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KISS1R Antagonism as a Promising Anti-Cancer Approach: An Overview on Function, Molecular Mechanisms and Therapeutic Potential

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Abstract

Herein, we explore the Kisspeptin 1 Receptor (KISS1R) as a potential therapeutic target for the restoration of drug sensitivity in Triple-Negative Breast Cancer (TNBC) clinical settings. Recent functional studies of KISS1R have revealed that the receptor plays a pro-metastatic role in TNBC. By the promotion of multidrug resistance proteins, KISS1R has been shown to regulate TNBC drug resistance. Chemotherapy remains the standard clinical procedure for the treatment of TNBC, however the ability of cancer cells to become resistant to chemotherapy continues to be a major obstacle. Thus, there is an intense interest around the development of novel, synthetic agents, which may synergise with current breast cancer therapeutic options. In this review, we aim to highlight the pro-metastatic mechanism of KP/KISS1R signalling and the therapeutic potential surrounding the receptor's attenuation. In addition, we explore the modes of KISS1R inhibition by drawing upon peptidic based antagonists, agonists and small molecule hit compounds.

Keywords: Triple-negative breast cancer; Chemoresistance; Kisspeptin; Kisspeptin 1 receptor; Antagonists.

Introduction

Global cancer statistics showed that there were approximately 2.2 million new cases of female Breast Cancer (BCa) and 684,996 associated deaths in 2020 [1]. BCa is the most common neoplasm among women and the leading cause of cancer in related mortalities [1]. The hypernym BCa describes a diverse heterogeneous tumour group with a highly variable treatment response. Interestingly, TNBC, a subtype of BCa, accounts for approximately 1 in 5 cases of BCa but is disproportionately responsible for all BCa-associated deaths [2]. Clinically, TNBC is defined by its lack of expression of the ER, PR, and HER2 receptors which are used as essential indicators to determine the optimal therapeutic protocols [3]. As reviewed by Yadav et al. patients with hormone dependent positive BCa benefit from chemo-targeted and hormone therapy,

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whereas treatment for TNBCs are currently limited to surgery and conventional cytotoxic agents [4].

In comparison to other BCa subtypes, TNBC tumours are frequently larger, less differentiated, and have a higher incidence of distant metastases, proliferation and recurrence [2,5]. TNBC is difficult to manage, with the disease characterised by poor prognosis due to limited treatment options and subsequent drug resistance [6]. Notwithstanding the current advances in cancer chemotherapy, the ability of cancer cells to become chemo-resistant continues to be a major obstacle in treating TNBC patients - especially in the metastatic setting [6]. Numerous mechanisms can lead to the development of chemoresistance, and the kisspeptin/kisspeptin 1 receptor (KP/KISS1R) is a recognised system in the investigation of TNBC drug-resistance (Figure 2) [7]. Thereby, there is an intense interest for novel agents which may synergise with current chemotherapeutic options and, consequently, attenuate the signalling potential of KISS1R. Currently, there are no small molecule KISS1R inhibitors reported against TNBC chemotherapeutic desensitisation, despite the evidence that the receptor may be a pharmaceutically attractive target for intervention against TNBC. In this paper, in addition to mapping the mechanistic pathway of KISS1/KISS1R expression, we explore the prospective implications of previously reported KISS1R antagonists and their effects in curtailing chemoresistance within TNBC.

KISS1R overview

Kisspeptin receptor 1 (KISS1R; aka GPR54, OT7T175, AXOR12) is a $G\alpha_{q/11}$-coupled G-Protein Coupled Receptor (GPCR) and a key regulator of the Hypothalamic-Pituitary-Gonadal (HPG) axis [8]. KISS1R is highly expressed in the brain, including the hypothalamus and pituitary gland as well as peripheral regions [8]. Kisspeptins (KPs), a product of the *KISS1* gene, are a group of peptide fragments that bind to and activate the KISS1R (Figure 1). The *KISS1* gene encodes a polypeptide consisting of 145 amino acids, known as the precursor peptide (KP-145) [9]. KP-145 gives rise to a secretory protein of 126 amino acids that is proteolytically cleaved into smaller fragments: KP-54 (aka Metastin), KP-14, KP-13 and KP-10 [8]. All peptide fragments bind to and activate the KISS1R with equal potency [10]. Each peptide fragment shares the same 10 terminal amino acid sequence, KP-10, the smallest fragment necessary for binding to and activating KISS1R [10]. The identification of the *KISS1* gene was initially championed for its anti-metastatic role, and *KISS1* activated KISS1R signalling has been shown to suppress cancer metastasis by inhibiting cancer cell migration and invasion [11]. Downregulation of *KISS1* was clinically established with a worse prognosis among those diagnosed with melanoma [11], colorectal [12], prostate [13], ovarian [14], lung [15], and bladder cancers [16]. Paradoxically, elevated KISS1R signalling appears to play a pro-metastatic role in some cancers such as breast and liver cancer [17,18]. Successive findings have likened the metastases of TNBC basal-like malignancies, and subsequent drug resistance, with the overexpression of *KISS1* [7]. As such, *KISS1* expression might be a useful predictive biomarker in medical outcomes.

