Priming strategy with sub-50 nanometer capsules and RNAi cargo ("GS-10") to enhance RNAi and immunotherapy responses

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#3746

HARNESSING RNAi IN CANCER

STRATEGY FOR ENHANCING MECHANISMS

BACKGROUND

To date, strategies for RNAi-treatment of solid tumor cancers have been sub-optimal due to, at least in part, the varied levels of RNA degradation machinery across cancer patients, tumors and tissues. For example, we have found wide variation in Ago2 protein levels, a key RISC component for RNA cleavage, in human head and neck cancer (HNC) tissues. Here we investigate a novel combination strategy for potentially overcoming challenges of RNAi therapy in solid tumors by:

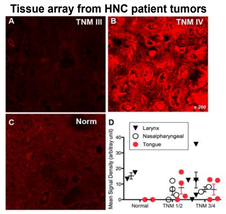
1) Enhancing intracellular delivery
2) Engaging and elevating Ago2 and Ago4 proteins, and
3) Modulating PD-L1 and PD-L2 levels

FIG. 1.

1A. Ago2 levels in tumors can vary significantly from patient to patient, suggesting potential implications for RNAi-mediated cancer therapies.

1B. Argonaute proteins have different functions in RNAi that can potentially be exploited as mechanistic targets.

Tissue array from HNC patient tumors

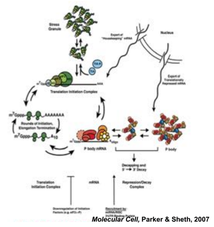


Initial observations of Ago2 in human HNC:

- No correspondence with tumor staging.
- No correspondence with baseline Ago2 levels in normal oral tissue.

Ago 2 level variability in HNC was investigated in an paraffin tissue array (Biomax) of duplicate cores from 10 HNC patient tumor samples and 2 normal oral mucosal tissues by confocal indirect immunofluorescence (Ago2 clone 11A9, Sigma), A-C. Fluorescence intensity was assessed as mean density by image analysis and is plotted in D.

RNAi and the life cycle of an mRNA



mRNA cleavage
Ago 2
RNAseH

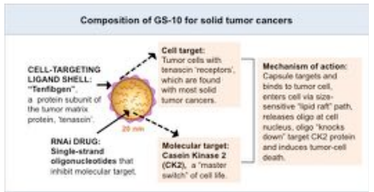
mRNA storage
Ago 1,3,4, sorting to G-bodies

Molecular Cell, Parker & Sheth, 2007

Current model describes mRNAs as cycling between translation, repression and degradation depending on relative abundance and competition of various "factors"

FIG. 2.

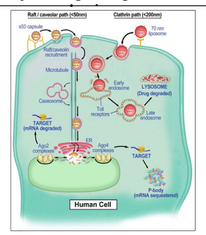
2A. GS-10 is a "synthetic phage" comprising a unique 20 nm drug delivery platform, applied to target and treat solid tumor cancers.



GS-10 is a tumor-targeted 20 nm crystalline capsule bearing single-strand RNAi oligos against Protein Kinase CK2 (CK2), and a ligand-coated shell derived from Tenascin-C (tenfibgen). TN-C is upregulated in chronic wounds and tumor matrix and is a promising ligand for the tumor throughout it's lifecycle (**1**). CK2 modulates > 300 proteins, and is not modulated by other kinases. The ultrasmall "s50" capsule size enables SQ or IV treatment, raft-mediated delivery, and ability to reach metastases without reliance on EPR.

Diffraction pattern indicates s50 crystallite nature

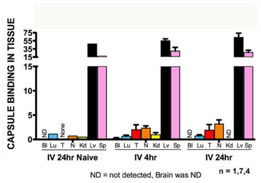
2B. GS-10 novel delivery + single-strand oligo cargo potentially addresses Ago2 variability with Ago2 / Ago4 / GW-182



s50 capsule mechanism of lipid raft uptake where multivalent, mechanically stable capsid-like capsule 1) engages multiple surface receptors, enters cell through lipid raft avoiding endosomes (**1**), 2) trafficks via microtubules to the perinuclear region (unpublished), 3) uncoats possibly by protease action to release oligo for RISC loading on or near nuclear membrane together with Ago 2 (**2**) or 4) Ago 4 (this poster). Ago2-complexes subsequently traffic to cytosol (**2**).

