Sustained Exposure to the Investigational Kisspeptin Analog, TAK-448, Down-Regulates Testosterone into the Castration Range in Healthy Males and in Patients With Prostate Cancer: Results From Two Phase 1 Studies

David B. MacLean, Hisanori Matsui, Ajit Suri, Rachel Neuwirth, and Marc Colombel

Takeda Pharmaceuticals International Co (D.B.M., A.S., R.N.), Cambridge, Massachusetts 02139; Takeda Pharmaceutical Company, Ltd (H.M.), Kanagawa 251-8555, Japan; and Hospital Edouard Herriot (M.C.), 69003 Lyon, France

Background/Objective: Kisspeptin-54, an endogenous naturally occurring ligand of the G protein-coupled receptor-54, stimulates GnRH-gonadotropin secretion and suppresses metastases in animal models of cancer but is subject to rapid degradation and inactivation. TAK-448 is an investigational oligopeptide analog of the fully active 10-amino acid C terminus of kisspeptin-54. This phase 1 study evaluated the safety, pharmacokinetics, and pharmacodynamics of TAK-448 in healthy subjects and patients with prostate cancer (PC).

Design: Healthy subjects aged 50 years or older received TAK-448 sc as a single-bolus or 2-hour infusion (0.01–6 mg/d; part A) and as a 14-day sc administration (0.01–1 mg/d; part B). In a subsequent, open-label, phase 1 study in PC patients aged 40–78 years, TAK-448 was given as a 1-month depot formulation.

Results: Eighty-two healthy subjects received TAK-448; 30 received placebo. Grades 1–2 adverse events were reported in 26% of subjects during TAK-448 treatment. All dosing regimens resulted in dose-proportional exposures. The maximum observed plasma concentration occurred after 0.25–0.5 hours, and median terminal elimination half-life was 1.4–5.3 hours. T increased approximately 1.3- to 2-fold by 48 hours after a single bolus or 2 hour injections, whereas during the 14-day infusion, at doses above 0.1 mg/d, T dropped to below-baseline values by 60 hours and reached a subsequently sustained below-castration level by day 8. In PC patients, T decreased to less than 20 ng/dL in four of five patients dosed with 12 or 24 mg TAK-448 sc-depot injections. The prostate-specific antigen decreased greater than 50% in all patients dosed with 24 mg.

Conclusions: Continuous TAK-448 infusion was well tolerated by healthy males and resulted in sustained T suppression. Depot injection in patients with PC similarly reduced T and resulted in prostate-specific antigen responses. (***J Clin Endocrinol Metab*** **99: E1445–E1453, 2014)**

Prostate cancer (PC) remains the most prevalent male cancer and the second leading cause of cancer death in men (1, 2). Most PC is diagnosed early (3), and localized disease may be initially treated with surgery and/or radiation therapy with curative intent. When disease recurs, evidenced most often by rising prostate-specific antigen (PSA) or in patients presenting with advanced/metastatic disease, androgen deprivation therapy is recommended (4). However, androgen deprivation therapy is not curative and progression to castration-resistant PC often re-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 27, 2013. Accepted April 14, 2014.
First Published Online April 24, 2014

Abbreviations: AUC, area under the plasma concentration time curve; BMI, body mass index; C_{max}, maximum observed plasma concentration; GPR-54, G protein-coupled receptor-54; KISS1, kisspeptin; PC, prostate cancer; PD, pharmacodynamic; PK, pharmacokinetic; PSA, prostate-specific antigen; SAE, serious adverse event; TEAE, treatment-emergent adverse event; T_{max}, maximum TAK-448 concentration.

quires additional experimental or recently approved endocrine or chemotherapy-based interventions (5–7).

Most cancer deaths result from complications caused by tumor cell metastasis rather than the original tumor growth. Recently metastasis suppressor genes, which inhibit the spread of cancers to secondary sites, have become the target of clinical and basic cancer research (8, 9). Kisspeptin (formerly known as metastin), an endogenous agonist ligand of the kisspeptin (KISS1) receptor OT7T175/G protein-coupled receptor 54 (GPR54), has demonstrated metastasis suppression in melanoma and breast cancer models (10–12). Kisspeptin has no direct toxic effect on cells lacking GPR54 expression but reduces proliferation in GPR54-positive cells by mechanisms that are not fully understood (13). Kisspeptin expression is down-regulated in PC and correlates with clinical stage; loss of kisspeptin correlates with enhanced metastatic capacity in PC cell lines (9).

Kisspeptin has a well-defined role in the regulation of hypothalamic-pituitary-gonadal function (13–15) and is a key regulator of GnRH and gonadotropin release (16–21). Administration of exogenous kisspeptin(45–54), the fully active C-terminal, 10-amino acid peptide, induces gonadotropin secretion in animal models and humans (16, 17, 19–22). However, full-length kisspeptin and kisspeptin(45–54) are subject to rapid degradation in vivo, resulting in a search for peptides with increased metabolic stability while retaining agonist activity (23, 24).

TAK-448 (Figure 1A) is an investigational oligopeptide analog of kisspeptin and a potent agonist of the GPR54 receptor (22). In animals, acute TAK-448 administration stimulates LH/FSH release, whereas continuous sc exposure rapidly down-regulates the pituitary-gonadal axis, with rapid reduction of T levels in a dose-dependent manner (22, 25) (Figure 1B). TAK-448 has exhibited potent antitumor activity in rat androgen-dependent prostate cancer models (26). Therefore, TAK-448 may be useful in the treatment of PC in humans.

This paper reports on a phase 1 study in healthy males evaluating the single-dose and 2-week multiple-dose safety, pharmacokinetic (PK), and hormone-release profile of TAK-448 administered as sc bolus or infusions. In addition, results are reported from a subsequent, open-label phase 1 study that explored the activity of TAK-448 administered as a single-dose, 1-month depot formulation in patients with PC.

