Degradation of the ETS transcription factor ERG by stabilized helical peptide (Helicon™) degraders enables pharmacological validation in ERG-fusion prostate cancer models

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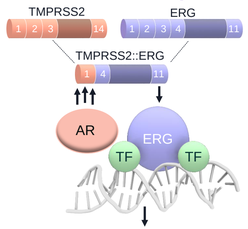
TMPRSS2-ERG Fusion in Prostate Cancer

TMPRSS2-ERG fusions occur in 40-50% of all prostate cancer (PCa) cases, appearing early in the tumorigenic process and persisting throughout treatment regimens. ETS-related gene (ERG) is an oncogenic ETS-family transcription factor normally expressed highly in the endothelium, hematopoietic stem cells, and adipose tissue. In PCa, N-terminal deleted ERG expression is driven by androgen receptor (AR) due to a fusion with the TMPRSS2 promoter. Expression of the ERG fusion promotes AR binding to a wider range of AR Response Elements, expanding AR functions in the tumor setting. ERG also alters gene expression more globally, by promoting stemness through its interactions with chromatin modifying enzymes, such as the BAF complex. This re-purposing of AR and chromatin modifiers increases proliferation and migration of these tumor cells.

The highly prevalent and specific ERG fusions observed in PCa patients, coupled with a central role for ERG in AR and PCa biology, highlight ERG as an attractive, as yet undrugged, target. We report here the discovery of stabilized peptide Helicons with bifunctional E3 ligase degrader activity that provide (to our knowledge) the first pharmacological proof of concept for ERG inhibition and dependency in preclinical ERG-fusion PCa models.

TMPRSS2-ERG Oncogene



Proliferation | Angiogenesis
Anti-apoptosis | Cell migration
Differentiation | Invasion
Genomic instability

Prostate Cancer Growth

ERG Dependency in VCaP Cells

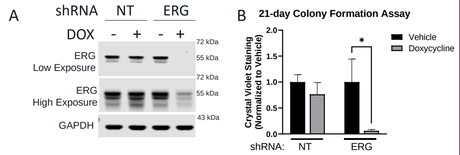
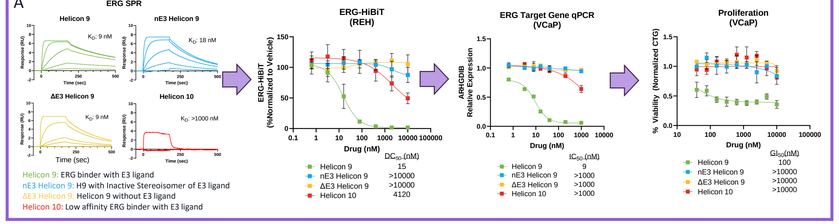


Fig. 1: VCaP cells, the only AR⁺ PCa cell line harboring the TMPRSS2-ERG fusion, are sensitive to genetic loss of ERG.[1] (A-B) VCaP cells stably expressing pLKO-Tet-on-puro with either Non-targeting (NT) shRNA or ERG shRNA were grown with 100 ng/mL doxycycline for 7 days and checked for ERG expression by Western blot (A) and 21-days in a colony formation assay (B). N = 3, *p<0.05, One-way ANOVA.

ERG Degraders Exhibit Nanomolar Potency *in vitro*

Compound Screening Cascade



Helicon 9: ERG binder with E3 ligand
nE3 Helicon 9: H9 with Inactive Stereoisomer of E3 ligand
ΔE3 Helicon 9: Helicon 9 without E3 ligand
Helicon 10: Low affinity ERG binder with E3 ligand