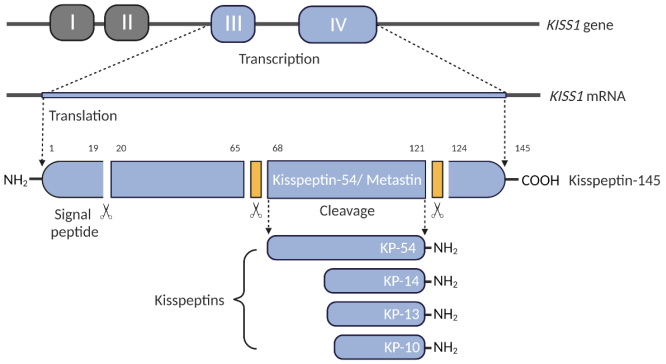

Figure 1: Kisspeptins (KPs) formation. The KISS1 gene is located in the long-arm of chromosome 1 (1q32-q41) and encodes a 145 amino-acid sequence (KP-145), which is subsequently cleaved into smaller C-terminal fragments: KP-54, KP-14, KP-13 and KP-10 [8]. All KP fragments possess biological activity and are endogenous ligands to the KISS1R.

Role of KISS1R in TNBC development

KISS1R signals through a plethora of diverse molecular mechanisms that have the potential to regulate the processes navigating clinical diagnoses of TNBCs. The underlying mechanisms by which the KP/KISS1R system regulates tumourigenesis in TNBC has been reviewed (Figures 2). Successive findings have likened the metastases of TNBC malignancies, and consequent drug resistance, with the overexpression of KISS1R.

To understand the mechanisms by which KISS1R stimulates BCa metastasis, Goertzen et al. examined the effects of KISS1R on invadopodia formation [19]. Goertzen et al. showed that G-protein dependent KISS1R promotes TNBC cell invasion by regulating the formation of invadopodia which are actin-rich protrusions that facilitate extracellular matrix degradation and cancer cell invasion [19]. Goertzen also demonstrated that KP-10 induced KISS1R signalling which, in turn, induced two key regulators of actin dynamics during invadopodia maturation - the dephosphorylation of cortactin and cofilin - through the adapter protein β-arrestin2 [19]. Treatment with the KISS1R ligand KP-10 in highly invasive TNBC cell lines (MDA-MB-231 and Hs578T) increased gelatin degradation by TNBC cells, indicating enhanced invadopodia formation and activity. KP-10 stimulation led to colocalisation of the invadopodia markers F-actin and cortactin at sites of gelatin degradation [19]. Accordingly, Blake and co-workers, demonstrated that overexpression of KISS1R, in SKBR3 FLAKISS1R cells, also increased actin stress fibre formation [7]. KISS1R signalling also stimulated phosphorylation of MT1-MMP, a membrane-type matrix metalloprotease that plays a critical role in ECM degradation at invadopodia [19]. Goertzen et al. showed that KISS1R localised to invadopodia structures in TNBC cells upon KP-10 stimulation where it colocalised with cortactin, MT1-MMP, and the KISS1R interacting protein β-arrestin2 [19]. Notably, β-arrestin2 is a key mediator of KISS1R's effects on invadopodia, for depletion of β-arrestin2 inhibited KP-10 induced invadopodia formation and the dephosphorylation of cortactin and cofilin. Moreover, it was demonstrated that KISS1R-stimlated invadopodia formation occurred through β-arrestin2 and ERK1/2 dependent pathways. Inhibition of the ERK1/2 pathway blocked KP-10 induced invadopodia formation and MT1-MMP phosphorylation [19].

Pampillo et al. demonstrated that β-arrestin2, in addition to GRK2, are critical regulators of early KISS1R signalling events, including desensitisation and coupling to the ERK1/2 MAPK pathway [20]. The study used HEK-293 cells to investigate the molecular regulation of KISS1R as well as genetically modified MDA-MB-231 cells, expressing lower levels of β-arrestin2 compared to controls [20]. GRK2 was found to stimulate the desensitisation of KISS1R in HEK 293 cells, and this occurred through a phosphorylation-independent mechanism [20]. In the MDA-MB-231 cells, β-arrestin2 was required for KISS1R activation of the ERK1/2 signalling pathway, suggesting β-arrestin2 mediates this aspect of KISS1R signalling in this breast cancer cell line [20].

