activated in a number of neuroendocrine tumors. The array analysis showed very high expression in most SCLC cell lines. Transfection with a reporter gene regulated by a 1.7 kb region of the INSM1 promoter (Li Q, *et al., BBRC.* **236**, p776, 1997) showed very high expression in most SCLC cell lines (3–12 times stronger than SV40) and no expression in other cell lines. Expression of the Herpes Simplex Thymidine kinase gene from the INSM1 promoter conferred cell death after treatment with the prodrug ganciclovir in SCLC cell lines with high expression of INSM1, but not in cell lines with low or no expression of INSM1. We have therefore demonstrated that the activity of INSM1 is sufficient for gene therapy approaches and that the cloned promoter region retains the SCLC cancer specificity.

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ARHI gene therapy and paclitaxel exert additive cytotoxicity for breast cancer cells through caspase-independent and caspase-dependent apoptotic mechanisms

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Background: ARHI is an imprinted tumor suppressor gene encoding a 26kD GTPase with 50–60% homology to ras and rap, whose expression is downregulated in breast and ovarian cancers. Re-expression of ARHI inhibits growth, decreases invasiveness, and induces apoptosis. Our recent work demonstrated that ARHI could induce apoptosis through a caspase-independent and calpain-dependent mechanism. Re-expression of ARHI can reduce tubulin expression and significantly inhibit mitosis in breast cancer cells. More effective and less toxic therapy is needed. Consequently, we have combined ARHI gene therapy with paclitaxel that induces apoptosis through caspase-dependent pathways.
Intratumoral injection of ARHI in an inducible binary adenoviral vector produced significant regression of established human breast cancer xenografts. To develop tumor specific expression, we have modified the first binary vector to contain a human telomerase reverse transcriptase (hTERT) promoter. As over 90% of breast cancers are positive for telomerase activity, more specific tumor cell killing might be established by this new delivery approach.
Material and Methods: Using a dual adenovirus system (Ad/ARHI/PGK-GV16, Ad/ARHI/hTERT-GV16), ARHI was re-expressed in breast cancer cells (MCF-7, SKBr3) that have lost ARHI expression. Cells were treated with paclitaxel alone and in combination with virus. Limiting dilution clonogenic assays and isobolographic analysis were used to measure clonogenic growth. For studies with ARHI driven by hTERT promoter, the sulforhodamine B (SRB) assay was used to measure cell growth.
Results: We have tested the effects of a combination of Ad/ARHI/GV16 and paclitaxel on breast cancer cell lines MCF-7 and SKBr3 by limiting dilution clonogenic assay. Additive cytotoxicity was produced with a combination of paclitaxel (5–10nM) and Ad/ARHI/PGK-GV16 (1000–2000 particles/cell). Clonogenic growth was only inhibited 40 to 90% by Ad/ARHI/GV16, and 90 to 99.9% by pacitaxel. Inhibition was greatly increased to more than 99.99% by a combination with both reagents.
Given the limited specificity of the adenovirus system and the need to develop strategies that could be used systemically, we have used the hTERT promoter to target ovarian and breast cancer cells. When ARHI gene was induced by Ad/hTERT-GV16, high levels of transgene expression and significant cell growth inhibition were observed in breast cancer cells. In contrast, only minimal toxicity was detected in normal human primary mammary epithelial cells after treatment with this vector.
Conclusion: A combination of paclitaxel and ARHI gene therapy is more effective than either agent alone for inhibiting breast cancer cells growth through a combination of caspase-dependent and caspase-independent mechanisms. Ad/hTERT-GV16 vector facilitates specific expression of ARHI gene in breast cancer cells.

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Mesothelial cells as target of antiangiogenic therapy for peritoneal metastasis

