Early Development of a KISS1/GPR54 Receptor Agonist for Prostate Cancer

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Introduction

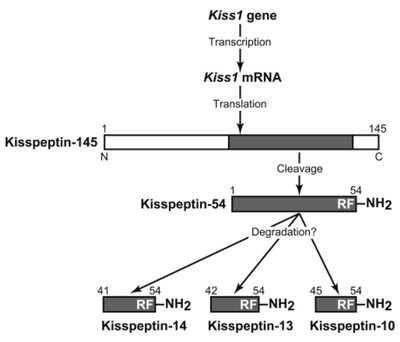


Figure 1: Products of the KISS1 gene.

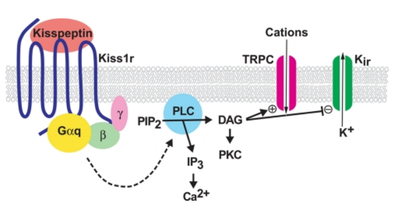


Figure 2: Proposed mechanism of neuronal depolarization by kisspeptin binding to its receptor.

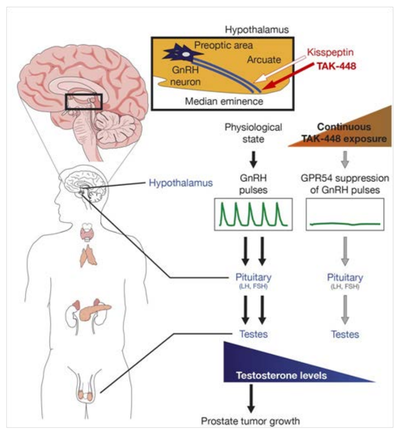
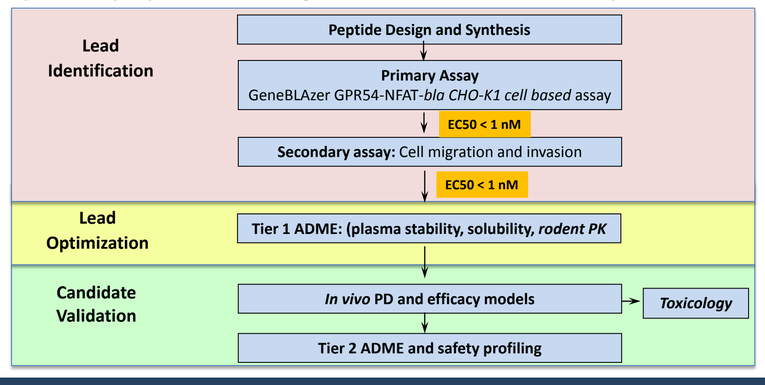


Figure 3: Effect of KISS1R agonists (e.g. TAK-448) on the Hypothalamic-Pituitary-Gonadal (HPG) axis.

- Kisspeptin is a C-terminal amidated 54 amino acid peptide first isolated from human placental tissue. It functionally interacts with the orphan G-protein-coupled receptor KISS1R (=GPR54), which is expressed throughout the central nervous system, endocrine, and gonadal tissues. Kisspeptin functionally stimulates the release of gonadotropin-releasing hormone (GnRH) from hypothalamic GnRH neurons.[1] In turn, GnRH activates pituitary release of Luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the circulation to regulate testicular testosterone levels.
- Compared to the full-length metastin protein, the N-terminally truncated peptide metastin (45–54) has 3–10 times higher receptor affinity and enhanced ability to increase intracellular Ca^{2+} concentration, which is essential for activation of protein kinases-dependent intracellular signaling critical for reproduction and cell migration.[2]
- KISS1R receptor agonists and antagonists have been explored to investigate the biology of targeting the KISS1 receptor. Reported metastin (45–54) analogues with higher agonist activity and improved metabolic stability suppress plasma testosterone in male rats with continuous subcutaneous administration.[3] In addition, a KISS1R agonist has reached phase II clinical

Research Rational and Objectives

- Continuous subcutaneous administration of KISS1R agonists induces a transient increase in plasma testosterone, followed by sustained (up to 4 weeks) reduction of plasma testosterone to castrate levels in a manner that is more rapid and profound than those induced by the GnRH agonist analogue leuprolide.[3]
- Hormonal therapy involving luteinizing hormone (LH)-releasing hormone agonists (LHRHs) is widely used as a systemic treatment for prostate cancer (recommended in the European Association of Urology guidelines on prostate cancer for use in patients with locally advanced and metastatic disease)[5],
- We sought to design novel peptides with increased activity on KISS1R (that regulates GnRH release), significantly enhanced plasma stability over previously reported KISS1R agonists, and show *in vivo* efficacy.



