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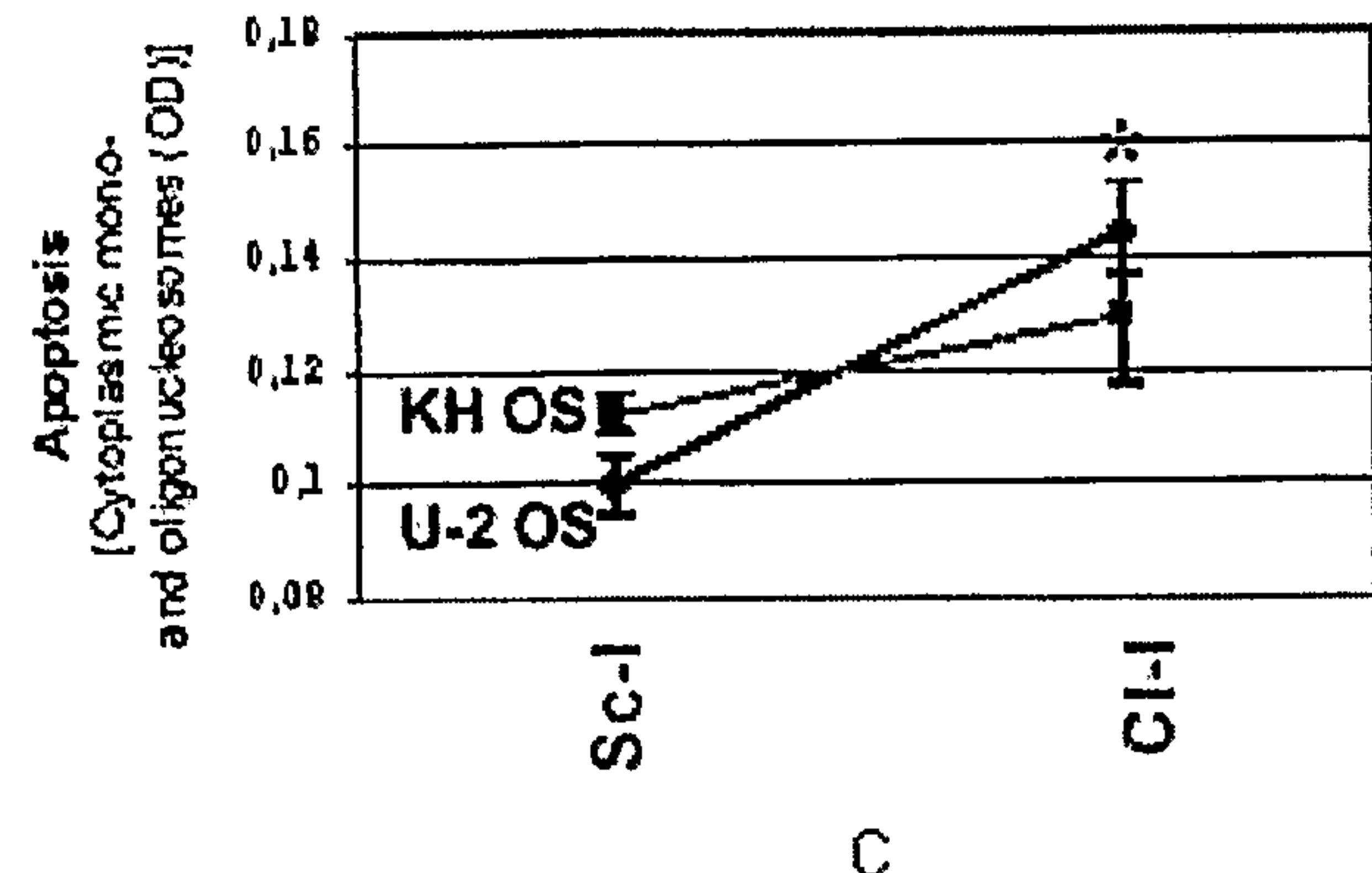
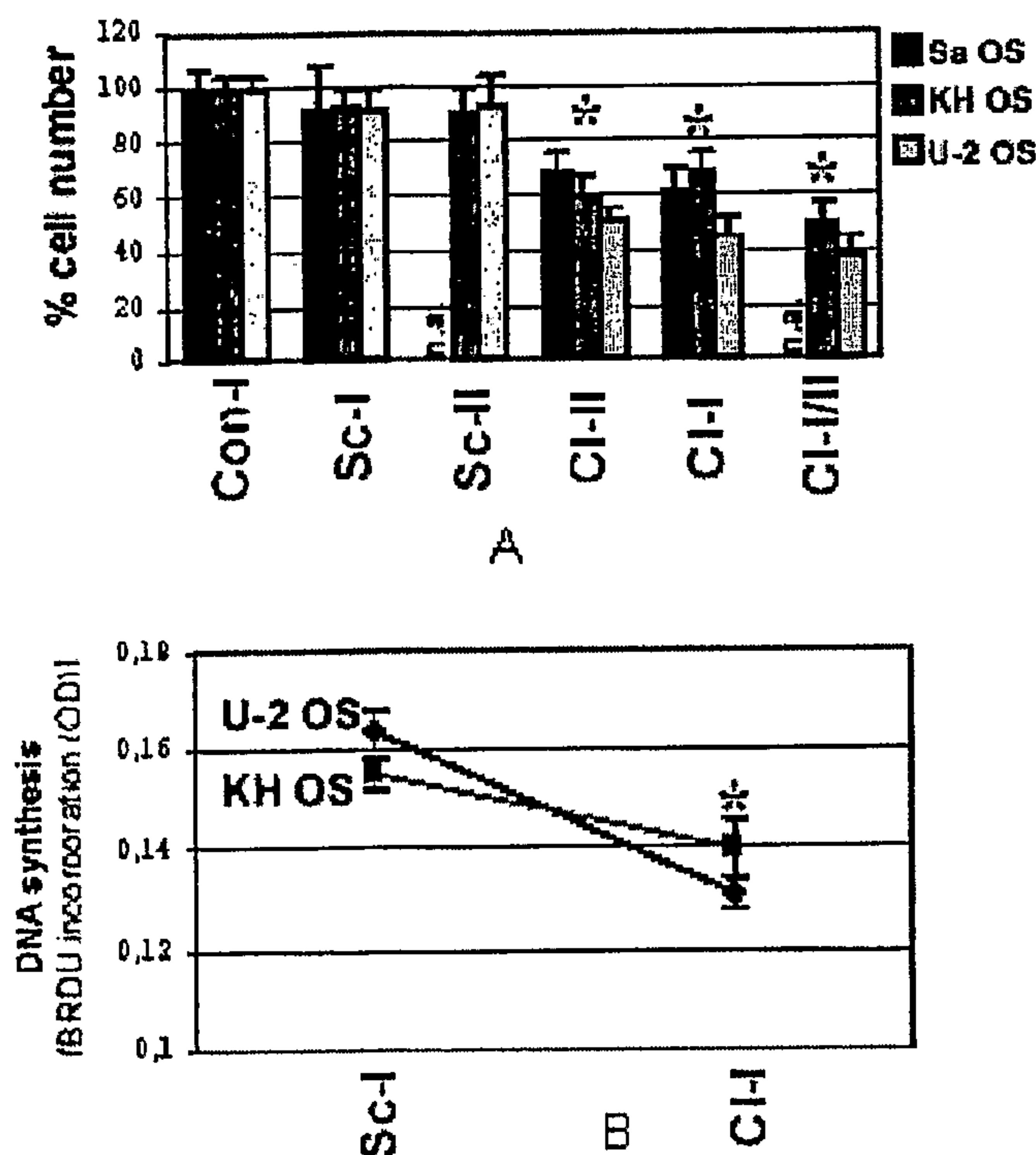
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(54) **Title: RNAI PROBES TARGETING CANCER-RELATED PROTEINS**



(57) **Abrégé/Abstract:**

RNAi sequences that are useful as therapeutics in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease. These sequences target clusterin, IGFBP-5, IGFBP-2, both IGFBP-2 and -5 simultaneously, Mitf, and B-raf. The invention further provides for the use of these RNAi sequences in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease, and a method of treating such conditions through the administration of the RNA molecules with RNAi activity to an individual, including a human individual in need of such treatment.

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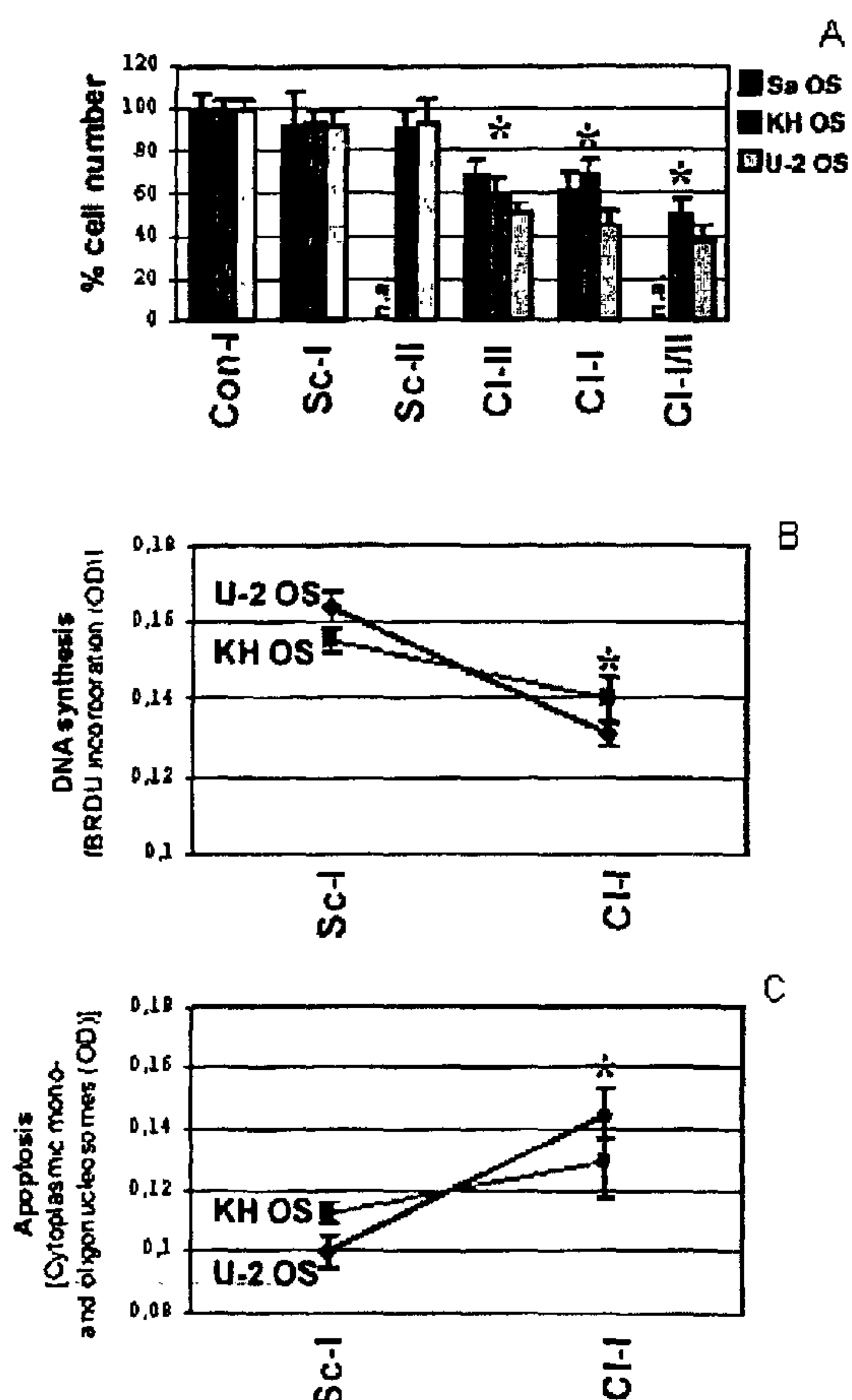
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(54) Title: RNAI PROBES TARGETING CANCER-RELATED PROTEINS



(57) Abstract: RNAi sequences that are useful as therapeutics in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease. These sequences target clusterin, IGFBP-5, IGFBP-2, both IGFBP-2 and -5 simultaneously, Mitf, and B-raf. The invention further provides for the use of these RNAi sequences in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease, and a method of treating such conditions through the administration of the RNA molecules with RNAi activity to an individual, including a human individual in need of such treatment.

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## RNAi PROBES TARGETING CANCER-RELATED PROTEINS

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Background of the Invention

10 This application relates to short double stranded RNAi probes useful in cancer therapy and treatment of other diseases. RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire et al. (1998) Nature 391, 806-811 ). dsRNA directs gene-specific, post-transcriptional silencing in many organisms, including vertebrates, and has  
15 provided a new tool for studying gene function. RNAi involves mRNA degradation, but many of the biochemical mechanisms underlying this interference are unknown. The use of RNAi has been further described in Carthew et al. (2001) Current Opinions in Cell Biology 13, 244-248, and Elbashir et al. (2001) Nature 411, 494-498.

20 Within any given mRNA molecule, there are sites which are affected by RNAi probes, and sites which are not. Thus, one cannot simply chop up the overall sequence into subsequences of appropriate lengths (for example, 21 to 23 base pairs) to arrive at functional RNAi-based therapeutics. Indeed, published US Patent Application 2002-0086356-A1 discloses a method for use in assessing where target sites might be  
25 located in a mRNA sequence, although this method is not the only approach to development of effective RNAi sequences.

Summary of the Invention

30 Some embodiments of the present invention provide RNAi sequences that are useful as therapeutics in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, lung

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cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease. These sequences target clusterin, IGFBP-5, IGFBP-2, both IGFBP-2 and -5 simultaneously, Mitf, and B-raf.

Some embodiments of the invention provide for the use of these RNAi sequences in the treatment of cancers of various types, including prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma; and Alzheimer's disease, and a method of treating such conditions through the administration of the RNA molecules with RNAi activity to an individual, including a human individual in need of such treatment.

In accordance with an aspect of the present invention, there is provided an RNA molecule having a sequence effective to mediate degradation or block translation of mRNA encoding clusterin when used as an RNA duplex, characterized in that the RNA molecule consists of a sequence or a duplex formed from a complementary pair of sequences selected from among Seq. ID Nos. 1, 2, 5, 6, 9, 10, 61 and 62.

In another aspect, the present invention provides a pharmaceutical composition comprising an RNA molecule of the invention and a pharmaceutically acceptable carrier.

In another aspect, the present invention provides an RNA molecule of the invention for use in mediating degradation or blocking translation of mRNA encoding clusterin.

In another aspect, the present invention provides an RNA molecule of the invention for use in mediating degradation or blocking translation of mRNA encoding clusterin.

In another aspect, the present invention provides an RNA molecule of the invention, or a pharmaceutical composition of the invention, for the treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

In another aspect, the present invention provides an RNA molecule of the invention, or a pharmaceutical composition of the invention, for use in the treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.



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In another aspect, the present invention provides a use of an RNA molecule of the invention in manufacture of a medicament for treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

In another aspect, the present invention provides an RNA molecule of the invention for use in manufacture of a medicament for treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

#### Brief Description of the Drawings

Fig. 1A shows relative growth rate, estimated by cell number counting of Sa OS, KH OS and U-2 OS cells following siRNA-mediated clusterin gene expression silencing.  $5 \times 10^3$  cells/cell line were seeded in 6 well plates and after siRNA treatment for 70 hours the total number of cells was counted. A significant reduction in the cell number, that is more pronounced in U-2 OS cells was observed in all clusterin knock-down cells.

Figs. 1B and C show endogenous DNA synthesis levels and spontaneous apoptosis in clusterin knock down KH OS and U-2 OS cells, as estimated by cell proliferation ELISA BrdU colorimetric immunoassay and a Cell Death detection ELISA photometric enzyme immunoassay, respectively. Clusterin knock-down was accompanied by reduced DNA synthesis and an enhanced rate of endogenous spontaneous apoptosis in both cell lines.

Figs. 2A and B show reduced clonogenic potential of clusterin knock down OS cells. After siRNA treatment for 70 hours,  $5 \times 10^3$  KH OS cells were seeded in 6 well plates, and the total number of cells was counted after 5 days of growth in complete medium (Fig. 2A). Fig. 2B shows the results of a comparable experiment with U-2 OS cells.

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Figs. 3A-F show the effect of DXR treatment in OS cells and cell sensitization to DNA damage and oxidative stress following siRNA-mediated clusterin knock down. The dark bars in Figs. 3A and B show the results when  $2 \times 10^4$  KH OS or U-2 OS cells were seeded in 6 well plates in complete medium, siRNA treated for 70 hours and then allowed to recover. The cells were then exposed to 0.35  $\mu$ M DXR for 24 hours, sub-cultured in complete medium for 72 hours and counted. The light bars in Figs. 3A and B show the results when  $2 \times 10^4$  KH OS or U-2 OS cells were seeded in 6 well plates in complete medium, siRNA treated for 70 hours and DXR was added directly to the transfection medium to a final concentration of 0.35  $\mu$ M. Cells were incubated in the drug containing transfection medium for 24 hours, washed, allowed to recover in complete medium for 72 hours and counted.

Fig. 4 shows quantitative analysis of sequence-specific clusterin gene silencing by siRNA in PC3 tumor cells.

Fig. 5 shows the effects of paclitaxel<sup>TM</sup> treatment on clusterin knock down PC3 cell growth and apoptosis. Cells were treated with 50 nM of the Cl-III, Cl-IV or scrambled control siRNA for 1 day. Two days following the siRNA treatment, cells were exposed to the indicated concentrations of paclitaxel for 48 hours, and cell viability was determined by an *in vitro* MTT assay.

Fig. 6 shows quantitative results of exposure of PC3 prostate cancer cells to CLU-5 siRNA (Seq ID NOs. 9 and 10).

Fig. 7 shows quantitative results of exposure of A549 lung cancer cells to CLU-5 siRNA (Seq ID NOs. 9 and 10).

Fig. 8 shows the reduction in clusterin transcript as a result of treatment of PC3 cells with clusterin-targeted siRNA as determined by RT-PCR.

Fig. 9 shows the reduction in clusterin transcript as a result of treatment of A549 cells with clusterin-targeted siRNA as determined by RT-PCR.

Fig. 10 shows the reduction in clusterin transcript as a result of treatment of PC3 cells with clusterin-targeted siRNA as determined by Northern blot.

Fig. 11 shows cell viability following treatment of PC3 cells with combinations of siRNA and Taxol<sup>TM</sup>.



Fig. 12 shows cell viability following treatment of A549 cells with combinations of siRNA and Taxol.

Fig. 13 shows the reduction in clusterin transcript as a result of treatment of OVCAR3 cells with clusterin-targeted siRNA as determined by Northern blot.

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Fig. 14 shows the reduction in clusterin transcript as a result of treatment of MDA-MB 231 cells with clusterin-targeted siRNA as determined by Northern blot.

Fig. 15 shows the reduction in clusterin transcript as a result of treatment of MDA-MB 231 cells with clusterin-targeted siRNA as determined by RT-PCR.

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Fig. 16 shows the reduction in clusterin transcript as a result of treatment of MCF-7 cells with clusterin-targeted siRNA as determined by Northern blot.

Fig. 17 shows the reduction in clusterin transcript as a result of treatment of MCF-7 cells with clusterin-targeted siRNA as determined by RT-PCR.

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Fig. 18 shows the reduction in the amount of clusterin protein in MCF-7 cells treated with the siRNA relative to the scrambled control.

Fig. 19 shows the reduction in IGFBP-2 transcript as a result of treatment of A549 cells with bispecific IGFBP-2 and -5-targeted siRNA as determined by RT-PCR.

Fig. 20 shows the reduction in IGFBP-5 transcript as a result of treatment of PC3 cells with bispecific IGFBP-2 and -5-targeted siRNA as determined by RT-PCR.

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Fig. 21 shows reduction in IGFBP-5 mRNA in PC3 cells.

Fig. 22 shows inhibitions of IGFBP-5 transcript in primary human bone fibroblast.

Fig. 23 shows growth inhibition of C42 cells by IGFBP-2/IGFBP-5 bispecific siRNA.

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Fig. 24 shows growth inhibition of A549 cells by IGFBP-2/IGFBP-5 bispecific siRNA.

### Detailed Description of the Invention

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The present invention relates to isolated RNA molecules which mediate



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RNAi. That is, the isolated RNA molecules of the present invention mediate degradation of mRNA that is the transcriptional product of the gene, which is also referred to as a target gene. For convenience, such mRNA may also be referred to herein as mRNA to be degraded. The terms RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) may be used interchangeably to refer to RNA that mediates RNA interference. These terms include double-stranded RNA, single-stranded RNA, isolated RNA (partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA), as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the RNA or internally (at one or more nucleotides of the RNA). Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. Collectively, all such altered RNAi compounds are referred to as analogs or analogs of naturally-occurring RNA. RNA of the present invention need only be sufficiently similar to natural RNA that it has the ability to mediate RNAi. As used herein the phrase "mediate RNAi" refers to and indicates the ability to distinguish which mRNA are to be affected by the RNAi machinery or process. RNA that mediates RNAi interacts with the RNAi machinery such that it directs the machinery to degrade particular mRNAs or to otherwise reduce the expression of the target protein. In one embodiment, the present invention relates to RNA molecules that direct cleavage of specific mRNA to which their sequence corresponds. It is not necessary that there be perfect correspondence of the sequences, but the correspondence must be sufficient to enable the RNA to direct RNAi inhibition by cleavage or lack of expression of the target mRNA.

As noted above, the RNA molecules of the present invention in general comprise an RNA portion and some additional portion, for example a deoxyribonucleotide portion. The total number of nucleotides in the RNA molecule is suitably less than 49 in order to be effective mediators of RNAi. In preferred RNA molecules, the number of nucleotides is 16 to 29, more preferably 18 to 23, and most preferably 21-23.

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A first group of RNA molecules in accordance with the present invention are directed to mRNA encoding clusterin, a protein also known as testosterone-repressed prostate message-2 (TRPM-2) or sulfated glycoprotein-2 (SGP-2). Clusterin is expressed in increased amounts by prostate tumor cells following androgen withdrawal.

5 Furthermore, it has been determined that antisense therapy which reduces the expression of clusterin provides therapeutic benefits in the treatment of cancer. In particular, such antisense therapy can be applied in treatment of prostate cancer and renal cell cancer. (PCT Patent Publication WO 00/49937 ).

10 Administration of therapeutic agents clusterin also can enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific RNA molecules which may be used to interfere with the expression of clusterin are listed in Table 1 and 7. (See, US Patent No.: 7,569,551).

15 These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of prostate cancer, sarcomas such as osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, colon cancer, ovarian cancer, anaplastic large cell lymphoma and melanoma.. In addition, clusterin has been shown to promote amyloid plaque formation and to be critical for neuritic toxicity in mouse models for Alzheimer's disease. (De Mattos et al., *Proc. Nat'l Acad. Sci. (USA)* 99: 10843-10848 (2002) ).