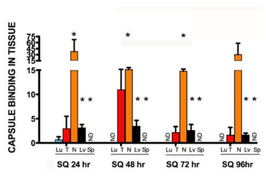
FIG. 3.

SQ delivery of tumor-targeted, tenfibgen-ligand capsules results in more favorable biodistribution profile than IV delivery in tumor-bearing mice – potentially due to lymphatic trafficking.



Fadu FoxN Dy165m NAA

Dy Spike Recovery in liver tissue (ng)	
Treat	Recovery
0	0
40	36
400	280



Bolus pharmacokinetics of s50 capsule delivery is followed via bulk neutron activation analysis (NAA) of Dy surrogate marker (Dy Dextran cargo) to show on a wet tissue basis: 1) NAA confirms SQ-administered capsule concentration peaks at 48 hours in primary tumor (dose ratio = 11 ± 6.2% vs. 1.9 ± 1.1%, IV (4 hr), 2) SQ s50 capsule (Dy) accumulation is 5 -10x larger than IV in nodal tumor for 72 hours post bolus dosing, 3) tenfibgen-coated capsules exhibit specific extracellular binding to collagen V-(+) fibers, abundant in livers and spleens of most mice strains, but for humans, found only in normal spleen and fibrotic lungs, liver (**3**). Nevertheless, SQ administration essentially avoids liver binding (0 – 3 %ID/gm wet wt), even in collagen V-(+) mice. In low collagen V mice, IV liver dose ratio ranges from 2-15% ID/g wet wt (data not shown).

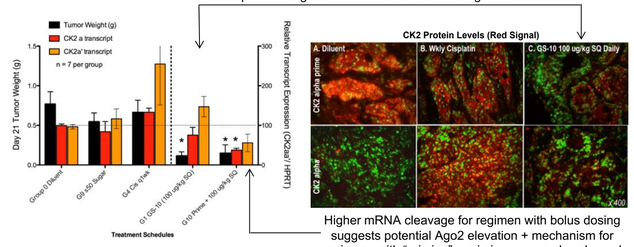
GS-10 ENGAGES MULTIPLE RISC MECHANISMS

IMMUNOCOMPROMISED, XENOGRAFT MODELS

FIG. 4.

RNAi mechanisms underlying GS-10 anti-tumor efficacy vary based on regimen.

Low mRNA cleavage, high protein knockdown suggests potential Ago4 mechanism for low-dose regimens



Higher mRNA cleavage for regimen with bolus dosing suggests potential Ago2 elevation + mechanism for regimens with "priming" – priming groups also showed significant reduction from diluent in brain burden.

Model: HPV-(+) SCC-47 HNC cells in NIH athymic outbred nude, 2e6 cells, wait two weeks, starting size: 6-80 cu. mm.

Cell stress + CK2 biology

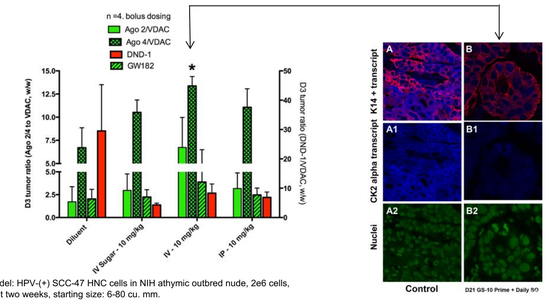
In CK2 biology, upon stress (here, cisplatin), the alternate catalytic kinase subunit (alpha or alpha prime) is rapidly upregulated and protein is recruited to the nucleus to overdrive inflammation and survival pathways (**4**).