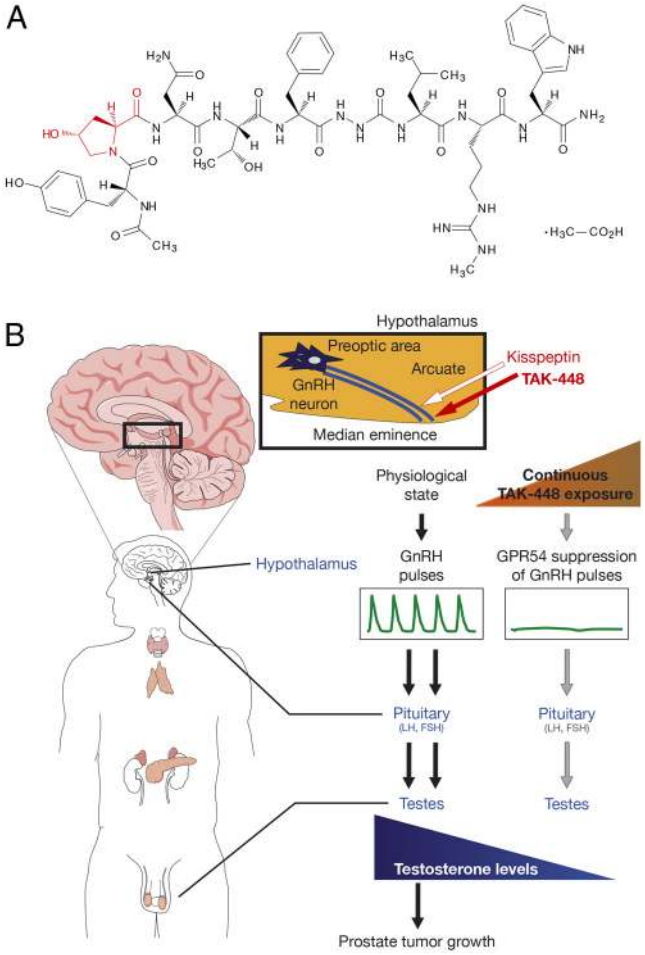

Figure 1. TAK-448 structure (A) and effect on the hypothalamic-pituitary-gonadal axis (B). At the normal physiological state, GnRH pulses in the hypothalamus lead to an elevation in T levels via stimulation of the pituitary. Continuous administration of TAK-448 suppresses GnRH pulses and T levels, primarily through the desensitization of the KISS1 receptor/GPR54. A low level of nonpulsatile GnRH leakage may be detected due to residual receptor activity (18).

Materials and Methods

Subjects

These studies were conducted according to the study protocols, the Declaration of Helsinki/good clinical practice, and all the applicable local and regional laws and regulations. For the two studies conducted in France, Institutional Review Boards under French Regulatory Authority Review approved all aspects of the study. All subjects and patients with PC provided written informed consent.

For investigations in healthy males, eligible subjects were aged 50 years or older, weighing 50 kg or greater, with a body mass indexes (BMI) between 18.5 and 32.0 kg/m^2 at screening. Key inclusion criteria were absence of clinically relevant disease or clinical conditions, LH, FSH, SHBG, and T levels within the normal range by conventional immunoassay, and normal clinical laboratory testing including liver function tests. Key exclusion criteria were a history of cancer; serum PSA greater than 4 ng/mL, use of any hormone preparation within 12 weeks for part A (single ascending dose), or within 4 weeks for part B (14 d administration).

In the subsequent study in patients with PC, the primary objectives were the safety and PK of TAK-448 after a single dose of a 1-month TAK-448 depot formulation (number

NCT01132404). Males aged 40–78 years were eligible to enroll if they had completed their primary treatment for cancer 6 months or longer prior to screening and were either on GnRH agonist therapy or a potential candidate for GnRH therapy based on evidence of biochemical (PSA) recurrent disease, which was nonmetastatic and relatively indolent. Due to additional uncertainty regarding the release profile of the formulation and its consequent therapeutic benefit, patients on concurrent GnRH therapy were eligible to remain on that therapy during evaluation of lower TAK-448 doses (6 and 12 mg). Patients not on GnRH were excluded if the baseline PSA was less than 2 ng/mL or PSA-doubling time were less than 3 months. For patients on established concurrent or intermittent GnRH-analog therapy, a PSA-doubling time of more than 4 months was required without a lower PSA limit.

Study designs

The studies were conducted in France with the study in healthy subjects at a single center and the subsequent study in men with PC at five centers.

The study in healthy subjects was a phase 1, randomized, placebo-controlled, double-blind ascending dose trial (Supplemental Table 1). All subjects entered the study unit the day prior to dosing and remained until 72 hours after the final dose. In part A, healthy subjects were enrolled into nine dose cohorts to receive TAK-448 or placebo single sc-bolus dose of 0.001, 0.003, 0.01, 0.03, 0.1, or 0.3 mg or as a 2-hour sc infusion of 1, 3, or 6 mg, in ascending dose order (Supplemental Table 1). Staggered enrollment occurred at each dose level to review preliminary safety results in two subjects prior to completion of the entire cohort. All injections were administered in the morning after a minimum 8-hour fast.

In part B, healthy subjects in sequential cohorts were randomized to active TAK-448 or placebo (3:1). On day 1, a 0.1 mg sc bolus dose of study drug or placebo was administered to mimic the usual bolus release of peptide that occurs during the first day after a depot administration due to relatively unbound peptide within the depot matrix. On day 2, a continuous infusion of study drug was initiated and continued for 13 days, with only brief interruptions for syringe changes (Supplemental Table 1). The sequential cohorts on days 2–14 received total per-day doses of 0.01, 0.1, 0.3, or 1 mg. All infusions were administered using standard insulin infusion pumps, with needle insertion into the abdomen sc space. Dose escalation between cohorts was based on data from at least six subjects at the previous dose, which included safety, PK, and endocrine profiles.

In the subsequent phase 1 study in men with PC, a single dose of TAK-448 was given as a 1-month depot formulation at 6, 12, or 24 mg in diluent/matrix volumes of 1.2 mL via sc injection at a lateral abdominal site. The initial three patients also received a contralateral, placebo injection. (Placebo injections were subsequently discontinued due to the presence in the formulation of a sterile precipitate that did not form in the TAK-448 suspension.) Based on initial PK and safety findings, additional patients were to be enrolled at additional dose levels with provisions for expansion of a given dose cohort to up to six patients.