20 Thus, the sequences of the invention can also be used in the treatment of Alzheimer's disease.



Table 1 -- Clusterin RNAi Sequences			
Target region of cDNA (nt)	487-505	1105-1123	1620-1638
sense siRNA	ccagagcucgcccucucac-dtdt (SEQ. ID No: 1)	gaugcucaacaccuccucc-dtdt (SEQ. ID No: 3)	cuaauucaauaaaacuguc-dtdt (SEQ. ID No: 5)
antisense siRNA	guagaagggcgagcucugg-dtdt (SEQ. ID No: 2)	ggaggagguguugagcauc-dtdt (SEQ. ID No: 4)	gacaguuuuauugaauuag-dtdt (SEQ. ID No: 6)
Target region of cDNA (nt)	HIV 1152-1176	53-71	not sequence specific
sense siRNA	uaauucaacaaaacugu-dtdt (SEQ. ID No: 7)	augaugaagacucugcugc-dtdt (SEQ. ID No: 9)	ugaaugaagggaacuaaccug-dtdt (SEQ. ID No: 11)

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antisense siRNA	acaguuuuguugaauua-dtdt (SEQ. ID No: 8)	gcagcagagucuucaucu- dtdt (SEQ. ID No: 10)	cagguuagucccucauuc -dtdt (SEQ. ID No: 12)
Target region of cDNA (nt)	not sequence specific	not sequence specific	
sense siRNA	cagaaauagacaaaguggg- dtdt (SEQ. ID No: 13)	acagagacuaagggaccag a-dtdt (SEQ. ID No: 15)	
antisense siRNA	ccccacuuugucuauuucug- dtdt (SEQ. ID No: 14)	acagagacuaagggaccag a-dtdt (SEQ. ID No: 16)	

Specific results relating to the use of the RNAi species shown in Table 7 are shown in the Figures. These results demonstrate the effectiveness of clusterin suppression by RNAi mediated processes to reduce the growth of and to promote apoptosis in osteosarcoma cells, thus demonstrating a further type of condition which can be treated in accordance with the invention. The results also demonstrate that RNAi treatment to reduce clusterin levels results in increased levels of p53 and reduced levels of bcl-2. Thus, in accordance with the invention, conditions in which active p53 or bcl-2 levels are affected due to regulation as opposed to a mutation that renders the tumor



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suppressor wholly or partially inactive are overcome by administration of an amount of clusterin RNAi effective to reduce the amount of clusterin present, and thus the undesirable clusterin-associated modulation of p53 and bcl-2 levels.

5 A second group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-5 (IGFBP-5). It has been shown that inhibition of IGFBP-5 expression can delay the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, provide a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast  
10 or prostate cancer and inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone. (Published PCT Application No. WO01/05435  
15 ). These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 2. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts.

Table 2 -- IGFBP-5 RNAi Sequences			
Target region of cDNA (nt)	44-61	876-895	not sequence specific
sense siRNA	augguguugcucaccgcg-dtdt (SEQ. ID No: 17)	cccugggcugcgagcugguc-dtdt (SEQ. ID No: 19)	gaggaaacugaggaccucg-g-dtdt (SEQ. ID No: 21)

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antisense siRNA	cgcgugagcaacaccau- dtdt (SEQ. ID No: 18)	gaccagcucgcagcccaggg -dtdt (SEQ. ID No: 20)	ccgagguccucaguuuccuc -dtdt (SEQ. ID No: 22)
Target region of cDNA (nt)	not sequence specific	850-568	1225-1243
sense siRNA	cucggauucucaugcaaggg -dtdt (SEQ. ID No: 23)	agcccucuccaugugcccc- dtdt (SEQ. ID No: 25)	gaagcugaccaguccaag- dtdt (SEQ. ID No: 27)
antisense siRNA	cccuugcuagagauuccgag -dtdt (SEQ. ID No: 24)	ggggcacauggagagggcu- dtdt (SEQ. ID No: 26)	cuuggacugggucagcuuc- dtdt (SEQ. ID No: 28)
Target region of cDNA (nt)	1501-1520		
sense siRNA	gcugccaggcauggaguacg -dtdt (SEQ. ID No: 29)		



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antisense siRNA	cguacuccaugccuggcagc -dtdt (SEQ. ID No: 30)		
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A third group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor binding protein-2 (IGFBP-2). It has been shown that inhibition of expression of IGFBP-2 delays the progression of prostatic tumor cells to androgen independence, and provides a therapeutic benefit for mammalian individuals, including humans, suffering from hormone-regulated cancer such as prostate or breast cancer. In addition, the compositions of the invention can be used to inhibit or delay the growth and metastatic progression of such cancers. (Published PCT Application No. WO02/22642). These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 3. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts.

Table 3 -- IGFBP-2 RNAi Sequences			
Target region of cDNA (nt)	118-138	1393-1411	906-924

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sense siRNA	augcugccgagagugggcu gcd-TdT (SEQ. ID No: 31)	ccccuguguccuuuugca- dTdT (SEQ. ID No: 33)	cugugacaagcauggccug- dTdT (SEQ. ID No: 35)
antisense siRNA	gcagcccacucucggcagca u-dTdT (SEQ. ID No: 32)	ugcaaaagggacacagggg- dTdT (SEQ. ID No: 34)	caggccaugcuugucacag- dTdT (SEQ. ID No: 36)
Target region of cDNA (nt)	525-542		
sense siRNA	gcgccgggacgccgagua- dTdT (SEQ. ID No: 37)		
antisense siRNA	uacucggcgucccggcgc- dTdT (SEQ. ID No: 38)		

A fourth group of RNA molecules in accordance with the present invention are directed to mRNA encoding insulin-like growth factor-2 and 5



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simultaneously (IGF-Bis). Inhibition of expression of both IGFBP-2 and IGFBP-5 can delay the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, provide a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast or prostate cancer and inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone potentially more effectively than the inhibition of either of these factors (Published PCT Application No. WO01/05435, Published PCT Application No. WO02/22642 ).

These same results are obtained using RNAi therapy in accordance with the invention using siRNA molecules having the sequences set forth in Table 4. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts.

Table 4 -- IGFBP-2 and IGFBP-5 Bispecific RNAi Sequences			
Target region of cDNA (nt)	BP5- 898-919 BP2 346-362	BP5 948-964 BP2 416-432	BP5 976-991 BP2 444-459
sense siRNA	ggagccgggcugcggcugc-dtdt (SEQ. ID No: 39)	cgugcggcgucacacc-dtdt (SEQ. ID No: 41)	ccaggggcugcgcugc-dtdt (SEQ. ID No: 43)
antisense siRNA	gcagccgcagcccgccucc-dtdt (SEQ. ID No: 40)	gguguagacgccgcacg-dtdt (SEQ. ID No: 42)	gcagcgcagccccugg-dtdt (SEQ. ID No: 44)

A fifth group of RNA or DNA antisense molecules in accordance with the present invention are directed to mRNA encoding the group of microphthalmia transcription factors (Mitf). Bcl-2 is regulated in melanoma and other cells by the master regulator Mitf which has been reported to modulate melanoma cell viability, lineage survival, and susceptibility to apoptosis (McGill et al. (2002) Cell 109, 707-718).

Mitf and Bcl-2 regulated by Mitf are expressed in increased amounts by various human tumors. RNAi or antisense therapy which reduces the expression of Mitf may provide therapeutic benefits in the treatment of cancer. In

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accordance with the invention, Mitf can also enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific antisense or RNA molecules which may be used to interfere with the expression of Mitf are listed in Table 5. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of melanoma, prostate cancer, renal cell carcinoma, bladder cancer, lung cancer, bone cancer and other tumors.

Table 5 -- Mitf RNAi Sequences			
Target region of cDNA (nt)	207-225	1287-1305	2172-2190
sense siRNA	ccgcugaagagcag caguu-dtdt (SEQ. ID No: 45)	augcaggcucgagcucau g-dtdt (SEQ. ID No: 47)	agauacaguaccccucua g-dtdt (SEQ. ID No: 49)
antisense siRNA	aacugcugcucuucagcg g-dtdt (SEQ. ID No: 46)	caugagcucgagccugca u-dtdt (SEQ. ID No: 48)	cuagagggguacuguauc u-dtdt (SEQ. ID No: 50)

A sixth group of RNA or DNA antisense molecules in accordance with the present invention are directed to mRNA encoding B-raf. B-raf is a key player in cellular signal transduction and is activated by somatic missense mutations in 66% of malignant melanomas and at lower frequencies in a wide range of human cancers (Davies et al. (2002) Nature 417, 949-954. ). RNAi or antisense therapy which reduces the expression of activated and/or non-activated B-raf may provide therapeutic benefits in the treatment of cancer. In accordance with the invention, reduction in expression of B-raf can also enhance sensitivity of cancer cells to chemotherapeutic agents and to radiotherapy both *in vitro* and *in vivo*. Sequences of specific antisense or RNA molecules which may be used to interfere with the expression of B-raf are listed in Table 6. These sequences can be used alone or in combination with other chemotherapy agents or apoptosis inducing treatment concepts in the treatment of melanoma, prostate cancer, renal cell carcinoma, bladder cancer, lung cancer, bone cancer



and other tumors.

Table 6 -- b-raf RNAi Sequences			
Target region of cDNA (nt)	362-380	1184-1202	2249-2267
sense siRNA	ucucuggggguucgguucu g-dtdt (SEQ. ID No: 51)	ccugucaauauugaugac u-dtdt (SEQ. ID No: 53)	cccuccuuguuucgggcu g-dtdt (SEQ. ID No: 55)
antisense siRNA	caguuccguuccccagag a-dtdt (SEQ. ID No: 52)	agucaucaauauugacag g-dtdt (SEQ. ID No: 54)	cagcccgauucaaggagg g-dtdt (SEQ. ID No: 56)

The siRNA molecules of the invention are used in therapy to treat patients, including human patients, that have cancers or other diseases of a type where a therapeutic benefit is obtained by the inhibition of expression of the targeted protein. siRNA molecules of the invention are administered to patients by one or more daily injections (intravenous, subcutaneous or intrathecal) or by continuous intravenous or intrathecal administration for one or more treatment cycles to reach plasma and tissue concentrations suitable for the regulation of the targeted mRNA and protein.

### Example 1

#### **Protocol for Transfection of LNCaP and PC3 cells with siRNA Duplexes**

##### 1) Cell preparation:

In each well of 6-well plate seed  $0.5 \times 10^6$  of LNCaP cells [PC3 cell at the density  $0.3 \times 10^6$  per well] in appropriate media containing 5% FBS without antibiotics [penicillin/ streptomycin]

Incubate the cells at 37E C in a humidified 5% CO2 incubator until they reach 40-50% confluence.

##### 2) si RNA preparation:



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Prepare the following si RNA dilution in microcentrifuge tubes. For each well :  
0.01-100 nM

3) Prepare the following transfection reagent dilution in microcentrifuge tubes:

For each well of 6-well plate dilute 4ml of OligoFECTAMINE™ Reagent into 11  
5 ml of OPTI-MEM™ and incubate 10 min at room temperature.

4) Combine the diluted OligoFECTAMINE™ to the diluted siRNA duplexes and  
mix gently by inversion.

5) Incubate 20 min at room temperature.

6) Remove the media from the well and replace it with 800 ml of Opti-MEM™.

10 7) Overlay the 200 ml of transfection complexes onto the cells.

8) Incubate 4 hrs at 37 degrees C in a CO<sub>2</sub> incubator.

9) add 500 ml of media containing 15 % FBS

10) after 24 hrs check gene expression by Real Time PCR or

11) check protein expression with Western Blot after 1, 6, 12, 24, 48, 72 and 96 hours

15

### Example 2

The human clusterin cDNA was manually scanned in order to identify  
sequences of the AA(N<sub>19</sub>)UU (N, any nucleotide) type that fulfill the required criteria for  
siRNA (Harbourn et al., J Cell Sci 114: 4557-4560 (2001)). Two such sequences with  
20 symmetric 2-nt 3' overhangs, were found 433 (C1-I) and 644 (C1-II) nts downstream of the  
CLU gene transcription initiation codon. Two additional oligonucleotides used targeted a  
region 1620 nts downstream of the CLU gene transcription initiation codon (C1-III) and  
the human CLU transcription initiation site (C1-V or C1-IV; throughout this document,  
the terms "C1-V" and "C1-IV" refer to the same sequences and are intended to be  
25 interchangeable) (see Table 7). BLAST analysis showed no homology with other known  
human genes. Selected RNA oligos were synthesized by Dharmacon Research, Inc.  
(Lafayette, CO), diluted at 20 :M final concentration in RNasefree ddH<sub>2</sub>O and stored at -  
20°C. The Scramble-I™ (Sc-I) (D-1200-20) and Scramble-II™ (Sc-II) (D-1205-20)  
oligonucleotides used were purchased from Dharmacon Research, Inc.

30 siRNA transfection of the C1-I, C1-II and scrambled RNA duplexes in exponentiary  
growing OS cells was performed as described (Harbourn et al., Tuschel et al.,

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Genes Dev. 13: 3191-3197 (1999)). Briefly, cells were seeded the day before siRNA transfection in 24-well plates containing 500 :l complete medium and were ~40-50% confluent during transfection. For the transfection mixture 100 nM of siRNA duplex per well were used. The RNA duplex was diluted in Opti-MEM<sup>®</sup> I (Gibco Life Technologies, Inc., San Diego, CA) serum free medium and transfection efficiency was enhanced by using the Oligofectamine<sup>™</sup> reagent (Invitrogen Life technologies Inc., San Diego, CA). When cells were treated with both Cl-I and Cl-II oligonucleotides, 100 nM of each siRNA duplex were used. Cell treatment with the siRNA oligonucleotides lasted for 2-3 days.

Alternatively, in PC3 cells Lipofectin<sup>™</sup> (Invitrogen Life technologies Inc., San Diego, CA) was used to enhance transfection with the Cl-III and CL-V oligonucleotides. PC3 cells were treated with 10, 50, or 100 nM of the RNA duplexes after pre-incubation with 4 :g/ml of Oligofectamine<sup>™</sup> reagent in serum free OptiMEM<sup>®</sup> I for 20 minutes. Four hours after starting the incubation, the medium containing the RNA duplexes and Lipofectin was replaced with standard tissue culture medium. Cells were treated once on day 1 and then harvested 48 h after treatment on day 3. In all cases controls used included: (a) the usage of the Sc-I and Sc-II RNA duplexes and (b) mock transfections in the absence of a nucleic acid (Con-I). CLU gene silencing was assayed by RNA blot analysis, immunoblotting analysis or confocal immunofluorescence.

Efficient silencing of the CLU gene expression in OS cells is achieved using siRNA. Treatment of the three OS cell lines with the Cl-I or the Cl-II siRNA oligonucleotides appeared to be quite effective and resulted in knocking down significantly the cellular CLU protein levels. Interestingly, the Cl-I oligonucleotide appeared to be slightly more effective than Cl-II in silencing the CLU gene. No CLU gene silencing was seen in the presence of the control Sc-I or the Sc-II oligonucleotides or at the absence of RNA duplexes from the transfection medium.

Next we addressed the issue of whether the CLU-specific siRNA oligonucleotides could also inhibit the accumulation of the CLU protein following cellular exposure to DXR. Exposure of the KH OS and U-2 OS cells to DXR for 24 h in the presence of the Cl-I, Cl-II or a mixture of both the Cl-I, Cl-II oligonucleotides



effectively abolished CLU protein accumulation. It is thus evident that in the presence of the Cl-I or the Cl-II oligonucleotides the cellular CLU protein cannot be induced after cell exposure to apoptosis inducing agents.

5     Example 3

Phenotypic effects in OS cells following CLU gene expression silencing by siRNA. The effects of CLU gene expression silencing in OS cells were studied by direct counting of the cells following siRNA, by recording cellular morphology and phenotype, as well as by clonogenic assays. CLU knock down in KH OS and Sa OS cells did not  
10     result in any visible phenotype. However, the CLU knock down cells were found to be significantly growth retarded as compared to their control counterparts (Fig. 1A). In contrast at the U-2 OS cells, that express the higher endogenous amount of the CLU protein, the effects of CLU knock down appeared quite significant. Specifically, CLU siRNA treated (for three days) U-2 OS cells lost their firm adherence to plastic and  
15     acquired a rounding shape. This phenotype was accompanied by a severe growth retardation effect. In order to study whether a combination of both Cl-I and Cl-II RNA duplexes would be more effective in inhibiting cell growth, we treated cells with both these oligonucleotides. For the KH OS and U-2 OS cells, only a slight increase in growth retardation was observed as compared to the Cl-I treated cells. Finally, in order to  
20     distinguish between the cytostatic and cytotoxic effects of CLU protein elimination we directly assayed CLU knock down KH OS and U-2 OS cells for DNA synthesis and endogenous spontaneous apoptosis. CLU knock down cells showed a reduced DNA synthesis rate (Fig. 1A) and higher levels of endogenous spontaneous apoptosis (Fig. 1B) as compared to their sibling controls. Effects were again more pronounced in U-2 OS  
25     cells. In summary, these results suggest that the reduced number of CLU knock down cells is due to a reduced rate of cell proliferation as well as to an increased level of spontaneous apoptosis.

The effect of CLU knock down in plating efficiency and growth following siRNA was also studied by clonogenic assays as recent studies have demonstrated that  
30     gene silencing is sustained for more than 7 cellular doublings (Harbourth et al.). KH OS



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and U-2 OS cells were selected for these assays since they represent two extreme opposite cases as far as the endogenous CLU amount and the intensity of CLU accumulation during stress are concerned. CLU knock-down KH OS cells when plated were firmly attached to the plastic (more than 90% of the seeded cells were attached) and only a few of the attached cells showed an abnormal morphology. However, the growth potential of the adherent cells was impaired as found 5 days post-plating after analyzing the total colony number and size of the formed colonies (Fig. 2A). CLU knocked-down U-2 OS cells were poorly attached to the plastic after trypsinization (only ~ 70% of the seeded cells were attached) and most of the adherent cells appeared quite abnormal in shape. Cells showed an extremely low proliferation potential (Fig. 2B) and after 9 days in culture only some small colonies could be seen.

#### Example 4

Sustained silencing of CLU gene expression in OS cells by siRNA results in significant sensitization to apoptosis induced by genotoxic and oxidative stress. Prior to CLU functional assays, we analyzed the DXR effects in OS cells since the drug-related reported effects vary in different cell-types (Gewritz et al., *Biochem. Pharmacol.* 57: 727-741 (1999)). As the DXR plasma concentration in treated patients fall into a range of 1-2 :M and decline into a range of 0.025-0.25 :M within 1 h (Muller et al, *Cancer Chemother. Pharmacol.*, 32: 379-384 (1993)) cells were treated with 0.35 and 1 :M of DXR. To analyze the extent of the DXR-mediated cell death we scored apoptosis by TUNEL. Attached cells following drug treatment for 24 h underwent significant morphological changes as compared to non-treated control cells. At this time cells exhibit an enlarged and flattened morphology that is reminiscent of a senescence-like phenotype, while a significant number of them are TUNEL positive. On-going apoptosis is also apparent 24 h later, verifying that apoptosis is a dynamic process that continues even after DXR removal from the medium. In agreement with the results obtained by TUNEL, DXR treatment was accompanied by PARP cleavage; the anti-apoptotic protein bcl-2 showed no altered expression in drug-treated KH OS and U-2 OS cells. Following DXR treatment, accumulation of p53 protein and its downstream effectors related to either

- 20 -

growth arrest (p21) or apoptosis (bax) was found only in U-2 OS cells indicating that the cytostatic and cytotoxic effects mediated by DXR in OS cell lines rely on both p53-dependent and p53-independent mechanisms.

Next, we followed two complementary approaches to study the effect of CLU knock down in OS cells exposed to DXR (Figs 3A and B). SiRNA treated cells were either re-plated in complete medium and were subsequently exposed to 0.35 :M DXR for 24 h, or they were exposed to 0.35 :M DXR in the presence of the Cl-II or Cl-I oligonucleotides. In both cases viable cells were counted 3 days post-DXR treatment. CLU knocked-down KH OS cells appeared more sensitive to DXR than their control counterparts (Fig. 3A-dark bars), whereas DXR treatment appeared significantly more effective when it was combined with the presence of the CLU-specific siRNA oligonucleotides in the medium (Fig. 3A-light bars). Similarly, CLU knocked-down Sa OS cells were more sensitive to the DXR treatment. In U-2 OS cells both strategies appeared very effective and CLU knock-down cells were significantly more sensitive to the drug as compared to controls (Fig. 3B). When DXR treatment was performed in the presence of the CLU-specific siRNA oligonucleotides, the CLU knock-down cells appeared to be significantly more sensitive to the drug (Fig. 3B-light bars) and a massive apoptosis was observed. Finally, when U-2 OS cells were treated with both the Cl-I and Cl-II oligonucleotides, cells were almost eliminated.

To understand the mechanism of cell sensitization following CLU knock down, we directly assayed the intensity of apoptosis induction right after cell exposure to agents inducing genotoxic (DXR) or oxidative stress (H<sub>2</sub>O<sub>2</sub>). As shown in Fig. 3C-F. Exposure of the CLU knock-down cells to either DXR or H<sub>2</sub>O<sub>2</sub> resulted in a significantly higher rate of apoptosis in both KH OS and U-2 OS cells. This observation suggests that CLU directly affects or interacts with the cellular machinery involved in apoptosis, by providing cytoprotective signals.

#### Example 5

OS cells sensitization to genotoxic and oxidative stress due to CLU gene silencing is related to activation of the cellular apoptotic machinery. By analyzing the



expression levels of other recently identified CLU protein forms, to our surprise, we found that the Cl-I and Cl-II oligonucleotides did not exert any significant effect on the putative 55 kDa n-CLU<sup>35</sup> CLU protein form in both KH OS and U-2 OS cells; a minor effect on the 49 kDa c-CLU<sup>49</sup> protein form level was detected despite the fact that the binding sites of the Cl-I or Cl-II oligonucleotides are common between the s-CLU and n-CLU mRNAs (Leskov et al., J. Biol. Chem. 278: 11590-16000 (2003)). We assume that the explanation of this effect relies on our observation that the n-CLU protein is extremely stable. Thus CL-I and CL-II oligonucleotides specifically knock-down the secreted CLU (s-CLU) protein form.

We then assayed the expression levels of several proteins involved in regulating apoptosis in human cells. CLU knock down in both KH OS and U-2 OS cells resulted in the down-regulation of the anti-apoptotic molecule bcl-2. No effect was detected on levels of Ku70, a protein implicated in DNA damage repair and signaling that, moreover, binds n-CLU (Yang et al., Proc. Nat'l Acad. Sci (USA) 97: 5907-5912 (2000)). Interestingly, in U-2 OS cells, which bear a functional p53 molecule, CLU knock down apart from bcl-2 down-regulation is also accompanied by p53 accumulation and up-regulation of its downstream pro-apoptotic effector, bax. Supportively, CLU knock-down U-2 OS cells when exposed to DXR showed a more intense and robust accumulation of the p53 protein as compared to the Sc-I treated cells. We suggest that sensitization of OS cells following CLU knock down largely depends on the activation of the cellular pro-apoptotic machinery. On-going studies in our laboratories are investigating the implication of CLU on the cell signaling cascades related to apoptosis regulation.

#### Example 6

Effects of CLU gene silencing in PC-3 prostate cancer cells were determined. Having established the significant effects of CLU knock down in OS cells, we then applied CLU siRNA in PC3 human prostate cancer cells. PC3 cells are p53 null (Rohlf, et al., Prostate 37: 51-59 (1998)) and express relatively low endogenous amount of the s-CLU protein form similar to the Sa OS cells. In PC3 cells, apart from employing



the Cl-I and Cl-II oligonucleotides, we also tested two additional CLU-specific siRNA oligonucleotides (Cl-III, Cl-V). From these oligos, Cl-V targeted the s-CLU transcription initiation site. Usage of the Cl-I and Cl-II oligonucleotides in PC3 cells resulted in similar effects to those described for OS cell lines. As it can be seen in Fig. 4 both the  
5 Cl-III and Cl-V oligonucleotides are quite effective in silencing CLU RNA and protein expression in a sequence-dependent but dose-independent manner. More specifically, treatment of the PC3 cells, for one day, with 10, 50 and 100 nM of either Cl-III or Cl-V siRMN oligonucleotides severely reduced CLU mRNA levels ranging from 60% to 98% (Fig. 4). This effect on mRNA levels was also evident at protein level. Additionally and  
10 in agreement with findings in the U-2 OS cells, CLU knock down in PC3 cells resulted in significant morphologic changes that resembled an on-going apoptosis.

