Identification of a GPC3 specific RNA aptamer and its use in cell specific targeted killing of GPC3 expressing tumors

Kristin M. Thompson, Christina McGuire, Jin Yuan, Aaron Ball, Justin Sonberg, Yuxun Wang, Shuhao Zhu
Guardian Therapeutics, Lexington, MA

Background

Aptamers are short oligonucleotides that have distinct three-dimensional shapes that specifically recognize molecular targets though electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions. Aptamers are generated against a target molecule using a process called SELEX[1]. SELEX is an iterative process of binding, elimination and amplification in which binding sequences are enriched as rounds are executed. This method allows the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules.

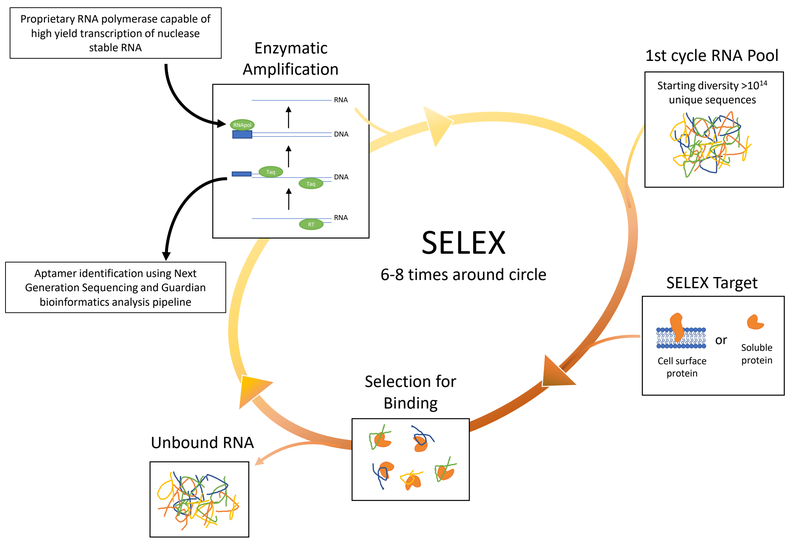
GPC3 is a heparan sulfate proteoglycan and cell surface oncofetal protein which is highly expressed on a variety of liver cancers, but not in healthy adult tissues making it suitable for targeted therapy as a tumor antigen[2].

Maitansine and its derivatives, drug maytansinoids, are membrane-permeable inhibitors of tubulin which can prevent cell division. DM1, *N*$^{2'}$-deacetyl-*N*$^{2'}$-(3-mercapto-1-oxopropyl)-maytansine], was developed to overcome systemic toxicity associated with maytansine and to enhance tumor-specific delivery[3]. DM1 can be conjugated to an amine group at the 5′ end of a synthetic oligo via the crosslinker SMCC.

Study aim: Determine if a GPC3 specific aptamer conjugated to a cytotoxin will selectively target and kill GPC3 expressing tumor cells.

GPC3 SELEX

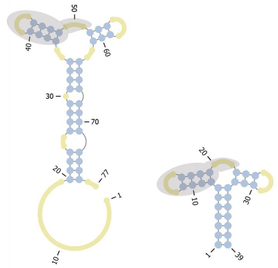
Two independent SELEX against campaigns against recombinant GPC3-Fc were carried out using a proprietary transcription enzyme that results in a highly nuclease resistant pool of RNAs. Each RNA library started with approximately 1x10^{14} unique sequences. The final round of PCR was subjected to Next Generation sequencing (~1 million sequences). The most abundant 192 sequences (clones) were made via chemical synthesis and used in screening for binding to recombinant GPC3 and cell expressed GPC3. Both SELEX campaigns resulted in a similar dominant group of related sequences that have high affinity and specificity to GPC3.



Aptamer Characterization and Optimization

A) Secondary structure prediction of a high affinity clone and final optimized aptamer. Grey highlighted area was conserved among all high affinity clones. Homology and covariation between clones was used to design minimized, optimized final aptamer. B) rGPC3 protein binding. Binding kinetics for the optimized aptamer were determined using Biacore. C) Endogenous GPC3 binding. Flow cytometry using GPC3 expressing tumor cells, HepG2 and Hep3B, and engineered HEK293 cells overexpressing GPC3 detecting binding of clone (1uM), aptamer (1uM) or anti-GPC3 antibody.

A)



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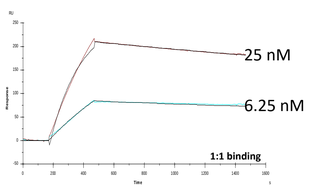
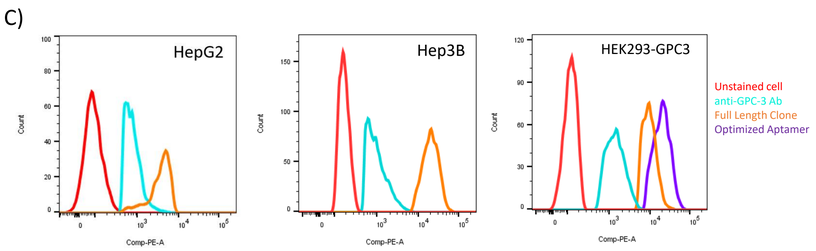


ka (1/Ms)	kd (1/s)	KD (nM)
1.99 x 10^5	1.46 x 10^{-4}	0.74

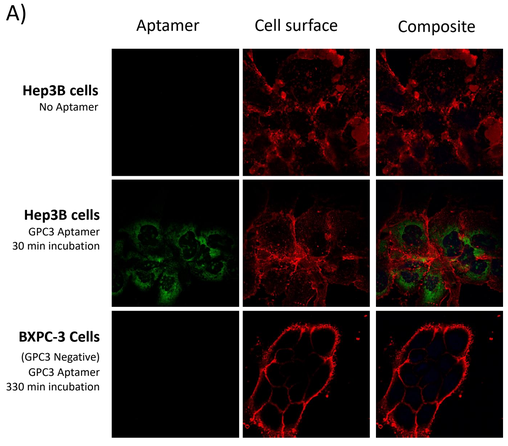
C)



