Systemic Gene Therapy with Tumor Suppressor TUSC2/FUS1 Nanoparticles for Recurrent/Metastatic Lung Cancer

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Abstract

Background: The tumor suppressor gene TUSC2/FUS1 (TUSC2) is frequently inactivated early in lung cancer development. TUSC2 mediates apoptosis in cancer cells but not normal cells by upregulation of the Apaf-1-dependent apoptotic pathway. DOTAP:cholesterol nanoparticles encapsulating a TUSC2 expression plasmid (DOTAP:chol-TUSC2) showed preferential uptake by cancer cells and prolonged survival in mouse xenograft metastatic lung cancer models. **Methods:** Patients with recurrent and/or metastatic lung cancer previously treated with platinum-based chemotherapy were treated with escalating doses of intravenous DOTAP:chol-TUSC2 every 3 weeks. The primary end point was assessment of DOTAP:chol-TUSC2 toxicity and determination of the MTD. TUSC2 plasmid expression in pretreatment and 24 hour posttreatment tumor specimens from subjects consenting to tumor biopsies was analyzed with quantitative RT-PCR analysis using a TUSC2 plasmid sequence-specific probe, in situ proximity ligation assay for TUSC2 protein, and apoptosis signaling nanoscale PCR array. **Results:** Thirty-one patients were treated at 6 dose levels ranging from 0.01 to 0.09 mg/kg and 7 had paired pre- and posttreatment biopsies. RT-PCR analysis detected high TUSC2 plasmid expression in 6 of 7 posttreatment tumor specimens but not in pretreatment specimens and negative controls. Immunohistochemical staining has been performed on one paired specimen, demonstrating low background TUSC2 protein staining in the pretreatment tissue compared with high intense TUSC2 protein staining in the posttreatment tissue. RT-PCR gene expression profiling analysis of apoptotic pathway genes in one paired specimen demonstrated significant upregulation and downregulation of genes involved in both the intrinsic and extrinsic apoptotic pathways. Among 4 patients treated without premedications, all 4 developed grade 2 or 3 fever. Among the 27 patients premedicated with dexamethasone and diphenhydramine, the highest fever was grade 2, which occurred in 2 subjects. The only dose-limiting toxicities were 2 episodes of transient grade 3 hypophosphatemia, resulting in an MTD of 0.06 mg/kg. Twenty-three patients who received two or more doses were evaluable for response, with 5 achieving stable disease (2.6-10.8 months) and 18 progressing. One patient with stable disease had evidence of a durable metabolic response on positron emission tomography imaging. The pretreatment apoptotic index was predictive of disease stability. Median survival time was 9.1 months. **Conclusions:** DOTAP:chol-TUSC2 can be safely administered intravenously in lung cancer patients and results in demonstrable gene and protein expression in tumors. It appears to regulate apoptotic pathway genes with evidence of antitumor activity. The MTD for phase II testing is 0.06 mg/kg every 3 weeks. Based on promising preclinical data, a trial in combination with erlotinib is planned..

Background

•TUSC2 is a 3p21.3 tumor suppressor gene with potent proapoptotic activity (Figure 1).

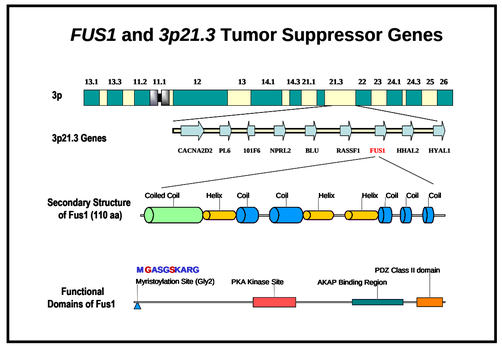
•TUSC2 knockout mice develop hemangiomas, and hemagiosarcomas.

•TUSC2 protein is expressed in normal bronchial epithelium, but frequently absent in primary lung cancers.

•DOTAP-cholesterol nanoparticle is a novel nonviral, systemic gene delivery system.

•Nude mouse lung cancer xenograft studies with DOTAP:chol-TUSC2 nanoparticles have demonstrated antitumor activity and increased survival.

Figure 1



Objectives

•Assess toxicity of intravenous DOTAP:Chol-TUSC2.

•To determine the maximal tolerated dose and recommended phase II dose.

•Assess expression of TUSC2 following intravenous delivery of DOTAP:Chol-TUSC2 in tumor biopsies:

- •Plasmid expression: vector-specific RT-PCR).
- •Protein expression: *In situ* proximity ligation assay

•Assess any anti-cancer activity for DOTAP:Chol-TUSC2; response rate, survival.

