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

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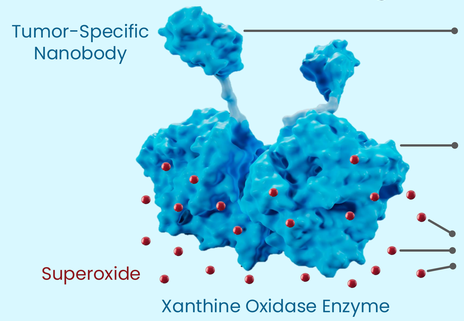
Summary

VISK-103 is a first-in-class anti-EGFR nanobody–enzyme fusion protein designed to induce localized oxidative stress and immunogenic cell death (ICD). VISK-103 generates reactive oxygen species, driving tumor-selective cytotoxicity and bystander killing while inducing calreticulin exposure (CALR), ATP release (eATP), and HMGB1 secretion. VISK-103 preferentially binds EGFR-expressing cells, induces sub-micromolar cytotoxicity in vitro, and achieves ~50% tumor growth inhibition with survival benefit in immunocompetent CRC models. These data support VISK-103 as an in situ tumor vaccination strategy capable of overcoming immune resistance in EGFR tumors.

What Makes Us Different?

A Novel Fusion Protein with a Three-Pronged Mechanism of Action



1. **Locking in Our Cancer Target:** **Tumor-targeting** nanobody* domain **inhibits tumor growth** with **high specificity**
2. **Triggering Cell Death:** Human xanthine oxidase produces **superoxide** to kill **target** and **surrounding cells**
3. **Educating the Immune System:** Extracellular superoxide triggers **Immunogenic Cell Death (ICD)** to stimulate the immune system and make "cold" tumors "hot"

*Nanobody: A single domain antibody, also known as VHH

Locking in Our Cancer Target & Triggering Cell Death

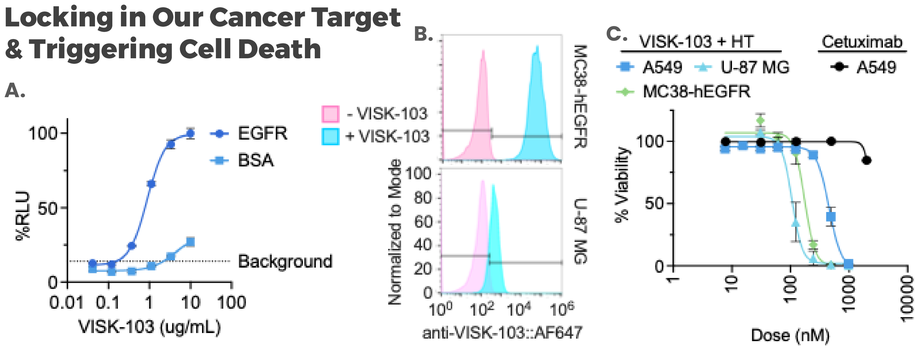


Figure 1. VISK-103 binds to EGFR and reduces viability of EGFR-expressing cells. **A.** VISK-103 binding to EGFR or BSA by ELISA. **B.** VISK-103 binding to EGFR-positive cells (MC38-hEGFR: CRC; U-87: GBM by flow cytometry. **C.** Cell viability of indicated cancer cell lines (A549: NSCLC) after 3 days of treatment with VISK-103 and hypoxanthine (HT) as measured by CellTiter-Glo 2.0 and normalized to vehicle control.

Triggering Cell Death: In Vivo Evidence

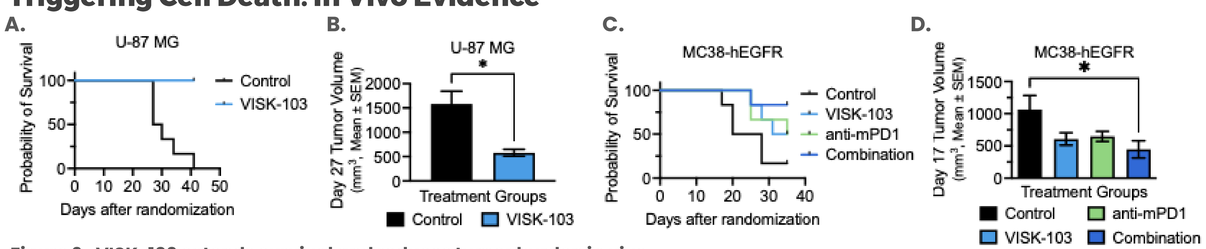


Figure 2. VISK-103 extends survival and reduces tumor burden in vivo.
A. Survival and **B.** tumor volume in B-NDG (NOD/SCID) mice bearing subcutaneous U-87 MG tumors treated with VISK-103 or vehicle control. Mice were co-treated with hypoxanthine and received four intratumoral doses administered every 3 days. Statistics: Welch's t-test; *p<0.05.
C. Survival and **D.** tumor volume in B-hEGFR (C57BL/6) mice bearing subcutaneous MC38-hEGFR tumors treated with VISK-103, anti-mPD-1, combination VISK-103 + anti-mPD-1, or vehicle control. All groups were co-treated with hypoxanthine. VISK-103 (or vehicle) and hypoxanthine were administered intratumorally; anti-mPD-1 (or vehicle) was administered intraperitoneally. Mice received two treatment cycles of four doses every 3 days (Days 0–9 and 21–30). Hypoxanthine was administered daily from days 25–34. Statistics: one-way ANOVA with Kruskal–Wallis test; *p<0.05.