Mechanism of Action: E3- and Proteosome-Dependent, On-Target Degradation

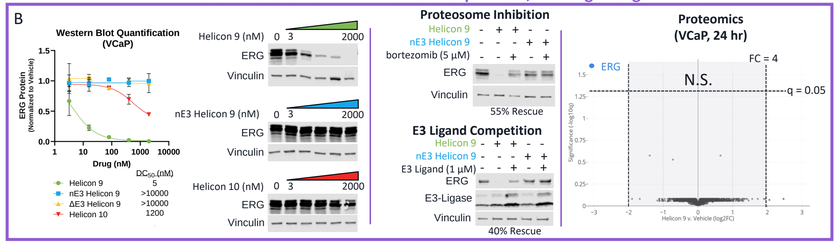
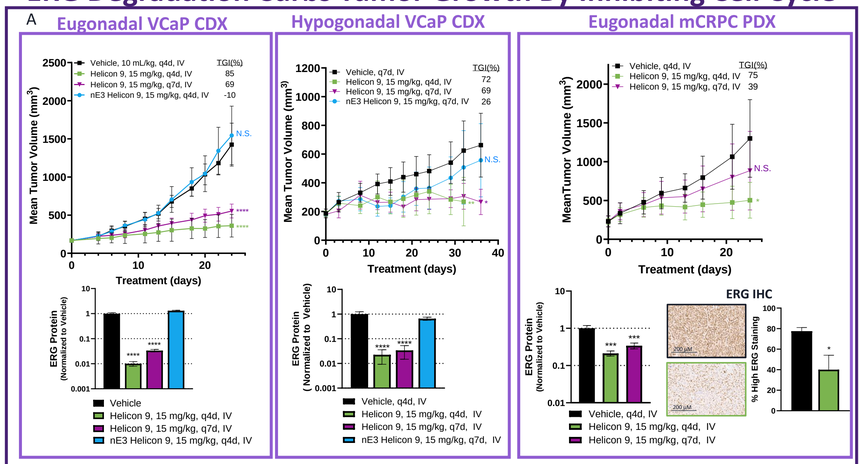


Fig. 2: ERG Degraders degrade ERG, inhibit ERG target gene expression, and proliferation through E3 ligase and UPS mediated degradation. (A) Screening assays demonstrate nanomolar affinity for ERG by SPR, on-target ERG degradation (24 hr, REH), ERG target gene modulation (24 hr, VCaP), and anti-proliferative effects (10 nM DHT + Helicon, 14-days, VCaP). N = 3, all data shown as mean ± S.D. (B) ERG degradation with Helicon 9 (100 nM, VCaP) can be rescued by treatment with bortezomib and excess E3 Ligand, demonstrating expected MoA. Volcano plot demonstrates no significant off-target protein changes with Helicon 9 treatment (N = 3). FC, fold-change

ERG Degradation Curbs Tumor Growth By Inhibiting Cell Cycle



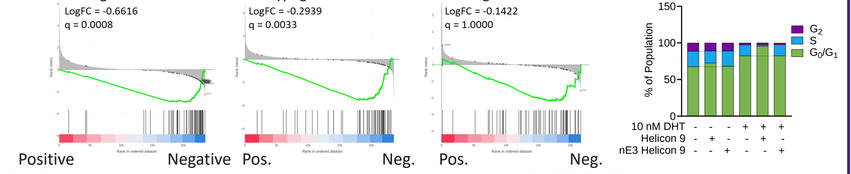
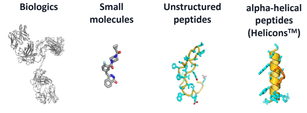


Fig. 4: Helicon 9 inhibits tumor growth in three xenograft models of TMPRSS2-ERG fusion prostate cancer. (A) Tumor growth inhibition (TGI) and ERG levels in eugonadal (L) and hypogonadal (middle) VCaP CDX, and Eugonadal PDX (R). N = 4-6 mice. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, N.S. not significant compared to vehicle control using ANOVA (Mixed-effects Model where required). Data presented as mean ± S.D. (B) GSEA from RNA sequencing of VCaP CDX reveals Helicon 9 downregulates Myc target genes. (C) Treatment of VCaP cells with Helicon 9 ± 10 nM DHT for 48 hrs demonstrates reduction in S-phase population and cell cycle arrest at G_0/G_1. Representative data from N = 3 experiments.

Discovery of ERG Binders and Conversion to ERG Degraders

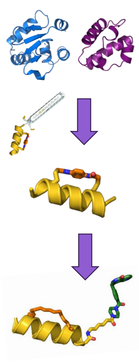
Helicons™ hit challenging intracellular proteins

Engaging intracellular targets without deep binding pockets like transcription factors remains a challenge in drug discovery. Small molecules are cell permeable, but do not have the contact area to engage targets without druggable pockets. Biologics and other peptide modalities have larger binding surface areas but are not cell permeable. Constrained alpha-helical peptides (Helicons™) are a demonstrated solution, combining cytosolic exposure with the ability to bind traditionally undruggable targets.



