**A First-in-Class Nanobody–Enzyme Platform for TME Reprogramming: Coupling EGFR-Targeted Cytotoxicity with Immunogenic Cell Death**

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**Abstract:**

Colorectal cancer (CRC) remains the second leading cause of cancer-related deaths worldwide. Microsatellite stable (MSS) CRC, which represents approximately 85% of cases, exhibits poor responses to immune checkpoint inhibitors due to immunologically "cold" tumor microenvironments (TME). To address this unmet need, we developed VISK-103, a novel nanobody–enzyme fusion protein designed to reprogram the TME through targeted cytotoxicity coupled with potent immune activation.

VISK-103 comprises an anti-EGFR VHH nanobody fused to a modified xanthine oxidase (XO) enzyme that catalyzes local generation of reactive oxygen species (ROS) upon intratumoral delivery. This targeted burst of ROS induces tumor-selective cytotoxicity with bystander effects on neighboring cancer cells. Critically, VISK-103-mediated oxidative stress triggers immunogenic cell death (ICD), characterized by surface exposure of calreticulin (CRT), extracellular ATP release, and HMGB1 secretion. These damage-associated molecular patterns (DAMPs) promote dendritic cell (DC) maturation, cross-presentation of tumor antigens to CD8+ T cells, and pro-inflammatory cytokine production, thereby converting cold tumors into immunologically inflamed lesions.

Biochemical characterization demonstrated that VISK-103 binds EGFR-expressing CRC cells 2- to 3-fold more than non-EGFR expressing cells while retaining enzymatic activity. VISK-103 treatment induced concentration-dependent cytotoxicity across a panel of CRC cell lines (sub micromolar $IC_{50}$). In syngeneic, immunocompetent mouse models of EGFR-high CRC, intratumoral VISK-103 administration resulted in approximately 50% tumor growth inhibition . Mechanistic studies in DC-tumor co-cultures revealed that VISK-103-treated tumor cells elicited >3-fold increases in IL-1β secretion and enhanced DC activation markers relative to oxaliplatin-treated controls, indicating superior ICD induction.

These data establish VISK-103 as a first-in-class therapeutic that integrates targeted enzyme-mediated cytotoxicity with potential for in situ tumor vaccination. The modular antibody–enzyme platform enables tumor antigen-agnostic immune priming and represents a promising strategy to overcome immune exclusion in solid tumors refractory to checkpoint blockade.