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TECHNOLOGY UPDATE

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Novel therapeutics based on genomics data, being released at an accelerated pace, will dramatically change the way cancer is diagnosed, classified and treated. Synthetic nucleic acid sequences (SNAS), the topic of this issue, represent but one of numerous approaches to modulate tumor-related pathways instead of indiscriminately killing both healthy and diseased cells. If the SNAS field is of interest to you, learn more about it, and stay updated and informed of new developments by subscribing to NEW MEDICINE's Oncology KnowledgeBASE (nm|OK) residing at oncologyknowledgebase.com. In addition to a comprehensive review of all aspects of oligonucleotides as therapeutic modalities, nm|OK provides information on competitive agents based on different technologies/mechanisms as well as new target opportunities. Call NEW MEDICINE at (949) 830-0448 for a no-obligation walk-through nm|OK, and a temporary pass for you and your colleagues to evaluate this unique resource at your leisure.

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

SYNTHETIC NUCLEIC ACID SEQUENCE CONSTRUCTS AS ONCOLOGY THERAPEUTICS — PART III

Synthetic nucleic acid sequences (SNAS) were originally considered an elegant new way to regulate oncogenic activity with minimal host toxicity. Also, SNAS may be relatively easily designed against any well characterized mRNA or gene target, making them broadly applicable to various cancer types. In a way, this technology sector closely resembles that of monoclonal antibodies (MAbs) that was initially also hailed as the ultimate anticancer modality, only to become a powerful laboratory tool, and then, finally, to return as a technology widely used to develop numerous therapeutic approaches for a variety of indications, including cancer.

Antisense constructs have been designed against virtually every target, and have been invaluable in research to validate targets, and the actions of numerous agents *in vitro* and in animal models. Yet, although an antisense construct has been commercialized for a noncancer application (Isis Pharmaceuticals' Vitravene for CMV retinitis), the utility of SNAS in oncology has not been conclusively demonstrated. As cancer monotherapy, SNAS approaches appear unlikely, but these constructs show promise in combination therapies (Exhibit 1). In addition, as regulatory agents, SNAS may present a significant opportunity as chronically administered agents, similar to vaccines, to prevent tumor recurrences. However, considerable research and development efforts will need to be directed toward improving the effectiveness and delivery approaches of these agents for any chronic application to be feasible.

MOLECULAR TARGETS IN ONCOLOGY

Virtually any molecular marker may be targeted by SNAS, and SNAS have been designed to modulate numerous molecular markers or pathways associated with malignancy, such as:

- oncogenes (c-myc, myb, HER2, etc.)
- growth factors (EGF, VEGF, etc.)
- receptor proteins (EGFr, VEGFr, etc.)
- relay molecules such as Ras
- downstream effector proteins such as PKA and PKC
- tumor suppressor genes such as p53 and BRCA1
- viruses associated with malignancy (EBV, HPV, HBV, HCV, etc.)
- other effector molecules

This last installment of the 3-part series of SNAS as cancer therapeutics, presents preclinical and clinical data on various SNAS constructs, including combination therapy approaches (Exhibit 1). The development status of SNAS constructs in oncology is presented in Exhibit 2. In this article, only SNAS in clinical trials are described in great detail. More complete information regarding all aspects of SNAS and their targets is found in NEW MEDICINE's oncology knowledgeBASE (nm|OK), residing at www.oncology-knowledgebase.com.

C-myc

The c-myc gene, and the expression of the c-Myc protein, are frequently altered in human cancers, but the precise role of c-myc in malignancy remains obscure. The c-myc protooncogene is overexpressed in a variety of human cancers, and appears to play an important role in the proliferation of tumor cells. Hence, c-myc antisense oligonucleotides should be able to inhibit the proliferation of human cancer cells expressing c-myc constitutively (Smith JB and Wickstrom E, Methods Enzymol 2000;314:537-80). C-myc is the focus of several oligonucleotide constructs that are currently being evaluated in clinical trials (Exhibit 2), following extensive preclinical testing.

C-myc inhibition is cytostatic for normal and malignant lymphoid cells, and some Burkitt's lymphoma cells can be specifically growth-arrested *in vitro* with c-myc antisense. C-myc antisense is particularly effective against several solid tumors, including human and rat glioblastoma. In solid tumors, c-myc antisense oligonucleotides have, in addition to antisense effects, a sequence-specific, non-antisense mediated effect on cellular attachment to extracellular matrix (ECM).

When immunocompetent mice, administered subcutaneous injections of tumor cells from a transgenic mouse model of Burkitt's lymphoma, were treated with either a DNA phosphorothioate oligonucleotide complementary to c-myc codons 1-5 (myc6), or other c-myc-related oligonucleotides, for 7 consecutive days, beginning 1 day after tumor cell transplantation, treatment with myc6 delayed tumor onset by 3 days, decreased total tumor mass at sacrifice (17 days after tumor cell transplantation) by 40% ± 16%, and decreased the splenic Myc-to-actin ratio. Inhibition of tumors by myc6 and an immunostimulatory oligonucleotide, both of which shared a dACGTT motif, was comparable. Administration of an oligonucleotide sequence complementary to c-myc codons 384-388 (myc55) delayed tumor onset by 5-6 days, decreased total tumor mass at sacrifice by 65% +/- 6%, and reduced the splenic Myc-to-actin ratio to below that produced by myc6. A 14-day treatment regimen of myc55, alternating with an immunostimulatory oligonucleotide, completely inhibited tumor formation in a B-cell NHL model during the therapeutic schedule. Such an approach may be used to increase the number and duration of complete remissions obtained after standard chemotherapy for B-cell lymphoma, and many other solid tumors and leukemias (Smith JB and Wickstrom E, JNCI, 5 Aug 1998;90(15): 1146-54, and Smith JB and Wickstrom E, Adv Exp Med Biol 1998;451:17-22).

Exhibit 1
Combination Cancer Therapies Involving SNAS

Combinations Being Evaluated in Clinical Trials*	Combinations Evaluated Preclinically
Inhibition of c-myc	
Combination increased cisplatin sensitivity in metastatic melanoma	Increase in the number and duration of CR obtained after standard chemotherapy for B-cell lymphoma, and many other solid tumors and leukemias; inhibition of CYP3A2, a liver enzyme, that regulates metabolism of about 60% of all FDA-approved chemotherapeutics may improve combination therapy
Inhibition of c-myb	
Ex vivo purging of marrow in BMT candidates who underwent myeloablative chemotherapy with busulfan and cyclophosphamide	
Inhibition of c-raf	
Combination with 5-FU and leucovorin	Combination with doxorubicin against MCF-7 breast cancer, with cisplatin or mitomycin C, in NCI-H69 sclc, with cisplatin against PC3 human prostate carcinomas, and with mitomycin C against NCI-H460 large-cell lung carcinoma xenografts
Inhibition of ras	
Combination with gemcitabine in advanced solid tumors, and advanced or metastatic pancreatic cancer	
Inhibition of protein kinase C (PKC)-α	
Combination with carboplatin and paclitaxel, or 5-fluorouracil (5-FU) and leucovorin in various types of metastatic cancer	Additive antitumor effects were found in 50% of combinations with standard chemotherapeutic agents (cisplatin, mitomycin-C, vinblastine, estracyt and doxorubicin) in animal studies in various xenografts, with vinblastine or cisplatin demonstrating super-additive antitumor activity against MCF-7 human breast and PC3 prostate carcinoma xenografts, and with doxorubicin against BT20 human breast carcinoma, resulting in complete responses, and with mitomycin-C, against NCI-H460 human large-cell carcinoma
Inhibition of Protein kinase A (PKA)	
Combination with the taxanes docetaxel and paclitaxel	Combination of chimeric MAb (C225) against EGFr and anti-Rla antisense caused regression of tumor xenografts; futhermore combination of this construct with ionizing radiation (RT) and MAb C225 potentiated the antitumor activity in colon cancer (GEO) xenografts, resulting in growth regression and a significant improvement in survival compared with controls; cooperative antitumor activity was observed with docetaxel in ZR-75-1 human breast cancer cells; double blockade of these pathways using MAb C225 with docetaxel achieved apoptosis of a majority of ZR-75-1 cells at suboptimal doses of the three agents
Inhibition of bcl-2	
Combination with paclitaxel in solid tumors, including androgen-independent prostate cancer; with docetaxel in androgen-independent, metastatic prostate cancer and advanced breast cancer; with mitoxantrone in metastatic, hormone-refractory prostate cancer (HRPC); with conventional chemotherapy or cyclophosphamide in chemoresistant NHL; with DTIC in advanced malignant melanoma; with irinotecan in metastatic or recurrent colorectal cancer; with other antineoplastic drugs and filgrastim (G-CSF) in refractory or relapsed acute myeloid leukemia (AML), or acute lymphoblastic leukemia (ALL)	Significal enhancement effectiveness of dacarbazine (DTIC); reduction of Bcl-2 in melanoma, and possibly also in other tumors,may be a novel and rational approach to improve chemo-sensitivity; combination with cyclophosphamide completely eliminated human NHL transplanted into SCID mice in highly synergistic manner, eradicating disease; synergistic antitumor activity was demonstrated when combined with doxorubicin; substantially enhanced paclitaxel and docetaxel chemosensitivity in a dose-dependent manner in androgen-independent, recurrent tumors and hormone-refractory tumors; combination with mitoxantrone in estrogen receptor (ER)-negative and positive breast cancer cell lines; combination with sub-optimal doses of docetaxel, paclitaxel, and cisplatin, resulted in synergistic anti-tumor effects and complete tumor regression

— continued on next page

Inhibition of ribonucleotide reductase (RNR)	
	Combination with 5-FU or vinblastine produced complete tumor regression in SCID mice bearing human xenografts of renal cancer and combination with mitomycin C in murine models of human melanoma and human colon cancer; antitumor effects of other other chemotherapeutics, including gemcitabine, estramustine, and paclitaxel were enhanced in a standard human colon carcinoma mouse model
Inhibition of murine double minute (mdm)2	
	Synergistic effects were seen with 5-FU, 10-hydroxycamptothecin, platinum derivatives, topotecan, doxorubicin and taxanes, and CPT-11
Inhibition of apoptosis proteins (IAPs) including X-linked IAP (XIAP)	
	Antisense targeting of XIAP increased cell death following irradiation
Inhibition of thymidylate synthase (TS)	
	Antisense downregulation of TS may prove useful in enhancing the efficacy of TS-targeted drugs
Inhibition of cytochrome P450 3A2 (CYP3A2)	
	Enhancement of the effectiveness of many currently approved drugs, including paclitaxel
Inhibition of telomerase	
	Antisense telomerase therapy may represent a new chemosensitization approach of tumors resistant to anticancer drugs

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), January 2001
**See Exhibit 2*

When intravenously administered to immunocompromised mice bearing human melanoma or leukemia xenografts, microencapsulated anti-c-myc oligonucleotides, developed by Alkermes (Cambridge, MA) scientists, were more effective than unencapsulated oligonucleotides, as was indicated by reduced tumor growth, decreased number of metastases, reduced c-myc expression, and increased survival in the melanoma model, as well as decreased metastatic potential and increased survival in the leukemia model (Putney SD, etal, Antisense Nucleic Acid Drug Dev, Oct 1999;9(5):451-8).

Inhibition of c-myc expression may also indirectly influence the expression of the cytochrome P450 3A2 (CYP3A2) isoform, a liver enzyme that regulates metabolism of about 60% of all FDA-approved drugs (Arora V, etal, J Pharmacol Exp Ther, Mar 2000;292(3):921-8). Therefore, antisense alteration of gene expression *in vivo* may improve the anticancer activity of drugs like paclitaxel without an increase in side effects.

Oncomyc-NG (AVI-4126), under development by AVI BioPharma (Portland, OR), is one of the company's NeuGene antisense constructs directed against c-myc that has been shown to inhibit cancer cell growth *in vitro* and in animal models (Knapp DC, etal, AACR00, Abs. 4083). A phase I clinical trial of a NeuGene antisense, Restin-AG, in development for the prevention of restenosis, which is based on the same technology as Oncomyc-NG, was initiated in late 1999 and completed in April 2000. This study involving 32 patients showed IV administration of NeuGene anti-c-myc agents to be safe and essentially nontoxic. A phase I clinical trial of oral delivery of Restin-NG was initiated in July 2000, and completed in November 2000. Although targeting c-myc expression in restenosis, these studies are applicable to both the cardiovascular and anticancer formulations of the drug. Patients in the study were treated with increasing doses of Restin-NG, with ranges exceeding those anticipated to be used in future phase II and phase III clinical trials; patients were then monitored closely for several weeks. Researchers performing the oral delivery phase I study did not identify any drug-related safety issues, nor were any alterations in organ systems noted.

INX-3280, under development by Inex Pharmaceuticals (Burnaby, British Columbia, Canada), is being evaluated in a phase I/II clinical trial in up to 30 patients with solid tumors and lymphoma, being conducted at the British Columbia Cancer Agency (Vancouver, Canada) and the Jewish General Hospital in Montreal, based on an IND filed in October 1999 and approval in December 1999 by the Therapeutic Products Programme of Health Canada. This is a combination regimen of INX-

3280 and cisplatin. INX-3280 was shown by researchers at the Regina Elena Cancer Institute (Rome, Italy) to increase cisplatin sensitivity in a human metastatic melanoma derived from a metastatic lymph node (LM) that was resistant to cisplatin. When cisplatin and INX-3280 were administered to the LM line, a decrease in Myc protein levels of approximately 50% was observed, accompanied by a 65% reduction in tumor weight, similar to the 70% reduction observed when a cisplatin-sensitive melanoma line, derived from a primary tumor (LP) from the same patient, was treated with cisplatin alone. Cisplatin treatment by itself was did not induce apoptosis in the LM line; however, apoptosis was evident both after treatment with INX-3280 alone and, more extensively, in combination with cisplatin. These results indicate that increased Myc expression observed in the LM line may be responsible for cisplatin resistance (Leonetti C, etal, Clin Cancer Res, Sep 1999;5(9):2588-95).

These findings are in line with other preclinical results showing that INX-3280, in combination with cisplatin, inhibits proliferation and colony formation of melanoma cells to a greater extent than that observed with either agent alone; inhibition was most effective when cisplatin was followed by the oligonucleotide. Cytometric analysis revealed that INX-3280 induced an accumulation of cells in S phase, and apoptosis in a fraction of the cells, detectable at day 5 after the beginning of treatment, while cisplatin induced a block in G2-M phase detectable at day 1, which was partially recovered, and apoptosis similar in extent to that induced by the oligonucleotide. Together, the two agents induced arrest in G2-M phase, which was delayed compared to that induced by cisplatin alone. The combination induced a higher percentage of apoptosis, evident at day 3 from the start of treatment, that correlated with a significant reduction in Bcl-2 expression. Mice bearing human melanoma xenografts, treated sequentially with cisplatin and INX-3280, demonstrated a higher inhibition of tumor growth, reduction in the number of lung metastases, and increase in life span compared with those treated with either agent alone (Citro G, etal, Cancer Res, 15 Jan 1998;58(2):283-9).

Treatment of three established human melanoma cell lines (M14, JR8, and PLF2) with INX-3280 inhibited the growth of all melanoma cultures *in vitro*, whereas treatment with 15-mer oligomers containing c-myc sense and two different scrambled sequences, had no effect. Oligonucleotide treatment resulted in reduced cellular levels of Myc protein, with growth inhibition associated with the induction of apoptosis. In mice bearing NG xenografts, treatment with INX-3280 resulted in a significant inhibition of tumor growth, a reduction in the number of lung metastases, and an increase in life span; *in vivo*, antitumor activity and toxicity were both dose and schedule dependent, and only antisense oligomers exhibited antitumor activity. Reduced levels of Myc protein and increased levels of apoptosis were also observed in NG tumor cells following antisense treatment (Leonetti C, etal, JNCI, 3 Apr 1996;88(7):419-29).

C-myb

The c-myb gene product is a DNA-binding protein thought to regulate cell proliferation and differentiation in blood cell development, and like the c-myc gene, the c-myb gene is classified as a protooncogene. The encoded protein, Myb, is preferentially expressed in hematopoietic cells, and has been identified in a variety of malignant and normal hematopoietic cell types. Inappropriate expression of the c-myb gene inhibits the differentiation of blood cells in the body, resulting in blood cancers such as leukemia (Gewirtz AM, Oncogene, 13 May 1999; 18(19):3056-62, and Agarwal N and Gewirtz AM, Biochim Biophys Acta, 10 Dec 1999;1489(1):85-96).

INX-3001, is being clinically evaluated for its ability to purge bone marrow cells of clonogenic chronic myelogenous leukemia (CML) tumor cells *ex vivo* as part of an NCI-sponsored phase II clinical trial (protocol IDs: UPCC-3492, NCI-H94-0532) of autologous bone marrow transplant in the treatment of patients with chronic or accelerated phase CML. Although Inex is not a sponsor of this trial, it is supplying INX-3001 to Dr. Gewirtz's group, which is conducting the trial at the University of Pennsylvania Cancer Center (Philadelphia, PA). In the early 1990s, Dr. Alan M. Gewirtz's laboratory at the University of Pennsylvania established human leukemia-SCID mouse chimeras with K562 cells expressing the c-myb protooncogene, and treated diseased animals with INX-3001, then known as LR-3001 (Ratajczak MZ, etal, PNAS USA, 15 Dec 1992;89(24):11823-7, and Gewirtz AM, Leuk Lymphoma 1993;11 Suppl 1:131-7).

In the clinical trial, patients who have had myeloablative chemotherapy with busulfan and cyclophosphamide undergo autologous bone marrow transplant using marrow treated *ex vivo* with INX-3001 and filgrastim (G-CSF). Approximately 40 patients are being accrued for this study. Inex will determine the drug's future development path after analyzing trial results.

In December 1994, Lynx Therapeutics (Hayward, CA), the original developer of this approach, had initiated a physician-sponsored *in vivo* phase I dose-escalation clinical trial of LR-3001, at the University of Pennsylvania, in the treatment of Ph1+ CML in accelerated phase or blast crisis, using repeated courses of 7-day IV infusion is of this agent. In another phase I clinical trial, initiated in May 1994, bone marrow of CML patients not elligible for allogeneic BMT, was purged *ex vivo* and cryopreserved. This purged marrow was then re-infused to patients who had undergone myoablative therapy. In this trial, 1/4 evaluable patients experienced a durable cytogenic response.

Raf

Raf comprises a family of serine/threonine kinases including A-raf, B-raf, and C-raf; these proteins are immediate downstream mediators of ras in cell growth/differen-

tiation-based signal transduction pathways. Raf mutations have been linked to a variety of malignancies and are a current focus in both *in vitro* and *in vivo* therapeutic targeting studies. The role that the resultant deregulation of c-raf plays in malignant transformation and uncontrolled proliferation has attracted particular attention.

ISIS 5132, a phosphorothioated antisense oligonucleotide under development by Isis Pharmaceuticals (Carlsbad, CA), was designed to be complementary to the 3' untranslated sequences of c-raf mRNA. It has been evaluated in animal studies, and is currently in phase II clinical trials in a variety of solid tumors as monotherapy, and in phase I trials in combination with cytotoxic chemotherapy. Although phase II clinical trials of ISIS 5132 as a single agent are ongoing, Isis has de-emphasized clinical testing with this compound in favor of its other antisense agents, particularly ISIS 3521 and ISIS 2503.

When ISIS 5132 was added to 16 ovarian cancer cell lines exhibiting c-Raf-1 protein expression in amounts varying some 24-fold, 4% to 100% growth inhibition was evident in 12 of the 16 cell lines at 200 nM after 72 hours, compared to 0%-20% inhibition by a mismatch oligonucleotide. Antisense treatment also resulted in a 50% to 90% reduction in c-Raf-1 (McPhillips F, etal, AACR00, Abs. 4082:642).

Combination of ISIS 5132 with doxorubicin demonstrated antitumor activity in MCF-7 breast cancer xenografts, implanted subcutaneously in Balb-C nude mice. The combination of ISIS 5132 with cisplatin or mitomycin C showed superadditive antitumor activities against NCI-H69 sclc resulting in complete tumor responses. Superadditive antitumor activity with tumor cures was also observed with ISIS 5132 in combination with cisplatin against PC3 human prostate carcinomas, and in combination with mitomycin C against NCI-H460 large-cell lung cancer xenografts. A sequence-specific antisense mechanism of action was suggested based upon the lack of action of a mismatched control oligomer as a single agent against NCI-H69 human sclc, and the lack of enhanced antitumor activity against NCI-H69 cancers when the mismatched control oligonucleotide was combined with mitomycin C or cisplatin (Geiger T, etal, Clin Cancer Res, Jul 1997;3:1179-85).

One of the most challenging problems with antisense therapies involve the mode of administration. Early phase I clinical trials used continuous IV infusion which proved cumbersome. In a phase I study conducted at Baylor University Medical Center (Houston, TX), ISIS 5132 was administered by multiple 21-day continuous IV infusions followed by 1 week of rest per cycle. Patients with advanced solid tumors were treated with doses ranging from 0.5 to 5.0 mg/kg/day. Among 34 patients participating in this study, Grade 4 thrombocytopenia was observed in 2 (1 at 1.0 mg/kg/day, also associated with sepsis, and 1 at 5.0 mg/kg/day). Two other patients experienced Grade 3 thrombocytopenia (1 related to sepsis), 1 patient had Grade 4 fever and Grade 3 hypotension, and 2 patients experienced Grade 3 fatigue. Tumor responses occurred in 2 patients, 1 with ovarian cancer at 3.0 mg/kg/day, who presented with a 97% decrease in CA125 for over 7 cycles, and 1 with renal cancer, whose disease stabilized for 9 months before progression (Holmlund J, etal, ASCO98, Abs. 811:210a, and Cunningham CC, etal, Clin Cancer Res, May 2000;6:1626-31).

In another phase I clinical trial, conducted at Thomas Jefferson University's Kimmel Cancer Center (Philadelphia, PA), 31 patients with refractory solid tumors were administered ISIS 5132 as a 2-hour IV infusion, 3 times weekly for 3 of every 4 weeks; doses were increased from 0.5 mg/kg to 6.0 mg/kg in 9 cohorts. Transient low-grade fevers and fatigue were common, but not dose limiting. A trend towards increased complement component C3a activation was observed, and although MTD was not reached, dose escalation was halted at 6.0 mg/kg, at which the maximum plasma concentrations of ISIS 5132 approached that associated with complement activation in primates. One patient with metastatic colon cancer demonstrated 90% decrease in peripheral blood cell expression of c-Raf-1 protein, accompanied by a 20% reduction in disease lasting for 24 weeks. Also, disease stabilized in 1 patient with renal cell carcinoma for 10 courses of treatment before progression (O'Dwyer PJ, etal, ASCO98, Abs. 810:210a, and Stevenson JP, etal, J Clin Oncol, Jul 1999;17(7):2227-36). A reduction to a median of 42% of baseline within 48 hours of dosing was observed in 13/14 evaluable patients. This reduction occurred at all dose levels >2.5 mg/kg, and did not appear to exhibit a dose-response relationship (O'Dwyer PJ, etal, Clin Cancer Res, Dec 1999;5:3977-82).

ISIS 5132 has also been administered in a phase I trial at the University of Chicago Medical Center (Chicago, IL) by weekly 24-hour IV infusion to patients with advanced solid tumors or lymphoma; doses increased from 6 to 30 mg/kg/week. Among 19 treated patients, the most common toxicity was low-grade fever (n=15), with 1 patient experiencing Grade 3 fever at 18 mg/kg. More serious toxicities occurred at 30 mg/kg, including a Coombs-positive hemolytic anemia and renal failure, possibly related to tumor progression. At a dose of 18 mg/kg/week, complement activation was observed with increasing levels of C3a, blood pressure, and activated partial thromboplastin time (aPTT); however, no clinically significant sequelae resulted. Transient thrombocytopenia was seen in 9/18 patients at all dose levels, and did not appear to be dose-dependent. No objective tumor responses were observed (Holmlund JT, etal, ASCO99, Abs. 605:157a).