To determine whether treatment of PC3 cells with CLU siRNA oligonucleotides could enhance the cytotoxic effects of chemotherapeutic drugs, PC3 cells were treated first with the Cl-III, Cl-V or the Sc-I siRNA oligonucleotides and then  
15 incubated with medium containing various concentrations of Paclitaxel for 2 days. An MTT assay was then performed to determine cell viability. As shown in Fig. 5, CLU siRNA treatment significantly enhanced chemosensitivity of Paclitaxel in a dose-dependent manner reducing the  $IC_{50}$  (the concentration that reduces cell viability by 50%) of Paclitaxel by more than 90%, whereas the scrambled siRNA had no effect.

Table 7: Sequence specific characteristics pf the Cl-I, Cl-II, Cl-III and Cl-V oligonucleotides. The Cl-I, Cl-II, Cl-III and Cl-IV clusterin specific siRNA oligonucleotides have GC/AT ratios of 57/43, 67/33, 24/76 and 43/57, respectively.		
	Cl-I	Cl-II
<i>Targeted region</i>	AAccagagctcgcccttctacTT (SEQ. ID No: 57)	AAgtcccgcacgtccgcagcTT (SEQ. ID No: 60)
Sense siRNA	ccagagcucgcccucucdTdT (SEQ. ID No: 58)	gucccgcaucguccgcagcdTdT (SEQ. ID No: 61)
Antisense siRNA	guagaagggcgagcucuggdTdT (SEQ. ID No: 59)	gcugcggacgaugcgggacdTdT (SEQ. ID No: 62)
	Cl-III	Cl-IV
<i>Targeted region</i>	AActaattcaataaaactgtcTT (SEQ. ID No: 63)	GCatgatgaagactctgtgcTG (SEQ. ID No: 66)
Sense siRNA	cuaauucaauaaaacugucdTdT (SEQ. ID No: 64)	augaugaagacucugcugc (SEQ. ID No: 67)
Antisense siRNA	gacaguuuuauugaauuagdTdT (SEQ. ID No: 65)	gcagcagagucuucaucu (SEQ. ID No: 68)

### Example 7

5                      8 species of siRNA targeting clusterin were formed as double-stranded RNA from Seq. ID NOs. 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, and 15 and 16, and labeled as CLU1-CLU 8, respectively. PC3 cells were transfected with various doses (10, 50 and 100 nM) of the 8 species of siRNA or scrambled control.

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Three days after treatment, proteins were extracted and analyzed by Western blotting for clusterin levels (MW = 40 and 60 kDa). Reduction in the amount of clusterin was observed with all eight species of siRNA, although the best results were obtained with CLU-5 (Seq ID Nos 9 and 10).

5                   Densitometric measurements were performed after normalization to a vinculin control the blots for cells treated with CLU-5 (Seq ID Nos. 9 and 10). The results are summarized in Fig. 6. "Oligo" cells were treated with oligoFECTAMINE™ only. As can be seen, a dose dependent response to the siRNA was observed.

10    Example 8

                  The experiment of Example 7 was repeated using A549 lung cancer cells in place of PC3 prostate cells, and comparable results were observed. Densitometric measurements were performed after normalization to a vinculin control the blots for cells treated with CLU-5 (Seq ID Nos. 9 and 10). The results are summarized in Fig. 7.

15    "Oligo" cells were treated with oligoFECTAMINE™ only. As can be seen, a dose dependent response to the siRNA was observed.

Example 9

                  PC3 cells were transfected with siRNA at levels of 10, 50 or 100 nM or

20    with 100 nM scrambled control. Two days after transfection, total RNA was extracted and the level of clusterin transcript was quantified by Real Time PCR. The results are shown in Fig. 8.

25    Example 10

                  The experiment of Example 9 was repeated using A549 cells in place of PC3 cells. The results are shown in Fig. 9.

30



Example 11

PC3 cells were treated (1 pulse) with CLU-3 (Seq ID NOs. 5 and 6) or CLU-5 (Seq ID Nos 9 and 10) or with a scrambled control at levels of 10, 50 or 100 nM, or with oligoFECTAMINE™ only. After two days total RNA was extracted and  
5 analyzed for clusterin and GADPH by Northern blotting. Densitometric measurement was performed. Fig. 10 presents results of these measurements after normalization to GADPH. Reduction in the amount of clusterin transcript was observed at all doses of CLU-3 and CLU-5 .

10 Example 12

PC3 cells were treated once with 25 nM human clusterin siRNAs (CLU-3 and CLU-5), a scrambled control, or oligoFECTAMINE™ only. After two days of treatment, the medium was replaced with medium containing various concentrations of Taxol (Paclitaxel). After three days of incubation, cell viability was determined by MTT  
15 assay. Fig. 11 summarizes the results. As can be seen, the siRNA and the Taxol worked in synergy to reduce the number of viable cells.

Example 13

The experiment of Example 12 was repeated with A549 cells in place of  
20 PC3 cells. The results are summarized in Fig. 12

Example 14

OVCAR3 ovarian cancer cells were treated once with 1, 10 or 50 nM CLU5, scrambled control or a vehicle only control. An untreated control was also run.  
25 After two days, total RNA was extracted and analyzed for clusterin and GAPDH mRNA by Northern blot. Densitometric measurements of clusterin mRNA levels after normalization to GAPDH mRNA are shown in Fig. 13. Substantial dose dependent reduction in the amount of clusterin transcript was observed.

Example 15

MDA-MB 231 human breast cancer cells were transfected with 5, 50 or 100 nM CLU-5 or a scrambled control, or with oligoFECTAMINE™ vehicle alone. Two days after transfection, RNA was extracted and analyzed for clusterin and GAPDH by Northern blotting. Densitometric measurements of clusterin mRNA levels after normalization to GAPDH mRNA are shown in Fig. 14. Substantial reduction in the amount of clusterin transcript was observed.

Example 16

The experiment of Example 15 was repeated, except that clusterin transcript was quantified using RT-PCR. The results are summarized in Fig. 15. Substantial reduction in the amount of clusterin transcript was observed.

Example 17

MCF-7 human breast cancer cells were transfected with 5, 25 or 50 nM CLU-5 or a scrambled control, or with oligoFECTAMINE™ vehicle alone. Two days after transfection, RNA was extracted and analyzed for clusterin and GAPDH by Northern blotting. Densitometric measurements of clusterin mRNA levels after normalization to GAPDH mRNA are shown in Fig. 16. Substantial dose-dependent reduction in the amount of clusterin transcript was observed.

Example 18

The experiment of Example 17 was repeated, except that clusterin transcript was quantified using RT-PCR. The results are summarized in Fig. 17. Substantial reduction in the amount of clusterin transcript was observed.

Example 19

MCF-7 cells were transfected with various doses (5 and 50 nM) of CLU-5 siRNA or scrambled control. Three days after treatment, proteins were extracted and



analyzed by Western blotting for clusterin levels (MW = 40 and 60 kDa). Fig. 18 shows the reduction in the amount of clusterin protein in cells treated with the siRNA relative to the scrambled control.

5     Example 20

Clusterin overexpressing LNCaP/T1 cells were transfected (1 pulse) with 10 nM CLU-5 siRNA or scrambled control. Three days after treatment, the proteins were extracted and analyzed by Western blotting for clusterin. No clusterin was detected in the cells treated with the siRNA.

10

Example 21

3 species of siRNA targeting IGFBP-2 and -5 were formed as double-stranded RNA from Seq. ID NOs. 39 and 40, 41 and 42, and 43 and 44 and labeled as BS-1-BS-3, respectively. A549 cells were transfected with various doses (10, 50 and 100 nM) of the 3 species of siRNA or scrambled control. A vehicle only control and an untreated control were also evaluated. Total RNA was extracted and analyzed by RT-PCR for IGFBP-2 transcript. As shown in Fig. 19, reduction in the amount of IGFBP-2 transcript was observed with all three species of siRNA.

15

20     Example 22

PC3 cells were transfected with various doses (10, 50 and 100 nM) of the 3 species of bispecies siRNA (BS-1, BS-2 and BS-3) or scrambled control. A vehicle only control was also evaluated. Total RNA was extracted and analyzed by RT-PCR for IGFBP-5 transcript. As shown in Figs. 20 and 21, reduction in the amount of IGFBP-5 transcript was observed with all three species of siRNA.

25

Example 23

Primary human bone fiborblasts were transfected with 50 nM of the 3 species of bispecific siRNA (BS-1, BS-2 and BS-3) or scrambled control. A vehicle only and an untreated control were was also evaluated. Total RNA was extracted and analyzed

30

by RT-PCR for IGFBP-5 transcript. As shown in Figs. 22, reduction in the amount of IGFBP-5 transcript was observed with all three species of siRNA.

#### Example 24

5 C42 cells (a sub-line of LNCaP prostate cancer cells) were treated with BS-1, BS-2, and BS-3, and growth inhibition was assessed using a crystal violet assay. The results are shown in Fig. 23.

#### Example 25

10 A549 lung cancer cells were treated with BS-1, BS-2, and BS-3, and growth inhibition was assessed using a crystal violet assay. The results are shown in Fig. 24.



## SEQUENCE LISTING

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JANSEN, Burkhard  
GLEAVE, Martin  
SIGNAEVSKY, Maxim  
BERALDI, Eliana  
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## CLAIMS

1. An RNA molecule having a sequence effective to mediate degradation or block translation of mRNA encoding clusterin when used as an RNA duplex, characterized in that the RNA molecule consists of a sequence or a duplex formed from a complementary pair of sequences selected from among Seq. ID Nos. 1, 2, 5, 6, 9, 10, 61 and 62.
2. The RNA molecule of claim 1, consisting of Seq ID No. 1, Seq ID No. 2 or a duplex RNA formed from Seq ID Nos. 1 and 2.
3. The RNA molecule of claim 1, consisting of Seq ID No. 5, Seq ID No. 6 or a duplex RNA formed from Seq ID Nos. 5 and 6.
4. The RNA molecule of claim 1, consisting of Seq ID No. 9, Seq ID No. 10 or a duplex RNA formed from Seq ID Nos. 9 and 10.
5. The RNA molecule of claim 1, consisting of Seq ID No. 61, Seq ID No. 62 or a duplex RNA formed from Seq ID Nos. 61 and 62.
6. A pharmaceutical composition comprising an RNA molecule as defined in any one of claims 1 to 5 and a pharmaceutically acceptable carrier for mediating degradation or blocking translation of mRNA encoding clusterin.
7. The pharmaceutical composition of claim 6, wherein the pharmaceutically acceptable carrier is a sterile injectable solution.
8. An RNA molecule as defined in any one of claims 1 to 5 for use in mediating degradation or blocking translation of mRNA encoding clusterin.
9. Use of an RNA molecule as defined in any one of claims 1 to 5 for use in mediating degradation or blocking translation of mRNA encoding clusterin.

10. Use of an RNA molecule as defined in any one of claims 1 to 5, or a pharmaceutical composition as defined in claim 6 or 7, for the treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

11. An RNA molecule as defined in any one of claims 1 to 5, or a pharmaceutical composition as defined in claim 6 or 7, for use in the treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

12. Use of an RNA molecule as defined in any one of claims 1 to 5 in manufacture of a medicament for treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

13. An RNA molecule as defined in any one of claims 1 to 5 for use in manufacture of a medicament for treatment of Alzheimer's disease or a cancer, wherein the cancer is prostate cancer, a sarcoma, osteosarcoma, renal cell carcinoma, breast cancer, bladder cancer, lung cancer, melanoma, colon cancer, ovarian cancer or anaplastic large cell lung cancer.

14. The use of claim 10 or 12, wherein the cancer is osteosarcoma, prostate cancer, lung cancer, ovarian cancer or breast cancer.

15. The RNA molecule of claim 11 or 13, wherein the cancer is osteosarcoma, prostate cancer, lung cancer, ovarian cancer or breast cancer.



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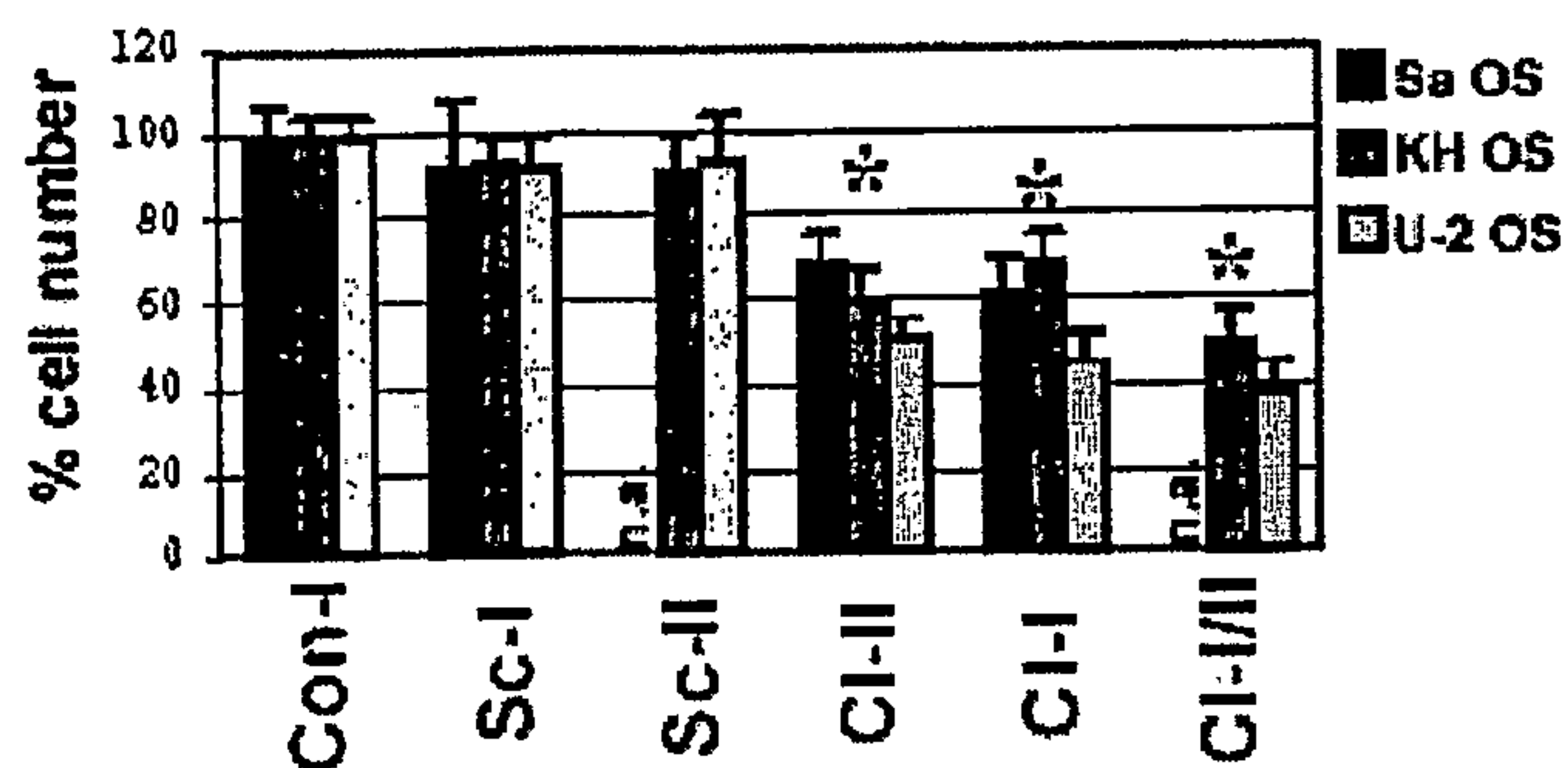


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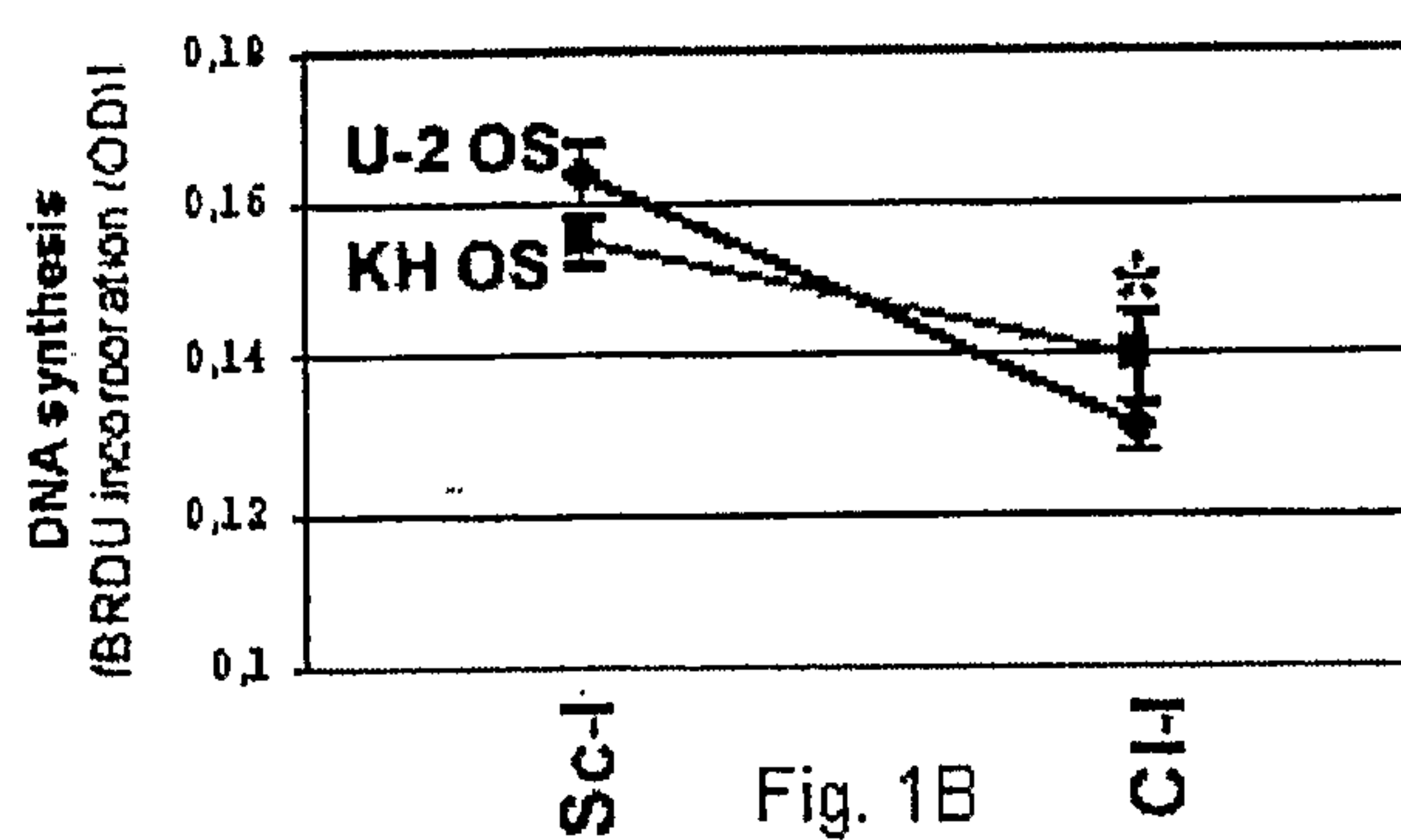


