Safety and Efficacy in Advanced Solid Tumors of a Targeted Nanocomplex Carrying the p53 Gene Used in Combination with Docetaxel: A Phase 1b Study

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Loss of p53 suppressor function, through mutations or inactivation of the p53 pathway, occurs in most human cancers. SGT-53 is a liposomal nanocomplex designed for systemic, tumor-targeting delivery of the wt p53 gene. In this nanodelivery system, an anti-transferrin receptor single-chain antibody fragment serves as the targeting moiety. In an initial phase 1 trial in patients with advanced solid tumors, SGT-53 demonstrated tumor-specific targeting, was shown to be well tolerated, and was associated with an antitumor effect in several patients. Our preclinical studies have also demonstrated enhanced antitumor activity with the combination of SGT-53 and docetaxel. Thus, this dose-escalation trial was undertaken to assess the combination of SGT-53 and docetaxel for safety and potential efficacy in 14 advanced cancer patients. Results reveal that the combination of SGT-53 (maximum dose, 3.6 mg DNA/infusion) and docetaxel (75 mg/m^2/infusion) was well tolerated. Moreover, clinical activity involving 12 evaluable patients was observed. Three of these patients achieved RECIST-verified partial responses with tumor reductions of –47%, –51%, and –79%. Two others had stable disease with significant shrinkage (–25% and –16%). These results support phase 2 testing of SGT-53 in combination with docetaxel.

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INTRODUCTION

The p53 gene (TP53) is an important tumor-suppressor gene in humans that has been implicated in a wide range of cellular processes (regulation of cell cycle checkpoints, cell death, senescence, DNA repair, maintenance of genomic integrity, and control of angiogenesis).[1–6] The loss of such critical tumor-suppressor activity is believed to be responsible for the involvement of p53 in a broad array of human tumors. Abnormalities in the p53 tumor-suppressor gene have been reported in over 60% of human cancers,[7–10] and in certain subtypes of cancer such as triple-negative breast cancer, lung cancers and high-grade serous ovarian tumors, p53 mutations can be found in up to 80% of patients.[11]

Loss of p53 function has been found to impact the efficacy of standard anticancer treatments, such as radiation and chemotherapy, *i.e.*, cells lacking p53 are more resistant to radiation and chemotherapy.[12,13] Because of the high frequency of mutation and/or inactivation of p53 in human cancers and its impact on response to standard therapies, p53 has been considered to be an important therapeutic target although p53-based therapy is challenging since a gain of p53 function rather than its inhibition is desirable. Restoration of p53 function has been demonstrated to enhance sensitivity to standard therapeutic modalities and to result in tumor regression in a number of different animal models.[14–19] A number of approaches have been employed to exploit p53 as a target for the treatment of cancer.[20] These include conversion of mutant p53 to a form with wild-type properties via small molecules such as PRIMA-1; prevention of p53 degradation by low-molecular-weight MDM2/4 antagonists which prevent MDM2-p53 interactions (*e.g.*, nutlins); exploitation of the principle of synthetic lethality gene (SLG); and gene therapy using delivery systems such as adenovirus and liposomes.[21,22]

Currently, ~80 gene therapy clinical trials employing p53 have been initiated or conducted worldwide and this number is likely to increase over the coming years. Introduction of wt p53 by various viral gene therapy delivery systems, in particular retroviral and adenoviral vectors, has been reported to suppress the growth of various types of malignancies in both *in vitro* and in xenografts in mouse models. The types of malignancies studied include leukemia, prostate, head and neck, colon, cervical, glioblastomas, breast, liver, ovarian, kidney, and lung tumors.[23–27] Several gene therapy clinical trials using adenoviral delivery to restore functional p53 have been initiated or conducted[28–32] in which intratumoral administration resulted in both complete local positron emission

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tomography/computed tomography (PET/CT)imaging response[33] and biopsy-documented pathological complete responses (when administered in conjunction with radiotherapy).[34] Recombinant adenoviral p53 vectors have also been studied in small clinical trials in patients with hepatocellular cancer, ovarian cancer, esophageal cancer, and gliomas.[35,36] Despite impressive local tumor responses, this approach involving viral delivery and intratumoral injection of the primary tumor lacks the ability to treat the metastatic disease that is actually responsible for most cancer deaths.[37] Gene therapy systems using nontargeted cationic liposomes that also involve intratumoral injection share this inability to treat metastases.

One of the major obstacles to effective p53 gene therapy is the development of a systemic gene delivery system that can specifically and efficiently reach both primary and metastatic tumor cells. A tumor-targeting immunoliposome complex (termed SGT or scL) for delivery of molecular medicines has been developed to address this need. In this nanosized complex, the payload is encapsulated within a cationic liposome the surface of which is decorated with a single-chain antibody fragment targeted to the transferrin receptor (TfR) as the targeting moiety (the single-chain antibody fragment is termed TfRscFv). The TfR is highly expressed on tumor cells, and the receptor and its ligand are internalized into the tumor cells via receptor-mediated endocytosis.[38] The specificity of this complex in targeting tumors in preference to normal cells has been well established both *in vitro* and *in vivo* (in mice).[39–48] We have shown that, when systemically administered, this nanosized complex can efficiently target both primary and metastatic tumors in animal models, including delivering the nanocomplexed payload across the blood–brain barrier to target brain tumors.[49–51]

SGT-53 is a nanocomplex that contains a normal human wt p53 cDNA. In preclinical studies, systemic administration of SGT-53 demonstrated not only the ability to target tumor cells throughout the body with exquisite specificity but also displayed significant antitumor activity. Furthermore, a variety of types of tumors treated with SGT-53 were shown to be sensitized to conventional radiation/chemotherapy,[17–19,41] resulting in tumor growth inhibition and long-term regression.[39,40]

SGT-53 recently completed a phase 1a human clinical trial as a single agent for systemic delivery of wt p53 for the treatment of solid tumors. In this first-in-man testing of SGT-53, the nanocomplex was very well tolerated at all of the doses tested which ranged from 0.6 to 3.6 mg DNA/infusion, including the highest dose (3.6 mg DNA/infusion). No maximum tolerated dose (MTD) was reached and 95% of the adverse events (AEs) experienced were grade 1 and 2. Moreover, an antitumor effect was observed in several patients, even at an SGT-53 dose as low as 0.6 mg DNA/infusion.[22] Significantly, using paired DNA polymerase chain reaction analysis of biopsied tumor and uninvolved skin, a tumor-selective, dose-dependent level of the transgene was detected in malignant metastatic tissue from three patients[22] but not in their normal tissue. As no MTD was reached in this phase 1a trial and a dose of 3.6 mg DNA/infusion was well tolerated, this dose was selected for use in this phase 1b combination trial.

Preclinical *in vivo* studies have demonstrated significantly enhanced antitumor response to the combination of SGT-53 and docetaxel compared to docetaxel alone. Based upon the positive results for safety in the phase 1a trial, and the significant efficacy observed in *in vivo* preclinical studies with the combination of SGT-53 and chemotherapeutic agents, including docetaxel, further clinical studies with SGT-53 in combination with docetaxel appeared warranted. Therefore, phase 1b testing was initiated to determine the safety of the combination of SGT-53 plus docetaxel and to assess the therapeutic potential of this combination treatment in patients. The results reported here demonstrated that the addition of SGT-53 to conventional docetaxel therapy did not increase the number or intensity of AEs compared to docetaxel alone. Furthermore, impressive patient responses were observed.

