**Title:** New advances in menopause symptom management

**Authors:** Kanyada Koysombat (MBBS BSc)1,2, Patrick McGown (MBBS BSc)1, Sandhi Nyunt (MBBS MSc)1,2, Ali Abbara (MBBS BSc PhD)1,2, Waljit S. Dhillo (MBBS BSc PhD)1,2

*This should include the full name and address of each author, together with their email address, telephone, and fax numbers. Degrees and affiliations of each author should also be provided. If more than one author is involved, please remember to specify who the corresponding author is.*

1. Section of Investigative Medicine, Imperial College London, London, United Kingdom
2. Department of Endocrinology, Imperial College Healthcare NHS Trust, London, United Kingdom

**Word count:** 6513 including references, excluding table and figure caption.

**Language style:** British English

**Corresponding Author:**

Professor Waljit S. Dhillo

Section of Endocrinology & Investigative Medicine,

Imperial College London, 6th Floor, Commonwealth Building,

Hammersmith Hospital, Du Cane Road,

London, W12 0NN, UK.

[w.dhillo@imperial.ac.uk](mailto:w.dhillo@imperial.ac.uk)

Tel: 0207 594 3487

Fax: 0207 594 3487

**Abstract** (149/150 words)

Vasomotor symptoms (VMS) are characteristic of menopause experienced by over 75% of postmenopausal women with significant health and socioeconomic implications. Although the average duration of symptoms is seven years, 10% of women experience symptoms for more than a decade. Although menopausal hormone therapy (MHT) remains an efficacious and cost-effective treatment, its use may not be suitable in all women, such as those at an increased risk of breast cancer or gynaecological malignancy. The neurokinin B (NKB) signaling pathway, together with its intricate connection to the median preoptic nucleus (MnPO), has been postulated to provide integrated reproductive and thermoregulatory responses, with a central role in mediating postmenopausal VMS. This review describes the physiological hypothalamo-pituitary-ovary (HPO) axis, and subsequently the neuroendocrine changes that occur with menopause using evidence derived from animal and human studies. Finally, data from the latest clinical trials using novel therapeutic agents that antagonise NKB signaling are reviewed.

**Key words:** menopause, neurokinin B, neurokinin 3 receptor antagonist, hot flushes, vasomotor symptoms

**Introduction**

Menopause, derived from the Greek terms *menos* (month) and *pauso* (to cease), marks the permanent cessation of menstruation. Hormonal changes that occur during the menopausal transition, particularly the decline in levels of oestrogens secondary to depletion of ovarian follicular activity, contribute to the pathogenesis of menopause-related symptoms. Vasomotor symptoms (VMS), a collective term to describe hot flushes / hot flashes and sweats, are considered as hallmark symptoms of the menopausal transition experienced by over three quarters of women and severely by 25% of women (1). The onset of menopause-related symptoms is highly variable and can begin well before menstrual irregularities, termed ‘perimenopause’ (2). The average duration of symptoms is 7 years (3), but symptoms last longer in a third of women, with 1 in 10 women experiencing symptoms for up to 12 years (4). Menopause thus bears personal, health, and economic implications, particularly in light of increasing average life expectancy (5), women’s key role in the work-place (6), and symptomatic burden experienced by women during the menopause transition.

Menopausal hormone therapy (MHT) (previously commonly known as ‘hormone replacement therapy’; HRT) comprises of an oestrogen, in combination with a progestogen if the uterus has not been removed. Oestrogen replacement forms the backbone of MHT and corrects the chief hormonal deficit associated with menopause responsible for the inappropriate activation of heat dissipation effectors and the subsequent occurrence of VMS. Together with lifestyle modifications, MHT is efficacious, cost-effective, and is the most commonly used treatment for the management of menopause-related symptoms (7).

Landmark studies on MHT in the early 2000s including the Women’s Health Initiative (WHI) trial and the Million Women Study (MWS) first suggested increased risks of cardiovascular disease and breast cancer associated with MHT (8,9), which resulted in profound reductions of MHT uptake (10). Since then, one follow-up study of the WHI trial suggested an increased risk of breast cancer only in combined MHT (oestrogen and progestogen) (11), whereas other reports have suggested an increased risk with both oestrogen-only and combined MHT in a duration-dependent manner (12). The complex body of evidence surrounding MHT contributes to the hesitancy around MHT use and prescription. Moreover, systemic MHT remains at least relatively contraindicated in a subpopulation of women with previous personal history, or at increased risk, of breast cancer or certain hormonally sensitive gynaecological malignancies (e.g. oestrogen sensitive endometrial sarcomas), and could be unsuitable for patients with hormone-dependent malignancies undergoing sex-steroid deprivation therapy (7). Although advances in the development of modern preparations of MHT, such as transdermal preparations, have led to improvements in adverse thrombogenic profiles associated with MHT (7), non-hormonal therapy remain a useful alternative treatment option to alleviate the symptomatic burden experienced by women during the menopause transition.

Here, we aim to first review the physiology of the hypothalamo-pituitary-ovary (HPO) axis which underpins the neuroendocrine regulation of the female reproductive axis. We shall then outline the neuroendocrine changes that occur with menopause using evidence derived from both animal and human studies. Novel therapeutic pathways targeting these neuroendocrine pathways will be reviewed together with evidence from clinical trials of novel compounds currently in development for management of VMS.

**I. The hypothalamo-pituitary-ovary axis**

The hypothalamo-pituitary-ovary (HPO) axis describes the regulation of the reproductive hormones produced by the hypothalamus, anterior pituitary gland, and the ovaries (**Figure 1**). Feedback from oestradiol (E2) on the HPO axis is critical for major events in female reproduction including regulation of the menstrual cycle, follicle growth, the mid-cycle LH surge and resultant ovulation, and formation of the corpus luteum. Depletion of ovarian follicles, sex-steroids, and inhibin B, during the menopausal transition results in marked increases in GnRH secretion from the hypothalamus and serum follicle stimulating hormone (FSH) (as well as luteinising hormone; LH) from the anterior pituitary. Due to loss of the inhibitory action of inhibin at the pituitary gland, in addition to loss of negative feedback from sex-steroids, FSH is markedly raised after the menopausal transition. The absence of oestrogen receptor α (ERα) (13) on GnRH neurons suggests the presence of afferent intermediary neurons that provide integrated feedback to GnRH neurons.

Neurokinin A (NKA) and Substance P (SP), which are encoded in humans by the *TAC1* gene, and Neurokinin B (NKB) encoded by the *TAC3* gene, are members of the tachykinin peptide family (14). The critical role of the decapeptide NKB in reproductive endocrinology was demonstrated in recent years following observations that inactivating variants of *TAC3* and *TAC3R* (a gene encoding the cognate neurokinin 3 receptor for NKB; NK3R) result in pubertal delay and congenital hypogonadotrophic hypogonadism (CHH) (15). In preclinical models, NKB is co-expressed with kisspeptin and dynorphin in neurons in the hypothalamic arcuate nucleus known as the Kisspeptin/Neurokinin B/Dynorphin (KNDy) neurons (16). KNDy neurons have emerged as afferent intermediary neurons that provide integrated neuroendocrine, sex-steroid, and metabolic feedback to GnRH neurons and are now recognised as key regulators of GnRH neuronal activity and subsequent pulsatile GnRH secretion (17). Numerous studies from both animal and human models (reviewed in subsequent sections) provide evidence for the critical role these KNDy neurons play in the sex-steroid feedback on GnRH neurons and downstream gonadotrophin secretion in the context of menopause.

