Development of Next Generation Galeterone Analogs (NGGAs) for Prostate Cancer Therapy

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Background: We recently developed clinical candidate galeterone (gal) which disrupts AR signaling via multiple mechanisms of action. Gal progressed into pivotal Phase III clinical trial in patients with metastatic castration-resistant PC (mCRPC) whose tumor cells express splice variant AR-V7 (ARMOR3-SV). However, the recent termination of this trial and the required 2550 mg/day high therapeutic dose of gal underscores the need to further systematic refinements to enable development of the next generation gal analogs (NGGAs) with enhanced efficacies and high therapeutic indices at low dose-administration expected to result in safer, more effective treatments across all stages/forms of PC. This study was designed to determine the therapeutic potential of lead NGGAs that simultaneously target both AR/AR-V7 and Mnk-eIF4E for the treatment of all forms of PC.

Methods: Using medicinal chemistry strategies, we designed and synthesized novel next generation galeterone analogs (NGGAs) with metabolically stable chemical moieties tethered at C-3 of gal. The effects of lead NGGAs on AR/AR-V7 and Mnk1/2 were evaluated in human PC LNCaP, CWR22Rv1 and drug-resistant cell lines. The antiproliferative effects of gal/NGGAs alone or in combination with PC standard of care drugs were assessed using a variety PC cell lines *in vitro*. Toxicity and pharmacokinetics studies were conducted in mice. The antitumor activities, including inhibition of AR/AR-V7, Mnk-eIF4E and mTORC1/4E-BP1/p70SK6 signaling, were investigated using human PC cell lines and cell line derived xenografts (CDX) tumors *in vivo*.

Results: We discovered that gal and its new more efficacious analogs (VNPP414 and VNPP433-3β) also effectively target oncogenic eukaryotic protein translation, via modulation of Mnk-eIF4E axis. These compounds also suppress oncogenic peIF4E via degradation of Mnk1 and 2, and as such, are also referred to as Mnk degrading agents (MNKDAs). We demonstrated that, gal and our new lead NGGAs degraded AR/AR-Vs and Mnk1/2, inhibited PSA synthesis and secretion, blocked cell cycle progression and growth of human PC cells in culture, induced apoptosis, and inhibited cell migration, invasion, and putative stem cell markers and reversed the expression of epithelial-to-mesenchymal transition (EMT), suggesting a direct inhibitory effect on the neoplastic process. In addition, the new NGGAs (alone and in combination) also inhibited the growth of gal-, enzalutamide-, docetaxel-, and mitoxantrone-resistant human PC cell lines. Importantly, initial *in vivo* testing showed that VNPP433-3β (at 7.53-fold lower equimolar dose than gal) markedly suppressed (84% vs. gal, 47%; $p < 0.01$) the growth of castration-resistant CWR22Rv1 xenograft tumors, with no apparent host toxicity.

Conclusions: In summary, our findings show that targeting AR/AR-V7 and Mnk1/2 for degradation represents an effective therapeutic strategy for PC/CRPC treatment and supports further development of VNPP433-3β towards clinical investigation.

Conflict of Interest: VCON, PP and FNM are co-inventors on pending patents of the NGGAs. There are no conflicts of interest for the other co-authors.

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