

## **Engineering hyaluronic acid binding cytokines for enhanced tumor retention and safety**

Elizabeth Fink

Many anticancer therapies are constrained by narrow therapeutic windows, with doses required for efficacy approaching those that induce systemic toxicity. As a result, considerable effort has focused on restricting drug activity to the tumor, where immune activation or cytotoxicity is desired, while limiting exposure to healthy tissues. Intratumoral administration was proposed to provide such spatial control, but therapeutic agents readily diffuse out of the tumor and enter systemic circulation, limiting the magnitude of local response while still potentiating on-target, off-tumor toxicity. To overcome this, intratumoral anchoring strategies have been developed to retain therapeutic payloads within the tumor microenvironment. Among these strategies, targeting extracellular matrix (ECM) components has been a widely used approach that has been shown to broaden the therapeutic windows of multiple immunotherapies.

ECM-targeted anchoring strategies have shown promise in preclinical models, but have yet to be widely validated in the clinic. Most studies to date have targeted a narrow set of ECM components, such as collagen and fibronectin, leaving much of the design space unexplored. While molecular weight and binder affinity predictably determine retention within these systems, it remains unclear how other ECM-specific factors influence the distribution and function of anchored cargo in the tumor microenvironment. This distinction is particularly relevant in the context of tumor heterogeneity, as ECM composition, architecture, and spatial relationships to tumor and immune cells vary both inter- and intratumorally. Thus, the choice of ECM target may influence not only overall retention but also the bioavailability of the therapeutic to target cells.

To explore these open questions, we focused on hyaluronic acid (HA), a highly abundant and dynamic glycosaminoglycan that is frequently enriched in solid tumors. Compared with collagen, HA occupies a distinct spatial and functional niche within tumors, providing the opportunity to test whether matrix target choice can meaningfully influence therapeutic performance. While collagen-binding strategies are among the most extensively characterized ECM anchoring approaches, HA targeting has been minimally explored, with prior work limited to peptide-binding approaches in isolated therapeutic contexts. Here, we engineered a HA-anchoring platform, using targeted protein engineering to optimize binding, stability, and fusion compatibility. We applied this platform to IL-12/IL-15 combination therapy, a regimen with a particularly narrow therapeutic window. We compared our HA-anchored cytokines to a well-established, size-matched collagen-anchored system to examine how ECM target influences therapy retention and function. We found HA anchoring significantly improved tumor retention and loading, which were concomitant with improved therapy tolerability and downstream immune activation. This work thus establishes HA-anchoring as a viable approach for the intratumoral retainment of anticancer therapeutics and underscores the importance of tailoring anchoring strategies to payload properties to achieve the desired localization, safety, and efficacy.