Cvetković et al. investigated the role of kisspeptins and their cognate receptor, KISS1R, in BCa metastasis [21]. The study demonstrated that KP-10 stimulation induces invasion of ER-negative MDA-MB-231 breast cancer cells *via* transactivation of the Epidermal Growth Factor Receptor (EGFR, aka HER1). The paper reports that exogenous expression of KISS1R in ER-negative SKBR3 breast cancer cells was sufficient to trigger invasion and induce extravasation *in vivo*. In contrast, KP-10 failed to transactivate the EGFR or stimulate invasiveness in ER-positive MCF7 and T47D breast cancer cells, thus, suggesting that ER negatively regulates KISS1R dependent breast cancer cell migration, invasion, and EGFR transactivation [21]. IQGAP1, an actin cytoskeletal binding partner of KISS1R, was shown to regulate EGFR transactivation in breast cancer. Cvetković and co-workers found that KP-10 induced EGFR phosphorylation was inhibited in IQGAP1 depleted cells (MDA-MB-231), suggesting that IQGAP1 is required for KISS1R induced EGFR activation [21]. It was demonstrated that KISS1R colocalises with IQGAP1 - in lamellipodia - at the leading edge of motile cells, indicating a potential role for IQGAP1 in transducing KISS1R signals to the cytoskeleton. The signalling of KISS1R can promote an invasive phenotype and was found to be regulated by the ER status of the breast epithelial cells. KISS1R expression was negatively regulated by E2 signalling through ER [21]. Overall, the findings strongly indicate that KISS1R mediated EGFR transactivation in breast cancer cells.

Blake et al. assessed the effect of KISS1R overexpression in TNBC cell lines, MDA-MB-231 and Hs578T, as well as non-malignant MCF10A and breast cancer SKBR3 cells [7]. The results demonstrated that KISS1R overexpression promoted Epithelial-Mesenchymal Transition (EMT)-like events, leading to increased tumour cell migration and invasion. Moreover, the overexpression of KISS1R in SKBR3 cells stimulated cell motility and scratch closure, independent of increased cell proliferation. Notably it was observed that overexpression of KISS1R in SKBR3 cells led to significant increases in the expression of pro-survival molecules AXL, AKT, ERK and the anti-apoptotic protein survivin [7]. Stimulation of TNBC MDA-MB-231 and Hs578T, which endogenously express KISS1R, with KP-10 led to an upregulation in AXL protein and mRNA expression. Conversely, ER-positive breast cancer cell lines, T47D and MCF7, which have minimal KISS1R expression, do not express AXL. Blake *et al*. found a significant increase in the expression of AXL in TNBC patients, which positively correlated with *KISS1* expression [7]. Immunofluorescence analysis of patient's tumours revealed punctate distribution of *KISS1*, robust localisation of KISS1R and AXL in tumour cells, and partial co-localisation in tumours and stromal cells. Depletion of KISS1R signalling inhibited metastatic TNBC cell migration, invasion, and malignant transformation. The findings suggest that *KISS1*, KISS1R and AXL are upregulated in invasive breast cancer cells, compared to non-invasive or weakly invasive breast cancer cells [7].

Dragan et al. identified the role of KISS1R as a novel regulator of TNBC metabolism and metastasis [22]. The study demonstrated that KISS1R overexpression changes the metabolic profile of TNBC cells, making them more dependent on glutamine for survival [22]. Metabolic analysis of human primary tumour biopsies revealed elevated levels of glutamate in tumours compared to ER-positive tumours. Dragan *et al.* also highlighted the overexpression of the MYC oncogene in TNBC, which promotes EMT and stimulates glutoaminolysis, leading to glutamine as a bioenergetic substate [22]. As such, KISS1R induces the expression of c-Myc to promote a glutamine-dependent phenotype and increase glutamine uptake in tumours. KISS1R induced c-Myc expression was dependent on ERK1/2 signalling. Inhibition of glutaminase or knockdown of c-Myc attenuated the growth and migration of KISS1R-overexpressing cells, demonstrating the importance of KISS1R-mediated glutamine metabolism for TNBC tumour progress [22].

The findings have important implications for understanding the molecular mechanisms underlying KISS1R function and for the development of potential therapeutic strategies targeting this receptor. Targeting the KP/KISS1R signalling axis could have therapeutic potential for TNBC.

