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PREMISE: An oncogene target is both causative and specific to only tumor cells, offers unrivaled specificity through their genetic distinctiveness, and presents a more effective approach toward treatment of cancer. We have developed and evaluated a strategy to block transcription of oncogenes such as *BRAF* V600E and *KRAS* G12D directly using modified complementary peptide nucleic acid (PNA) oligomer conjugates, thus targeting oncogenesis specifically and effectively causing inhibition of tumor growth. PNA oligonucleotides bind to DNA over 1000-fold more avidly than its native complement, are resistant to enzymatic degradation, and when conjugated to delivery peptides can be made nuclear and cell membrane permeable.

PROOF OF PRINCIPLE: *In vitro* studies reveal concomitant dose-dependent suppression of cell proliferation and oncogene-transcribed mRNA upon exposure to (*BRAF* V600E or *KRAS* G12D) oncogene sequence-specific complementary PNA-delivery peptide conjugates with an IC50 range of 250 to 500 nM. Despite WT and mutant target sequences differing only by a single base pair, there is no inhibition of WT cell growth at these concentrations, and mutant mRNA transcription is exclusively down-regulated without suppression of heterogeneously expressed WT gene. Moreover, *in vivo* animal trials show tumor growth delay, extensive necrosis, and regression. This therapeutic was well tolerated without associated weight loss. By H&E staining, tumor tissue from trials shows ablation and a marked decrease in cellularity upon exposure to PNA-peptide conjugate. Similarly quantitative measurement shows a 2.5-fold decrease in Ki67 and a 3-fold increase in TUNEL expression. Additionally, suppression of KRAS G12D transcription is allele-specific in our *in vitro* studies.

PROPOSED WORK: This provides a new means to develop PNA-delivery peptide conjugates as targeted therapeutics across a broad range of oncogenes that drive cancer cell growth. As 90% pancreatic adenocarcinomas and 40% of GI tumors overall are driven by the *KRAS* G12D oncogene, we are currently focusing upon improving *KRAS* G12D-specific PNA therapeutic for these cancers. Features that further stabilize PNA oligomer binding will be appended to the current design. This includes cell-based screens utilizing RAS-dependent cell lines evaluated by assays based upon RAS-effector and RAS-lipid interactions, RAS-dependent growth and localization, MEK/ERK activation, and RAS-effector binding. Intracellular localization of KRAS, BRET assays monitoring for live cell RAS interactions with RAF and RALGDS, and biosensor-innate cell lines reporting ERK activity to monitor the RAS/RAF/MEK/ERK pathway would also be valuable to evaluate the array of the PNA oligomer modifications. Furthermore evaluation by organoids, 3D models of patient-derived xenografts, and mouse embryonic fibroblasts dependent upon exogenous RAS genes offer a means to follow cell fates through direct suppression of allele-specific KRAS transcription.