In preclinical models, the arcuate KNDy neurons project to the key preoptic thermoregulatory areas, the median preoptic nucleus (MnPO) and medial preoptic area (MPA) (18). Integration of thermosensory information from warm-sensitive cutaneous sensors in the MnPO and MPA ultimately influence thermoeffectors such as autonomic cutaneous vasodilation and cold-seeking behaviour (18). Furthermore, NK3R are expressed by neurons in the MnPO; taken together this intricate anatomical and functional connection between the arcuate and preoptic area suggests that a possible mechanism underlying the pathogenesis of hot flushes occur at the level of the hypothalamus through increased NKB signaling (18). Therefore, antagonising the action of NKB in these signaling pathways can provide a novel non-steroid-based therapeutic option for the management of VMS.

**II. Neuroendocrine changes in animal models**

Evidence for the potential of blocking NKB signalling to ameliorate VMS was first deciphered in preclinical models. Measurement of skin-tail temperature in rats was used as a proxy for hot flushes in humans, because the tail functions as the rat’s primary heat exchange organ (19), analogous to the primary mechanism of hot flush in women namely cutaneous vasodilation (18). In ovariectomised rodents, the temperature threshold at which heat-defence mechanisms are activated is reduced (20) and skin-tail temperature is increased to effect heat loss (21). Conversely, skin-tail temperature is reduced, and activation of heat-defence mechanisms is restored to higher thresholds when oestrogen replacement is provided to ovariectomised rats (20), mirroring the improvement in VMS reported in women treated with MHT.

Evidence from animal models have furthered our understanding of the neuroendocrine changes in menopause including the influence of the kisspeptin, NKB and dynorphin signalling pathway on the pathogenesis of menopausal hot flushes. Indeed, co-localisation of kisspeptin, NKB and dynorphin in the arcuate nucleus are conserved amongst most mammalian species including the sheep (22), mouse (23), rat (24), cow (25), goat (26) and non-human primates (27). Ovariectomised cynomolgus monkeys demonstrated marked neuronal hypertrophy and increased NKB and kisspeptin gene expression in the arcuate nucleus, as seen in the hypothalamus of post-menopausal women (28,29). Replacement of oestrogen to ovariectomised mammals was able to revert the heightened NKB and kisspeptin gene expression to their baseline levels (29). This finding is replicated in ovariectomised mice where KNDy neuronal cell size decreases following oestrogen replacement (30). This indicated that the neuronal hypertrophy and changes in KNDy gene expression are the result of oestrogen withdrawal specifically, rather than part of the ageing process (31). Furthermore, ablation of KNDy neurons led to consistent decreased tail skin vasodilatation indicating that KNDy neurons facilitate cutaneous vasodilation, an important heat dissipation effector in rats (19). Notably, ablation of KNDy neurons partially blocked the thermoregulatory effect of E2 (blocked reduction of skin-tail temperature during the light phase but not during the dark phase) supporting the role of KNDy neurons modulation of body temperature in conjunction with E2 (19).

The MnPO has been postulated as the anatomic site that provides integration between the reproductive (through projections from arcuate KNDy neurons) and thermoregulatory axes. Focal microinfusion of senktide, a selective NK3R agonist, into the hypothalamic MnPO resulted in a rapid, dose-dependent reduction in core temperature independent of oestrogen replacement (32). Additionally, administration of an NK3R agonist to mice increased skin-tail temperature and reduced core temperature, in keeping with heat dissipation effector activation, and synonymous with VMS (33). This effect in mice is partially mediated by oestrogen administration (33), and becomes more sensitised in rodents in rapid dramatic oestrogen withdrawal states such as post-ovariectomy (34). Modulation of body temperature through the NKB/NK3R pathway supports the hypothesis that hypothalamic NKB neurons are involved in the generation of VMS.

Furthermore, pharmacological administration of a neurokinin receptor antagonist cocktail (with affinity for neurokinin receptors 1, 2 and 3) into the MnPO region prevented the increase in skin-tail temperature heat dissipation response in mice, which is likely to have occurred from KNDy neurons (34). Additionally, neurokinin deficient mice lack a pulsatile LH surge (35), which is strongly associated with hot flush timing in humans (36,37). Likewise, administration of a neurokinin 1 receptor (NK1R) antagonist in monkeys reduced the amplitude and duration of the LH surge (38). These findings highlight that the NKB/NK3R/NK1R signalling pathway plays a key role in the pathogenesis of hot flushes, and that modulation of this pathway is a promising area for the delivery of novel treatment options for VMS.

**III. Neuroendocrine changes in postmenopausal women**

Cessation of ovarian function and depletion of ovarian follicular activity during the menopausal transition result in reduction of gonadal sex-steroids and inhibin B production with a corresponding increase in GnRH secretion and gonadotrophins from the loss of negative feedback (**Figure 1**). Occurrence of VMS, both subjectively reported and objectively measured, demonstrated close temporal relationship to LH pulsatile secretions (36), and significant positive correlations were observed between simultaneous skin temperature measurements and circulating LH levels (37). However, as hot flushes can occur in women post-hypophysectomy with low circulating concentrations of serum gonadotrophins suggests that LH is not directly causative of hot flushes (39). Two models of hypothalamic dysfunction including isolated gonadotrophin deficiency (representing defective GnRH secretion or function), and functional hypothalamic amenorrhoea (FHA; representing defective neurotransmitter input to GnRH neurons) were used to further examine possible hypothalamic mechanisms underlying hot flushes (40). Women with isolated gonadotrophin deficiency experienced similar rates of hot flushes compared to postmenopausal women (0.76 ± 0.03 *vs* 0.75 ± 0.04 flushes/hour) thus suggesting that alteration of GnRH synthesis and/or release is not involved in the generation of hot flushes (40). Similarly, women with FHA, a condition thought to arise from dysfunctional input to GnRH neurons, despite marked hypooestrogenism (equivalent to that occurring post-oophorectomy) reported no symptoms resembling hot flushes (40). Interestingly, women with hypergonadotrophic hypogonadism secondary to gonadal dysgenesis, who have pulsatile gonadotrophin release similar to that seen in postmenopausal women, also do not experience hot flushes, even at advanced age (36). However, if exogenous oestrogen is administered in these patients for several months and then discontinued, hot flushes were experienced for the first time. Taken together, these findings suggest that menopausal hot flushes manifest as consequence of oestrogen withdrawal, mediated through functional changes of oestrogen-sensitive afferent intermediary neurons that provide input and control GnRH pulsatile release from GnRH neurons.

Sheehan and Kovács first described marked morphological changes observed within the hypothalamus following the menopause in 1966 (41). Pioneering studies from Rance and colleagues have since compared postmortem hypothalamic tissues from premenopausal and postmenopausal subjects (28,29,42). Within the infundibular nucleus of postmenopausal women (equivalent to arcuate nucleus in animal models), a subset of neurons expressing ER𝛼 (42), *NKB*, *SP* (28), and *KISS1* mRNA (29) demonstrated hypertrophic morphology compared to premenopausal state. Furthermore, *KISS1* and *TAC3* gene expression was found to be increased, whilst *prodynorphin* mRNA expression was reduced (43). This differential change in expression suggests increased stimulatory action by kisspeptin and NKB action, and reduced basal inhibitory action from dynorphin, on gonadotrophin secretion after the menopause (43).