Fig. 1B

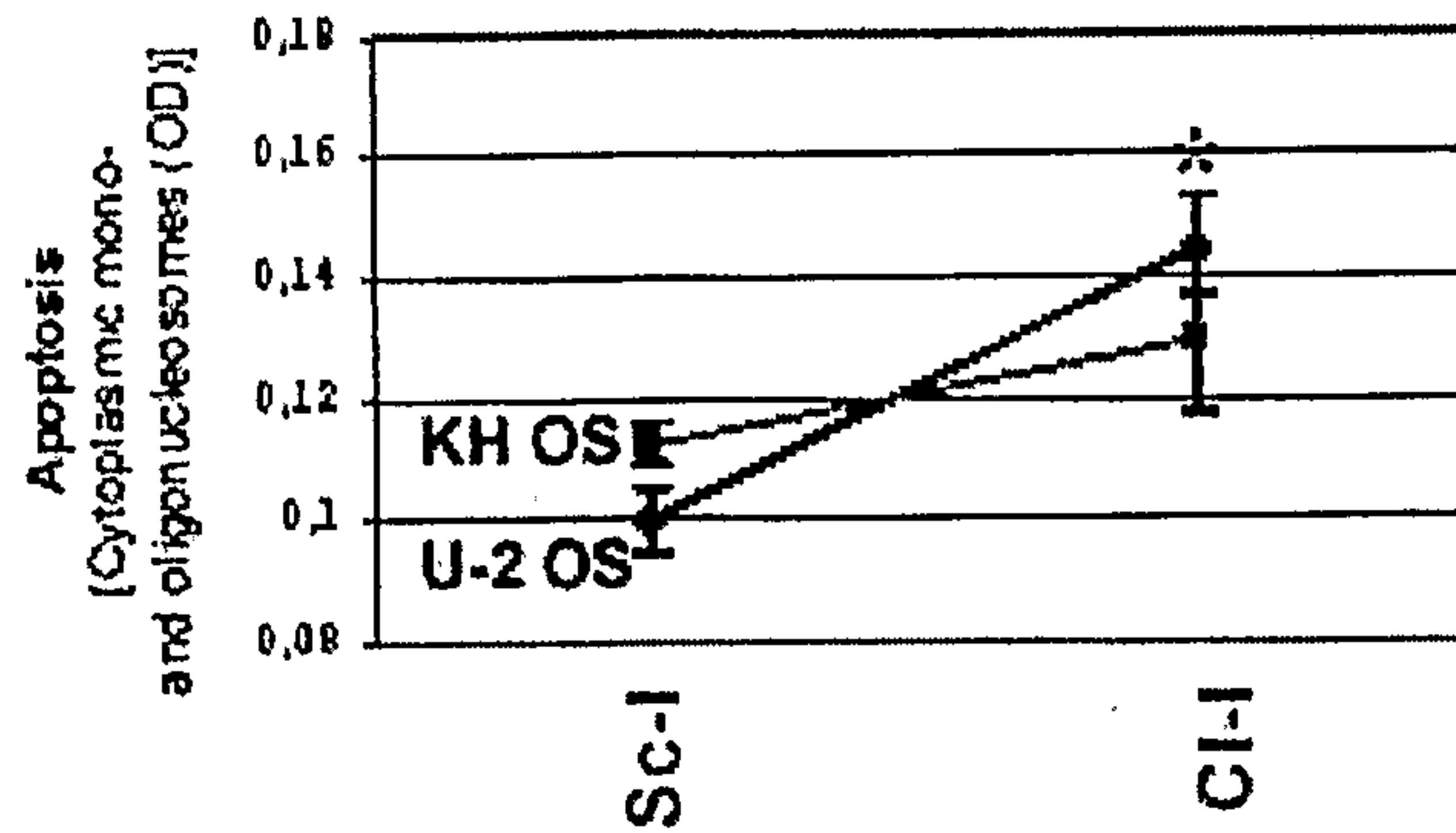


Fig. 1C

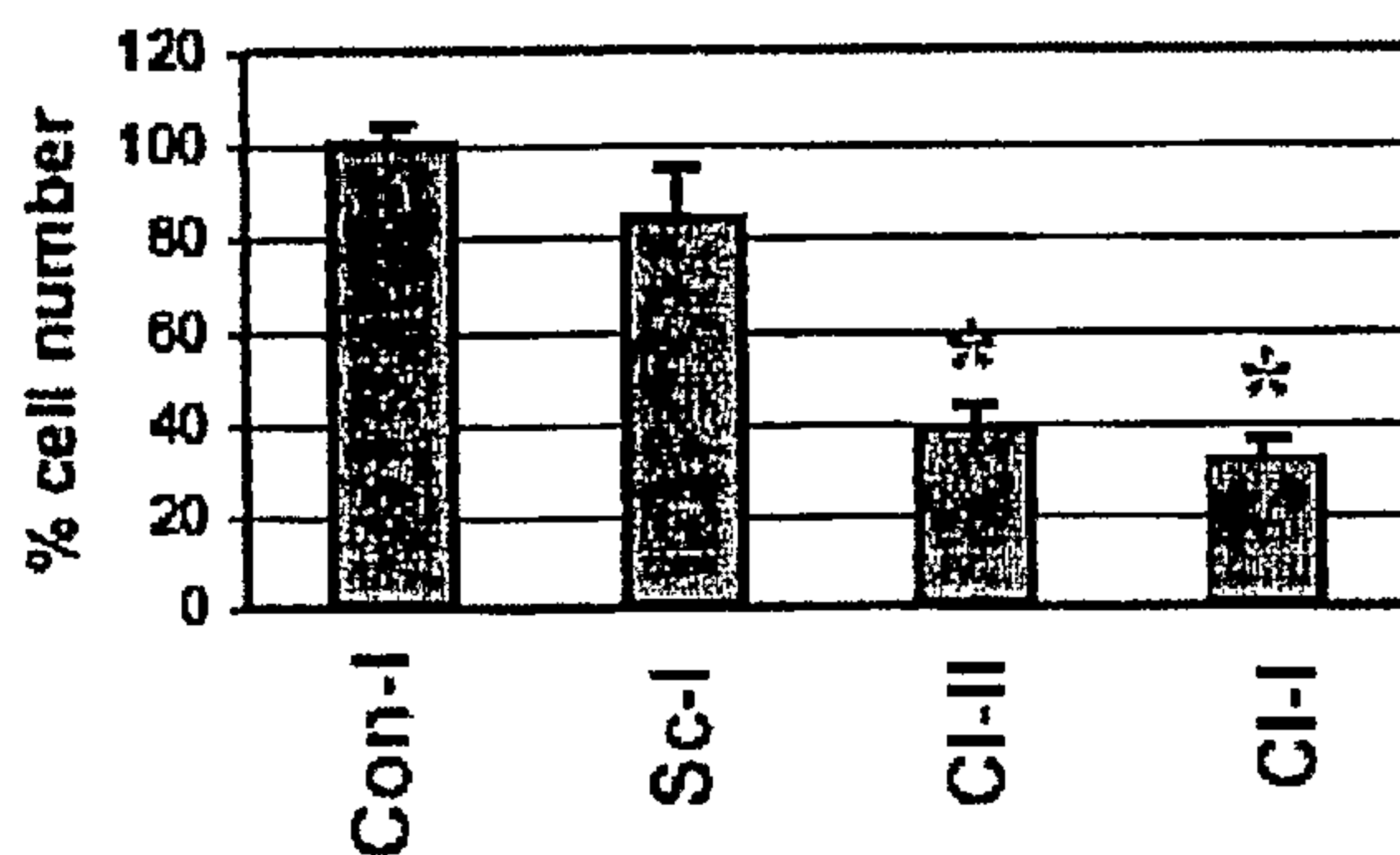


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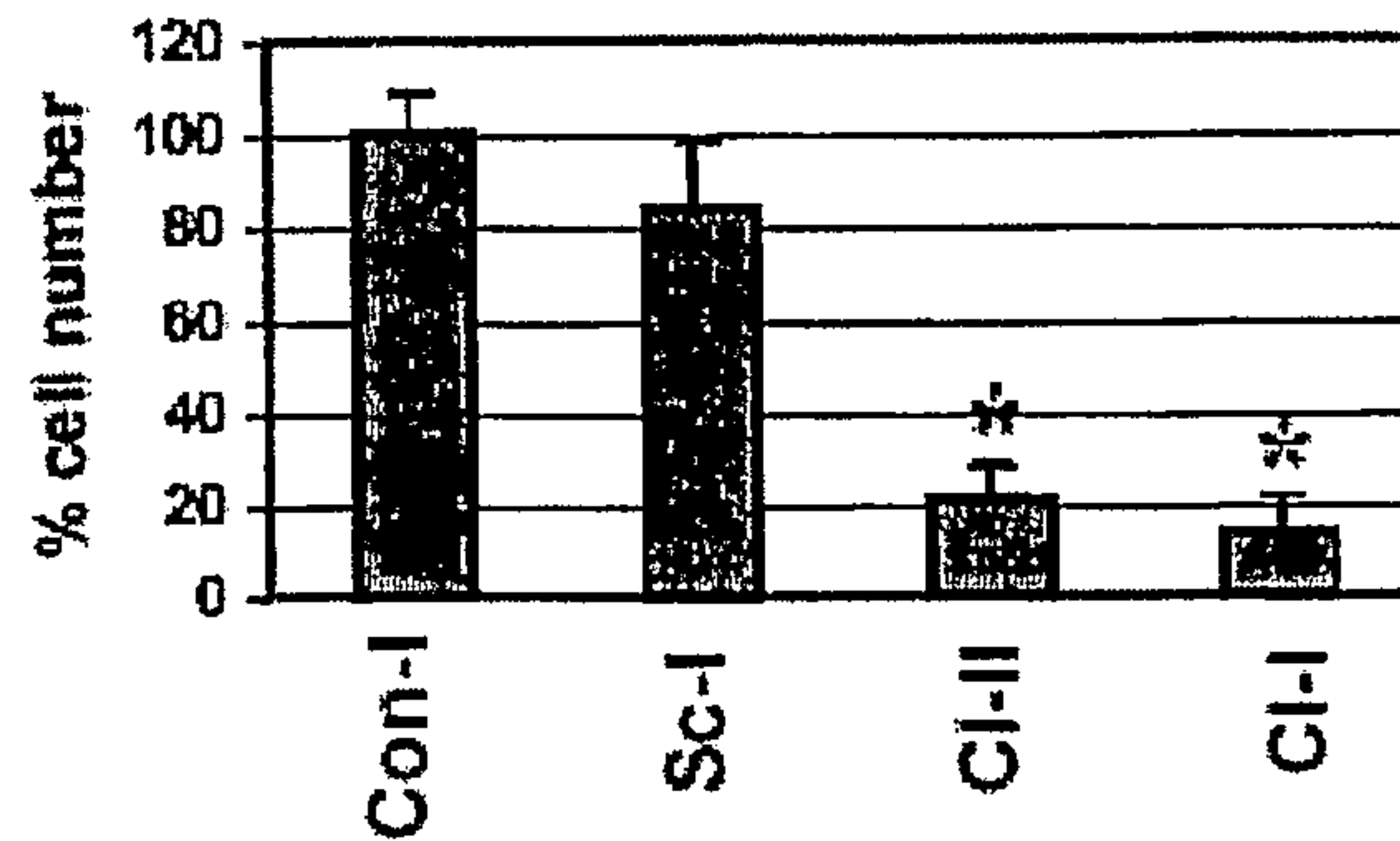


Fig. 2B

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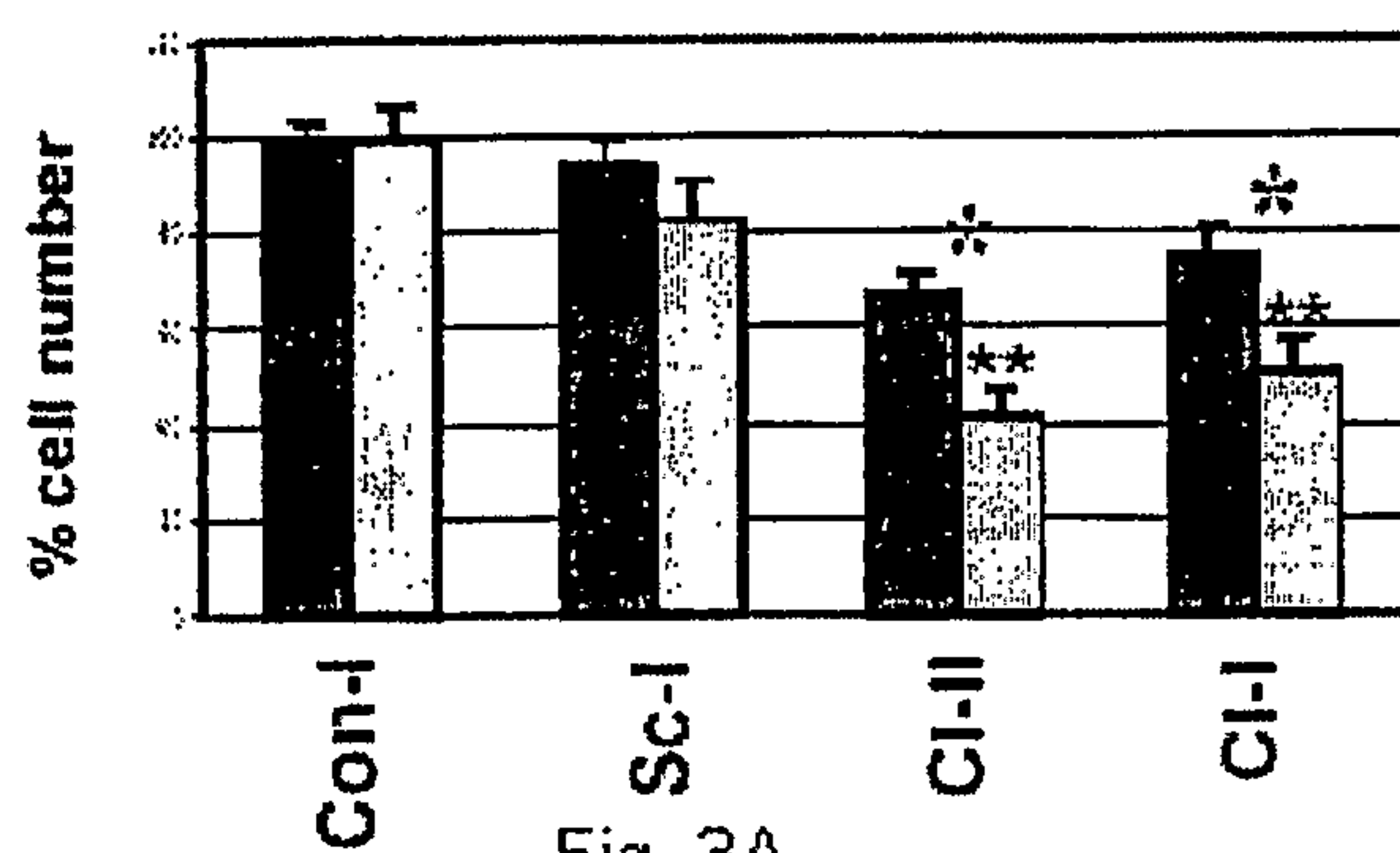


