Raptamer-Drug Conjugates as Molecularly Targeted Cancer Therapeutics

Stephanie P. Vega1,2, Nancy Ward 1,2, Michael J. Heffernan1,2, Daniel Ciznadija3, Dev Chatterjee1, Atul Varadhachary1, Mike Ritchie3

1 Fannin, Houston, Texas, 2 Raptamer Discovery Group, Houston, Texas 3 Champions Oncology, Rockville, Maryland

Abstract presentation number: 456

Introduction

COF-01 is a validated target in patient-derived tumors. We recently identified COF-01 (designated COF-01 as the proprietary code name) to be upregulated in many tumor samples derived from patients with non-small cell lung cancer (NSCLC) and head and neck (H&N) cancer. This dataset was established from DIA-label free quantitative proteomics characterization of a large bank of low passage Patient Derived Xenograft (PDX) models. Importantly, the majority of these PDX models are derived from patients who were treated, and progressed on, standard of care (SOC) therapy, suggesting overexpression of COF-01 in patient populations with high unmet medical need. Subsequent immunohistochemistry (IHC) evaluation of COF-01 in these tumor samples validated the findings from proteomics.

Targeted cancer therapy: Antibody-drug conjugates

Antibody-drug conjugates (ADCs) are a class of therapeutics that include an antibody moiety for tumor targeting, and a drug payload tethered to the antibody through a chemical linker. Unlike chemotherapy, ADCs are targeted to kill tumor cells while sparing healthy cells. While ADCs have scored some notable successes, development of efficacious ADCs still presents considerable challenges. The use of aptamers as a targeting moiety, holds the promise of building RapDCs (Raptamer-Drug Conjugates) as a novel strategy for COF-01-targeted anti-cancer therapeutics.

Materials and Methods

Methods

Figure 1. Library beads and a tagged target(s) are incubated and pulled down with magnetic particles to recover sequences. Putative Raptamers are released into solution and are ready for amplification and next-generation sequencing. Raptamer candidates are synthesized at a small scale (50 nmol) to test their binding affinities on using biolayer interferometry (Octet®). Top candidates will then be synthesized at a medium scale for further testing.

Results

Binding affinities of COF-01 Raptamers

Figure 2. Raptamer candidates were synthesized to test binding affinity using biolayer interferometry (Octet®). Raptamers were screened using a single concentration of COF-01 (500 nM). Representative graphs for Raptamers 18, 23, 24, 27, 29, and 30 are shown.

Figure 3. Binding curves were performed using a range of COF-01 protein concentrations (500, 200, 125, 62.5, 31.3, 15.6, and 7.81 nM). Representative graphs for Raptamer 18 and Raptamer 27 are shown.

In vitro efficacy of RapDCs in COF-01-positive and COF-01-negative human cancer cells

Figure 4. In vitro efficacy of Raptamer 12-MMAE RapDC (1 and 2) in COF-01-positive human skin cancer cells, gastric cancer cells, pancreatic cancer cells and COF-01-negative lung cancer cells. Cytotoxicity was evaluated using the Cell Titer Glo® luminescent assay after incubating cells with RapDCs for 4 days and IC50 values calculated for each RapDC.

Efficacy of RapDCs in COF-01-positive human skin cancer cells

Efficacy of RapDCs in COF-01-negative human skin cancer cells

Efficacy of RapDCs in COF-01-positive human gastric cancer cells

Efficacy of RapDCs in COF-01-negative human gastric cancer cells

Efficacy of RapDCs in COF-01-positive human pancreatic cancer cells

Efficacy of RapDCs in COF-01-negative human pancreatic cancer cells

Efficacy of RapDCs in COF-01-positive human lung cancer cells

Efficacy of RapDCs in COF-01-negative human lung cancer cells

Discussion

• We have generated high affinity Raptamers against a novel therapeutic target, COF-01.
• RapDCs were developed using a multi-arm backbone that allowed for direct conjugation of Raptamers and a single MMAE-linker compound (lysosomally cleavable, valine-citrulline linker).
• We have examined the in vitro efficacy of COF-01 RapDCs. There was a significant and specific decrease in cell viability of COF-01-positive human cancer cells when compared to COF-01-negative control cells.
• Our next studies involve examining the in vivo efficacy of RapDCs in cell-derived xenograft models of various types of cancers.

• We can rapidly generate high-affinity and specific Raptamers against a range of targets.

Acknowledgements

Fannin would like to acknowledge Aurigen Pharmaceutical Services for synthesizing the COF-01 Raptamers, the Raptamer 12-MMAE RapDCs and other RapDC variations we will soon be testing.

References

4. Chen, Y., Heffernan, M. J., & Ritchie, M. (2022). Optimization strategies for developing antibody drug conjugates. Molecules (Basel, Switzerland) 2022, 27(10), 1926. Summary of various therapeutic targets with corresponding natural aptamers vs. Raptamers and the type of modifications that were incorporated. Raptamer modifications can improve binding affinity down to the pM range.

Table showing various therapeutic targets with corresponding natural aptamers vs. Raptamers and the type of modifications that were incorporated. Raptamer modifications can improve binding affinity down to the pM range.