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

C Lu, DJ Stewart, L Ji, R Ramesh, G Jayachandran, MI Nunez, II Wlstuba, JJ Erasmus, JJ Lee, NS Templeton, JD McMannis, JA Roth.

Univ of Texas MD Anderson Cancer Center, Houston, TX; Gradalis Inc., Carrollton, TX.

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:cholesterol/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:cholesterol/TUSC2 every 3 weeks. The primary endpoint was the assessment of DOTAP:cholesterol/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 protein expression in 6 of 7 posttreatment tumors 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 sample with high intense TUSC2 protein staining in the posttreatment tissue sample. 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 febrile reaction was grade 1, 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 months) and 2 patients, demonstrate low protein staining in pretreatment tissues and high protein staining in posttreatment tissues (Figure 1).

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 tumors with 94% purity after treatment (transgene specific RT-PCR).
• TUSC2 protein is expressed in posttreatment tumors (in situ proximity ligation assay).
• Median survival in chemotherapy-naive patients population is encouraging.

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

References

• Itu et al. Mol Ther 2003;7:409-418
• Ivanova et al. J Pathology 2007;211:591-601
• Kondo et al. Oncogene 2001;20:6259-6262
• Lerman et al. Cancer Res 2000;60:6116-6133
• Ramesh et al. Mol Ther 2003;13:337-350
• Tamin et al. Nat Biotechnol 1997;15:547-562

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

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<tr>
<th>Plt</th>
<th>Dose level (mg/kg)</th>
<th>Tumor Biopsy Site</th>
<th>Pre vs Post</th>
<th>Gene Expression (mg/mg tissue)</th>
<th>Copy Number (per mg tissue)</th>
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</table>

Figure 1

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