Role of KISS1R in the acquisition of chemoresistance in TNBC

There is some evidence to suggest, as established by Huijbregts et al. and, that regulation of 17β-estradiol (E2) mediates the expression of *KISS1* in MDA-MB-231 cells [23]. Thus, the depletion of E2, brought upon by chemotherapy, supresses the downregulation of *KISS1* expression [24]. Treatment with E2 was shown to dissuade *KISS1* mRNA as well as KISS1R protein levels. It should be noted that higher levels of KP/KISS1R are expressed, clinically, upon exposure to chemotherapy. Levels of mRNA KISS1 are upregulated in TNBC cells which has been implicated in the increased protein/ KISS1R signalling as a factor that may induce breast cancer progression and chemoresistance [24].

IQGAP1 is a multidomain protein that scaffolds multiple signalling pathways [21]. The scaffold role of IQGAP1 is emerging as key in the P13K-AKT pathway [6]. In agreement, Pan et al. showed that overexpression of IQGAP1 enhances AKT activation [25]. Studies have demonstrated that AKT is the key signal transduction protein that phosphorylates a triad of downstream effectors, namely, Nrf2, NF-κB and mTOR [6]. The P13K-AKT-mTOR (PAM), NF-κB and KEAP1-Nrf2 pathways enhanced chemotherapeutic drug resistance *via BCRP* transcription, thus, inducing the ATP-Binding Cassette (ABC) transporter family [6].

Blake et al. demonstrated that KISS1R signalling promotes the expression of the drug efflux transporter, ABC-G member 2 (ABCG2, BCRP), in metastatic TNBC cell lines [7]. KISS1R expression led to a reduction in the cellular accumulation of doxorubicin, indicating a potential mechanism of drug resistance. Notably, the increase in expression of KISS1R was correlated with a heightened expression levels of BCRP mRNA and protein compared to control cells, while pharmacological inhibition of KISS1R decreased BCRP mRNA levels. Additionally, cells expressing KISS1R in TNBC tu-

mour biopsies also expressed BCRP, reinforcing the association that KISS1R signalling promotes drug resistance by upregulating BCRP expression [7]. Blake et al. also identified AXL as a signalling partner in the KISS1R pathway, leading to increased signalling *via* AKT, and possibly regulating BCRP expression *via* an AXL/EMT-dependent mechanism [7]. The significant expression of AXL in TNBC patients, which positively correlated with *KISS1* expression, may confer drug resistance in TNBC patients, as AXL overexpression was linked to poor TNBC patient prognosis, and has been shown to promote breast cancer drug resistance [7].

KISS1R expression plays a cardinal role in breast cancer resistance to chemotherapy. Drug resistance and tumourigenesis in TNBC, *via* KISS1R, can occur through various mechanisms (efflux drug transporter, BCRP, promotion of AXL expression and activity, PAM, NF-κB and KEAP1-Nrf2 pathways) including altered expression and activation of KISS1R signalling, interaction with other signalling pathways, and genetic alteration affecting KISS1R function.

Antagonistic agents: inhibition of KISS1R expression

Attenuation of KISS1R signalling *via* peptidic antagonists

Several preclinical studies have indicated that KISS1R inhibition or KISS1R stimulation can be reliant on its cognate KPs. KP analogues have been synthesised based on the structure of human KP-10, as this is the smallest fragment needed to bind to and activate KISS1R. In general, the antagonistic efficacy of KP-10 analogues have been assessed upon their ability to regulate KP-stimulated PLC-β mediated Inositol Phosphate (IP) and intracellular calcium release [26]. KISS1R's capacity to signal *via* G-protein independent pathways attracts many secondary effectors (Figure 2). In consequence, recent reports assess an antagonist's value upon its ability to inhibit downstream mediators [7,21,26]. Systematic substitution of amino-acids in KP-10, established by Roseweir et al., elucidated the functional potency of peptide-based KISS1R antagonists [26]. Four effective antagonists were created, whereby, valuable information regarding structural-activity relationship-derived pharmacophores and a consensus model for KP-10 anta-