PK sampling and analyses

TAK-448 plasma and urine concentrations were measured by validated liquid chromatography with tandem mass spectroscopy. For data analysis, concentrations below the lower limit of quantification (5 pg/mL) were set to zero.

PK blood sampling schedules are detailed in Supplemental Table 2. In part A, urine samples were collected predose (−12 to 0 h) and during five predefined time periods up to 72 hours after the dose. PK analyses included a maximum observed plasma concentration (C_{max}), area under the plasma concentration time curve on day 1 ($AUC_{0-24\ h}$), AUC through day 29 ($AUC_{0-29\ d}$), AUC to the time of last quantifiable concentration ($AUC_{0\text{-last}}$), maximum TAK-448 concentration (T_{max}), and time to last quantifiable concentration.

Pharmacodynamic (PD) sampling and analyses

PD sampling schedules are detailed in Supplemental Table 2 and included sampling for LH, FSH, T, SHBG, prolactin, TSH, corticotrophin, cortisol, dehydroepiandrosterone sulfate, dehydroepiandrosterone, dihydrotestosterone, and androstenedione.

Gonadotropins were measured by conventional RIA. T was assayed using conventional immunoassay for part A (single bolus administration; assay range 20–1200 ng/dL) and liquid chromatography with tandem mass spectroscopy for part B (continuous infusion) and in PC patients by a central laboratory (ICON plc; assay range 2–300 ng/dL). All assays were fully validated and had acceptable within- and between-assay coefficient of variations.

Safety

Safety variables included treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), clinical laboratory tests (hematology, serum chemistry, and urinalysis), vital signs (pulse oximetry, 12 and 2 lead electrocardiograms, and 24 h, 3-lead Holter electrocardiogram during screening) and physical examinations. Adverse events were graded by intensity, coded using MedDRA version 12.0, and assessed for relatedness to the study drug.

Results

Parts A and B in healthy volunteers

In part A (single ascending dose), 82 healthy male subjects were enrolled; mean age was 59.8 years, mean BMI was 25.6 kg/m^2, and all subjects were white with one black/African (details in Supplemental Table 3). Fifty-nine subjects received a single sc bolus injection of TAK-448 or a single sc 2-hour continuous infusion and 23 received placebo. In part B (14 d administration), 30 healthy subjects were enrolled; mean age was 60.8 years, mean BMI was 25.3 kg/m^2, and all but one were white. Twenty-three subjects received TAK-448 and seven subjects received placebo.

LH and T concentration profile (PD)

In normal subjects, the overall pattern of LH and T concentrations after single-dose injections (part A) were remarkably similar across the broad dose range of 0.001 (1 μg) to 6 mg. In all cases, an initial abrupt LH rise was followed by sustained LH concentration increases

