PROTOCOL SUMMARY

Evaluating Alternative Medical Therapies in Primary Hyperparathyroidism

NCT02525796

2/2/2021

<u>AIM:</u> To conduct a double-blinded, placebo-controlled, randomized intervention study to investigate whether mineralocorticoid receptor (MR) antagonism, alone or in combination with cinacalcet, is an effective therapy for primary hyperparathyroidism (P-HPTH).

- *<u>Hypothesis:</u>* The aim will test the hypothesis that MR antagonism is a mechanism to lower parathyroid hormone (PTH) in primary hyperparathyroidism (P-HPTH).
- *<u>Study Design:</u>* Sixty subjects with P-HPTH will be enrolled to randomly receive eplerenone (a potassium-sparing diuretic that directly blocks the MR), amiloride (a potassium-sparing diuretic that *does not* directly block the MR), or placebo for 4 weeks +/- 3 days. Thereafter, *all* subjects will receive cinacalcet therapy (a calcimimetic that lowers PTH) *in addition* to their randomized intervention for an additional 2 weeks +/- 3 days.
- *<u>Anticipated Results:</u>* In this novel proof-of-concept study, eplerenone therapy will lower PTH, serum calcium, and markers of bone resorption in P-HPTH, when compared to placebo. The PTH response to amiloride will resemble that of placebo, suggesting that the eplerenone mediated reductions in PTH are specific to interactions with the MR. Combination therapy with eplerenone + cinacalcet will result in additive or synergistic reductions in PTH, when compared to placebo + cinacalcet or placebo + amiloride.
- *<u>Implications:</u>* MR antagonism (alone or in combination with cinacalcet) may be a mechanism to lower PTH and calcium in P-HPTH, thereby identifying a new potential option in the limited medical therapies for P-HPTH.

BACKGROUND AND SIGNIFICANCE

This proposal will investigate a novel endocrine relationship between the renin-angiotensin-aldosterone system (RAAS), the mineralocorticoid receptor (MR), and parathyroid hormone (PTH) that can be targeted to improve human skeletal health.

♦ **<u>The RAAS and skeletal health:</u>** The RAAS physiologically regulates sodium homeostasis and plays a pathophysiologic role in propagating cardiovascular disease; aldosterone (ALDO) and the mineralocorticoid receptor (MR) are the crucial mediators of these RAAS-associated effects. In this context, the use of RAAS inhibitors and MR antagonists has revolutionized the approach to cardiovascular disease. These pharmacologic therapies that inhibit the RAAS and block ALDO

have significantly advanced public health efforts to mitigate and treat hypertension, heart failure and death. However, it is clear that interrupting the RAAS has many *pleiotropic benefits* *beyond* cardiovascular health alone. One key example is the link between inappropriately high RAAS activity and the risk for developing skeletal diseases. Observational studies have shown that high ALDO levels in primary aldosteronism are associated with a high likelihood of developing osteoporosis and fragility fracture. Conversely, MR antagonism to block ALDO in small studies has been associated with protection from fracture. **Our published and preliminary data suggest that ALDO, via stimulation of the MR, can raise PTH levels and that blocking the MR may lower PTH levels.**

♦ **PTH and skeletal health:** PTH regulates calcium and bone homeostasis; however, persistent elevations in PTH decrease bone integrity and increase risk for fragility fractures. One common disorder of continuously elevated PTH is primary hyperparathyroidism (P-HPTH). Recent trends in the U.S. suggest that the incidence of P-HPTH is higher than previously believed, with >100,000 new cases annually with a rapidly rising prevalence (76 to 233 cases per 100K, from 1995-2010). Despite this alarming rise in P-HPTH prevalence, its etiology remains largely unknown. Surgical parathyroidectomy is recommended when P-HPTH is sufficiently advanced to induce clinically detectable end-organ pathology. Surgery can cure P-HPTH, *but not in all cases*; the large variation in surgical practice patterns at low-volume centers is associated with higher rates of operative failure (>5%), complications (>2%), re-operation (>5%), and mortality (>1%). The future of consistently safe and effective parathyroid surgery is likely to involve highly specialized centers performing more resource-intensive procedures. Further, for many older patients (with high cardiovascular risk), or those with recurrent disease, non-surgical therapies may be preferred to minimize risk. *Therefore, new alternative non-surgical therapies for P-HPTH are much needed*. Although cinacalcet (a calcimimetic) can lower PTH levels in P-HPTH, trials have failed to show it can improve skeletal and cardiovascular endpoints. Importantly, P-HPTH is a chronic disease, that is often monitored for years prior to definitive attempts at surgical therapy; during this monitoring time, medical therapies capable of lowering PTH levels could lower the risk of incident sequalae such as osteoporosis, nephrocalcinosis, and hypercalcemia. **Our data strongly support a novel modifiable interaction between ALDO, the MR, and PTH that may represent a target for medical therapies to lower PTH, and skeletal and cardiovascular disease risk, in P-HPTH.**