RESULTS

Patient characteristics

A total of 14 patients with metastatic, refractory cancer who had been previously treated with standard therapies and who had no further standard-of-care options were entered into this phase 1b trial (**Table 1**). All of these patients (median age of 58.5 years (range 33–77years)) had received prior chemotherapy with a variety of agents. A total of 11 (78.6%) had received at least one taxane-based regimen: 8 of the 11 (72.7%) with paclitaxel and 3 of the 11 (27.3%) with docetaxel. Among these 11, 3 (27.3%) had prior treatment with both paclitaxel and docetaxel. These patients suffered from a variety of tumor types. The primary disease site, as well as the sites of the target and nontarget lesions, including metastases, is given in **Supplementary Table S1**. The patients were enrolled in cohorts with increasing doses of SGT-53 (2.4 or 3.6 mg DNA/infusion) and/or docetaxel (40, 60, or 75 mg/m^2) (**Table 2**) following the treatment schedule given in **Table 3**. These DNA doses are equivalent to preclinical DNA doses of 10 and 15 μg/mouse, respectively, which had been found to be efficacious in animal studies. Patients received a median of 10 infusions of SGT-53 and 3 infusions of docetaxel. Two patients received two cycles of treatment for a total of 20 infusions of SGT-53 and 6 of docetaxel. A treatment summary for each of the patients, including cohort, tumor type, and number and doses of SGT-53 and docetaxel administered, is given in **Table 4**.

Safety

All 14 patients enrolled in the trial received at least one infusion of SGT-53 and docetaxel and were included in the evaluation of safety. No treatment-related deaths occurred. There were 106 incidents of AEs classified as at least possibly related to SGT-53 over the course of the study. The frequency of AEs that occurred more than once (as percent of total incidences) is given in **Supplementary Table S2**.

A listing of the AEs that occurred in ≥14% of the patients are given in **Table 5**. The majority of the AEs reported were grades 1 and 2. They consisted primarily fever (reported in 43% of patients), intermittent diarrhea (reported in 36% of patients), and anemia, chills, dehydration, and thrombocytopenia all reported in 29% of patients. Grade 1/2 hypotension, intermittent fatigue, and tachycardia were observed in 21% of the patients. Tachycardia was only recorded in patients in cohort 3. With regards to grade 3/4 AEs, only anemia, neutropenia, and hypophosphatemia occurred in >10% of the patients. However, none of the grade 3/4 AEs recorded were classified as definitely related to SGT-53. Moreover,

Table 1 Demographics and characteristics

	Cohort 1 ($n = 4$)	Cohort 2 ($n = 4$)	Cohort 3 ($n = 6$)	Total No. of patients ($n = 14$)
Sex, *n* (%)				
Female	4 (100%)	1 (25%)	4 (66.7%)	9 (64.3%)
Male	0	3 (75%)	2 (33.3%)	5 (35.7%)
Race/ethnicity, *n* (%)				
Caucasian	4 (100%)	4 (100%)	5 (83.3%)	13 (92.9%)
African American	0	0	0	0
Hispanic	0	0	0	0
Asian	0	0	1 (16.7%)	1 (7.1%)
Age				
Mean (SD)	51.8 (12.6)	64.5 (9.5)	59 (12.1)	58.5 (11.8)
Median	57	63.5	55.5	57.5
Min, Max	33, 60	54, 77	44, 75	33, 77
ECOG score, *n* (%)				
0	3 (75%)	3 (75%)	2 (33.3%)	8 (57.1%)
1	1 (25%)	1 (25%)	4 (66.7%)	6 (42.9%)
Primary tumor type, *n* (%)				
Anal	1 (25%)	0	0	1 (7.1%)
Breast	0	0	2 (33.3%)	2 (14.3%)
Endometrial	1 (25%)	0	0	1 (7.1%)
Esophagus	0	1 (25%)	0	1 (7.1%)
Extrahepatic bile duct	0	0	1 (16.7%)	1 (7.1%)
Lung	0	1 (25%)	1 (16.7%)	2 (14.3%)
Ovarian	1 (25%)	0	2 (33.3%)	3 (21.4%)
Pancreas	1 (25%)	0	0	1 (7.1%)
Scalp	0	1 (25%)	0	1 (7.1%)
Uterus	0	1 (25%)	0	1 (7.1%)
Prior therapy, *n* (%)				
Chemotherapy	4 (100%)	4 (100%)	6 (100%)	14 (100%)
Taxol	4	2	4	10 (71.4%)
Taxotere	1	2	1	4 (28.6%)
Radiation therapy	2 (50%)	2 (50%)	3 50%)	7 (50%)
Hormonal therapy	1 (25%)	0	1 (16.7%)	2 (14.3%)
Vaccine therapy	1 (25%)	0	0	1 (7.1%)
Other[a]	0	1 (25%)	1 (16.7%)	2 (14.3%)

[a]Details given in **Supplementary Table S3**.

Table 2 Cohort information

Cohort number	SGT-53 dose/ infusion (mg)	Docetaxel dose/ infusion (mg/m²)	Number of patients (*n*)
1	2.4	40 (cycle 1) 60 (cycles 2 and 3)	4
2	2.4	75 (all cycles)	4
3	3.6	75 (all cycles)	6

Table 3 Schedule of administrations

Week	Days					
	1	2	3	4	5	
1	SGT-53		Doc	SGT-53		Cycle 1
2	SGT-53					
3	SGT-53					
4	SGT-53		Doc	SGT-53		Cycle 2
5	SGT-53					
6	SGT-53					
7	SGT-53		Doc	SGT-53		Cycle 3

only three grade 4 AEs were categorized as even possibly or probably related to SGT-53, one incidence each of neutropenia (patient ID #02-024), thrombocytopenia (patient ID #02-031), and leukopenia (patient ID #02-031). This was the only instance of leukopenia that occurred in the trial.

There were six serious adverse events reported during this study. Of these, five were classified as completely "unrelated" (patient ID #02-018, 02-019, 02-024, 02-029) or "unlikely to be related" (patient ID #02-037) to the SGT-53 study drug. One (patient ID #02-018) was classified as "possibly related" due to an unplanned hospitalization for nausea, vomiting, and neutropenia. While unable to rule out an involvement of SGT-53 in this serious adverse event, the neutropenia was considered to be more likely related to administration of docetaxel.

Only two patients experienced a dose-limiting toxicity (DLT) event during this study: patient ID #02-018 for neutropenia and #02-024 for neutropenia/syncopal episode. After these DLTs in the first patient in cohorts 1 and 2, respectively, three more patients were enrolled in each cohort and no additional DLTs occurred. More significantly, there were no DLTs in cohort 3, the highest levels of both SGT-53 (3.6 mg DNA/infusion) and docetaxel (75 mg/m²/infusion). Therefore, no MTD was reached in this trial, and the cohort 3 dose would be the recommended phase 2 dose.

Tumor responses

Of the 14 patients enrolled in the study, 12 were evaluable for tumor response. One patient (patient ID #02-018) went off study after one dose each of SGT-53 and docetaxel because of a DLT and was not evaluable. A second patient, ID #02-024 who had received two doses of SGT-53 and one of docetaxel, was also removed from study due to the occurrence of a DLT. The remaining 12 patients had measurable disease as per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.0.

The patient responses are given in **Table 6**. Of these 12, two achieved RECIST partial response (PR) (02-023, 02-026), one had an unverified PR (02-029) and two (02-033, 02-035) were classified as stable disease (SD) with significant shrinkage but not >30%. Four additional patients (02-020, 02-030, 02-036, and 02-037) were classified as having SD at the end of treatment but without tumor shrinkage. Patient ID #02-026 (metastatic adenocarcinoma of the esophagus) received a second round of treatment and maintained 58% tumor reduction with no disease progression for ~13 months.

The CT response for this patient showing the decrease in tumor signal after the combination of SGT-53 and docetaxel is shown in **Figure 1**. Patient ID #02-033 (male metastatic breast cancer) also received two rounds of treatment. After one round of treatment there was a 25.3% decrease in the sizes of the tumors (primary and metastatic) by RECIST, which was maintained at 23% after the second round. Photographic documentation of the decrease in the primary tumor in this patient over the time of treatment is shown in **Figure 2a**. Even after ~3 weeks, *i.e.*, only the first cycle, tumor shrinkage was evident. By the end of the second round of treatment at 15 weeks, a dramatic change was evident.

