Selective KRAS(G12D)-Binding Monobodies for the Treatment of Cancer

A selective, efficacious and versatile treatment for KRAS(G12D)-driven cancers

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Technology

The Koide research group at NYU Langone Health is a pioneer and world expert in engineering monobodies, which are synthetic binding proteins constructed on the fibronectin type III domain scaffold, for therapeutic and diagnostic applications. They have developed affinity-matured monobodies that preferentially bind to the prevalent RAS mutant isoform KRAS(G12D) for the treatment of KRAS(G12D)-driven cancers. As described in published proof-of-concept studies (*Akkapeddi et al. PNAS 2023*), the monobodies preferentially engaged KRAS(G12D) in the GDP or GTP-bound nucleotide states with low nanomolar affinity, while displaying significantly weaker binding (micromolar range and above) to nucleotide-bound KRAS wild-type (WT) and other RAS mutant isoforms. The most promising clone (12D4) bound to KRAS-GDP and KRAS-GTP with binding affinities (KD) of 18nM and 9.8nM, respectively. Additionally, crystal structures of multiple monobody:KRAS(G12D) complexes revealed the monobodies to engage KRAS(G12D) at the Switch II pocket (in an atypical “open” conformation) and provided a framework for structure-guided affinity maturation. In functional studies, intracellularly expressed monobodies colocalized with KRAS(G12D) and demonstrated effective and selective inhibition of oncogenic KRAS-mediated signaling activity. When tested in a conditionally-inducible mouse xenograft model, clone 12D4 reduced the growth of KRAS(G12D)-driven tumors (Pa14C), but not KRAS(G12R)-driven tumors (PSN1), demonstrating its specificity and efficacy in vivo. Taken together, these affinity-maturated monobody clones represent promising therapeutic candidates for selective inhibition of KRAS(G12D)-driven cancers.

Background

KRAS is one of three major isotypes of the GTPase RAS, which collectively regulate critical cellular signaling events. Activating, missense mutations of RAS genes (KRAS, HRAS, and NRAS) are frequently associated with human cancers and play important roles in oncogenic transformation, with more than 25% of all human cancers containing missense RAS mutations. The G12D mutation is among the most common oncogenic KRAS mutations, and is particularly prevalent in challenging cancers, including pancreatic and colorectal cancer. While there are KRAS(G12D) inhibitors currently in preclinical and clinical development, such as Mirati’s MRTX1133 small molecule inhibitor and other cyclic peptide inhibitors, the monobodies described here afford unique advantages and meaningfully contribute toward addressing the unmet need.

Development Stage

Researchers have shown anti-KRAS(G12D)-driven tumor specificity and efficacy in *in vivo* xenograft models and are seeking a commercial partner to progress this technology to a pre-

IND application.

Applications

- **Therapeutic treatment:** Monobodies are expected to treat KRAS(G12D)-mediated cancers such as pancreatic and colorectal cancer, when administered via:
  - Peptide format
  - Polynucleotide format using lipid nanoparticle delivery
  - bioPROTAC format (as either a peptide or polynucleotide)
- **KRAS(G12D) drug development:** For use in high-throughput competition screens for new small molecule or biologic inhibitors of KRAS(G12D)
- **Research reagent tool:** For use in KRAS(G12D) mechanistic studies

Advantages

- **High selectivity:** Monobodies preferentially bind to KRAS(G12D) over KRAS(WT) or other mutant RAS isoforms
- **High affinity:** Monobodies bind to KRAS(G12D) with low-nanomolar affinity
- **Flexible and adaptable:** Monobodies are highly stable and free of endogenous cysteine residues, which offer high flexibility in conjugation. Monobodies, in peptide or polynucleotide format, can be conjugated with various agents for a variety of applications and delivery approaches
- **Accompanying structural information:** Crystal structures of monobody:KRAS(G12D) complexes can be leveraged for further monobody development

Development Stage

Researchers have shown anti-tumor specificity and efficacy in NRAS-mutant tumor cell lines and are confirming these findings in *in vivo* xenograft models.

Intellectual Property

NYU has filed a U.S. non-provisional patent application (as of 12/13/25) covering the composition of the anti-KRAS mutant-specific monobodies and their method of use.

References

1. Akkapeddi, Padma et al. , https://pubmed.ncbi.nlm.nih.gov/37399416/