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(71) Applicant *(for all designated States except US)*: **BIONICHE LIFE SCIENCES INC.** [CA/CA]; 231 Dundas Street, Belleville, Ontario K8N 5J2 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PHILLIPS, Nigel, C.** [CA/CA]; 101 Seigniory Avenue, Pointe-Claire, Québec H2R 1J6 (CA). **FILION, Mario, C.** [CA/CA]; 1145 Jean de Brebeuf, Laval, Québec H7E 3Y6 (CA).

(74) Agent: **BERESKIN & PARR**; 40 King Street West, Box 401, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

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(54) Title: HYALURONIC ACID IN THE TREATMENT OF CANCER

(57) Abstract: The present invention relates to a composition and method comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier, wherein the purified HA and the second anti-neoplastic agent are administered to a mammal having cancer in an amount effective to treat the cancer.

HYALURONIC ACID IN THE TREATMENT OF CANCER

This application claims benefit from United States provisional application no. 60/173,375, filed December 28, 1999 which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to hyaluronic acid and a second anti-neoplastic agent in the treatment of cancer.

BACKGROUND OF THE INVENTION

Hyaluronic acid (hereinafter, "HA") is a glycosaminoglycan with repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine that exists as a high molecular mass polymer (10^6 to 10^7 Da) in its native form (Laurent et al. FASEB J. 6:2397, 1992). HA is a major non-structural component of connective tissue and is important for maintaining extracellular matrix architecture and for promoting cell motility, adhesion and proliferation (Entwistle, J. Cell. Biochem. 61:569, 1996).

The effects of both low molecular mass HA ($\leq 5 \times 10^5$ Da) and high molecular mass HA ($\geq 5 \times 10^5$ Da) on normal cells has been studied extensively (McKee et al. J. Biol. Chem. 272:8013, 1997; Hodge-Dufour J. Immunol. 159:2492, 1997; Rooney et al. Int. J. Cancer 60:632, 1995). However, little is known about the effects of HA on malignant cells. *In vitro*, HA (>0.320 mg/ml) inhibited proliferation of B16F10 murine melanoma cells by 50 to 90%. *In vivo*, HA (1 mg/ml) administrated over 7 days by an Alzet osmotic pump into the immediate vicinity of a B16F10 murine melanoma tumor, reduced tumor volume >85%. *In vivo*, HA (>750 mg/kg) administered with various other therapeutic agents over various periods of time, reduced or eliminated rectal, gastric, breast, prostate and endometrial cancers (PCT/CA/00283). *In* vivo, hyaluronan (HA) (7.5 mg/kg) administered with 2.5 mg/kg of the lipophilic, tubulin-stabilizing, chemotherapeutic drug paclitaxel (TAXOL®) decreased tumor mass of colon 26-cells seeded into BALB/c mice. It was proposed that the water-insoluble paclitaxel binds to hydrophobic patches on HA and that the HA binds to HA receptors on the surface of malignant cells and, thereby,

delivers the paclitaxel directly to the malignant cells (PCT/CA98/00660). That is, HA functions as a delivery agent for the paclitaxel and the efficiency of this delivery depends on the expression of HA cell surface receptors such as CD44. However, as colon-26 cancer cells express high levels of HA receptors, HA alone significantly inhibits the growth of these cancer cells (Freemantle et al. Int. J. Tissue React. 17:157, 1995).

Cancer is an aberrant net accumulation of atypical cells that results from an excess of cell proliferation, an insufficiency of cell death, or a combination of the two. Cell proliferation is characterized by replication of total cellular DNA and the division of one cell into two cells (Hochhauser D. Anti-Cancer Chemotherapy Agents 8:903, 1997). Cell death is affected by immune-mediators including, but not limited to, IL-6 and IL-12 that initiate cytolytic processes and that promote apoptosis, and from apoptosis inducers that directly initiate pathways leading to cell death (Muzio et al. Cell 85:817, 1996; Levine, A. Cell 88:323, 1997).

Current cancer treatments act by inhibiting proliferation of cancer cells or by inducing apoptosis in cancer cells. However, many of these treatments have proven to be less than adequate for clinical applications and, at standard dosages, are inefficient or toxic, have significant adverse side-effects, result in development of drug resistance or immunosensitization, are debilitating and compromise the quality of life of the patient. Moreover, the costs of these treatments are substantial, both to the individual patient and to society.

Therefore, there is a continuing need for novel cancer treatments that inhibit proliferation of cancer cells, induce apoptosis in cancer cells, are effective at dose regimens associated with minimal toxicity, and are cost effective.

SUMMARY OF THE INVENTION

The present invention fulfills these needs by providing a composition and method comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier, wherein the HA and the second anti-neoplastic agent act synergistically to potentiate each other's effect on cancer cells.

HA is a nontoxic anti-neoplastic agent that acts synergistically with a second anti-neoplastic agent including, but not limited to, a toxic chemotherapeutic drug to

inhibit proliferation and induce apoptosis in cancer cells. As the HA and the toxic chemotherapeutic drug potentiate each other's effect on cancer cells, the standard dose of the toxic chemotherapeutic drug can be reduced without compromising the therapeutic effectiveness of the cancer treatment. Moreover, as HA is inexpensive and as most chemotherapeutic drugs are expensive, the combined use of HA and a chemotherapeutic drug can reduce significantly the cost of cancer treatment. The increase in dose effectiveness, decrease in toxicity and decrease in cost address long felt unfulfilled needs in the medical arts and provide important benefits for mammals, including humans.

