Structure-guided engineering fine-tunes pharmacokinetics, tolerability, and anti-tumor profile of anti-frizzled antibody

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Aberrant activation of Wnt signaling occurs frequently in cancer, but therapeutic targeting of this pathway is complicated by its role in maintaining tissue homeostasis. Here, we evaluated the anti-tumor efficacy of antibodies that target Wnt receptors Frizzled (Fz) 1, 2, 4, 5, 7, and 8, but found these to cause gastrointestinal toxicity or have poor exposure in mice. Crystal structures of three antibodies in complex with Fz's revealed they block a hydrophobic site on Fz involved in interacting with the Wnt palmitoyl moiety. We next used structure-guided design to fine-tune *in vivo* tolerability. An engineered variant with affinities to Fz4, Fz5, and Fz7 in the 13-138 nM range retained *in vitro* potency, and was well tolerated *in vivo*. This antibody significantly inhibited tumor growth in a HPAFII xenograft model. Our data inform on the development of anti-Fz cancer therapeutics that require balance specificities to navigate *in vivo* exposure, tolerability, and efficacy.