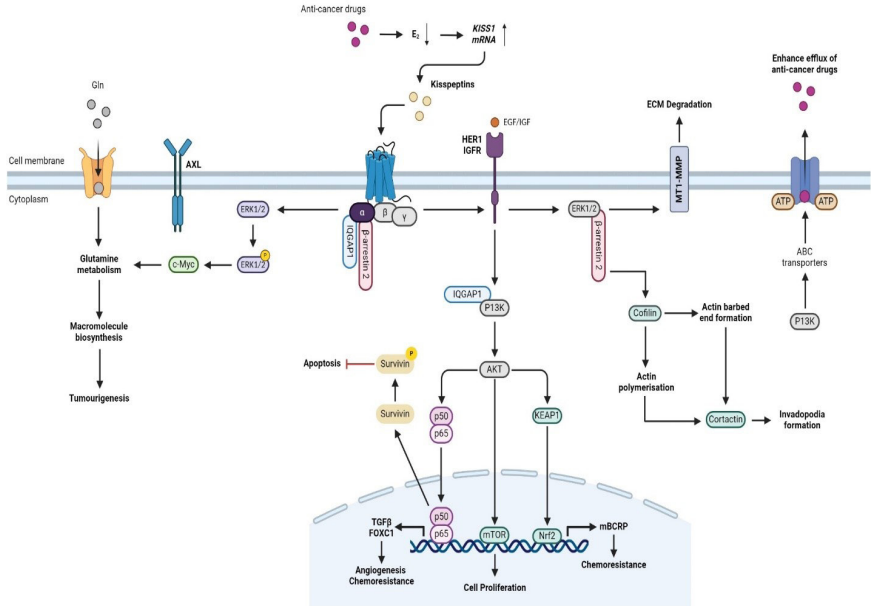

Figure 2: Schematic diagram highlighting vital KISS1R signalling pathways within TNBC. The regulation of oestradiol (E2), *via* chemotherapy, mediates the expression of *KISS1.* KISS1R can signal *via* a G-protein independent pathway to activate IQGAP1, β-arrestin2 and HER1. KISS1R signalling, *via* β-arrestin2, regulates Extracellular Matrix (ECM) degradation, and the phosphorylation levels of invadopodia proteins cofilin and cortactin. The KISS1R signalling mechanism *via* IQGAP1 regulates the P13K-AKT-mTOR (PAM), and KEAP1-Nrf2 pathways. Subsequent transcription of target gene, *BCRP*, enhance the efflux of chemotherapeutics *via* ABC transporters, including BCRP. Moreover, KISS1R expression is implicated in the upregulation of the pro-survival protein survivin and tumourigenesis *via* c-Myc.

Table 1: Reported KISS1R ligands: (i) amino-acid sequences of four key KISS1R peptidic antagonists; (ii) fluorescently labelled KP agonists.

	Name	Sequence	Reference
Agonist	Kisspeptin-10	H-Tyr^1-Asn^2-Trp^3-Asn^4-Ser^5-Phe^6-Gly^7-Leu^8-Arg^9-Phe^{10}-NH_2	[10]
	TAK-448	Ac-d-Tyr^1-Hyp^2-Asn^3-Thr^4-Phe^5-azaGly^6-Leu^7-Arg(Me)^8-Trp^9-NH_2	[27,28]
Antagonist	Peptide-230	Ac-d-Tyr^1-Asn^2-Trp^3-Asn^4-Gly^5-Phe^6-Gly^7-Leu^8-Arg^9-Phe^{10}-NH_2	[26]
	Peptide-234	Ac-d-Ala^1-Asn^2-Trp^3-Asn^4-Gly^5-Phe^6-Gly^7-d-Trp^8-Arg^9-Phe^{10}-NH_2	
	Peptide-273	Ac-d-Ala^1-Asn^2-Trp^3-Asn^4-d-Ser^5-Phe^6-Gly^7-d-Trp^8-Arg^9-Phe^{10}-NH_2	
	Peptide-276	Ac-d-Ala^1-Asn^2-Trp^3-Asn^4-Gly^5-Phe^6-Gly^7 -Leu^8-Arg^9-Phe^{10}-NH_2	

gonism were attained (Figure 3) [26]. Of those four antagonists, P-234 was the most efficacious, inhibiting KP-stimulated calcium release with an IC_{50} of 10 nM). In comparison, P-273 had a lower binding affinity than the decamer, KP-10, and P-230 displayed weak peptide-induced IP inhibition. Notwithstanding a complete reduction of KISS1R Ca^{2+} mobilisation, P-276 exhibited an idiosyncratic agonistic effect when administered at higher concentrations. Thus, *in vitro* structure-activity data demonstrates that a KP amino-acid substitution from Tyr^1, Ser^5 and Leu^8 to D-Ala^1, Gly^5 and D-Trp^8 is critical for KISS1R antagonism. The data also purports that Asn^2-Trp^2, Phe^6 and Arg^9-Phe^{10} are the five essential amino acid residues involved in receptor binding [26].

Based on its structure-activity profile and therapeutic potential, P-234 has become the *de-facto* agent in mediating KP/KISS1R expression in various *in vivo* and *in vitro* systems [7,19,26]. In TNBC, the role of P-234/KISS1R signalling has not been fully elucidated, although studies have confirmed how inhibition of endogenously expressed KISS1R cells significantly attenuate TNBC tumourigenesis.