A phase II randomized study of ISIS 3521 and ISIS 5132 in the treatment of hormone-refractory prostate cancer is being conducted by the National Cancer Institute of Canada (protocol ID: CAN-NCIC-IND111), to enroll between 15 to 30 chemotherapy-naive patients who may continue LH-RH therapy while participating in this trial. Patients are treated either with IV ISIS 3521 or ISIS 5132

for 21 days, repeated every 4 weeks in the absence of unacceptable toxicity or disease progression. Follow-up is being conducted every 4 weeks, and then every 3 months until disease relapse or progression. There are no current plans to develop ISIS 5132 as a single agent for this indication beyond the conclusion of this trial, as may also be the case in ovarian cancer where phase II trials are also ongoing.

A phase II randomized, multicenter clinical trial (protocol IDs: 199/13258, E-3197) of ISIS 3521 and ISIS 5132, in women with previously treated metastatic breast cancer, was initiated in May 1998 by the Eastern Cooperative Oncology Group (ECOG) under the direction of Dr. William John Gradishar. Patients are stratified according to the number of prior therapies for metastatic breast cancer (1 versus 2), and randomized to 1 of 2 treatment arms, either ISIS 5132, administered IV continuously over 21 days (Arm I, enrollment closed 22 December 1999), or ISIS 3521, administered IV continuously over 21 days (Arm II). Treatment repeats every 4 weeks in the absence of disease progression or unacceptable toxicity, and after every 2 courses of therapy, patients are evaluated for response. Patients are followed every 3 months for the first 2 years, every 6 months the next 3 years, then annually thereafter. The projected accrual for this study is a maximum of 68 patients, 34 per treatment arm. There are no current plans to develop ISIS 5132 as a single agent for this indication beyond the conclusion of this trial.

ISIS 5132 is also being evaluated in combination with other chemotherapeutics. In an ongoing phase I clinical trial, being conducted at the University of Pennsylvania Cancer Center, 14 patients with refractory cancers have been treated with ISIS 5132 at doses of 1.0 to 3.0 mg/kg/day, delivered as a 21-day IV infusion, in combination with 5-FU (425 mg/m^2) and leucovorin (20 mg/m^2) as an IV bolus, on days 1-5, every 4 weeks; DLT in the form of neutropenia (Grade 4, n=3), thrombocytopenia (Grade 4, n=1), and mucositis (Grade 4, n=2) at the 3.0 mg/kg/day dose level precluded further escalation. Eight patients treated at the 2.0 mg/kg/day dose level experienced toxicities that included neutropenia, thrombocytopenia, diarrhea, and mucositis, but these were not dose-limiting. Disease stabilized in 3 patients (1 each with renal cell, colon, and pancreatic cancer) lasting 6 cycles (Stevenson JP, etal, AACR-NCI-EORTC99, Abs. 579).

LE-AON is a liposome-encapsulated oligonucleotide, being developed by NeoPharm (Lake Forest, IL), in collaboration with Georgetown University (Washington, DC). In return for sponsorship of supportive research, NeoPharm has received exclusive licenses to manufacture and sell products incorporating the University's electrostatic liposome encapsulation technology. NeoPharm is also obligated to pay the University royalties on commercial sales of products incorporating this liposome technology, and to make certain advance payments, which will be credited against future royalties. An IND to initiate a phase I clinical trial with LE-AON at Georgetown University Medical Center and Fox-Chase Temple Cancer Center (Philadelphia, PA) in patients with radiation-resistant solid tumors was filed in July 2000 and trials are expected to begin in early 2001.

Ras

Ras and Ras-subfamily proteins (N-ras, H-ras, Ka-ras, and Kb-ras), known collectively as p21ras, are small guanine nucleotide-binding proteins that have been clearly implicated in signal transduction and regulation of growth stimulatory signaling pathways, playing a pivotal role in the regulation of cell proliferation and cell death. Ras proteins transduce biological information from the cell surface to cytoplasmic components within cells. The signal is transduced to the cell nucleus through second messengers, and it ultimately induces cell division (Scharovsky OG, etal, J Biomed Sci, Jul-Aug 2000;7(4):292-8). Activation of ras genes has been associated with tumorigenesis and enhanced proliferation of tumors. Ras is mutated in about one third of all human cancers, and the many activating mutations in ras in human tumors make this an attractive target for SNAS approaches (Exhibit 2).

ISIS 2503, under development by Isis Pharmaceuticals, hybridizes to the translation initiation region of human H-ras mRNA resulting in RNase H-mediated cleavage of the target mRNA. According to the company, ISIS 2503 has displayed antitumor activity against a wide range of human cancer cell lines, and in human tumor types in mouse models. So far, the tumor type identified as being most sensitive to this oligonucleotide *in vitro*, is non-small-cell lung cancer (nsclc), although ISIS 2503 has also been shown to have potent antitumor activity against other tumor types, including bladder, breast and colon adenocarcinomas.

When ISIS 2503 was administered once daily by tail vein injection to mammary tumor-bearing MMTV-H-ras transgenic mice at doses of 20 and 50 mg/kg/day, tumor growth inhibition was observed after 6 days of treatment, with identical efficacy for the 2 dose levels, while a mismatched control oligonucleotide analog exhibited no effect on tumor growth. Also, after 10 days of treatment, treated animals demonstrated slower tumor growth recovery compared to controls (Petit T, etal, AACR99, Abs. 136:20).

Interestingly, the strain of mouse used in preclinical studies with ISIS 2503 affected the sensitivity of xenografted tumors to the oligomer, an important factor in experimental chemotherapy studies. Scientists at the Institute of Cancer Research (Sutton, Surrey, UK) and Isis treated two colonies of athymic nude mice, ICR (a closed outbred colony) and Balb-C, bearing subcutaneously implanted human MDA-MB-231 breast tumor xenografts, with ISIS 2503 injected IV at 25 mg/kg/day. After 21 days of treatment, tumors were 36% of control tumor volumes in the Balb-C mice, while no growth delay was observed in the ICR mice. Also, H-ras message decreased to 66% (day 8) and 68% (day 22) of control tumors in treated Balb-C mice,

with no decrease observed in the treated tumors in ICR mice. Aside from significant thrombocytopenia observed in the Balb-C mice, with a concurrent decrease in RBC counts on day 22, no other significant differences were observed in the two strains (Orr RM, etal, AACR-NCI-EORTC99, Abs. 591).

Two phase I clinical trials, varying in the scheduling of administration of ISIS 2503 monotherapy, have been conducted in patients with advanced solid tumors, refractory to standard chemotherapy. In a phase I clinical trial, performed at Indiana University (Indianapolis, IN), 19 patients, most with colon cancer, were administered ISIS 2503 at doses ranging from 3 mg/kg to 24 mg/kg via a 24-hour IV infusion, weekly, for three weeks. Three patients developed a hemolytic uremic syndrome during the first infusion; in 2 cases, the toxicity was self limiting and resolved in 3-7 days. The third episode resulted in acute renal failure requiring dialysis. Since two episodes occurred at the 24 mg/kg level, the MTD was determined to be 18 mg/kg. Other toxicities included mild fevers and Grade 2 allergic rashes. One Grade 3 thrombocytopenia case was seen coincident with reversible renal insufficiency. Pharmacokinetics showed linear and dose-related increases in ISIS 2503 serum concentrations; no clinical evidence of complement activation was observed. One patient with melanoma experienced a minor response in a liver metastasis lasting 27 weeks (Gordon MS, etal, ASCO99, Abs. 604:157a).

In a phase I clinical trial, conducted by the Physician Reliance Network (PRN) Research (Dallas, TX), ISIS 2503 was administered to 22 patients with advanced cancers at doses ranging from 1.0 mg/kg/day to 10.0 mg/kg/day by 14-day continuous IV infusion, followed by a 7-day rest. Toxicity included Grade 2 fevers in 2 patients at 10.0 mg/kg, and Grade 2 fatigue in 3 patients at 4.5 mg/kg or 10.0 mg/kg. Grade 2 thrombocytopenia, associated with bacteremia, was observed in 1 patient at 2.0 mg/kg. Pharmacokinetics demonstrated dose-related increases in steady state levels of ISIS 2503. Although no objective clinical responses were seen, disease stabilized in 4 patients for 6-10 cycles of treatment, including 1 patient with pancreatic cancer whose disease stabilized for 8 treatment courses administered over 6 months. While MTD was not defined, a dose of 6.0 mg/kg/day using this treatment schedule was chosen for phase II trials (Dorr A, etal, ASCO99, Abs. 603:157a).

A multicenter, phase II clinical trial of ISIS 2503, as first-line therapy of patients with previously untreated Stage IV or recurrent colorectal carcinoma is in progress. As of May 2000, 17 patients were treated with 38 cycles of ISIS 2503 at 6 mg/kg/day by 14-day continuous IV infusion, repeated every 21 days. Patients are evaluated for tumor response after every 3 treatment cycles, and treatment continues in patients with objective response or stable disease until disease progression. Toxicity has been limited to Grade 1-2 fever in the first 24-48 hours after starting the infusion in several patients, and Grade 1 thrombocytopenia in 3 patients. The best responses to date have been SD in 2 patients (6 and 3+ cycles), while disease progressed in 5 patients. Accrual is continuing to evaluate the activity of single-agent ISIS 2503 in this patient population.

A multicenter, phase II study of ISIS 2503 in patients with advanced (Stage IVa, III or IVb) adenocarcinoma of the pancreas was initiated in July 2000 (protocol IDs: UAB-9915, NCI-G00-1730, ISIS-2503-CS5, UAB-F990526011). Study objectives include determination of the response rate, and time-to-disease progression, as well as characterization of the safety profile for ISIS 2503 at the recommended 6 mg/kg/day dose administered by continuous IV infusion for 14 days, with treatment continuing every 21 days for a minimum of 3 courses in the absence of unacceptable toxicity, or disease progression. Projected accrual for this study is 14 to 27 patients. A multicenter, phase II trial of ISIS 2503 in patients with nsclc is also enrolling subjects (protocol ID: ISIS-2503-CS-7). ISIS 2503 will be administered at the same dose level and schedule as above to 15 to 30 patients.

Combination trials with ISIS-2503 and various chemotherapeutics are also ongoing. A phase I clinical study (protocol ID: ISIS-2503-CS3) to define the toxicity, pharmacokinetics and clinical activity of the combination of ISIS 2503 and gemcitabine (Gemzar; Lilly), in patients with advanced solid tumors, was initiated at the Mayo Clinic (Rochester, MN) under the direction of Alex A. Adjei, MD. As of May 2000, 11 patients had been treated with 28 courses, of gemcitabine (1000 mg/m^2), administered IV on days 1 and 8, and 2 escalating doses of ISIS 2503 (4 and 6 mg/kg/day) administered as a 14-day continuous IV infusion starting on day 1, and repeating every 21 days in the absence of disease progression or unacceptable toxicity. Common but non-dose-limiting toxicities have been hematologic, manifesting as neutropenia (Grade 2, n=1; Grade 3, n=1; and Grade 4, n=3) and thrombocytopenia (Grade 1, n=5; Grade 2, n=3; and Grade 3, n=2). Mild-to-moderate non-hematologic toxicities included anorexia, nausea and fatigue. Although data is limited, ISIS 2503 does not appear to alter the pharmacokinetics of gemcitabine. So far, 1 mixed response was observed in a heavily pretreated patient with metastatic breast cancer (Adjei AA, etal, ASCO00, Abs. 722:186a).

A multicenter, phase II clinical trial (protocol ID: NCCTG-N0043) also involving the combination of gemcitabine with ISIS 2503 in the treatment of advanced or metastatic pancreatic cancer was to begin enrolling patients in early 2001. Study objectives are to determine the response rate as well as 6-month and overall survival rates in patients treated with this regimen. A total of 20-44 patients will be accrued for this study, under the direction of Dr. Michael J. O'Connell of the North Central Cancer Treatment Group (NCCTG).

All the above studies involve or have involved IV formulations of ISIS 2503. In June 2000, Isis announced that a phase I study had been conducted demonstrating that

oral formulation of antisense drugs is achievable. At the joint meeting of the American Society for Pharmacology and Experimental Therapeutics (ASPET), and the American Society for Biochemistry and Molecular Biology (ASBMB), that convened in Boston, MA, in June 2000, scientists from OraSense (Carlsbad, CA), a joint venture of Isis and Elan (Dublin, Ireland), presented results of a dose-escalation study in 16 healthy volunteers that evaluated the impact of various absorption enhancers on the uptake of a solution formulation of ISIS 2503, delivered intrajejunally. The proprietary formulations tested produced therapeutically relevant blood levels of ISIS 2503, and analysis of plasma drug concentrations indicated that 10% to 15% bioavailability in human tissue is attainable. Abstracts for the ASBMB/ASPET 2000 meeting are presented in the 11 May 2000 issue of FASEB Journal (volume 14, issue 8).

Protein Kinases

Protein kinases (PK) are important molecules in the intracellular transduction of signals (growth factors, hormones, and neurotransmitters) that bind at the plasma membrane. At the molecular level, they serve as effective targets for drug design because overexpression of a variety of isoforms of the different protein kinase classes has been implicated in unregulated growth. The protein serine/threonine kinase A (PKA) and PKC types are targets particularly emphasized in current SNAS designs. In *in vitro*, xenograft, and clinical studies that are ongoing, antisense approaches appear to be effective against cancers that overexpress protein kinases.

PKA is involved in mediating intracellular responses to external signals that are involved in cell proliferation and oncogenesis. PKA is present in mammalian cells in two distinct isoforms with identical catalytic subunits but different cAMP-binding regulatory subunits, defined as RI in PKA type I (PKAI) and RII in PKA type II (PKAII); enhanced expression of the RIa subunit of PKAI is transiently detected in normal cells exposed to mitogenic stimuli, and detected in human cancer cell lines, and primary tumors, particularly in the most aggressive ovarian and breast cancers.

PKC comprises a family of ubiquitously expressed, closely related phospholipid-dependent enzymes that regulate cell growth and differentiation, and is believed to play an important role in tumorigenesis (Uchida N, etal, Oncol Rep, Jul-Aug 2000;7(4):793-6). In laboratory studies PKC was shown to stimulate the activities of urokinase plasminogen activator, matrix metalloproteinases and cell adhesion molecules, all of which are known to increase invasiveness in human cancer cell lines (Stoll BA, Eur J Cancer Prev, Apr 2000;9(2):73-9). At least 12 different isoenzymes of this kinase have been identified that are believed to play distinct regulatory roles. However, the nuclear PKC isoform α (PKC-α) is apparently both necessary and sufficient to promote cell cycle progression, and decreased PKC-α expression results in a significant decrease in cell proliferation *in vitro*. It has been suggested that PKC-α activity controls cell cycle progression through the upregulation of p21(Waf1/Cip1), which facilitates active cyclin-CDK complex formation (Besson A and Yong VW, Mol Cell Biol, Jul 2000;20(13):4580-90). Perturbations of PKC-α expression have been implicated in the growth and progression of various human tumors, including ovarian, liver, breast, gastric, brain, and colon cancer.

ISIS 3521, under development by Isis Pharmaceuticals, targeting PKC-α, was found to inhibit the growth of T-24 bladder carcinoma, A549 human lung carcinoma, and Colo 205 colon carcinoma xenografts in nude mice, in a dose-dependent fashion, with ID_{50} values for growth inhibition ranging (depending on cell line) between 0.06 and 0.6 mg/kg, daily, when administered IV. Three control oligonucleotides had no effect on the growth of the tumors at doses as high as 6 mg/kg (Dean N, etal, Cancer Res, 1 Aug 1996;56(15):3499-507). ISIS 3521 has also been extensively evaluated in nude mice, transplanted subcutaneously with a variety of human tumors, including breast, prostate, large-cell lung and small-cell lung cancer, and melanoma, in combination with standard chemotherapeutics (cisplatin, mitomycin-C, vinblastine, estracyt and doxorubicin). Additive antitumor effects were found for half of the combinations studied, with the combination of ISIS 3521 and vinblastine or cisplatin demonstrating superadditive antitumor activity against MCF-7 human breast carcinoma, and PC3 prostate carcinoma xenografts, resulting in complete responses. When combined with adriamycin, ISIS 3521 resulted in superadditive antitumor effects against BT20 human breast carcinomas with complete tumor responses, and in combination with mitomycin-C, superadditive antitumor effects with cures were observed against NCI-H460 human large-cell lung carcinomas. Two 20-mer control oligonucleotides were completely inactive as single agents against A549 and NCI-H69 human lung carcinomas, and the antitumor activity of cisplatin against NCI-H69 human sclc was slightly inhibited by one of the control oligomers, indicating that the mechanism of action of ISIS 3521 is indeed sequence-dependent (Geiger T, etal, Anticancer Drug Des, Jan 1998;13(1):35-45).

ISIS 3521 is currently in clinical trials both as a single agent as well as in combination with standard chemotherapeutic regimens. In a phase I clinical trial, conducted at Stanford University Medical Center (Stanford, CA) using a 21-day, continuous IV infusion dosing schedule, 21 patients with relapsed or refractory cancers (pancreatic, colon, stomach, lung, breast, and ovarian cancer and lymphoma) were administered ISIS 3521 at doses increasing from 0.5 to 3.0 mg/kg/day, with treatment repeating after a 7 day rest if well tolerated. Grade 2 thrombocytopenia was seen in 2 patients at 0.5 mg/kg/day to 1.5 mg/kg/day, and Grade 3 thrombocytopenia occurred in 1 patient at 2.0 mg/kg/day on day 7 of infusion. Platelet counts returned to normal in all 3 patients by the next treatment cycle. At 3.0 mg/kg/day, Grade 3 and Grade 4 DLT developed. Of the 4

Exhibit 2
Selected Syntheic Nucleic Acid Sequence (SNAS) Constructs in Oncology

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Status ❑ Indications➢Location
Aegera Therapeutics ❑ Canadian Genetic Diseases Network		Antisense oligos targeting mRNA encoding for the inhibitors of apoptosis proteins (IAPs), including X-linked IAP (XIAP) ❑ injection	Research (ongoing 11/00)➢Canada ❑ solid tumor
Alkermes		Phosphorothioate antisense oligo directed against the c-myc proto-oncogene, complexed with zinc carbonate and encapsulated in biodegradable, injectable microspheres ❑ injection	Preclin (no activity reported 10/99)➢USA ❑ melanoma, leukemia
AstraZeneca ❑ Isis Pharmaceuticals	ODN 83	Phosphorothioated 20-mer antisense oligo complementary to thymidylate synthase mRNA ❑ injection	Preclin (o12/00)➢USA ❑ solid tumors
Aventis Pharmaceuticals ❑ Ludwig Institute for Cancer Research, U L'Aquila, U College of London	HMR-5543, 5543-ODN	Antisense oligo targeting αV integrin gene ❑ injection	Research (o10/00)➢Europe ❑ solid tumors
AVI BioPharma ❑ Pharma-Eco, Boston BioSystems, Lorus Therapeutics, DepoMed	AVI-4126 ❑ Oncomyc-NG (Restin-NG)	20-mer antisense morpholino oligo targeting c-myc protooncogene mRNA; part of the NeuGene technology platform ❑ IV, PO	Phase I (completed 4/00)➢USA ❑ solid tumors
AVI BioPharma ❑ U Nebraska	TAG-6	Phosphorothioate oligo that acts as a telomere mimic, inhibiting telomerase activity ❑ IV, transdermal	Preclin (o12/00)➢USA ❑ cancer
AVI BioPharma ❑ Lorus Therapeutics, DepoMed		Antisense morpholino oligo targeting bcl-2 mRNA; part of the NeuGene technology platform ❑ injection	Research (o10/00)➢USA ❑ cancer
AVI BioPharma ❑ Lorus Therapeutics, DepoMed		Antisense morpholino oligo targeting the bcr-abl oncogene; part of the NeuGene technology platform ❑ injection	Research (o10/00)➢USA ❑ chronic myelogenous leukemia (CML)
AVI BioPharma ❑ Lorus Therapeutics, DepoMed, U Nebraska	AVI-3172	Antisense morpholino oligo targeting the liver enzyme cytochrome P450 3A2 (CYP3A4) mRNA; part of the NeuGene technology platform ❑ transdermal	Preclin (c12/00)➢USA ❑ cancer
AVI BioPharma ❑ Lorus Therapeutics, DepoMed, U Nebraska		Antisense morpholino oligo targeting p53 mRNA; part of the NeuGene technology platform ❑ intraperitoneal (IP)	Preclin (o12/00)➢USA ❑ cancer
Coley Pharmaceutical Group (CpG) ❑ Ludwig Institute for Cancer Research, U Iowa, Glaxo SmithKline, Isis Pharmaceuticals	CpG 7909	Synthetic CpG-containing DNA mimic capable of stimulating cellular and humoral immune responses ❑ IV, intradermal, intratumoral	Phase I/II (o10/00)➢USA ❑ relapsed or refractory non-Hodgkin's lymphoma (NHL); phase I/II (o10/00)➢Europe ❑ malignant melanoma, metastatic, basal cell carcinoma
Coley Pharmaceutical Group (CpG) ❑ U Iowa, Ludwig Institute for Cancer Research, Isis Pharmaceuticals	CpG 8916	Synthetic CpG-containing DNA mimic capable of stimulating innate immunity through activation of natural killer (NK) cells ❑ injection	Preclin (o10/00)➢USA ❑ melanoma, solid tumors
Coley Pharmaceutical Group (CpG) ❑ U Iowa, Ludwig Institute for Cancer Research, Isis Pharmaceuticals	CpG 8954	Synthetic CpG-containing DNA mimic capable of inducing *in vivo* interferon-α (IFN-α) production ❑ injection	Preclin (o10/00)➢USA ❑ solid tumor

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Coley Pharmaceutical Group (CpG) ❑ Ludwig Institute for Cancer Research, U Iowa, Isis Pharmaceuticals	CpG DNA	Synthetic oligos containing cytidine-phosphate-guanosine (CpG) dinucleotide motif; can act as DNA mimics with immunostimulatory activity ❑ IP, IV, intradermal, intratumoral	(See descriptions for CpG 7909, CpG 8916, and CpG 8954)
Columbia University	Ad-Rbz-Bcl-2 ❑ 001430	Adenoviral-mediated delivery of specific hammerhead ribozyme against bcl-2 mRNA ❑ injection	Preclin (o10/00)➢USA ❑ hormone-refractory prostate cancer
Columbia University	001863	Antisense oligos directed against bcl-xL mRNA ❑ injection	Research (o10/00)➢USA ❑ prostate and bladder cancer
Cytoclonal Pharmaceutics (CPI) ❑ U Texas		Antisense phosphorothioate oligo directed against bcl-2 mRNA; part of the OASIS technology platform ❑ injection	Research (o10/00)➢USA ❑ cancer
Cytoclonal Pharmaceutics (CPI) ❑ U Texas		Antisense phosphorothioate oligo directed against c-Raf-1 mRNA; part of the OASIS technology platform ❑ injection	Research (o12/00)➢USA ❑ cancer
Cytoclonal Pharmaceutics ❑ U Texas		Antisense phosphorothioate oligo directed against the a isoform of protein kinase C, PKC-α; part of the OASIS technology platform ❑ injection	Research (o10/00)➢USA ❑ cancer
Exiqon ❑ U Copenhagen, Karolinska Institute, Danish Cancer Society, Proligo		Locked nucleic acid (LNA) oligos; high-affinity, nuclease-resistant DNA analogs	Research (o10/00)➢Denmark, Sweden ❑ cancer
Gemini Technologies ❑ Cleveland Clinic Foundation, NIH	2-5A-anti-hTR	2-5A (2',5'-oligoadenylate)-linked, 19-mer antisense oligo targeting mRNA for the RNA component (hTR) of human telomerase ❑ injection	Peclin (o12/00)➢USA ❑ solid tumors
Gemini Technologies ❑ Cleveland Clinic Foundation, NIH		2-5A (2',5'-oligoadenylate)-linked antisense oligo targeting bcr-abl fusion mRNA ❑ *ex vivo*	Research (nar1998)➢USA ❑ CML
GeneSense Technologies ❑ AVI BioPharma, U Manitoba	GTI 2501	Phosphorothioate antisense oligo directed against the R1 subunit of ribonucleotide reductase (RNR) ❑ injection	Preclin (o12/00)➢Canada ❑ solid tumors
GeneSense Technologies ❑ AVI BioPharma, U Manitoba, Proligo	GTI 2040	Phosphorothioate 20-mer antisense oligo directed against the R2 subunit of ribonucleotide reductase (RNR) ❑ IV	Phase I/II (o12/00)➢USA ❑ solid tumors
Genta ❑ Molecular Biosystems, NCI, U Pennsylvania, Avecia LifeScience Molecules	Augmerosen ❑ G3139 ❑ Genasense	An 18-mer fully phosphorothioated antisense oligo targeting bcl-2 gene; the lead compound of the Anticode technology platform ❑ subcutaneous, IV	Phase I/II (b8/97, o10/00)➢USA ❑ solid tumors; phase I/IIa (b5/99, o10/00)➢USA (combination) ❑ metastatic, androgen-independent prostate cancer and advanced breast cancer; phase I (b11/98, o10/00)➢Canada (combination) ❑ metastatic, hormone-refractory prostate cancer; phase II (b1/99, o10/00)➢UK ❑ relapsed low-grade or follicular NHL; phase I/IIa (o10/00)➢Canada (combination) ❑ relapsed NHL; phase III (b3/00, o10/00)➢USA, Canada, Europe ❑ metastatic malignant melanoma; phase I/II (b4/00 and b6/00, o10/00)➢USA (combination) ❑ advanced, recurrent, sclc and recurrent colorectal cancer; phase I (o12/00)➢USA (combina-