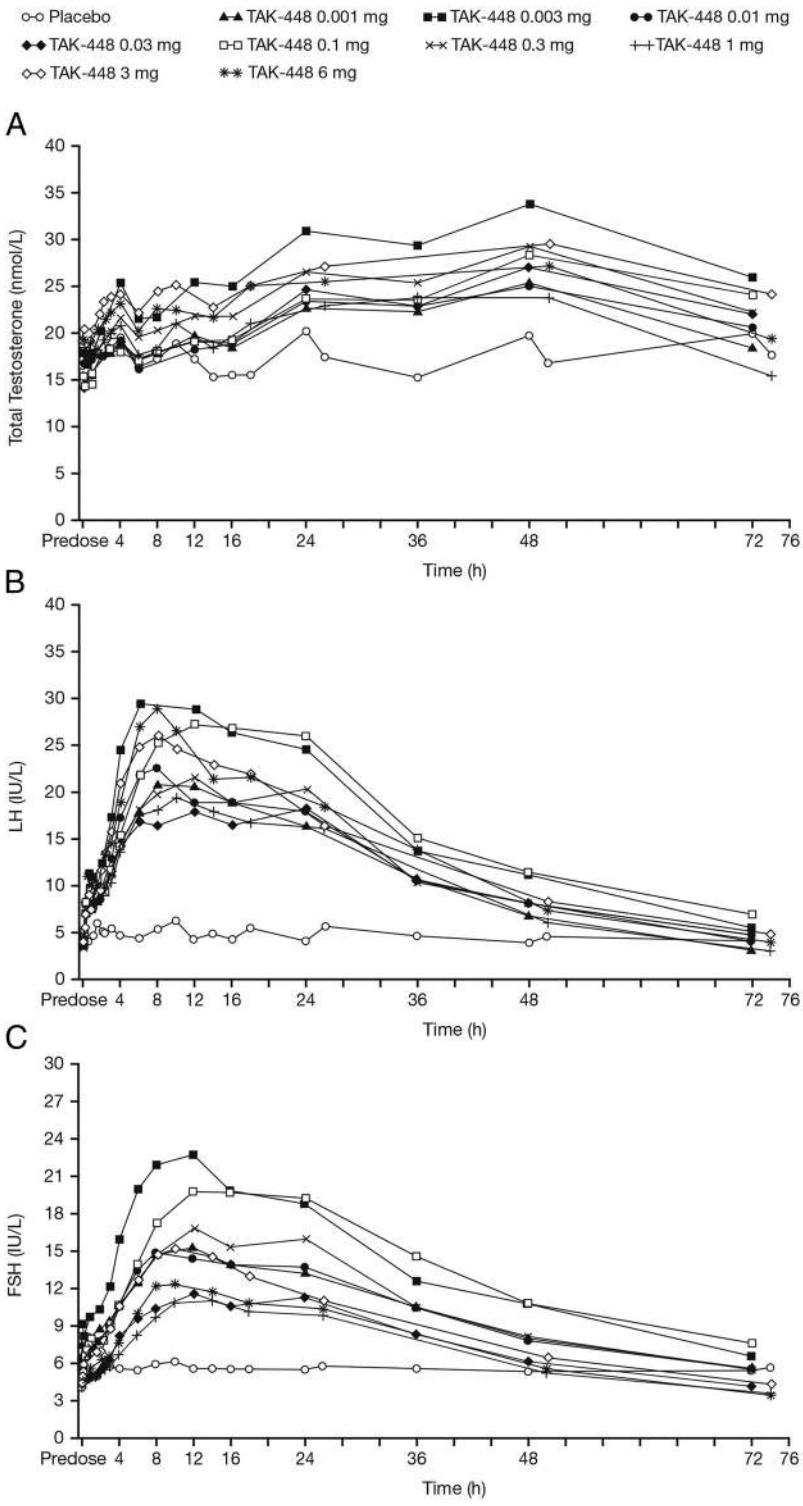

Figure 2. Part A: mean serum concentration-time profiles of total T (A), LH (B), and FSH (C) in healthy subjects receiving TAK-448 as a single-bolus dose (0.001–0.3 mg) or a 2-hour infusion (1–6 mg).

through the subsequent 12–48 hours (Figure 2). Although statistical inferences were not possible with the large overlap among doses and small cohort sizes, there is no suggestion that doses greater than 0.03 mg are more effective than the lower doses. Increases in serum T were consistent with but lagged behind the LH rise and subsequent return to baseline. Similar to the LH results, the data do not suggest that doses greater than 0.03 mg are more effective at initiating or prolonging the increased T concentrations. There were no changes in the pooled data from subjects (one to two per dose cohort) receiving placebo. The pattern of the LH/T release was not appreciably altered in subjects receiving 2-hour infusions (Figure 2). However, variability in the T concentration over time was generally less pronounced in those high dose groups.

In part B, after the 0.1-mg sc bolus injection on day 1, the expected acute rise in LH and T were observed in subjects randomized to active TAK-448 (Figure 3). However, within 24 hours of initiating continuous sc infusion, LH and T concentrations declined to below baseline values by day 2, reached nadir values by day 4, and remained low throughout the remainder of the infusion period. T levels returned to the normal range by 14 days after discontinuation of the infusion (Figure 3A).

The lowest infusion dose of 0.01 mg/d was associated with incomplete LH/T suppression (Figure 3). However, regardless of dose level, all subjects had at least one time point at which T concentration was below castration level (<1.74 nmol/L or 50 ng/dL). Except for one subject in the 0.3 mg/d cohort, all subjects in the 0.1, 0.3, and 1 mg/d cohorts had T concentrations below the castrate level during most of the 14-day sc infusion, and a majority of values were below 0.7 nmol/L (20 ng/dL). Changes in FSH in response to single-bolus injection or continuous infusion in general paralleled those of LH.

Other endocrine markers

In normal subjects, after a single-bolus dose or a 2-hour infusion of TAK-448 (part A), there were no relevant

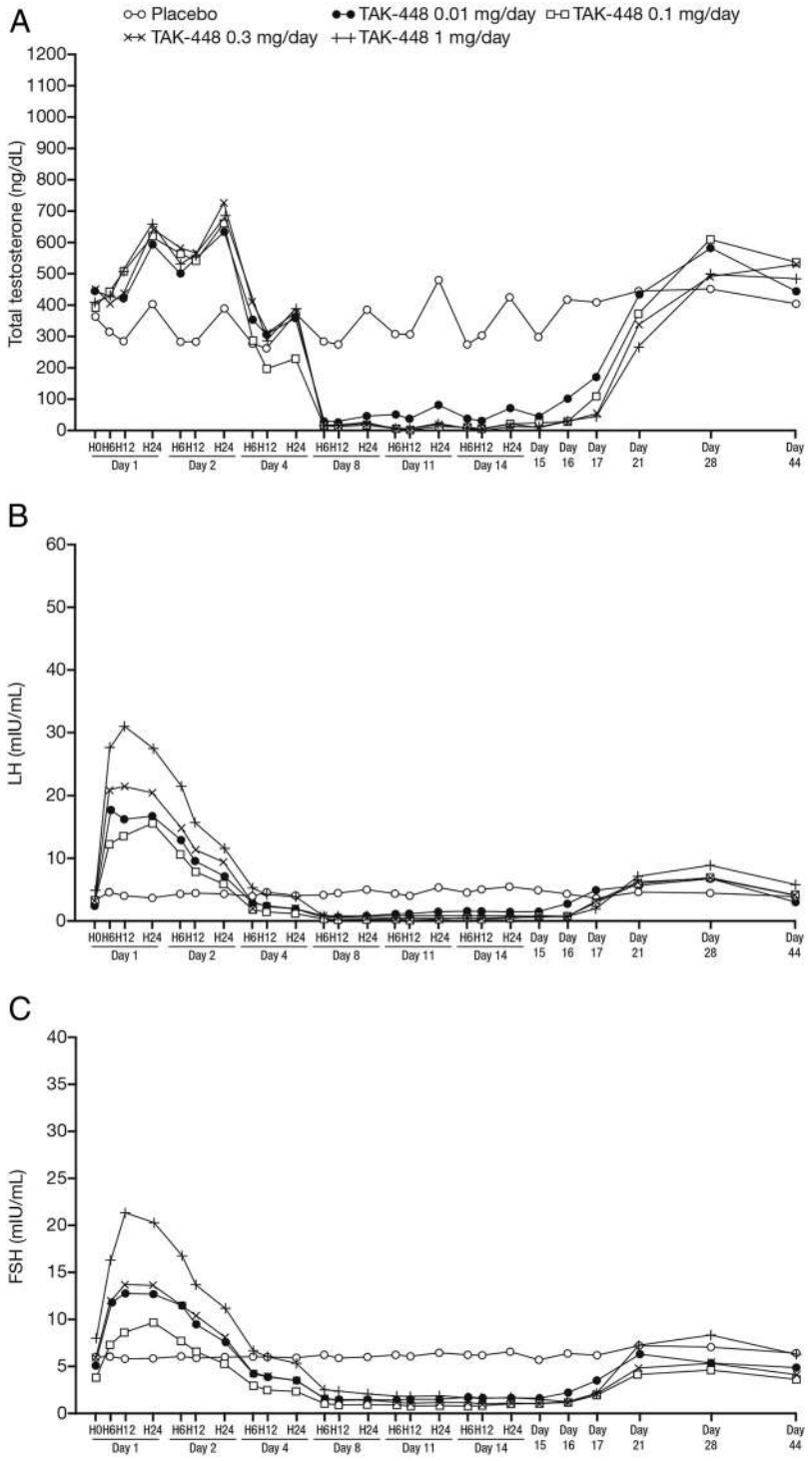

Figure 3. Part B: mean serum concentrations of T (A), LH (B), and FSH (C) after TAK-448 administered as a single-bolus dose (d 1) and followed by a 13-day infusion (d 2–14) in healthy subjects.

changes in prolactin, TSH, or corticotrophin, suggesting that TAK-448 is specific for the GnRH/LH axis.