Educating the Immune System

The ICD Cascade: From Death to Immunity

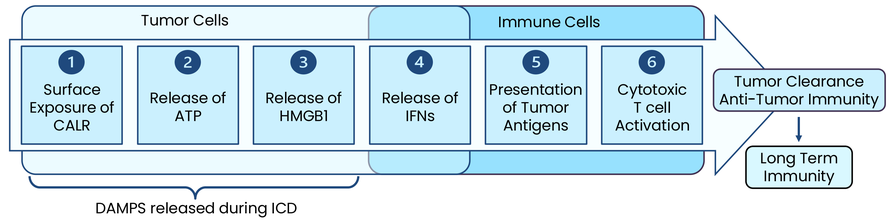


Figure 3. The ICD cascade from: from death to immunity. Schematic of immune activation. Damage-associated molecular patterns (DAMPs) released from tumor cells undergoing ICD promote dendritic cell maturation, cytotoxic T cell activation, and durable anti-tumor immunity.

Immunogenic Cell Death

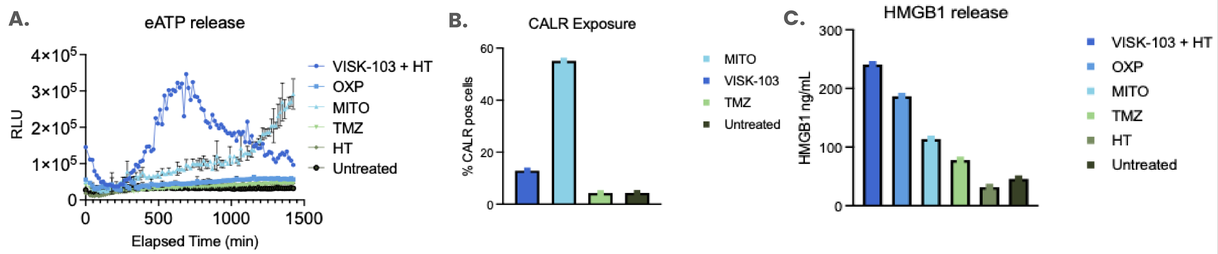


Figure 4. VISK-103 induces markers of ICD in GBM cells. U-87 MG cells were treated with the indicated drugs for 24 hours and assessed for **A.** eATP release (RealTime-Glo™). **B.** CALR surface expression (flow cytometry) or **C.** HMGB1 release (Lumit™). OXP = oxaliplatin; TMZ = temozolomide; MITO = mitoxantrone; HT = hypoxanthine. All drugs were treated at 10 uM except for HT (1 mM) and VISK-103 (3 uM in A. and 1 uM in B. and C.).

Immune Engagement

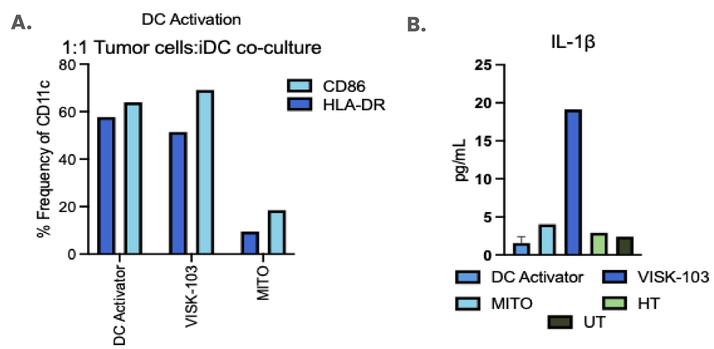


Figure 5. VISK-103 promotes dendritic cell activation and pro-inflammatory cytokine release.
Human monocyte-derived immature dendritic cells (iDCs) were co-cultured with treated tumor cells at a 1:1 ratio to assess immune engagement. **A.** Flow cytometric analysis of DC activation markers showing increased frequency of CD11c⁺ DCs expressing CD86 and HLA-DR following exposure to VISK103-treated tumor cells, comparable to a DC activator control and markedly higher than ICD inducer control (Mitoxantrone). **B.** Cytokine analysis of co-culture supernatants demonstrates significantly elevated IL-1β secretion in response to VISK103-treated tumor cells compared to controls.

Conclusion

VISK-103 couples **EGFR-targeted cytotoxicity** with **robust induction of immunogenic cell death (ICD)**, driving both direct tumor killing and activation of anti-tumor immune responses. Through localized oxidative stress within EGFR-expressing tumors, VISK-103 induces hallmark ICD signals, including **calreticulin exposure**, **extracellular ATP release**, and **HMGB1 secretion,** that promote dendritic cell activation and immune engagement. In vivo, VISK-103 significantly **reduces tumor burden and extends survival** in both immune-deficient and immune-competent tumor models. Notably, **combination treatment with PD-1 blockade further enhances anti-tumor efficacy,** supporting a role for adaptive immune involvement downstream of VISK-103–mediated tumor cell death. Together, these findings position **VISK-103 as a first-in-class nanobody–enzyme fusion therapeutic** capable of converting immunologically "cold" tumors into immune-responsive "hot" lesions and provide a strong rationale for continued translational development and rational combination strategies.

Questions? Contact Bushra@viska.bio to learn more.