In order to evaluate the NKB/NK3R pathway in menopause, NKB was administered through peripheral intravenous infusion to healthy premenopausal women during the follicular phase in a randomised, double-blinded, placebo-controlled trial (44). Intravenous NKB, at the highest tolerated dose of 5.12 nmol/kg/hr, resulted in increased occurrence of hot flushes, both subjectively and objectively (increased mean heart rate and skin temperature, both measures by similar magnitudes to natural menopausal flushes) in healthy women (44) but resulted in no significant changes in serum gonadotrophins or E2 (44,45). Meta-analysis of three genome-wide association studies (GWAS) of European American, African American, and Hispanic American postmenopausal women aged 50 to 79 years was conducted to demonstrate the effect of genetic variation in relation to VMS (46). In the meta-analysis, 14 single-nucleotide polymorphisms (SNPs) were identified to be associated with VMS (46). These SNPs were all located on chromosome 4 in the *TACR3* locus, which suggested that genetic variation in *TACR3* may contribute to an increased risk of VMS (46). Collectively, this implicates the importance of NKB signalling as a mediator of menopausal flushing and sparked interest to further explore the potential of antagonism of the NKB pathway at its cognate receptor, NK3R, to inhibit the occurrence of VMS.

**IV. Antagonism of NKB/NK3R pathway as a novel treatment for menopausal hot flushes**

The first trial of NK3R antagonism as novel treatment for menopausal hot flushes in women was published in 2017 (47); since then, multiple compounds which target the NKB pathway have been developed (**Table 1**).

*a. Pavinetant (MLE4901)*

In 2017, a phase 2, proof-of-concept, randomised, double-blinded, placebo-controlled, crossover trial of an oral NK3R antagonist (MLE4901, Pavinetant) was conducted in 28 healthy post-menopausal women aged 40-62 years who experienced ≥7 hot flushes per 24 hours (47). The study intervention group received oral MLE4901 40 mg twice daily for 4 weeks, following a 2-week washout period, with subsequent cross-over with MLE4901 treatment for 4 weeks in the placebo arm (47). Treatment with MLE4901 resulted in a significant reduction in the number and frequency of hot flushes compared to placebo (47). Subjective reporting of hot flushes was reduced (45 % decrease) concordant with the reduction in objective measurement of skin conductance monitoring (43% decrease) (47). The severity (41% decrease) and interference (58% decrease) of weekly hot flushes were also decreased with MLE4901 compared to placebo (47). Consistent improvements in hot flush frequency, severity and interference with daily activities were also reported in a separate cohort of 11 postmenopausal women (8 with symptomatic hot flushes) following one week of MLE4901 treatment, with positive symptomatic effects appreciable from the second day of treatment (48). These studies therefore highlighted the rapid relief of VMS and pioneered treatment of menopausal hot flushes without the need for oestrogen-based treatments.

In one report, MLE4901 did not result in significant changes in the number of LH pulses but led to increased LH pulse amplitude, and improved orderliness of LH pulses, compared to placebo (47). In another report, MLE4901 treatment for 7 days resulted in an overall suppressive effect of LH secretion (pre-treatment 29.5 ± 4.1 *vs* 24.4 ± 3.8 IU/L on day 7, *p* < 0.05) (48). Basal LH secretion was reduced (549.0 ± 70.8 *vs* 366.1 ± 92.1 IU/L per 6 hrs, *p* = 0.006), and although LH pulse frequency did not change overall, sub-analysis of 8 women with symptomatic hot flushes demonstrated a reduction in LH pulse frequency (from 1.0 ± 0.1 to 0.7 ± 0.1 pulses per hr) (48). No change in E2 (47) or FSH (48) levels were observed. The authors postulated that reductions in LH pulses in symptomatic postmenopausal women following administration of NK3R antagonists could arise from changes in hypothalamic GnRH pulse release and that this could independently exert positive effects on control of VMS. Overall, treatment with MLE4901 was well-tolerated, however three participants developed a rise in transaminase levels up to 6x upper limit of normal and subsequently its development has been discontinued.

*b. Fezolinetant (ESN364)*

Fezolinetant (ESN364) is a selective and reversible antagonist of NK3R. A twelve-week, phase 2a, double-blinded, randomised, placebo-controlled study from eight Belgian centres demonstrated that twice daily Fezolinetant significantly reduced total VMS scores compared to placebo (-26.5 *vs* -12.2; *p <* 0.001) (49). The frequency of moderate / severe VMS was also reduced (Fezolinetant 5.7 episodes per week *vs* placebo 39.0 episodes per week) from the first day of treatment (49). Fezolinetant led to a 93% reduction in VMS frequency from baseline to week 12 *vs* 46% with placebo (49). Furthermore, Fezolinetant resulted in improvements in sleep quality, reduction of overall daily interference and climacteric symptoms. At 3 hours post Fezolinetant dose (peak drug levels), plasma LH levels decreased by 49.8% *vs* 16.4% with placebo relative to baseline consistent with the proposed mechanism of action of KNDy neuron inhibition (49). No effect on E2, FSH and sex hormone binding globulin (SHBG) levels were observed (49).

In a subsequent phase 2b trial (VESTA trial), the effect of different Fezolinetant doses and dosing regimens on the frequency and severity of postmenopausal flushing was evaluated in a randomised, double-blinded, placebo-controlled, dose-ranging, parallel group study in 51 sites in the United States (50). Postmenopausal women aged 40-65 years (*n*=356) were randomly assigned to doses of Fezolinetant between 30mg once daily to 90mg twice daily or placebo for 12 weeks (50). All Fezolinetant regimens significantly reduced the frequency of moderate/severe VMS at weeks 4 and 12 compared to placebo, with >2 VMS episodes reduced per day at all doses except the lowest twice daily dose (15mg twice daily) (50). Similarly, all Fezolinetant regimens significantly reduced the severity of moderate / severe VMS at week 4, and Fezolinetant 60 mg twice daily, 90 mg twice daily, and 60 mg once daily significantly reduced VMS severity relative to placebo at week 12 (50). The effect was observable by the end of the first week of treatment, congruous with the outcomes of previous NK3R trials.

SKYLIGHT 1, 2 and 4 are phase 3 trials of Fezolinetant treatment (30mg or 45mg daily *vs* placebo for 12 weeks) conducted across multiple international locations with majority of sites in the United States. Consistent with earlier trials, Fezolinetant treatment resulted in a significant reduction in VMS frequency and severity at 4 and 12 weeks compared to placebo [**Table 1**] (51). SKYLIGHT 2 investigated the persistence of Fezolinetant efficacy over 52 weeks (52). Improvements in VMS frequency and severity, as previously described at 12 weeks with Fezolinetant, were maintained throughout the 52-week total study duration. Improvements in VMS were seen within the first week of treatment and further reductions were observed beyond 12 weeks of treatment (52).

Treatment-emergent adverse events including endometrial hyperplasia and malignancy were primary end points of SKYLIGHT 4 (53). Treatment-emergent adverse events occurred in 64.1% after placebo, 67.9% after Fezolinetant 30mg, and 63.9% after Fezolinetant 45mg following a 1-year period of Fezolinetant use (53). The incidence of endometrial hyperplasia or malignancy was within the prespecified limits and there was no significant change from baseline endometrial thickness between Fezolinetant and placebo-treated participants, and no effect on bone health  (53). Notably, hepatic safety profile was given important consideration in SKYLIGHT 4 and participants with a variety of potential liver related risk factors such as obesity and non-alcoholic fatty liver disease were recruited in the trial (53). Overall, 98.2% of participants in the Fezolinetant group had liver function test results within the normal range compared with 99.0% in the placebo group. Furthermore, there was no evidence of liver function impairment or liver-associated symptoms, including no Hy's law cases to indicate drug-induced liver injury (53). Overall, SKYLIGHT 4 confirmed the safety and tolerability of Fezolinetant and the data supported its continued development. At present, a New Drug Application for Fezolinetant has been submitted for consideration by the Food and Drug Administration (FDA).