Changes in serum 5α-dihydrotestosterone concentrations generally were similar to those of serum T at all doses of TAK-448. In part B, the mean serum 5α-dihydrotestosterone and androstenedione concentrations decreased to values below baseline during the continuous TAK-448 infusion and returned to near baseline values by 7 days after infusion discontinuation.

Results after 1-month depot injection in patients with PC

In the TAK-448 depot formulation study, nine evaluable patients, aged 58–73 years, were enrolled (three in each of the 6 mg, 12 mg, and 24 mg dose groups; Supplemental Table 4). All three patients in the 6 mg dose group and one of three in the 12 mg dose group were on concomitant GnRH therapy for indolent biochemical (PSA) disease recurrence, and thus, evidence of TAK-448-mediated T lowering was not discernible. In one of two GnRH-naïve patients at 12 mg, and in all three GnRH-naive patients at 24 mg, after the TAK-448 administration, T decreased to less than 20 ng/dL over the first month and began to return to normal levels during month 2 (Figure 4). Similar reductions occurred in LH. A greater than 50% PSA decrease from baseline was seen at month 1, day 29, in all three patients in the 24 mg dose group, which were considered consistent with the observed changes in T.

Pharmacokinetics

Plasma PK parameters for TAK-448 administered as a single-bolus dose or as a 2-hour infusion in normal subjects (part A) are shown in Table 1 and Figure 5A.

Mean exposure to TAK-448 (C_{max}, AUC, median terminal elimination half-life) increased in proportion to the dose between 0.001 and 0.3 mg (Table 1 and Figure 5A). The maximum concentration of TAK-448 was reached 0.25–0.5 hours after a single-bolus dose and decreased thereafter; TAK-448 was not detectable in plasma after 24 hours. Mean apparent oral clearance and T_{max} were similar at all doses. After a 2-hour infusion, T_{max} for TAK-448 was reached at approximately 2.25 hours after the start of infusion (ie, 0.25 h after the end of infusion; Table 1 and Figure 5A).

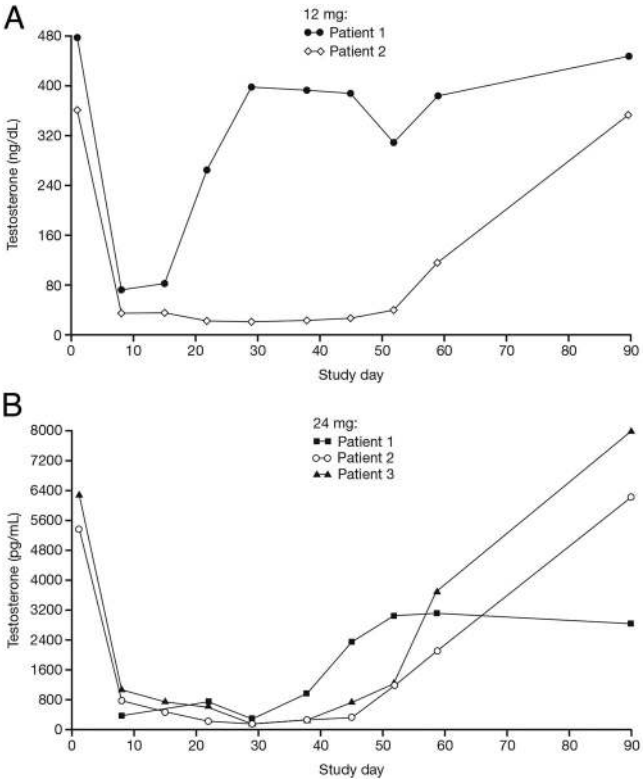

Figure 4. Patients with PC not receiving GnRH: aggregate individual serum T concentration-time curves after TAK-448 administration at 12 mg (A) and 24 mg (B).

Mean exposure increased in proportion to the dose (Table 1), and the median terminal elimination half-life was lower for the 1-mg infusion than for the 3- and 6-mg infusions. Apart from T_{max}, all other plasma PK parameters generally showed a consistent behavior across the dose levels, with generally low intersubject variability.

Statistical analysis of dose proportionality indicated that the increase in exposure to TAK-448 in normal subjects (C_{max} and $AUC_{0\text{-}inf}$) was proportional to the administered dose. The 95% confidence interval (CI) for the estimated slope of the regression line for $AUC_{0\text{-}inf}$ for all cohorts and the C_{max} for the bolus cohorts as well as the 2-hour infusion cohorts were within the equivalence limits of 0.8–1.25 (Supplemental Table 5). The CI included 1, indicating dose proportionality.

In part B (14 d administration), the mean plasma PK parameter values (C_{max}, T_{max}, and $AUC_{0\text{-}last}$) for TAK-448 administered as a single 1-mg bolus (d 1) followed by a 13-day infusion (d 2–14) are summarized in Supplemental Table 6. On days 2–14, the steady-state concentration increased in an apparent dose-proportional fashion over the dose range studied with low intersubject variability throughout the infusion period (Figure 5B). In patients with PC and not receiving concurrent GnRH analogs, the T-lowering results observed at the 24-mg dose level was associated with relatively low TAK-448 concentrations (10–300 pg/mL), confirming findings observed in the 14-day study in healthy subjects.