Accordingly, it is an object of the present invention is to provide a composition and method effective to treat cancer in a mammal, including a human.

Another object is to provide a composition and method that reduces the toxic side-effects of cancer treatments.

Another object is to provide a composition and method that reduces the cost of cancer treatments.

Another object is to provide a composition and method, wherein two or more anti-neoplastic agents act synergistically on cancer cells.

Another object is to provide a composition and method that inhibits proliferation of cancer cells.

Another object is to provide a composition and method that induces apoptosis in cancer cells.

Another object is to provide a composition and method that potentiates the effect of chemotherapeutic drugs on cancer cells.

Another object is to provide a composition and method that potentiates the effect of anti-neoplastic nucleic acids on cancer cells.

Another object is to provide a composition and method that potentiates the effect of anti-neoplastic bacterial DNAs on cancer cells.

Another object is to provide a composition and method that potentiates the effects of anti-neoplastic bacterial DNA-bacterial cell wall complexes on cancer cells.

Another object is to provide a composition and method that potentiates the effect of anti-neoplastic bacterial cell wall extracts on cancer cells.

Another object is to provide a composition and method that potentiates the effect of anti-neoplastic synthetic oligonucleotides on cancer cells.

Another object is to provide a composition and method that stimulates the production of cytokines by immune system cells.

Another object is to provide a composition and method that stimulates the production of IL-6 by immune system cells.

Another object is to provide a composition and method that stimulates the production of IL-12 by immune system cells.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

DETAILED DESCRIPTION OF THE INVENTON

The present invention provides a composition comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier, wherein the HA and the second anti-neoplastic agent act synergistically to potentiate each other's effect on cancer cells. The present invention also provides a method, wherein a composition comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier is administered to a mammal having cancer in an amount effective to treat the cancer.

As used herein, "hyaluronic acid (HA)" refers to hyaluronan, hyaluronate, salts of HA, homologues, analogues, derivatives, complexes, esters, fragments and subunits of HA that have been tested and found suitable for use in a mammal, including a human.

As used herein, "anti-neoplastic agent" refers to any agent that inhibits the growth or metastases of a cancer.

As used herein, "chemotherapeutic drug" refers to any drug approved by a regulatory agency of a country or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia to treat cancer in a mammal, including a human.

As used herein, "non-lipophilic" refers to a chemotherapeutic drug having greater than zero solubility in water.

As used herein, "standard dose" refers to the dose or dose range suggested in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia to treat cancer in a mammal, including a human.

As used herein, "synergism" refers to the coordinated action of two or more agents on the growth or metastases of a cancer.

As used herein, "potentiates" refers to a degree of anti-cancer activity that is greater than additive.

As used herein "toxic" refers to the adverse side-effects of an anti-neoplastic agent as included in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia.

HA is highly viscous, highly electronegative and highly hydrophilic. Various methods for the isolation, purification and fractionation of HA are known to those skilled in the art. In addition, molecular mass fractions of purified HA can be purchased from commercial sources including, but not limited to, Fluka Chemical Corporation (Ronkonkoma, NY, USA), Genzyme Corporation (Cambridge, MA, USA), Lifecore Inc. (Chaska, MN, USA), Hyal Pharmaceutical Corporation (Mississauga, Ontario, Canada) and Bioniche Life Sciences, Inc. (Belleville, Ontario, Canada).

Anti-neoplastic agents include, but are not limited to, chemotherapeutic drugs, biologicals, immunostimulants, cytokines, antigens, antibodies, nucleic acids, synthetic oligonucleotides, vaccines, aptamers nucleic acids, antisense nucleic acids, immunomodulators, telomerase inhibitors, caspase activators, apoptosis inducers, cyclin inhibitors, CDK inhibitors, stable triple helix forming agents, genetically engineered, biologically engineered and chemically synthesized agents, agents that target cell death molecules for activation or inactivation, and combinations thereof.

Chemotherapeutic drugs include, but are not limited to, DNA-alkylating agents, DNA-cross-linking agents, antibiotic derivatives, topoisomerase inhibitors, tubulin stabilizers, tubulin destabilizers, antimetabolites, nitrogen mustard derivatives, steroids, hormone antagonists, protein kinase inhibitors, HMG-CoA inhibitors, metaloproteinase inhibitors, angiogenesis inhibitors, CDK inhibitors, cyclin inhibitors, caspase inhibitors, RNA, antisense RNA, DNA, antisense DNA, bacterial extracts, bacterial DNA, bacterial DNA-bacterial cell wall complexes, synthetic

oligonucleotides, molecular biologically modified viral and bacterial components, and combinations thereof.