A related phase 3 study of Fezolinetant 30mg once daily *vs* placebo, the MOONLIGHT trials, was conducted in Asian women across 48 locations exclusively in Asia. MOONLIGHT 1 (54) evaluated the efficacy of Fezolinetant 30mg daily *vs* placebo for 12 weeks with an unblinded, controlled 12-week extension phase in 302 post-menopausal women. MOONLIGHT 1 did not demonstrate significant difference in moderate-severe VMS frequency and severity with Fezolinetant use compared to placebo (55). MOONLIGHT 3 (56) is currently underway to investigate the long-term safety profile of Fezolinetant (30mg once daily) *vs* placebo over 52 weeks in Asian women.

*c. Elinzanetant (NT-814)*

Elinzanetant is a dual NK1R and NK3R antagonist which was initially developed for the treatment of addiction disorders (57). More recently, the RELENT-1 and SWITCH-1 trials have investigated the use of Elinzanetant as a therapeutic agent for the treatment of menopausal hot flushes, given that dual NK1R/NK3R antagonism has the potential to decrease GnRH pulse frequency by blocking the effects of endogenous SP and NKB on the reproductive axis respectively (58). Although NK1R has been proposed to contribute to the pathogenesis of VMS, the predominant action on VMS associated with Elinzanetant is likely to be mediated via NK3R antagonism as NK1R antagonism alone is unlikely to fully alleviate VMS but may provide additional benefits on sleep and anxiolytic effects in postmenopausal women (14).

RELENT-1 (59) was a dose-ranging study of 76 postmenopausal women with moderate/severe hot flushes, where participants were randomised to four sequential dose cohorts of once daily Elinzanetant (50mg, 100mg, 150mg and 300mg) *vs* placebo for 14 days (59). Participants were asked to complete a symptom diary before and after treatment of hot flush frequency and waking due to night sweats (59). Higher dose regimens of ≥150mg Elinzanetant once daily were associated with significant reductions *vs* placebo in hot flush frequency (84% reduction with 150mg dose (*p* < 0.001); 66% reduction with 300mg dose (*p* = 0.022); 37% reduction with placebo) and in waking due to night sweats (81% reduction with 150mg dose (*p* < 0.001); 63% reduction with 300mg dose (*p* = 0.031); 32% reduction with placebo) at the end of the 14 day period (59). Improvements were present from the first week indicating immediate response (59). Average severity also improved compared to placebo in the 150mg group, although not in the other groups. The most common side effect was mild somnolence and headache, more frequently in the 300mg treatment group, likely due to increased NK1R antagonist action (60). No significant safety concerns were identified.

SWITCH-1 (61) was a subsequent larger multi-centre, dose-ranging trial involving 199 participants with ≥7 moderate/severe hot flushes per day, which investigated the effects of Elinzanetant on VMS severity, sleep, and quality of life. Post-menopausal women were randomised into cohorts of once daily Elinzanetant (40mg, 80mg, 120mg and 160mg) *vs* placebo for 12 weeks, with interim analyses at 1, 2, 4, 8 and 12 weeks (61). Results demonstrated improvements *vs* placebo in daily VMS frequency for higher doses of Elinzanetant (120mg and 160mg) from week 1 throughout 12 weeks of treatment, with statistically significant improvements at both primary endpoints for the 120mg dose (61). Night-time awakening secondary to VMS was also significantly reduced *vs* placebo for the 120mg dose in weeks 1, 2, 4 and 8, but not at week 12 or when using any other doses (61). Significant improvements in sleep (measured via the Insomnia Severity Index and Pittsburgh Sleep Quality Index), and quality of life (using the MenQoL instrument) were also demonstrated for both the 120mg and 160mg doses compared to placebo, but this effect was not present at lower doses. Subsequently, improvements in sleep and quality of life returned towards baseline 4 weeks after discontinuation of treatment (61). All doses were well-tolerated, with the most frequent adverse effects being mild or moderate headache, somnolence, and diarrhoea.

Given the promising yield from the above two studies, a Phase 3 trial (OASIS-1) is currently being conducted to examine the efficacy and safety of Elinzanetant in treating VMS.

*d. SJX-653*

A phase 1, randomised, placebo-controlled, double-blinded and single ascending dose study of SJX-653, a centrally acting, competitive NK3R antagonist, was conducted in 7 cohorts of 6 healthy men aged 18-45 years (62). The aim was to characterise the pharmacodynamics, pharmacokinetics, safety, and tolerability profile of SJX-653 in healthy men as a proof-of-mechanism study. SJX-653 resulted in marked, but reversible suppression of gonadotrophins and testosterone with approximately 70% suppression of both LH and testosterone after a single oral dose (dose range 0.5-90mg) (62). As other NK3R antagonists have demonstrated reduction in menopausal hot flushes following similar reductions in LH and testosterone in men, this led to the extrapolation that SJX-653 may be an effective treatment of menopausal hot flushes (62). Subsequently, a phase 2 trial to assess the efficacy of SJX-653 in postmenopausal women with moderate to severe VMS was conducted in healthy postmenopausal women (63). However, the trial was terminated early as the primary outcome of safe and efficacious treatment of VMS was not met and its further development has now been discontinued (63).

Lastly the effects of targeting the dynorphin pathway has recently been described in preclinical models (64). As described previously, dynorphin regulates KNDy neurons through inhibitory collaterals and kappa opioid receptors, thus this pathway offers an alternative strategy to regulate increased KNDy neuronal activity seen in menopause (64). Peripherally restricted kappa receptor agonists (PRKAs) which acts on the dynorphin pathway has recently been demonstrated to inhibit LH secretion and reverse changes in body temperature in ovariectomised murine models (64). PRKAs may thus provide a novel treatment option in the management of VMS however further preclinical and clinical studies are needed.

**Summary** (250/250 words)

Menopause-related symptoms are common and affect over 70% of women during the menopausal transition with important health and socioeconomic implications. Although MHT remains an efficacious and cost-effective treatment, its use remains at least relatively contraindicated in some women with a previous personal history or in those at increased risk of breast cancer and gynaecological malignancies. Data from animal models and post-mortem hypothalamic studies from postmenopausal subjects paved our understanding of the role of the NKB pathway in the pathogenesis of VMS. NK3R and NK1R antagonists have provided promising data on improvement in the frequency and severity of hot flushes, and improvements in sleep quality, and overall markers of quality of life. To date, four novel neurokinin receptor antagonists that target the NK3R and NK1R have been evaluated in human clinical trials; Pavinetant (MLE4901), Fezolinetant (ESN364), Elinzanetant (NT-814) and SJX-653. Antagonism of the neurokinin B pathway resulted in rapid and sustained relief of menopausal hot flushes and at present phase 3 trials on Fezolinetant and Elinzanetant are currently underway at multiple international centres to provide data on long-term safety and efficacy. While the therapeutic benefit is demonstrated by all agents in the class, concerns regarding the hepatic safety profile appears to be specific to each agent and not a general class effect for NK3R antagonists.

In summary, antagonism of the NKB pathway demonstrate clear effectiveness for the management of menopausal hot flushes and is likely to provide an effective, non-hormonal treatment strategy for the management of VMS that are likely to be clinically available within the coming year.

**Practice Points:**

* Vasomotor symptoms are common and affect over 70% of peri-/post-menopausal women.
* Shared-decision making is recommended for the management of menopausal symptoms.
* Menopausal hormone therapy (MHT) / hormone replacement therapy (HRT) remains an effective treatment for management of menopausal symptoms in conjunction with life-style changes. However, MHT may not be a suitable treatment option for every woman.
* Antagonism of NKB/NK3R/NK1R pathway offers a novel, non-hormonal treatment for menopausal hot flushes.
* Fezolinetant (ESN394) and Elinzanetant (NT-814) are both being investigated in phase 3 trials with potential to become treatment options for menopausal hot flushes in the near future.

