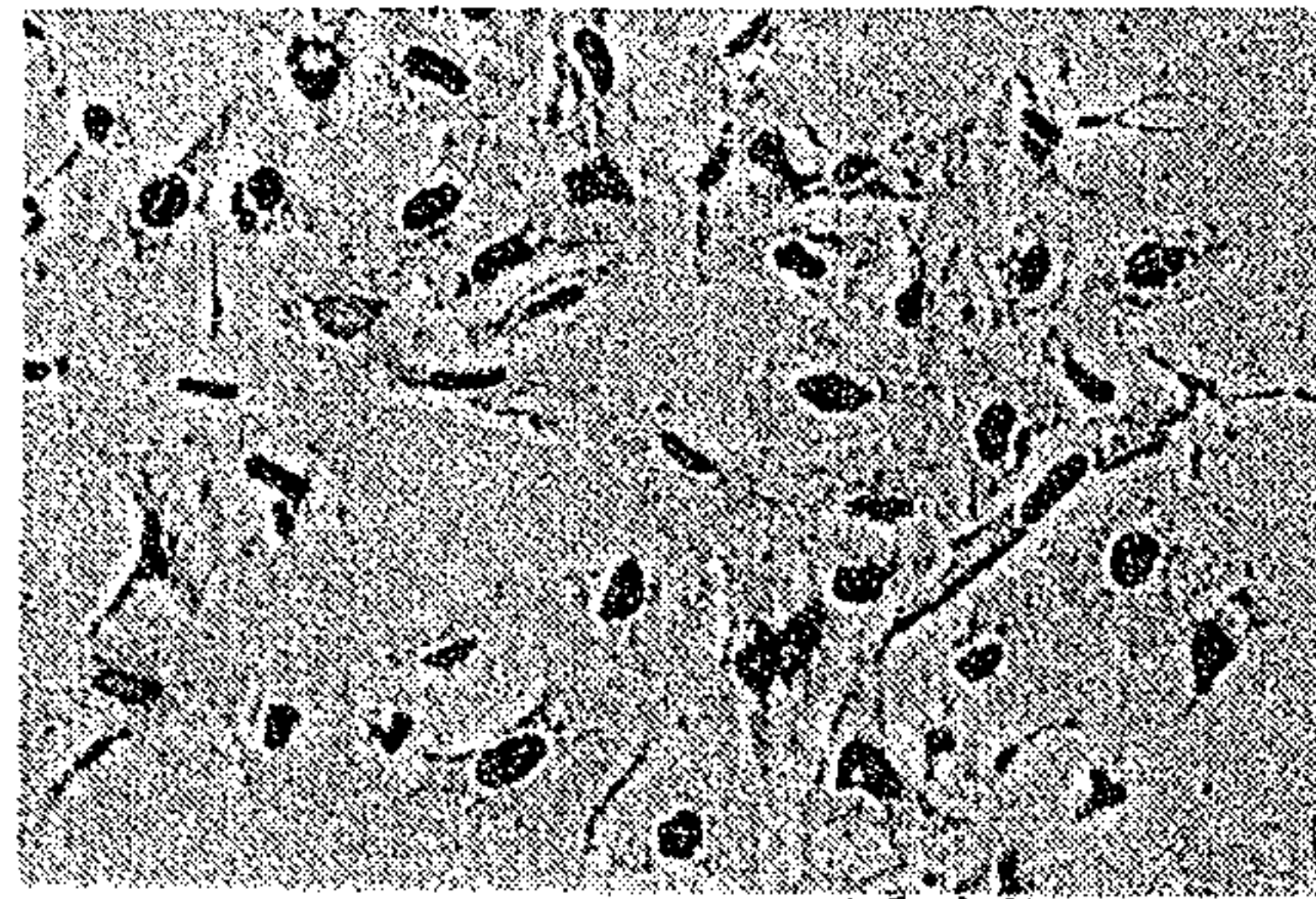


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Control