Fig. 3A

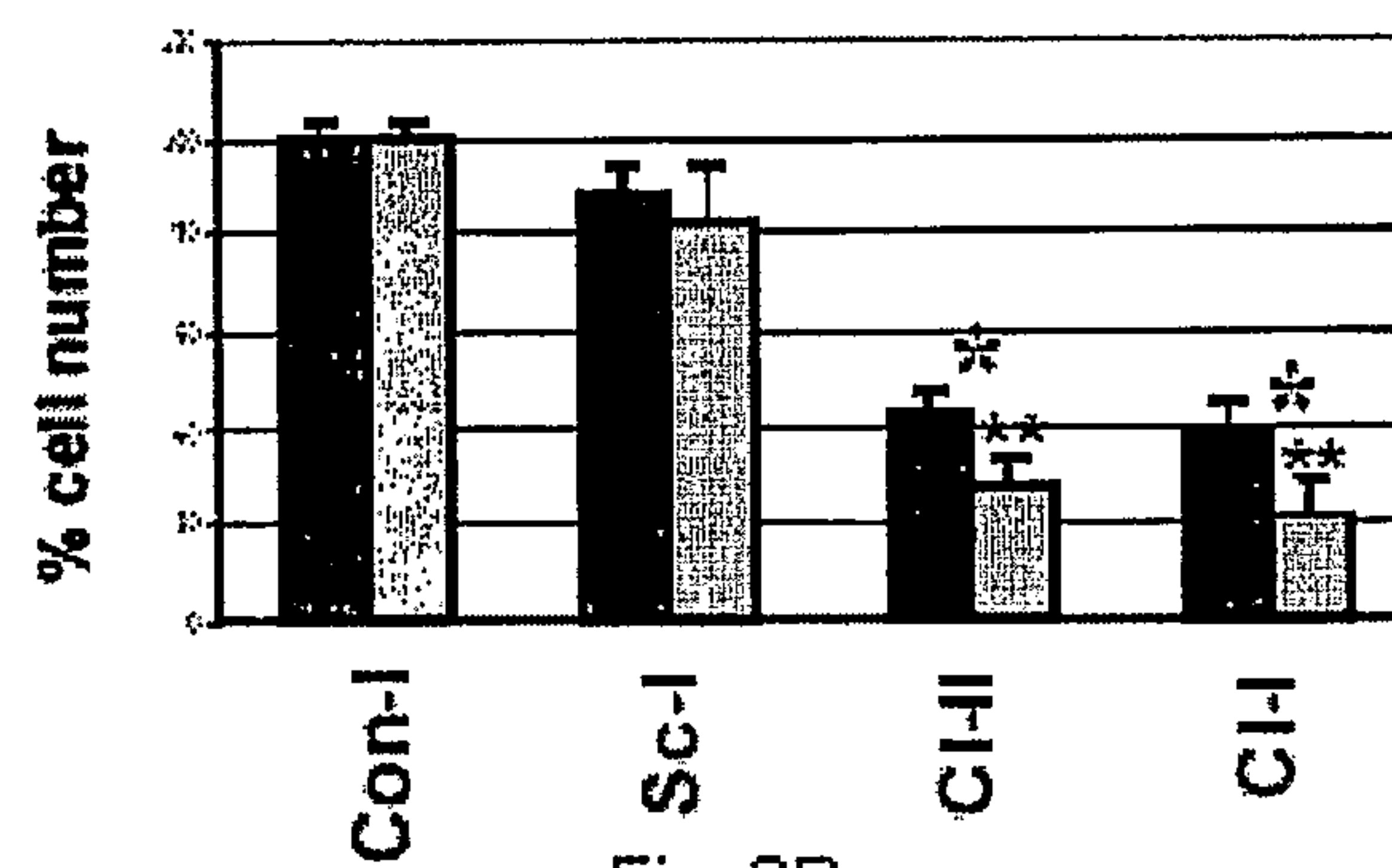


Fig. 3B

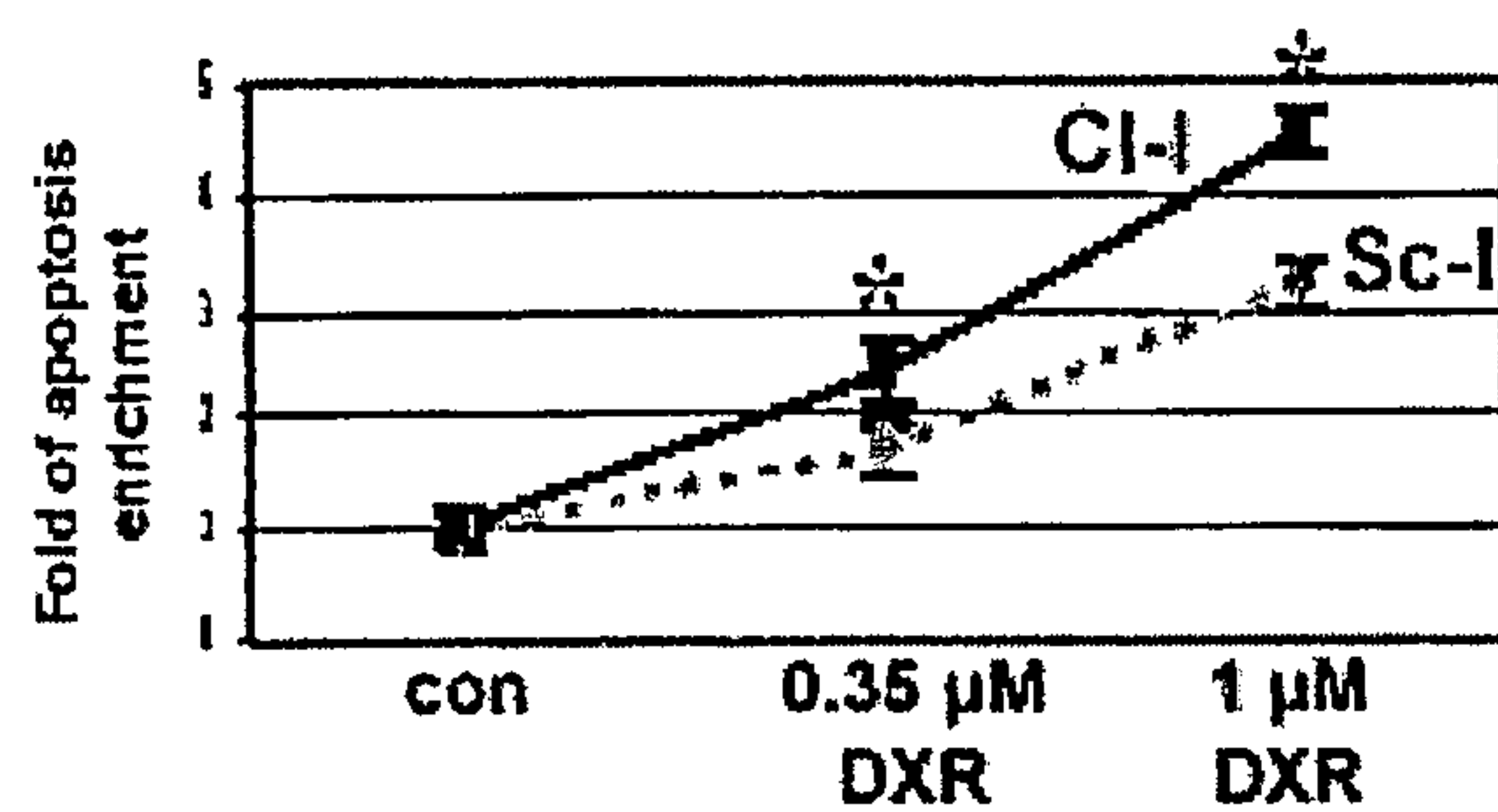


Fig. 3C

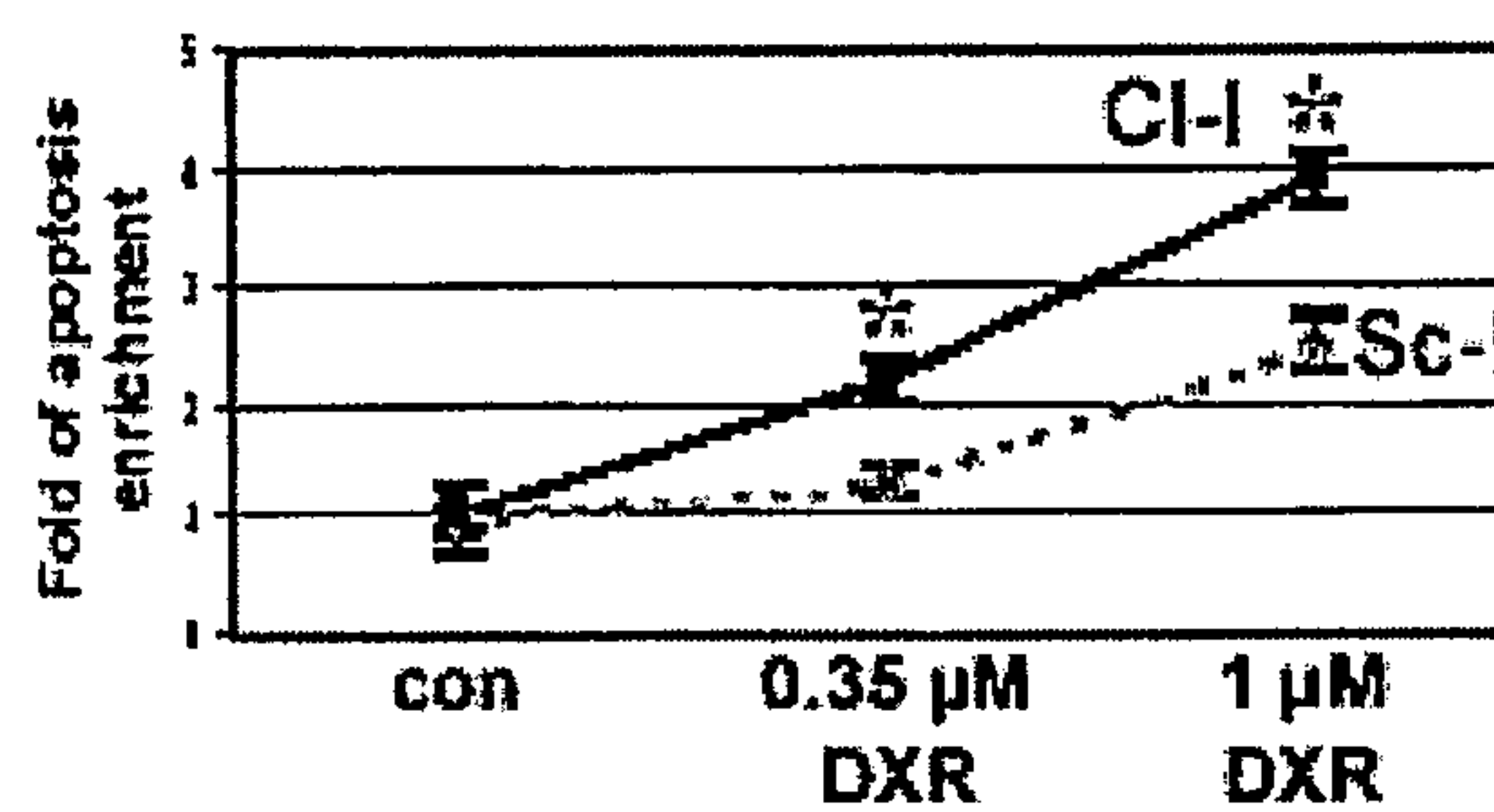


Fig. 3D

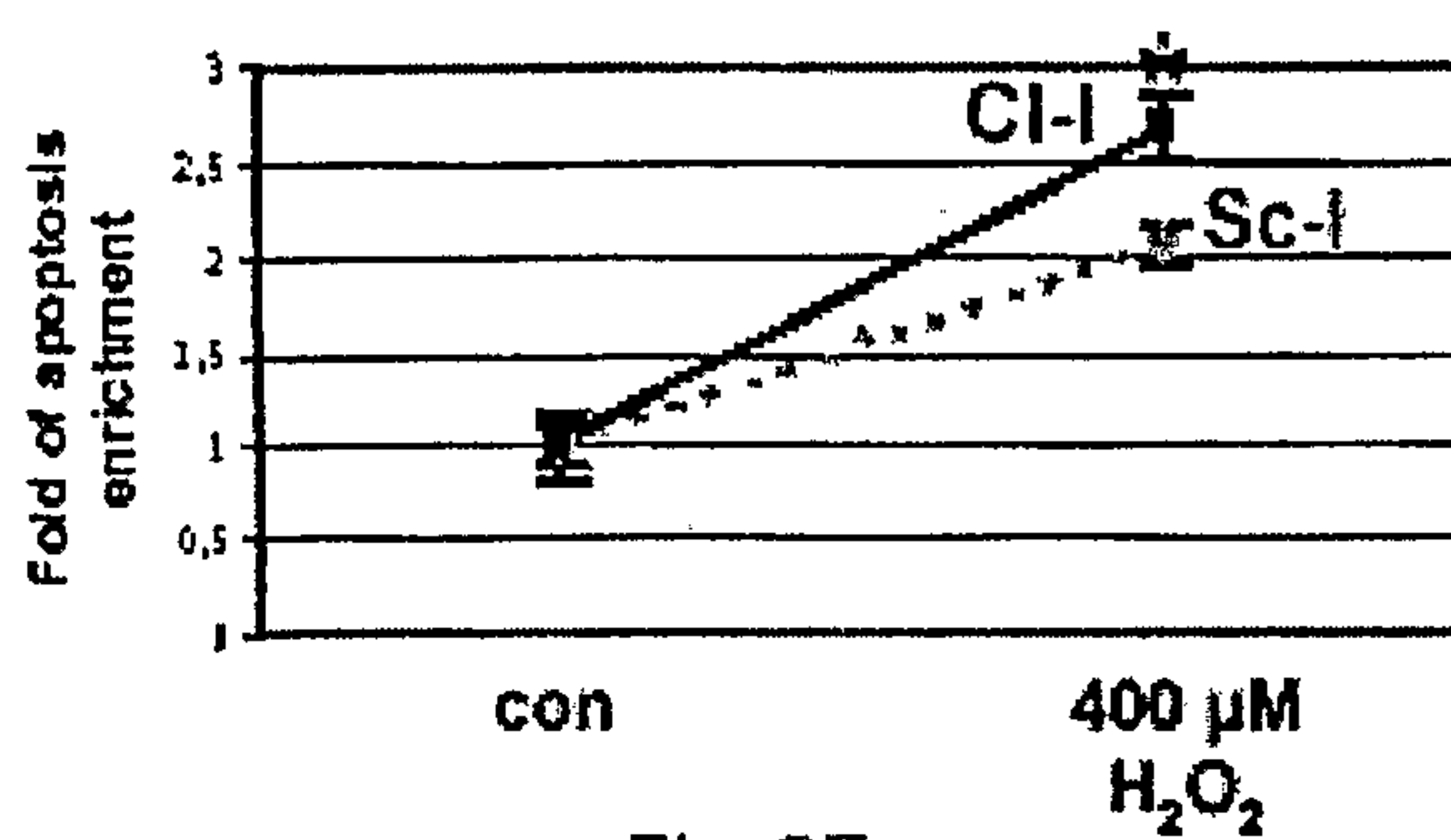


Fig. 3E

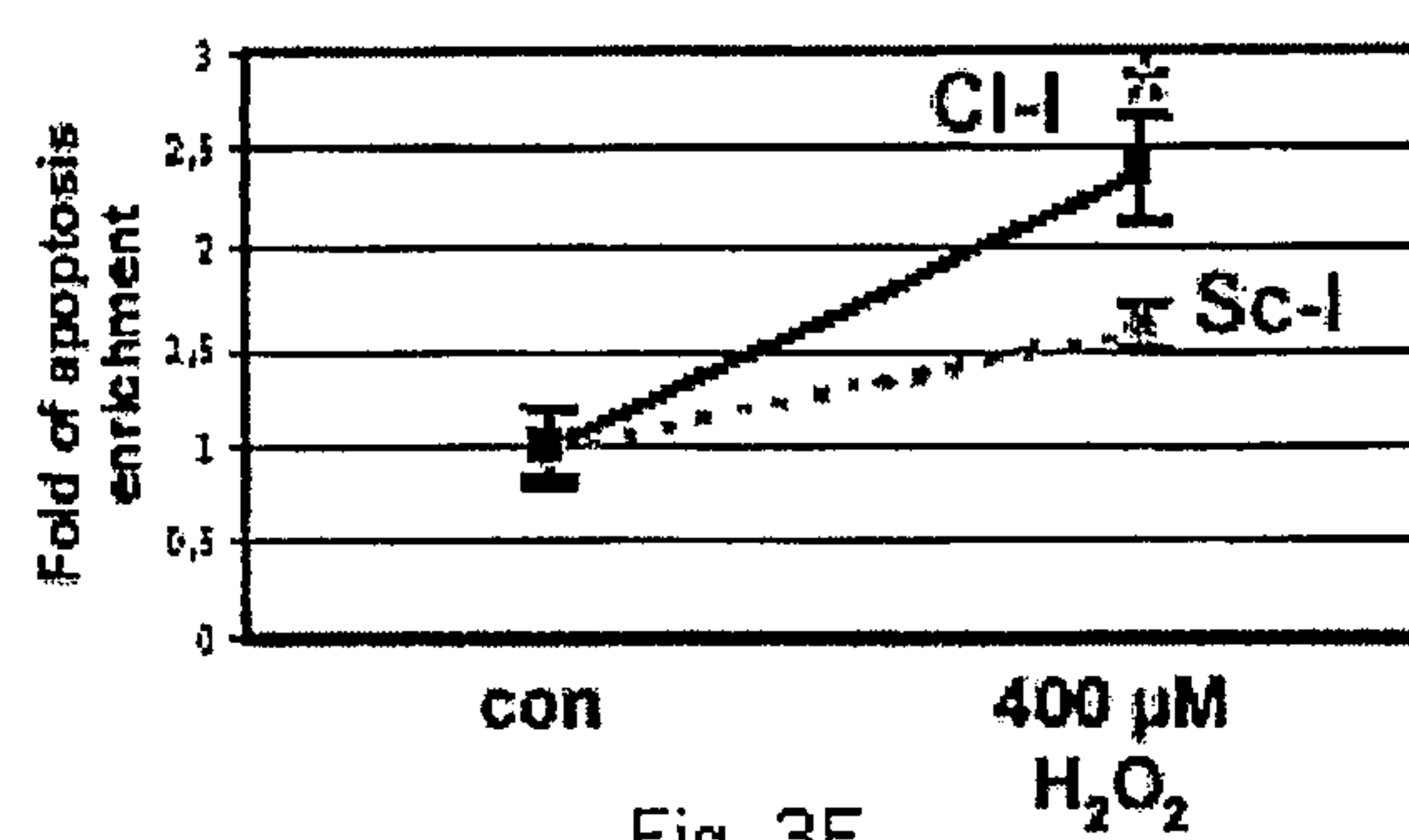


Fig. 3F

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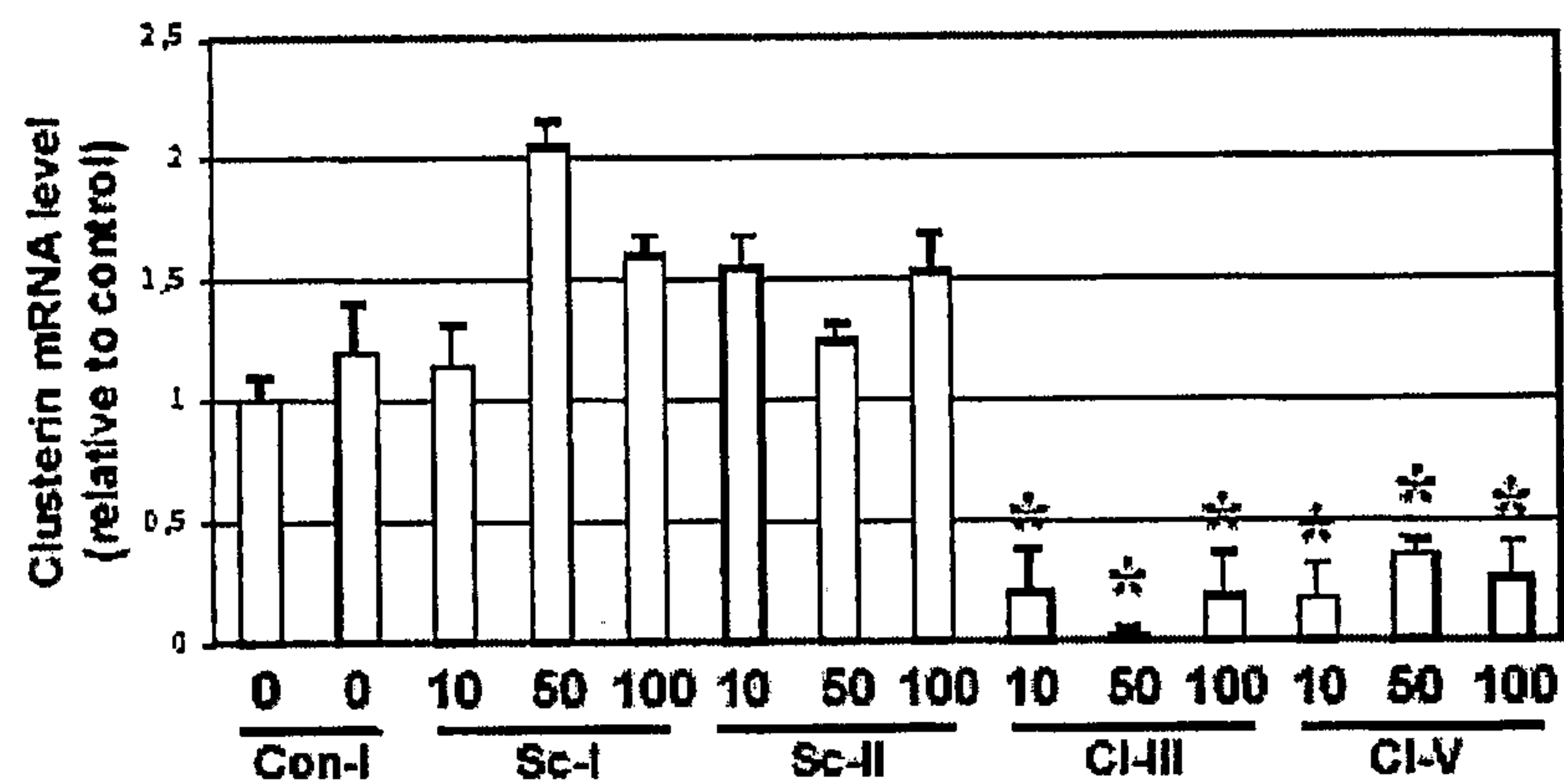


Fig. 4

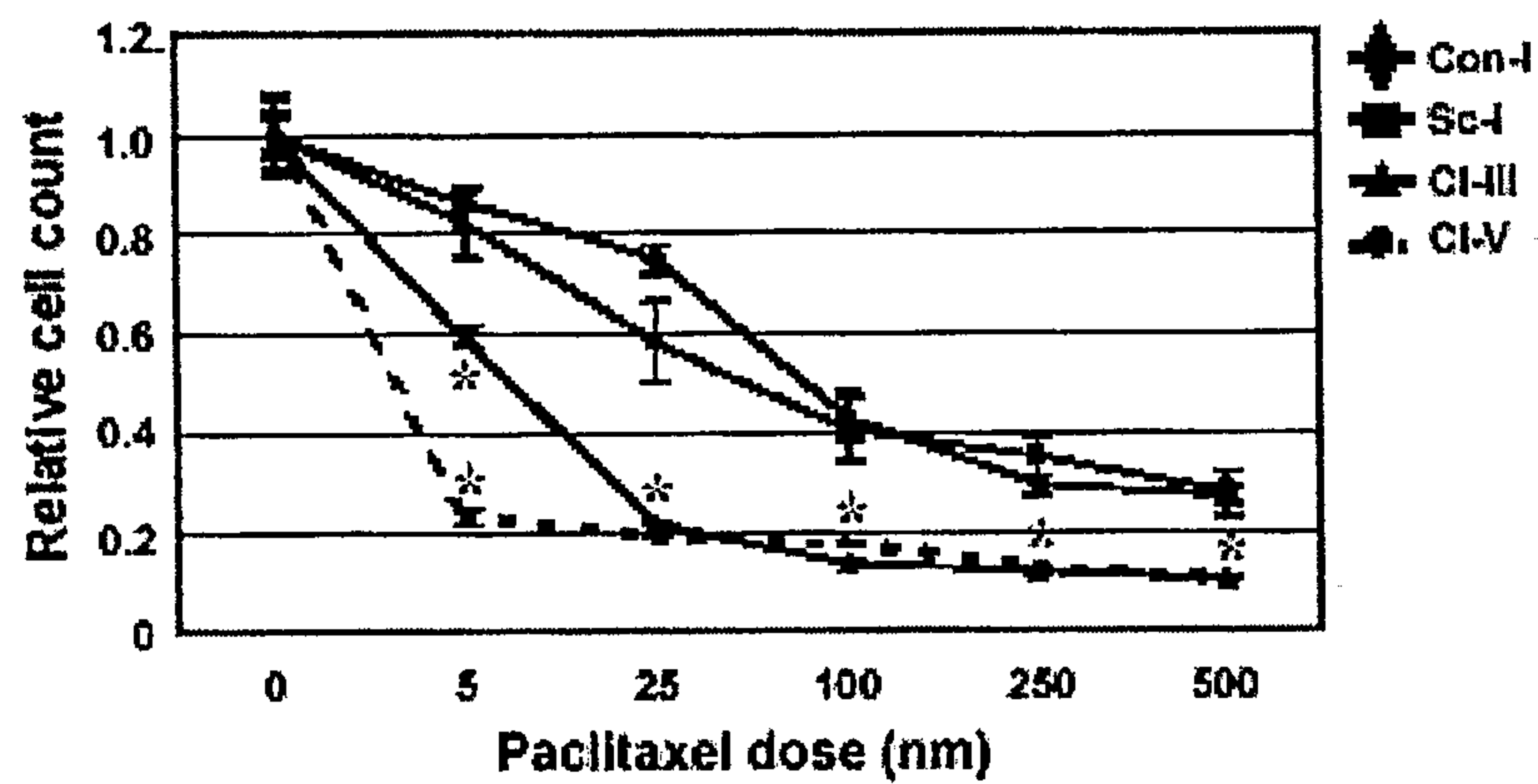


Fig. 5



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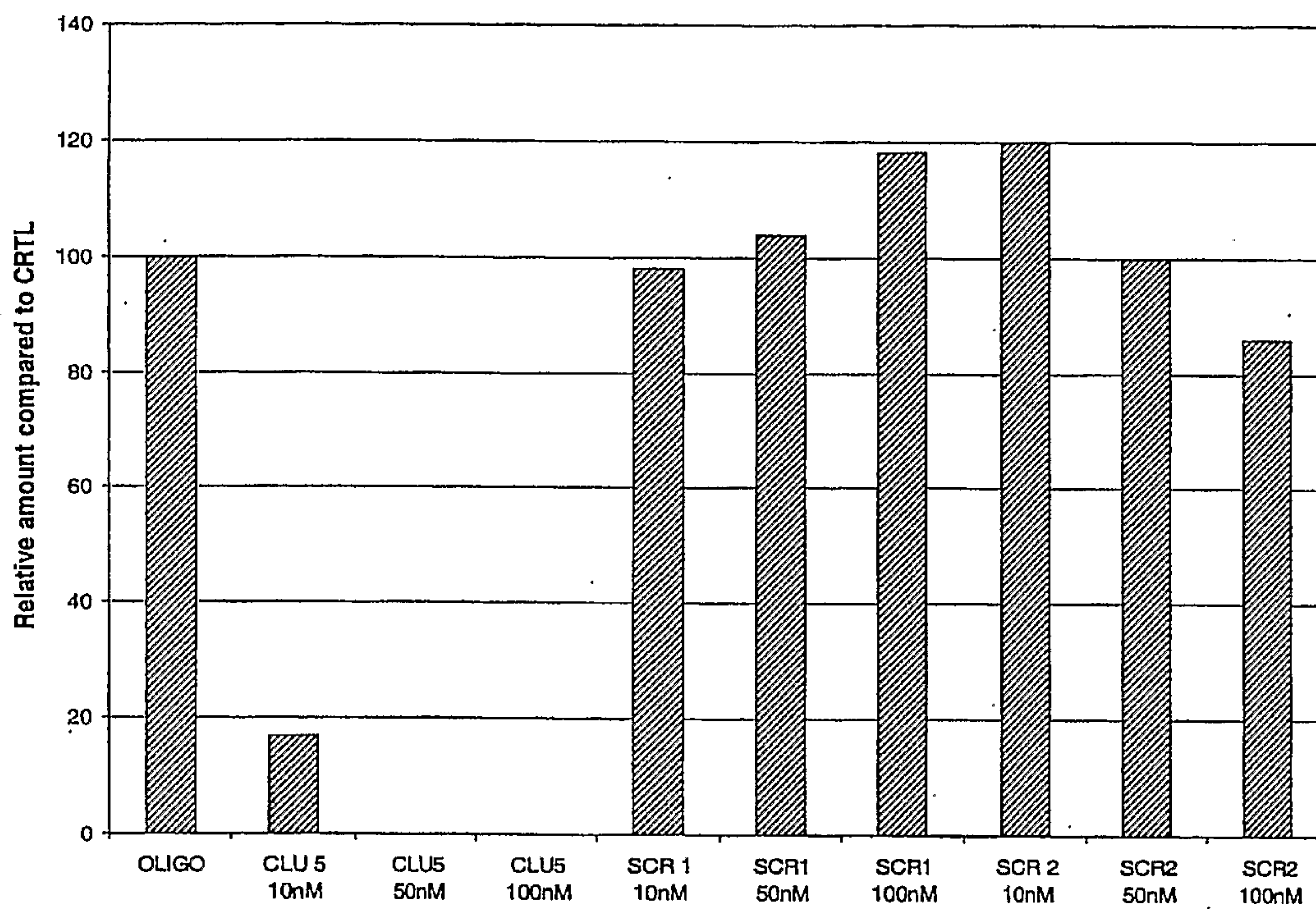


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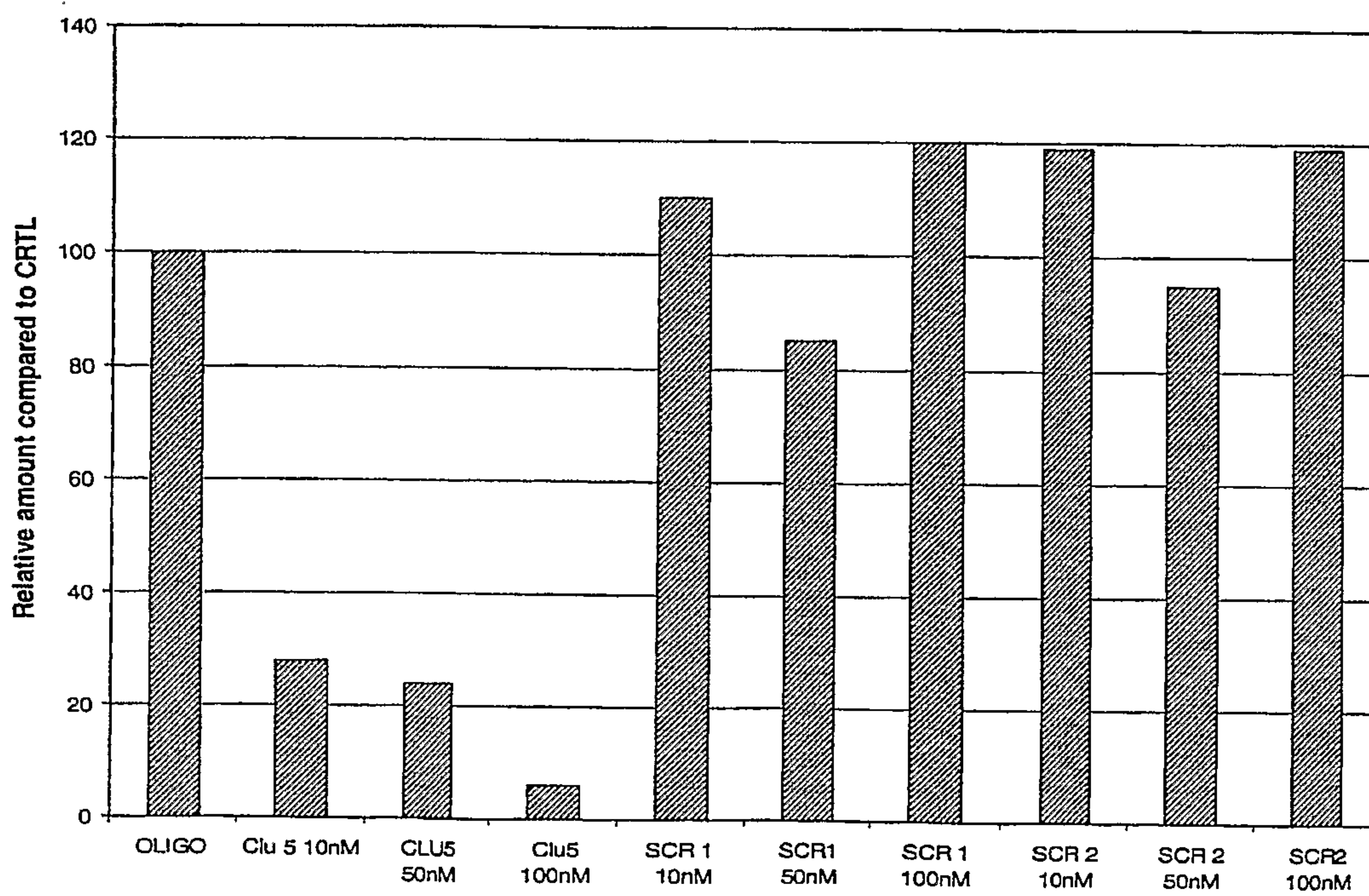


