

Molecular Cancer Therapeutics

Large Molecule Therapeutics

Targeted Drug Delivery with an Integrin-Binding Knottin–Fc–MMAF Conjugate Produced by Cell-Free Protein Synthesis

Nicolas V. Currier, Shelley E. Ackerman, James R. Kintzing, Rishard Chen, Maria Filsinger Interrante, Alexander Steiner, Aaron K. Sato, Jennifer R. Cochran

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Abstract

Antibody–drug conjugates (ADC) have generated significant interest as targeted therapeutics for cancer treatment, demonstrating improved clinical efficacy and safety compared with systemic chemotherapy. To extend this concept to other tumor-targeting proteins, we conjugated the tubulin inhibitor monomethyl-auristatin-F (MMAF) to 2.5F–Fc, a fusion protein composed of a human Fc domain and a cystine knot (knottin) miniprotein engineered to bind with high affinity to tumor-associated integrin receptors. The broad expression of integrins (including $\alpha\beta3$, $\alpha\beta5$, and $\alpha5\beta1$) on tumor cells and their vasculature makes 2.5F–Fc an attractive tumor-targeting protein for drug delivery. We show that 2.5F–Fc can be expressed by cell-free protein synthesis, during which a non-natural amino acid was introduced into the Fc domain and subsequently used for site-specific conjugation of MMAF through a noncleavable linker. The resulting knottin–Fc–drug conjugate (KFDC), termed 2.5F–Fc–MMAF, had approximately 2 drugs attached per KFDC. 2.5F–Fc–MMAF inhibited proliferation in human glioblastoma (U87MG), ovarian (A2780), and breast (MB-468) cancer cells to a greater extent than 2.5F–Fc or MMAF alone or added in combination. As a single agent, 2.5F–Fc–MMAF was effective at inducing regression and prolonged survival in U87MG tumor xenograft models when administered at 10 mg/kg two times per week. In comparison, tumors treated with 2.5F–Fc or MMAF were nonresponsive, and treatment with a nontargeted control, CTRL–Fc–MMAF, showed a modest but not significant

therapeutic effect. These studies provide proof-of-concept for further development of KFDCs as alternatives to ADCs for tumor targeting and drug delivery applications. *Mol Cancer Ther*; 15(6); 1291–300. ©2016 AACR.

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Footnotes

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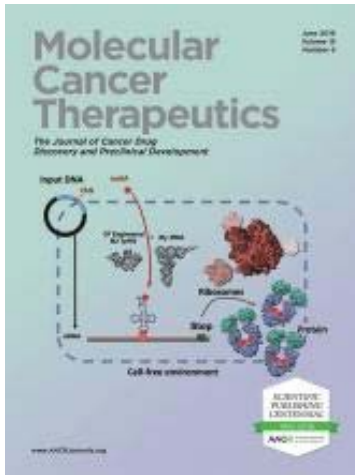
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