As demonstrated by Goertzen et al., KISS1R stimulates invadopodia formation *via* the co-localisation of actin and cortactin [19]. Moreover, they observed how down-stream mediators – namely, β-arrestin2 and ERK1/2 - are implicated in MT1-MMP phosphorylation. Goertzen et al. showed how P-234 mediated KISS1R attenuation may impede tumour cell invasion, extra-cellular membrane degradation and metastasis. The KISS1R antagonist, P-234 inhibited KP-10 induced invadopodia formation demonstrating the specificity of KISS1R in regulating this process [19].

KISS1R's capacity to promote drug resistance, as assessed *in vitro* by Blake et al., is mediated by numerous key effectors [7]. Nonetheless, Blake's comprehensive insight in the downstream effects of KISS1R overexpression heighten the antagonistic effects of P-234. The researchers found that SKBR3FLAG-KISS1R cells displayed increased cell survival in the presence of the chemotherapy drug doxorubicin, compared to control cells [7]. When the KISS1R antagonist, P-234 was used to attenuate KISS1R activity, the dose response curve shifted to the left, indicating increased cell death, similar to the response observed in control cells. This effect was also observed in other cell lines, including MCF10A breast cells expressing KISS1R and metastatic TNBC MDA-MB0231 cells. From the data provided by Blake et al. P-234 was shown to attenuate chemotherapeutic drug desensitisation and drug efflux (BCRP; Figure 2), up-regulation of pro-survival pathways (survivin, AXL, ERK, AKT; Figure 2), increase in HER1 transactivation, and an increase in TNBC cell motility [7].

Interestingly, P-234 based KISS1R suppression is marginal compared to more profound attenuation by chronic administration of KP agonist analogues. Matsui et al. highlighted how TAK-448 (Table 1) significantly supresses HPG axis response primarily through the desensitisation of KISS1R *via* β-arrestin. Sustained stimulation of KISS1R typically affects receptor desensitisation, causing receptor loss from the cell surface [27,28]. Taken together, these results suggest that KISS1R is a regulator of TNBC progression and targeting the receptor, *via* a suitable peptidic ligand, could have therapeutic potential.

Even so, therapeutic use of the known receptor ligands, such as P-234, would be complicated because they would need to be given parenterally, and stability of peptides are problematic because KPs have been shown to degrade by the action of serum proteases [26]. As such, peptides may degrade over the course of the induction period such that the actual concentration of the antagonist is diminished across time. Hence, mimics of P-234 needs to avoid such shortcomings.

Following on from the pharmacophore model, developed by Roseweir et al. [26], we can begin to identify non-peptidic modulators of KISS1R, as potential drug candidates, as discussed below.

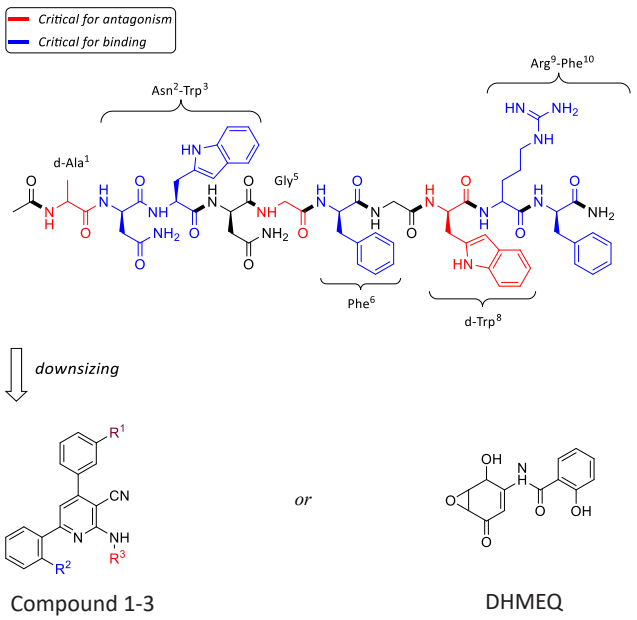

Figure 3: Downsizing of peptidic based antagonist, P-234, to 2-acylamino-4,6-diphenylpyridine or DHMEQ templates [29,31].