Safety

In parts A and B (single bolus or 2 h and 14 d infusions), 21 of 82 receiving TAK-448 (26%) experienced at least one TEAE, compared with 3 of 30 subjects receiving placebo (10%) (Supplemental Table 7). In part A with 7 of 59 subjects receiving TAK-448 (12%) and 1 of 23 subjects receiving placebo (4%), at least one TEAE was considered related to the study drug. In part B, 14 of 23 subjects receiving TAK-448 and one of seven receiving placebo (14%) experienced at least one TEAE considered related to the study. All TEAEs were mild or moderate in intensity and no adverse events grade 3 or greater or SAEs were reported. There were no TEAEs leading to the discontinuation of the study drug and no deaths occurred during the study. The most common adverse events in part A were diarrhea, postural dizziness, headache, hyperhydrosis, and hot flush. Adverse events in part B were similar to part A and included injection site reaction (Supplemental Table 7) as well as vasomotor and related symptoms associated with acute T lowering.

The TAK-448 1-month depot was well tolerated, with no adverse events related to the rapid-release phase of the depot formulation that occurred during day 1. All nine treated patients experienced at least one AE after the TAK-448 depot administration. The most common TEAEs (after either TAK-448 or placebo injections) were grade 1 injection site reactions: erythema (n = 6, 67%), induration (n = 5, 56%), hematoma (n = 3, 33%), and pain (n = 3, 33%). Most of these reactions occurred at the site of the TAK-448 injections with the exception of hematoma and pain (n = 1 each) occurring at the site of placebo injection. Other TEAEs were either nonspecific or related to acute T lowering (ie, asthenia, hot flush). There were no grade 3 or greater adverse events, no SAEs, and no deaths during the study.

Discussion

Kisspeptin-GPR54 signaling in the hypothalamus has a pivotal role in the regulation of GnRH-gonadotropin secretion. Based on evidence that kisspeptin-stimulated LH/FSH release can be inhibited by GnRH antagonists and that kisspeptin does not directly stimulate LH/FSH release from the pituitary, kisspeptin activation of GPR54 occurs primarily on GnRH-containing neurons, which thereby controls secretion from pituitary gonadotropes. In this study, using the kisspeptin oligopeptide analog TAK-448, we confirmed the potent stimulatory role of kisspeptin

Table 1. Geometric Mean (Percentage Coefficient of Variation) Plasma PK of TAK-448 Among Healthy Subjects After a Single-Bolus Dose or 2-Hour Infusion

	TAK-448 (Bolus)						TAK-448 (2 Hour Infusion)		
Parameter	0.001 mg (n = 7)	0.003 mg (n = 7)	0.01 mg (n = 7)	0.03 mg (n = 7)	0.1 mg (n = 7)	0.3 mg (n = 6)	1 mg (n = 6)	3 mg (n = 6)	6 mg (n = 6)
C_{max}, pg/nL	13.3 (19)	42.4 (30)	140.1 (44)	340.2 (18)	1382 (27)	4349 (34)	13 051 (21)	39 403 (15)	82 478 (23)
$AUC_{0\text{-inf}}$, h/pg · mL	47.5 (34)	122.2 (16)	371.0 (27)	1108.1 (21)	3944 (15)	13 214 (25)	43 825 (26)	14 1489 (11)	317 753 (26)
$AUC_{0\text{-last}}$, h/pg · mL	26.7 (22)	100.9 (20)	345.2 (29)	1070 (21)	3888 (15)	13 156 (25)	43 656 (26)	14 1393 (11)	317 667 (26)
$t_{½}$, h[a]	1.8 (1.2–4.6)	1.4 (1.2–2.3)	2.2 (1.8–2.7)	2.6 (1.5–3.1)	3.1 (2.3–3.6)	3.5 (3.0–4.1)	3.6 (3.5–3.8)	5.2 (3.2–5.9)	5.3 (4.6–5.6)
T_{max}, h[a]	0.5 (0.25–1.0)	0.75 (0.5–1.0)	0.5 (0.25–0.75)	0.5 (0.25–0.75)	0.5 (0.25–0.75)	0.625 (0.25–0.75)	2.25 (2.0–2.25)	2.25 (2.25–2.5)	2.25 (2.0–2.5)
CL/F, L/h	21.1 (34)	24.5 (16)	27.0 (27)	27.1 (21)	25.4 (15)	22.7 (25)	22.8 (26)	21.2 (11)	18.9 (26)
Vz/F, L	63.9 (27)	55.9 (34)	85.6 (35)	96.0 (15)	108.0 (21)	114.2 (33)	120.0 (28)	149.9 (29)	141.0 (29)

Abbreviations: $AUC_{0\text{-inf}}$, area under the plasma concentration-time curve from time 0 to infinity; $AUC_{0\text{-last}}$, AUC from time 0 to the time of last quantifiable concentration; CL/F, apparent oral clearance; $t_{½}$, terminal elimination half-life; T_{max}, time to reach C_{max}; Vz/F, apparent volume of distribution.

[a] Median (range).

and, for the first time in humans, demonstrated with continuous dosing a profound down-regulation of the gonadotropin-testicular axis. Continuous infusion of TAK-448, as well as changes after the depot administration, results initially in a transient modest rise in LH/T occurring during the first 24 hours, followed by a subsequent rapid down-regulation of LH/T that is fully achieved within approximately 4 days. The findings suggest thatTAK-448 may have a useful role as a novel rapid inhibitor of the hypothalamic-pituitary-gonadal axis for sex steroid deprivation therapy.

