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Preclinical development of RAG1-40-31L: A novel small activating RNA-lipid conjugate targeting tumor suppressor gene p21 for treatment of non-muscle invasive bladder cancer.

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Background: Loss-of-function of tumor suppressor genes are the dominant driving force in tumorigenesis. Restoration in function and/or expression of these genes holds tremendous therapeutic potential in cancer treatment. Bladder cancer is the 9th leading cause of cancer-related deaths in the United States in which ~75% of all newly diagnosed cases are non-muscle invasive bladder cancer (NMIBC). Standard-of-care includes intravesical installation of Bacillus Calmette-Guerin following tumor resection with an expected failure rate of ~50% within 6 months for patients with high-grade tumors. RNA activation (RNAa) is a mechanism in which short RNA duplexes termed small activating RNA (saRNA) induce gene transcription by targeting non-coding regulatory sequence (*i.e.*, promoters) embedded in chromatin. Thus, saRNA-induced overexpression of p21$^{WAF1/CIP1}$ (p21) – a tumor suppressor of several anti-growth pathways – may hold therapeutic potential in bladder cancer. Through conjugation of a novel lipid (*i.e.*, C5X5), we have created a p21-targeting saRNA drug candidate referred to as RAG01-40-31L for delivery to bladder cancer cells *in vivo*. The aim of this work was to characterize the intravesical administration of RAG01-40-31L on p21 induction and its anti-tumor potential in NMBIC models. **Methods:** *In vitro* expression analyses were performed to investigate p21 induction via saRNA in KU7 and T24 bladder cancer cell lines by RT-qPCR and immunoblot analysis. Cell cycle analysis and tumorigenicity assays examined impact of RAG1-40-31L on cancer cell growth. Orthotopic human bladder tumors were established in female BALB/c nude mice using the recombinant cell line KU7 engineered to overexpress luciferase reporter. Tumor growth was monitored in live animals via luciferase bioluminescence using an *in vivo* imaging system. Plasma and tissue were harvested for drug exposure and expression analytics. **Results:** *In vitro* analyses indicated RAG1-40-31L activated p21 expression by approximately 6-fold and inhibited bladder cancer cell growth (*i.e.*, cell cycle arrest, apoptosis, and senescence) in a dose-dependent manner with low nanomolar potency. Intravesical administration of RAG1-40-31L (3 times per week for 2 weeks) inhibited growth of orthotopic tumors by 65% in mouse bladder models. Single-dose pharmacokinetics demonstrated a half-life ($t_{1/2}$) of ~114 h with minimal systemic exposure (~0.05% of that of bladder tissue). **Conclusions:** These data constitute a preclinical proof-of-concept for saRNA as a novel p21-targeting modality in the treatment of cancer. The C5X5 lipid conjugate appears to be a viable approach to enhance oligonucleotide delivery and activity in bladder cancer. Preclinical safety assessment studies to enable clinical trials of RAG01-40-31L are currently underway. Research Sponsor: Ractigen Therapeutics.