"bifunctional" shRNA Strategy for Cancer Gene and Cell Therapy

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RNA interference (RNAi) mediated targeted gene silencing has been demonstrated to be highly effective and specific in broad applications. Optimal silencing requires that the RNAi payload be internalized into the target cells and the active RNAi moiety(ies) enter into the RNA-induced silencing complex (RISC). At least four different Argonaute protein (Ago1-4)- RISC complexes have been identified in the mammalian system with target RNA endonuclease activity limited to the Ago2 complex. The RNA-binding affinities of the different Ago proteins vary with miRNA structure motif and between miRNA and siRNA. We have recently developed a novel "bifunctional" shRNA strategy for a broader spectrum of differential Ago binding and RISC incorporation. This novel formulation is highly specific and durable in target gene knockdown with significantly enhanced potency compared to siRNA. We have successfully applied this strategy against multiple targets for cancer gene therapy applications. Using colon cancer cell line CCL-247 (which overexpresses stathmin 1 (STMN1)), we demonstrated that "bifunctional" shRNA targeting STMN1 effectively knocked down STMN1 expression within 24 hour post transfection and persisted beyond 72 hours *in vitro*. Dose response data showed the IC_{50} for the "bifunctional" shRNA 5 logs lower than the siRNA targeted to the same sequence. In addition, a significantly lower dose was required for the "bi-functional" design to achieve protein expression knockdown over time when compared with conventional shRNA. Likewise, when targeting pancreatic and duodenal homeobox 1 (PDX-1) for insulinoma and pancreatic cancer, we found the "bifunctional" design to be highly effective (85-90% target protein knockdown within 48 hours) and sequence specific (selective for target site sequence with 89% homology and single mismatch at the seed region). The "bifunctional" strategy was demonstrated to be effective using an intratumoral administration of the anti-STMN1 lipoplex formulation in a xenograft osteosarcoma tumor model. *In vivo* evaluation of efficacy with systemic application of "bifunctional" anti-PDX-1 lipoplex formulation for insulinoma is currently underway. An autologous cancer vaccine strategy, FANG, comprised of patient tumor cells combining "bifunctional" knockdown of furin expression (to effect inhibition of TGF-β1 and –β2 expression) in combination with GM-CSF transgene expression is currently in Phase I clinical trial. In summary, we have demonstrated the specificity, effectiveness, durability, and potency of the "bi-functional" shRNA effector strategy. Encapsulated in effective delivery vehicles, the "bifunctional" strategy can be used to target single or multiple cancer relevant targets with exquisite specificity across the breadth of personalized cancer gene therapy and cancer vaccine applications.