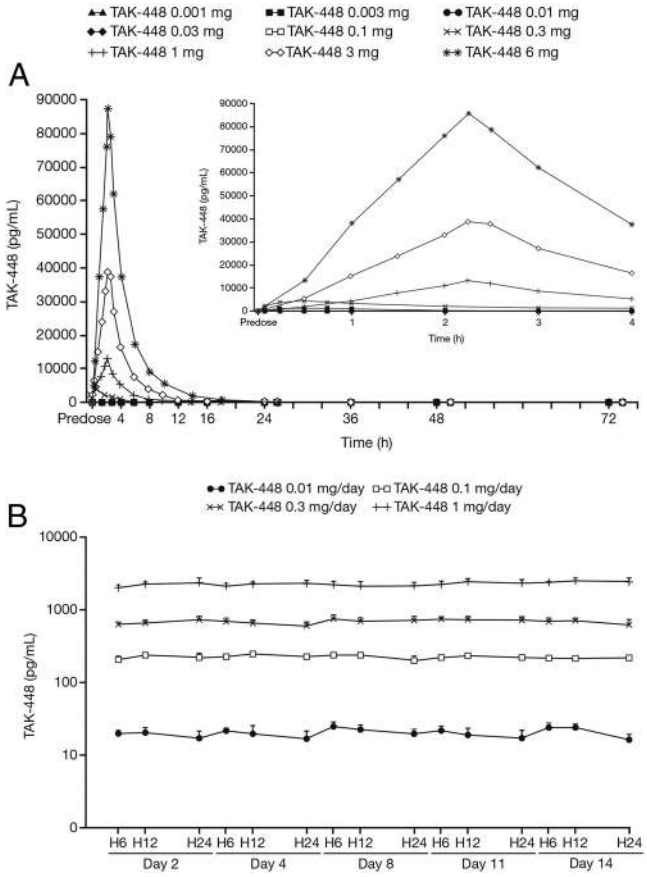

Figure 5. Mean plasma concentrations of TAK-448 up to 72 hours after the administration of a single-bolus dose in healthy subjects (part A of study; inset shows concentrations after 4 h) (A); and in healthy subjects during a 13-day sc infusion of TAK-448 (d 2–14), by treatment group (part B of study) (B).

In part A, single-dose TAK-448 was effective at the lowest dose (1 μg) for acute LH stimulation and subsequent downstream increase in T. Estimated plasma concentrations at this low dose are well below the in vitro estimated IC_{50}, 23 μmol/L (95% CI 210–250) (26). In addition, such low concentrations might not be expected to efficiently access hypothalamic neuronal (GnRH) cell bodies. The findings suggest, but do not address, that TAK-448 is binding and activating GnRH nerve endings within the median eminence, which is fully exposed to the systemic circulation (27). In addition, the PK/PD relationships in the single-dose studies demonstrate that the stimulatory effects of TAK-448 on LH/T release persist well after the disappearance of detectable plasma TAK-448 concentrations. Consistent with other studies in cycling women, kisspeptin may have a prolonged tonic effect on GnRH pulses (28). Results of single TAK-448 injections, as observed in part A, reinforce the observations of George et al (29) that the kisspeptin/GPR54 receptor acutely is a potent stimulant of LH release. Furthermore, George et al reported that differences between TAK-448 and native kisspeptin-10 on the dose response and results of bolus vs infusion administration are likely due to differences in potency and intrinsic half-life (stability) of the native peptide vs the synthetic analog (29).

In part B with continuous TAK-448 infusion, LH and T secretions were profoundly down-regulated at a continuous infusion of low daily doses and the associated steady-state concentrations of TAK-448. The minimum estimated mean steady-state concentration value associated with castration-level T suppression in all subjects

throughout the final week of dosing was 228 pg/mL at an infusion dose of 0.1 mg/d. Thus, it is more likely that continued exposure above a supraphysiological threshold level is likely required for desensitization. In men, as reported by George et al (29), there was loss of pulsatility and reduced LH response with higher dose kisspeptin infusions, thus also suggesting that desensitization is likely a function of both peak and duration of (continuous) exposure. Kisspeptin infusion studies in women suggest that desensitization may be more likely to occur in women with disturbed (hypothalamic amenorrhea) (30) vs normal regulation of the hypothalamic-pituitary-gonadal axis (31). Whether there is differential vulnerability to kisspeptin desensitization in men, eg, as a result of age or metabolic status, will require further study.

In patients with PC, the TAK-448 1-month depot resulted in highly variable and relatively low concentrations of TAK-448. Further work with this specific formulation was subsequently discontinued. However, the PK and T-lowering results observed at the 24-mg dose level, in patients not receiving concurrent GnRH analogs, confirmed the findings observed in the 14-day study in healthy subjects.

Based on a variety of nonclinical evidence (22, 25), the apparent desensitization associated with continuous TAK-448 administration occurs by a different mechanism compared with GnRH-agonist analogs, which may cause desensitization of the GnRH signaling pathways in the pituitary (30). The finding suggests that rapid down-regulation of GnRH/LH secretion occurs in response to continuous TAK-448 exposure, similar to previous findings in male animals (22, 25).

TAK-448 was well tolerated in healthy male subjects or patients with PC over a wide dose and exposure range, suggesting that GPR54 receptor function is physiologically very specific and that full activation of the receptor is well tolerated. Nondose-related orthostatic hypotension was attributed to the sedentary state of inpatient volunteers. Diarrhea or more profound dizziness, with associated bradycardia, was reported at the highest bolus dose levels (>3 mg/d) and occurred at TAK-448 concentrations greater than 30 000 pg/mL; these may not be related to specific GPR54 activation. Other side effects in patients with PC were attributable to the injection site itself or to the effects of T lowering into the castration range.

The kisspeptin peptides were originally discovered in nonmetastasizing melanoma cell lines and thus originally named metastin. Kisspeptin was subsequently identified in other local tumor types including breast and PC (9, 32). However, the role of this signaling system in tumor biology remains controversial. In this study, the observed changes in PSA were consistent with corresponding reductions in T. However, no additional inferences regarding antitumor activity are possible based on the single 1-month depot administration. Rather, longer-term studies that include a GnRH comparator arm would be required to detect additional antitumor activity of TAK-448 beyond those effects due directly to medical castration.

Acknowledgments

We acknowledge the writing assistance of Stephen Mosley and Dawn L. Lee (FireKite Ltd) in the development of this manuscript.

Trial identifications were C18001 and; C18002 (EUCTR2009-017668-18-FR.

The study was registered at clinicaltrials.gov with the number of NCT01132404.