Pharmaceutically acceptable carriers include liquid carriers, solid carriers, or both. Liquid carriers include, but are not limited to, water, saline, physiologically acceptable buffers, aqueous suspensions, oil emulsions, water in oil emulsions, water-in-oil-in-water emulsions, site-specific emulsions, long-residence emulsions, sticky-emulsions, microemulsions and nanoemulsions. Preferred aqueous carriers include, but are not limited to, water, saline and physiologically acceptable buffers. Preferred non-aqueous carriers include, but are not limited to, a mineral oil or a neutral oil including, but not limited to, a diglyceride, a triglyceride, a phospholipid, a lipid, an oil and mixtures thereof. Solid carriers are biological carriers, chemical carriers, or both and include, but are not limited to, particles, microparticles, nanoparticles, microspheres, nanospheres, minipumps, bacterial cell wall extracts, and biodegradable or non-biodegradable natural or synthetic polymers that allow for sustained release of the composition (Brem et al. J. Neurosurg. 74: 441, 1991).

Cancers include, but are not limited to, squamous cell carcinoma, fibrosarcoma, sarcoid carcinoma, melanoma, mammary cancer, lung cancer, colorectal cancer, renal cancer, osteosarcoma, cutaneous melanoma, basal cell carcinoma, pancreatic cancer, bladder cancer, brain cancer, ovarian cancer, prostate cancer, leukemia, lymphoma and metastases derived therefrom.

Preferably, the molecular mass of the HA used is between about 1×10^3 and 1×10^7 Da, more preferably between about 5×10^4 and 1×10^6 Da, and most preferably between about 1×10^4 and 8×10^5 Da. Preferably, the amount of HA administered per dose is from about 0.001 to 25 mg/kg, more preferably from about 0.01 to 15 mg/kg, and most preferably from about 0.1 to 10 mg/kg. The amount of anti-neoplastic agent administered per dose depends on the anti-neoplastic agent used and is preferably about 5 to 75% of the standard dose, more preferably from about 5 to 50% of the standard dose, and most preferably from about 5 to 10% of the standard dose.

Routes of administration include, but are not limited to, oral, topical, subcutaneous, transdermal, subdermal, intra-muscular, intra-peritoneal, intra-vesical, intra-articular, intra-arterial, intra-venous, intra-dermal, intra-cranial, intra-lesional,

intra-tumoral, intra-ocular, intra-pulmonary, intra-spinal, placement within cavities of the body, nasal inhalation, pulmonary inhalation, impression into skin, electrocorporation, osmotic minipumps, and through a cannula to the site of interest.

Depending on the route of administration, the volume per dose is preferably about 0.001 to 100 ml per dose, more preferably about 0.01 to 50 ml per dose, and most preferably about 0.1 to 30 ml per dose. The dose can be administered in a single treatment or in multiple treatments on a schedule and over a period of time appropriate to the cancer being treated, the condition of the recipient, and the route of administration. Moreover, the HA can be administered before, at the same time as, or after administration of the anti-neoplastic agent as long as both are administered within a 24 hour time period.

In an example, 100 mg of $<1.5 \times 10^4$ Da HA + 4 mg/kg of the antimetabolite fluorinated pyrimidine 5-fluorouracil (hereinafter, "5-FU"; standard dose 12mg/kg) are administered intravenously to a mammal having cancer in a number of doses and over a period of time effective to treat the cancer. In another example, 100 mg of $5.0\text{-}7.5 \times 10^5$ Da HA + 2 mg/kg of 5-FU are administered intratumorally to a mammal having a cancer in a number of doses and over a period of time effective to treat the tumor. In another example, 100 mg of $5.0\text{-}7.5 \times 10^5$ Da HA + 1.2 mg/kg of 5-FU are administered intratumorally to a mammal having a cancer in a number of doses and over a period of time effective to treat the tumor. In another example, 100 mg of $1\text{-}3 \times 10^5$ Da HA + 10 mg/m^2 of the alkylating agent cisplatin (PLATINOL®; hereinafter, "CIS"; standard dose 100 mg/m^2) is administered intravenously to a mammal having cancer in a number of doses and over a period of time effective to treat the cancer. In another example, 100 mg of $3\text{-}5 \times 10^5$ Da HA + 36 mg/m^2 of the DNA cross-linker carboplatin (PARAPLATIN®; standard dose 360 mg/m^2) is administered intravenously to a mammal having a cancer in a number of doses and over a period of time effective to treat the cancer.

The amount of HA per dose, the particular second anti-neoplastic agent used, the amount of the second anti-neoplastic agent per dose, the dose schedule and the route of administration should be decided by the practitioner using methods known to those skilled in the art and will depend on the type of cancer, the severity of the cancer, the location of the cancer and other clinical factors such as the size, weight

and physical condition of the recipient. In addition, *in vitro* assays may be employed to help identify optimal ranges for HA + anti-neoplastic agent administration.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example 1

Cells

All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were cultured in the medium recommended by the ATCC.

Table 1 shows the cell lines, their origins and their properties.

Table 1

Cell lines

CELL LINE	ORIGIN	PROPERTIES
MCF-7	Human breast cancer	Caspase 3-negative; estrogen-dependent
PC-3	Human prostate cancer	p53 mutated; androgen-independent (hormone refractory)
LNCaP	Human prostate cancer	TGF-beta 1 receptor-negative; androgen-dependent
Du-145	Human prostate cancer	Fas-resistant; Rb-mutated, p53 mutated; androgen independent
T-24	Human bladder cancer	p53 mutated
RT-4	Human bladder cancer	N.D.
UMUC-3	Human bladder cancer	P-glycoprotein over-expression
HT-1376	Human bladder cancer	p53 and p21 (waf-1) mutated
HT-1080	Human fibrosarcoma	N.D.
B-16F1	Murine melanoma	N.D.