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			tion) ❑ refractory acute AML, and refractory acute lymphoblastic leukemia (ALL); preclin (o10/00)➢Austria ❑ skin cancer, Merkel cell carcinoma; preclin (o12/00)➢Netherlands ❑ multiple myeloma; phase I (b01/01)➢USA (monotherapy) and phase III (b2/01)➢USA, Canada, Europe (combination) ❑ relapsed chronic lymphocytic leukemia (CLL)
Genta ❑ U North Carolina (terminated 11/1998), Molecular Biosystems		Fully phosphorothioated antisense oligos targeting focal adhesion kinase (FAK) mRNA; part of the Anticode (antisense) technology program ❑ injection	Preclin (discontinued 11/98)➢USA ❑ solid tumors
Genta ❑ Johns Hopkins U, Polska Akademia Nauk Centrum Badan Molekularnych I Makromolekularnych		Advanced-generation antisense oligos containing chirally pure methylphosphonate backbones; part of Genta's Anticode technology platform	Research (o10/00)➢USA ❑ cancer
Geron ❑ Kyowa Hakko Kogyo, Pharmacia (terminated 01/01), U Texas Southwestern Medical Center (UTSW), U California, U Colorado		Phosphoramidate antisense oligo targeting the RNA component (hTR) of telomerase ❑ injection	Research (o12/00)➢USA ❑ solid tumors
Humboldt-U zu Berlin	HP-DS1	Hairpin-derived twin ribozyme ❑ injection	Research (o10/00)➢Germany ❑ cancer
Hybridon	GEM 231 (formerly HYB 165)	End-modified, antisense 18-mer, mixed-backbone RNA/DNA oligo targeting the RIα cAMP-binding regulatory subunit of protein kinase A type I (PKAI) ❑ IV, intraperitoneal, PO	Phase I (combination) and phase II (o12/00)➢USA ❑ refractory solid tumors
Hybridon	GEM 220	End-modified, antisense 21-mer, mixed-backbone RNA/DNA oligo targeting vascular endothelial growth factor (VEGF) mRNA ❑ IV	Preclin (o1/01)➢USA ❑ solid tumors
Hybridon ❑ Genzyme Molecular Oncology, Searle (terminated 3/00), Louisiana State U, U Alabama, U Naples		Antisense mixed-backbone RNA/DNA oligo targeting the mdm2 oncogene ❑ injection	Preclin (o11/00)➢USA ❑ solid tumors
Hybridon ❑ Louisiana State U		Antisense mixed-backbone RNA/DNA oligos targeting cellular E6-AP ubiquitin ligase and the mdm2 oncogene ❑ injection	Research (o11/00)➢USA ❑ cervical cancer
Immusol	Id4 gene	Regulators and therapeutic molecules, including ribozymes, that increase the activity of the BRCA-1gene; identification of Id4 as a regulator of BRCA1 expression	Research (o01/01)➢USA ❑ breast and ovarian cancer
INEX Pharmaceuticals ❑ Duke U, Lynx Therapeutics	INXC-erbB2	Antisense oligo targeting erbB-2 (HER2/neu) mRNA, encapsulated in a Transmembrane Carrier System (TCS)	Preclin (suspended 12/98)➢ Canada ❑ breast cancer
INEX Pharmaceuticals ❑ Lynx Therapeutics, Regina Elena Cancer Institute	INX-3280 (formerly LR-3280)	Unencapsulated 15-mer phosphorothioate antisense oligo directed against the c-myc protooncogene ❑ IV	Phase I/II (o11/00)➢Canada ❑ solid tumors
INEX Pharmaceuticals ❑ Lynx Therapeutics		Phosphorothioate antisense oligo directed against VEGFr mRNA, encapsulated in TCS ❑ injection	Research (o1/01)➢Canada ❑ cancer

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INEX Pharmaceuticals ❑ Lynx Therapeutics, Regina Elena Cancer Institute	INXC-6295	15-mer phosphorothioate antisense oligo directed against the c-myc protooncogene, encapsulated in TCS ❑ IV	IND (approved 7/99)➢Canada ❑ solid tumors
INEX USA ❑ Lynx Therapeutics	INX-3001 (formerly LR-3001)	Unencapsulated 24-mer phosphorothioate antisense oligo directed against the c-myb protooncogene ❑ IV, *ex vivo*	Phase I (o11/00)➢USA ❑ accelerated phase or blast crisis CML; phase II (o11/00)➢ USA ❑ CML (*ex vivo* treatment)
INEX USA ❑ Thomas Jefferson U, Lynx Therapeutics	INX-4437	Unencapsulated antisense oligo targeting the gene encoding for the receptor that binds insulin-like growth factor type 1 (IGF-1r) ❑ injection	Preclin (s1999)➢USA ❑ solid tumor; phase I (c3/99)➢USA ❑ brain cancer
INEX USA ❑ Lynx Therapeutics	INXC-3001	24-mer phosphorothioate antisense oligo directed against the c-myb protooncogene, encapsulated in TCS ❑ IV	Preclin (o11/00)➢Canada ❑ leukemia
INEX USA ❑ Lynx Therapeutics	OL(1)p53 ❑ INX-3523 (formerly LR-3523)	Unencapsulated 20-mer antisense phosphorothioate oligo directed against p53 mRNA ❑ IV, *ex vivo*	Phase I (s1999)➢USA ❑ hematologic malignancies and *ex vivo* bone marrow purging
Introgen Therapeutics ❑ Aventis Pharmaceuticals, U Texas M.D. Anderson Cancer Center (UTMDACC)	RV-AS-K-ras, LNSX-AS-K-ras ❑ INGN-111	Retroviral vector-mediated transduction of K-ras antisense RNA	Phase I (s11/99)➢USA ❑ non-small-cell lung cancer (nsclc)
Introgen Therapeutics ❑ Aventis Pharmaceuticals, UTMDACC	AD-ScFV-ras ❑ INGN-212	Adenoviral K-ras H322a antisense vector	Preclin (o12/00)➢USA, Europe ❑ solid tumor; phase I (s11/99)➢ Europe ❑ colorectal cancer
Isis Pharmaceuticals ❑ Novartis Pharmaceuticals (terminated 11/99)	ISIS 3521, ISI641A, CGP64128A	20-mer antisense phosphorothioate antisense oligo inhibitor of PKC-α gene expression ❑ IV	Phase I (c8/99)➢USA ❑ relapsed or refractory solid tumors; phase II (o12/00)➢USA ❑ low-grade, refractory NHL; phase II (b8/97, o12/00)➢USA, Canada, Europe ❑ ovarian cancer; phase II (o12/00) ➢Canada ❑ hormone-refractory prostate cancer; phase I (o12/00) ➢USA (combination) ❑ advanced solid tumors, phase II (o12/00) ➢USA ❑ adult, recurrent brain cancer; phase III (o12/00)➢USA, Europe ❑ advanced nsclc; phase II (o12/00)➢USA ❑ breast cancer
Isis Pharmaceuticals ❑ Novartis Pharmaceuticals (terminated 11/99)	ISIS 5132, ODN698A, CGP69846A	20-mer antisense phosphorothioate oligo inhibitor of c-Raf-1 mRNA ❑ IV, PO	Phase I (c8/99)➢USA ❑ advanced solid tumors; phase II (o12/00)➢Canada, USA ❑ hormone-refractory prostate cancer; ovarian cancer, metastatic, refractory breast cancer; phase I (o12/00)➢USA (combination) ❑ advanced solid tumors
Isis Pharmaceuticals ❑ Elan	ISIS 2503	20-mer antisense phosphorothioate oligo inhibitor of H-ras mRNA ❑ IV, injection, PO	Phase I (c5/99)➢USA ❑ advanced solid tumors; phase II (o12/00)➢ USA ❑ colon and pancreatic cancer and nsclc; phase I (p12/00) ➢USA (combination) ❑ advanced solid tumor; phase II (p12/00)➢ USA ❑ locally advanced or metastatic pancreatic cancer
Isis Pharmaceuticals ❑ Elan		Second-generation, 2'-methoxyethyl-modified phosphorothioate antisense inhibitor of tumor necrosis factor-α (TNF-α) ❑ IV, PO	Research (o12/00)➢USA ❑ solid tumors

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Isis Pharmaceuticals ❑ Gilead Sciences	ISIS 16009	Antisense oligo directed against bcl-xL mRNA ❑ injection	Research (o10/00)➢USA ❑ solid tumors
Isis Pharmaceuticals		Advanced-generation methoxyethyl-modified phosphorothioate oligo targeting bcl-x pre-mRNA; induces expression of proapoptotic Bcl-xS protein ❑ injection	Research (o10/00)➢USA ❑ cancer
Isis Pharmaceuticals	ISIS 16601, ISIS 16609	Second-generation, 2'-methoxyethyl-modified 20-mer phosphorothioate antisense oligos targeting Wilm's tumor gene (WT1) RNA ❑ injection	Research (o12/00)➢USA, UK ❑ leukemia
Kennedy Krieger Research Institute	U1-ribozymes	Anti-c-met chimeric transgene consisting of U1 small nuclear RNA, a hammerhead ribozyme, and antisense sequences designed to inhibit expression of scatter factor/hepatocyte growth factor (SF/HGF or HGF/SF) ❑ intratumoral	Preclin (12/00)➢USA ❑ glioblastoma
Medical U South Carolina	Ad-aCaSm	Antisense oligo targeting cancer-associated Sm-like (CaSm) oncogene	Preclin (o10/00)➢USA ❑ pancreatic cancer
Medizyme Pharmaceuticals	Herzyme	Synthetic hammerhead ribozyme engineered to promote sequence-specific catalytic cleavage of HER2/neu mRNA in trans ❑ subcutaneous	IND (3/01)➢Canada ❑ breast cancer
MethylGene ❑ MGI Pharma	MG98	Second-generation RNA/DNA phosphorothioate antisense oligo targeting mRNA for human DNA-MeTase (DNA methyltransferase) ❑ IV, intraperitoneal (IP)	Phase I (b2/99, o11/00)➢USA, Canada ❑ solid tumors; phase II (b11/00)➢USA, Canada ❑ head and neck cancer
National Cancer Institute (NCI)		Antisense oligos directed against bog (B5T over-expressed gene) gene (also referred to as RBBP9) to restore sensitivity to TGF-β 1 in cancer cells overexpressing Bog protein ❑ injection	Research (o12/00)➢USA ❑ hepatocellular carcinoma
National Cancer Institute (NCI)		Antisense oligos and ribozymes complementary to human folate receptor-α (ahFR) mRNA ❑ injection	Research (o10/00)➢USA ❑ ovarian and cervical cancer
National Cancer Institute (NCI) ❑ Genta		Cyclic AMP response element (CRE)-decoy phosphorothioated aptamer ❑ injection	Research (o11/00)➢USA ❑ solid tumors, breast cancer
National Cancer Institute (NCI)	R434 (ribozyme), AntiE6 (antisense oligo)	Hairpin ribozymes and antisense oligos targeting HPV-16 E6/E7 mRNA ❑ injection	Preclin (o11/00)➢USA ❑ cervical cancer
NeoPharm ❑ Georgetown U, Isis Pharmaceuticals	LE-AON (Liposomal Encapsulated Antisense OligoNucleotide)	Liposome encapsulated formulation of antisense phosphorothioate oligo inhibitor of c-Raf-1 mRNA ❑ IV	IND (f7/00)➢USA ❑ radiation-resistant solid tumors
NeXstar Pharmaceuticals ❑ EyeTech Pharmaceuticals	NX 1838	Nuclease-resistant, RNA aptamer specific for the VEGF165 isoform of VEGF ❑ injection	Preclin (o1/01)➢USA ❑ cancer
Novartis Pharma		Bispecific antisense oligo directed against bcl-2 mRNA and bcl-xL mRNA ❑ injection	Research (o10/00)➢Switzerland ❑ solid tumor
Pharmacyclics ❑ U Texas	Dy-Tex	Ribozyme analog comprised of phosphoramidate derivative of dysprosium (III) texaphyrin covalently bound to a synthetic oligo ❑ injection	Research (o10/00)➢USA ❑ cancer
Ribozyme Pharmaceuticals (RPI) ❑ Chiron, U Colorado		Synthetic hammerhead ribozyme engineered to promote sequence-specific catalytic cleavage of ras mRNA in *trans* ❑ injection	Research (o12/00)➢USA ❑ solid tumors

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Ribozyme Pharmaceuticals (RPI) ❑ Chiron, U Colorado		Synthetic hammerhead ribozyme engineered to promote sequence-specific catalytic cleavage of c-myb mRNA in *trans* ❑ injection	Research (o11/00)➢USA ❑ solid tumors
Ribozyme Pharmaceuticals (RPI) ❑ Chiron, U Colorado, Competitive Technologies	RPI.4610 ❑ Angiozyme	Nuclease-stabilized synthetic hairpin ribozyme targeting mRNA of the Flt-1 (VEGFr1) receptor subtype for VEGF ❑ IV, subcutaneous, IP	Phase I/II (c11/00)➢USA ❑ solid tumors; phase II (b2/01)➢USA ❑ breast cancer; phase II (p2/01) ❑ renal, lung and colorectal cancer
Somagenics		Transplatin-modified (platinated) phosphorothioate oligo complemntary to vascular cell adhesion molecule-1 (VCAM-1) mRNA ❑ injection	Research (o12/00)➢USA ❑ solid tumors
Tel Aviv U ❑ National Institutes of Health (NIH)		Antisense oligo complementary to activity-dependent neurotrophic factor III/activity-dependent neuroprotective protein (ADNF III/ADNP) ❑ injection	Research (o10/00)➢Israel, USA ❑ solid tumors
Thomas Jefferson U		Antisense phosphorothioate oligo targeting the bcr-abl oncogene ❑ injection	Preclin (o10/00)➢USA (combination) ❑ CML
Thomas Jefferson U		Antisense DNA directed against the c-myc protooncogene ❑ injection	Preclin (o11/00)➢USA ❑ solid tumors, lymphoma, leukemia
U Alabama	GRO29A and GRO15A	Guanosine-rich oligos that bind to nucleolin, and abundant protein of the nucleolus implicated in cell proliferation ❑ injection	Preclin (nar1999)➢USA ❑ solid tumors
U Florida		Antisense RNA to aldehyde dehydrogenase class-1 (ALDH-1) ❑ injection	Research (o10/00)➢USA ❑ solid tumors, leukemia
U Massachusetts	Snorbozyme	Nucleolar RNA:ribozyme *trans*-acting hybrid ❑ injection	Research (o10/00)➢USA ❑ cancer
U Massachusetts		Peptide nucleic acid (PNA) oligos labeled with gamma-emitting radioisotopes for use as radiopharmaceuticals ❑ IV	Preclin (o10/00)➢USA ❑ solid tumors
U Massachusetts		Antisense oligos complementary to the mRNA of Epstein-Barr virus immediate-early replication genes BZLF1 and BRLF1 ❑ injection	Research (o10/00)➢USA ❑ cancer
U Texas M.D. Anderson Cancer Center (UTMDACC)	Ad5CMV-alphaVEGF	Recombinant adenoviral vector carrying the coding sequence for wild-type VEGF 165 isoform (VEGF165) cDNA in an antisense orientation ❑ injection	Preclin (o12/00)➢USA ❑ brain cancer
U Tsukuba (Japan)	Maxizyme	Heterodimer hammerhead ribozyme targeting bcr-abl oncogene ❑ injection, *ex vivo*	Preclin (o10/00)➢Japan ❑ CML
ValiGen ❑ Thomas Jefferson U	Chimeraplasts	Synthetic, self-complementary oligos composed of both RNA and DNA residues, capable of effecting site-directed genetic repair in mammalian cells *in vitro* and *in vivo* ❑ IV	Research (o10/00)➢USA ❑ cancer
Yale U ❑ NCI		Triplex-forming oligos (TFOs) designed to induce targeted intrachromosomal gene modification and DNA repair through site-specific recombination ❑ *ex vivo*	Research (o10/00)➢USA ❑ cancer

*Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|**OK**), January 2001*

*Note: Subscribers to NEW MEDICINE's Oncology KnowledgeBASE (nm|**OK**) may maintain this information perpetually updated by logging on to www.oncologyknowledgebase.com and entering the term "synthetic nucleic acid sequence" in the Drug Type field.*

ovarian cancer patients, 3 demonstrated evidence of tumor response, with 1 PR lasting 11 months, and 2 patients experiencing a decrease in CA125 of 40% and 76%, lasting 5 and 7 months, respectively. One patient with lymphoma had a PR, and disease stabilized in 1 patient with nsclc for 8 months. Results of this study suggested an optimal dose of 2.0 mg/kg/day when administered over a period of 21 days (Sikic BI, etal, ASCO97, Abs. 741:212a, and Yuen AR, etal, Clin Cancer Res, Nov 1999;5:3357-63).

Under another dosing schedule, as part of a phase I clinical trial, conducted at PRN Research and Baylor University Medical Center, 36 patients with a variety of cancers that were refractory to standard chemotherapy were administered ISIS 3521 at doses ranging from 0.15 to 6.0 mg/kg/day, as thrice-weekly IV infusions, each of a 2-hour duration, repeated for 3 of every 4 weeks. Toxicities were generally mild, with Grade 3/4 nausea most prevalent; Grade 2/3 thrombocytopenia occurred at 5.0 mg/kg/day. Transient, asymptomatic dose-dependent elevation of aPTT and C3a was also observed; dose escalation was terminated at 6 mg/kg/day because peak plasma levels approached the level (6 μM) associated with complement activation in primates. Two patients with non-Hodgkin's lymphoma (NHL) who completed 18 and 9 cycles of therapy, experienced CR, with no recurrence of disease in either patient after 21 and 14 months, respectively. Disease stabilized for 8 months in one patient with nsclc (Nemunaitis J, etal, ASCO97, Abs. 870:246a, Nemunaitis J, etal, ASCO98, Abs. 812:211a, and Nemunaitis J, etal, J Clin Oncol, Nov 1999;17(11):3586-95).

With an alternative dosing schedule of weekly 24-hour continuous infusions at doses ranging from 6 to 24 mg/kg, 11 patients with a refractory solid tumors experienced dose-dependent steady-state levels of ISIS 3521 that were higher than those observed with the 21-day infusion schedule. Grade 3 toxicities included fevers (n=1) and bleeding (n=1) at 18 mg/kg, and chills at 24 mg/kg (n=1). No clinical evidence of complement activation was seen. However, transient activation of complement split products, as well as increases in aPTT and PT were observed, particularly at doses of 18 mg/kg or higher. Disease stabilized for over 3 months in one patient with colon cancer (Advani R, etal, ASCO99, Abs. 609:158a, Advani R, etal, ASCO00, Abs. 809:208a).

In a phase I clinical trial, 18 patients (chemotherapy-naive=11) with metastatic cancer of various types such as nsclc (n=12), small-cell cancer of the cervix (n=2), esophageal cancer (n=1), sarcoma (n=1) and cancer of unknown primary (n=2), were treated with fixed doses of carboplatin and paclitaxel (175 mg/m^2), over 3 hours on cycle 1, and with ISIS 3521 by continuous IV infusion on days 1-14, and carboplatin and paclitaxel on day 4, on cycle 2; treatment was repeated at 21-day intervals, and doses of ISIS 3521 were escalated among patient cohorts from 1.0 to 2.0 mg/kg/day, while the dose of carboplatin was increased from an AUC of 5 to 6. According to final results reported by investigators from Stanford University Medical Center and Isis, the combination of ISIS 3521, carboplatin and paclitaxel was well tolerated and active in patients with advanced cancer. Toxicities during the first 2 cycles included Grade 3 diarrhea (n=1), Grade 3/4 neutropenia (n=12), Grade 3 thrombocytopenia (n=3), and Grade 3 fatigue (n=1), with no difference in toxicity between cycles 1 and 2; no DLT was observed during treatment at the maximum doses of ISIS 3521, carboplatin and paclitaxel, after 77 cycles of therapy. Among 16 evaluable patients assessed after cycle 3, 7 displayed PR, with all responses occurring among 10 patients with nsclc (Sikic BI, etal, ASCO99, Abs. 1718:445a, Yuen A, etal, AACR-NCI-EORTC99, Abs. 580:118, and Yuen A, etal, ASCO00, Abs. 1802:459a).

In an ongoing phase I clinical trial involving patients with advanced solid tumors, the safety and pharmacokinetics of ISIS 3521, administered over 21 days by continuous infusion, starting at 1.0 mg/kg/day and escalating to 2.0 mg/kg/day in subsequent cycles, is being assessed in combination with 5-FU (425 mg/m^2) and leucovorin (20 mg/m^2), administered on days 1-5 of each 28-day cycle. In a preliminary report on 10 patients having been administered 19 cycles of ISIS-3521 at doses of 1.0 (n=3), 1.5 (n=3) and 2.0 (n=4) mg/kg/day, combined with 5-FU and leucovorin, researchers at the University of Chicago Medical Center and Isis noted that MTD had not been reached. Two patients exhibited Grade 2 thrombocytopenia in cycles 1 and 2; nonhematologic toxicity included Grade 2 fatigue (n=5), mucositis (n=2), tumor pain (n=2), and anorexia/nausea (n=1 each). No cumulative toxicity was seen in patients treated with over 2 cycles of this regimen. Antitumor activity was observed with the combination, with 2 PR, one in a patient with flavopiridol-refractory colon cancer after 4 cycles of treatment, and the other in a patient with metastatic adenocarcinoma of unknown primary after >5 cycles. However, a randomized trial will be required to assess the additional effect of the antisense oligomer itself (Mani S, etal, ASCO99, Abs. 608:158a).

Based on results from phase I clinical trials demonstrating that ISIS 3521 was well tolerated and exhibited encouraging antitumor activity in advanced solid tumors, phase II clinical trials were initiated to evaluate the drug as single-agent therapy, and in combination with standard chemotherapeutics, for the treatment of various solid tumors, including ovarian, prostate, breast, brain, and lung cancer. These trials are being conducted at about 20 sites in North America and Europe.

In a multicenter phase II clinical trial, initiated in August 1997, ISIS 3521 has demonstrated a favorable safety profile and promising activity in patients with chemotherapy-sensitive as well as chemotherapy-resistant ovarian cancer. Among 14 patients with chemotherapy-sensitive disease, 4 patients experienced either a PR or long-term stabilized disease. Furthermore, among 20 patients with chemotherapy-resistant ovarian cancer, 4 experienced either a PR or their disease stabilized for a long term.

As noted earlier, a phase II randomized study of ISIS 3521 and ISIS 5132 in the treatment of hormone-refractory prostate cancer is being conducted by Canada's NCI, and a phase II randomized, multicenter study of ISIS 3521 and ISIS 5132 in women with previously treated metastatic breast cancer was initiated by the Eastern Cooperative Oncology Group in May 1998 (see discussion for ISIS 5132).