Address all correspondence and requests for reprints to: David B. MacLean, MD, Takeda Pharmaceuticals International Co, 35 Landsdowne Street, Cambridge, MA 02139. E-mail: david.maclean@takeda.com.

This work was supported by Takeda Pharmaceuticals International Co. Writing support was funded by Millennium: The Takeda Oncology Company.

Disclosure Summary: D.B.M., A.S., and R.N. are employees of Takeda Pharmaceuticals International Co, Cambridge, Massachusetts; H.M. is an employee of Takeda Pharmaceutical Company Ltd, Fujisawa, Japan. M.C. has received research funding from Millennium: The Takeda Oncology Company.

References

1. **Center MM, Jemal A, Lortet-Tieulent J, et al.** International variation in prostate cancer incidence and mortality rates. *Eur Urol*. 2012; 61:1079–1092.
2. **Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D.** Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
3. **Cooperberg MR, Lubeck DP, Meng MV, Mehta SS, Carroll PR.** The changing face of low-risk prostate cancer: trends in clinical presentation and primary management. *J Clin Oncol*. 2004;22:2141–2149.
4. **National Comprehensive Cancer Network.** *Clinical Practice Guidelines in Oncology. Prostate Cancer*. Version 2. Fort Washington, PA: National Comprehensive Cancer Network; 2013.
5. **de Bono JS, Oudard S, Ozguroglu M, et al.** Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet*. 2010;376:1147–1154.
6. **de Bono JS, Logothetis CJ, Molina A, et al.** Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med*. 2011; 364:1995–2005.
7. **Scher HI, Fizazi K, Saad F, et al.** Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012; 367:1187–1197.
8. **Li N, Wang HX, Zhang J, Ye YP, He GY.** KISS-1 inhibits the proliferation and invasion of gastric carcinoma cells. *World J Gastroenterol*. 2012;18:1827–1833.
9. **Wang H, Jones J, Turner T, et al.** Clinical and biological significance of KISS1 expression in prostate cancer. *Am J Pathol*. 2012;180: 1170–1178.

10. **Lee JH, Miele ME, Hicks DJ, et al.** KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst*. 1996; 88:1731–1737.
11. **Lee JH, Welch DR.** Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, *KiSS-1*. *Cancer Res*. 1997;57:2384–2387.
12. **Lee JH, Welch DR.** Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. *Int J Cancer*. 1997;71:1035–1044.
13. **Kotani M, Detheux M, Vandenbogaerde A, et al.** The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*. 2001; 276:34631–34636.
14. **Murphy KG.** Kisspeptins: regulators of metastasis and the hypothalamic-pituitary-gonadal axis. *J Neuroendocrinol*. 2005;17:519–525.
15. **Nash KT, Phadke PA, Navenot JM, et al.** Requirement of KISS1 secretion for multiple organ metastasis suppression and maintenance of tumor dormancy. *J Natl Cancer Inst*. 2007;99:309–321.
16. **Dhillo WS, Chaudhri OB, Patterson M, et al.** Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab*. 2005;90:6609–6615.
17. **Gottsch ML, Cunningham MJ, Smith JT, et al.** A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*. 2004;145:4073–4077.
18. **Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T.** Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. *Biochem Biophys Res Commun*. 2004;320:383–388.
19. **Messager S, Chatzidaki EE, Ma D, et al.** Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci USA*. 2005;102:1761–1766.
20. **Navarro VM, Castellano JM, Fernandez-Fernandez R, et al.** Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. *Endocrinology*. 2004;145:4565–4574.
21. **Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM.** Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci USA*. 2005;102:2129–2134.
22. **Matsui H, Tanaka A, Yokoyama K, et al.** Chronic administration of the metastin/kisspeptin analog KISS1-305 or the investigational agent TAK-448 suppresses hypothalamic pituitary gonadal function and depletes plasma testosterone in adult male rats. *Endocrinology*. 2012;153:5297–5308.
23. **Asami T, Nishizawa N, Ishibashi Y, et al.** Serum stability of selected decapeptide agonists of KISS1R using pseudopeptides. *Bioorg Med Chem Lett*. 2012;22:6391–6396.
24. **Asami T, Nishizawa N, Ishibashi Y, et al.** Trypsin resistance of a decapeptide KISS1R agonist containing an Nomega-methylarginine substitution. *Bioorg Med Chem Lett*. 2012;22:6328–6332.
25. **Tanaka A, Matsui H, Asami T, et al.** Suppression of testosterone release by chronic administration of investigational novel metastin analogs in male dogs and monkeys, and in healthy male volunteers. Paper presented at: 22nd European Organisation for Research and Treatment of Cancer-National Cancer Institute-American Association for Cancer Research Symposium on Molecular Targets and Cancer Therapeutics; Berlin, Germany: November 16–19, 2010.
26. **Matsui H, Masaki T, Akinaga Y, et al.** Anti-tumor growth effect of TAK-683, a metastin analogue, in preclinical androgen-dependent prostate cancer models. Paper presented at: 22nd European Organisation for Research and Treatment of Cancer-National Cancer Institute-American Association for Cancer Research Symposium on Molecular Targets and Cancer Therapeutics; Berlin, Germany: November 16–19, 2010.
27. **Uenoyama Y, Inoue N, Pheng V, et al.** Ultrastructural evidence of kisspeptin-gonadotrophin-releasing hormone (GnRH) interaction in the median eminence of female rats: implication of axo-axonal regulation of GnRH release. *J Neuroendocrinol*. 2011;23:863–870.
28. **Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB.** Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. *J Clin Endocrinol Metab*. 2012;97:E1458–E1467.
29. **George JT, Veldhuis JD, Roseweir AK, et al.** Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. *J Clin Endocrinol Metab*. 2011;96:E1228–E1236.
30. **Jayasena CN, Nijher GM, Abbara A, et al.** Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. *Clin Pharmacol Ther*. 2010;88:840–847.
31. **Dhillo WS, Chaudhri OB, Thompson EL, et al.** Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *J Clin Endocrinol Metab*. 2007;92:3958–3966.
32. **Zajac M, Law J, Cvetkovic DD, et al.** GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness. *PLoS One*. 2011;6:e21599.