Example 2

Reagents

HA, purified from *Streptococcus sp.*, was obtained from Lifecore Inc. (Chaska, MN, USA) and was dissolved in sterile saline at 0.8 mg/ml (CYSTISTAT®,

Bioniche Life Sciences Inc., Belleville, Ontario, Canada) or at 10.0 mg/ml (SUPLASYN®, Bioniche Life Sciences Inc., Bellville, Ontario, Canada).

Mycobacterium phlei-DNA (hereinafter, "M-DNA") and M-DNA-*Mycobacterium phlei* cell wall complex (hereinafter, "MCC") were prepared as in US Application No. 09,129,312 (incorporated by reference herein).

Example 3

Preparation of HA of $\leq 5.0 \times 10^5$ Da

HA of $\leq 5.0 \times 10^5$ Da was prepared from HA of 5.0-7.5 x 10^5 Da by digestion with hyaluronidase type IV-S derived from bovine testes (Sigma-Aldrich Canada, Oakville, Ontario, Canada) for 60 minutes at 37°C, by sonication on ice (Branson Sonifier Model 450, Danbury, CT, USA) for 20 minutes at maximal intensity, or by autoclaving (Amsco-Steris International, Model 2002, Mentor, OH, USA) for 30 minutes.

The HA obtained was electrophoresed in 0.5% agarose gels prepared in TAE buffer (40 mM Tris, 20 mM acetic acid and 2.0 mM EDTA, pH 7.9) for 3 hours at 100 V (Lee et al. Anal. Biochem. 219:278, 1994). The molecular mass distribution of the HA was visualized using 0.005% of the cationic dye Stains-All (1-Ethyl-2-[3-(1-ethylnapthol[1,2-d]thiazolin-2-ylidene)-2-methylpropenyl]napthol[1,2-d]thiazolium Bromide; Sigma-Aldrich, Oakville, Ontario, Canada) and the gel photo was scanned using 1D software (Advance American Biotechnology, Fullerton, CA, USA). The molecular mass of HA was $<1.5 \times 10^4$ Da after hyaluronidase, about 1.0-3.0 x 10^5 Da after sonication, and about 3.0-5.0 x 10^5 Da after autoclaving.

Example 4

Measurement of cell proliferation

Cell proliferation was measured using dimethylthiazoldiphenyltetrazolium (MTT) reduction (Mosman et al. J. Immunol. Methods 65:55, 1983). Unless otherwise stated, 100 µl of 5 mg/ml of MTT (Sigma-Aldrich, St. Louis, MO, USA) dissolved in PBS was added into each well. After 4 h, medium was removed from each well, 1.0 ml of acid-isopropanol (0.04 N HCl in isopropanol) was added and reduced MTT was solubilized by mixing. Absorbency of the reaction product was measured at a wavelength of 570 nm using a multiplate spectrophotometer reader (Elx800 Model, Bio-TEK Instruments Inc., Winooski, Vermont, USA).

Example 5

Inhibition of cell proliferation with HA

Unless stated otherwise 1.0 x 10^5 cells/ml were seeded in 6-well flat-bottom tissue culture plates and were maintained for 48 h at 37° C in a 5% CO_2 atmosphere.

PC-3, LNCaP and Du-145 human prostate cancer cells; MCF-7 human breast cancer cells; HT-1080 human fibrosarcoma cancer cells; B16F1 murine melanoma cells; and, UMUC-3, RT-4, HT-1376 and T-24 human bladder cancer cells were incubated with 0.8, 8.0 and 80 µg/ml of 5.0-7.5 x 10^5 Da HA (Table 2).

Table 2

Inhibition of cell proliferation with 0.5-7.5 x 10^5 Da HA

CELLS	% INHIBITION		
	0.8 µg/ml	8.0 µg/ml	80.0µg/ml
PC-3	15	26	30
LNCaP	7	13	26
Du-145	6	15	38
MCF-7	19	24	35
HT-1080	4	4	36
B16F1	20	20	30
UMUC-3	10	10	2
RT-4	12	13	5
HT-1376	0	3	1
T-24	10	8	0

As shown in Table 2, inhibition of proliferation of PC-3, LNCaP, Du-14, MCF-7, HT-1080 and B16F1 cancer cells increased with increasing concentrations of HA, whereas. inhibition of proliferation of UMUC-3, RT-4, HT-1376 and T-24 cancer cells did not increase with increasing concentrations of HA.

PC-3, LNCaP and Du-145 human prostate cancer cells were incubated with 8.0 µg/ml of 5.0-7.5 x 10^5 Da HA or of <1.5 x 10^4 Da HA (Table 3).