Moreover, although not evaluable by RECIST after going off study due to a DLT as described above, there is photographic evidence of tumor response in patient ID #02-024. This individual was diagnosed with angiosarcoma of the scalp that was not considered as targetable tumor by RECIST and metastatic disease to the lymph nodes. This patient received only two infusions of SGT-53 (2.4 mg DNA/dose) and one infusion of 75 mg/m² of docetaxel before going off study. A representative image of the effect of the combination of SGT-53 and docetaxel on the angiosarcoma lesions in this patient is shown in **Figure 2b**. As shown in this photograph, the tumor has begun to respond and reduce in size.

Although the tumors shown in **Figure 2** did not demonstrate RECIST criteria of response, both demonstrated reduction in erosive nature of tumor growth.

Table 4 Patient summary

Patient ID (cohort #)	Tumor type	SGT-53 dose, mg (No. of infusions)	Docetaxel dose, mg/m² (No. of infusions)	Length of exposure (days)
02-018 (1)	Adenocarcinoma of the pancreas	2.4 (1)	40 (1)	7
02-019 (1)	Adenocarcinoma of the endometrium	2.4 (7)	40 (1); 60 (1)	44
02-020 (1)	High-grade serous carcinoma of the ovary	2.4 (10)	40 (1); 60 (2)	46
02-023 (1)	Poorly differentiated squamous cell carcinoma of the anus	2.4 (10)	40 (1); 60 (2)	45
02-024 (2)	Angiosarcoma of the scalp	2.4 (2)	75 (1)	3
02-025 (2)	Adenocarcinoma of the lung	2.4 (10)	75 (3)	45
02-026 (2) Round 1	Adenocarcinoma of the esophagus	2.4 (10)	75 (1); 60 (2)	45
02-026 (2) Round 2		2.4 (10)	60 (3)	44
02-029 (2)	Endometrial cancer of the uterus	2.4 (9)	75 (2)	42
02-030 (3)	Adenosquamous carcinoma of the lung	3.6 (10)	75 (1); 60 (2)	52
02-031 (3)	Malignant serous tumor of the ovary	3.6 (11)	75 (2); 60 (1)	67
02-033 (3) Round 1	Poorly differentiated adenocarcinoma of the breast	3.6 (12)	75 (3)	57
02-033 (3) Round 2		3.6 (10)	75 (3)	45
02-035 (3)	Cholangiocarcinoma of the extrahepatic bile duct	3.6 (10)	75 (3)	45
02-036 (3)	Papillary serous carcinoma of the ovary	3.6 (8)	75 (2)	36
02-037 (3)	Invasive ductal carcinoma of breast	3.6 (10)	75 (3)	45

Table 5 Adverse events at least possibly related to SGT-53 treatment in ≥14% of patients

Adverse event	Cohort 1: 2.4 mg DNA/infusion C1: Docetaxel 40 mg/m² ≥ C2: Docetaxel 60 mg/m²		Cohort 2: 2.4 mg DNA/infusion docetaxel 75 mg/m²		Cohort 3: 3.6 mg DNA/infusion docetaxel 75 mg/m²		Total patients (*N* = 14)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2 (*n* (%))	Grade 3/4 (*n* (%))
Anemia	0	0	1	1	3	2	4 (29%)	3 (21%)
Chills	2	0	0	0	2	0	4 (29%)	0
Dehydration	0	0	1	0	3	0	4 (29%)	0
Diarrhea	0	0	2	0	0	0	2 (14%)	0
Elevated aspartate transaminase	0	0	1	0	1	0	2 (14%)	0
Fever	2	0	1	0	3	0	6 (43%)	0
Hypotension	1	0	1	0	1	0	3 (21%)	0
Intermittent diarrhea	2	0	0	0	3	0	5 (36%)	0
Intermittent fatigue	1	0	1	0	1	0	3 (21%)	0
Neutropenia	0	1	1	1	0	1	1 (7%)	3 (21%)
Tachycardia	0	0	0	0	3	0	3 (21%)	0
Thrombocytopenia	1	0	0	0	3	1	4 (29%)	1 (7%)

Table 6 Response summary

Patient ID	Tumor type	RECIST		Tumor marker (if applicable)		Survival (days from consent)
		% Change	Best response	Marker	% Change	
02-018	Pancreatic	N/A[a]	PD	N/A		36
02-019	Endometrial	124%	PD	N/A		79
02-020	Ovarian	8%	SD	CA 125	6.9%	128
02-023	Anal	−47%	PR	N/A		461
02-024	Angiosarcoma	N/A[b]		N/A		827
02-025	Non-small cell lung cancer	24%	PD	N/A		127
02-026	Esophageal	−79%	PR	N/A		772
02-029	Uterine	−51%	PR*	N/A		91
02-030	Adenocarcinoma	0 (only nontarget lesions)	SD	N/A		Unknown[c]
02-031	Ovarian	50.80%	PD	N/A		152
02-033	Breast	−25.3%	SD	CA 27–29	−66.0%	256
02-035	Extra-hepatic bile duct	−16%	SD	N/A		496
02-036	Ovarian	13%	SD	CA 125	−57.0%	300
02-037	Breast	7.30%	SD	CA 27–29	−37.8%	94

PD, progressive disease; SD, stable disease; PR, partial response.
[a]Patient went off treatment after one infusion of SGT-53. [b]Patient went off treatment after two infusions of SGT-53. [c]Patient lost to follow-up. *Not verified.

Another indicator of the response to the combination therapy of SGT-53 and docetaxel is shown by changes in tumor markers (**Table 6**). Tumor markers were available and values obtained for four of five patients with either ovarian carcinoma (CA125) or breast cancer (CA27-29). All four of these patients showed tumor response by RECIST (SD). With the exception of patient ID #02-020 in cohort 1 (ovarian cancer), these patients also had significant decreases in their tumor markers (38–66% decrease from baseline). However, even in patient ID #02-020, the increase in CA125 was a modest 7%.

DISCUSSION

Cancer radiotherapy and chemotherapeutic agents cause DNA damage that triggers apoptotic cell death via a pathway involving p53. Thus, the lack of a functional p53 pathway present in many cancers confers resistance to both radiotherapy and chemotherapy.[12,13] Such a correlation between expression of mutant p53 and resistance to chemotherapy has been observed in a number of different types of solid tumor including ovarian,[52] gastric, and colorectal,[53] as well as in hematological malignancies.[54]

The use of a combination of therapies to treat cancer is now standard. The rationale behind these new combinations is based upon the heterogeneity of tumors. Using agents that work through different mechanisms may not only avoid the development of resistance, but may result in additive or even synergistic responses. This approach includes not only combinations of chemotherapeutic agents, but also use of chemotherapeutics with other classes of agents,[55] such as biological agents that target biochemical pathways such as Herceptin, oncogenes, or tumor-suppressor genes. Restoration of p53 function through methods such as the small molecule PRIMA-1 analog APR-246 has resulted in synergy with chemotherapeutic drugs including adriamycin and danorubicin *in vitro* and *in vivo*.[56] Chemosensitization after restoration of p53 function has also been observed in human clinical trials including a randomized phase 2 study of INGN-225, wherein a p53-modified adenovirus-transduced dendritic cell vaccine appeared to sensitize small cell lung cancer to subsequent chemotherapy.[57]

In preclinical studies, we have shown that systemic administration of SGT-53 targeted and sensitized various types of cancer, including brain tumors, to conventional radiation and chemotherapies.[17–19,41,50,51] In this nanocomplex, the wt p53 gene is under the control of a modified promoter which results in high levels of expression of exogenous wt p53. Using this construct in the SGT-53 nanocomplex in our preclinical studies, we have demonstrated sensitization to radiation/chemotherapy and tumor responses irrespective of the p53 status of the tumors. Thus, in this trial the p53 status of the patients was not determined.