In a phase II clinical trial involving 15 patients with nsclc, 13 benefited from ISIS-3521 treatment through objective responses or stabilized disease lasting from > 2 months to > 13 months; 8 (53%) of the 15 patients experienced PR. As of November 1999, survival results from the study demonstrated that 7/15 (47%) patients lived one year or more with the longest survival at 21 months following study entry. After an average of 8 months of follow-up, 90% of the patients were alive and under evaluation.

As of November 2000, among 48 patients with Stage IIIb or Stage IV nsclc enrolled in completed phase I and II studies, 83% have benefited from treatment with ISIS 3521 through objective responses or stable disease. Two patients experienced CR, 18 PR, 4 of 48 (8%) had a minor response, and disease stabilized in 16 (9%). The average survival time was over 19 months, with a range of 3 months to more than 26 months; average survival time with currently approved therapies is approximately 8 months. Toxicities consisted of Grade 3/4 neutropenia and thrombocytopenia (Yuen A, etal, ASCO00, *ibid*, and Yuen A, etal, NCI-EORTC-AACR00, Abs. 529).

Because of the promising activity demonstrated among patients with nsclc in phase I and II trials, a phase III randomized study of ISIS 3521 at 2.0 mg/kg/day for 14 days, in combination with carboplatin (AUC of 6) and paclitaxel (175 mg/m^2), with treatment repeated every 21 days until maximum benefit, was initiated in September 2000 in patients with advanced nsclc, with the first patient starting the dosing protocol in October 2000. This multicenter trial is expected to involve 600 patients in approximately 60 clinical centers throughout the USA and Europe; patients will be randomized to chemotherapy alone and chemotherapy in combination with ISIS 3521. In November 2000, Isis announced that the FDA had granted fast track review status to ISIS 3521 for the treatment of nsclc.

A phase II study at the NABTT CNS Consortium (Baltimore, MD) is evaluating the response rate, time to progression, toxicity, and pharmacokinetics of ISIS 3521, administered at 2.0 mg/kg/day by continuous IV infusion for 21 days/month, in patients with recurrent, high-grade astrocytomas. Among 22 patients accrued to the first stage of this trial (median age=46), 4 patients had recurrent anaplastic oligodendrogliomas, 2 anaplastic astrocytomas, and 16 glioblastoma multiforme; 3 patients had no prior chemotherapy, 14 had one prior regimen, and 5 had two, while 17 patients were taking P450-inducing anticonvulsants. Toxicities were mild, reversible, and uncommon, with Grade 3 thrombocytopenia occurring in 3 patients, and Grade 4 SGOT in 1 patient; no coagulopathy or CNS bleeding was observed.

Although, in preclinical studies, ISIS 3521 was shown to cause regression of intracerebral and subcutaneous U-87 glioblastoma, there was no evidence of clinical benefit from ISIS 3521 in this trial; median time to progression was 35 days after entering the protocol, and MST was 93 days. The rapid deterioration seen in these patients may be the result of tumor growth and/or an effect of ISIS 3521 on blood-brain barrier integrity as 1 patient improved clinically after therapy was discontinued (Alavi JB, etal, ASCO00, Abs. 647:167a).

To determine whether poor drug delivery may explain the observed lack of efficacy of ISIS 3521 in patients with recurrent high grade astrocytomas, adult Fischer rats (n=22), implanted stereotactically with intracranial 9L-gliosarcoma, were administered ISIS 3521 either intravenously or intra-arterially. Seven days after implantation, one group of rats was administered tail vein injections of ISIS 3521 (20 mg/kg) while the other the same dose by intracarotid injection. Animals were sacrificed at 2 and 8 hours following drug administration, and examined for drug levels in the brain and kidneys. The kidney was found to contain the highest concentrations of ISIS 3521, with intact oligonucleotide concentrations 2 hours following IV injection about 40 times higher than the *in vitro* IC_{50} for ISIS 3521 for PKC-α mRNA and protein expression. The concentration of ISIS 3521 in brain tumor was about 40%-60% of the kidney concentrations 2 and 8 hours after IV or intra-arterial administration. The results of this study indicate that ISIS 3521 enters U-87 brain tumors in amounts that are many fold higher than the *in vitro* IC_{50}, that it does not cross intact blood-brain barrier, and that IA administration does not improve the delivery to brain tumors. Substantial concentrations of ISIS 3521 in this model suggests that poor drug delivery is probably not an explanation for the lack of efficacy in recent clinical trials (Jana P, etal, ASCO00, Abs. 660:170a).

A recent *in vitro*/animal study focused upon the potential of an antisense-oriented PKC-α cDNA-inserted plasmid as a therapeutic alternative to ISIS 3521. Given the observed overexpression of PKC-α in human lung carcinoma cells (LTEPa-2), clones were designed with an inserted antisense expression vector in hopes of controlling the endogenous PKC-α level and, thus, curbing cell proliferation. Upon determining that antisense PKC- α mRNA levels were high in vector-transfected cells, PKC-α protein was assayed showing a 3.5-fold lower level and a 60% decrease of enzyme activity in plasmid-containing cells compared to controls. These antisense PKC-α-expressing cells also inhibited growth rate and colony forming ability when compared to non-transfected control cell lines. Consistently, antisense expression in a murine host inhibited tumorgenicity; 40 days after injection, tumor mass was significantly smaller. This study, in addition to further characterizing of PKA-α's role in transcriptional control,

provides a novel recombinant antisense technology as a means of inhibiting PKC-α expression in lung carcinoma cells. Further exploration of this method is ongoing (Wang X, etal, Exp. Cell Research, 1999;250:253-63).

GEM 231 is a mixed-backbone RNA/DNA hybrid oligonucleotide that is specific for the RIα subunit of PKA, under development by Hybridon (Millford, MA). Overexpression of the RIα regulatory subunit of the PKAI isoform is associated with cell proliferation as well as neoplastic transformation, and is linked to poor prognosis in a variety of cancers making it a target for cancer therapy. Sequence-specific inhibition of RIα gene expression by antisense oligonucleotides has been shown to result in the suppression of RIα protein and PKAI, accompanied by the growth arrest of cancer cells *in vitro* and *in vivo*. The suppression of RIα by antisense is accompanied by rapid, compensatory increase in the half-life of the growth-inhibitory and differentiation-inducing RIIβ protein, via its stabilization in the PKAII holoenzyme complex, and persistent down-regulation of PKAI. This compensatory stabilization of RIIβ protein appears to represent a biological mechanism of RIα antisense that ensures depletion of PKAI and sustained inhibition of tumor growth (Cho-Chung YS, etal, AACR99, Abs. 3401:515, Cho-Chung YS, etal, Front Biosci, 1 Dec 1999;4:D898-907, and Cho YS, etal, PNAS USA, 18 Jan 2000;97(2):835-40).

PKA has been shown to be involved in the phosphorylation of bcl-2 as well as bcl-2-related targets, and Dr. Giampaolo Tortora of Universitá Federico II (Naples, Italy) and associates have shown that the double blockade of PKAI and bcl-2 by antisense exerts a cooperative effect on both growth inhibition and apoptosis of different human cancer cells *in vitro*. In addition, when GEM 231 was administered orally in combination with an antisense oligonucleotide targeting bcl-2 mRNA administered intraperitoneally, a significant cooperative antitumor effect and a prolongation of survival in nude mice bearing human cancer xenografts was observed (Tortora G, etal, AACR00, Abs. 4073:641).

In vitro, GEM 231 has been shown to inhibit tumor cell growth or colony formation of at least twelve different human tumor cell lines including colon, breast, ovary, prostate, and lung cancer, and lymphoma, and leukemia. *In vivo*, GEM 231 has been shown active in a dose-dependent fashion after oral dosing or intraperitoneal administration in animal models implanted with human tumor xenografts, including colon (LS174T, GEO), breast (MDA-MB-468), lung (A549), and ovarian (OVCAR-8) cell lines (Cho-Chung YS, Antisense Nucleic Acid Drug Dev, Fall 1996;6(3):237-44, Cho-Chung YS, Proc Assoc Am Physicians, Jan 1997;109(1):23-32, Zhang R, etal, AACR98, Abs. 3522:518, Miller WR, etal, AACR98, Abs. 4399:646, Alper O, etal, Oncogene, 2 Sep 1999; 18(35):4999-5004, and Wang H, etal, PNAS USA, 23 Nov 1999;96(24):13989-94). This and other recent experimental evidence also point to a functional interaction between PKAI and the epidermal growth factor receptor (EGFr) in the emergence and proliferation of certain malignancies.

Overexpression of EGF-related growth factors such as transforming growth factor (TGF)-α and amphiregulin and/or their specific receptor, EGFr, has been detected in several types of human cancers, including breast, lung, and colorectal cancer, suggesting the blockade of EGFr activation as a potential anticancer therapy. Experimental evidence indicates functional cross-talk between ligand-induced EGFr activation and PKAI expression and function; PKAI is overexpressed and activated following TGF-α-induced transformation in several rodent and human cell line models. PKAI is also involved in intracellular mitogenic signaling following ligand-induced EGFr activation. An interaction between EGFr and PKAI has been shown to occur through direct binding of the RIα subunit to the Grb2 adaptor protein. In this respect, PKAI seems to function downstream of the EGFr, and experimental evidence suggests that PKAI is acting upstream of the mitogen-activated protein kinase pathway (Ciardiello F and Tortora G, Clin Cancer Res, Apr 1998;4(4):821-8).

Taking advantage of the functional interaction between the EGFr and PKAI pathways, Dr. Tortora and associates have obtained cooperative inhibition of renal cancer growth *in vitro* and in SCID mice bearing ACHN human renal cancer xenografts using a chimeric MAb (C225) directed against EGFr and HYB 190, the anti-RIα antisense predecessor to GEM 231. Interestingly, while the combination treatment caused regression of tumor xenografts, single-agent treatment only delayed tumor growth (Ciardiello F, etal, JNCI, 15 Jul 1998;90(14):1087-94).

More recently, potentiation of antitumor activity was observed in mice bearing human colon cancer (GEO) xenografts exposed to ionizing radiation therapy (RT) in combination with MAb C225 and GEM 231. Long-term tumor growth regression was obtained following combination treatment, which produced a significant improvement in survival compared with controls, an RT-treated group, or a group treated with MAb C225 plus GEM 231 alone. All mice in the combination treatment group were alive 26 weeks after tumor cell injection, and 50% of mice in this group were alive and tumor-free after 35 weeks (Bianco C, etal, Clin Cancer Res, Nov 2000;6(11):4343-50).

GEM 231 has been shown to exhibit cooperative antitumor activity with docetaxel in ZR-75-1 human breast cancer cells. On the basis of the demonstration of a structural and functional relation between PKAI and EGFr, Dr. Tortora's laboratory has combined double blockade of these pathways using GEM 231 and MAb C225 with docetaxel to achieve apoptosis of a majority of ZR-75-1 cells at suboptimal doses of the three agents (Tortora G, etal, Clin Cancer Res, Apr 1999;5(4):875-81).

A phase I clinical trial of GEM 231 as monotherapy in patients with refractory solid tumors, initiated in January 1998 at Georgetown University Medical Center's Lombardi

Cancer Center, was completed in October 1998. In this study, 13 evaluable patients with nsclc (n=4), renal cell carcinoma (RCC; n=3), sarcoma (n=3), and other malignancies (n=3), were treated twice-weekly with escalating doses (20, 40, 80, 160, 240, and 360 mg/m^2) of GEM 231 administered as a 2-hour IV infusion over periods of up to 10 weeks duration. Single doses were well tolerated up to a level of 360 mg/m^2 (equivalent to 7.3 to 9.2 mg/kg), while 240 mg/m2 was determined to be the MTD for repeated administration. Treatment-related toxicities included Grade 1/2 fever and flu at the 240 mg/m^2 dose level over an 8-week regimen. Further DLT included a transient elevation of aPTT in 1 patient, and reversible Grade 3 transaminase increase at 240 and 360 mg/m^2. Although this study was not designed to demonstrate efficacy, 1 patient with colon cancer treated at 360 mg/m^2 demonstrated stabilization of a previously rising CEA at the end of the treatment period (Chen H, etal, ASCO99, Abs. 610:159a, and Chen HX, etal, Clin Cancer Res, Apr 2000;6(4):1259-66).

Having demonstrated enhanced cooperative activity in preclinical testing, two phase I clinical trials were initiated in July 1999 at the Weiler Hospital of the Albert Einstein College of Medicine, a division of Montefiore Medical Center (Bronx, NY), to evaluate the safety and preliminary antitumor activity of GEM 231 in combination with taxanes in patients with recurrent or refractory solid tumors. Both trials are under the direction of Dr. Sridhar Mani. One trial (protocol IDs: AECM-1199906197, NCI-G00-1666, HYBRIDON-231-100A) will evaluate GEM 231 in combination with docetaxel (Taxotere; Aventis Pharmaceuticals), while the other (protocol IDs: AECM-1199906196, NCI-G00-1665, HYBRIDON-231-100B) combines GEM 231 with paclitaxel (Taxol; Bristol-Myers Squibb Oncology). Patients will be treated by docetaxel (paclitaxel) IV over 1 (3) hour(s) on day 1, immediately followed by GEM 231 IV over 2 hours on days 1, 4, 8, and 11 (1, 4, 8, 1, 15, and 18), with treatment continuing every 3 weeks in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are to be treated with escalating doses of GEM 231 and docetaxel (or 1 of 2 doses of paclitaxel) until MTD is determined. Patients will be followed monthly for 3 months, and a maximum of 1 patient will be accrued per week for this study until the MTD is reached.

Reporting at the 11th NCI-EORTC-AACR Symposium on 10 of 12 patients with chemotherapy refractory advanced cancer having been administered 18 or more cycles of paclitaxel plus GEM 231, Dr. Mani noted that 6 patients were treated with schedule A involving paclitaxel (140-175 mg/m^2) combined with twice weekly GEM 231 (220-240 mg/m^2), while 4 patients were treated with schedule B involving paclitaxel (175 mg/m^2) with GEM 231 (240 mg/m^2). The MTD for schedule A was 175 mg/m^2 of paclitaxel and 240 mg/m^2 of GEM 231 while for schedule B MTD had not been reached. One patient developed fatal intrapulmonary hemorrhage attributed to progressive disease in the context of persistent aPTT elevation. DLT included Grade 3/4 transaminitis (n=3) after infusion dose 2, 6, and 8 of GEM 231. Other Grade 3 toxicities included hyperglycemia (n=1, cycle 2) and diarrhea (n=1, cycle 1). Hematologic toxicity was mild. One patient developed Grade 2 thrombocytopenia in cycle 1. Transient aPTT elevations were observed in 3 patients with each dose of GEM 231 that normalized to baseline within 3 days. Cumulative transaminitis was noted in 2 patients treated with over 4 doses of GEM 231 (Mani S, etal, NCI-EORTC-AACR00, Abs. 534).

In another report at the 11th NCI-EORTC-AACR Symposium on 12 of 14 patients with chemotherapy refractory advanced cancer having been administered 18 or more cycles of docetaxel plus GEM 231, Dr. Mani noted that 6 patients each were treated with schedule A involving docetaxel (50 mg/m^2) with twice weekly GEM 231 (220 mg/m^2) without a scheduled break, or with schedule B involving docetaxel (50-75 mg/m^2) with GEM 231 (220 mg/m^2). The MTD for schedule A was determined to be 50 mg/m^2 of docetaxel and 220 mg/m^2 of GEM 231; MTD for schedule B had not been reached. DLT included Grade 3/4 transaminitis (n=2) after infusion dose 4 and 8 of GEM 231; other Grade 3 toxicities included hyperglycemia (n=1, cycle 1 and 2), and neutropenia (n=1, cycle 1). Hematologic toxicity was mild. One patient with hepatoma had clinically insignificant intraperitoneal blood with normal coagulation parameters while on GEM 231 therapy; 3 patients had transient aPTT elevations with each dose of GEM 231 that normalized to baseline within 3 days. Cumulative transaminitis was noted for 1 patient treated with over 4 doses of GEM 231 (Mani S, etal, NCI-EORTC-AACR00, Abs. 535).

A limited phase II clinical effort has been underway since October 1998, with GEM 231 monotherapy, to explore the utility of various alternative dosing schedules (ranging from 24-hour to 14-days continuous infusion). This experience will allow optimal coordination with different established antitumor therapies, and will assist in the selection of initial tumors to treat in expanded phase II trials.

Bcl-2

Bcl-2-related proteins consititute one of the most biologically important classes of apoptosis regulating molecules. This family of proteins contains at least 14 members, of which some function as apoptosis antagonists (Bcl-2, Bcl-xL, Bcl-w, Bfl-1, Bag-1, Mcl-1 and A1), while others promote cell death (Bax, Bak, Bcl-xS, Bad, Bik, Bid and Hrk). Apoptosis has been shown to be regulated by several signals, including the bcl-2 gene. Bcl-2 interacts with other gene products, such as Bax, through the production of different dimers, which leads to the regulation of apoptosis. Bcl-2 homodimers block apoptosis, whereas Bax homodimers promote apoptosis. Bax can bind to Bcl-2 protein and neutralize the ability of Bcl-2 to block cell death, and the ratio of Bcl-2 to Bax is thought to determine

the susceptibility of a cell to death. Excessive expression of Bcl-2 is associated with chemotherapy resistance in many cancers including malignant gliomas and melanomas, NHL, multiple myeloma and breast, prostate, liver and lung cancer (Miyashita T, etal, J Biol Chem, 3 Nov 1995;270(44):26049-52).

Various preclinical evaluations have confirmed the effectiveness of bcl-2 inhibition as an anticancer strategy and various SNAS strategies are being employed to inhibit bcl-2 and its related proteins (Exhibit 2). When human glioma cell lines were treated with 4 different oligonucleotides designed to be specific for the bcl-2 mRNA translation start site (AS-1), a downstream coding region in close proximity to the start site (AS-2), a 3' end coding region (AS-3), and a mismatched control sequence, cellular proliferation was effectively inhibited with AS-1 and AS-2, and this reduction was amplified when the oligonucleotides were used in combination with etoposide (Chen ZG, etal, AACR99, Abs. 4171:632). Consistent with initial predictions, when combination treatments were employed, there was a concomitant decrease in Bcl-2 and apoptotic blebbing in these cells. In another similar *in vitro* study, a decline in Bcl-2 was measured in a prostate cancer epithelial cell line transfected with a bcl-2 antisense gene, and growth factor withdrawal as well as doxorubicin-inducing apoptotic events were confirmed (Campbell MJ, etal, BJC 1998;77(5):739-44).

Genasense (G3139), a fully phosphorothioated 18-mer antisense oligonucleotide, designed to hybridize to the 3' untranslated region of bcl-2 mRNA, under development by Genta (Lexington, MA), has been shown to effectively down-regulate the expression of bcl-2, resulting in increased chemosensitivity and decreased growth of a variety of tumors. G3139 has been evaluated as a single-agent, and in combination studies with other antitumor drugs, and continues to be the focus of a variety of clinical trials.

Despite containing two CpG motifs resembling the consensus sequence for immune stimulation, no consistent evidence has been presented to support a cell-mediated (TH1) response, or natural killer (NK) cell activation for G3139 in humans as was observed with CpG-containing oligonucleotides in rodents. Rather, G3139 has been shown to bind in a sequence-specific fashion to the open reading frame of bcl-2 mRNA, causing antisense-mediated inhibition of Bcl-2 protein production, leading to increased tumor cell apoptosis and enhanced chemosensitivity (Waters JS, etal, ASCO00, Abs. 48:14a).

Investigators from the University Medical Center (Utrecht, the Netherlands) report that G3139 exhibits significant activity against multiple myeloma cell lines and myeloma cells taken directly from patients. Results demonstrate a time- and dose-dependent uptake of G3139 into myeloma cells. Following uptake, levels of Bcl-2 mRNA and protein were consistently reduced after approximately 48 hours. Myeloma cells were also tested with a standard chemotherapy drug (doxorubicin), alone or in combination with G3139; the antisense treated cells showed significantly higher levels of cell death relative to controls (van de Donk N, etal, ASH00, Abs. 3273:757a).

In a study of immune-deficient mice bearing human breast cancer MDA435/LCC6 xenografts, G3139 treatment lasting >3 weeks reduced Bcl-2 protein levels by up to 97%, which correlated with histologic evidence of tumor cell death during the first week of treatment. However, expression of Bcl-2 returned to pretreatment levels during the second week of the 3 week treatment schedule in some xenografts, and in all cases tumors were re-established after antisense treatment was completed, implicating resistance to G3139 or possibly outgrowth of resistant subclones. When G3139 was combined with doxorubicin (either free drug or a sterically stabilized liposomal formulation), synergistic antitumor activity was demonstrated, while the agents showed only modest antitumor activity when used alone, and a control (non-Bcl-2 specific) antisense drug was ineffective. Pharmacokinetic analysis revealed that plasma doxorubicin levels were unaffected by G3139 treatment, whereas elevated tumor drug levels were obtained with systemic G3139 administration compared with drug treatment in the absence of G3139, suggesting possible positive G3139-drug interactions at the tumor site (Lopes de Menezes DE, etal, AACR00, Abs. 4079:642, and Lopes de Menezes DE, etal, Clin Cancer Res, Jul 2000;6(7):2891-902).

At the University of British Columbia (Vancouver, British Columbia, Canada) and British Columbia Cancer Agency, scientists treated 518A2 melanoma cells with bcl-2 antisense encapsulated in programmable fusogenic vesicles (PFV). PFV are liposomes composed of non-bilayer lipid components stabilized by the inclusion of an exchangeable PEG-lipid conjugate. Vesicle destabilization by loss of the PEG-lipid results in recovery of the inherent fusogenic character, so that PFV can be designed to display a long circulation lifetime after IV injection, high accumulation at disease sites, and full bioavailability of an encapsulated compound. At a concentration of 0.5 mM, an encapsulated bcl-2 antisense oligomer resulted in about 20% reduction of bcl-2 mRNA level after 48 hours of incubation. In contrast, free antisense bcl-2 did not affect the gene expression, and encapsulated control antisense (reverse) led to a non-specific increase in mRNA level (Hu Q, etal, AACR00, Abs. 2063:325).

In June 2000, Genta announced that G3139, in combination with cyclophosphamide (Cytoxan; Bristol-Myers Squibb), completely eliminated human NHL transplanted into immune-deficient mice. While low-dose cyclophosphamide alone produced only short-term effects, the combination of both drugs was highly synergistic. Eradication of disease was documented by both histologic and molecular criteria. No effects, either alone or in combination, were observed when reverse-matched or mismatched control antisense compounds were used.

In an animal model of human melanoma, pretreatment of mice with G3139 significantly enhanced effectiveness of dacarbazine (DTIC). In 10 of 13 pretreated animals tumors were completely eliminated. Also, in a separate experiment, G3139 monotherapy was significantly more efficacious in reducing tumor growth than three other control treatments. According to investigators at the Department of Clinical Pharmacology, University of Vienna, in Austria, reduction of bcl-2 expression by antisense oligonucleotides improves chemosensitivity of human melanoma grown in SCID mice. Findings suggest that reduction of Bcl-2 in melanoma, and possibly also in other tumors, may be a novel and rational approach to improve chemosensitivity and treatment outcome (Jansen B, etal, Nature Medicine, 1998 Feb, 4(2):232-4).

Using a murine model of human Merkel cell carcinoma, researchers at the University of Vienna have also shown that G3139 is both highly effective and superior to cisplatin (Platinol; Bristol-Myers Squibb) in the treatment of this disease. Merkel cell carcinoma is an aggressive neuroendocrine skin tumor with a high metastatic potential. Thought to derive from the neuroendocrine (Merkel) cells of the skin, Merkel cell carcinomas express high levels of the Bcl-2 oncoprotein in contrast to fetal and especially adult Merkel cells. After 28 days, cisplatin treatment had no effect in reducing tumor size compared with controls, whereas G3139 resulted in a 90% reduction in average tumor size. Moreover, some of the G3139-treated animals were apparently cured of the disease. G3139 also appeared to be better tolerated, and while cisplatin treatment resulted in an average weight loss of 10%, animals treated with G3139 showed normal or improved weight throughout the period of cancer treatment (Schlagbauer-Waadl H, etal, J Invest Dermatol, Apr 2000;114(4):725-30).