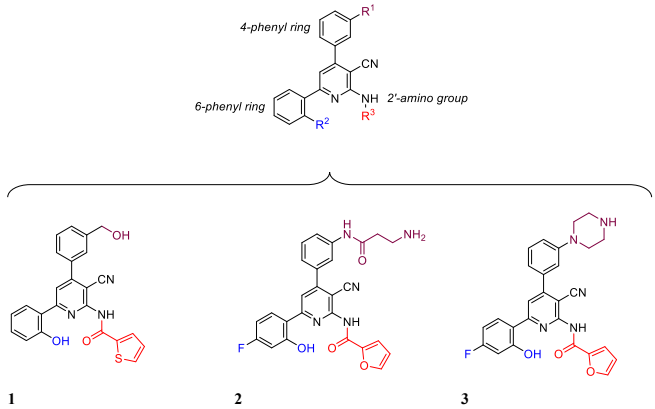

Figure 4: The 2-acylamino-4,6-diphenylpyridine template supported the design and synthesis of compound 1-3. Contrary to Kobayashi et al. this paper explores the potential of utilising the inhibition of KISS1R as a means of restoring drug sensitivity to those suffering from TNBC [29,30].

Attenuation of KISS1R signalling *via* small molecular antagonists

Kobayashi et al., identified compound 1 (Figure 4) as their most potent antagonist (58 % Ca^{2+} inhibition) of KISS1R-metastin signalling within the HPG axis *via* a high-throughput screen of their proprietary compounds [29]. They established a small molecular, non-peptide-based, antagonist based on a 2-acylamino-3-cyano-4,6-diphenylpyridine scaffold, wherefrom a structure-activity relationship of the 2-amino group, 4-phenyl and 6 phenyl rings were founded. In their investigations of this pyridine scaffold, introduction of para-substituted or ortho-substituted hydroxy acetophenones, within a four component synthetic methodology, identified that a 2'-postion hydroxy group on the 6-phenyl ring was essential for the binding to KISS1R [29]. Moreover, introduction of an electron-withdrawing group on the 4'-position of the 6-phenyl ring improved potency in Kobayashi's series. Initially, they revealed how 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives containing a primary amine on the 3'-subsituent on the 4-phenyl ring, and the presence of a 2-furoyl group, gave rise to the most active antagonist of all derivatives tested (compound 2) [29]. Thereafter, in a strategy to enhance Blood-Brain-Barrier (BBB) penetration, the primary amine on compound 2 was replaced with a cyclic amine to afford antagonist, 3 (IC_{50} of 0.93 µM) [30]. Although compound 3 showed encouraging levels of KISS1R binding affinity *in vitro*, as well as some limited activity after dosing *in vivo*, compound 3 did not antagonise calcium release to the same extent as P-234 [30].

Lin et al. examined the anti-cancer activity of the NF-κB inhibitor Dehydroxymethylepoxyquinomicin (DHMEQ) on mouse plasmacytoma SP2/0 cells [31]. To investigate the role of KISS1R, Lin et al. performed knockdown experiments in SP2/0 cells using KISS1R targeted siRNA [31]. The KISS1R knockdown was found to suppress the cellular invasion of SP2/0 cells, suggesting that KISS1R plays an essential role in the invasion of these plasmacytoma cells. Moreover, the inhibitory effect of DHMEQ on invasion was not further enhanced by KISS1R knockdown, indicating that the suppression of KISS1R expression is a key mechanism by which DHMEQ inhibits the invasive behaviour of SP2/0 cells. In addition to DHMEQ's anti-invasive effects, the study also demonstrated that DHMEQ was able to enhance the cytotoxicity of the chemotherapeutic agent melphalan in SP2/0 cells. DHMEQ synergistically increased melphalan induced apoptosis, as evidenced by elevated levels of cleaved caspase-3. Further analysis showed that DHMEQ inhibited the expression of several NF-κB dependent anti-apoptotic proteins [31].

Conclusion and future outlook

Characterised by poor survival rates, high instance of distance metastases, and limited therapeutic options, TNBC is the most aggressive form of BCa [1]. Thus, there is a particular medical interest to elucidate the molecular drivers behind TNBC chemoresistance. KISS1R signals through a plethora of diverse molecular mechanisms that have the potential to regulate the processes navigating TNBC clinical outcomes [32]. There is growing evidence to support the notion that KISS1R plays a cardinal role in the late-stage progression of malignant, solid-tumour progression. A summary of the significance of KISS1R overexpression and inhibition in TNBC is shown in Figure 5.

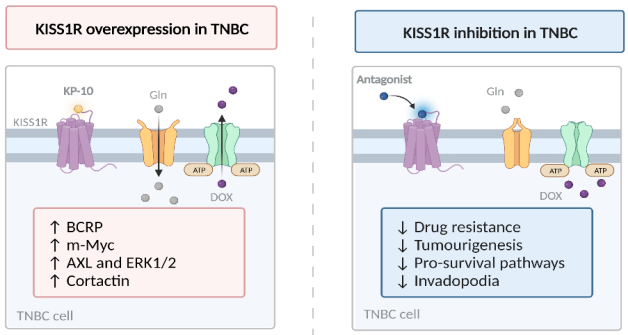

Figure 5: Significance of KISS1R overexpression and inhibition in TNBC.