Table 3

Inhibition of cell proliferation

CELLS	% INHIBITION	
	5.0-7.5 x 10^5 Da HA 8.0 µg/ml	<1.5 x 10^4 Da HA 8.0 µg/ml
PC-3	26	5
LNCaP	13	24
Du-145	15	37

PC-3 cancer cell proliferation was inhibited more by 5.0-7.5 x 10^5 Da HA, whereas LNCaP and Du-145 cancer cell proliferation was inhibited more by <1.5 x 10^4 Da HA.

Example 6

HA potentiation of the anti-neoplastic effect of M-DNA and MCC

Unless stated otherwise 2.0 x 10^4 cells/ml were seeded in 24-well plates and were maintained for 48 h at 37° C in a 5% CO_2 atmosphere. MTT was used at 50 µl per well.

T-24 human bladder cancer cells, PC-3 and Du-145 human prostate cancer cells, and MCF-7 human breast cancer cell were incubated with saline or with 80.0 µg/ml of 5.0-7.5 x 10^5 Da HA + 1.0 µg/ml of M-DNA (Table 4).

Table 4

HA potentiation of M-DNA inhibition of cell proliferation

CELLS	% INHIBITION 5.0-7.5 x 10^5 Da HA	
	Saline	80.0 µg/ml
T-24 + saline	0	0
T-24+ M-DNA	28	36
PC-3 + saline	0	30
PC-3 + M-DNA	10	50
Du-145 + saline	0	38
Du-145+ M-DNA	11	58
MCF-7+ saline	0	35
MCF-7 + M-DNA	13	52

As shown in Table 4, HA potentiated the anti-neoplastic effect of M-DNA on T-24, PC-3, Du-145 and MCF-7 cancer cells.

HT-1376, RT-4 and T-24 human bladder cancer cells were incubated with 0.8 µg/ml of 5.0-7.5 x 10^5 Da HA + 1.0 µg/ml of MCC (Table 5).

Table 5

HA potentiation of MCC inhibition of cell proliferation

CELLS	% INHIBITION	
	Saline	5.0-7.5 x 10^5 Da HA at 0.8 µg/ml
HT-1376 + saline	0	3
HT-1376 + MCC	21	34
RT-4 + saline	0	13
RT-4 + MCC	16	37
T-24 + saline	0	8
T-24 + MCC	31	45

As shown in Table 5, HA potentiated the anti-neoplastic effect of MCC on H-1376, RT-4 and T-24 cancer cells.

Example 7

HA potentiation of the anti-neoplastic effect of synthetic oligonucleotides

MCF-7 human breast cancer cells (2.5 X 10^5 cells/ml) were incubated as in Example 6 with 0.0, 0.01 and 0.1 µg/ml of 5.0-7.5 x 10^5 Da HA + 100 µg/ml of the synthetic 6 base oligonucleotide $GG(GT)_1GG$ (SEQ ID NO:1) or + 100 µg/ml of the synthetic 27 base oligonucleotide $(GT)_{13}G$ (SEQ ID NO:2) (Table 6). MTT was used at 50 µl per well.

Table 6

HA potentiation of inhibition of synthetic oligonucleotide inhibition of cell proliferation

SEQUENCES 100 µg/ml	% INHIBITION 5.0-7.5 x 10^5 Da HA		
	0.0 µg/ml	0.01 µg/ml	0.1 µg/ml
	0	6	5
$GG(GT)_1GG$-(6 bases) - SEQ ID NO:1	20	34	33
$(G_1T)_{13}G$-(27 bases) - SEQ ID NO:2	44	51	59

As shown in Table 6, 0.01 μg/ml HA and 0.1 μg/ml HA potentiated the anti-neoplastic activity of 6 base $GG(GT)_1GG$ (SEQ ID NO:1) and 0.01 μg/ml HA potentiated the antineoplastic activity of 27 base $(G_1T)_{13}G$ (SEQ ID NO:2).

Example 8

HA potentiation of the anti-neoplastic effect of chemotherapeutic drugs

RT-4 human bladder cancer cells and MCF-7 human breast cancer cells were incubated in as in Example 6 with 0.0, 0.008 or 0.08 μg/ml of 5.0-7.5 x 10^5 Da HA + 0.1 μg/ml of CIS and with + 1.0 μg/ml or + 10 μg/ml of 5-FU (Table 7).

Table 7

HA potentiation of chemotherapeutic drug inhibition of cell proliferation*

CELLS	% INHIBITION 5.0-7.5 x 10^5 Da HA		
	Saline	0.008 μg/ml	0.08 μg/ml
RT-4 + saline	0	0	0
RT-4 + CIS at 0.1 μg/ml	2	13	15
RT-4 + 5-FU at 1.0 μg/ml	14	18	19
MCF-7 + saline	0	12	12
MCF-7 + 5-FU at 10 μg/ml	18	32	31

As shown in Table 7, 0.008 μg/ml and 0.08 μg/ml HA potentiated the anti-neoplastic effect of 0.1 μg/ml CIS and of 1.0 μg/ml 5-FU on RT-4 cancer cells and the anti-neoplastic effect of 10 μg/ml of 5-FU on MCF-7 cancer cells.