Treatment of androgen-independent chemoresistant mouse Shionogi tumor cells with murine antisense bcl-2 oligonucleotides decreased expression of bcl-2 mRNA approximately 85% compared with treatment with a mismatch control oligomer, whereas paclitaxel treatment did not alter bcl-2 mRNA expression despite inducing Bcl-2 protein phosphorylation. Antisense treatment also substantially enhanced paclitaxel chemosensitivity in a dose-dependent manner, and characteristic apoptotic DNA laddering and cleavage of poly (adenosine diphosphate-ribose) polymerase were demonstrated only after combined treatment. Adjuvant *in vivo* administration of antisense oligomer and micellar paclitaxel following castration, resulted in a statistically significant delay of androgen-independent, recurrent tumors compared with administration of either agent alone. Combination therapy also statistically significantly inhibited growth of established hormone-refractory tumors compared with treatment with either agent alone (Miayake H, etal, JNCI, 5 Jan 2000;92(1):34-4, and Miayake H, etal, Cancer Res, 15 Aug 1999;59(16):4030-4). Similar results were observed *in vitro* and *in vivo* in murine Shionogi tumor models with murine antisense bcl-2 oligonucleotides in combination with docetaxel (Gleave ME, etal, Urology, Dec 1999;54(6A Suppl):36-46).

When athymic male mice bearing subcutaneous human prostate LNCaP tumor xenografts, were castrated and injected intraperitoneally with G3139, a 10%-50% decrease in precastrate tumor volume was observed compared to a gradual 5-fold increase in tumor volume in control mice 12 weeks after castration. After decreasing 70% by 1 week after castration, PSA increased 1.6-fold above precastrate levels in 11 weeks in controls while staying 30% below precastrate levels in antisense-treated mice. In a second group of experiments, LNCaP tumor growth and serum PSA levels were 90% lower in mice treated with G3139 compared with mismatch or reverse polarity oligomer controls (Gleave M, etal, Clin Cancer Res, Oct 1999;5(10):2891-8).

In nude mice subcutaneously implanted with human prostate LNCaP tumors and then castrated, mean tumor volumes at 14 weeks post-castration were 66% of baseline for combination therapy with G3139 and paclitaxel, compared to 2.5- and 2.9-fold increases over baseline for paclitaxel alone and G3139 alone, respectively. Mean PSA values mirrored tumor volume at 14 weeks for all three cohorts. Because murine tolerability for the combination of paclitaxel and G3139 (a human bcl-2 sequence) could not be assessed in the LNCaP xenograft model, the combination of paclitaxel and a murine sequence bcl-2 antisense oligonucleotide was administered to mice bearing the androgen-independent Shionogi tumor, and found to be tolerable at effective doses (Tolcher AW, etal, AACR98, Abs. 2837:417). A similar result was observed when G3139 was combined with mitoxantrone (Tolcher A, etal, AACR99, Abs. 3198:484).

Antisense G3139 and control (reverse or two-base mismatch) oligonucleotides were also tested against two estrogen receptor (ER)-negative breast cancer cell lines (MDA-MB-231, MDA-MB-435) and one ER-positive cell line (MDA-MB-361) *in vitro* as well as against these tumor cell xenografts in mice; all three cell lines express a high level of Bcl-2 protein. Treatment with G3139 induced apoptosis and inhibited cell growth *in vitro*, and significantly enhanced apoptosis and growth inhibition were observed in G3139-treated cells following exposure to docetaxel or paclitaxel; these effects were not observed in cells treated with control oligonucleotides. Treatment with G3139 alone inhibited xenograft tumor formation *in vivo* by 60% to 90% in a dose-dependent manner, and when G3139 was combined with suboptimal doses of several different chemotherapy drugs, synergistic antitumor effects and complete tumor regression were observed with docetaxel, paclitaxel, and cisplatin; mice remained tumor free for more than 5 months (Yang D, etal, AACR99, Abs 4814:729).

In a murine model of human follicular lymphoma, SCID mice bearing established DoHH2 human lymphoma xenografts were treated for up to 21 days with G3139;

treated animals became disease free, remaining so at 30 days post-treatment, with no observed toxicity. In contrast, all animals treated with control sequences continued to exhibit disease. Treatment effects were strongly dependent on dose, duration, and route of administration, with subcutaneous infusion resulting in less excretion and metabolism of the administered dose compared with IV bolus (Raynaud FI, etal, J Pharmacol Exp Ther, Apr 1997;281(1):420-7).

In August 1997, a phase I/II dose escalation study (protocol IDs: MSKCC-97096, NCI-G97-1337, GENTA-G3139-97/01) of G3139 in the treatment of advanced solid tumors, including androgen-independent prostate cancer, was initiated at Memorial Sloan-Kettering Cancer Center (MSKCC; NY, NY), with Howard I. Scher, MD, Chief of the Genitourinary Oncology Service at MSKCC, as the Study Chair. In phase I, with a targeted accrual of 42 patients, G3139 is administered IV for 14-21 days to cohorts of 3 patients, followed by a 1-2 week rest; patients are initially treated with one course of G3139 alone and if tolerated, are administered G3139 in combination with paclitaxel, weekly, in subsequent courses. Patients are administered up to 3 courses of therapy, and dose escalation in a new cohort is undertaken after at least 3 patients treated at a given dose complete 14 days of drug treatment and 2 weeks of follow-up. In phase II, with a projected accrual of 15 patients, G3139 is administered continuously over 21 days in combination with weekly paclitaxel at one dose level below MTD. In the absence of toxic effects and disease progression, patients are treated with up to 2 more courses of therapy, and are followed every 3 months until disease progression.

In reporting on 35 patients having been treated in this trial (prostate cancer=23, RCC=4, prostate and renal=3, and 1 each with unknown primary, sarcoma, and esophageal, bladder, and rectal cancer), of which 27 were administered G3139 alone at 0.6-4.1 mg/kg/day, and 8 were administered G3139 alone for 1 cycle at 4.1 mg/kg/day, 5.3 mg/kg/day, and 6.9 mg/kg/day, followed by G3139 plus paclitaxel (100 mg/m^2), weekly x 3 for 2 cycles, 2 patients (6%) experienced port-related clots or infections. Drug-related Grade 3 adverse events were leukopenia, fatigue, and rash in 1 (3%) patient each; there were no Grade 4 drug-related events. Additional events noted with paclitaxel were Grade 1/2 mucositis (n=2, 6%), leukopenia (n=3, 9%), thrombocytopenia (n=2, 6%), abnormal LFT (n=3, 9%), and hyperglycemia (n=4, 11%); 2 of 3 patients in the 6.9 mg/kg/day G3139 cohort developed Grade 1/2 transaminitis on day 14, at full and again at modified doses. Western blots of peripheral blood lymphocytes in patients treated at a dose of 4.1 mg/kg/day revealed that Bcl-2 protein expression decreased within one week, with the peak effect seen at 8-15 days. A radiographic and clinical response to treatment was seen in 1 patient treated at 6.9 mg/kg/day, another experienced pain relief, while disease stabilized in 3 patients with RCC. Patient accrual is continuing and further study is ongoing to establish whether the time needed to achieve peak effect of protein expression decreases at higher doses (Scher HI, etal, ASCO00, Abs. 774:199a).

In an earlier report on 15 evaluable patients (prostate cancer=11, RCC=2, and prostate and RCC=2), administered G3139 for 14 days (cohorts of 3 patients each were treated with 0.6, 1.3, 1.7 mg/kg/day; 6 patients were treated with 2.3 mg/kg/day), followed by 4 weeks of observation, no toxicities exceeded Grade 1/2 were observed with the exception of 1 patient, being treated at the 2.3 mg/kg/day level, who developed Grade 3 neutropenia that resolved in 24 hours. Disease stabilized in 2 patients with RCC and in one with prostate cancer with 3, 3 and 2 treatments, respectively (Morris MJ, etal, ASCO99, Abs 1243:323a).

Increased expression of the bcl-2 gene has been observed in prostate cancer cells after androgen withdrawal, and has been associated with the development of androgen independence and chemoresistance. In July 2000, Genta launched of two prostate-specific phase I/II clinical trials as an extension of its ongoing phase I/II study of G3139 in advanced solid tumors. The similarly designed trials use G3139 in combination with docetaxel in the treatment of androgen-independent, metastatic prostate cancer, and are being conducted by Dr. Anthony W. Tolcher of the Cancer Therapy Research Center (San Antonio, TX) and by Dr. Howard I. Scher at MSKCC. The objective of these studies is to determine whether G3139 can enhance docetaxel cytotoxicity and delay disease progression.

In November 2000, data presented at the New Drugs in Cancer Therapy Initiatives Symposium held in Amsterdam, the Netherlands, indicated early positive results for G3139 in the treatment of advanced prostate cancer. As reported by Dr. Tolcher, while several patients among the first 13 enrolled in the trial had failed treatment with another taxane, 4 of the first 8 patients who had not been previously treated with a taxane showed objective antitumor responses on the combined G3139/docetaxel program. These responses included more than 10-fold reductions in serum PSA, as well as major shrinkage of liver and lymph node metastases. Side-effects of the combination were similar to those expected from the use of docetaxel alone.

A phase I/II dose-escalation trial of G3139 in combination with mitoxantrone in patients with metastatic, hormone-refractory prostate cancer (HRPC), was initiated in November 1998, at the British Columbia Cancer Agency with Dr. Richard Klasa as the PI. G3139 (0.6 to 3.1 mg/kg/day) was administered on an outpatient basis with a 14-day continuous IV infusion every 28 days, and mitoxantrone (4 to 12 mg/m^2) as an IV bolus on day 8. In an interim report, 18 patients having completed at least 1 treatment cycle, at doses of G3139 up to 3.1 mg/kg and mitoxantrone up to 12 mg/m^2, and evaluable for toxicity among 21 treated at seven dose levels, demonstrated tran-

sient toxicities which included Grade 3 neutropenia, and Grade 2 lymphopenia, as well as Grade 1 fatigue, arthralgia and myalgia. Antitumor activity was observed in 8 patients who demonstrated reduction of tumor size, improved tumor-related symptoms, reduction of serum tumor markers, and/or stabilization of previously progressive disease (Kim NC, etal, ASCO00, Abs. 1299:330a).

In May 1999, a phase I/II dose-escalation study was initiated at Georgetown University Medical Center under the direction of Dr. Daniel F. Hayes, to determine the MTD and pharmacokinetics of the combined therapy of G3139 and docetaxel in advanced breast cancer (and other solid tumors). In a 28-day cycle, G3139 was administered as a 21-day continuous infusion in addition to weekly docetaxel (35 mg/m^2) on days 8, 15 and 22. In an interim report on 8 evaluable patients (breast cancer=5, and 1 each head and neck cancer, nsclc, and sclc), all with documented Bcl-2 expression in their tumors, treated with G3139 at escalating doses of 1 mg/kg/day, 2 mg/kg/day and 3 mg/kg/day, Grade 3 thrombocytopenia was observed in 1 patient (3 mg/kg/day) with sclc and a history of pelvic radiation who was found to have myelophthisis with extensive tumor infiltration. Transient Grade 3 thrombocytopenia was also seen in 1 patient during intrapatient dose escalation. Other side effects included Grade 1/2 fatigue, Grade 1 mucositis, Grade 2 neutropenia (n=1), and Grade 1 transaminitis (n=1). Serial analysis of the peripheral blood lymphocytes demonstrated Bcl-2 downregulation at G3139 doses >2 mg/kg/day; at dose level of 3 mg/kg/day, maximum Bcl-2 reduction could be seen by day 3 of the G3139 infusion regimen. Tumor response was observed in 2 patients with breast cancer (Chen HX, etal, ASCO00, Abs. 692:178a).

An NCI-sponsored phase I/II clinical trial (protocol IDs: UCCRC-10017, NCI-T98-0091) of G3139 in combination with paclitaxel in recurrent sclc was initiated in April 2000, at the University of Chicago Cancer Research Center, with Charles M. Rudin, MD, as Study Chair. This study will enroll 19 to 33 patients with recurrent sclc, who are to be stratified according to whether they were previously exposed to taxanes. Treatment consists of IV G3139 administered continuously for 1 week, followed by 2 weeks of rest, with IV paclitaxel administered over 3 hours on day 6 of each course. Treatment lasts for a minimum of 2 courses in the absence of disease progression or unacceptable toxicity, and intrapatient dose escalation is allowed. Trial objectives include assessment of feasibility of paclitaxel administration during continuous IV delivery of G3139, and toxicity as well as evaluation of any correlation between Bcl-2 expression and treatment efficacy.

In late 1995, a phase I/II clinical trial of G3139 in the treatment of drug-resistant NHL was initiated at the Royal Marsden Hospital (Sutton, Surrey, UK), and the Institute of Cancer Research, under the direction of Drs. David Cunningham and Justin Waters. The phase I portion of this study was completed in 1997. A phase II study was initiated in January 1999 to examine the safety and efficacy of G3139 when combined with conventional chemotherapy in the treatment of chemoresistant NHL.

According to results involving 21 NHL patients previously treated with several conventional chemotherapy regimens, entered into the phase I/II study, treatment was well tolerated at G3139 doses up to 108.4 mg/m^2/day, with no significant systemic toxicity observed (8 cohorts of patients were treated with a 14-day subcutaneous infusion of G3139 at doses ranging from 4.6 to 195.8 mg/m^2/day). Local skin inflammation at the infusion site was seen in all patients, but was manageable in all but 2. DLT was represented by thrombocytopenia, fever and lethargy. In addition, one patient treated at 147.2 mg/m^2/day developed Grade 3 hypotension and fever. MTD was 147.2 mg/m^2/day (approximately 4 mg/kg/day). Of 20 patients evaluable for response, there was 1 CR (30+ months), 2 MR, and disease stabilized in 9, and progressed in 9. Circulating lymphoma cells were reduced in 5 of 12 assessable patients, and lymphoma-related symptoms were reduced in 6 of 9 evaluable patients. Bcl-2 protein was shown to be reduced after treatment in 7 of 16 assessable patients. No evidence for a TH1 response or NK cell activation was found in 4 patients assessed for immune response (Waters JS, etal; ASCO99, Abs 11:4a, and Waters JS, etal, J Clin Oncol, May 2000;18(9):1812-23).

In preliminary reports on the phase I study published in 1997 and 1998, no treatment-related toxicities except local inflammation at the infusion site were observed among 9 patients administered G3139 as a daily subcutaneous infusion for 2 weeks, with dose escalation from 4.6 mg/m^2/day to 73.6 mg/m^2/day. CT scans revealed a reduction in tumor size in 2 patients (MR + CR). The number of circulating lymphoma cells decreased in 2 patients during treatment, and serum concentrations of lactate dehydrogenase fell in 4 patients, with symptoms improving in 2. Bcl-2 protein levels declined in 2/5 patients. The mean terminal half-life of G3139 was 9.4 hours as evaluated by noncompartmental analysis, and elimination half-life was 18.2 hours using compartmental modeling (Webb A, etal, Lancet, 19 Apr 1997, 349(9059):1137-41, and Raynaud F, etal, AACR98, Abs. 3543).

In January 1999, Genta initiated a phase I/IIa clinical trial of G3139 in patients with relapsed NHL, at the British Columbia Cancer Agency under the direction of PI, Dr. Richard Klasa, to evaluate the safety and efficacy of G3139 in combination with cyclophosphamide.

In October 1999, the FDA assigned fast-track designation to G3139 for use with dacarbazine (DTIC) in the treatment of malignant melanoma. In March 2000, Genta initiated of a randomized, multicenter, open-label phase III clinical trial (designated GM-301) of G3139 in combination with DTIC in the treatment of patients with advanced malignant melanoma. This trial is being conducted at major cancer centers and oncology practices in North America and Europe. Patients will be randomly assigned to

either DTIC (1000 mg/m^2), infused over 60 minutes (Arm A), or to G3139 (7 mg/kg/day), administered as a continuous IV ambulatory infusion, daily, for 5 days, followed immediately by DTIC (1000 mg/m^2) infused over 60 minutes (Arm B). Treatment in this study is provided in 21-day cycles. According to Dr. Raymond P. Warrell, Jr, Chairman of Genta, in remarks made in September 2000 at the Anticancer Biopharmaceuticals conference in San Francisco, long-term survival of patients in the original phase I/II study (initiated at the University of Vienna in June 1998) now exceeds a median of one year, and patient accrual to the phase III trial remains on target for completion by mid-summer 2001.

With follow-up extending beyond one year, Dr. Burkhard Jansen of the University of Vienna has reported highly encouraging rates of durable response and survival from the phase I/II clinical trial. Treatment of malignant melanoma with G3139 plus DTIC resulted in downregulation of the Bcl-2 protein in 10 of 12 evaluable patients, with 20%-70% (median 40%) reductions in protein levels in tumor biopsy samples compared to baseline. In this trial, G3139 was administered by either continuous IV infusion or twice daily subcutaneous injections, and was combined with DTIC (800 to 1000 mg/m^2), which was started on day 5 of G3139 therapy. Antisense therapy was well tolerated, with no overlapping toxic reactions observed in patients treated with monthly cycles for up to 1 year. Side effects of G3139 included transient fever, flushing, and a rash in 2 patients that resolved with antihistamines. Also, 2 patients treated with G3139 at a dose of 6.5 mg/kg/day developed transient liver function abnormalities of uncertain drug relation. Six of 14 (43%) evaluable patients responded to treatment, including 1 biopsy-proven CR in a 90-year old woman with metastatic disease, 2 PR, and 3 minor responses, with several responses sustained for over 1 year. The MST in this generally heavily pretreated patient population was 7+ months (Jansen B, etal, AACR00, LB23, and Jansen B, etal, Lancet, 18 Nov 2000;356:1728-33).

At a follow-up of >6 months of patients in the phase I/II trial, Dr. Jansen reported that survival was predicted to exceed that observed in other recent studies of DTIC monotherapy. Several patients had survived with stable or responsive disease, and the median survival had not been reached. Prolonged antitumor activity, lasting over one year in some cases, was observed in patients treated second-line after relapse from other first-line therapies. Favorable safety and pharmacologic profiles had been noted for G3139 with both subcutaneous and IV administration, and no patients had discontinued G3139 therapy because of adverse events (Jansen B, etal, ASCO99, Abs 2049:531a).

In June 2000, the NCI commenced a phase I/II dose escalation study (protocol IDs: SACI-IDD-98-32, NCI-T98-0094, UTHSC-IDD-98-32) of G3139 in combination with irinotecan (Camptosar; Pharmacia) for the treatment of metastatic or recurrent colorectal cancer. The study is being conducted under a CRADA at the San Antonio Cancer Institute (SACI; San Antonio, TX) and Ireland Cancer Center (Cleveland, OH), with Dr. Anthony W. Tolcher of SACI as Study Chair. In this multicenter study, patients are administered IV G3139, continuously, on days 1-7, and IV irinotecan over 90 minutes on day 6; treatment continues every 21 days in the absence of unacceptable toxicity or disease progression. Cohorts of 3-6 patients will be administered escalating doses of G3139 and irinotecan until MTD is determined; once the MTD is determined, additional patients will be accrued to undergo treatment with G3139 and irinotecan at the recommended phase II dose. Patients are followed every 30 days until toxicity resolves. The projected accrual is 18 patients for the phase I portion of this study, and 55 patients for the phase II portion.

In October 1999, a phase I dose-escalation trial (protocol IDs: OSU-99H0257, NCI-T99-0057, OSU-9977) of G3139 in combination with other antineoplastic drugs for the treatment of refractory or relapsed acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) was initiated by the NCI at Arthur G. James Cancer Hospital (Columbus, OH) and Ohio State University (Columbus, OH) under the direction of Dr. Guido Marcucci, Study Chair. Patients are administered IV G3139, continuously, on days 1-10, and filgrastim (G-CSF), subcutaneously, beginning on day 5 and continuing until blood counts recover; patients are treated with fludarabine IV over 30 minutes followed 3.5 hours later by cytarabine (cytosine arabinoside) IV over 4 hours on days 6-10. Patients who achieve CR are treated with a second course beginning 4 weeks after completion of the first course, and patients who achieve CR and have a matched sibling or unrelated bone marrow donor may undergo allogeneic bone marrow transplantation. Cohorts of 3-6 patients are treated with escalating doses of fludarabine and cytarabine until MTD is determined. This study has a projected accrual of a maximum of 24 patients.

According to preliminary results of the phase I study of G3139 in acute leukemia, among 10 enrolled patients, there were 5 clinical responses; 3 CR in patients treated with a second course of therapy, with 2 demonstrating no evidence of disease (NED) at day 53 and 111. Among 2 patients with NED, but persistent neutropenia/thrombocytopenia at day 52 and 55, 1 continued with NED at day 76. The responses were especially noteworthy as 3 of the 5 responding patients had previously undergone high-dose therapy with cytarabine. One patient demonstrated leukostasis on day 6, and was taken off the study (Marcucci G, etal, ASH00, Abs. 513:119a).

In February 2001, Genta launched a randomized phase III clinical trial with Genasense in chronic lymphocytic leukemia (CLL), to be conducted at approximately 60 centers in the USA, Canada and Europe. This trial will test whether the addition of Genasense improves response rates, response duration, and quality of life when added to

standard second-line combination treatment involving fludarabine and cylcophosphamide used in all patients enrolled in the study. The trial will enroll approximately 200 patients with relapsed CLL.

Vascular Endothelial Growth Factor (VEGF)

VEGF is a multifunctional, homodimeric cytokine and potent permeability factor, whose upregulation leads to neovascularization, thereby providing oxygen and nourishment to tumor cells, and allowing them to gain access to the circulation and to metastasize. While various receptors, such as neuropilin-1 and -2, can bind VEGF, the angiogenic activity of VEGF is mediated primarily through two structurally related transmembrane, tyrosine kinase receptors that are present almost exclusively on endothelial cells, kinase insert domain-containing receptor or KDr (VEGFr2) and Flt-1 (VEGFr1), with target validation studies implicating KDr as the primary driver of angiogenesis in up to 80% of all solid tumors. Four different aminoacids of VEGF, 121, 165, 189, 206, have been identified, based on alternative exon binding.

Angiozyme, under development by Ribozyme Pharmaceuticals (RPI; Boulder, CO), in partnership with Chiron (Emeryville, CA), is a nuclease-stabilized hairpin ribozyme targeting Flt-1 mRNA and is the first chemically synthesized ribozyme that specifically inhibits Flt-1 to be studied in human clinical trials.

Intravenous or SC administration of Angiozyme in murine cancer models, including mice bearing Lewis lung carcinomas, has resulted in a reduction of primary tumor growth and metastatic burden. Angiozyme has been shown to be well absorbed after SC administration in both monkeys and mice, with clearance from plasma in mice attributed to a combination of renal elimination, metabolism, and distribution into tissue. In both *in vitro* and *in vivo* genotoxicity tests, Angiozyme has proven negative in mice and cynomolgus monkeys. However, repeated daily SC dosing of up to 300 mg/m^2 over 28 days in monkeys and mice did result in dose-dependent accumulation of basophilic, cytoplasmic granules in kidney (monkeys and mice), and hypertrophy of hepatic Kupffer cells (monkeys), and signs of local irritation were observed at the injection sites in both species. These findings are similar to effects seen with other oligonucleotides, and resolve partially or completely within four weeks of the end of treatment, and are not associated with impairment of renal or hepatic function (Sandberg JA, etal, AACR00, Abs. 2074:327, and Sandberg JA, etal, Antisense Nucleic Acid Drug Dev, Jun 2000;10(3):153-62).

In examining routes of administration that might be useful for systemic delivery of this ribozyme, because of rapid and extensive absorption after extravascular injections in animal models, either intraperitoneal or SC administration was considered to be best for pharmacodynamic studies of Angiozyme (Sandberg JA, etal, Antisense Nucleic Acid Drug Dev, Jun 1999;9(3):271-7). In a phase Ia trial (Study 9801), which enrolled healthy volunteers, 4 subjects were administered a single dose of Angiozyme of 10 or 30 mg/m^2 IV and 20 mg/m^2 SC in each of 3 cohorts. In a phase Ib trial (Study 9901), in patients diagnosed with solid malignant tumors, 4 patients each were administered Angiozyme by a 4-hour IV infusion, or by SC bolus at dose levels of 100 mg/m^2 and 300 mg/m^2 respectively. Angiozyme was well tolerated by all subjects, with no serious adverse drug-related events reported in either trial. The pharmacokinetics of Angiozyme were linear over the range tested, and the absolute bioavailability following SC administration was estimated to be 74%-90% (Parker VP, etal, ASCO00, Abs. 703:181a).