**Research Agenda:**

* Evaluation of the effects of NK3R antagonists on postmenopausal women from various ethnic backgrounds (e.g. the MOONLIGHT trials were conducted in women from Asian backgrounds). Similar trials will be beneficial for postmenopausal women from other ethnic backgrounds.
* The long-term effect of NK3R antagonist treatment in phase 3 and phase 4 clinical trials.
* Trials are needed of NK3R antagonists in women with VMS due to cancer treatments.
* Further pre-clinical and clinical studies are needed for peripherally restricted kappa receptor agonists (PRKAs) for treatment of VMS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Study** | **Study design** | **Total no. participants** | **Subjects** | **Intervention** | **Outcomes** |
| **Pavinetant (MLE4901)** | | | | | |
| Prague *et al.* (2017) (47) | Single-centre, phase 2, randomised, double-blinded, placebo-controlled, single-centre, crossover trial | 37 women in intention to treat (ITT) analysis  28 women in per-protocol analysis | Healthy women aged 40-62 years; ≥7 hot flushes/24hrs | MLE4901 40mg BD *vs* placebo for 4 weeks with 2 weeks washout | Total weekly number of hot flushes:  MLE4901 19.35 (95% CI 15.99–23.42) vs placebo 49.01 (95% CI 40.81–58.56); *p*<0.0001.  45% decrease (95% CI 22-67).  Weekly hot flush severity:  MLE4901 3.27 (95% CI 2.92–3.66) *vs* placebo 5.70 (95% CI 5.09–6.38); *p*<0.0001.  41% decrease (95% CI 32–49). |
| Skorupskaite *et al.* (2018) (48) | Single-centre intervention study | 11 women  8 women with symptomatic hot flushes) | Healthy women aged 46-62 years | MLE4901 40mg BD for 1 week | Reduction in VMS frequency:  MLE4901 3.4 ± 1.2 *vs* placebo 1.0 ± 0.6 hot flushes/day; *p* = 0.008.  Improvement hot flushes severity from moderate to mild:  MLE4901 mean severity 2.1 ± 0.2/3 *vs* placebo 1.4 ± 0.1/3; *p* = 0.03. |
| **Fezolinetant (ESN364)** | | | | | |
| Depypere *et al.* (2019) (49) | Multi-centre, phase 2a, double-blinded, randomised, placebo-controlled study | 80 women –  Fezolinetant; *n*=40  Placebo; *n*=40 | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 90mg BD *vs* placebo for 12 weeks | Reduction in frequency of moderate / severe VMSs at week 12:  Fezolinetant 5.7 episodes/week (95% CI 2.4, 9.1) *vs* placebo 39.0 episodes/week (95% CI 26.6, 51.5).  Reduction in total VMS score *vs* placebo at week 12:  Fezolinetant -26.5 (95% CI -30.8, -22.2) *vs* placebo -12.2 (95% CI -16.5, -7.8); *p* < 0.001. |
| **VESTA**  Fraser *et al.* (2020) (50) | Multi-centred, phase 2b, double-blinded, randomised, placebo-controlled, dose-ranging, parallel group | 356 women (287 completed study) –  Fezolinetant 15mg BD; *n*=45  Fezolinetant 30mg BD; *n*=44  Fezolinetant 60mg BD; *n*=45  Fezolinetant 90mg BD; *n*=44  Fezolinetant 30mg OD; *n*=45  Fezolinetant 60mg OD; *n*=45  Fezolinetant 120mg OD; *n*=44  Placebo; *n*=44 | Healthy women aged 40-65 years with ≥50 moderate/severe hot flushes per week | Fezolinetant 15, 30, 60, 90 mg BD or 30, 60, 120 mg OD *vs* placebo for 12 weeks | Reduction in moderate/severe VMS frequency:  Fezolinetant −1.9 to −3.5/day at week 4  Fezolinetant −1.8 to −2.6/day at week 12 (all *p <*0.05 *vs* placebo).  Mean difference in VMS severity score:  Fezolinetant -0.4 to -1.0 at week 4 (all doses *p* < 0.05 *vs* placebo).  Fezolinetant -0.2 to -0.6 at week 12 (*p* < 0.05 for 60mg and 90mg BD and 60mg OD). |
| **SKYLIGHT 1**  Lederman *et al.* (2022) (51,65) | Phase 3, double-blinded, randomised, placebo-controlled | 527 women –  Placebo; *n*=175  Fezolinetant 30mg OD; *n*=176  Fezolinetant 45mg OD; *n*=176 | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 30mg or 45mg OD *vs* placebo for 12 weeks | VMS frequency; least squares mean (standard error) reductions  Fezolinetant *vs* placebo:  Fezolinetant 30mg at week 4  –1.87 (0.42); p < 0.001  Fezolinetant 45mg at week 4  –2.07 (0.42); p < 0.001  Fezolinetant 30mg at week 12  –2.39 (0.44); p < 0.001  Fezolinetant 45mg at week 12  –2.55 (0.43); p < 0.001  VMS severity; least squares mean (standard error) reductions Fezolinetant *vs* placebo:  Fezolinetant 30mg at week 4  –0.15 (0.06); p = 0.012  Fezolinetant 45mg at week 4  –0.19 (0.06); p = 0.002  Fezolinetant 30mg at week 12  –0.24 (0.08); p = 0.002  Fezolinetant 45mg at week 12  –0.20 (0.08); p = 0.007 |
| **SKYLIGHT 2**  Johnson *et al.*  (2023) (52) | Phase 3, double-blinded, randomised, placebo-controlled | 484 women –  Fezolinetant 30mg; *n*=166  Fezolinetant 45mg; *n*=167 Placebo/Fezolinetant 30mg; *n*=76 Placebo/Fezolinetant 45mg; *n*=75 | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 30mg or 45mg OD *vs* placebo for 12 weeks with a 40 week active treatment extension  Completers in the placebo cohort were re-randomised to Fezolinetant 30mg or 45mg OD for 40 additional weeks. | VMS frequency; least squares mean (standard error) reductions Fezolinetant *vs* placebo:  Fezolinetant 30mg at week 4  –1.82 (0.46); p < 0.001  Fezolinetant 45mg at week 4  –2.55 (0.46); p < 0.001  Fezolinetant 30mg at week 12  –1.86 (0.55); p < 0.001  Fezolinetant 45mg at week 12  –2.53 (0.55); p < 0.001  VMS severity; least squares mean (standard error) reductions Fezolinetant *vs* placebo:  Fezolinetant 30mg at week 4  –0.15 (0.06); p < 0.05  Fezolinetant 45mg at week 4  –0.29 (0.06); p < 0.001  Fezolinetant 30mg at week 12  –0.16 (0.08); p < 0.05  Fezolinetant 45mg at week 12  –0.29 (0.08); p < 0.001 |
| **SKYLIGHT 4**  Neal-Perry *et al.* (2023) (53) | Phase 3, double-blinded, randomised, placebo-controlled, 52-week safety study | 1830 women –  Placebo; *n*=610  Fezolinetant 30mg; *n*=611,  Fezolinetant 45mg;  *n*=609 | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 30mg or 45mg OD *vs* placebo for 52 weeks | Treatment-emergent adverse events (*n*=1830):  Placebo; 64.1%  Fezolinetant 30mg; 67.9%  Fezolinetant 45mg; 63.9%  Endometrial hyperplasia (*n*=599):  Placebo; 0.0%  Fezolinetant 30mg; 0.0%  Fezolinetant 45mg; 0.5%  Endometrial malignancy (*n*=599):  Placebo; 0.0%  Fezolinetant 30mg; 0.5%  Fezolinetant 45mg; 0.0%  Liver enzyme elevations >3X upper limit of normal (*n*=1762):  Placebo; 1.0%  Fezolinetant 30mg; 1.4%  Fezolinetant 45mg; 2.0%  No Hy's law cases were reported |
| **MOONLIGHT1**  (Press release only) | Phase 3, double-blinded, randomised, placebo-controlled, conducted exclusively in Asia | 302 women | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 30 mg OD *vs* placebo for 12 weeks, with an unblinded non-controlled extension phase for additional 12 weeks | Fezolinetant 30 mg daily did not meet primary pre-defined end point for efficacy which is reduction in moderate-severe VMS frequency and severity |
| **MOONLIGHT3**  (Ongoing trial) | Phase 3, single-arm study, 52-week safety study in mainland China | 150 women | Healthy women aged 40-65 years with ≥7 moderate/severe hot flushes per day | Fezolinetant 30 mg OD for 52 weeks, with a follow-up visit three weeks after the last dose. | Detailed report is not available yet |
| **Elinzanetant (NT-814)** | | | | | |
| **RELENT-1**  Trower *et al.* (2020) (59) | Phase 2, multi-centred, double-blinded, randomised, placebo-controlled | 76 women (74 completed study) – Placebo; *n*=18  Elinzanetant 50mg; *n*=15  Elinzanetant 100mg; *n*=15  Elinzanetant 150mg; *n*=15  Elinzanetant 300mg; *n*=13 | Healthy women aged 40-65 with an average of 7 to 20 moderate to severe hot flushes per day | Elinzanetant 50, 100, 150 mg or 300mg OD *vs* placebo for 14 days | Reductions in moderate/severe hot flush frequency at 2 weeks:  Elinzanetant 50mg 24% *vs* placebo 37%; *p* = 0.048  Elinzanetant 100mg 59% *vs* placebo 37%; *p* = 0.155  Elinzanetant 150mg 84% *vs* placebo 37%; *p* < 0.001  Elinzanetant 300mg 66% *vs* placebo 37%; *p* = 0.022 |
| **SWITCH-1**  Simon *et al.* (2023) (61) | Phase 2, multi-centre, double-blinded, adaptive-randomisation, placebo-controlled | 199 women (180 completed study) –  Placebo; *n*=47  Elinzanetant 40mg; *n*=31  Elinzanetant 80mg; *n*=17  Elinzanetant 120mg; *n*=52  Elinzanetant 160mg; *n*=52 | Healthy women aged 40-65 yrs with ≥7 moderate/severe hot flushes per day | Elinzanetant 40, 80, 120 mg or 160mg OD *k* placebo for 12 weeks | Daily VMS frequency; least squares mean (standard error) reductions:  Elinzanetant 40mg at week 4  -1.52 (1.17); p = 0.19  Elinzanetant 80mg at week 4  –1.29 (1.43); p = 0.37  Elinzanetant 120mg at week 4  –3.93 (1.02); p < 0.001  Elinzanetant 160mg at week 4  –2.63 (1.03); p = 0.01  Daily VMS frequency; least squares mean (standard error) reductions:  Elinzanetant 40mg at week 12  -1.67 (1.32); p = 0.21  Elinzanetant 80mg at week 12  –0.77 (1.62); p = 0.64  Elinzanetant 120mg at week 12  –2.95 (1.15); p = 0.01  Elinzanetant 160mg at week 12  –1.78 (1.19); p = 0.13 |

