Discovery of Helicon Peptides for the Selective Degradation of the Agonist-Bound Conformation of Androgen Receptor (AR^{ON}) in Prostate Cancer

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Background:

Stabilized helical peptides (Helicons™) have been demonstrated to bind to intracellular targets at binding sites on proteins that are generally regarded as undruggable e.g., FOG-001 inhibits the b-catenin-TCF4 interaction and is currently being evaluated in the clinic in advanced solid tumors. Helicons can also be converted to degraders, resulting in targeted ubiquitin-proteosome degradation of proteins that play key driver roles in tumorigenesis.

One such driver is the androgen receptor (AR), which remains highly relevant in the initiation and progression of prostate tumorigenesis. Currently approved agents and the majority of those in development bind to the inactive conformation of AR that is not androgen bound (AR^{OFF}). This mechanism serves to prevent the activation of the protein, rather than targeting it in the active, agonist-bound conformation that is responsible for driving transcription and tumorigenesis. Tumors that retain AR pathway dependence while treated with these inhibitors respond with adaptive activation of AR signaling, which in turn is associated with relapse and disease progression.

Methods:

To address the limitations of current AR^{OFF} agents, we developed Helicon peptide degraders that are designed to selectively target the agonist-bound, transcriptionally active conformation of AR (AR^{ON}). AR^{ON} degraders are expected to degrade AR regardless of mutations in the androgen binding pocket and work better in AR-amplified cells compared to AR^{OFF} agents as only a fraction of total AR is in the ON state. Hence, both major mechanisms of resistance to current AR antagonists are expected to be addressed by AR^{ON} degraders. Using a combination of *de novo* computational and structure-based molecular design, we discovered compounds that bind to the AR ligand-binding domain (LBD) in its agonist-bound conformation, at a site distinct from the ligand binding pocket targeted by AR^{OFF} agents.

Results:

AR^{ON} Helicon binders were engineered as E3-specific proteasome-mediated degraders exhibiting potent ternary complex formation, and selective degradation of AR in prostate cancer cell lines. In these models, degradation of AR leads to potent suppression of transcription and proliferation. Notably, the AR^{ON} degraders remained efficacious in low-androgen conditions where only a minority of AR exists in the agonist-bound state, suggesting that this agonist-bound pool may be critical for maintaining proliferation. Importantly, the distinct binding sites of AR^{ON} Helicons versus existing AR^{OFF} inhibitors and degraders predicts that combining these agents will allow more complete inhibition of AR. Indeed, combination with

standard-of-care inhibitors such as enzalutamide revealed additive anti-proliferative effects, likely stemming from a more comprehensive blockade of both AR^{ON} and AR^{OFF} pools.

Conclusion:

These findings demonstrate that selective degradation of the AR^{ON} pool has the potential to provide a mechanistically novel and therapeutically promising strategy to suppress AR-dependent tumorigenesis across androgen states, including models of castration resistance.

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Conflicts of Interest Disclosure Statement:

All authors are employees of Parabilis Medicines Inc.