Materials and Methods

Peptide design:
Lead peptides were 6 - 9 amino acids in length and were designed and optimized using a combination of unnatural amino acid replacements, novel peptide backbone modifications, architecture changes, N-terminal capping, C-terminal capping and/or conjugation strategies.

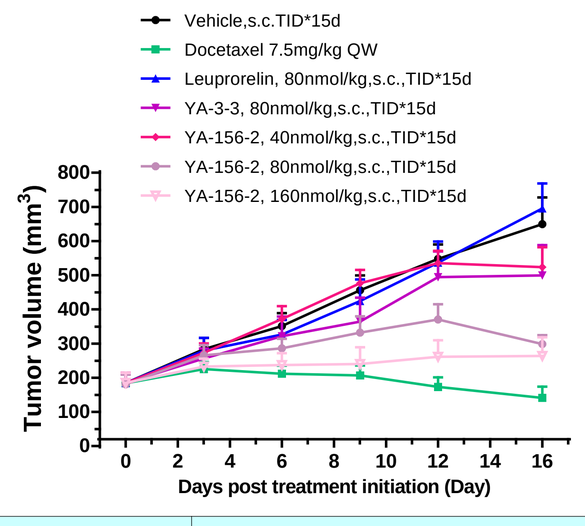
Peptide synthesis:
All peptides and non-commercially available building blocks were synthesized at ChemPartner Shanghai. Peptides were synthesized using CTC or MBHA resin. Couplings were performed with 3 equiv. of amino acid derivative / coupling reagent / base or additive (5 equiv.). Fmoc-deprotection was carried out using 20 % piperidine in DMF. Peptides were cleaved from the resin using TFA cocktail. Peptides were purified by reverse-phase HPLC (RP-HPLC). Purity QC requirement was ≥ 95% as estimated by RP-HPLC and identity was confirmed by mass spectrometric analysis.

YA-156-2 Lead Compound Summary

Study	Result	Comments
Primary Assay	EC50 = 0.33 nM	Beta lactamase reporter assay with CHO-K1 cell line expressing hKiSS1R
Secondary Assay	IC50 = 0.008 nM	Cell migration assay ; GPR54 CHO cells
Plasma stability (Rat, mouse, human)	T1/2 = 44.38 hr; 28.84 hr; 115.80 hr	
Plasma protein binding (fraction unbound)	0.6 % unbound	
Cyp Inhibition	> 10 μM	
PAMPA; Log D	LogPe = -8.86; Log D = 3.42	LogPe value <-5 shows low permeability
HLM metabolic stability	T1/2 = 21.83 min; Cl = 79.64 mL/min/kg)	Prone to liver metabolism
Solubility of TFA salt form (0.9% NaCl; 5% glucose; water)	0.63 mg/mL; 21.17 mg/mL; 27.0 mg/mL	
Mouse PK	T1/2 = 2.99 hr; CL = 0.0688 L/h/kg; urine fraction 0.167	IV dose at 1 mg/kg in male C57BL6 mice; low kidney excretion
In Vivo Efficacy	TGI = 75% w/80 nmol/kg TID and TGI = 85% w/160 nmol/kgTID ; significant tumor weight reduction	Good anti-tumor efficacy with 80 nmol/kg and 160 nmol/kg TID dosing in s.c. x1LnCaP mouse efficacy model

YA-156-2 Reduces Tumor Growth

Tumor Volume change of the mice in treatment of x1LNCaP s.c xenografts (BALB/c nude, n=8) (2017/12)