Hence, rapid knockdown is important in suppressing tumor adaptation. More rapid kinetics from Ago2-mediated cleavage may be beneficial in this respect and prompted further investigation of "priming".

FIG. 5.

Acute Study: GS-10 loading dose activates RISC proteins to "Prime" tumor for enhanced RNAi efficacy.

At D3 , Priming regimen elevates Ago 4 proteins; other RISC proteins trend upward.
At D21 (from Fig. 4 study), FISH shows reduction of alternate kinase transcript and shift from "active" perinuclear localization to "diffuse" cytoplasmic localization consistent with sequestration.



Model: HPV-(+) SCC-47 HNC cells in NIH athymic outbred nude, 2e6 cells, wait two weeks, starting size: 6-80 cu. mm.

Novel findings regarding DND-1

DND-1 is present and is modulated downward in human xenograft HNC tumor primed with bolus IV GS-10.

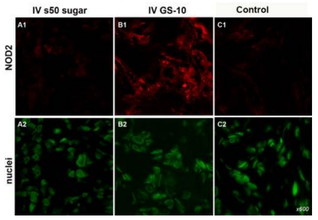
DND-1(for animals, Dead End homolog-1) is an RNA-binding protein known to negatively regulate miRISC activity in mammalian germ cells (5), but, is otherwise poorly studied. However, in plants, following viral infection or stress, DND-1 (for plants, defense not death) is downregulated by activation of R(resistance)-proteins, the plant orthologs of the NOD cytosolic PAMP (RNA) receptors to activate RNAi through HDAC19-co-mediated transcriptional corepression (6).

FIG. 6.

Consistent with tissue-specific s50 targeting, GS-10 engages the innate immune system in a tumor-specific manner.

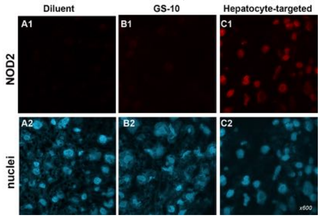
GS-10 but not sugar-bearing capsules elevate NOD2 in tumor.

NOD2 levels in tumor tissue 72 hours after priming IV dosing at 10 mg/kg



ASOR-ligand (hepatocyte) RNAi capsules but not GS-10 elevates NOD2 in liver.

NOD2 levels in matching liver tissue. Results confirmed by western blot.



- NOD proteins are elements of the innate immune system that serve as cytosolic receptors for viral RNA.
- NOD2 is key element of the inflammasome and as such serves as a sensor for intracellular stress, e.g. crystalline particles, elevated ROS and pH shift, initiating engagement of the innate immune system through inflammasome and consequent IL-1ß activation.

Rationale for transition of GS-10 studies to murine syngeneic (CT26) model

- GS-10's tenfibgen ligand targets tumors and inflamed fibroblasts in tumor tissue
- GS-10's human CK2 molecular sequence matches xenograft tumor, not mouse fibroblasts
- Result: Migrating fibroblasts post-treatment confounded analyses re: anti-tumor efficacy
- Strategy: Move to syngeneic tumor, analog drug to properly analyze in relevant conditions

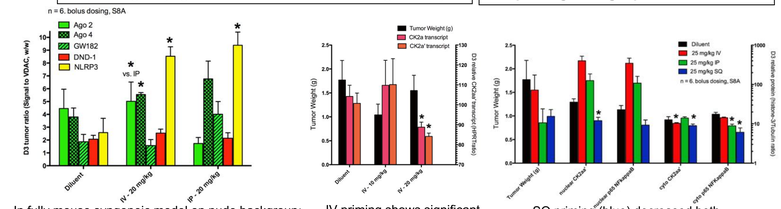
GS-10 PRIMES RISC, MODULATES PD-L1 + 2

IMMUNOCOMPROMISED, SYNGENEIC MODEL

FIG. 7.