Based upon the findings in the literature, the positive results of the phase 1a trial, as well as our preclinical studies, further clinical studies with SGT-53 in combination with chemotherapy appeared warranted.

Docetaxel is frequently used in combination with other chemotherapeutic agents[58] and more recently has been combined with biological agents in order to enhance efficacy. These include the insulin-like growth factor-1 inhibitor figitumumab against solid tumors[59]; trastuzumab and pertuzumab for treatment of epidermal growth factor receptor 2–positive breast cancer[60]; and rituximab (with or without Ibrutinib) against B-cell non-Hodgkin lymphoma.[61] Our own preclinical studies showed that systemically administered tumor-targeted delivery of the wt p53 gene in combination with docetaxel resulted in enhanced long-term survival in a mouse model of metastatic melanoma[39] and substantial inhibition of tumor growth in mouse models of prostate, ovarian, and lung cancers (unpublished data). Thus, this phase 1b trial was undertaken to assess the safety and therapeutic potential of SGT-53 when used in combination with docetaxel for the treatment of a spectrum of solid tumors.

The primary objective of this study was to determine the safety of the combination of SGT-53 and docetaxel. As was observed in the previous single-agent trial of SGT-53,[22] overall SGT-53 was found to have a good safety profile at therapeutic doses and no MTD was reached. A similar toxicity profile was observed by Lu *et al.*[62] in a phase 1 study of a DOTAP:Cholesterol nanoparticle delivering the tumor-suppressor gene TUSC2(FUS1).

As indicated in **Table 5** and **Supplementary Table S2**, the preponderance of AEs experienced by the patients were grade 1/2. Of these, fever, chills, hypotension, and even fatigue were expected based upon the results of the single-agent trial of SGT-53.[22] Furthermore, with the exception of anemia and thrombocytopenia, the vast majority of the grade 1/2 AEs presented as only one occurrence/patient.

There was a single instance of grade 1/2 neutropenia in cohort 2. In addition, grade 3/4 neutropenia was reported in 21% of patients (one instance in a single patient from each cohort). In the previous phase 1a trial where SGT-53 was administered as a single agent, seven patients received SGT-53 at DNA doses of either 2.4 mg (one patient) or 3.6 mg (six patients), the same DNA doses of SGT-53 being tested here. However, no grade3/4 occurrences of neutropenia were reported when SGT-53 was tested as a single agent. In contrast, neutropenia is the most prominent toxicity associated with docetaxel and can be dose-limiting. Neutropenia occurs in virtually all patients given 60–100 mg/m^2 of docetaxel and grade 4 neutropenia occurs in 75% of patients given 60 mg/m^2.[63,64] Thus, while involvement of SGT-53 cannot be ruled out, it is more likely that the neutropenia observed in the current trial was related to the administration of docetaxel.

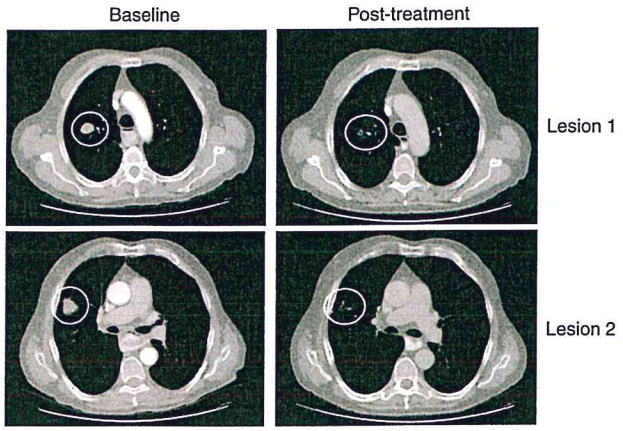

Figure 1 CT scan of lungs in patient ID #02-026 (metastatic adenocarcinoma of the esophagus) showing the tumor response after treatment with the combination of SGT-53 and docetaxel. Baseline: The circled areas show two individual metastatic target lesions prior to treatment; **post-treatment**: a significant decrease (78%) in size of both lesions is evident in this scan obtained 1 month after the end of the first round of treatment.

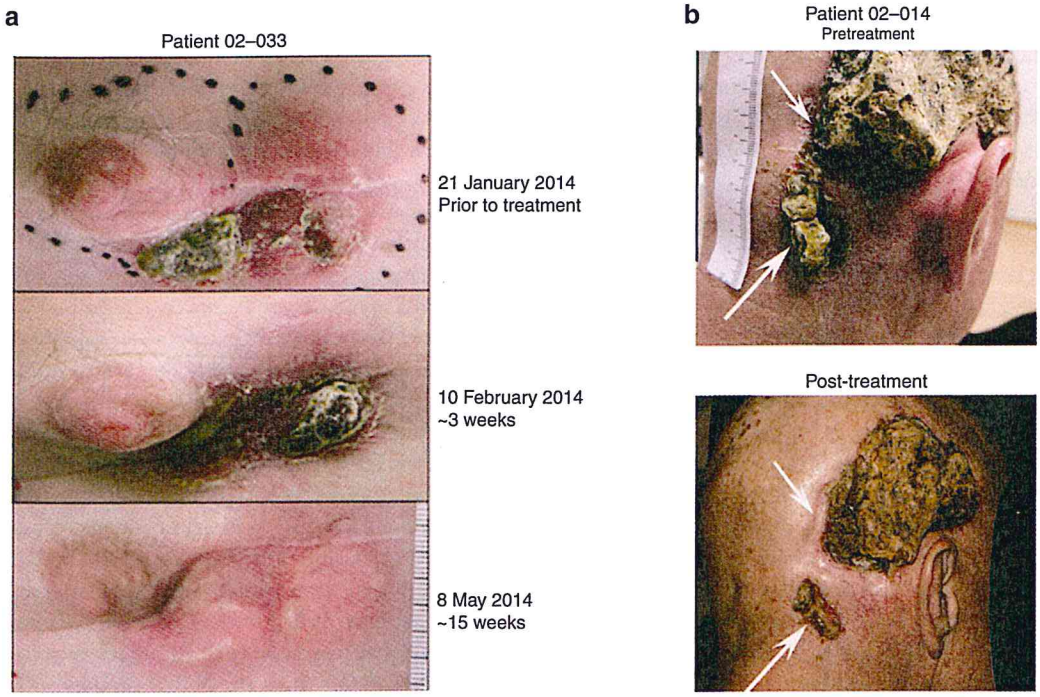

Figure 2 Photographic evidence of tumor response in two patients. (a) Patient ID #02-033 (male metastatic breast cancer). Top panel shows primary cutaneous tumor on the breast prior to treatment. The image in the middle panel, obtained half-way through the first cycle of treatment, shows the beginning of tumor response. The bottom panel shows the dramatic change evident in the primary tumor at the end of the second round of treatment. **(b)** Patient ID #02-024 (angiosarcoma of the scalp). This patient received only two infusions of SGT-53 (2.4 mg DNA/ dose) and one infusion of docetaxel (75 mg/m^2) before going off study. The arrows indicate areas of significant change (shrinkage and drying) of the tumor after going off study. Moreover, although not evaluable by RECIST after going off study due to a dose-limiting toxicity as described above, there is photographic evidence of tumor response in patient ID #02-024. This individual was diagnosed with angiosarcoma of the scalp which was not considered as targetable tumor by RECIST and metastatic disease to the lymph nodes. This patient received only two infusions of SGT-53 (2.4 mg DNA/dose) and one infusion of 75 mg/m^2 of docetaxel before going off study. A representative image of the effect of the combination of SGT-53 and docetaxel on the angiosarcoma lesions in this patient is shown in **b**. As shown in this photograph, the tumor has begun to respond and reduce in size.