Encouraging results from phase I and phase I/II clinical trials of Angiozyme in the treatment of patients with refractory solid tumors set the stage for phase II trials in breast, lung, colorectal, and renal cancer as well as malignant melanoma. In November 2000, results of an open-label, dose-escalation phase I/II safety and biological efficacy study of Angiozyme in patients with refractory solid tumors were reported at the annual EORTC Conference. This trial was initiated in November 1999 at the Taussig Cancer Center of the Cleveland Clinic Foundation (Cleveland, OH), with Dr. Ernest Borden as PI. Angiozyme was administered to 31 patients with progressive cancer at doses of up to 300 mg/m^2/day via SC injection for at least 29 days. Patients were observed at the clinic for 24 hours after the initial treatment, and again on day 28. Angiozyme was well-tolerated, and pharmacokinetic analyses demonstrated good bioavailability using SC injections. Patients without progressive disease were allowed to continue daily injections of Angiozyme past the 29-day trial period at the physician's option. As of November 2000, disease stabilized in 17 (61%) evaluable patients for periods ranging from 1 to 8 months and 4 patients remained on Angiozyme. Two patients have had minor clinical responses, including 1 case of central turmor necrosis.

A phase II clinical trial of Angiozyme in patients with breast cancer was initiated in February 2001 under PI Dr. Gabriel Hortobagyi of the M.D. Anderson Cancer Center. Phase II clinical trials of Angiozyme in the treatment of renal, lung, and colorectal cancer as well as malignant melanoma are pending.

DNA-MeTase (DNA Methyltransferase)

When DNA-MeTase is overactivated in cancer, it silences certain tumor suppressor genes, permitting cancer cells to grow unchecked. Inhibition of the genetic overexpression of DNA-MeTase may lead to reactivation of tumor suppressor genes, and subsequent suppression of cancer cell growth (Szyf M, Pharmacol Ther 1996;70(1):1-37, and Siu LL, etal, ASCO00, Abs. 733:189a).

MG98, a phosphorothioate-modified, antisense oligonucleotide directed against DNA-MeTase mRNA, developed by MethylGene (Montreal, Quebec, Canada), was shown to reduce the level of DNA-MeTase mRNA, to

inhibit DNA-MeTase activity, and to inhibit anchorage independent growth of Y1 adrenocortical carcinoma cells *ex vivo* in a dose-dependent manner. Injection of DNA-MeTase antisense oligomers IP inhibits growth of Y1 tumors in syngeneic LAF1 mice, reduces the level of DNA-MeTase, and induces demethylation of the adrenocortical-specific gene C21 and its expression in tumors *in vivo* (Ramchandani, S, etal, PNAS USA, 21 Jan 1997; 94(2):684-9).

Based on an IND application submitted in December 1998, in February 1999, a dose-escalation phase I clinical trial (protocol IDs: CAN-NCIC-IND125, METHYL-MG98-002) was initiated at the Johns Hopkins Oncology Center (Baltimore, MD), Ottawa Regional Cancer Centre (Ottawa, Ontario, Canada), British Columbia Cancer Agency, and Princess Margaret Hospital (Toronto, Canada), with Johns Hopkins' Dr. Ross C. Donehower as Study Chair. Patients with advanced solid tumors are administered MG98 IV over 2 hours, twice-weekly, for 3 weeks, with courses of treatment repeated every 4 weeks, and continuing in the absence of disease progression or unacceptable toxicity. The dose of MG98 is escalated in cohorts of 1-6 patients until MTD is determined. Patients are followed at week 4, and then at least every 3 months until relapse. The projected accrual for this study is approximately 20 patients.

As reported at the 11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy in Amsterdam, among 14 patients with solid cancers treated with up to 37 cycles of MG98, at dose levels ranging from 40 to 360 mg/m^2/day, 2 of 9 patients demonstrated significant sustained suppression of DNA-MeTase mRNA at 80 mg/m^2/day. One patient with RCC has had a durable PR lasting more than 9 months, and disease stabilized in 2 mesothelioma patients for more than 6 and 14 months, respectively. MG98 has been generally well tolerated, with toxic effects including Grade 1 or 2 fatigue, anorexia, fever and chills, and elevated liver enzymes. Patient accrual is continuing at 360 mg/m^2/day (Stewart D, etal, NCI-EORTC00, Abs. 528, and Davis AJ, etal, NCI-EORTC00, Abs. 257).

In an interim report on 9 patients with solid cancers having been administered 10 courses of MG98 at dose levels of 40 mg/m^2/day, 80 mg/m^2/day, 160 mg/m^2/day, and 240 mg/m^2/day, dose-limiting Grade 3 drug-related elevation in transaminases was encountered in 2 of 2 patients at the 240 mg/m^2/day dose level. Gradual decline of the transaminases towards baseline values occurred upon drug discontinuation, without clinically significant sequelae. Other nonhematologic toxicities were Grade 1 or 2 and included fever, sweats, fatigue, and transient rise in PTT. No evidence of complement activation was seen. Hematologic toxicities have been minimal and consisted mainly of delayed Grade 1 thrombocytopenia. Biologically relevant concentrations for the inhibition of human DNA-MeTase mRNA were achievable at the lowest dose level evaluated, and mean steady state concentrations attained at the higher dose levels were approximately 10-fold the IC_{50} values for inhibition of both target mRNA and protein *in vitro* (Siu LL, etal, *ibid*).

In November 2000, MethylGene and MGI Pharma (Bloomington, MN) initiated a phase II trial in the USA and Canada with MG98 in recurrent or metastatic SCCHN. Up to 30 patients may be enrolled in this multicenter trial, which is under the direction of Dr. Andrew Maksymiuk, principal investigator at CancerCare Manitoba, the provincial Canadian center in Winnipeg for cancer treatment.

Ribonucleotide reductase (RNR)

Ribonucleotide reductase (RNR) is rate limiting for the synthesis of DNA. The active enzyme is composed of two dissimilar components, R1 and R2, encoded by different genes. The activity of RNR, and therefore DNA synthesis and cell proliferation, is controlled in proliferating cells by the synthesis and degradation of the R2 component of RNR. R2 can cooperate with a variety of well-known oncogenes to dramatically elevate the malignant characteristics of tumor cells, including tumor growth rates and metastatic potential (Fan H, etal, PNAS USA, 26 Nov 1996;93(24):14036-40; Fan H, etal, Cancer Res, 15 Apr 1998;58(8):1650-3).

GTI 2040, under development by Lorus (Markham, Ontario, Canada), specifically reduces R2 mRNA expression and protein levels, and has demonstrated significant tumor suppressive abilities when used as a single agent in standard mouse models of human tumors of the colon, pancreas, liver, lung, breast, kidney, ovary, brain, prostate, and skin (Young AH, etal, AACR00, Abs. 4084:643). In November 1999, Lorus reported results of antitumor studies with GTI 2040, in combination with certain chemotherapeutic agents, in murine models bearing human kidney and colon cancer, and melanoma xenografts. When SCID mice bearing a human kidney cancer xenograft were treated with a combination of GTI 2040 and 5-FU or vinblastine, complete tumor regression was observed in all treated mice. Similar tumor regression was observed in studies combining GTI 2040 and mitomycin C in murine models of human melanoma and human colon cancer. GTI 2040 also significantly enhanced the antitumor effects of other chemotherapeutics, including gemcitabine, estramustine, and paclitaxel in a standard human colon carcinoma mouse model. In toxicology studies using rhesus monkeys, GTI 2040 was well tolerated, even at dose levels exceeding that which is expected to have antitumor activity in human clinical trials (Fitsialos DP, etal, AACR00, Abs. 3133:491).

In December 1999, the FDA approved an IND application, filed in November 1999, for initiation of clinical trials of GTI 2040 in the treatment of refractory solid tumors. In February 2000, a phase I/II dose-escalation trial of GTI 2040 began at the University of Chicago Cancer Research Center, under the direction of Dr. Richard Schilsky, to determine optimum dosing levels. The trial will enroll 20 to 25 patients to be administered GTI 2040 by continuous

IV infusion for 21 days, to be repeated for another 21 days after 1 week's rest. Based on promising interim results from this trial, expected to be reported in the first quarter of 2001, Lorus hopes to advance GTI 2040 into phase II clinical testing in the second quarter of 2001; the phase II studies will also be conducted under the direction of Dr. Schilsky.

Insulin-like Growth Factor (IGF)-I, -II and IGF-Ir

The insulin-like growth factors (IGF-I and IGF-II) play a major role in regulating normal growth and differentiation during the fetal, neonatal and pubertal periods of mammalian development, and have been found to play an important role in malignant transformation and in protecting tumor cells from apoptosis. The cancer-related functions of these ligands are mediated exclusively through their interaction with the type I IGF receptor (IGF-Ir) which is expressed on many human tumor types (Whitesell L, etal, AACR00, Abs. S33:894).

Antisense-mediated down-regulation of IGF-I and IGF-II as well as IGF-Ir, results in considerable apoptosis of tumor cells *in vitro* and *in vivo*, leading to a disruption of the transformed phenotype in a variety of carcinomas. Because binding of IGF-I to IGF-Ir is required for the onset and maintenance of the human hepatocellular carcinoma (HCC) phenotype, an antisense strategy has been developed as a potential therapeutic modality in liver cancer. HCC tumors were produced by subcutaneous injection of an IGF-I-expressing HCC cell line into syngeneic rats (Lafarge- Frayssinet C, etal, Cancer Gene Therapy 1997;4(5):276-85). An episomal vector-containing human hepatic-derived antisense IGF-I cDNA placed under control of the zinc-inducible mouse metallothionein promoter, transfected into this HCC cell line, resulted in inhibition of growth and in *in vivo* tumor regression. A concomitant increase in major histocompatibility antigen I (MHC-I) expression suggests that IGF-I has an inhibitory effect on MHC-I presentation, and that suppression of IGF-I leads to T lymphocyte activation, and subsequent tumor regression. A follow-up study confirmed the onset of massive apoptosis *in vitro* of HCC cells containing the cDNA for antisense IGF-I, following transcriptional activation by the introduction of zinc (Ellouk-Achard S, etal, J Hepatology 1998;29:807-18). Distinct morphological characteristics of apoptosis, cytoplasmic vacuolization, condensation of nuclei, and internucleosomic DNA fragmentation were observed in HCC, but not in control cell lines.

The expression of IGF-1r has also been demonstrated in ovarian cancer cells, and when an IGF-Ir antisense oligonucleotide was tested *in vitro* in the OVCAR-3 ovarian cancer cell line (Muller M, etal, Int'l J Cancer 1998;77:567-71), it effectively reduced cellular proliferation, while sense and mismatch oligonucleotides did not disrupt cell growth. A reduction in both receptor protein and mRNA levels following treatment was observed, suggesting a specificity of the antisense oligomer action. Effective cell proliferation inhibition was achievable with an oligonucleotide concentration of 50 nM when complexed with the cationic lipid carrier Lipofectin.

INX-4437, a phosphorothioate-modified oligonucleotide directed against IGF-Ir, developed by Inex Pharmaceuticals, has demonstrated antisense-mediated downregulation of IGF-Ir in rat glioblastoma cells. This effect was observed to correspond with massive apoptosis of tumor cells *in vivo*, and disruption of tumorigenesis in a nude mouse model of malignant glioma administered INX-4437. In addition to apoptosis, an antitumor effect was elicited in immunocompetent mice resulting in the prevention of tumor recurrence (Resnicoff M, Int J Mol Med, May 1998;1(5):883-8). In a physician-sponsored phase I clinical trial at Thomas Jefferson University Hospital and Kimmel Cancer Center, 12 therapy-resistant patients with malignant gliomas were treated *ex vivo* with INX-4437. Treatment-related toxicities were minimal following subcutaneous implantation of autologous glioma cells treated *ex vivo* with INX-4437. Encouraging radiographic and clinical responses were observed in 60 % of the patients; 2 experienced disease remission at 6 and 14 months post-treatment (Resnicoff M, etal, AACR99, Abs. 4816:729). Inex has suspended clinical work with INX-4437 in this indication to better focus on its other antisense development activities, in particular INX-3280 and INX-3001.

OTHER POTENTIAL SNAS INTERVENTIONS IN ONCOLOGY

SNAS are uniquely suited to modulate, correct or inhibit gene function at the gene or transcription level, and may thus interfere in many signaling pathways implicated in oncogenesis and metastasis. Although in most cases SNAS do not have a single action, but participate in cascade of secondary events to bring about the final desired effect, their activities may be defined as primary and secondary endpoints. The activities of novel SNAS are described below according to their primary endpoint.

Oncogene Inhibition

Oncogene inhibition is an anticancer approach that has been demonstrated in numerous animal studies. SNAS have been designed against various oncogenes, with several undergoing clinical trials. Development efforts in SNAS inhibition of the c-myc and c-myb oncogenes are described above. Additional targeted oncogenes (Exhibit 2) include cancer-associated Sm (CaSm), HER2/neu, and several others described below.

Oncogene bcr-abl, formed when the abl proto-oncogene translocates from chromosome 9 to chromosome 22, the site of the bcr gene, creating the Philadelphia (Ph) chromosome, has been the target of various SNAS approaches. Bcr-abl is expressed only in leukemic cells, typically as one of three main fusion protein variants, p210 splice 1 or splice 2, or p190. Most patients with CML express the p210 variant of Bcr-Abl, and CML cell lines expressing the Bcr-Abl fusion protein are resistant to the

induction of apoptosis by a number of agents and conditions, suggesting that bcr-abl acts as an antiapoptosis gene in CML cells. This effect appears to be dependent on the Abl kinase activity of Bcr-Abl. In addition to SNAS approaches, inhibition of bcr-abl tyrosine kinases is being pursued using other methodologies (see FO, pp 242-253), with the most prominent being the Novartis' 2-phenylaminopyrimidine derivative, STI571, in phase II clinical trials in CML and related malignancies.

In preclinical evaluations in the past, SNAS targeting bcr-abl have been used alone as well as in combination with other chemotherapeutics, including cyclophosphamide (Skorski T, etal, JNCI, 15 Jan 1997;89(2):124-33), to assess the validity of an SNAS approach in treating CML and related malignancies. However, one of the problems of targeting bcr-abl by antisense is the fact that most primitive CML cells do not express this protein, and an antisense oligonucleotide (G-1128) under development by Genta in the early 1990s, that downregulated bcr-abl mRNA, failed to show effectiveness in phase I clinical trials conducted at M. D. Anderson Cancer Center, in the *ex vivo* purging of leukemic cells in autologous bone marrow transplant (autoBMT) procedures for treating CML.

Yet, in an approach under development by Gemini Technologies (Cleveland, OH), a binary molecule, created by attaching 5'-phosphorylated 2'-5'-linked oligoadenylate (2-5A) to an antisense oligonucleotide that targets either the fusion site or the translation start sequence of bcr-abl mRNA, has shown promise as a bone marrow purging agent. By attaching 2-5A to an antisense oligomer, RNase L is recruited and activated at the site of the targeted mRNA sequence, resulting in specific and effective RNA degradation at an accelerated rate, compared to that achievable by the oligonucleotide alone. This SNAS construct has demonstrated selective degradation of the targeted RNA sequences and decreases in p210 bcr-abl kinase activity levels in the human CML cell line, K562. The 2-5A-linked antisense chimeras also suppressed growth of K562 cells, while having substantially reduced effects on the promyelocytic leukemia cell line, HL60.

Similar results were observed in primary CML cells isolated from patient bone marrow. The specificity of this approach was demonstrated with control oligonucleotides, including chimeras containing an inactive dimeric form of 2-5A, antisense lacking 2-5A, and chimeras with altered sequences containing several mismatched nucleotides; the control oligonucleotides had either reduced or no effect on CML cell growth and bcr-abl mRNA levels. These findings suggest the potential for these compounds as *ex vivo* purging agents of autologous hematopoietic stem cell transplants from CML patients (Maran A, etal, Blood, 1 Dec 1998;92(11):4336-43).

A gene therapy approach has also been attempted to target all cells incorporating bcr-abl fusion oncogene that uniquely distinguishes them from normal cells. Investigators have designed a retroviral vector for specific inhibition and tagging of Bcr-Abl p190 cells, that encodes both antisense transcripts specific for the fusion junction, to act as the specific antitumor agent, and a truncated human CD5 cDNA, to allow for selection of the infected cells. The picornavirus internal ribosome-entry site was incorporated in the constructs to coexpress the antisense molecule with the truncated human CD5 gene (Garcia-Hernandez B and Sanchez-Garcia I, Molecular Medicine, Jan 1996;2(1):124-33).

Murine double-minute 2 (mdm2) oncogene has been shown to be amplified or overexpressed in many human cancers and is often associated with poor prognosis. The Mdm2 oncoprotein regulates the p53 tumor suppressor gene through a negative feedback mechanism by binding to the p53 protein, inhibiting p53 functions as a transcription factor, and promoting the ubiquitination and proteasome-dependent degradation of p53, possibly by acting as a ubiquitin ligase (Pochampally R, etal, Oncogene, 19 Nov 1998;17(20):2629-36, Chen L, etal, Mol Med, Jan 1999;5(1):21-34, Lu W, etal, Oncogene, 13 Jan 2000;19(2):232-40, and Zhang R and Wang H, Curr Pharm Des, Mar 2000;6(4):393-416). Mdm2 oncoprotein also interacts with other cellular proteins involved in cancer progression, such as E2F-1, and appears to facilitate G1 to S phase transition by activation of E2F-1 (Teoh G, etal, Blood, 1 Sep 1997;90(5):1982-92).

In research conducted at the University of Alabama at Birmingham (UAB), in collaboration with Hybridon, scientists have used an antisense oligonucleotide based on Hybridon's proprietary mixed-backbone phosphorothioate oligonucleotide chemistry to inhibit Mdm2 oncoprotein expression in various human cancer cell lines, including those containing wild-type p53 (LS174T, A549, MCF-7, LNCap) and those containing mutant p53 (DLD-1, DU145, MDAMB-468, PANC-1. The *in vivo* antitumor activity of the oligomer was demonstrated in nude mice bearing LS174T and DLD-1 xenografts in a dose-dependent manner. Both *in vitro* and *in vivo* antitumor activities were associated with repression of Mdm2 expression. Although activation of p53 was found in antisense-treated cell lines with wild-type p53, no change in p53 levels was found with cells containing mutant p53, suggesting Mdm2's role in tumor growth through p53-dependent and independent pathways. Also, *in vivo* synergistic effects of the antisense compound and chemotherapeutic agents 5-FU and 10-hydroxycamptothecin were observed, regardless of p53 status (Wang H, etal, AACR00, Abs. 2474:390).

Hybridon, UAB and Louisiana State University (Baton Rouge, LA) researchers have observed significant *in vitro* antitumor activities in human osteosarcoma SJSA and choriocarcinoma JAR cell lines, in a time-, concentration-, and sequence-dependent manner using anti-mdm2 antisense oligonucleotides. Intraperitoneal administration of these constructs also caused dose-dependent antitumor activity in nude mice bearing SJSA, JAR, or A549 lung cancer xenografts. In addition, synergistically therapeutic

effects were demonstrated in nude mice with antisense Mdm2 inhibition and use of the DNA-damaging agents doxorubicin and 10-hydroxycamptothecin (Wang H, etal, Int J Oncol, Oct 1999;15(4):653-60, and Zhang R, etal, AACR99, Abs. 3197:484).

In research conducted at the Louisiana State University Medical Center, an antisense phosphorothioate oligodeoxynucleotide targeting mdm2 mRNA was found to effectively inhibit Mdm2 oncoprotein expression in tumor cells containing mdm2 gene amplifications; *in vitro* antisense inhibition of Mdm2 was associated with a decrease in Mdm2-p53 complex formation, increase in p53-inducible gene expression, increase in p53 transcriptional activity, and enhancement of p53-mediated apoptotic cell death induced, in a synergistic fashion, by the DNA-damaging chemotherapeutic agent CPT-11 (Chen L, etal, PNAS USA, 6 Jan 1998;95(1):195-200).

In collaborative research conducted at the University of Naples in Italy, a mixed-backbone phosphorothioate anti-mdm2 oligonucleotide was shown to cause reduction of Mdm2 oncoprotein expression as well as parallel induction of p53 and p21, and arrest growth of various human cancer cells *in vitro*. The oligomer also exhibited a synergistic growth inhibitory effect when combined with different classes of antitumor agents, such as platinum derivatives, topotecan, doxorubicin and taxanes. Antitumor activity was observed *in vivo* in nude mice xenografted with human cancer cells and treated with the antisense compound. Compared to control untreated mice or to mice treated with a mismatched oligomer, treatment with the anti-mdm2 oligonucleotide reduced tumor size by 50%. A cooperative antitumor effect was obtained in combination with cisplatin or topotecan (Tortora G, etal, AACR99, Abs. 4820:730, and Tortora G, etal, Int J Cancer, 1 Dec 2000;88(5):804-9).

Modulation of Tumor Suppressor Genes

SNAS have also been designed that modulate directly or indirectly the activity of tumor suppressor genes, including p53 (Exhibit 2).

BRCA1, is a tumor suppressor gene that is downregulated in various cancers. For instance, although 90% of all breast and ovarian cancers have a normal BRCA1 gene, its expression is downregulated by the dominant negative transcriptional regulator Id4, that prevents normal production of BRCA1. Using Inverse Genomics, a ribozyme-based proprietary target validation process, Immusol (San Diego, CA) announced in January 2001 the discovery of the role for the Id4 gene in BRCA1 expression. According to these findings, modulation of Id4 expression results in inversely regulated expression of BRCA1, and that inhibition of the Id4 gene with a ribozyme upregulates production of BRCA1 in cancer cells, reversing their malignant properties. Also, cells with increased expression of Id4 exhibited anchorage-independent growth. Because Id4 is a crucial gene in the regulation of BRCA1 expression, it may be important for the BRCA1 regulatory pathway involved in the pathogenesis of sporadic breast and ovarian cancer, making Id4 a potentially attractive drug target (Beger C, etal, PNAS USA, 2 Jan 2001;98(1):130-135).

Modulation of Signal Transduction Pathways

SNAS may play a role in modulating various signal transduction pathways implicated in tumorigenesis, tumor progression and metastasis. Currently, SNAS constructs interfering with the ras pathway are in clinical trials, as described above.

Epidermal growth factor (EGF) and transforming growth factor (TGF)-α exhibit 30% to 40% amino acid homology, and compete for EGFr binding. EGFr, the prototypal member of the EGF superfamily of receptors, is capable of extensive cross-activation upon binding to its ligands, including TGF-α; upon activation, EGFr tyrosyl-phosphorylates itself and numerous intermediary effector molecules, initiating a myriad of signaling pathways. TGF-α and EGFr are coexpressed at elevated levels (mRNA and protein) in a number of human tumors and tumor cell lines, suggesting an autocrine growth pathway involving TGR-α and EGFr. Therefore, downmodulation of one and/or the other may inhibit tumor growth. SCCHN overexpresses the EGFr/TGF-α receptor-ligand pair, and when 6 SCCHN and control cell lines were treated with antisense oligonucleotide specific for TGF-α mRNA, a decrease in TGF-α protein was observed in both the cancer and control cells (Grandis JR, etal, J Cellular Biochemistry 1998;69:55-62). However, growth rate was only reduced in transformed cells. This lends credence to the premise that SCCHN cells can experience unregulated proliferation when an autocrine stimulation of EGFr by TGF-α occurs, and that antisense SNASs directed to TGF-α mRNA may be an effective intervention in this setting.

In another test involving this receptor-ligand pair, an EGFr antisense-expression plasmid, under transcriptional control of the U6 small nuclear RNA (snRNA) promoter, was constructed as an *in vivo* challenge of SCCHN (He Y, etal, JNCI, 15 Jul 1998;90(14):1080-6). This plasmid DNA design was complexed with a DC-chol cationic liposome-mediated carrier, and injected into subcutaneously grafted human SCCHN tumors in nude mice. Antitumor effects, observed in inoculated xenografts, included inhibition of growth, suppression of EGFr protein expression, and enhanced level of apoptosis. These results document the potential effectiveness of an *in situ* antisense oligonucleotide expression system in the treatment of specific cancers, including SCCHN.