**RI_α
Antisense
Oligo**



**Random
Oligo**

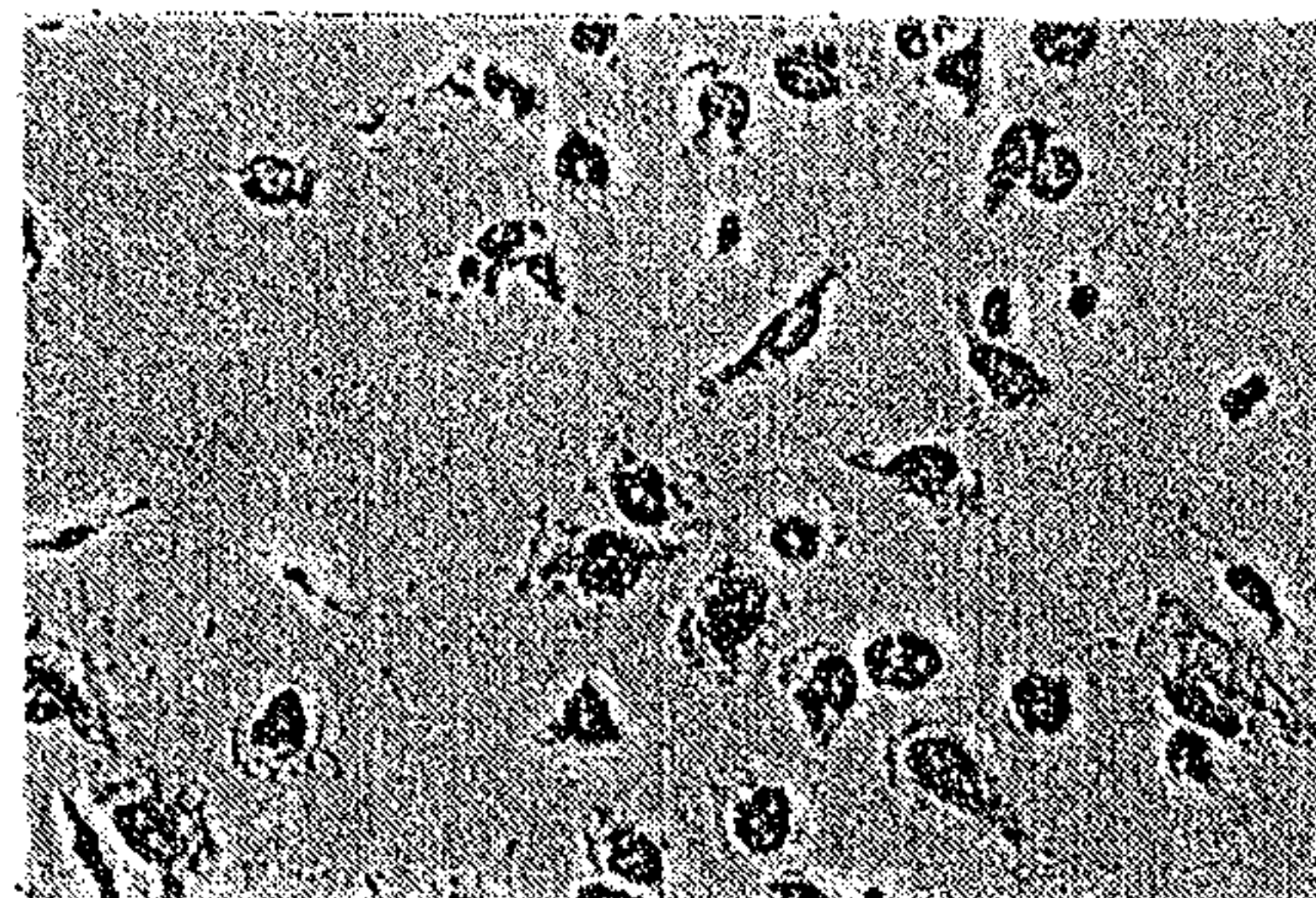
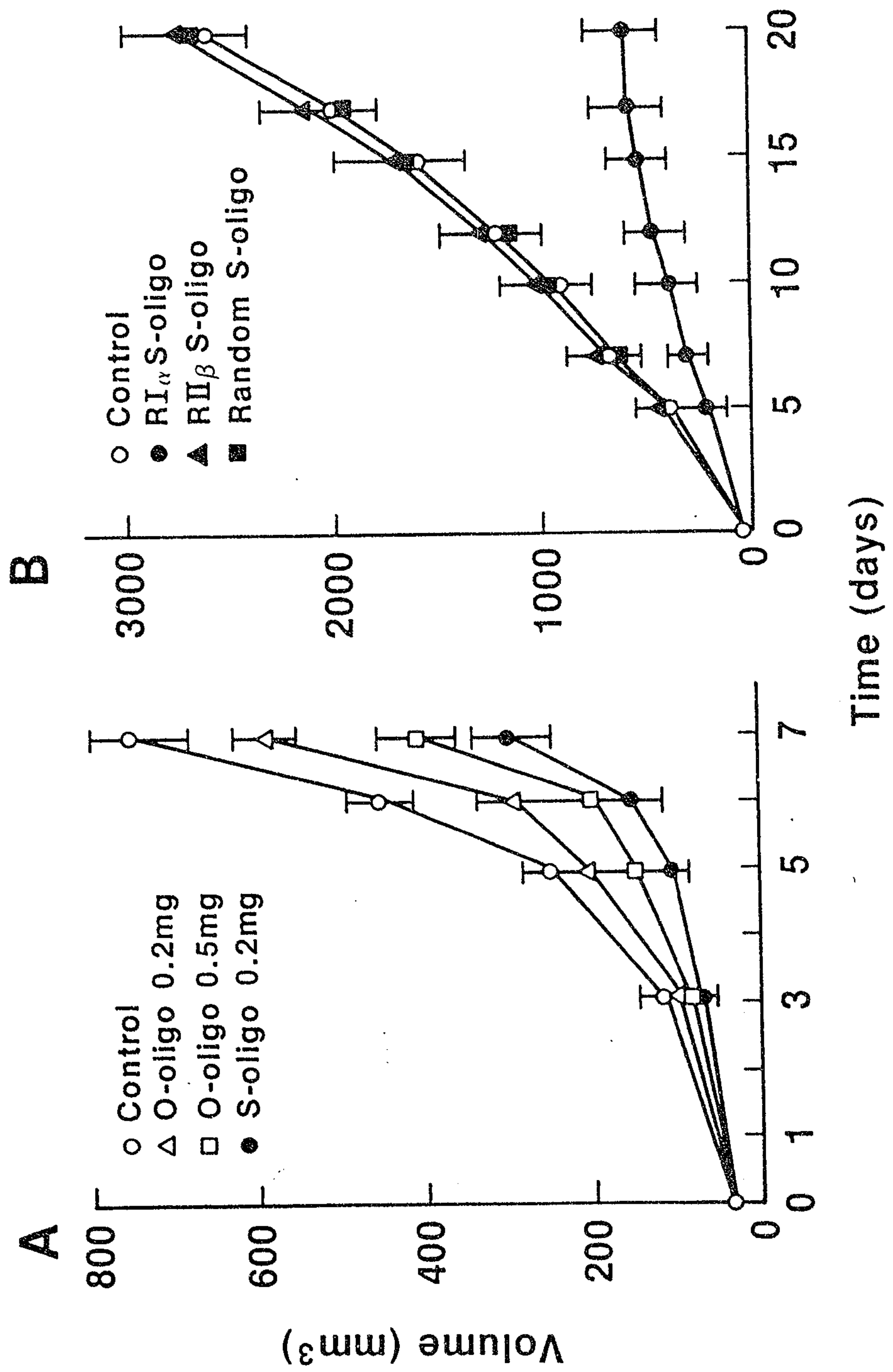


FIG.6

*Kirby, Eades, Gale,
Baker & Potvin*

FIG. 7



Kirby, Lades, Gale,
Baker & Potvin