Helicons™ degrader discovery process

D-peptide ERG binders were discovered through screening phage display libraries of alpha-helical peptides against synthetic mirror image ERG structured domains and were converted into ERG degraders through attachment of E3 ligands.



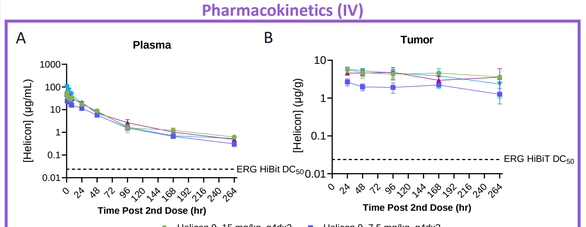
Two structured domains of ERG, the ETS domain (blue) and PNT domain (purple) were synthesized as D-proteins through single-shot fast flow synthesis.[4]

Helical L-peptide binders to synthetic D-ERG were identified through phage display of peptides constrained into an alpha-helix by chemical crosslinking (orange).

Mirror-image D-peptides bind to natural ERG and are converted into ERG degraders by appending E3 ligands (green).

ERG Degraders Decrease ERG in Prostate CDX by >90%

Pharmacokinetics (IV)



C

Group	Plasma C_0 (μg/mL)	Plasma $t_{1/2}$ (h)	Plasma $AUC_{(0\text{-}264)}$ (h*μg/mL)	Tumor C_{24} (μg/mg)	Tumor $AUC_{(24\text{-}264)}$ (h*μg/mg)
Helicon 9, 15 mg/kg q4dx2	75.5	122	1459	6.66	1202
Helicon 9, 15 mg/kg q7dx2	71.1	69.8	1490	4.63	1040
Helicon 9, 7.5 mg/kg q4dx2	88.6	73.5	947	3.60	541
nE3 Helicon 9, 15 mg/kg q4dx2	160	NR, Rsq <0.90	1797	6.45	1126

Pharmacodynamics

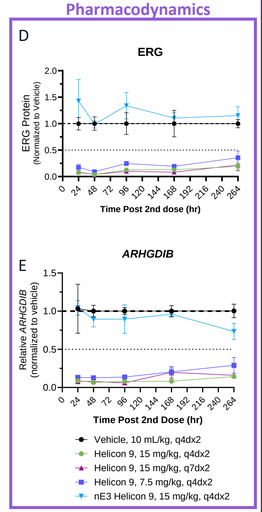


Fig. 3: Helicon 9 administered intravenously distributes to VCaP xenografts and dose-dependently degrades ERG. (A-C) PK in plasma and tumor of NSG mice with VCaP CDX tumors. Helicon 9 is 0.21% free in mouse plasma. (D) PD of ERG levels in tumor as measured by Western Blot, normalized to vinculin and matched vehicle control. (E) qPCR of ERG target gene *ARHGDIB*, normalized to *ACTB* and vehicle at each timepoint. N = 3 mice/ timepoint. Data presented as mean ± S.D.

Conclusions

- We show a first-in-class ERG degrader, demonstrating Helicon™ peptide target engagement and E3-dependent degradation *in vitro*. *In vivo*, over 90% of tumor ERG is degraded with corresponding inhibition of its target gene expression until 11 days post-dosing. In three relevant prostate models, ERG degradation correlates with TGI.
- Degradation of ERG results in downstream target gene expression loss as well as cell cycle arrest. In VCaP CDX and a PDX mCRPC model, inhibition of Myc-dependent cell cycle gene expression correlates with efficacy and consistent inhibition of cell cycle is observed in VCaP cells *in vitro*.
- Our data supports the hypothesis that degrading ERG in ERG⁺ mCRPC is an effective therapy for these patients with unmet need.

References

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4. Callahan, A.J., Gandhesiri, S., Travaline, T.L. *et al.* Mirror-image ligand discovery enabled by single-shot fast-flow synthesis of D-proteins. *Nat Commun* **15**, 1813 (2024).