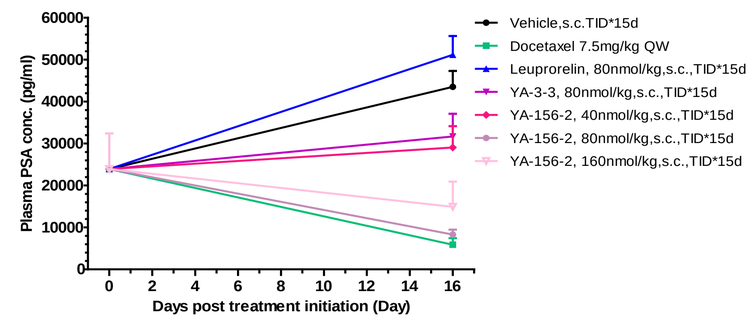


Group	TGI(100%)				
	D3	D6	D9	D12	D16
1 vehicle **saline**	-	-	-	-	-
2 Docetaxel 7.5mg/kg, QW	58.06%	83.49%	91.50%*	102.84%	109.23%
3 Leuprorelin 80nmol/kg, TID	5.05%	15.03%	11.83%	3.24%	-9.72%
4 YA-3-3 80nmol/kg, TID	28.50%	18.65%	34.12%	15.06%	32.47%
5 YA-156-2 40nmol/kg, TID	11.23%	-11.98%	-7.37%	3.60%	27.20%
6 YA-156-2 80nmol/kg, TID	14.70%	37.12%	44.50%	45.50%	74.69%
7 YA-156-2 160nmol/kg, TID	52.26%	68.76%	79.71%	79.04%	83.09%

* Numbers labeled in red are statistically significant

YA-156-2 Reduces Plasma PSA levels

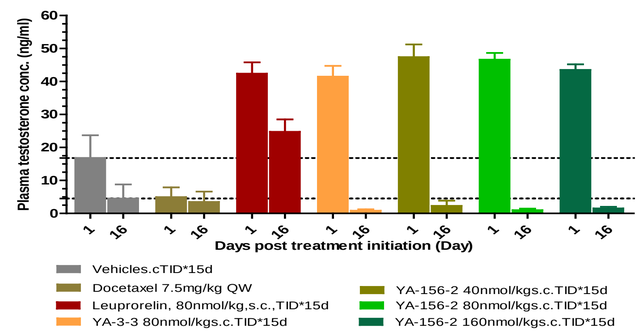
Plasma PSA level in the 5th efficacy study on x1LNCaP s.c xenograft model (CPB-P15-5602, 201712)



The plasma samples on day 0 were collected from ungrouped mice.
The 11 groups shared the data on day 0 as base level.

YA-156-2 Reduces Testosterone

Plasma testosterone level in the 5th efficacy study on x1LNCaP s.c xenograft model (CPB-P15-5602, 201712)
(at **4hrs** post the first dose on D1 and last dose on D16)



On Day 16, Plasma testosterone level decreased after its Day1 rise with YA-3 and YA-156 treatment, suggesting functional target engagement of KISS1 receptor.

In Vivo Data Summary

- YA-156-2 at 80 and 160 nmol/kg TID treatment showed significant anti-tumor efficacy in the x1LNCaP s.c xenograft tumors, with TGI values of 74.69% and 84.96% on Day 16. The reference compound, TAK-448, was less effective compared with YA-156-2 at equal molar dosage.
- At Day 16 post treatment, plasma PSA levels in YA-156-2 (80 nmol/kg and 160 nmol/kg) treated groups decreased significantly, suggesting that YA-156 is more efficacious than TAK-448 in treated groups at the same molar dosage.
- Plasma testosterone level was significantly decreased by TAK-448 and YA-156-2 after 16 days of treatment (following their initial rise on Day 1, as expected with KISS1R target engagement).
- YA-156-2 plasma exposure correlates well with its effect of inhibition on PSA and testosterone showing good PD/PK correlation.

Conclusion

- Kisspeptin is a member of the RFamide neuropeptide family that is implicated in gonadotropin secretion. KISS1 expression in the brain has a prognostic relevance in prostate cancer. KISS1 expression levels correlate with invasiveness in several human cancers.
- Low or lost KISS1 expression is associated with higher tumor grade, increased metastatic potential, and a poor prognosis; therefore, a receptor agonist may have therapeutic significance.
- Chronic administration of kisspeptin analogues (such as YA-156-2 in this study) may hold promise as a novel therapeutic approach in hormone-related diseases such as prostate cancer. Further studies are in progress to evaluate the lead KISS1R agonist peptides in multiple indications.

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