Fig. 7

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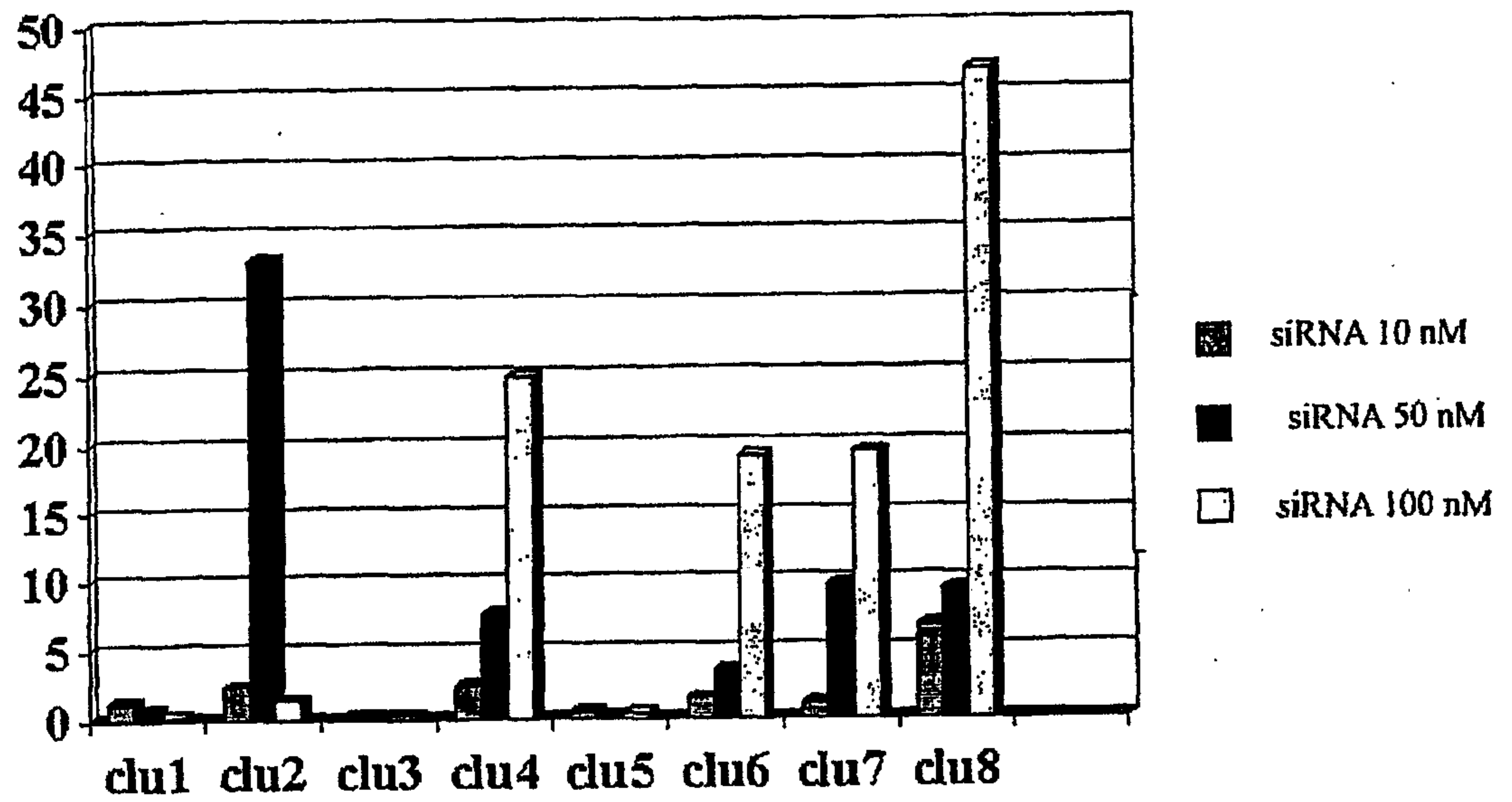


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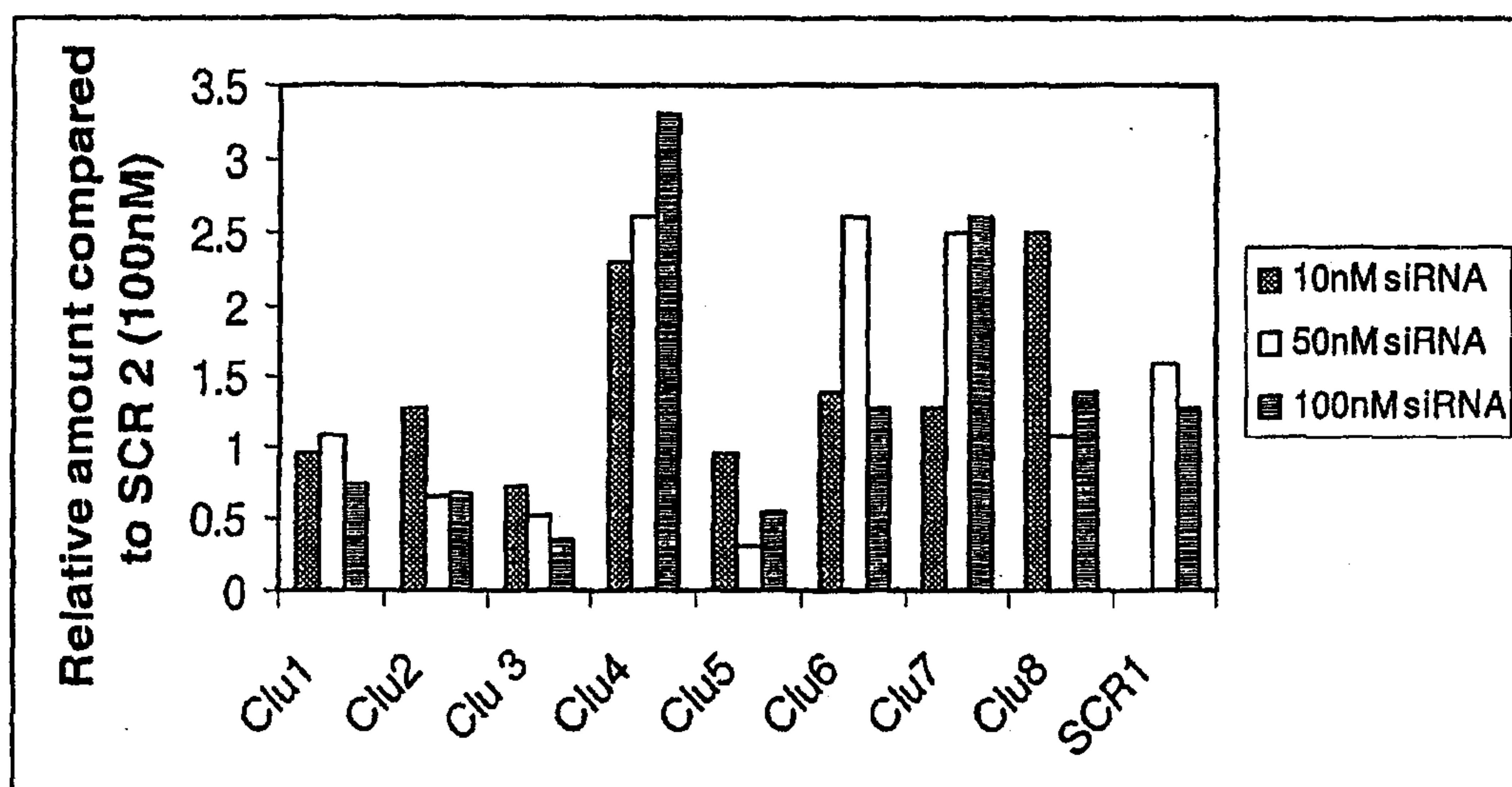


Fig. 9

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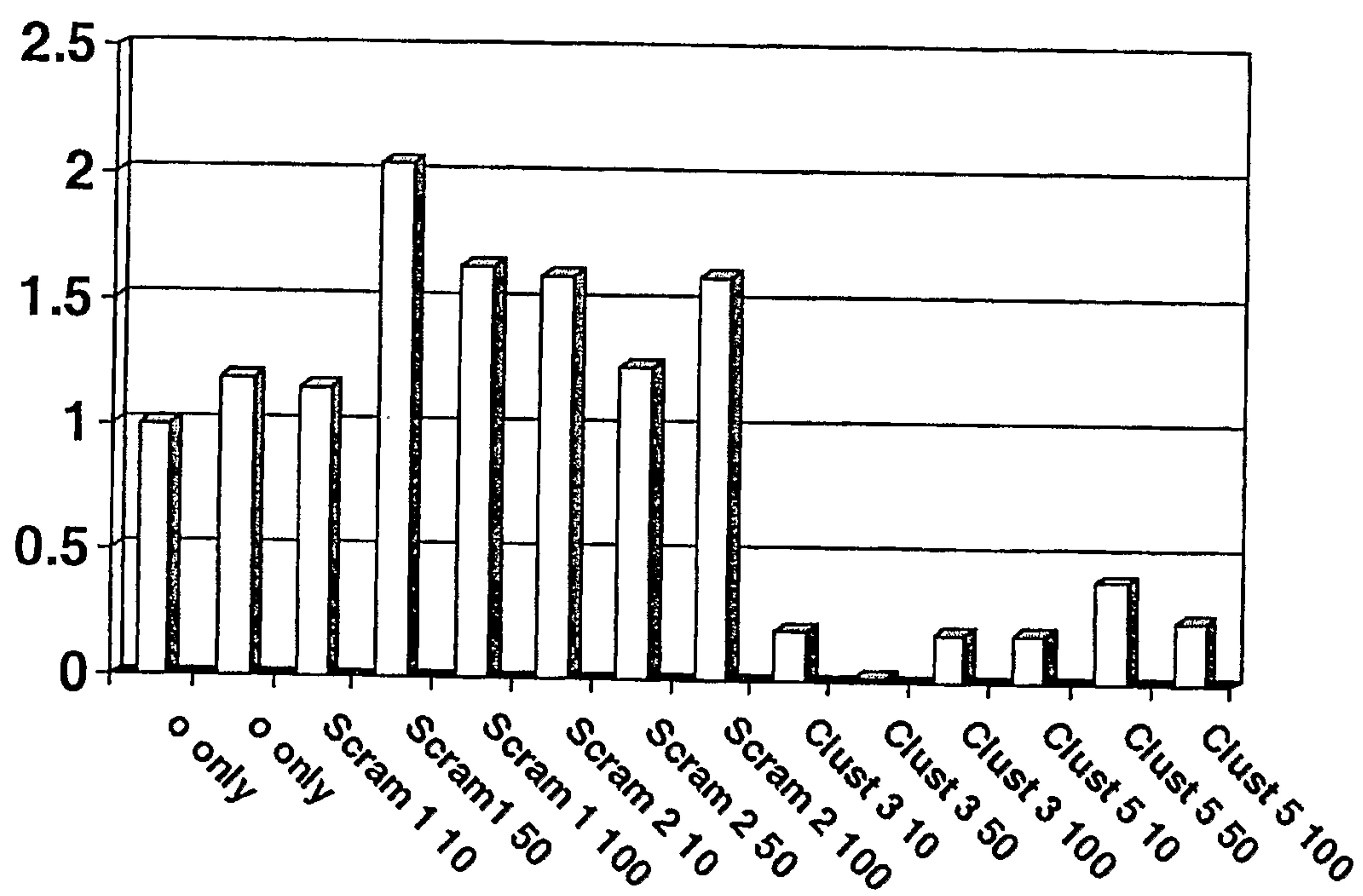


Fig. 10



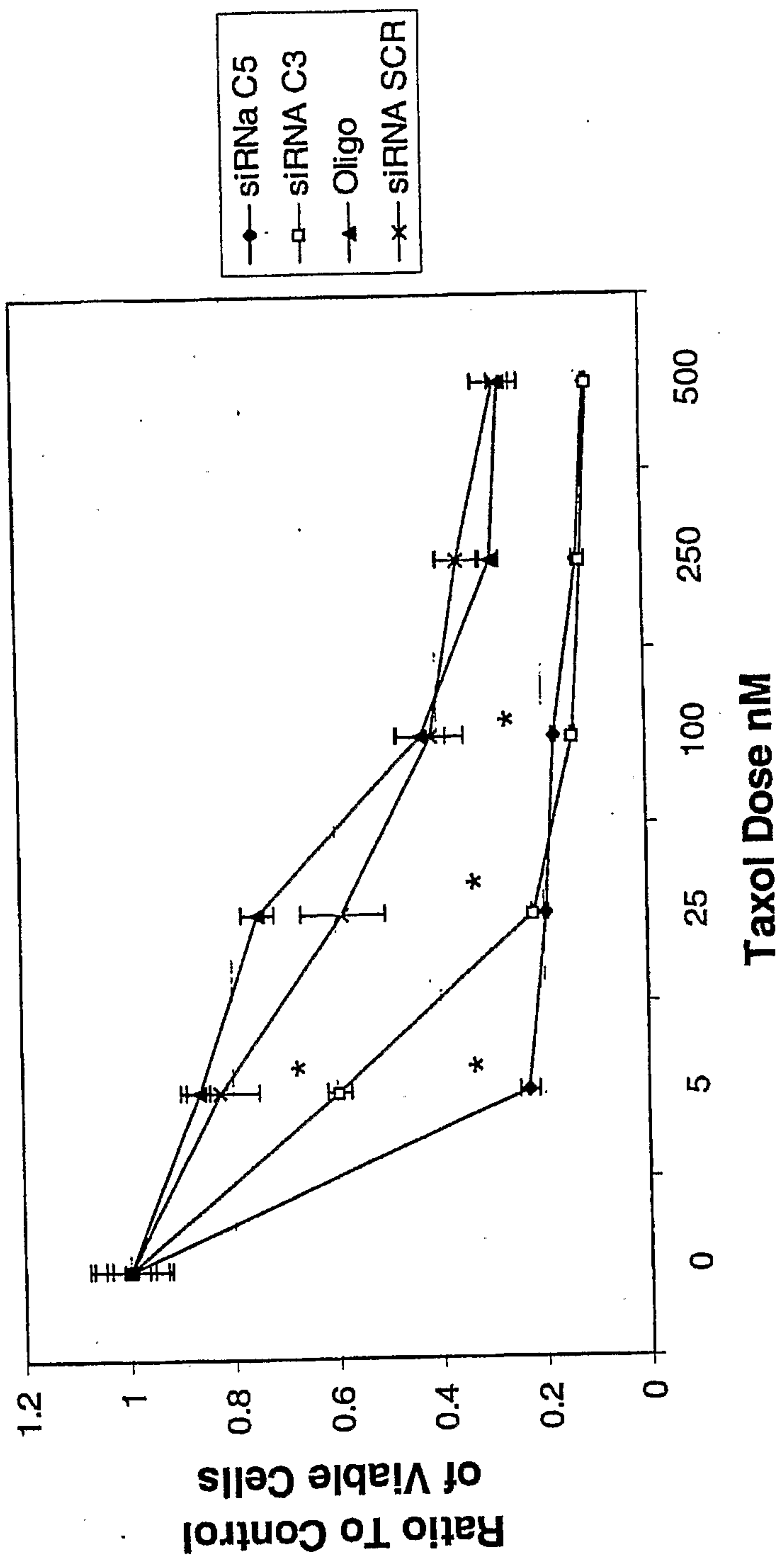


Fig. 11

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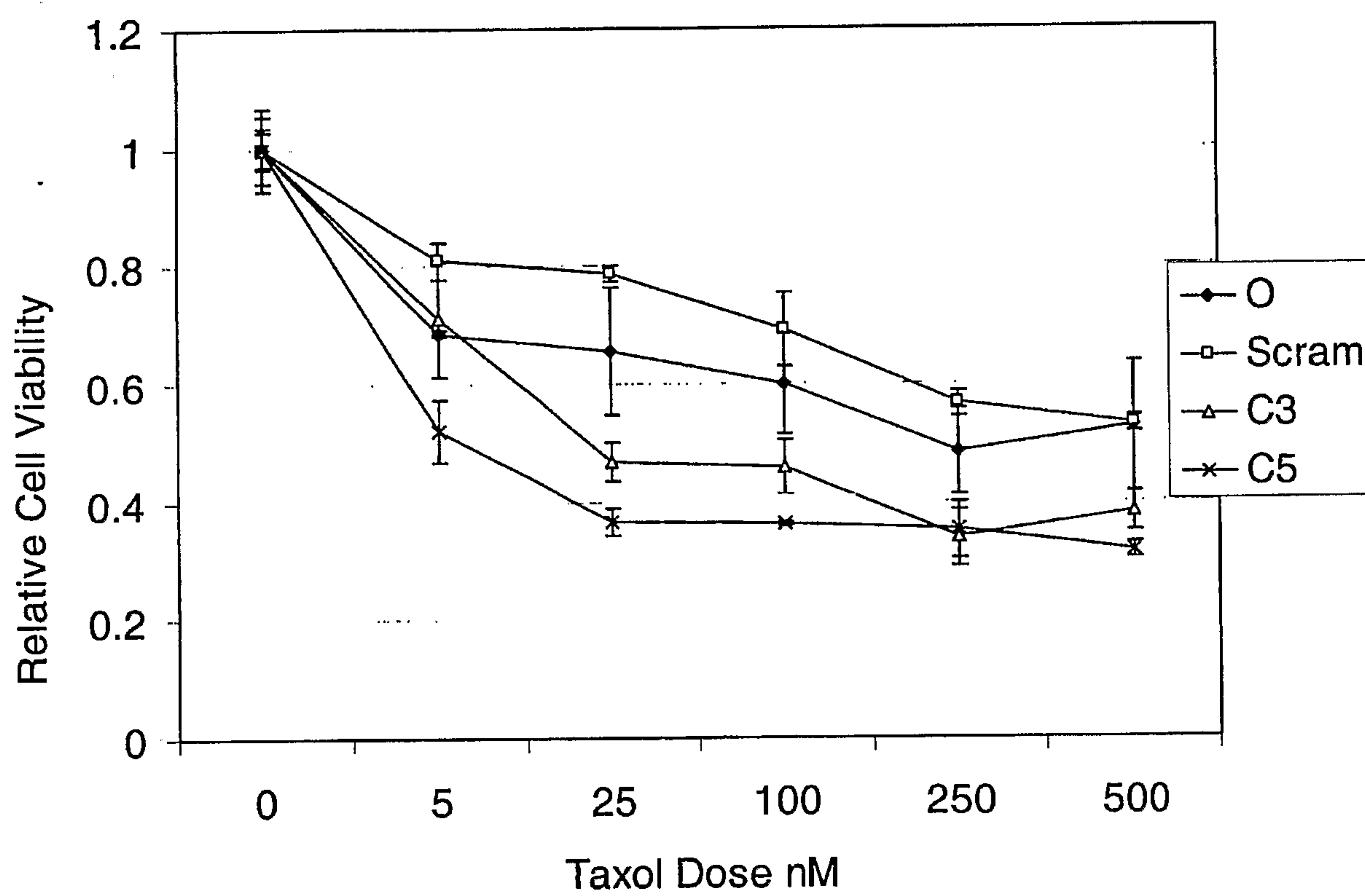


Fig. 12

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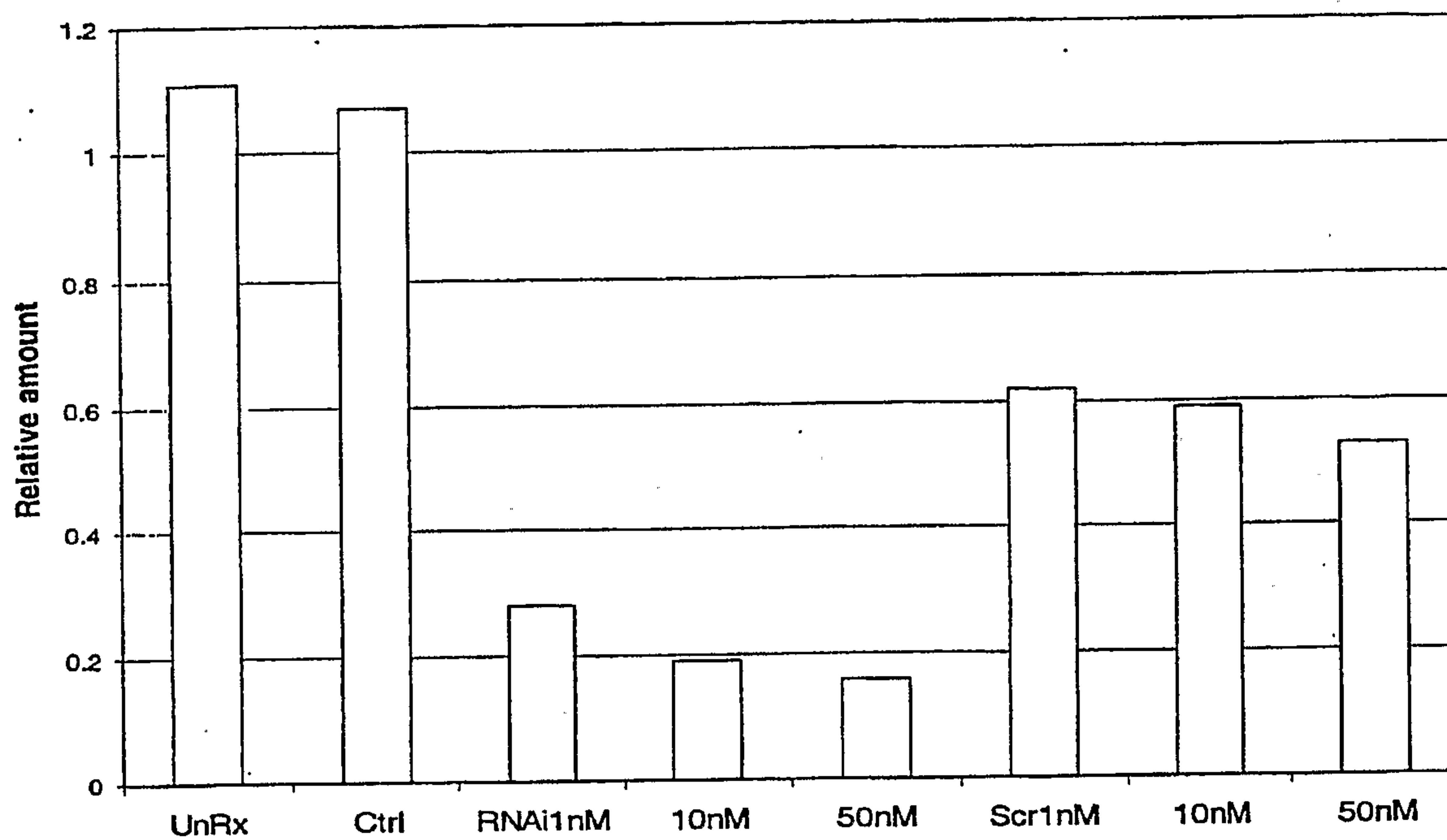


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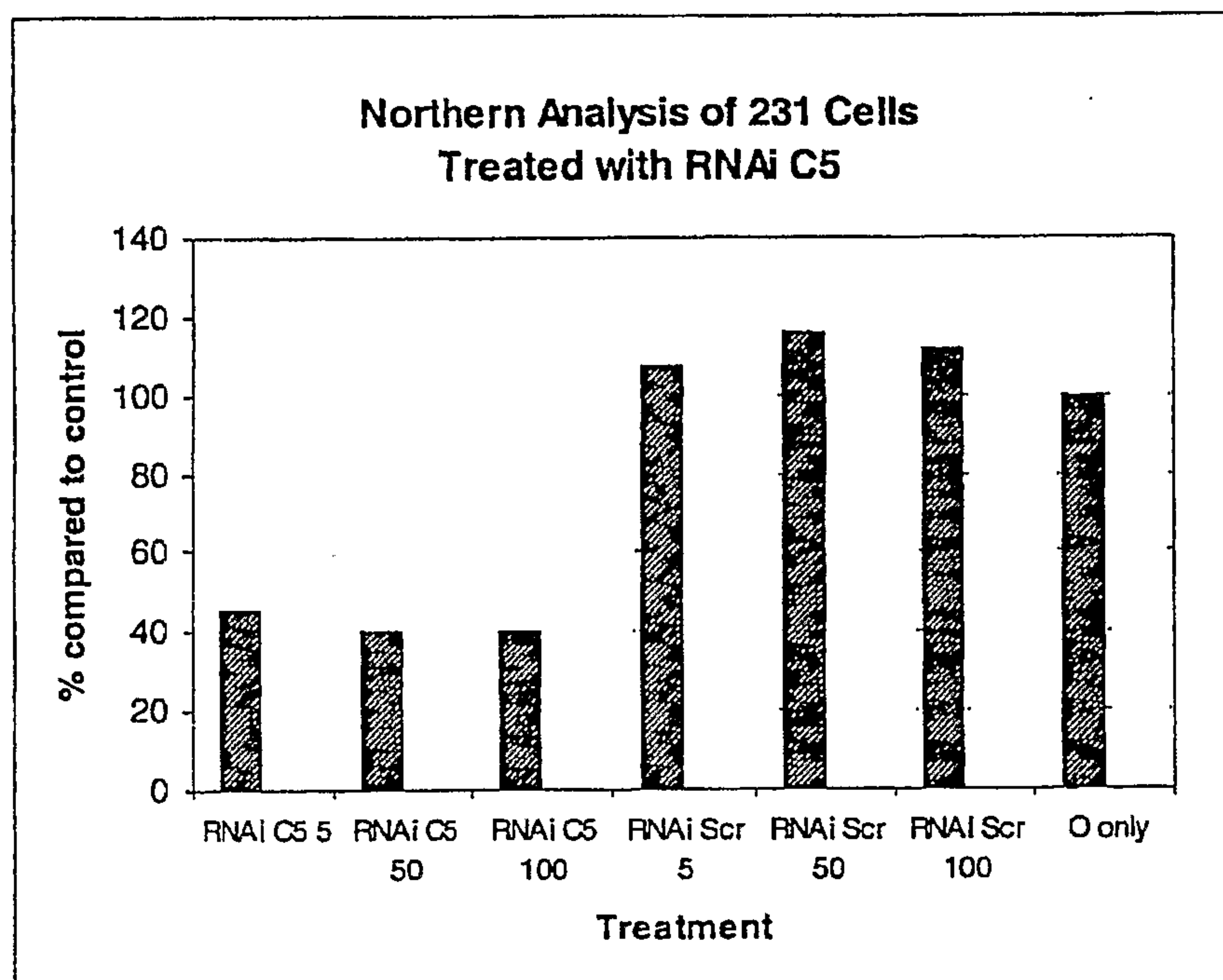