While further studies are required, to expand the structure-activity relationships around families of small, non-peptidic compounds, the data outlined in this review purports that substrates such as, Compounds 1-3 and (-) DHMEQ, could feasibly be developed into alternatives to existing peptide-based KISS1R antagonists. Dissimilar to its peptide counterpart, which needs to be administered by injection, small molecule antagonists are more likely to be orally active and would therefore be more amendable to patient compliance in the clinic [29-31].

Going forward, the principal objective should be to evaluate the viability of KISS1R antagonism, as a therapeutic option in TNBC. We are highly engaged in this area and will report on our results with novel small molecule structure-activity relationships in due course [33]. Initially, more highly selective and potent KISS1R inhibitors should be developed to obtain better tolerability and anticancer efficacy. The question of whether KISS1R antagonists can reduce angiogenesis and hence stop TNBC cells spreading should also be addressed in the future [34,35]. In addition, a study around Kisspeptin regulating cell invasion and migration in endometrial cancer has been reported [36], which raises the need for an effect on cancer cell invasion and migration to be demonstrated with K1SS1R antagonists.

Secondly, optimised combination regimens, with existing chemotherapeutics (i.e., doxorubicin), need to be investigated for the best synergic effect. This will help to determine if KISS1R antagonists can effectively restore sensitivity to patients receiving established chemotherapies (TNBC often develops resistance to chemotherapy). Also, the prospect of utilising KISS1R antagonists in combination with standard chemotherapeutic agents, such as anthracyclines and taxanes, to potentially enhance the efficacy of these drugs is an enticing research area. Thirdly, TNBC is characterised by a subpopulation of cancer stem cells that contribute to recurrence and resistance to therapy. KISS1R antagonists may have the potential to inhibit the renewal and maintenance of cancer stem cells, thereby reducing the likelihood of recurrence and improving long-term survival rates. It may also be worth investigating the use of KISS1R antagonists as an adjuvant therapy post-surgery, i.e. is it possible to eliminate residual cells and reduce chance of recurrence (something that is high in TNBC patients)? Moreover, KISS1R inhibitor resistance should also be investigated along with a greater understanding of the mechanism by which TNBC cells acquire drug resistance.

Additional studies should be conducted to identify reliable biomarkers, to predict antagonistic response and to minimise any adverse effects. Finally, can KISS1R expression levels serve as a biomarker to predict response to KISS1R antagonist therapy? Patients with higher KISS1R expression might benefit more from targeted therapy, i.e. more personalised clinical treatment. *KiSS-1* expression has been reported as a potentially useful prognostic marker in gastric cancer, and the hypothesis that this could be applied to breast cancer is worthy of future study [37].

Abbreviations: ABC: ATP Binding Cassette; ABCG2: ATP Binding Cassette G2; AKT: Protein Kinase B; ATP: Adenosine 5' Triphosphate; BBB: Blood-Brain Barrier; Bca: Breast Cancer; BCRP: Breast Cancer Resistant Protein; C-Myc: Cellular Myelocytomatosis; DAG: Diacylglycerol; E2: 17β-Estradiol; ECM: Extracellular Matrix; EFG: Epidermal Growth Factor; ER: Oestrogen Receptor; ERK1/2: Extracellular Signal-Regulated Kinases 1 and 2; GDP: Guanosine Diphosphate; Gln: Glutamine; GPCR: G-Protein Coupled Receptor; HER1: Human Epidermal Growth Factor Receptor 1; HER2: Human Epidermal Growth Factor Receptor 2; HPG: Hypothalamic-Pituitary-Gonadal; IC50: Inhibitory Concentration (Half-Maximal); IP: Inositol Phosphate; IP3: Inositol Trisphosphate; IQGAP1: IQ Motif Containing Gtpase Activating Protein 1; KEAP1: Kelch-Like ECH-Associated Protein 1; KISS1R: Kisspeptin 1 Receptor; KP: Kisspeptin; MT1-MMP: Membrane Type 1 Matrix Metalloproteinase; Mtor: Mechanistic Target Of Rapamycin; Nrf2: Nuclear Factor-Erythroid Factor 2-Related Factor 2; P13K: Phosphoinositide 3-Kinases; PIP2: Phosphatidylinositol 4,5-Bisphosphate; PKC: Protein Kinase C; PLC-B: Phospholipase C-B; PR: Progesterone Receptor; TNBC: Triple-Negative Breast Cancer.

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