Study Design

•Recurrent or metastatic non-small cell or small cell lung cancer patients who have received prior platinum-based chemotherapy

•PS ≤ 1, adequate hematologic, hepatic, renal function

•Initial dose level: 1/20 LD10 in mice = 0.02 mg/kg

•DOTAP:chol-TUSC2 administered intravenously over 30 min every 3 weeks, maximum 6 doses.

•Optional pre- and 24 hour post-treatment biopsies for first dose only.

•Tumor assessments (CT scan) every 6 weeks

•Dose escalation determined by modified continual reassessment method

Results

•31 pts enrolled, all evaluable for cycle 1 toxicity.

•Median age 60 yrs; 52% male; 42% ≥ 3 prior regimens

•Dose levels (mg/kg): 0.01, 0.02, 0.03, 0.06, 0.09.

•Patients treated without premedications (N=4) all developed fever (2 grade (G) 2, 2 G3).

•Patients treated with premedications (N=27, dexamethasone, diphenhydramine) did not develop G3 fever (7 G1, 2 G2).

•DLT with premedications: 2 pts with G3 hypophosphatemia at dose levels 0.06 and 0.09 mg/kg, resulting in an MTD of 0.06 mg/kg.

•23 pts evaluable for response: 5 stable disease, 18 progressive disease. Median survival (N=31): 9.1 mo

•Pre- and post-treatment biopsies obtained in 7 patients. TUSC2 plasmid expression detected in 6 post-treatment specimens (Table 1).

•Proximity ligation assay, performed on paired biopsies from 2 patients, demonstrate low protein staining in pretreatment tissues and high protein staining in post-treatment tissues (Figure 1)

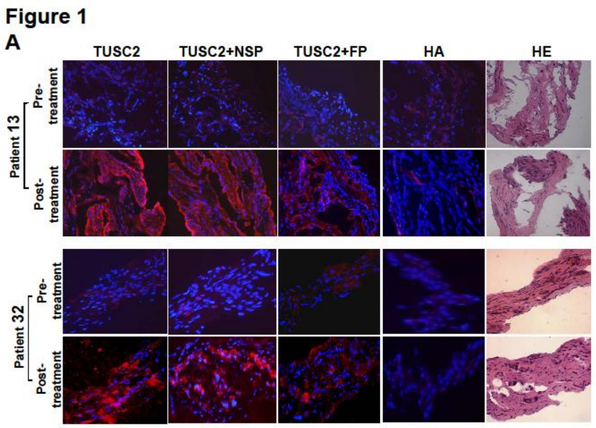
•RT-PCR expression profiling of apoptotic pathways in the paired biopsy of 1 patient demonstrated significant upregulation and downregulation of intrinsic and extrinsic apoptotic pathways.

•1 patient with stable disease demonstrated metabolic response on PET scan

Table 1. TUSC2 gene expression in pre- and post-treatment biopsy specimens

Pt	Dose level (mg/kg)	Tumor Biopsy Site	Pre vs Post	Gene Expression (pg/mg tissue)	Copy Number (per mg tissue)
1	0.02	Lung	Pre	0	0
		Lung	Post	2.0×10^{-5}	4.44
7	0.01	Lung	Pre	0	0
		Lung	Post	3.6×10^{-6}	0.89
13	0.02	Lung	Pre	0	0
		Lung	Post	3.0×10^{-5}	6.22
20	0.06	Liver	Pre	0	0
		Liver	Post	0	0
25	0.09	Skin	Pre	0	0
		Skin	Post	8.0×10^{-6}	1.90
26	0.06	Lung	Pre	0	0
		Lung	Post	4.0×10^{-5}	8.76

Figure 1



NSP non-specific peptide; FP TUSC2 peptide; HA non-specific control ab

Conclusions

•Intravenous administration of the DOTAP-cholesterol:TUSC2 nanoparticle is feasible in patients with advanced lung cancer with relatively low toxicity.

•The use of dexamethasone and diphenhydramine premedications appears to be effective in reducing febrile reactions.

•The plasmid containing TUSC2 is detected in tumor tissue 24 hours after treatment (transgene specific RT-PCR).

•TUSC2 protein is expressed in post-treatment tumors (*In situ* proximity ligation assay)

•Median survival in this heavily pretreated population is encouraging.

•Based on promising preclinical data, a trial in combination with erlotinib is planned.

References

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