Fig. 14



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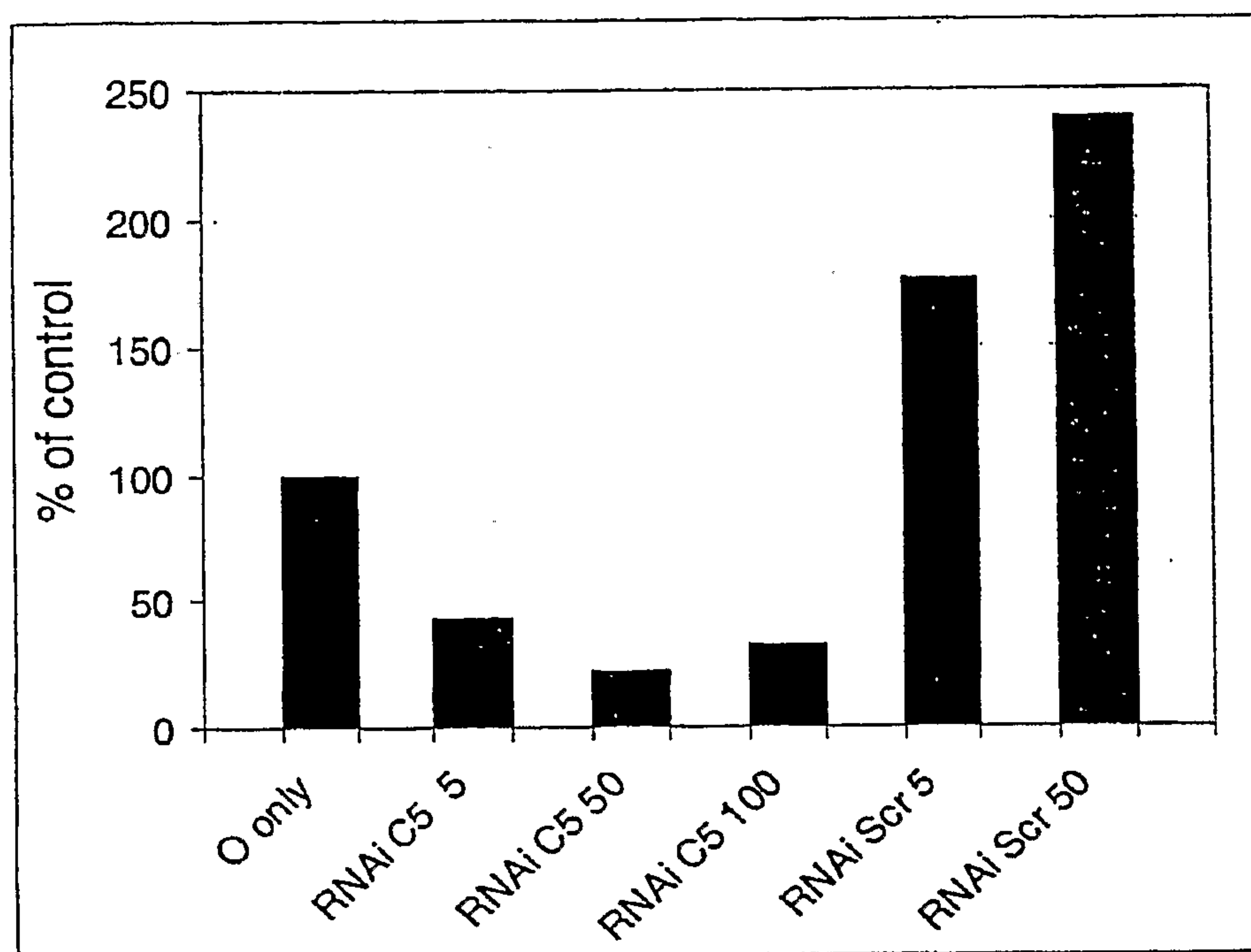


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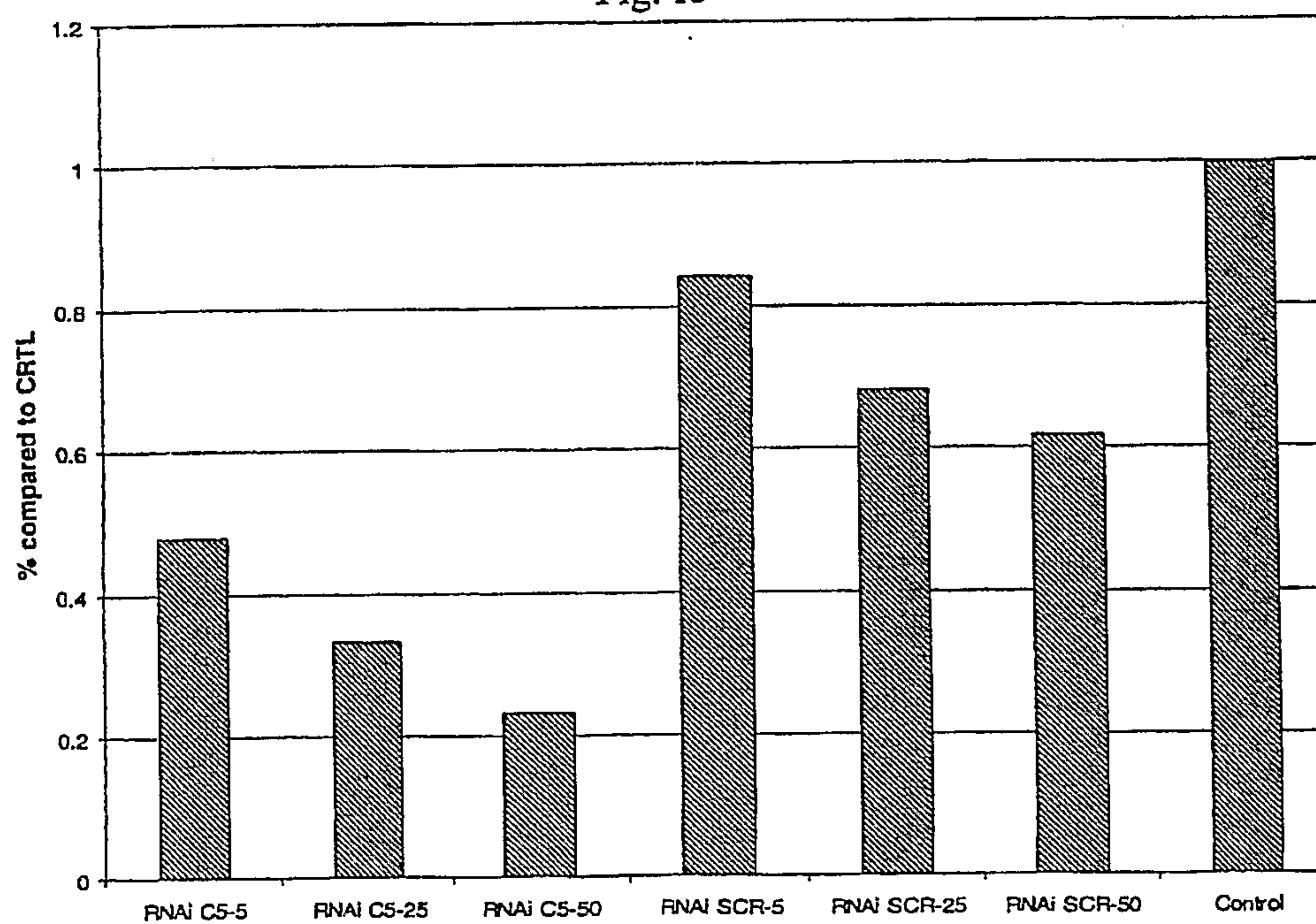
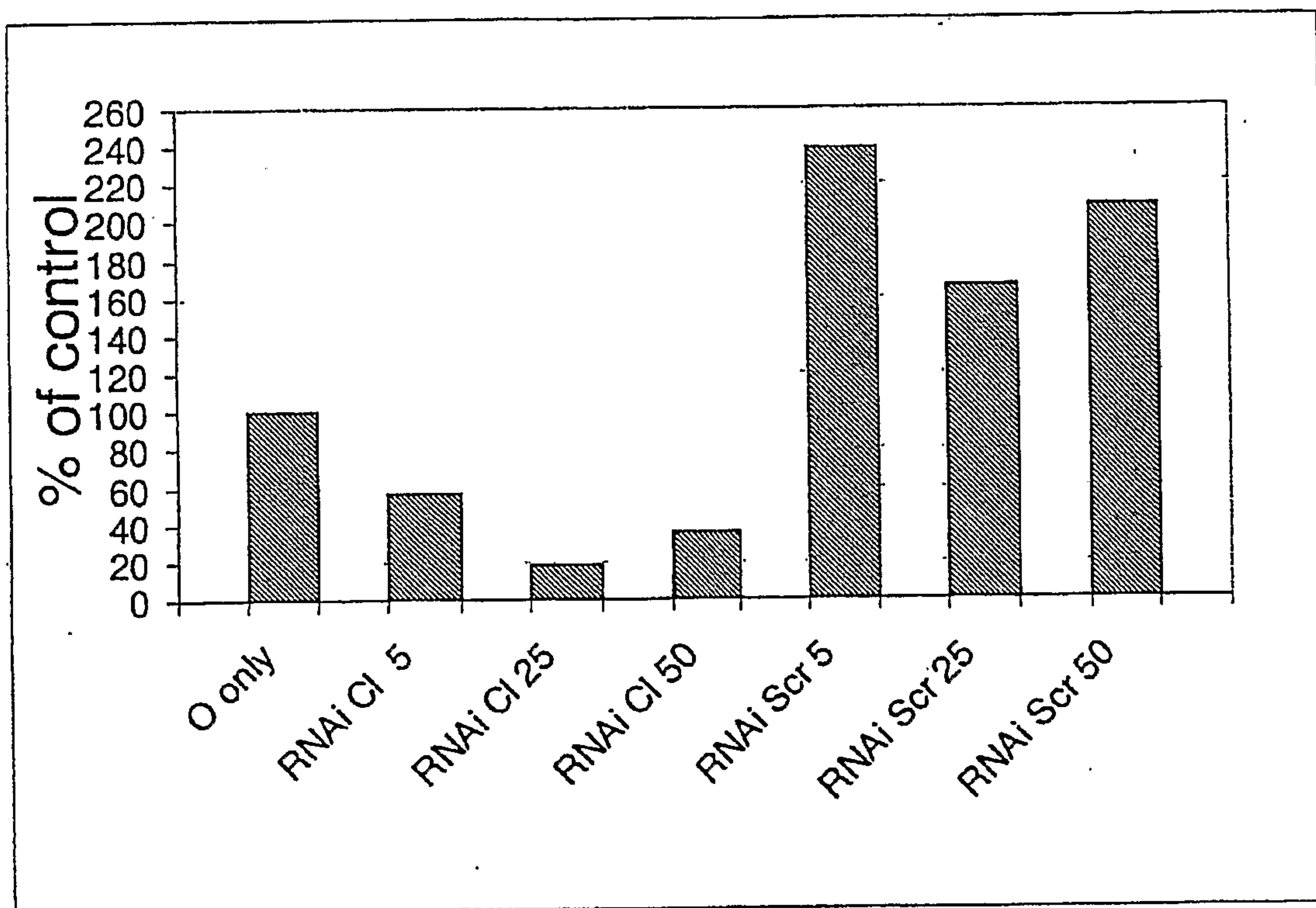


Fig. 16



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Clusterin Levels From Western Fig. 17

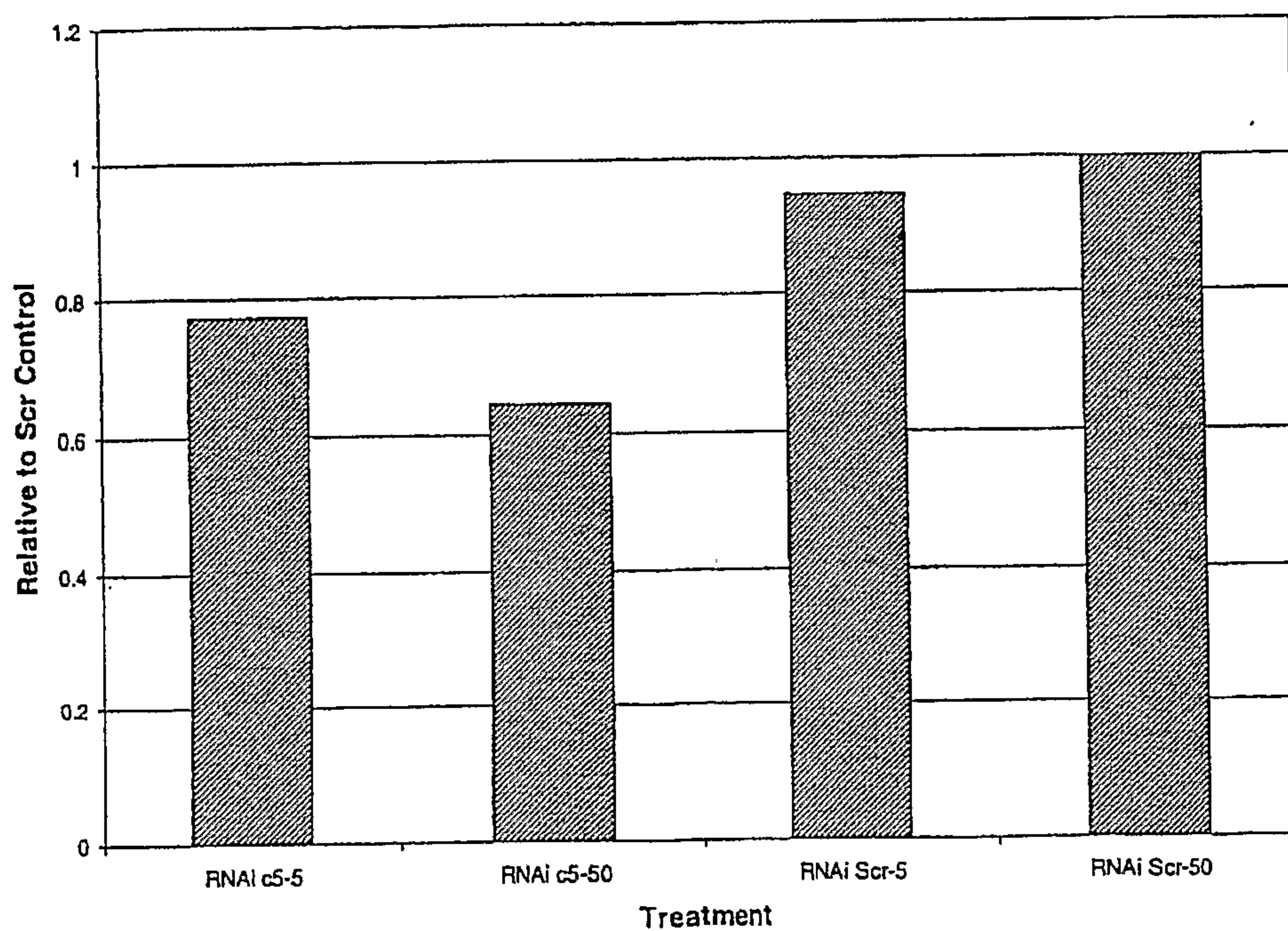


Fig. 18

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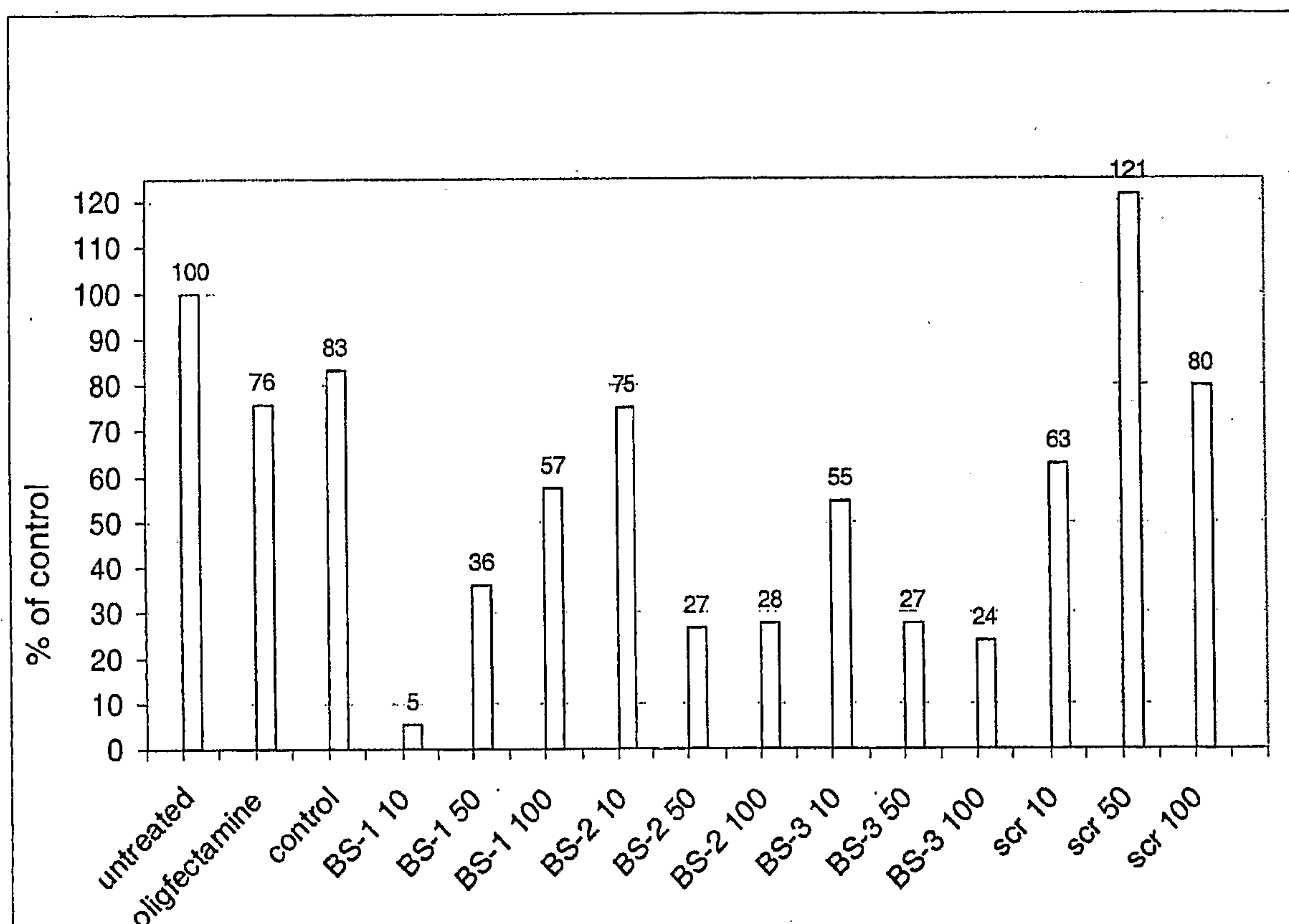


Fig. 19



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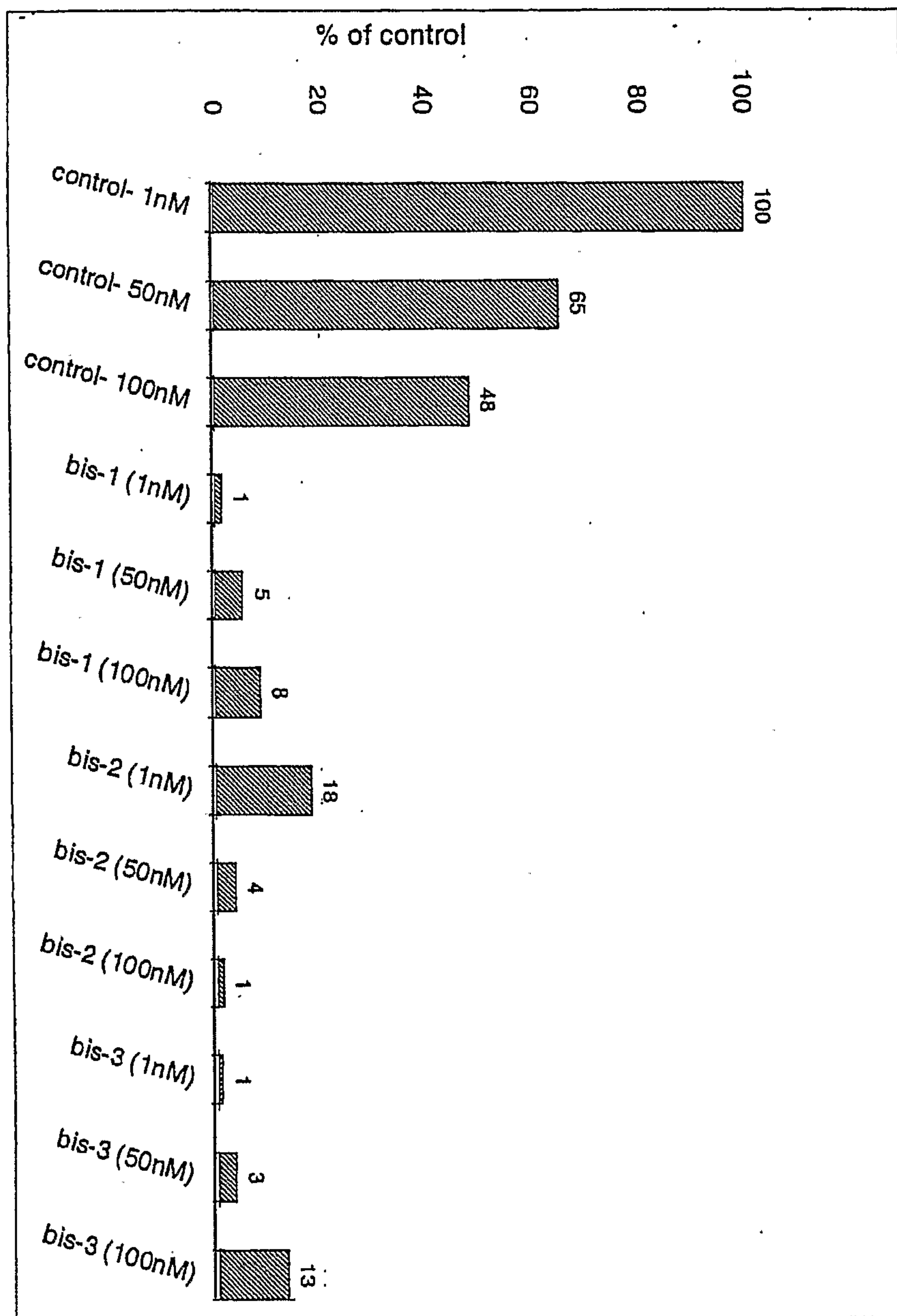