In addition to SCCHN, and a variety of other carcinomas, EGFr/TGF-α overexpression also plays an important role in the progression of malignant gliomas. At a concentration of 5 μM, a liposomally enveloped (Lipofectin) antisense EGFr oligonucleotide significantly inhibited the growth of 3 malignant glioma cell lines *in vitro* compared to cells exposed to a sense EGFr oligomer (Sugawa N, etal,

J Neuro-Oncology, Sept1998;39:237-44). An enzyme-linked immunosorbent assay (ELISA) also showed significant suppression of protein tyrosine kinase activity in cells treated with this 20-base-sequence oligomer.

In another study, when rats with well-established C6 glioma tumor foci were treated intratumorally with EGFr antisense cDNA mediated by Lipofectamine, mean survival time compared to that of rats implanted stereotaxically to the right caudate nucleus with wild-type C6 cells and C6 cells transfected with EGFr antisense cDNA. Mean survival time of control animals was 17.3 days, whereas that of the transfected and antisense-treated animals was significantly prolonged to over 200 days. MRI demonstrated a distinct cerebral tumor in wild-type C6-injected rats and control rats of the treated group, but the tumor foci were not found in the transfected rats and disappeared almost completely in the antisense-treated rats (Liu X, etal, Chung Hua Chung Liu Tsa Chih, Nov 1998;20(6):422-4, and Pu P, etal, J Neurosurg, Jan 2000;92(1):132-9).

The brain metastatic variant of human nsclc cell line H226Br, also exhibits elevated EGFr levels associated with tumorigenesis and cellular proliferation. When H226Br cells were transfected with an antisense EGFr cDNA/cytomegalovirus promoter plasmid construct, there was a significant decline in EGFr expression and cell growth rates. The transfected cells also demonstrated a prolonged G_2-M cell cycle phase, an observation that is consistent with a downregulation of cell proliferation (Fang K and Chen M, Int'l J Cancer 1999;81:471-8).

When the effects of TGF-α on cell growth were studied in human glioma U251 cells transfected with antisense TGF-α vectors (pcDNAI.neo), several antisense clones showed a significant decrease in growth rate in serum-free medium but not in medium containing 10% fetal bovine serum (FBS), compared with those of parental cells and clones from sense or vector-only transfectants. Antisense clones also produced fewer and smaller colonies in anchorage-independent growth assays. Moreover, there was a reduction in TGF-α expression in these antisense clones at both the protein and mRNA levels, as determined by ELISA and reverse transcriptase polymerase chain reaction (RT-PCR) analysis.

A U251 clone transfected by TGF-α antisense in a different vector (pMT/Ep) also showed a significant suppression in cell growth and TGF-α mRNA level. Finally, transfected clones with either vector system showed decreased tumorigenicity in nude mice. In summary, a strong correlation between the inhibition of glioma cell growth and TGF-α expression was obtained from two different plasmid vectors, indicating that the expression of TGF-α could be specifically and effectively downregulated by a TGF-α antisense vector, leading in turn to growth inhibition. These studies suggest that TGF-α plays an essential role in controlling human glioma cell proliferation and may serve as a potential target for treatment of malignant glioma (Tang P, etal, J Neurooncol, Jun 1999;43(2):127-35).

Prevention of Metastasis

SNAS may also play a significant role in preventing cell adhesion and inhibiting angiogenesis that are the main pathways promoting metastasis. SNAS inhibitors of VEGF receptors, described above, represents but one approach to tumor angiogenesis inhibition and metastasis prevention. Other approaches involve prevention of cell adhesion by inhibition of various mediators of cell-extracellular matrix (ECM) signaling events.

The α V integrins, which dimerize with β subunits to form receptors for vitronectin, fibrinogen and fibronectin substrates as well as bone sialoproteins, have been shown to play an essential role in epithelial- and hematopoietic-derived cell migration, cell growth and tumor invasion/metastasis (Huang S, etal, Oncogene, 6 Apr 2000;19(15):1915-23, and Dallabrida SM, etal, J Biol Chem, 13 Oct 2000;275(41):32281-8). The antisense oligomer 5543-ODN (HMR-5543), under development by Aventis Pharmaceuticals (Frankfurt am Main, Germany), has been used to inhibit α V integrin synthesis in the human breast cancer cell line MDA-MB231. Following a 72-hour exposure to a concentration of 1 mM α V mRNA, transcription and protein expression were reduced by 55% and 65%, respectively, while both control sense and mismatch reagents were inactive. The antisense oligonucleotide also disrupted α V-mediated adhesion structures as demonstrat- ed by the induction of dose- and time-dependent inhibition of MDA-MB231 adhesion to serum, vitronectin, fibrinogen, and fibronectin substrates. In addition, antisense treated cells showed evidence of programmed cell death with the appearance of apoptotic bodies. MDA-MB231 cells express a mutant form of p53, and immunofluorescence revealed an increased nuclear translocation of p53 suggesting activation of the protein; this translocation did not lead to significant changes in the expression of the cyclin dependent kinase inhibitor, p21(WAF1/CIP1), the cell survival factor Bcl-2, or the pro-apoptotic factor Bax (Townsend PA, etal, Eur J Cancer, 1 Feb 2000;36(3):397-409).

Focal adhesion kinase (FAK) is a 125-kDa nonreceptor member of the tyrosine kinase superfamily and a major phosphotyrosine-containing protein that is a mediator of cell-ECM signaling events. FAK is found in cell-matrix attachment sites (focal adhesions) and is activated on integrin-ligand binding, and by other signaling pathways. FAK has been shown to be a key regulator in cell adhesion, motility, and invasion, in part by mediating cell contact induction of VEGF transcription (Renshaw MW, etal, J Cell Biol, 1 Nov 1999;147(3):611-8, Maung K, etal, Oncogene, 18 Nov 1999;18(48):6824-8, and Sheta EA, etal, JNCI, 5 Jul 2000;92(13):1065-73). Overexpression of the fak gene and elevation of FAK has been observed in many cancer cell lines, including those derived from lung, breast, colon, thyroid and ovarian carcinoma, but FAK is only weakly expressed, if at all, in normal and benign tissues as well as

in noninvasive neoplastic tissues. Antisense oligonucleotides specific for fak mRNA have been used to attenuate FAK expression in tumor cells with elevated levels of this protein, resulting in a loss of attachment for the cells as well as the induction of apoptosis (Xu LH, etal, Cell Growth Differ, Apr 1996;7(4):413-8). Normal human fibroblasts, which did not express high levels of FAK protein, did not lose their attachment or become apoptotic with antisense treatment, suggesting that FAK may be a rational gene-directed target for disrupting tumor cell growth.

Enhancement of Apoptosis

Induction of apoptosis is a critical step in anticancer therapies and an important target of SNAS-based therapies. In addition to SNAS strategies to inhibit bcl-2, discussed above, oligos are being investigated in various other apoptosis-related pathways.

Inhibitors of apoptosis proteins (IAP) includes X-linked IAP (XIAP), a key regulator of programmed cell death activated by various apoptotic triggers (Holcik M and Korneluk RG, Mol Cell Biol, Jul 2000;20(13):4648-57). A distinct role for XIAP in radiation-resistant phenotype of human cancers has been demonstrated, with acute low-dose ionizing irradiation resulting in the translational upregulation of XIAP that correlates with an increased resistance to radiation. Also, transient overexpression of XIAP renders human carcinoma cells resistant to low-dose gamma irradiation. In contrast, antisense targeting of XIAP has been observed to result in XIAP downregulation accompanied by increased cell death following irradiation (Holcik M, etal, Oncogene, 24 Aug 2000;19(36):4174-7).

Telomerase Inhibition

Telomerase is an RNA-dependent DNA polymerase, or ribonucleoprotein enzyme, whose reactivation has been observed in over 85% of human primary malignancies. Telomerase synthesizes new 5'-d(TTAGGG)-3' hexameric repeats (telomeres) at the 3' ends of chromosomes. Telomeres are key genetic elements involved in the cellular aging process, protecting chromosomes from degradation and fusion. Abnormal telomerase upregulation has been associated with cell immortality, and although not sufficient in itself to induce neoplasia, is thought to be essential in maintaining the proliferative capacity of tumor cells (Yang J, etal, J Biol Chem, 10 Sep 1999;274(37): 26141-8). Consequently, antitelomerase therapy is actively being investigated as a means of preventing tumor-cell growth. One promising mechanism of telomerase inhibition is the use of antisense oligonucleotides.

Antisense inhibition of telomerase increases susceptibility of human malignant glioblastoma cells to cisplatin-induced apoptosis. When cisplatin-resistant U251-MG human malignant glioblastoma cells, with high telomerase activity, were treated with a 2-5A-linked, 19-mer antisense oligonucleotide targeting mRNA of the RNA component (hTR) of human telomerase (2-5A-anti-hTR), not only was telomerase activity decreased but susceptibility to cisplatin-induced apoptotic cell death was increased, suggesting that antisense telomerase therapy may represent a new chemosensitisation approach to tumors resistant to anticancer drugs (Kondo Y, etal, Oncogene, 30 Apr 1998;16(17):2243-8).

In another study, treatment with 2-5A-anti-hTR alone was shown to effectively suppress malignant glioma cell growth and survival *in vitro*, with most cells undergoing programmed cell death within 14 days after treatment. Antisense treatment of intracranial malignant human glioma xenografts in nude mice was also shown to significantly reduce tumor mass after a 14-day period (Kondo S, etal, Oncogene, 25 Jun 1998;16(25):3323-30).

Complexing 2-5A-anti-hTR with a cationic liposome enhanced its antitumor effect, reducing the viability of 5 malignant glioma cell lines to 20%-43% within 4 days. This intervention did not influence the viability of cultured astrocytes lacking telomerase. Treatment of intracranial malignant gliomas in nude mice with the liposome-complexed oligomer was also therapeutically effective compared with a control (Kondo Y, etal, Oncogene, 27 Apr 2000;19(18):2205-11, and Mukai S, etal, Cancer Res, 15 Aug 2000;60(16):4461-7). Treatment of prostate cancer cells with 2-5A-anti-hTR in the presence of a cationic liposome reduced cell viability of tumor cell lines tested to 9%-18% within 6 days, whereas normal fibroblast cells were resistant to treatment. The cytotoxic effect was mainly attributable to induction of apoptosis by activated caspase family members. Treatment of subcutaneous prostate tumor xenografts in nude mice with 2-5A-anti-hTR was also shown to significantly suppress tumor growth through induction of apoptosis (Kondo Y, etal, Oncogene, 27 Apr 2000, *ibid*).

When the ovarian cancer cell line HEY-1B was treated for 7 days with 2-5A-linked antisense oligonucleotides directed against 4 different hTR sequences, a decrease in telomerase activity and profound apoptotic cell death was observed in the tumor cells regardless of hTR target site. No effect on viability was seen in normal ovarian epithelial cells treated with the oligomers. Control oligonucleotides also had no effect on cell viability in either cell line (Kushner DM, etal, Gynecol Oncol, Feb 2000;76(2):183-92).

Modulation of Chemotherapeutics

The most important role of SNAS in cancer therapy will probably evolve as part of a combination therapy with traditional treatment modalities, acting as chemoradiosensitizers and modulators of the activities of standard chemotherapeutics. One such approach involves downregulation of thymidylate synthase (TS), a long-standing target of chemotherapeutic agents because of its central position in the pathway of DNA synthesis. TS is a critical enzyme for DNA replication and cell growth because it is the only *de novo* source of thymidine nucleotide precursors

for DNA synthesis. Thus, antisense downregulation of TS may prove to be a useful approach to inhibiting human tumor cell proliferation and/or enhancing the efficacy of TS-targeted drugs. When transfected into 5-fluorodeoxyuridine (5-FUdR)-resistant HeLa cells, ODN 83, a 2'-methoxyethoxylated, phosphorothioated antisense oligonucleotide under development by AstraZeneca (London, UK) and directed against TS mRNA, was shown to decrease TS mRNA levels by approximately 70% within 24 hours, slowing cell proliferation by up to approximately 40%-50%, and sensitizing resistant cell lines to 5-FUdR by 50%-60% (Ferguson PJ, etal, AACR99, Abs. 139:21, and Ferguson PJ, etal, AACR00, Abs. 4087:643).

In another approach, AVI BioPharma has used SNAS to inhibit cytochrome P450 3A2 (CYP3A2) mRNA. CYP3A2 cytochrome is a liver enzyme that regulates metabolism of about 60% of all FDA-approved drugs. Preclinical data demonstrated that passive transdermal delivery of AVI BioPharma's AVI-3172 results in oligonucleotide concentrations sufficient to alter gene expression *in vivo* (Brand RM and Iversen PL, Adv Drug Deliv Rev, 31 Oct 2000;44(1):51-7). Preclinical studies of AVI-3172 were completed in December 2000, demonstrating the potential of this agent to enhance the effectiveness of many currently approved drugs, including paclitaxel [Brand RM, etal, American Association of Pharmaceutical Scientists (AAPS) 2000, Abs. 1131].

Immunotherapy

Synthetic oligonucleotides containing the cytidine-phosphate-guanosine (CpG) dinucleotide motif can act as DNA mimics with potent immunostimulatory activity (see FO, pp 1276). In work conducted at the NCI, researchers investigated a CpG-containing oligonucleotide administered as an immune response stimulant to athymic mice, for its antitumor activity in human glioma xenografts. Mice were administered a single intraperitoneal injection of 1 mg phosphorothioate oligo, followed 24 hours later by subcutaneous inoculation of U87 glioma cells. Among control animals, all mice developed tumors weighing between 0.3 and 0.9 gm after 5 weeks, whereas of the oligo-treated mice, 4/5 had either no tumors, or significantly smaller tumors. In a second experiment, tumors grew to an average of 2.19 gm in 8 control mice, while 5/9 oligo-treated mice showed no evidence of tumor, and a 6th animal had a tumor weighing < 0.1 gm. In animals treated with a single injection of oligo 2 weeks following tumor inoculation, tumors completely regressed in 3/9 oligo-treated animals at 5 weeks, while disease stabilized in an additional 3/9 treated mice. In all controls, tumors increased in size by more than 10-fold during this period. Experiments performed in SCID mice suggested that the dramatic antitumor activity of this oligonucleotide was mediated by host NK cells (Connell YS and Neckers LM, AACR99, Abs. 1982:299).

At the University of Iowa (Iowa City, IA), immunization of mice with a CpG-containing oligonucleotide increased immune response to antigen plus soluble GM-CSF, or an antigen/GM-CSF fusion protein, and shifted the response to a Th1 response as indicated by production of antigen-specific IgG2a. The oligo enhanced the production of IL-12 by bone marrow derived dendritic cells, particularly when cells were also pulsed with antigen/GM-CSF fusion protein. Also, *in vivo* injection of antigen-pulsed dendritic cells with oligomer enhanced the antigen-specific Th1 response. These findings indicate a CpG-containing oligonucleotide is capable of enhancing an antigen-specific immune response when used in combination with other adjuvants, and can shift the response from a Th2 to a Th1 response (Weiner GJ, etal, AACR98, Abs. 3747:551).

Coley Pharmaceutical (Wellesley, MA), in collaboration with investigators at the University of Iowa, has developed several CpG-containing synthetic oligonucleotides as potential immunotherapeutics. A phase I/II open-label clinical trial was initiated in April 2000 at the University of Iowa Cancer Center to evaluate the safety, tolerability and immune activation of CpG 7909 (Exhibit 2) in patients with relapsed or refractory NHL. According to the protocol, 24 patients are being treated with weekly IV infusions of CpG 7909 for three weeks. A phase I/II European clinical trial of CpG 7909, administered intradermally as monotherapy in malignant melanoma patients, was initiated in the third quarter of 2000 and, at the same time, another phase I/II European clinical trial was initiated to study CpG 7909 as an intratumoral injection for the treatment of metastatic melanoma or basal cell carcinoma. CpG 7909 is a pan-activating oligonucleotide, boosting antibody-based as well as cellular immune responses to antigens presented by tumor cells.

Another CpG-containing oligomer being developed by Coley, is CpG 8954 that contains a proprietary CpG motif capable of inducing *in vivo* production of significant quantities of IFN-α. IFN-α activation results in multiple immunoregulatory effects that have been shown to contribute to antitumor activity. However, while recombinant IFN-α is currently available, its widespread use has been hindered by dose-limiting side effects. Because CpG 8954 induces natural IFN-α production *in vivo*, it may result in enhanced anticancer activity without the severe and limiting side effects of recombinant IFN-α. Another Coley construct, CpG 8916, is a synthetic DNA mimic containing a proprietary CpG sequence optimized to stimulate innate immunity through activation of natural killer (NK) cells.

Viral Inhibition

Several viruses such as hepatitis B and C (HBV and HCV), human papillomavirus (HPV), and Epstein Barr virus (EBV) have been implicated in tumorigenesis. SNAS are particularly suited as inhibitors of viral proteins/transcriptional factors.

DIAGNOSTIC, RESEARCH AND DRUG DISCOVERY APPLICATIONS

A discussion of the applications of SNAS other than as oncology therapeutics is beyond the scope of this report. However, it is in these areas that the contribution of SNAS methodologies has been most pronounced to date, creating a significant demand for "off-the-shelf" oligonucleotide constructs, and spawning a fledgeling manufacturing sector in the USA, Europe and Asia. Increased use of SNAS for research, microarray development, diagnostics, drug discovery/target validation, and clinical trials, has created a demand for mass production of these constructs. Among the many manufacturers of SNAS are:

- Avecia LifeScience Molecules (Manchester, UK and Milford, MA), a unit of Avecia (Wilmington, DE), formerly Zeneca Specialties, a fine and specialty chemicals company that has entered this field by the acquisition of Hybridon Specialty Products (HSP) for $15 million in September 2000, and Boston BioSystems in August 1999; outside the USA, Avecia operates a DNA medicines manufacturing facility at Grangemouth, UK, opened in May 1999, dedicated to the production of oligonucleotides
- MWG-Biotech (Ebersberg, Germany and High Point, NC), that through its Genomic Services Division is able to produce 15,000 constructs per day, provides both standard and customized SNAS synthesis services on a commercial scale; in 2001 the company launched a high-density oligonucleotide chip array that incorporates the complete 6,000-gene yeast genome
- Larova Biochemie (Teltow, Germany)
- Proligo, founded in 1998 by the divestiture of NeXstar Technology Products Business Unit (Boulder CO), and its subsidiary, Proligo Biochemie (Hamburg, Germany), are leading suppliers of key ingredients for oligonucleotide manufacturers; the company recently launched LNA nucleic acids for the genomics and genetic medicines market
- Genset Oligos, a division of Genset (Paris, France)
- Illumina (San Diego, CA), that claims that its proprietary process, Oligator, can produce >2 million high-putity SNAS annually

Another company, Tangerine Technologies (Philadelphia, PA) has introduced an Internet-based resource, OligoBuyer, a software program allowing scientists to purchase complex strands of DNA online.

DEVELOPERS OF SNAS-BASED ONCOLOGY DRUGS

There are four types of commercial entities participating in this sector, developers/manufacturers of human therapeutics, developers/manufacturers of laboratory diagnostics, contract manufacturers of SNAS in large enough volumes for clinical and other applications, and suppliers of SNAS for research. Several of the leading developers of therapeutic SNAS are profiled below.

atugen

atugen (Berlin, Germany) is a biotechnology company spun out of the target discovery and validation business of Ribozyme Pharmaceuticals (RPI) in 1998. In July 1999, atugen established a North American research center in Boulder, CO. The company uses the GeneBloc functional genomics technology platform, exclusively licensed from PRI, to identify and validate new human health therapeutic targets. Initial financing for atugen's first three years of operation totaled more than $20 million, and derived from a variety of sources, including venture capital, the German government, local government, banks, and RPI. As part of its formation, atugen acquired Transgenics Berlin-Buch, providing atugen with advanced DNA microinjection technology for the production of transgenic rodents that allow for early and rapid *in vivo* assessment of the effect of inhibiting expression of a targeted gene sequence.

GeneBlocs are small, synthetic, 23-mer DNA/RNA hybrid macromolecules that bind to any region of specific target mRNA, inducing its destruction by RNase H. They are constructed with a minimum content of phophorothioated antisense DNA to provide stability without toxicity. Protective end groups and naturally occurring modifications to the RNA moieties provide further stability. The effects of a GeneBloc are sustained over several days, although after the first 5 days, they gradually disappear, allowing normal translation of mRNA to resume and normal biological function to be restored. Among its many collaborations, atugen entered into a strategic alliance with GPC Biotech (Martinsried, Germany) to provide major life sciences companies with a fully integrated functional genomics and proteomics platform for the rapid identification and validation of novel drug discovery targets. The combined technology platform has applications in most major disease areas including oncology.

Also, in March 2000, atugen entered into a research collaboration with Axys Pharmaceuticals (South San Francisco, CA) to provide gene target validation services through its USA subsidiary; financial terms were not disclosed. Axys will provide atugen with gene targets in oncology, and atugen will develop GeneBloc reagents against these targets to allow evaluation of gene function, and their potential as therapeutic drug targets; atugen will perform quantitative RNA analysis and technology transfer so as to enable Axys to use the best GeneBlocs, proprietary delivery vehicles, and protocols, for phenotypic analysis.

In January 2001, Phogen (Cambridge, UK) and atugen entered into a research collaboration to evaluate Phogen's VP22-based Vectosome technology for the intracellular delivery of GeneBloc molecules, for applications in the field of functional genomics. atugen has an option to license VP22 protein for use in target validation screens. The financial terms of the deal were not disclosed. At the same time, atugen obtained a non-exclusive license of a

broad patent portfolio on antisense oligonucleotides from Genta (see below).

AVI BioPharma

AVI BioPharma (AVI), previously known as AntiVirals, is developing an array of SNAS constructs in the fields of oncology (Exhibit 2), infection and cardiovascular medicine. In July 2000, AVI entered into a 5-year agreement with Lorus Therapeutics, through Lorus' wholly owned subsidiary GeneSense Technologies, to evaluate and codevelop antisense drug therapies for cancer as well as infectious diseases. Under terms of the agreement, each company retains an ownership interest in any jointly developed compound, and drugs discovered by this collaboration may also be developed independently by any of the two companies with royalty payments to the other party. AVI is contributing its NeuGene-based antisense backbone to this venture, while Lorus is making available a series of proprietary cancer and infectious disease targets, and is spearheading the collaborative research program by performing molecular, cell and animal biology experiments.

AVI has also forged various collaborations aimed at improving delivery of its SNAS constructs. In June 2000, AVI entered into a joint collaboration with DepoMed (Menlo Park, CA) to investigate the feasibility of controlled oral delivery of AVI's proprietary NeuGene antisense agents using DepoMed's Gastric Retention (GR) controlled release system. DepoMed's GR system is designed to be retained in the stomach for an extended period of time while it delivers the incorporated drug, allowing enhanced absorption of drugs preferentially absorbed in the upper gastrointestinal tract. DepoMed's GR system provides the potential for oral delivery of drugs that are poorly soluble or insoluble, including drugs that normally require injection.

AVI is also collaborating with Dr. Rhonda M. Brand and her associates at the University of Nebraska (Lincoln, NE) on the development of a transdermally administered version of AVI-3172. This work combines AVI's NeuGene antisense technology with the passive transdermal peptide delivery strategy developed in Dr. Brand's laboratory, and based on an iontophoresis approach that was shown to enhance transdermal delivery of phosphorothioate oligomers across hairless mouse skin. To achieve passive transdermal transport, oligomers with neutral charge have been combined with propylene glycol (90%) and linoleic acid (10%). The oligomers are designed to exhibit hydrophobic helical properties at pH <6, penetrating the acidic environment of the epidermis and then changing conformation to hydrophilic helices at pH 7 (Brand RM, AAPS98, Abs. 1214). *In vivo* iontophoretic delivery of a C5-propyne-modified phosphorothioate oligonucleotide targeted to the translational start site of CYP3A2 mRNA for 3.5 hours, significantly reduced enzyme levels in the liver of treated rats (Brand RM and Iversen PL, Adv Drug Deliv Rev, 31 Oct 2000;44(1):51-7).