Other common side effects associated with docetaxel administration include diarrhea (in up to 43% of patients), anemia (in up to 94% of patients), thrombocytopenia (in up to 39% of patients), nausea/vomiting, sensory neurotoxicity, stomatitis, skin toxicity, and alopecia.[63,65] Two of the most prominent AEs reported in this trial were anemia and intermittent diarrhea. However, as there were no reports of anemia in the trial of SGT-53 as a single agent,[22] it is likely that SGT-53 treatment did not contribute to the occurrences of anemia observed here. Similarly, the percentage of patients who experienced diarrhea or thrombocytopenia in this trial is also in line with that reported in patients treated with docetaxel alone, suggesting that these AEs were also most likely associated with docetaxel treatment.

Therefore, with regards to safety, the data indicate that the addition of SGT-53 to standard docetaxel treatment does not appear to increase the intensity or number of the known docetaxel-related side effects. Correspondingly, the addition of standard doses of docetaxel to SGT-53 treatment does not heighten the mild toxicities observed for SGT-53 alone.

In addition to the safety of the combination therapy, the results of this small phase 1b trial also suggest some potential therapeutic benefit. Two (17%) achieved PR as per RECIST criteria. In addition, one had an unverified PR, two (17%) were classified as SD with significant shrinkage but not >30%, and four (33%) had SD at the end of treatment without tumor shrinkage. Moreover, of these nine patients six (67%), including two of the three with a PR (patient ID #02-023 and #02-029), and a patient with SD (patient ID #02-033 who had tumor decrease of 25.3%) had received and failed previous chemotherapy with taxol. Patient ID #02-024, in whom there was photographic evidence of tumor response, had also failed prior taxol therapy. Thus, the percent of patients showing at least SD in response to treatment with SGT-53 and docetaxel, ~67% of whom had failed prior taxane treatment, suggests the promise of this SGT-53 combination strategy as a means to increase the response to chemotherapy. The dose used here was 3.6 mg DNA/infusion. It is possible that in future trials, a higher dose would result in additional responses.

Although this phase 1b study was small, the positive results obtained warrant further investigation of the combination of SGT-53 and chemotherapy in larger phase 2 trials.

MATERIALS AND METHODS

Study population. The intended study population included subjects with biopsy-confirmed diagnosis of solid tumors who had exhausted or declined all treatment options that would provide substantive palliation and have tumors for which docetaxel is an appropriate therapy. The study participants were required to have measurable disease on imaging studies and/or physical examination and life expectancy of >12 weeks. The study population was registered through the Mary Crowley Cancer Research Center, Dallas, Texas.

Study design and objectives. This trial (Trial Registration ID: NCT00470613) was an open-label, single-center, sequential dose-escalating phase 1 trial evaluating the safety and potential activity of the combination of SGT-53 and docetaxel in subjects with solid tumors and who had been offered all standard or approved therapies. The primary objectives of this phase 1b study were to evaluate the safety of SGT-53 in combination with docetaxel and to assess the potential therapeutic benefit of this combination therapy on tumor size or progression.

SGT-53 (at a starting DNA dose of 2.4 mg/infusion) was administered in a standard 3×3 dose-escalation design in combination with docetaxel (at a starting dose of 40 mg/m², cohort 1, cycle 1). This protocol allowed for both inter- and intrapatient dose escalations. SGT-53 was administered weekly, or biweekly, while docetaxel was administered once every 3 weeks based upon the standard of care when using this chemotherapeutic agent. No subject initiated therapy until all preceding subjects had completed all first cycle study agent dose administrations.

Patient accrual would proceed at a rate of 3 weeks between each cohort level cycle 1 and with 3 weeks between the first and the subsequent two patients within each cohort following cycle 1. There would be no interpatient dose escalation (new cohort initiated) until all preceding subjects had completed all first cycle study agent dose administrations.

Patients were treated using standard Fibonacci dose escalation. For cohort 1, which allowed an intrapatient dose escalation if the patient does not experience a DLT in cycle 1, three patients would be enrolled. A cycle is defined as one docetaxel treatment within 3 weeks. Patients completing cohort 1, cycle 1 without DLT at docetaxel 40 mg/m² dose escalated to docetaxel at 60 mg/m² in cycles 2 and 3. If one of three patients experienced a DLT in cohort 1, cycle 1 then three additional patients would be enrolled at that cohort dose and intrapatient dose escalation discontinued. If any of the three additional patients experienced a DLT, the study would be put on hold and dosing parameters re-evaluated. Cohort 2 (SGT-53 at 2.4 mg DNA/dose and docetaxel at 75 mg/m²) would open 3 weeks after demonstration of 0/3 or ≤1/6 DLTs at docetaxel at 60 mg/m² in cohort 1. If one of three patients in cohort 2 experienced a DLT, then three additional patients would be enrolled at that cohort dose. If any of the three additional patients experienced a DLT, the previous dose level would be considered the MTD. In the absence of a ≥1/6 DLTs in cohort 2, cohort 3 would open. In cohort 3, the dose of SGT-53 would increase to 3.6 mg DNA/infusion, and docetaxel would remain at 75 mg/m². Patients completing cohort 3, cycle 1 without DLT at docetaxel 75 mg/m² will continue on docetaxel 75 mg/m² in cycles 2 and 3. If any of the patients experience a DLT at 75 mg/m² docetaxel, the dose of docetaxel for that patient will be reduced to 60 mg/m² while the dose of SGT-53 remained at 3.6 mg DNA. Three additional patients would be enrolled. If any of these three patients experience a DLT in cycle 1, the study would be terminated and the recommended phase 2 dose will be SGT-53 2.4 mg DNA/infusion and docetaxel 75 mg/m². If the highest scheduled dose level (SGT 3.6 mg DNA/infusion and docetaxel 75 mg/m²) is reached in the absence of a DLT, a total of six patients will be enrolled at this dose level.

Eligibility criteria. Eligible subjects met the following inclusion criteria: biopsy confirmed diagnosis; had been offered all standard or approved therapies; had solid tumors that could be measured on physical examination or by radiographic imaging studies; had a tumor for which docetaxel would be an appropriate therapeutic agent; were 18 years old or older; had an Eastern Cooperative Oncology Group (ECOG) performance study of 0–2; were able to give protocol-specific informed consent; had recovered from previous therapy (previous docetaxel allowed if treatment concluded >6 months prior to study entry); female subjects of childbearing potential must have had a negative pregnancy test within 7 days before initiation of study drug (postmenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential); male and female subjects of reproductive potential must have agreed to use birth control measures (*e.g.*, condoms or birth control pills) to avoid pregnancy throughout the study and for 3 months following discontinuation of the study drug; have adequate organ function characterized by ≤grade 1 scores defined by CTCAE v3.0 and laboratory values within the following criteria: hemoglobin ≥10.0 gm/dl; absolute neutrophil count (ANC) >1,500/mm³; white blood cell count >3,000/mm³; platelet count ≥100,000/mm³; prothrombin time/partial thromboplastin time (PT/PTT) <1.5 times

the upper limit of normal (ULN); LDH <3 times the ULN; total bilirubin ≤ULN; aspartate transaminase and alanine transaminase <2.5 × ULN with alkaline phosphatase ≤2.5 × ULN; creatinine ≤1.5 mg/dl or creatinine clearance ≥50 ml/minute.