**Table 1:** Summary of NK1R and NK3R antagonist clinical trials on the severity and frequency of menopausal hot flushes.

BD, twice daily; CI, confidence interval; ITT, intention to treat; *n*, number of participants; OD, once daily; VMS, vasomotor symptoms; *vs*, versus

**Figure caption**

**Figure 1:**

Cessation of ovarian function and depletion of ovarian follicular activity, characteristic of menopause, result in reduction of gonadal sex-steroids and inhibin production from the ovaries with a corresponding increase in hypothalamic GnRH secretion and gonadotrophins from the anterior pituitary gland from the loss of negative feedback. Within the arcuate nucleus (homologue to the infundibular nucleus in human) the KNDy neurons demonstrate hypertrophic morphology compared to premenopausal state. Due to the loss of negative feedback, there are compensatory increments in the secretion of kisspeptin and NKB and reduced basal inhibitory action from dynorphin. KNDy neurons have been demonstrated in preclinical models to project to the median preoptic nucleus (MnPO), a hypothalamic region important for integration of thermosensory information from warm-sensitive cutaneous sensors, mediation of efferent neural pathways controlling heat-defence effectors and ultimately control of thermoeffectors such as autonomic cutaneous vasodilation and cold-seeking behaviour. NK3R are expressed by neurons within the MnPO and arcuate nucleus thus antagonism of these NK3Rs can therefore offer a novel non-steroid-based therapeutic option for the management of VMS.

E2, oestradiol; GnRH, gonadotrophin releasing hormone; FSH, follicle stimulating hormone; KNDy; kisspeptin, neurokinin, dynorphin; LH, luteinising hormone; MnPO, median preoptic nucleus; NK3R, neurokinin 3 receptor; PRG, progesterone.

**Acknowledgements**

This work was supported by grants from the National Institute of Health Research (NIHR), the NIHR/Wellcome Trust Imperial Clinical Research Facility, and the NIHR Imperial Biomedical Research Centre. The Section of Endocrinology and Investigative Medicine was funded by grants from the Medical Research Council (MRC), , NIHR and was supported by the NIHR Biomedical Research Centre Funding Scheme. The views expressed are those of the authors and not necessarily those of the MRC, BBSRC, the NHS, the NIHR, or the Department of Health. The authors acknowledge infrastructure support for this research from the NIHR Imperial Biomedical Research Centre (BRC). K.K. is supported by NIHR Academic Clinical Fellowship Award (ACF-2021-21-001). A.A. is supported by an NIHR Clinician Scientist Award (CS-2018-18-ST2-002). W.S.D. is supported by an NIHR Senior Investigator Award. Figure created with BioRender.

**Conflict of interest statement**

WSD and AA have conducted consultancy work for Myovant Sciences Ltd. WSD has also conducted consultancy work for KaNDy therapeutics.

**References:**

1. Hickey M, Szabo RA, Hunter MS. Non-hormonal treatments for menopausal symptoms. BMJ. 2017 Nov 23;j5101.

2. Gracia CR, Freeman EW. Onset of the Menopause Transition. Obstetrics and Gynecology Clinics of North America. 2018 Dec;45(4):585–97.

3. Avis NE, Crawford SL, Greendale G, et al. Duration of Menopausal Vasomotor Symptoms Over the Menopause Transition. JAMA Internal Medicine. 2015 Apr 1;175(4):531.

**4. \*National Institute for Health and Care Excellence (NICE). Menopause: diagnosis and management . National Institute for Health and Care Excellence (NICE) . 2015;**

5. Lobo RA, Gompel A. Management of menopause: a view towards prevention. The Lancet Diabetes & Endocrinology. 2022 Jun;10(6):457–70.

6. Brewis J, Beck V, Davies A, Matheson J. The effects of menopause transition on women’s economic participation in the UK. 2017;

**7. \*Hamoda H, Panay N, Pedder H, et al. The British Menopause Society &amp; Women’s Health Concern 2020 recommendations on hormone replacement therapy in menopausal women. Post Reproductive Health. 2020 Dec 12;26(4):181–209.**

8. Writing Group for the Women’s Health Initiative Investigators. Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women: Principal Results From the Women’s Health Initiative Randomized Controlled Trial. JAMA: The Journal of the American Medical Association. 2002 Jul 17;288(3):321–33.

9. Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. The Lancet. 2003 Aug;362(9382):419–27.

10. McIntosh J, Blalock SJ. Effects of media coverage of Women’s Health Initiative study on attitudes and behavior of women receiving hormone replacement therapy. American Journal of Health-System Pharmacy. 2005 Jan 1;62(1):69–74.