Example 9

CD44 cell surface receptors and HA inhibition of proliferation

CD44 is a cell surface HA receptor that has multiple variants (Screaton et al. Proc. Natl. Acad. Sci. USA, 89:12160, 1992). CD44 variants are selectively expressed in human tumors and are over-expressed on numerous tumor cell lines (Naot et al. Adv. Cancer Res. 71:241, 1997). It has been suggested that CD44 receptors on cancer cells enables HA to deliver effective amounts of the highly lipophilic chemotherapeutic drug paclitaxel (TAXOL®) into cancer cells at low paclitaxel dosage amounts because the paclitaxel binds to the HA that, in turn, binds to the CD44 receptors (PCT/CA98/00660).

To determine if HA binding to CD44 receptors correlates with inhibition of cell proliferation, CD44 receptor expression was detected by flow cytometry (FCM)

using the fluorescent anti-HA receptor monoclonal antibody FITC-CD44 (clone G44-26 (C26), Pharmingen, Mississauga, Ontario, Canada). Briefly, cells were pelleted by centrifugation at 180 x G for 5 min at RT, washed twice in PBS and incubated with FITC-CD44 at the concentration recommended by the manufacturer for 20 min at 4° C in the dark. The cells were then washed twice in PBS by centrifugation and cell fluorescence was measured at 488 nm excitation and 530 nm emission (FL1 detector). Data were analyzed on a FACSCALIBUR using the program CELLQUEST (Becton Dickinson, San Jose, CA, USA).

CD44 expression and the inhibition of proliferation by 5.0-7.5 x 10^5 Da HA were measured using Jurkat T cell leukemia cells; MCF-7 human breast cancer cells; RT-4, T-24, HT-1376 and UMUC-3 human bladder cancer cells; and, PC-3, Du-145 and LNCaP prostate cancer cells (Table 8)

Table 8

CD44 cell surface expression and HA inhibition of cell proliferation

	CD44 expression in mean fluorescent units		5.0-7.5 x 10^5 Da HA - 80 µg/ml
CELLS	Unstained cells	Cells + anti-CD44	% inhibition of proliferation
HT-1376	4	200	1
RT-4	4	60	5
T-24	3	1800	0
UMUC-3	3	1800	2
PC-3	6	400	30
Du-145	3	450	38
MCF-7	3	70	35
Jurkat T	3	3	0
LNCaP	6	6	26

As shown in Table 8, expression of CD-44 receptors by cancer cells did not correlate with inhibition of proliferation by HA. PC-3 cancer cells express CD44 receptors, whereas LNCaP cancer cells do not express CD44 receptors (Lokeshwar et al. Anticancer Res. 15:1191, 1995). However, as shown in Table 8, HA inhibition of proliferation of PC-3 cancer cells (30%) and of LNCaP cancer cells (26%) was not significantly different.

Example 10

Induction of cytokine production

Peripheral blood mononuclear cells (hereinafter, "PBMCs") were isolated from the blood of 5 healthy humans by Ficoll-Hypaque (Amersham Pharmacia Biotech, Baie d'Urfée, Québec, Canada) density gradient centrifugation of whole blood. Stimulation of IL-6 and IL-12 production by the immune system cells was determined using the appropriate commercial ELISA (BioSource, Camarillo, CA). Results are expressed as the "fold" (x) increases in cytokine production by treated cells compared to control cells.

PBMCs were incubated with 1 mg/ml of 5.0-7.5 x 10^5 Da and production of IL-6 and IL-12 was determined (Table 9).

Table 9

Cytokine production

Individual	IL-6	IL-12
1	3.8x	25.2x
2	1.8x	7.1x
3	4.1x	26.5x
4	1.4x	10.0x
5	6.3x	8.5x

As shown in the Table 9, HA stimulated both IL-6 and IL-12 production by immune system cells.

PBMCs, isolated from the blood of individual #1, were incubated with 0.008, 0.04, 0.2 and 1 mg/ml of 5.0-7.5 x 10^5 Da (Table 10)

Table 10

Cytokine production

5.0-7.5 x 10^5 Da HA (mg/ml)	IL-6	IL-12
0.008	2.4x	4.9x
0.040	3.5x	13.8x
0.200	2.9x	8.5x
1.000	3.8x	25.2x

As shown in the Table 10, HA at 0.008, 0.04, 0.2 and 1 mg/ml, stimulated both IL-6 and IL-12 production by immune system cells.

Example 11

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC on PC3 tumors in mice

PC-3 human prostate cancer cells are implanted subcutaneously into 224 male nude BALB/c mice. The mice are divided into 28 groups of 8 mice (Table 11).

Table 11

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC

	Saline	M-DNA or MCC 0.02 mg/kg	M-DNA or MCC 0.2 mg/kg	M-DNA or MCC 2.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Groups 13 and 25
HA at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Groups 14 and 26
HA at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Groups 15 and 27
HA at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC are administered intravenously on day 0 and at 3-day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastases are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice. Groups 6-8, 10-12, 14-16, 18-20, 22-24 and 26-28 have less tumor mass and fewer metastases than Groups 5, 9, 13, 17, 21 and 25 mice.

Example 12

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC on LNCaP tumors in mice

LNCaP human prostate cancer cells (CD44 null) are implanted subcutaneously into 224 male nude BALB/c mice. The mice are divided into 28 groups of 8 mice (Table ~~11~~12).