Subjects with the following characteristics were excluded: those with prior hypersensitivity reaction to docetaxel; or with signs and symptoms consistent with an active infection; fever (>38.1 °C); known HIV infection; fasting glucose levels >180 mg/dl; uncontrolled diastolic blood pressure of >90 mm Hg resting at baseline despite medication; an abnormal stress echocardiogram or unfavorable results; have hematological malignancies, known cardiac disease, or a history of cardiac disease; uncontrolled congestive heart failure; unstable angina; significant baseline neuropathies (>grade 2 based upon CTCAE v 3.0); requirement for renal dialysis; requirement for systemic steroids within 30 days prior to study entry; receiving hematopoietic growth factors; receiving anticoagulants other than to maintain patency of venous access lines (≤2 mg warfarin); received an investigational drug within 30 days prior to study entry; received radiation treatment <4 weeks prior to study entry; prior exposure to gene vector delivery products; or received treatment with chemotherapeutic agents <4 weeks prior to study entry.

The study protocol was approved by the Mary Crowley Cancer Research Center (MCCRC) Institutional Review Board, and all patients gave written informed consent before any study procedures were performed. The study was conducted to Good Clinical Practice specifications in accordance with the Declaration of Helsinki and its amendments.

Investigational product. SGT-53 is a ligand-targeted liposomal-plasmid DNA nanocomplex consisting of three components: (i) a plasmid DNA encoding the normal human wt p53 gene, (ii) the targeting component: a recombinant single-chain fragment (scFv) derived from a monoclonal antibody against the human TfR that is known to be elevated on the surface of cells from a variety of tumor types, and (iii) a cationic liposome (DOTAP:DOPE). The complex for this clinical trial was prepared under cGMP conditions by simple mixing of the components in a defined ratio and in a specific order. The complex is formulated with sucrose (10% final concentration) as an excipient. The complex was then vialed and lyophilized. After the lyophilized SGT-53 complex is reconstituted with sterile water for injection, the solution is added to a 5% dextrose intravenous drip for administration.

Treatment. SGT-53 and docetaxel were administered following the schedule given in **Table 3**.

SGT-53 was administered at the MCCRC outpatient clinic once or twice weekly for 7 weeks for a total of 10 infusions. Infusion occurred over 2 hours. In the event of an infusion reaction, the infusion was discontinued, appropriate medication administered, and the infusion resumed at a slower infusion rate. A final volume of 150–225 ml of SGT-53 in 5% dextrose was infused intravenously via a newly inserted venous access line or following flushing of an intact central venous line. Infusion was completed within 8 hours of dilution of study agent in dextrose.

For the first infusion only of SGT-53, subjects were monitored for at least 23 hours, and up to 48 hours, from the time of admittance to MCCRC outpatient clinic. The subjects were monitored in the MCCRC outpatient clinic for the first 8 hours after completion of the infusion. In addition, subjects received i.v. fluids for 2 hours after completion of the first infusion, with continued i.v. access for rapid fluid management if necessary. After 8 hours, subjects were then transferred and admitted to Medical City Hospital for the remainder of the observation period to monitor and manage any reactions to SGT-53. During this time, subjects continued i.v. access with maintenance of the 20-gauge catheter for rapid fluid management if necessary. At the end of the 23–48 hours observation period, subjects were discharged if medically stable.

For subsequent infusions, subjects were infused with SGT-53 in the MCCRC outpatient infusion unit and monitored for 2 hours post-infusion, with continued i.v. fluids during this time, before discharge if medically stable.

All patients received dexamethasone 8 mg i.v. 1 hour ± 5 minutes prior to dosing, and a combination of histamine H1 and H2 blockers (*e.g.*, Benadryl 25 mg and Pepcid 20 mg, both i.v.) 30 ± 15 minutes and indocin 25 mg, p.o. ~30 ± 5 minutes prior to receiving SGT-53. All patients also received acetaminophen 650 mg p.o. just prior to SGT-53 administration as prophylaxis for pyretic reactions. Four hours after completion of each infusion of SGT-53, subjects received acetominophen, 650 mg p.o.

On day 3 of weeks 1, 4, and 7, subjects with any SGT-53 related toxicities that have resolved to ≤grade 1 received docetaxel at 40, 60, or 75 mg/m^2 i.v. (depending on cohort) in 250 ml 0.9% sodium chloride infused over 1 hour. Treatment was administered in 21-day cycles until disease progression or a maximum of three cycles. Pretreatment with corticosteroids was performed according to the recommendations on the Information for Prescribers (Package Insert) for docetaxel. Dexamethasone 8 mg p.o. was administered 12, 8, and 1 hours before each treatment with docetaxel. Subjects were observed for 4 hours following docetaxel administration.

Safety evaluation. Clinical and laboratory assessments for safety were performed before enrolment and at various times throughout the treatment. Physical examination was performed at the start of each cycle and at the end of treatment. Vital signs, hematology, blood chemistry, AE, and concomitant medication queries were obtained at each visit. Urine analysis was performed weekly. ECGs were taken 2 hours after each infusion of SGT-53. All assessments were obtained at follow-up.

AEs were graded according to the CTCAE v3.0 (http://ctep.cancer.gov/forms/CTCAEv3.pdf). The following grade 2 events defined toxicities that would lead to withholding infusions of SGT-53: grade 2 total hyperbilirubinemia, aspartate transaminase, alanine transaminase, dyspnea, and disseminated intravascular coagulation (DIC) if not resolved to ≤grade 1 in 2 weeks. For each of these grade 2 events, the dose of SGT-53 and docetaxel could be held for up to 2 weeks. If the grade 2 event resolved within 2 weeks, then infusion of SGT-53 and docetaxel resumed. For events other than the above, subjects who develop a grade 2 toxicity (except for alopecia) discontinue twice-weekly infusions of SGT-53 until the toxicity level reduces to at least grade 1. If the toxicity did not resolve within 1 week, study drug would be discontinued.

For the purposes of this study, a DLT is defined as all grade 3 or grade 4 toxicities (as defined by CTCAE v3.0) within the first cycle (3 weeks) of SGT-53 administration, and that was determined to be at least probably related to study drug (SGT-53), with the exception of grade 3 fever and chills, or hypotension, which responds promptly to treatment or neutropenia lasting <7 days, with no fever. Any study participant who experienced a DLT discontinued receiving SGT-53. In addition, subjects who received <4 of 10 of the intended drug infusions, due to experiencing grade 2 events that did not resolved as described above and that were determined to be probably related to SGT-53, were considered to have experienced a DLT.

Patients forced to discontinue docetaxel due to a docetaxel-related toxicity (*e.g.*, grade 3,4 hypersensitivity) following cycle 1 continued on SGT-53 as per protocol and are considered as evaluable. Patients remained on the docetaxel dose level assigned to them at the time of enrollment, except in cases where docetaxel dose modification was indicated. Patients must have had an ANC ≥1.5 × 10^9/l and a platelet count ≥100 × 10^9/l on day 1 of each cycle. If a patient had an ANC <1.5 × 10^9/l, treatment with both SGT-53 and docetaxel was held, and a weekly complete blood count, differential, and platelet count obtained. Study medication could resume if ANC had recovered within 1–2 weeks, with or without administration of colony stimulating factor. A grade 4 thrombocytopenia, grade 3 nonhematologic toxicity, elevated total bilirubin would result in a 25% reduction in the dose of docetaxel. In the event of any grade 4 nonhematologic and grade 3 or 4 peripheral neuropathy, all further therapy would be discontinued and the subject removed from protocol.

If total bilirubin is >ULN on treatment day (unless elevation is known to be due to Gilbert's disease), docetaxel treatment would be held until total bilirubin is ≤ULN (maximum 2 weeks), and then treatment continued at a 25% dose reduction. If bilirubin did not return to ≤ULN, treatment with study medication would be discontinued.

In the event that aspartate transaminase and/or alanine transaminase and/or alkaline phosphatase levels were abnormal in the absence of progressive disease, the dose of docetaxel would be reduced by 25% after recovery. No re-escalation and only one dose reduction would be allowed.