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Background: Peritoneal mesothelial cells (PMC) have the capacity to produce large amounts of VEGF. Although VEGF in ascites has been considered to mainly originate from tumor cells, the contribution of VEGF derived from host cells including PMC has also been considered. We first assessed the production of VEGF by cultured PMC in vitro. Then, we evaluated the ratio of host-derived VEGF using in a nude mouse model, and finally evaluated the effectiveness of gene transduction of soluble Flt-1 targeted to PMC against peritoneal dissemination.
Methods: VEGF in the culture supernatants of isolated PMC was measured by ELISA. With the same method, human- and mouse-VEGF were measured separately in ascitic fluid of nude mice that had been intraperitoneally inoculated with a human gastric cancer cell line, MKN45, at various time points. Finally, we evaluated whether transfer of the sFlt-1 gene specifically to PMC using an adenovirus vector could inhibit tumor formation in the peritoneal cavity.
Results: PMC produced similar amounts of VEGF to gastric cancer cells, which was significantly augmented by the addition of fibroblast growth factor (FGF) or lysophosphatidic acid (LPA). Of VEGF in ascitic fluid at 3 weeks after MKN45 inoculation, 12.8% was derived from mouse cells. At 6 week, however, the ratio of mouse-derived VEGF was reduced to 5.0%, suggesting that the ratio of host-derived VEGF may be higher in the earlier phase. When adenovirus expressing sFlt-1 was intraperitoneally (ip) administered in vivo, a high level of sFlt-1 protein could be detected in peritoneal lavage for 8 weeks, with a significant reduction in the number of intraperitoneal metastatic nodules and prolonged survival of nude mice that were ip inoculated with MKN45 3 days later.
Conclusion: PMC-derived VEGF has an essential role in the development of peritoneal metastasis and thus could be an attractive target for antiangiogenic gene therapy.

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Interim evaluation of a multi-institution phase I/II study of antisense oligonucleotide GTI-2040 (G) and capecitabine (C) in patients with metastatic renal cell carcinoma (mRCC)

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Introduction: G is a 20-mer phosphorothioate oligonucleotide complementary to the R2 subunit of ribonucleotide reductase that has broad-spectrum activity in human xenograft models including RCC models. 5-fluorouracil (5FU) has some activity in renal cell carcinoma (RCC) and G plus 5FU has cooperative activity against RCC in SCID mice.
Methods: An open label, non-randomized phase **I/II** study of G plus C was conducted to determine the toxicity and objective response rate in patients with metastatic RCC. G was dose escalated in the phase **I** portion and administered continuously via central venous catheter by a portable infusion pump for 21 days with C at 1660 mg/m^2/d orally divided into two daily doses followed by 7 days of rest during each 28 day treatment cycle. Pharmacokinetic samples were collected from all phase **I** pts and 6 additional pts treated at the phase **II** dose. Only toxicities occurring during cycle 1 were considered for determining dose limiting toxicity (DLT).
Results: To date, 29 pts [M: F ratio 18:11; median KPS 80 (70–100); median age 59 (37–73)] have received a total of 88 cycles of therapy. Twenty-six pts had prior systemic therapy (25/26 prior immunotherapy, 14/26 received $\geqslant$ 2 systemic therapies); 13 had prior radiotherapy and 19 had prior nephrectomy. None of the 3 pts treated at dose level 0 (G 145 mg/m^2/d) and only 1 of 6 pts treated at dose level 1 (G 185 mg/m^2/d) experienced DLT (grade 3 diarrhea). Since dose level 1 is also the recommended phase **II** dose for single agent G, the phase **II** portion of the study was conducted at this dose. The major toxicities in both the phase **I** and **II** portions included Grade 4: pancytopenia (1), pulmonary embolism (1) and bone pain (1); Grade 3: thrombocytopenia (4), lymphopenia (3), anemia (2), neutropenia (2), nausea/emesis (2), infections (3), fatigue (2); neuropathy, thrombosis, dehydration, hypophosphatemia, diarrhea (1 each). Additional common grade 1/2 toxicities include: nausea (62%), fatigue (45%), emesis (35%), hematologic toxicity (28%), anorexia (28%), and diarrhea (24%). Amongst the 25 response-evaluable pts at the phase **II** dose; 13 (52%) had stable disease (SD) as best response (median duration: 4 months, range 2–10), and 1 durable (8 months) partial response was observed. At the phase **II** dose, the pt with PR experienced a unidimensional tumor reduction of 39%, and the pt with the longest duration SD had a 23% tumor reduction. One additional pt at dose level 0 also had SD and a 13% decrease in tumor size. Pharmacokinetic studies are ongoing and completed analysis will be presented.
Conclusions: The combination of G and C is tolerated at the recommended phase **II** dose with expected toxicities. This G + C trial is ongoing and overall activity and objective response will be assessed when the Phase **II** efficacy timepoint is achieved. An additional G + cytokine combination study is being planned for mRCC patients.