Fig. 20

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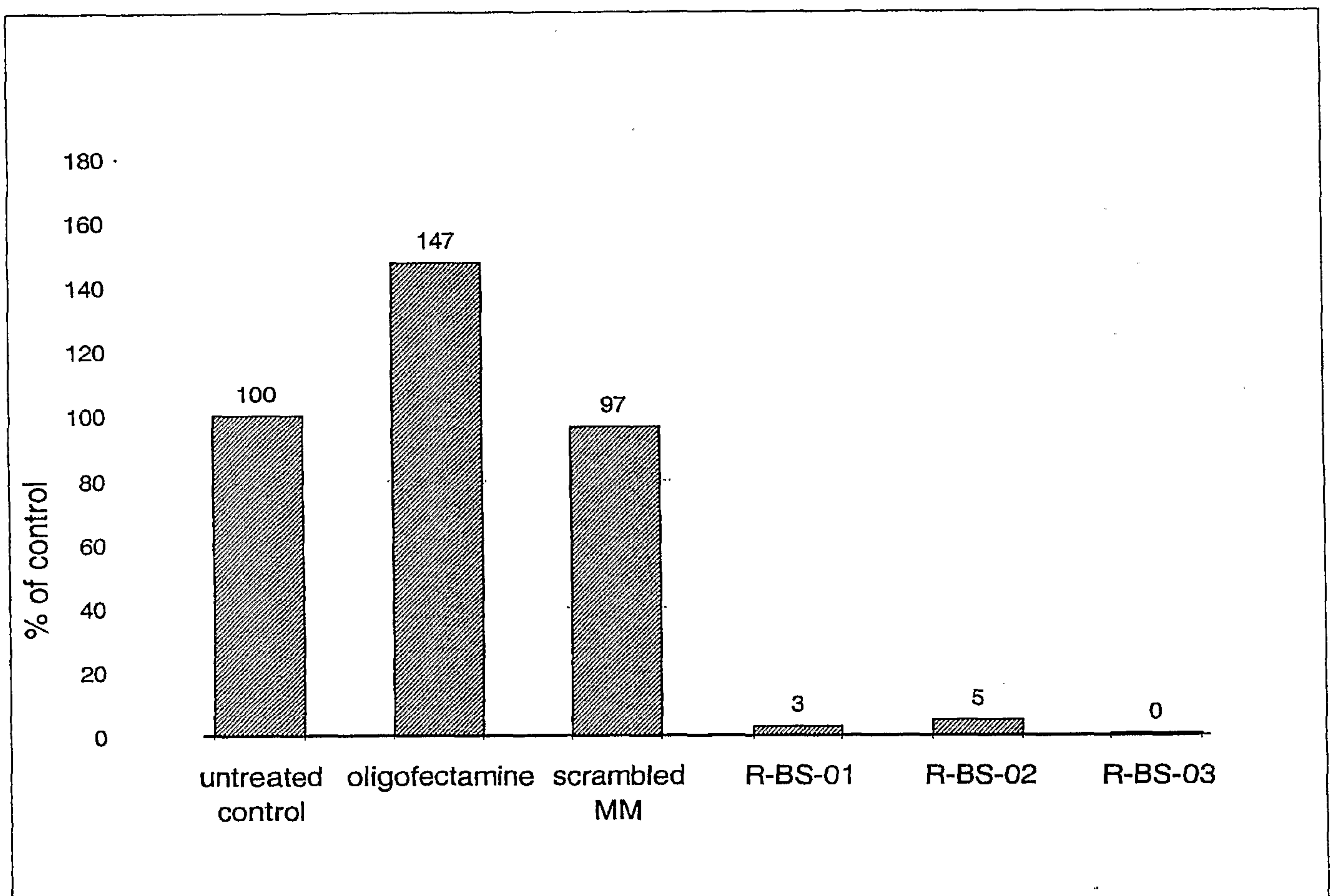


Fig. 21

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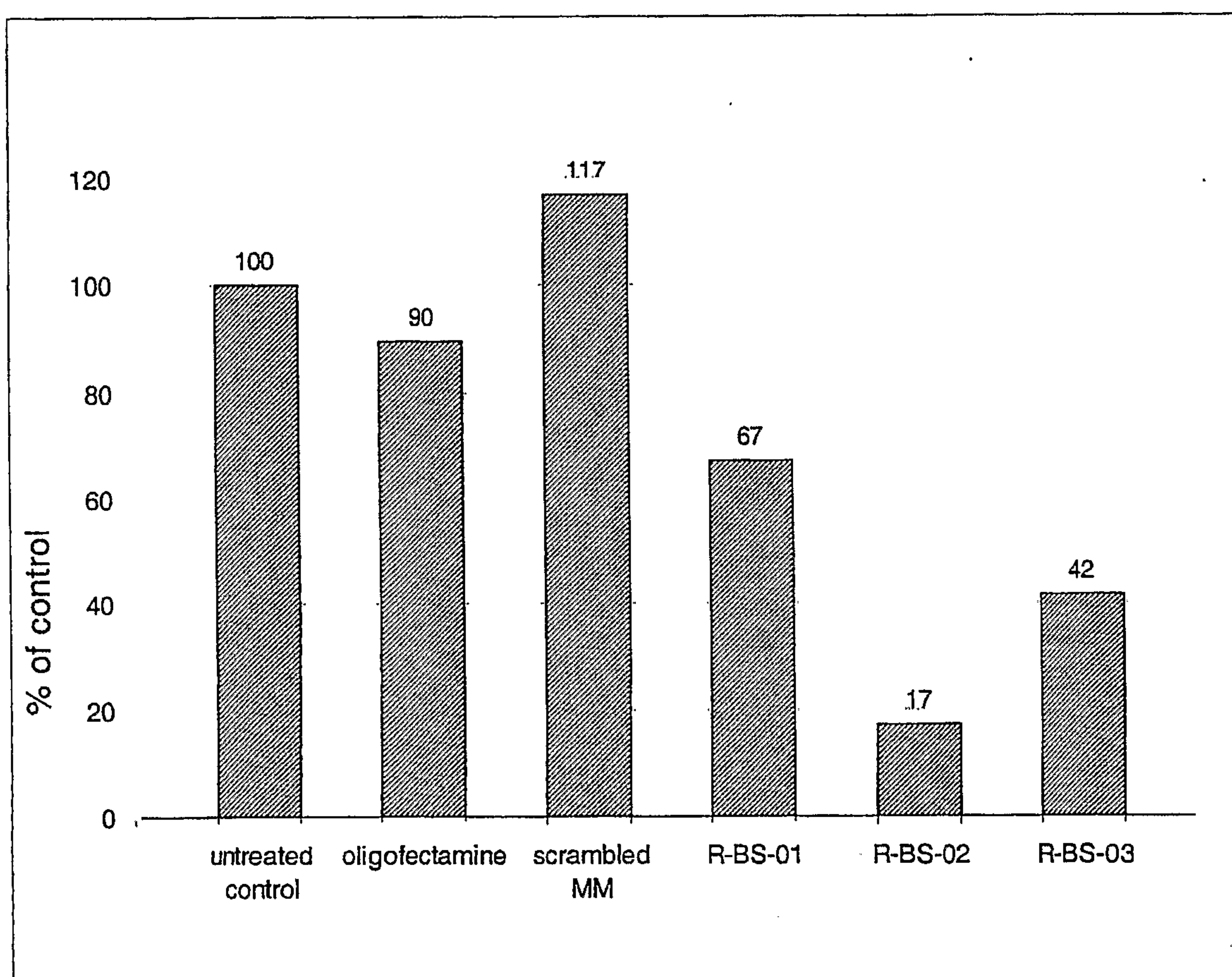


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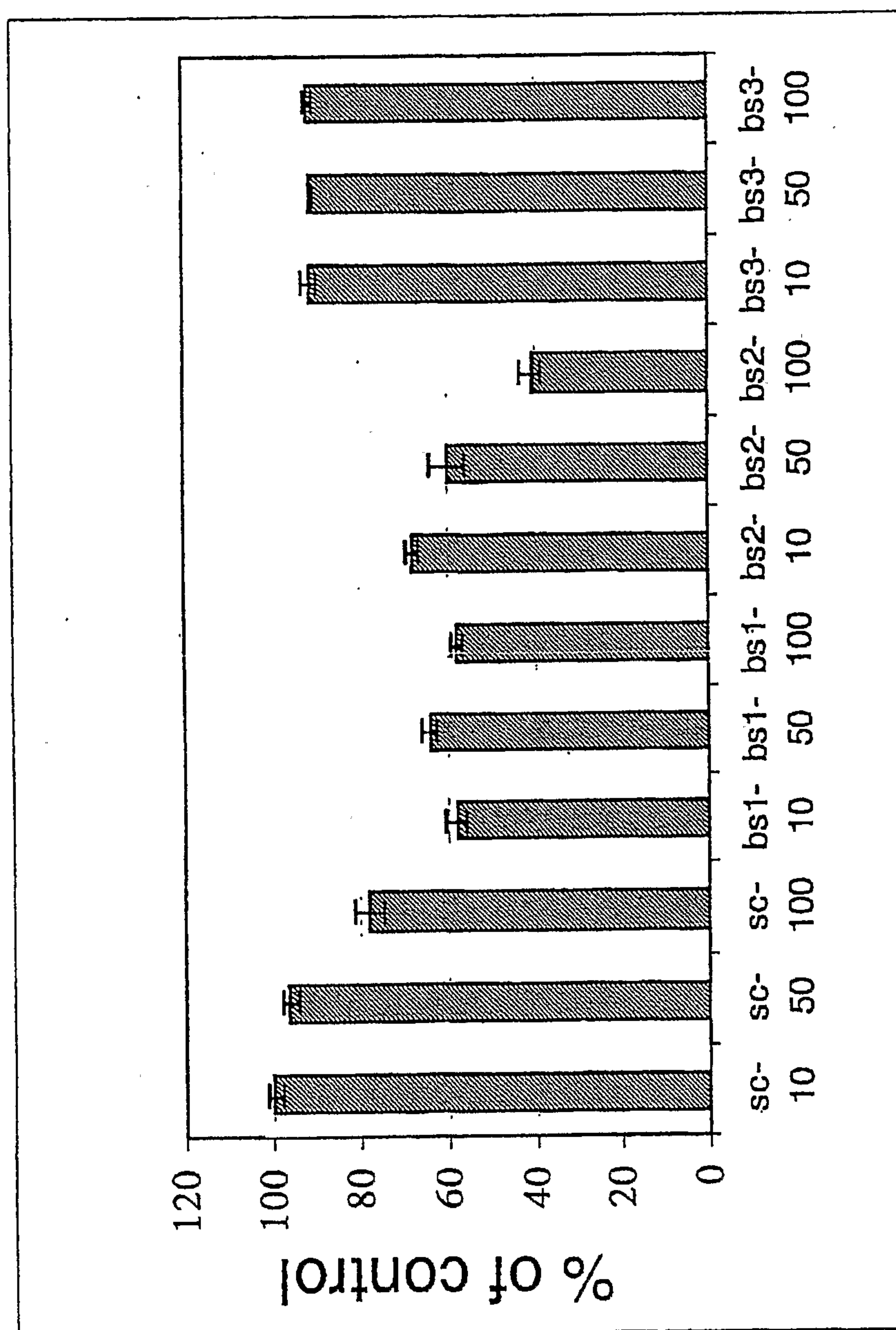


Fig. 23

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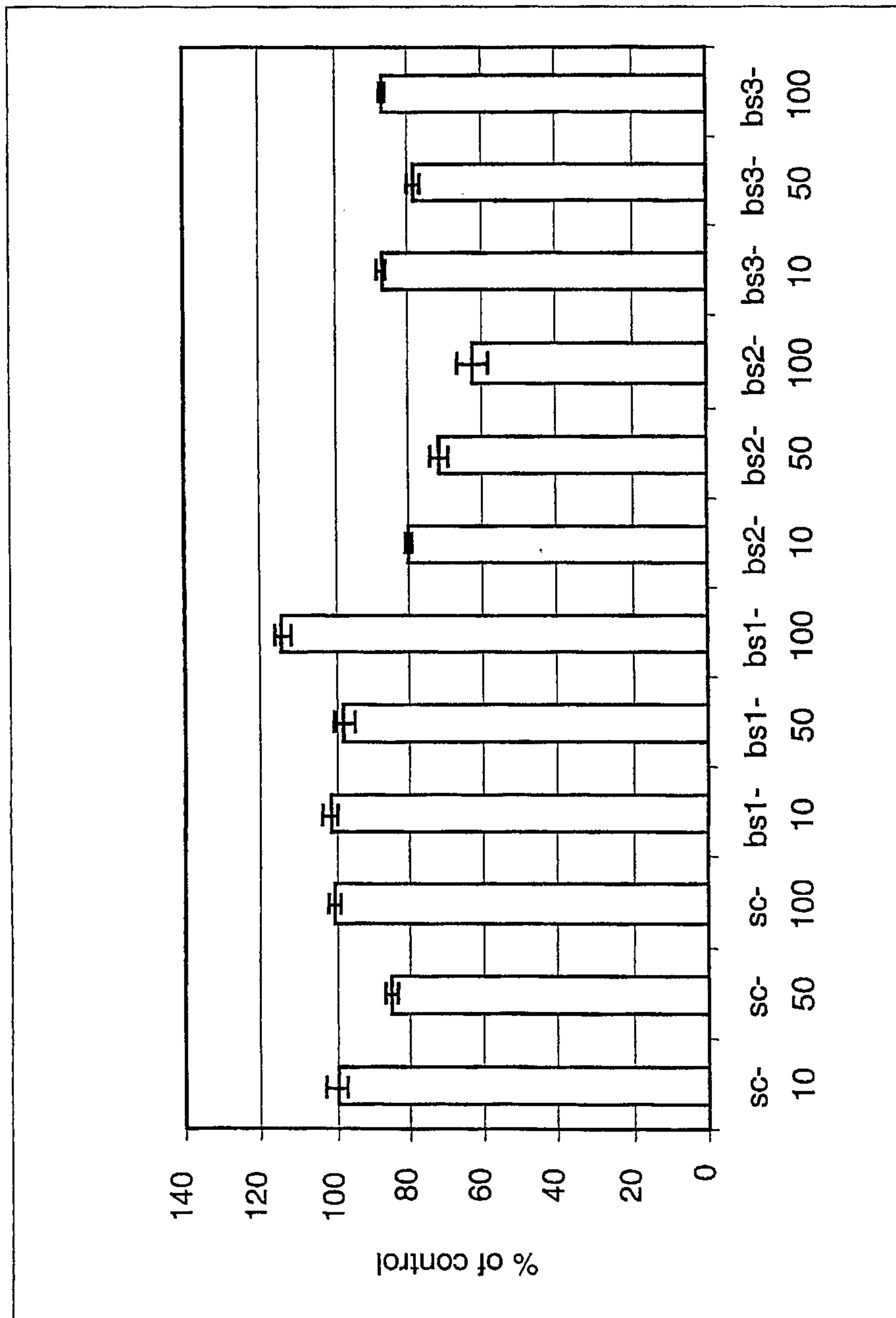
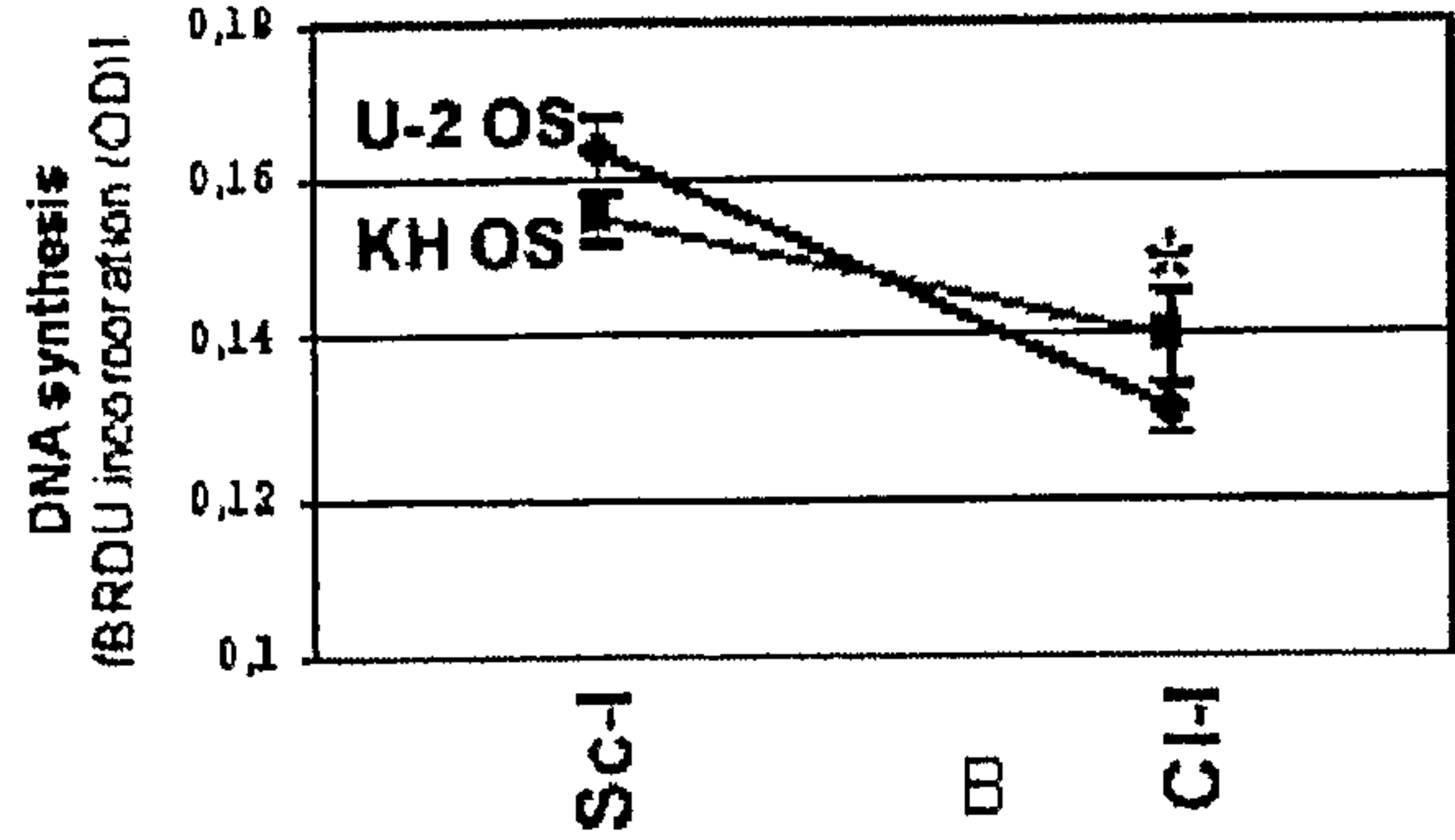
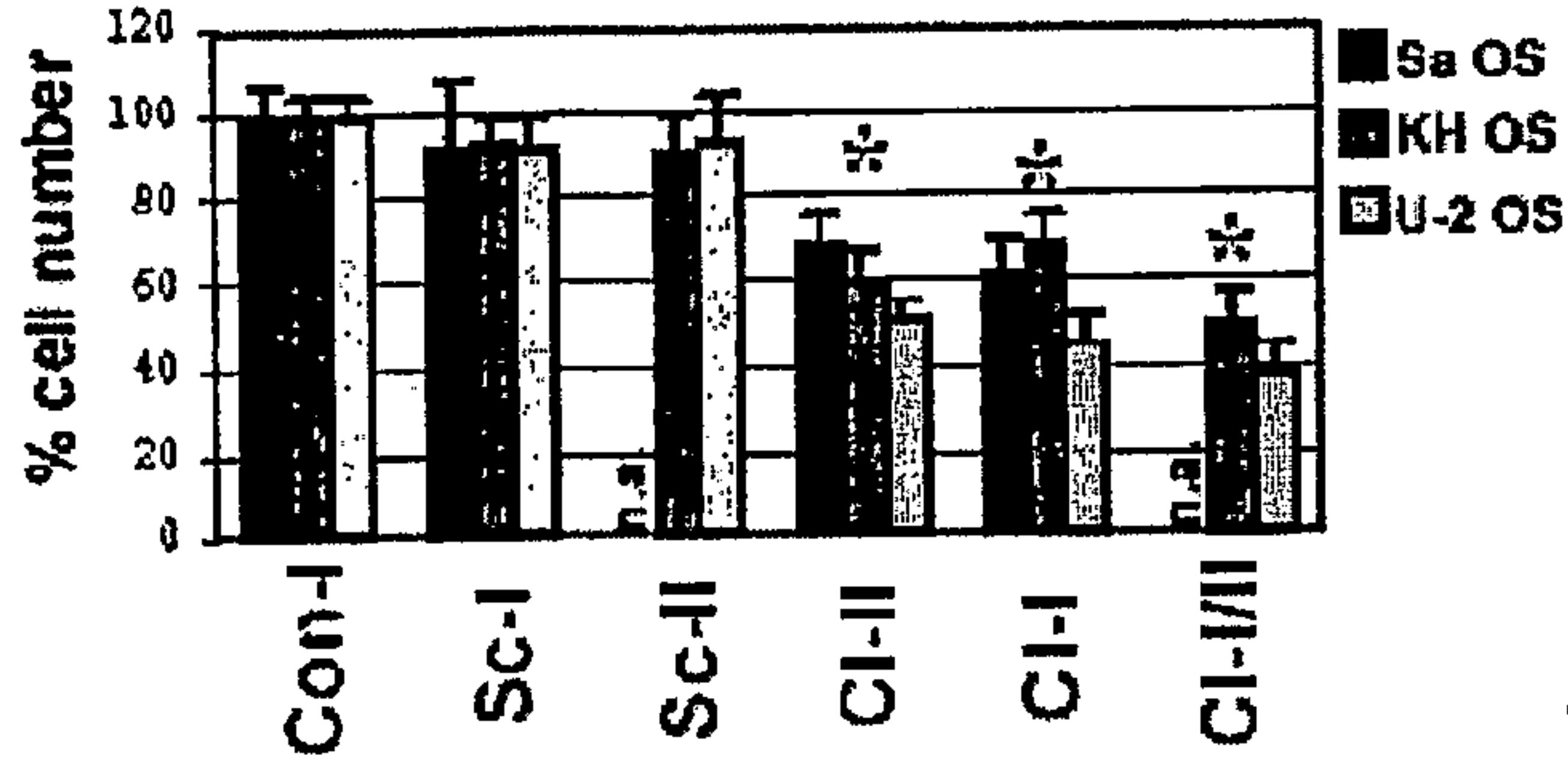


Fig. 24



**Apoptosis**  
[Cytoplasmic mono-  
and oligonucleosomes (OD)]