Tumor response. Participants were evaluated for response if they received at least one cycle of treatment. Measurable disease was defined as any lesion with clearly defined borders that could be measured with calipers on physical exam or a radiologic median such as on X-rays, computerized tomography, or magnetic resonance imaging. Malignant hepatomegaly, previously irradiated lesions, and lesions visible on the bone scan will not be considered measurable. Standard tumor measurement procedures were followed. Unequivocal new lesion(s) on radiographic imaging were considered to indicate progressive disease. The RECIST 1.0 criteria published in the 2 February 2000 issue of *JNCI*[66] was utilized to evaluate response.

SUPPLEMENTARY MATERIAL

Table S1. Sites of target and nontarget lesions.
Table S2. Adverse events occurring more than once and at least possibly related to SGT-53 treatment as number of incidences/patient.
Table S3. Other therapies.

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REFERENCES

1. Dameron, KM, Volpert, OV, Tainsky, MA and Bouck, N (1994). Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* **265**: 1582–1584.
2. Velculescu, VE and El-Deiry, WS (1996). Biological and clinical importance of the p53 tumor suppressor gene. *Clin Chem* **42**(6 Pt 1): 858–868.
3. Wiman, KG (2010). Pharmacological reactivation of mutant p53: from protein structure to the cancer patient. *Oncogene* **29**: 4245–4252.
4. Hainaut, P and Wiman, KG (eds) (2005). *25 Years of p53 Research*, Springer: Dordrecht. pp. 446.
5. Green, DR and Kroemer, G (2009). Cytoplasmic functions of the tumour suppressor p53. *Nature* **458**: 1127–1130.
6. Vousden, KH and Prives, C (2009). Blinded by the light: the growing complexity of p53. *Cell* **137**: 413–431.
7. Levine, AJ, Momand, J and Finlay, CA (1991). The p53 tumour suppressor gene. *Nature* **351**: 453–456.
8. Hollstein, M, Rice, K, Greenblatt, MS, Soussi, T, Fuchs, R, Sørlie, T *et al.* (1994). Database of p53 gene somatic mutations in human tumors and cell lines. *Nucleic Acids Res* **22**: 3551–3555.
9. Sherr, CJ and McCormick, F (2002). The RB and p53 pathways in cancer. *Cancer Cell* **2**: 103–112.
10. Gudkov, AV and Komarova, EA (2003). The role of p53 in determining sensitivity to radiotherapy. *Nat Rev Cancer* **3**: 117–129.
11. Kandoth, C, McLellan, MD, Vandin, F, Ye, K, Niu, B, Lu, C *et al.* (2013). Mutational landscape and significance across 12 major cancer types. *Nature* **502**: 333–339.
12. Lai, D, Visser-Grieve, S and Yang, X (2012). Tumour suppressor genes in chemotherapeutic drug response. *Biosci Rep* **32**: 361–374.
13. Knappskog, S and Lønning, PE (2012). P53 and its molecular basis to chemoresistance in breast cancer. *Expert Opin Ther Targets* **16 (Suppl 1)**: S23–S30.
14. Martins, CP, Brown-Swigart, L and Evan, GI (2006). Modeling the therapeutic efficacy of p53 restoration in tumors. *Cell* **127**: 1323–1334.
15. Xue, W, Zender, L, Miething, C, Dickins, RA, Hernando, E, Krizhanovsky, V *et al.* (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**: 656–660.
16. Ventura, A, Kirsch, DG, McLaughlin, ME, Tuveson, DA, Grimm, J, Lintault, L *et al.* (2007). Restoration of p53 function leads to tumour regression *in vivo*. *Nature* **445**: 661–665.
17. Xu, L, Pirollo, KF, Rait, A, Murray, A, Chang, EH (1999). Systemic p53 gene therapy in combination with radiation results in human tumor regression. *Tumor Targeting* **4**: 92–104.
18. Xu, L, Pirollo, KF and Chang, EH (1997). Transferrin-liposome-mediated p53 sensitization of squamous cell carcinoma of the head and neck to radiation *in vitro*. *Hum Gene Ther* **8**: 467–475.
19. Xu, L, Pirollo, KF, Tang, WH, Rait, A and Chang, EH (1999). Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck cancer xenografts. *Hum Gene Ther* **10**: 2941–2952.
20. Hoe, KK, Verma, CS, Lane, DP (2014). Drugging the p53 pathway: understanding the route to clinical efficacy. *Nat Rev Drug Discov* **13**: 217–36
21. Duffy, MJ, Synnott, NC, McGowan, PM, Crown, J, O'Connor, D and Gallagher, WM (2014). p53 as a target for the treatment of cancer. *Cancer Treat Rev* **40**: 1153–1160.
22. Senzer, N, Nemunaitis, J, Nemunaitis, D, Bedell, C, Edelman, G, Barve, M *et al.* (2013). Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors. *Mol Ther* **21**: 1096–1103.
23. Nabeya, Y, Loganzo, F Jr, Maslak, P, Lai, L, de Oliveira, AR, Schwartz, GK *et al.* (1995). The mutational status of p53 protein in gastric and esophageal adenocarcinoma cell lines predicts sensitivity to chemotherapeutic agents. *Int J Cancer* **64**: 37–46.
24. Fujiwara, T, Grimm, EA, Mukhopadhyay, T, Zhang, WW, Owen-Schaub, LB and Roth, JA (1994). Induction of chemosensitivity in human lung cancer cells *in vivo* by adenovirus-mediated transfer of the wild-type p53 gene. *Cancer Res* **54**: 2287–2291.
25. Clarke, AR, Purdie, CA, Harrison, DJ, Morris, RG, Bird, CC, Hooper, ML *et al.* (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **362**: 849–852.
26. Yonish-Rouach, E, Resnitzky, D, Lotem, J, Sachs, L, Kimchi, A and Oren, M (1991). Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* **352**: 345–347.
27. Lowe, SW, Bodis, S, McClatchey, A, Remington, L, Ruley, HE, Fisher, DE *et al.* (1994). p53 status and the efficacy of cancer therapy *in vivo*. *Science* **266**: 807–810.
28. Nemunaitis, JM and Nemunaitis, J (2008). Potential of Advexin: a p53 gene-replacement therapy in Li-Fraumeni syndrome. *Future Oncol* **4**: 759–768.
29. Nemunaitis, J and Nemunaitis, J (2011). Head and neck cancer: response to p53-based therapeutics. *Head Neck* **33**: 131–134.
30. Nemunaitis, J, Clayman, G, Agarwala, SS, Hrushesky, W, Wells, JR, Moore, C *et al.* (2009). Biomarkers predict p53 gene therapy efficacy in recurrent squamous cell carcinoma of the head and neck. *Clin Cancer Res* **15**: 7719–7725.
31. Senzer, N and Nemunaitis, J (2009). A review of contusugene ladenovec (Advexin) p53 therapy. *Curr Opin Mol Ther* **11**: 54–61.
32. Nemunaitis, J, Swisher, SG, Timmons, T, Connors, D, Mack, M, Doerksen, L *et al.* (2000). Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. *J Clin Oncol* **18**: 609–622.
33. Senzer, N, Nemunaitis, J, Nemunaitis, M, Lamont, J, Gore, M, Gabra, H *et al.* (2007). p53 therapy in a patient with Li-Fraumeni syndrome. *Mol Cancer Ther* **6**: 1478–1482.
34. Swisher, SG, Roth, JA, Komaki, R, Gu, J, Lee, JJ, Hicks, M *et al.* (2003). Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy. *Clin Cancer Res* **9**: 93–101.
35. Yang, ZX, Wang, D, Wang, G, Zhang, QH, Liu, JM, Peng, P *et al.* (2010). Clinical study of recombinant adenovirus-p53 combined with fractionated stereotactic radiotherapy for hepatocellular carcinoma. *J Cancer Res Clin Oncol* **136**: 625–630.
36. Tazawa, H, Kagawa, S and Fujiwara, T (2013). Advances in adenovirus-mediated p53 cancer gene therapy. *Expert Opin Biol Ther* **13**: 1569–1583.
37. Yilmaz, M and Christofori, G (2010). Mechanisms of motility in metastasizing cells. *Mol Cancer Res* **8**: 629–642.
38. Daniels, TR, Bernabeu, E, Rodríguez, JA, Patel, S, Kozman, M, Chiappetta, DA *et al.* (2012). The transferrin receptor and the targeted delivery of therapeutic agents against cancer. *Biochim Biophys Acta* **1820**: 291–317.
39. Xu, L, Tang, WH, Huang, CC, Alexander, W, Xiang, LM, Pirollo, KF *et al.* (2001). Systemic p53 gene therapy of cancer with immunolipoplexes targeted by anti-transferrin receptor scFv. *Mol Med* **7**: 723–734.
40. Xu, L, Huang, CC, Huang, W, Tang, WH, Rait, A, Yin, YZ *et al.* (2002). Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. *Mol Cancer Ther* **1**: 337–346.
41. Rait, AS, Pirollo, KF, Xiang, L, Ulick, D and Chang, EH (2002). Tumor-targeting, systemically delivered antisense HER-2 chemosensitizes human breast cancer xenografts irrespective of HER-2 levels. *Mol Med* **8**: 475–486.
42. Pirollo, KF, Zon, G, Rait, A, Zhou, Q, Yu, W, Hogrefe, R *et al.* (2006). Tumor-targeting nanoimmunoliposome complex for short interfering RNA delivery. *Hum Gene Ther* **17**: 117–124.
43. Pirollo, KF, Rait, A, Zhou, Q, Hwang, SH, Dagata, JA, Zon, G *et al.* (2007). Materializing the potential of small interfering RNA via a tumor-targeting nanodelivery system. *Cancer Res* **67**: 2938–2943.
44. Hwang, SH, Rait, A, Pirollo, KF, Zhou, Q, Yenugonda, VM, Chinigo, GM *et al.* (2008). Tumor-targeting nanodelivery enhances the anticancer activity of a novel quinazolinone analogue. *Mol Cancer Ther* **7**: 559–568.
45. Pirollo, KF, Dagata, J, Wang, P, Freedman, M, Vladar, A, Fricke, S *et al.* (2006). A tumor-targeted nanodelivery system to improve early MRI detection of cancer. *Mol Imaging* **5**: 41–52.
46. Freedman, M, Chang, EH, Zhou, Q and Pirollo, KF (2009). Nanodelivery of MRI contrast agent enhances sensitivity of detection of lung cancer metastases. *Acad Radiol* **16**: 627–637.
47. Yang, C, Rait, A, Pirollo, KF, Dagata, JA, Farkas, N and Chang, EH (2008). Nanoimmunoliposome delivery of superparamagnetic iron oxide markedly enhances targeting and uptake in human cancer cells *in vitro* and *in vivo*. *Nanomedicine* **4**: 318–329.
48. Xu, L, Frederik, P, Pirollo, KF, Tang, WH, Rait, A, Xiang, LM *et al.* (2002). Self-assembly of a virus-mimicking nanostructure system for efficient tumor-targeted gene delivery. *Hum Gene Ther* **13**: 469–481.
49. Kim, SS, Rait, A, Rubab, F, Rao, AK, Kiritsy, MC, Pirollo, KF *et al.* (2014). The clinical potential of targeted nanomedicine: delivering to cancer stem-like cells. *Mol Ther* **22**: 278–291.