Table 12

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC

	Saline	M-DNA or MCC 0.02 mg/kg	M-DNA or MCC 0.2 mg/kg	M-DNA or MCC 2.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Groups 13 and 25
HA at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Groups 14 and 26
HA at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Groups 15 and 27
HA at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC are administered intravenously on day 0 and at 3-day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastases are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice. Groups 6-8, 10-12, 14-16, 18-20, 22-24 and 26-28 have less tumor mass and fewer metastases than Groups 5, 9, 13, 17, 21 and 25 mice. These results show that treatment outcome does not depend on CD44 expression by the cancer cells.

Example 13

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC on B16F1 tumors in mice

B16F1 cancer cells are implanted intravenously into 224 female C57BL/6 mice. The mice are divided into 28 groups of 8 mice (Table 13).

Table 13

Effect of saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC on B16F1 tumors

	Saline	M-DNA or MCC 0.02 mg/kg	M-DNA or MCC 0.2 mg/kg	M-DNA or MCC 2.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Groups 13 and 25
HA-N at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Groups 14 and 26
HA-N at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Groups 15 and 27
HA-N at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, M-DNA, MCC, HA+M-DNA and HA+MCC are administered intratumorally in saline on day 0 and at 3 day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastases are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice. Groups 6-8, 10-12, 14-16, 18-20, 22-24 and 26-28 have less tumor mass and fewer metastases than Groups 5, 9, 13, 17, 21 and 25 mice.

Example 14

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on PC3 tumors in mice

PC-3 cancer cells are implanted subcutaneously into 224 male nude BALB/c mice. The mice are divided into 28 groups of 8 mice (Table 14).

Table 14

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on PC3 tumors

	Saline	CIS or 5-FU 0.1 mg/kg	CIS or 5-FU 1.0 mg/kg	CIS or 5-FU 10.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Group 13 and 25
HA-N at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Group 14 and 26
HA-N at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Group 15 and 27
HA-N at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, CIS, 5-FU, HA+CIS and HA+5FU are administered intravenously in saline on day 0 and at 3 day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastases are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice. Groups 6-8, 10-12, 14-16, 18-20, 22-24 and 26-28 have less tumor mass and fewer metastases than Groups 5, 9, 13, 17, 21 and 25 mice.

Example 15

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on LNCaP tumors in mice

LNCaP cancer cells (CD44 null) are implanted subcutaneously into 224 male nude BALB/c mice. The mice are divided into 28 groups of 8 mice (Table15).

Table 15

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on LNCaP tumors

	Saline	CIS or 5-FU 0.1 mg/kg	CIS or 5-FU 1.0 mg/kg	CIS or 5-FU 10.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Group 13 and 25
HA-N at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Group 14 and 26
HA-N at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Group 15 and 27
HA-N at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, CIS, 5-FU, HA+CIS and HA+5FU are administered intravenously in saline on day 0 and at 3 day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastasis are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice. Groups 6-8, 10-12, 14-16, 18-20, 22-24 and 26-28 have less tumor mass and fewer metastases

than Groups 5, 9, 13, 17, 21 and 25 mice. These results show that treatment outcome does not depend on CD44 expression by the cancer cells..

Example 16

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on B16F1 tumors in mice

B16F1 cancer cells are implanted subcutaneously into 224 female C57BL/6 mice. The mice are divided into 28 groups of 8 mice (Table 16).

Table 16

Effect of saline, HA, CIS, 5-FU, HA+CIS and HA+5-FU on B16F1 tumors

	Saline	CIS 0.01 mg/kg or 5-FU1.0 mg/kg	CIS 0.1 mg/kg or 5-FU 10.0 mg/kg	CIS 1.0 mg/kg or 5-FU 100.0 mg/kg
Saline	Group 1	Groups 5 and 17	Groups 9 and 21	Group 13 and 25
HA-N at 16.0 mg/kg	Group 2	Groups 6 and 18	Groups 10 and 22	Group 14 and 26
HA-N at 1.6 mg/kg	Group 3	Groups 7 and 19	Groups 11 and 23	Group 15 and 27
HA-N at 0.16 mg/kg	Group 4	Groups 8 and 20	Groups 12 and 24	Groups 16 and 28

Saline, HA, CIS, 5-FU, HA+CIS and HA+5FU are administered intravenously in saline on day 0 and at 3 day intervals for 4 weeks (10 injections) at which time the mice are sacrificed and tumor mass and number of metastases are determined. Groups 2 to 28 mice have less tumor mass and fewer metastases than Group 1 mice.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

We claim:

1. A composition comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier, wherein the HA potentiates the effect of the second anti-neoplastic agent on cancer cells.

2. The composition of claim 1, wherein the second anti-neoplastic agent is selected from the group consisting of a bacterial DNA, a bacterial DNA-bacterial cell wall complex, a synthetic oligonucleotide and a chemotherapeutic drug.

3. The composition of claim 2, wherein the bacterial DNA is *Mycobacterium phlei* DNA (M-DNA).

4. The composition of claim 2, wherein the bacterial DNA-bacterial cell wall complex is *Mycobacterium phlei* DNA (M-DNA)-*Mycobacterium phlei* cell wall complex (MCC).

5. The composition of claim 2, wherein the chemotherapeutic drug comprises ≤10% of a standard dose of the chemotherapeutic drug.