Cellular Uptake and Tumor Targeting

A) Confocal imaging of Hep3B (GPC3+) and BxPC-3 (GPC3-) cells. Cells were treated for 30 minutes with FAM-labeled aptamer, then washed with 0.5nM NaCl to remove surface-bound aptamer. The plasma membrane was stained with WGA. B) Specifically targeting Hep3B tumor. Hep3B and A549 cells were implanted into different nude mice. FAM-labeled aptamer was tail vein injected at 0.45mg/kg Fluorescent signal was specifically detected at Hep3B implanted tumor from 90 to 150 minutes, but not on A549 implanted tumor.

A)



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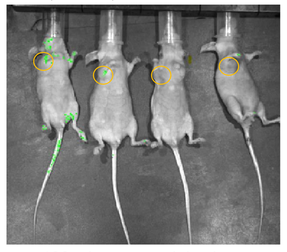
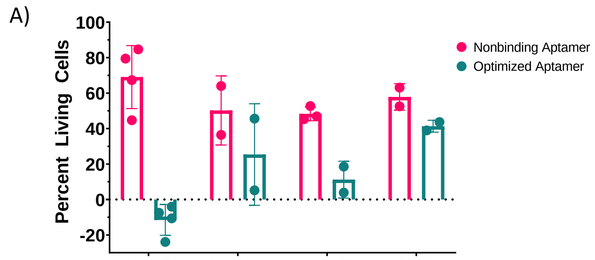


120 minutes post injection

DM1 Conjugated Aptamer, GRX54

A) In vitro killing of cells expressing GPC3. A 3-day treatment with 100nM of the optimized aptamer with DM1 conjugated to the 5′ end, GRX54, selectively kills GPC3 expressing liver tumor cells, but not GPC3 minus A549 cells. Conversely a nonbinding aptamer conjugated with DM1 does not have the same selective killing effect. B) GRX54 treatment reduces tumor volume in multiple tumor xenograft models. NCI-H446 xenograft, Balb/c nude mice. Relative tumor volume growth after 28 days of treatment. GRX54: 10mg/kg i.p. 7 day on/7 off and 5 on/5 off. Irinotean: 40mg/kg i.v. q4d C) Hep3B xenograft, Balb/c nude mice. Relative tumor volume growth after 28 days of treatment. GRX54: 10mg/kg i.p. 7 on/14 off, 5 on/9 off, 3 on/4 off. Sorafenib: 10 and 30mg/kg p.o. daily.

A)



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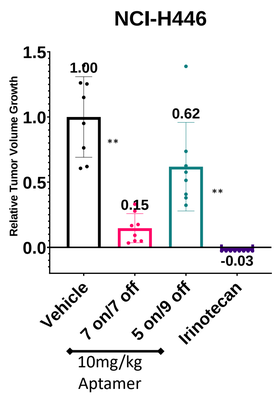

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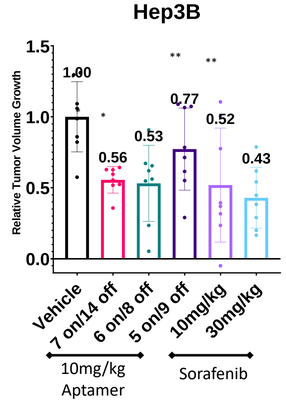

*P<0.05, **P<0.01 significantly different from vehicle (Dunnett's multi-comparison test)

Discussion

Here we describe a nuclease stable, fully modified 2'OMethyl aptamer that recognizes GPC3 with high affinity and specificity. A conjugate comprising a GPC3 aptamer and DM1 was used to specifically target GPC3 expressing cells in vitro and in vivo. The aptamer-drug conjugate (GRX54) is capable of selectively killing a variety of GPC3 expressing liver cells in vitro and has significantly less toxicity in cells not expressing GPC3. The anti-tumor effect of GRX54 was tested in several tumor xenograft mouse models: Hep3B xenograft, Balb/c nude mice and NCI-H446 xenograft, Balb/c nude mice. GRX54 was administered at different doses via intraperitoneal injection (IP). For each group, mice were administered with vehicle or GRX54 once a day (QD) for 3, 5 or 7 days. Tumor weight and growth were measured. An anti-cancer drug Sorafenib Tosylate (30mg/kg, oral administration, once a day - Hep3B xenograft mouse model) or irinotecan (40mg/kg, i.p. twice a day for 4 days - NCI-H446 xenograft mouse model) were used as positive controls. GRX54 decreases Hep3B tumor volume in a Balb/c nude mouse xenograft model by ~30-45% depending on treatment schedule and NCI-H446 tumor volume by~45-90% depending on treatment schedule. Together, our data show that GRX54 is a novel GPC3-targeting molecule capable of reducing tumor size with IP injection.

References
1. Ellington and Szostak, Nature, 1990; 346: 818-822
2. Nishida and Kataoka, Cancers, 2019; 11(9): 1339
3. Chari et al., Cancer Res, 1992; 52: 127–131

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