11. Chlebowski RT, Anderson GL, Aragaki AK, et al. Association of Menopausal Hormone Therapy With Breast Cancer Incidence and Mortality During Long-term Follow-up of the Women’s Health Initiative Randomized Clinical Trials. JAMA. 2020 Jul 28;324(4):369.

12. Collaborative Group on Hormonal Factors in Breast Cancer. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. The Lancet. 2019 Sep;394(10204):1159–68.

13. Wolfe A, Wu S. Estrogen Receptor-β in the Gonadotropin-Releasing Hormone Neuron. Seminars in Reproductive Medicine. 2012 Jan 23;30(01):23–31.

14. Abbara A, Phylactou M, Dhillo WS. Commentary on “Pharmacodynamic Activity of the Novel Neurokinin-3 Receptor Antagonist SJX-653 in Healthy Men.” The Journal of Clinical Endocrinology & Metabolism. 2021 Jan 23;106(2):e1028–30.

15. Young J, Bouligand J, Francou B, et al. TAC3 and TACR3 Defects Cause Hypothalamic Congenital Hypogonadotropic Hypogonadism in Humans. The Journal of Clinical Endocrinology & Metabolism. 2010 May 1;95(5):2287–95.

16. Goodman RL, Lehman MN, Smith JT, et al. Kisspeptin Neurons in the Arcuate Nucleus of the Ewe Express Both Dynorphin A and Neurokinin B. Endocrinology. 2007 Dec 1;148(12):5752–60.

17. Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proceedings of the National Academy of Sciences. 2005 Feb 21;102(5):1761–6.

**18. \*Rance NE, Dacks PA, Mittelman-Smith MA, et al. Modulation of body temperature and LH secretion by hypothalamic KNDy (kisspeptin, neurokinin B and dynorphin) neurons: A novel hypothesis on the mechanism of hot flushes. Frontiers in Neuroendocrinology. 2013 Aug;34(3):211–27.**

19. Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, et al. Role for kisspeptin/neurokinin B/dynorphin (KNDy) neurons in cutaneous vasodilatation and the estrogen modulation of body temperature. Proceedings of the National Academy of Sciences. 2012 Nov 27;109(48):19846–51.

20. Dacks PA, Rance NE. Effects of Estradiol on the Thermoneutral Zone and Core Temperature in Ovariectomized Rats. Endocrinology. 2010 Mar 1;151(3):1187–93.

21. Berendsen HHG, Weekers AHJ, Kloosterboer HJ. Effect of tibolone and raloxifene on the tail temperature of oestrogen-deficient rats. European Journal of Pharmacology. 2001 May;419(1):47–54.

22. Goodman RL, Lehman MN, Smith JT, et al. Kisspeptin Neurons in the Arcuate Nucleus of the Ewe Express Both Dynorphin A and Neurokinin B. Endocrinology. 2007 Dec 1;148(12):5752–60.

23. Navarro VM, Gottsch ML, Chavkin C, et al. Regulation of Gonadotropin-Releasing Hormone Secretion by Kisspeptin/Dynorphin/Neurokinin B Neurons in the Arcuate Nucleus of the Mouse. The Journal of Neuroscience. 2009 Sep 23;29(38):11859–66.

24. True C, Kirigiti M, Ciofi P, et al. Characterisation of Arcuate Nucleus Kisspeptin/Neurokinin B Neuronal Projections and Regulation during Lactation in the Rat. Journal of Neuroendocrinology. 2011 Jan;23(1):52–64.

25. Hassaneen A, Naniwa Y, Suetomi Y, et al. Immunohistochemical characterization of the arcuate kisspeptin/neurokinin B/dynorphin (KNDy) and preoptic kisspeptin neuronal populations in the hypothalamus during the estrous cycle in heifers. Journal of Reproduction and Development. 2016;62(5):471–7.

26. Wakabayashi Y, Nakada T, Murata K, et al. Neurokinin B and Dynorphin A in Kisspeptin Neurons of the Arcuate Nucleus Participate in Generation of Periodic Oscillation of Neural Activity Driving Pulsatile Gonadotropin-Releasing Hormone Secretion in the Goat. The Journal of Neuroscience. 2010 Feb 24;30(8):3124–32.

27. Ramaswamy S, Seminara SB, Ali B, et al. Neurokinin B Stimulates GnRH Release in the Male Monkey (Macaca mulatta) and Is Colocalized with Kisspeptin in the Arcuate Nucleus. Endocrinology. 2010 Sep 1;151(9):4494–503.

28. Rance NE, Young WS. Hypertrophy and Increased Gene Expression of Neurons Containing Neurokinin-B and Substance-P Messenger Ribonucleic Acids in the Hypothalami of Postmenopausal Women. Endocrinology [Internet]. 1991 May 1 [cited 2023 Mar 1];128(5):2239–47. Available from: https://academic.oup.com/endo/article/128/5/2239/2534979

29. Rometo AM, Krajewski SJ, Voytko M Lou, Rance NE. Hypertrophy and Increased Kisspeptin Gene Expression in the Hypothalamic Infundibular Nucleus of Postmenopausal Women and Ovariectomized Monkeys. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2007 Jul 1 [cited 2023 Mar 1];92(7):2744–50. Available from: https://academic.oup.com/jcem/article/92/7/2744/2598866

30. Cholanian M, Krajewski-Hall SJ, McMullen NT, Rance NE. Chronic Oestradiol Reduces the Dendritic Spine Density of KNDy (Kisspeptin/Neurokinin B/Dynorphin) Neurones in the Arcuate Nucleus of Ovariectomised Tac2-Enhanced Green Fluorescent Protein Transgenic Mice. Journal of Neuroendocrinology. 2015 Apr;27(4):253–63.

**31. \*Rometo AM, Krajewski SJ, lou Voytko M, Rance NE. Hypertrophy and Increased Kisspeptin Gene Expression in the Hypothalamic Infundibular Nucleus of Postmenopausal Women and Ovariectomized Monkeys. The Journal of Clinical Endocrinology & Metabolism. 2007 Jul 1;92(7):2744–50.**

32. Dacks PA, Krajewski SJ, Rance NE. Activation of Neurokinin 3 Receptors in the Median Preoptic Nucleus Decreases Core Temperature in the Rat. Endocrinology. 2011 Dec 1;152(12):4894–905.

33. Krajewski-Hall SJ, Blackmore EM, McMinn JR, Rance NE. Estradiol alters body temperature regulation in the female mouse. Temperature. 2018 Jan 2;5(1):56–69.

**34. \*Padilla SL, Johnson CW, Barker FD, et al. A Neural Circuit Underlying the Generation of Hot Flushes. Cell Reports. 2018 Jul;24(2):271–7.**

35. León S, Fergani C, Talbi R, et al. Tachykinin Signaling Is Required for Induction of the Preovulatory Luteinizing Hormone Surge and Normal Luteinizing Hormone Pulses. Neuroendocrinology. 2021;111(6):542–54.

36. Casper RF, Yen SSC, Wilkes MM. Menopausal Flushes: a Neuroendocrine Link with Pulsatile Luteinizing Hormone Secretion. Science [Internet]. 1979 [cited 2023 Mar 1];205(4408):823–5. Available from: https://www.science.org/doi/10.1126/science.462193

37. Tataryn I V., Meldrum DR, Lu KH, et al. LH, FSH and skin temperature duing the menopausal hot flash. The Journal of Clinical Endocrinology & Metabolism [Internet]. 1979 Jul 1 [cited 2023 Mar 1];49(1):152–4. Available from: https://academic.oup.com/jcem/article/49/1/152/2679283

38. Kerdelhué B, Williams RF, Lenoir V, et al. Variations in Plasma Levels of Substance P and Effects of a Specific Substance P Antagonist of the NK&lt;sub&gt;1&lt;/sub&gt; Receptor on Preovulatory LH and FSH Surges and Progesterone Secretion in the Cycling Cynomolgus Monkey. Neuroendocrinology. 2000;71(4):228–36.