6. A method, wherein a composition comprising purified HA, a second anti-neoplastic agent and a pharmaceutically acceptable carrier is administered to a mammal having cancer in an amount effective to treat the cancer.

7. The method of claim 6, wherein the second anti-neoplastic agent is selected from the group consisting of a bacterial DNA, a bacterial DNA-bacterial cell wall complex, a synthetic oligonucleotide and a chemotherapeutic drug.

8. The method of claim 7, wherein the bacterial DNA is *Mycobacterium phlei* DNA (M-DNA).

9. The method of claim 7, wherein the bacterial DNA-bacterial cell wall complex is *Mycobacterium phlei* DNA (M-DNA)-*Mycobacterium phlei* cell wall complex (MCC).

10. The method of claim 7, wherein the chemotherapeutic drug comprises ≤10% of a standard dose of the chemotherapeutic drug.

11. A method, wherein a composition comprising purified HA is administered to a mammal in an amount effective to stimulate immune system cells to produce cytokines.

12. The method of claim 11, wherein the cytokines are selected from the group consisting of IL-6 and IL-12.

SEQUENCE LISTING

<110> Bioniche Life Sciences Inc.

Phillips, Nigel

Filion, Mario

<120> Hyaluronic Acid in the Treatment of Cancer

<130> 6857-22

<150> US 60/173,375
<151> 1999-12-28

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01562

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 00 41730 A (MEDITECH RESEARCH LIMITED) 20 July 2000 (2000-07-20) claims 1-12	1,2,5-7, 10-12
X	WO 99 02151 A (HYAL PHARMACEUTICALCORPORATION) 21 January 1999 (1999-01-21) claims 1-10	1,2,5-7, 10-12
X	WO 98 17320 A (HYAL PHARMACEUTICAL CORPORATION) 30 April 1998 (1998-04-30) claims 1-60	1,2,5-7, 10-12
X	WO 95 30423 A (NORPHARMCO INC.) 16 November 1995 (1995-11-16) claims 1-19	1,2,5-7, 10-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2001	28/05/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Siatou, E

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INTERNATIONAL SEARCH REPORT

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PCT/CA 00/01562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 20115 A (MILES INC.) 15 September 1994 (1994-09-15) the whole document -----	1-12

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/01562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0041730 A	20-07-2000	AU 2423100 A	01-08-2000
WO 9902151 A	21-01-1999	CA 2208924 A	09-01-1999
		AU 8203198 A	08-02-1999
WO 9817320 A	30-04-1998	WO 9407505 A	14-04-1994
		WO 9526193 A	05-10-1995
		WO 9529683 A	09-11-1995
		WO 9530423 A	16-11-1995
		WO 9606622 A	07-03-1996
		EP 0952855 A	03-11-1999
		US 5792753 A	11-08-1998
		US 6103704 A	15-08-2000
		US 5834444 A	10-11-1998
		US 5614506 A	25-03-1997
		US 5827834 A	27-10-1998
		US 5910489 A	08-06-1999
		US 6022866 A	08-02-2000
		US 5990095 A	23-11-1999
		US 6194392 B	27-02-2001
		US 5852002 A	22-12-1998
		US 5830882 A	03-11-1998
		US 5817642 A	06-10-1998
		US 5811410 A	22-09-1998
		US 6017900 A	25-01-2000
		US 5962433 A	05-10-1999
		US 5977088 A	02-11-1999
		US 5824658 A	20-10-1998
		US 6087344 A	11-07-2000
		US 5972906 A	26-10-1999
		US 5817644 A	06-10-1998
WO 9530423 A	16-11-1995	CA 2122519 A	30-10-1995
		AU 696373 B	10-09-1998
		AU 2402395 A	29-11-1995
		CN 1151118 A	04-06-1997
		CZ 9603089 A	14-01-1998
		EP 0760667 A	12-03-1997
		HU 75868 A	28-05-1997
		JP 9512797 T	22-12-1997
		SK 137996 A	05-08-1998
		WO 9407505 A	14-04-1994
		WO 9526193 A	05-10-1995
		WO 9529683 A	09-11-1995
		WO 9606622 A	07-03-1996
		WO 9817320 A	30-04-1998
		EP 0952855 A	03-11-1999
		US 5792753 A	11-08-1998
		US 6103704 A	15-08-2000
		US 5834444 A	10-11-1998
		US 5614506 A	25-03-1997
		US 5827834 A	27-10-1998
		US 5910489 A	08-06-1999
		US 6022866 A	08-02-2000
		US 5990095 A	23-11-1999
		US 6194392 B	27-02-2001
		US 5852002 A	22-12-1998
		US 5830882 A	03-11-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int...ational Application No
PCT/CA 00/01562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9530423 A		US 5817642 A	06-10-1998
		US 5811410 A	22-09-1998
		US 6017900 A	25-01-2000
		US 5962433 A	05-10-1999
		US 5977088 A	02-11-1999
		US 5824658 A	20-10-1998
		US 6087344 A	11-07-2000
		US 5972906 A	26-10-1999
		US 5817644 A	06-10-1998
WO 9420115 A	15-09-1994	AU 6362594 A	26-09-1994