50. Kim, SS, Rait, A, Kim, E, Pirollo, KF, Nishida, M, Farkas, N *et al.* (2014). A nanoparticle carrying the p53 gene targets tumors including cancer stem cells, sensitizes glioblastoma to chemotherapy and improves survival. *ACS Nano* **8**: 5494–5514.
51. Kim, SS, Rait, A, Kim, E, Pirollo, KF and Chang, EH (2015). A tumor-targeting p53 nanodelivery system limits chemoresistance to temozolomide prolonging survival in a mouse model of glioblastoma multiforme. *Nanomedicine* **11**: 301–311.
52. Farrand, L, Kim, JY, Byun, S, Im-aram, A, Lee, J, Suh, JY *et al.* (2014). The diarylheptanoid hirsutenone sensitizes chemoresistant ovarian cancer cells to cisplatin via modulation of apoptosis-inducing factor and X-linked inhibitor of apoptosis. *J Biol Chem* **289**: 1723–1731.
53. Hamada, M, Fujiwara, T, Hizuta, A, Gochi, A, Naomoto, Y, Takakura, N *et al.* (1996). The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colorectal cancers. *J Cancer Res Clin Oncol* **122**: 360–365.
54. Cheung, KJ, Horsman, DE and Gascoyne, RD (2009). The significance of TP53 in lymphoid malignancies: mutation prevalence, regulation, prognostic impact and potential as a therapeutic target. *Br J Haematol* **146**: 257–269.
55. Frei, E, Eder, JP (2003). Combination chemotherapy. In: Kufe, DW, Pollock, RE, Weichselbaum, RR *et al.* (eds). *Holland-Frei Cancer Medicine*, 6th edn. BC Decker: Hamilton (ON).
56. Bykov, VJ and Wiman, KG (2014). Mutant p53 reactivation by small molecules makes its way to the clinic. *FEBS Lett* **588**: 2622–2627.
57. Chiappori, AA, Soliman, H, Janssen, WE, Antonia, SJ and Gabrilovich, DI (2010). INGN-225: a dendritic cell-based p53 vaccine (Ad.p53-DC) in small cell lung cancer: observed association between immune response and enhanced chemotherapy effect. *Expert Opin Biol Ther* **10**: 983–991.
58. Kavanagh, JJ (2002). Docetaxel in the treatment of ovarian cancer. *Oncology (Williston Park)* **16**(6 Suppl 6): 73–81.
59. Molife, LR, Fong, PC, Paccagnella, L, Reid, AH, Shaw, HM, Vidal, L *et al.* (2010). The insulin-like growth factor-I receptor inhibitor figitumumab (CP-751,871) in combination with docetaxel in patients with advanced solid tumours: results of a phase Ib dose-escalation, open-label study. *Br J Cancer* **103**: 332–339.
60. Giordano, SH, Temin, S, Kirshner, JJ, Chandarlapaty, S, Crews, JR, Davidson, NE *et al.*; American Society of Clinical Oncology. (2014). Systemic therapy for patients with advanced human epidermal growth factor receptor 2-positive breast cancer: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol* **32**: 2078–2099.
61. Younes, A, Thieblemont, C, Morschhauser, F, Flinn, I, Friedberg, JW, Amorim, S *et al.* (2014). Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1b study. *Lancet Oncol* **15**: 1019–1026.
62. Lu, C, Stewart, DJ, Lee, JJ, Ji, L, Ramesh, R, Jayachandran, G *et al.* (2012). Phase I clinical trial of systemically administered TUSC2(FUS1)-nanoparticles mediating functional gene transfer in humans. *PLoS One* **7**: e34833.
63. http://www.drugs.com/pro/taxotere.html.
64. https://www.drugbank.ca/system/fda_labels/DB01248.pdf?1265922807.
65. de Weger, VA, Beijnen, JH and Schellens, JH (2014). Cellular and clinical pharmacology of the taxanes docetaxel and paclitaxel—a review. *Anticancer Drugs* **25**: 488–494.
66. Therasse, P, Arbuck, SG, Eisenhauer, EA, Wanders, J, Kaplan, RS, Rubinstein, L *et al.* (2000). New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**: 205–216.