IV priming + syngeneic model → mRNA cleavage at Day 3

SQ priming → target protein knockdown



In fully mouse syngeneic model on nude background, IV priming increases Ago2/4 40-50% against diluent, and 3x against IP or diluent at 3 days post-priming.

In this model DND-1 shows no change, but NLRP3/NALP3, a downstream signaling component of NOD2 indicates inflammasome activation, despite no serum cytokines at 24 hrs

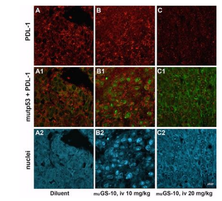
IV priming shows significant 20-25% mRNA cleavage, relative to 0% cleavage at D3 in xenograft model. CK2 half-life is dysregulated in tumors to GT 7 days

SQ priming (blue) decreased both nuclear/cytosolic CK2aa' and major target, p65 NFKappa-B, consistent with PK data (Fig. 3) showing increased drug accumulation. Will investigate SQ priming further.

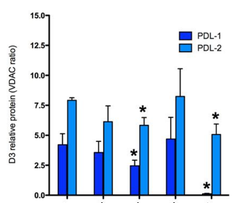
Model: CT26.CL25 murine colon carcinoma cells in CRL FoxN outbred nude, 1e5 cells, wait 10 dats, starting size: 150-350 cu. mm.

FIG. 8.

GS-10 Priming decreases both PD-L1 and PD-L2 on tumor cells at Day 3 via IV or SQ route.



Mutant p53, a sensitive target of anti-CK2 strategies, was modestly modulated by IV priming and has been reported to regulate PD-L1 through miR34a (7). Potentially GS-10 engages other mechanistic pathways (e.g., NFKappa-B.).



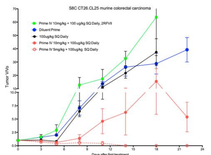
- SQ priming decreased PD-L1 97% and corresponded with significant inhibition of nuclear CK2 and NFKappa-B (Fig. 7). PDL ligands are known to increase by inflammation in normal biology.
- PD-L2 may be regulated differently from PD-L1 as both IP and SQ priming decreased PD-L2 similarly while SQ was significantly more effective in decreasing PD-L1.
- Retrospective analysis of chronically treated tumor tissue indicate PDL ligands can be reduced further and in a sustained fashion by continued dosing (data not shown).

Model: CT26.CL25 murine colon carcinoma cells in CRL FoxN outbred nude, 1e5 cells, wait 10 dats, starting size: 150-350 cu. mm.

IMMUNOCOMPETENT, SYNGENEIC MODEL

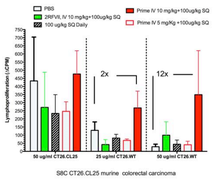
FIG. 9.

GS-10 is effective as a single agent in prime/maintenance dose regimen.

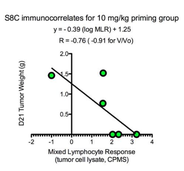


In this tumor line, priming was required for anti-tumor efficacy, potentially due to low Ago2 baseline

5 mg/kg group tumors cleared by 14 days



In mixed lymphocyte proliferation studies (MLR) at Day 21, only 10 mg/kg showed splenocyte response to challenge from CT26.wt (not Bgal-(+)) tumor cell lysate prepared by sonication in PBS.



In subanalysis of 10 mg/kg priming group MLR response, an inverse correlation was found between the magnitude of MLR response and D21 tumor weights and V/Vo. Higher MLR responses also cleared faster.

❑ Additional studies will be examining mechanisms and efficacy in murine syngeneic, immunocompetent models.

Model: n = 5, 7e5 CT26.cl25 cells in CRL Balb/C mice, wait 5 days til 80 cu. mm flank tumors.

CONCLUSIONS

- **A priming strategy can significantly enhance RNAi tumor-killing capabilities**
- **Combination of single-strand oligos + sub-50 nanometer capsules potentially increases access to Ago mechanisms**
- **Casein Kinase 2 is a potentially attractive target for modulating PD-L1 and PD-L2**

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