39. Mulley G, Mitchell JR, Tattersall RB. Hot flushes after hypophysectomy. BMJ. 1977 Oct 22;2(6094):1062–1062.

40. Gambone J, Meldrum DR, Laufer L, et al. Further Delineation of Hypothalamic Dysfunction Responsible for Menopausal Hot Flashes. The Journal of Clinical Endocrinology & Metabolism [Internet]. 1984 Dec 1 [cited 2023 Mar 1];59(6):1097–102. Available from: https://academic.oup.com/jcem/article/59/6/1097/2676078

41. Sheehan HL, Kovács K. The Subventricular Nucleus of the Human Hypothalamus. Brain [Internet]. 1966 Sep 1 [cited 2023 Mar 3];89(3):589–614. Available from: https://academic.oup.com/brain/article/89/3/589/313103

42. Rance NE, Mc mullen NT, Smialek JE, et al. Postmenopausal Hypertrophy of Neurons Expressing the Estrogen Receptor Gene in the Human Hypothalamus. The Journal of Clinical Endocrinology & Metabolism [Internet]. 1990 Jul 1 [cited 2023 Mar 1];71(1):79–85. Available from: https://academic.oup.com/jcem/article/71/1/79/2652468

43. Rometo AM, Rance NE. Changes in Prodynorphin Gene Expression and Neuronal Morphology in the Hypothalamus of Postmenopausal Women. Journal of Neuroendocrinology [Internet]. 2008 Dec 1 [cited 2023 Mar 2];20(12):1376–81. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2826.2008.01796.x

**44. \*Jayasena CN, Comninos AN, Stefanopoulou E, et al. Neurokinin B Administration Induces Hot Flushes in Women. Scientific Reports. 2015 Feb 16;5(1):8466.**

45. Jayasena CN, Comninos AN, de Silva A, et al. Effects of Neurokinin B Administration on Reproductive Hormone Secretion in Healthy Men and Women. The Journal of Clinical Endocrinology & Metabolism. 2014 Jan 1;99(1):E19–27.

**46. \*Crandall CJ, Manson JE, Hohensee C, et al. Association of genetic variation in the tachykinin receptor 3 locus with hot flashes and night sweats in the Women’s Health Initiative Study. Menopause. 2017 Mar;24(3):252–61.**

**47. \*Prague JK, Roberts RE, Comninos AN, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. The Lancet. 2017 May;389(10081):1809–20.**

48. Skorupskaite K, George JT, Veldhuis JD, et al. Neurokinin 3 Receptor Antagonism Reveals Roles for Neurokinin B in the Regulation of Gonadotropin Secretion and Hot Flashes in Postmenopausal Women. Neuroendocrinology. 2018;106(2):148–57.

49. Depypere H, Timmerman D, Donders G, et al. Treatment of Menopausal Vasomotor Symptoms With Fezolinetant, a Neurokinin 3 Receptor Antagonist: A Phase 2a Trial. The Journal of Clinical Endocrinology & Metabolism. 2019 Dec 1;104(12):5893–905.

50. Fraser GL, Lederman S, Waldbaum A, et al. A phase 2b, randomized, placebo-controlled, double-blind, dose-ranging study of the neurokinin 3 receptor antagonist fezolinetant for vasomotor symptoms associated with menopause. Menopause. 2020 Apr;27(4):382–92.

51. Lederman S, Shapiro CM M, Stute P, et al. Phase 3 Study of Fezolinetant for Treatment of Moderate-to-Severe Vasomotor Symptoms Associated With Menopause [A132]. Obstetrics & Gynecology. 2022 May;139(1):39S-39S.

**52. \*Johnson KA, Martin N, Nappi RE, et al. Efficacy and Safety of Fezolinetant in Moderate to Severe Vasomotor Symptoms Associated With Menopause: A Phase 3 RCT. The Journal of Clinical Endocrinology & Metabolism. 2023 Feb 3;**

**53. \*Neal-Perry G, Cano A, Lederman S, et al. Safety of Fezolinetant for Vasomotor Symptoms Associated With Menopause. Obstetrics & Gynecology. 2023 Mar 9;**

54. ClinicalTrials.gov. A Study to Find Out if Fezolinetant Helps Reduce Moderate to Severe Hot Flashes in Women in Asia Going Through Menopause (Moonlight 1) [Internet]. [cited 2023 Mar 11]. Available from: https://clinicaltrials.gov/ct2/show/NCT04234204

55. Beaudoin FL, McQueen RB, Wright A, et al. Fezolinetant for Moderate to Severe Vasomotor Symptoms Associated with Menopause: Effectiveness and Value. 2022 Dec.

56. ClinicalTrials.gov. A Study to Assess the Safety and Tolerability of Fezolinetant in Women Seeking Treatment for Relief of Vasomotor Symptoms (VMS) Associated With Menopause (Moonlight 3) [Internet]. [cited 2023 Mar 11]. Available from: https://clinicaltrials.gov/ct2/show/NCT04451226

57. Ridler K, Gunn RN, Searle GE, et al. Characterising the plasma-target occupancy relationship of the neurokinin antagonist GSK1144814 with PET. Journal of Psychopharmacology. 2014 Mar 15;28(3):244–53.

58. Pawsey S, Mills EG, Ballantyne E, et al. Elinzanetant (NT-814), a Neurokinin 1,3 Receptor Antagonist, Reduces Estradiol and Progesterone in Healthy Women. The Journal of Clinical Endocrinology & Metabolism. 2021 Jul 13;106(8):e3221–34.

59. Trower M, Anderson RA, Ballantyne E, et al. Effects of NT-814, a dual neurokinin 1 and 3 receptor antagonist, on vasomotor symptoms in postmenopausal women: a placebo-controlled, randomized trial. Menopause. 2020 May;27(5):498–505.

60. Ratti E, Carpenter DJ, Zamuner S, et al. Efficacy of Vestipitant, A Neurokinin-1 Receptor Antagonist, in Primary Insomnia. Sleep. 2013 Dec 1;36(12):1823–30.

61. Simon JA, Anderson RA, Ballantyne E, et al. Efficacy and safety of elinzanetant, a selective neurokinin-1,3 receptor antagonist for vasomotor symptoms: a dose-finding clinical trial (SWITCH-1). Menopause. 2023 Mar;30(3):239–46.

62. Anderson RA, Cormier J, Thieroff-Ekerdt R, et al. Pharmacodynamic Activity of the Novel Neurokinin-3 Receptor Antagonist SJX-653 in Healthy Men. The Journal of Clinical Endocrinology & Metabolism. 2020 Dec 1;105(12):e4857–65.

63. Anderson RA, Millar RP. The roles of kisspeptin and neurokinin B in GnRH pulse generation in humans, and their potential clinical application. Journal of Neuroendocrinology. 2021 Dec 28;

64. McCarthy EA, Dischino D, Maguire C, et al. Inhibiting Kiss1 Neurons With Kappa Opioid Receptor Agonists to Treat Polycystic Ovary Syndrome and Vasomotor Symptoms. The Journal of Clinical Endocrinology & Metabolism. 2022 Jan 1;107(1):e328–47.

65. Lederman S, Ottery FD, Cano A, et al. Fezolinetant for treatment of moderate-to-severe vasomotor symptoms associated with menopause (SKYLIGHT 1): a phase 3 randomised controlled study. The Lancet. 2023 Mar;