Novel therapy targeting mutant-KRAS^{G12D} and galectin-1 in pancreatic cancer

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Introduction: In pancreatic ductal adenocarcinoma (PDAC), low patient survival rate remains a problem. The activating point mutation of KRAS on codon-12 is present in 70–95% of PDAC cases and so far, no success has been achieved to inhibit KRAS. KRAS^{G12D} regulates cell proliferation, differentiation, apoptosis; recent preliminary and published studies show high Galectin-1 (Gal-1) levels in both PDAC and stromal cells, which modulate tumor microenvironment and metastasis. Therefore, we have developed a novel combination therapy for PDAC by targeting mutated KRAS^{G12D} and Gal-1 to target both proliferation and metastasis in PDAC. This includes the delivery of KRAS^{G12D} inhibiting siRNA (siKRAS^{G12D}) using a superparamagnetic iron oxide nanoparticle (SPION) and a galectin inhibitor.

Methods: Our patented SPION nano-formulation was used to deliver siKRAS^{G12D} and investigated in conjunction with Gal-1 inhibitor for its anticancer efficacy. Particles were investigated for size, physico-chemical characterization (Dynamic light scattering), hemocompatibility (hemolysis assay) and the complexation of siKRAS (gel retardation assay). Cellular internalization and uptake of the particles were investigated. Anti-cancer efficacy was determined using *in vitro* functional assays for cell viability (MTT), migration (Boyden chambers), invasion (Matrigel), clonogenicity, tumor spheroid formation, and in a mouse model.

Results: Our results demonstrate optimal particle size/zeta potential of SP-siKRAS formulation. SP-siKRAS efficiently internalized in PDAC cells and suppressed KRAS^{G12D} as well as its downstream targets, YAP and PDL-1. Combined targeting of siKRAS and Gal-1 inhibited cell proliferation. It inhibited cell proliferation, clonogenicity, migration, and invasion of PDAC cells. This resulted in activation of death related mechanisms, such as Bax, bcl-2, PARP cleavage in KRAS^{G12D} cells. Interestingly, the formulation was highly effective in inhibiting KRAS^{G12D} and growth of tumor spheroid in 3D cell models, which recapitulate the heterogeneity and pathophysiology of PDAC. This further provides a clinical validation demonstrating potential of SP-siKRAS particles to efficiently silence KRAS expression. SP-siKRAS also exhibited hemocompatibility and stability suggesting its potential of silencing KRAS without being toxic to the body. The formulation efficiently exhibited KRas^{G12D} silencing and inhibited tumor growth and metastasis in nude mice.

Conclusion: This gene therapy targeting KRAS G12D mutation with a Gal-1 inhibition has a potential to modulate the oncogenic network and tumor microenvironment resulting in the repression of growth, metastasis, chemoresistance, and improvement in patient survival. This study will develop a novel sustainable therapeutic approach to target PDAC growth and improve patient survivability.