Coley Pharmaceutical Group

Coley Pharmaceutical Group, formerly known as CpG ImmunoPharmaceuticals, is a private company established in 1997 to develop therapeutic and prophylactic products that harness the immune system to treat cancer, allergy, asthma and infectious diseases, and to accelerate recovery of immune system function after cancer chemotherapies, or other immunosuppressive treatments. The technology relating to immunomodulatory CpG-containing oligonucleotides was developed by Dr. Arthur M. Krieg at the University of Iowa, and was subsequently licensed to Coley Pharmaceutical.

In September 2000, Coley announced the purchase of patents from Isis Pharmaceuticals covering the use of phosphorothioate oligonucleotides for activation of the immune system. This transaction allows Coley to fully control the patents that were originally licensed in 1998 for a limited scope of use, at which time Coley paid Isis $5 million in cash and issued preferred stock. In exchange for all rights to this group of immunomodulation patents and in lieu of future payments owed under the original agreement, Coley will pay Isis a total of $10.7 million. A portion of the fee, $3.7 million, is being paid to repurchase the Coley preferred stock from Isis that was granted to Isis as part of the original patent license fee. Should Isis decide to license future technologies, it will provide Coley with a first right to an exclusive license of additional immunostimulatory oligonucleotide technology. Additionally, Coley will not work on antisense oligonucleotides and Isis will not work on immunostimulating (non-antisense) oligonucleotides.

In June 2000, Coley Pharmaceutical and the Ludwig Institute for Cancer Research (Hilden, Germany) announced the establishment of a clinical research collaboration for the development and evaluation of CpG DNA-based cancer products. Ludwig researchers will conduct early clinical studies designed to examine the safety and potential utility of CpG DNA-based products as standalone treatments for cancer as well as immune adjuvants for potential cancer vaccines. Under the terms of the agreement, Coley Pharmaceutical will supply the Ludwig Institute with preclinical results and clinical supplies of its products, while Ludwig plans to conduct and fund early clinical studies involving CpG DNA as a therapeutic agent in multiple cancer indications, including melanoma, myeloma, and RCC. Researchers at Ludwig will also evaluate the safety and utility of CpG DNA as an immune adjuvant in combination with the Institute's proprietary tumor-specific antigens. Coley will retain the rights to all CpG DNA-based products resulting from these trials.

In January 2000, Glaxo SmithKline (Brentford, Middlesex, UK) and Coley entered into a collaborative licensing agreement for the development of infectious disease vaccines incorporating CpG DNA. The agreement grants Glaxo SmithKline a worldwide coexclusive license to CpG 7909 and other CpG-based immune stimulants for

a number of therapeutic and prophylactic infectious disease indications. Coley Pharmaceutical received an upfront $8.0 million payment, and could receive up to $64 million in future research funding and milestone payments as well as royalties on sales of products developed through the collaboration.

Cytoclonal Pharmaceutics

Cytoclonal Pharmaceutic's (Dallas, TX) antisense agent program derives from its OASIS technology platform, developed in collaboration with the University of Texas (Austin, TX). OASIS represents a genome library of optimized antisense reagents, including several cancer-related genes/proteins such as bcl-2, c-raf and PKC-α. In June 1996, Cytoclonal entered into an agreement with the Board of Regents of the University of Texas whereby it obtained an exclusive, royalty-bearing license to manufacture, have manufactured, use, sell and/or sublicense products related to a USA patent application filed in March 1997 concerning a system and method for assessing the minimum number of RNA:DNA sequence combinations whose properties need to be determined for selecting SNAS that will form the most stable hybrids among all those possible in a given target mRNA sequence. A patent, #5,856,103, on this technology, which forms the basis of Cytoclonal's OASIS library of antisense reagents, was subsequently issued in January 1999 to Dr. Donald M. Gray and Dr. Chris L. Clark, which was assigned to the University of Texas, and became part of the Cytoclonal license agreement.

EpiGenesis Pharmaceuticals

EpiGenesis Pharmaceuticals (Cranburry, NJ) has developed a rapid, target validation approach, Respirable Antisense Oligonucleotides (RASON), that uses desadenosine respirable antisense oligonucleotides for direct application to the lung via aerosol inhalation. RASON is an enabling technology for oligonucleotide-based target validation in the hyper responsive airway. EpiGenesis uses its proprietary Oligosurf technology for ultrafast screening of oligonucleotide libraries to identify the most active antisense constructs.

Currently, the company is evaluating, in a phase I clinical trial, EPI 2010 that attenuates production of the adenosine A1 receptor in the cells lining the lung, as a treatment for asthma. The company's RASON technology and its in *in vivo* models of respiratory disease may also provide the foundation for therapeutic approaches in cancer, particularly for primary and/or metastatic lung cancer.

Gemini Technologies

Gemini Technologies, a wholly owned subsidiary of Atlantic Technology Ventures (formerly Atlantic Pharmaceuticals), has an exclusive worldwide sublicense from the Cleveland Clinic Foundation to USA patents #5,583,032, issued in December 1996, and #5,677,289, issued in October 1997, as well as related USA patent applications and corresponding foreign applications, concerning 2-5A chimeric antisense technology and its use for the selective degradation of targeted RNA. This sublicense includes rights to intellectual property obtained by the Foundation through an interinstitutional agreement with the NIH, the coassignee of the patents. Gemini's sublicense extends until the expiration of the underlying patent rights, and provides for payment of royalties to the Cleveland Clinic Foundation based on sales of products and processes incorporating licensed technology.

In August 2000, Gemini Technologies was awarded a $750,000 Small Business Innovation Research (SBIR) phase II grant by the National Institute for Allergy and Infectious Diseases (NIAID) to fund a preclinical efficacy study using aerosolized 2-5A for the inhibition of respiratory syncytial virus (RSV) in monkeys. This funding also provides money for toxicologic and pharmacologic studies needed to file an IND application to begin clinical studies in humans.

Genta

Genta is one of the original developers of antisense methodologies to treat cancer. Genta has exclusively licensed USA patents #4,507,433 and #4,469,863, granted in March 1985 and September 1984, respectively, from Johns Hopkins University (Baltimore, MD) covering methylphosphonate technology developed by Drs. Paul O. P. Ts'o and Paul Miller. The idea of using antisense oligonucleotides to downregulate Bcl-2 protein expression was first suggested by Dr. John C. Reed, formerly with the La Jolla Cancer Research Foundation (La Jolla, CA), and now scientific director of the Burnham Institute (La Jolla, CA). Dr. Reed received USA patent #5,734,033 in March 1998 on this concept, which was originally assigned to the University of Pennsylvania and subsequently licensed to Genta.

In April, 1999, Genta entered into a CRADA with the NCI for the development of G3139 as an anticancer agent. Under the CRADA, the NCI and Genta are collaborating in conducting clinical trials of G3139 in colorectal cancer, sclc, and relapsed or resistant leukemia.

In June 2000, Genta obtained both exclusive and nonexclusive rights, on a royalty-free basis, to antisense oligonucleotide technology developed by Molecular Biosystems (San Diego, CA), in return for cash and shares of its common stock. Some of the fundamental claims relating to the composition and use of antisense oligonucleotides in gene downregulation are covered in USA patents #5,023,243 and #5,919,619, issued to Molecular Biosystems in June 1991 and July 1999, respectively.

In December 2000, Genta signed a 2-year manufacturing agreement with Avecia for continued development aimed at improving manufacturing methods to increase yields and lower long-term manufacturing costs of Genasense.

In October 2000, Genta announced that it had outlicensed its antisense patent portfolio to Oasis Biosciences

(San Diego, CA); this nonexclusive license agreement includes upfront payments in cash and future royalties on product sales. This is the second transaction of this type for Genta, which announced antisense patent out-licensing to Sequitur (Natick, MA) under a similar arrangement in August 2000. Genta retains rights to the patents for its own use as well as an exclusive right to grant sublicenses without restriction to other users.

Genta has an exclusive worldwide license to USA patent #5,936,080, covering improved synthetic methods for obtaining chirally pure methylphosphonates, developed by Drs. Wojciech and Lucyna Wozniak of the Polska Akademia Nauk (Lodz, Poland). Advanced-generation chirally pure methylphosphonate backbones are believed to significantly reduce both the required human dose and side effects of antisense drugs, relative to current constructs. The major advantages of advanced-generation technologies compared with existing second- generation phosphorothioate compounds include a 1000-fold increase in mRNA binding affinity *in vitro*, improved stability because of *in vivo* resistance to degradation, lower toxicity attributed to enhanced specificity and reduced dose, and lower overall costs.

In fiscal 2000, Genta licensed new patents involving decoy aptamers and executed a new CRADA agreement with the NIH, which extends the company's chemistry expertise onto a new technology platform.

In January 2001, Genta completed a nonexclusive licensing agreement for a portfolio of its antisense patents to atugen. The agreement includes an upfront cash payment, yearly cash maintenance payments, and royalties on product sales. Fundamental claims in the Genta patent portfolio that was licensed involve methods, compositions, and processes for *in vitro* and *in vivo* inhibition of protein synthesis by antisense oligonucleotides. This portfolio incorporates exceptionally broad claims for most pharmaceutical uses, as well as many diagnostic applications. Genta has retained rights to these patents for its own use, and an exclusive right to grant unrestricted sublicenses to other users.

Hybridon

Hybridon was one of the original developers of antisense-based therapeutics. Its involvement in the field was bolstered significantly when, in January 1996, the then Searle (Skokie, IL) pharmaceutical division of Monsanto entered into a collaborative agreement with Hybridon to conduct research and development of therapeutic antisense compounds directed at up to eight molecular targets in inflammatory diseases, with Searle having the option to designate up to six additional molecular targets, including mdm2 for oncology application. Searle was to fund research and development, with Hybridon having responsibility for medicinal chemistry and manufacturing. The companies also had an option to form a 50/50 joint venture. As part of the collaborative agreement, Searle purchased 200,000 shares of common stock in Hybridon's 1996 IPO, and under a revised 1998 agreement, Searle was to pay Hybridon an aggregate amount of $14 million, representing $7 million in research payments and $7 million in equity investment. From 1998 through January 2000, Searle was making annual research payments to Hybridon of $600,000. However, in March 2000, Searle elected not to continue its collaboration with Hybridon and returned all Hybridon licenses granted to Searle, granting Hybridon the use of Searle's agreement-related patent rights, including all antisense rights relating to mdm2. A royalty is to be paid to Searle if antisense compounds discovered under the collaboration are successfully commercialized.

Hybridon has exclusive rights to USA patent #6,013,786, issued in January 2000, covering antisense oligonucleotides complementary to a portion of the mdm2-encoding RNA and methods for using such oligomers as analytical and diagnostic tools as well as potential therapeutic agents. This patent was issued to Dr. Jiandong Chen of Louisiana State University, Dr. Ruiwen Zhang of the University of Alabama at Birmingham (UAB), and Dr. Sudhir Agrawal of Hybridon. Dr. Ruiwen Zhang's laboratory at UAB has received funding from the NCI under grant number 1R01CA80698-01, for the April 1999-March 2003 period, to investigate the function of the mdm2 oncogene in tumor growth and the potential value of mdm2 as a drug target for cancer therapy.

Immusol

Immusol, a private company, is developing technologies for the discovery of novel targets for drug screening, target validation, and development of therapeutics for a variety of indications, including cancer. These programs are based on Immusol's ribozyme technologies which provide the company with multiple distinct product development and partnering opportunities from a single platform.

The company's ribozyme-based technology, Inverse Genomics, uses a library of randomized, hairpin-shaped ribozymes to enhance its ability to sift through the entire genome and rapidly isolate genes that may be useful as drug targets. This approach addresses the problem of drug target identification from the opposite direction of traditional discovery programs. Instead of starting with the entire human genome to identify genes that control a biological feature with therapeutic potential, using Inverse Genomics an investigator starts with a biological feature with therapeutic potential and works backward, identifying only those genes that are potentially viable therapeutic targets. Immusol is also focusing on the application of its ribozyme technology to enhance the early stages of drug discovery by providing a rapid analysis of gene function *in vivo.*

In February 2001, Immusol and Chugai Biopharmaceuticals (CBI, San Diego, CA), a wholly owned subsidiary of Chugai Pharmaceutical (Tokyo, Japan), entered into a functional genomics collaboration for drug discovery, using Immusol's gene inactivation technology to support Chugai's discovery and validation of drug target

genes that may result in methods to prevent/treat cancer metastasis. The collaboration also targets newly discovered genes from Chugai's wholly owned subsidiary, Chugai Research Institute for Molecular Medicine and Chugai's research center, Fuji Gotemba Laboratories.

Inex Pharmaceuticals

Inex's involvement in the SNAS sector began in February 1998, when it acquired the portfolio of phosphorothioate antisense patents and licenses, and a therapeutic oligonucleotide manufacturing facility from Lynx Therapeutics (Hayward, CA) for $3 million in cash, 1.2 million shares of Inex common stock, and royalties on future sales of products based on this technology. In addition, Lynx agreed to assign a royalty-bearing license to Inex for its phosphoroamidate chemistry for certain therapeutic applications in the fields of cancer and inflammation to be defined later.

Before this acquisition, Inex had made several attempts to develop SNAS agents with collaborators by contributing its Transmembrane Carrier Systems (TCS) technology for the delivery of oligonucleotides. TCS is a proprietary lipid envelope-based drug delivery system, designed to optimize the delivery of IV drugs to sites of tumors. In the TCS approach, drug is encapsulated in small multilamellar vesicle structures (approximately 100 nm diameter) that form spontaneously upon the entrapment of large quantities of the drug within lipid bilayers. As a result of small size and neutral charge at physiological pH, TCS-encapsulated drug exhibits extended blood circulation times, and enhanced accumulation in tumor tissue. The targeted delivery improves drug effectiveness and reduces side effects to healthy tissues. TCS technology has been applied to a wide range of therapeutic agents including conventional small molecule drugs as well as oligonucleotides and gene plasmids (Leonetti C, etal, AACR00, Abs. 4080:642, and Mui B, etal, Biochim Biophys Acta, 25 Aug 2000;1467(2):281-92).

Isis Pharmaceuticals

Isis is a leader in SNAS development with several products for a number of indications in clinical development. Although the company has experienced a number of setbacks in this field, including the November 1999 decision by Novartis Pharma to terminate its September 1990 collaboration for the development of ISIS 3521 and ISIS 5132, and somewhat curtailed its broad oncology-related effort, Isis is still actively pursuing the development of a number of SNAS-based agents against cancer and other targets.

In addition to its drug development activities, Isis has created two divisions focused on more broadly capitalizing on its expertise in RNA. Ibis Therapeutics is a division focused on the discovery of small molecules that bind to RNA, and GeneTrove is a division that focuses on gene functionalization using antisense to assist the pharmaceutical industry in validating and prioritizing potential gene targets. Among Isis' partners in this program are AstraZeneca, Abbott Laboratories, Aventis, AstraZeneca and R.W. Johnson Pharmaceutical Research Institute (PRI), a Johnson & Johnson company.

In December 1998, Isis Pharmaceuticals purchased from Gilead Sciences (Foster City, CA) the holdings of its antisense patent estate, including patents and patent applications covering a broad proprietary suite of antisense chemistry and antisense drug delivery systems. Under the agreement, Isis purchased the rights to all Gilead intellectual property involving antisense chemistry and applications for $6 million, payable in 4 installments over 3 years. In July 1990, Gilead had entered into a research collaboration with Glaxo to develop genetic code blocker technology, which was subsequently terminated in June 1998, with the two companies each retaining rights to jointly developed technology.

In December 1998, Isis and AstraZeneca established a 3-year, worldwide research collaboration focused on the development and commercialization of novel antisense-based cancer drugs. Under the terms of this collaboration, Isis will create and, with AstraZeneca, screen antisense-based candidates for undisclosed targets on an exclusive basis. AstraZeneca is responsible for the development of any potential drug candidates. Isis will receive from AstraZeneca a technology access fee, annual research funding, and milestone payments for any drugs progressing into clinical development, as well as royalties on sales of any commercialized drugs. AstraZeneca has exclusive worldwide rights to develop and commercialize drug candidates emerging from this collaboration. The collaboration may be extended beyond its initial 3-year term.

In April 1999, Isis and Elan formed a new subsidiary, OraSense, to develop platform technology for the oral delivery of antisense drugs. Isis is the majority shareholder in OraSense, owning 80.1% of the company's outstanding common stock. Elan made a $27 million equity investment in Isis, consisting of $15 million of common stock purchased at a premium to market, and $12 million of convertible exchangeable preferred stock. Elan also received warrants exercisable in five years, and has the right to convert the preferred stock into either an ownership interest in Isis or in OraSense. The first oral drug OraSense is working on is ISIS 104838, Isis' antisense inhibitor of tumor necrosis factor-α (TNF-α), for the treatment of inflammatory diseases. OraSense has also conducted a limited phase I study evaluating the impact of various absorption enhancers on the uptake of a solution formulation of ISIS 2503 delivered intrajejunally.

Lorus Therapeutics

Lorus Therapeutics (formerly Imutec Pharma) entered the SNAS drug development sector through the acquisition of GeneSense Technologies in November 1999. GeneSense's antisense platform derives from an exclusive worldwide license from the University of Manitoba (Winnipeg, Manitoba, Canada) and the Manitoba Cancer Treatment and Research Foundation, entered into in 1997,

giving the company access to the antisense technology developed by Drs. Jim A. Wright and Aiping H. Young, cofounders of GeneSense.

In October 2000, Lorus announced that it had formed a strategic supply alliance with Proligo (Boulder, CO), a joint venture between Germany's SKW Trostberg (51%) and Gilead Sciences (49%). Proligo will invest the necessary resources to increase production capacity so as to supply the higher volumes of GTI 2040 required for multicenter phase II clinical trials.

MethylGene

MethylGene, a private company, was founded in January 1996 as a Hybridon spinout. Currently, Hybridon holds a minority ownership position in the company. MethylGene is a chemistry-driven drug discovery company engaged in the application of mechanism-based small-molecule and mRNA-inhibitor drug design technologies to discover, develop and commercialize novel medicines for the treatment of disease for which there are currently limited or no effective treatment, in particular, cancer and infectious diseases.

In August 2000, MethylGene entered into a North American license, research and development agreement for MG98 and novel small molecule inhibitors of DNA-MeTase with MGI Pharma (Minneapolis, MN). Under terms of the agreement, MGI Pharma will make an initial cash payment to MethylGene followed by certain milestone payments based on progress toward receiving North American approvals. MGI Pharma will fund the ongoing development of MG98 and the DNA-MeTase small-molecule inhibitors and make an equity investment in MethylGene. MethylGene will also receive royalties on net sales, and retained a copromotion right in North America, and all rights outside of North America. Initial and milestone cash payments by MGI Pharma would amount to approximately $16 million for each of the two development programs up to the time of regulatory approvals. In addition, MGI Pharma is to invest $6.8 million in MethylGene stock by March 31, 2001.

In October 2000, MethylGene completed a new round of private funding in the aggregate amount of $18.6 million in support of its drug discovery and drug development programs. This financing, combined with previous rounds of financing, brought the total equity investment and equity commitments in the company to $53 million. This financing complements the Technology Partnerships Canada investment of $4.77 million in support of MG98 development, and the company's previous announcement of its agreement with MGI Pharma.

Ribozyme Pharmaceuticals (RPI)

Ribozyme Pharmaceuticals (RPI) is a public company founded in 1992 to capitalize on the broad potential of ribozymes for use as human therapeutics and in drug discovery for the identification of gene function and therapeutic target validation. RPI's core technology is based on intellectual property relating to the alteration of the nucleic acid sequence of deleterious target molecules by ribozyme-catalyzed *trans*-splicing, patented by Dr. Thomas R. Cech and Dr. Bruce A. Sullenger (USA patent #5,667,969 and #5,869,254, issued in September 1997 and February 1999, respectively) of the University of Colorado (Boulder, CO). The Cech/Sullenger technology was assigned to the University's affiliate, University Research Corporation (URC), which subsequently gave licensing rights to Competitive Technologies (Fairfield, CT). United States Biochemical (USB) subsequently licensed the technology and RPI entered into a license with URC and sublicenses with USB as well as Competitive Technologies, in effect securing exclusive, worldwide rights (excepting noncommercial academic research) to make, use, and sell ribozymes and ribozyme-based products covered by the Cech/Sullenger patents. The URC license and USB sublicense are fully paid by RPI. The Competitive Technologies sublicense provides for royalty payments on sales of ribozyme products based on the licensed technology. RPI may grant technology sublicenses subject to the payment of fees and/or royalties to Competitive Technologies on revenues derived from such sublicenses.

In August 1999, RPI acquired all of the ribozyme-based intellectual property assets of Innovir Laboratories, now Nexell Therapeutics (Irvine, CA). The acquisition included 28 patents and patent applications plus 2 trademarks bringing RPI's total portfolio to more than 100 issued or allowed patents plus at least 100 additional patents under consideration. This intellectual property package deals with ribozyme motifs, uses of oligonucleotides for therapeutics, target validation and diagnostics, chemical modifications of oligonucleotides, and oligonucleotide delivery, detection, manufacturing and purification. The acquisition is part of the closing of Innovir's ribozyme-based research and manufacturing operations. Among the acquired patent estate was an allowed patent application for ribozyme molecules that can be regulated, Regulatable Nucleic Acid Therapeutics (RNAT). RNAT makes it possible to maximize ribozymes' ability to prevent or halt cells' excess production of proteins, which causes many diseases. RNAT uses a substance that binds to ribozymes, modulating the ability of the ribozyme to cleave, and thereby inactivate, a targeted RNA molecule. Another allowed patent application involved enhancement of the delivery of oligozymes and other oligonucleotides to hepatocytes (liver cells) using hemeliposomes which consist of lipid particles that are coupled to heme, a hemoglobin component that binds to liver cells with high affinity. Because of this binding action, therapeutic oligozymes that are mixed with hemeliposomes can be targeted to diseased liver cells. An additional allowed patent application involved production of a delta virus-based vector for the delivery of oligonucleotide-based therapeutics to cells and organs to treat viral infections. Delta virus is a small virus that infects the liver and can divide only in cells already infected with hepatitis B virus.

RPI is developing Angiozyme in partnership with Chiron, as part of a collaboration established in July 1994, involving use of ribozymes in the treatment and/or diagnosis of ophthalmologic diseases, cancer, and viral infections. The agreement calls for exclusive collaboration on up to five specific targets selected by Chiron, including Angiozyme, and provides that RPI and Chiron will, in general, share equally in the development costs and net profits of any jointly developed products.

RPI retains the right to manufacture synthetic ribozyme products resulting from the collaboration, whether jointly developed, or developed by Chiron or RPI individually. Chiron holds exclusive marketing rights for all jointly developed products, subject to a copromotion agreement for sales in North America and Europe. In the Far East, Chiron has exclusive marketing rights with the right to sublicense, although RPI retains the right to share in 50% of the profits. The collaborative term for jointly developed products is 30 years, and the license for solely developed products is 15 years after first commercial sale or the duration of patent protection, whichever is longer.

In January 2000, RPI and Elan established a joint venture, Medizyme, to combine RPI's SNAS technology with Elan's drug delivery expertise. Under terms of the transaction, RPI licensed Herzyme to Medizyme and contributed $12.0 million in initial funding in exchange for 80.1% of Medizyme's capital stock. This initial funding derived from the sale to Elan by RPI of its Series A convertible preferred stock. Elan provided Medizyme with $3.0 million in initial funding in exchange for 19.9% ownership. Elan has licensed its Medipad drug delivery technology to Medizyme in return for a license fee. RPI and Elan have estimated that Medizyme will require approximately $15 million in funding to cover future operating and product development costs. Elan has made a $12 million credit facility available to RPI on a drawdown basis, for RPI to use to fund its portion of Medizyme operating costs over a 30 month period. Elan may ultimately convert this debt into RPI Series B convertible preferred stock at a 50% premium to the average value of the stock in the 60 trading days prior to the time of the applicable drawdown on the credit facility. Elan purchased $5 million-worth of RPI common stock, and has committed to purchase an additional $5 million within 15 months at a per share price based on the achievement of milestones. Elan has also received warrants to additional purchase shares of RPI common stock, and has the right to exchange the Series A preferred stock for additional capital stock of Medizyme or RPI common stock.

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