♦ **The ALDO-MR-PTH Interaction:** We have developed strong evidence (see detailed protocol) to support a modifiable interaction that may address the aforementioned public health problems: **an endocrine relationship whereby ALDO stimulates PTH secretion via the MR expressed in parathyroid tissue.** Our published and preliminary data suggest that this relationship: **1)** May represent a novel determinant of PTH secretion and development of P-HPTH; **2)** Can be interrupted with a RAAS inhibitor or MR antagonist; **3)** and may be a modifiable target to reduce the risk of skeletal outcomes that are exacerbated by excess PTH, such as low bone mineral density and fragility fractures. This proposal will investigate the novel interplay between the RAAS and PTH using an innovative proof-of-concept clinical trial. The demonstration that MR antagonists can treat P-HPTH by lowering PTH (and calcium and bone turnover) could have significant public health implications.

RESEARCH DESIGN AND METHODS

This is an interventional study where up to 60 subjects with active and untreated P-HPTH will be evenly randomized to receive eplerenone, amiloride or placebo in a double-blinded fashion. It is anticipated that up to 75 subjects will need to be enrolled to account for participant non-completion and withdrawals. After 4 weeks +/- 3 days of treatment, each study arm will receive cinacalcet in addition to their blinded experimental therapy. There are 3 main study visits as well as additional safety visits, which are described below. All visits will occur in the outpatient clinical research centers at BWH.

SAMPLE SIZE:

A sample size of up to 60 total subjects has been chosen using very conservative calculations stemming from our preliminary data (see Detailed Protocol). Therefore, at most there will be 20 subjects per study arm; however, our sample size calculations suggest that we are likely to be adequately powered with a significantly smaller sample size as well. These sample size calculations to achieve sufficient power to observe the primary and secondary outcomes of the study:

- **Primary Outcome:** Change in PTH levels (ΔPTH) following randomized drug intervention (**Figure; visits A=>B**).
- **Secondary Outcomes:** Change in serum iCa and markers of bone resorption following randomized drug intervention (**Figure; visits A=>B**).
- Exploratory Outcome 1: ΔPTH in response to amiloride therapy (**Figure; visits A=>B**)
- Exploratory Outcome 2: ΔPTH in response to combination drug therapy with EPL+ cinacalcet (**Figure; visits A=>B**).
- Exploratory Outcome 3: Correlation of eplerenone-induced ΔPTH with MR expression from adenomatous/ hyperplastic parathyroid glands of subjects that undergo parathyroidectomy following study completion.

INCLUSION CRITERIA: The target study population is healthy individuals with **a physician diagnosis of active and untreated P-HPTH**. P-HPTH will be classified as: Serum calcium > upper limit of reference range (ULRR) **AND** serum PTH > ULRR; or Serum Calcium > ULRR **AND** serum PTH > 30 pg/mL; or Serum Calcium within 0.2 mg/dL of the ULRR and PTH>ULRR. Subjects with multiple endocrine neoplasia or recurrent P-HPTH following parathyroidectomy will be included since their response to PTH lowering therapy does not differ when compared to sporadic forms of P-HPTH. Subjects with hypertension will be included if their blood pressure is <150/90 mmHg. However, since it is known that ACE inhibitors and ARB's can influence PTH and that the combination of MR antagonists and ACEi/ARB medications can increase the risk of electrolyte disturbances, subjects on an ACEi or ARB will be asked to discontinue these medications for 2 weeks prior to study initiation and during the course of the study. We expect it to be very rare that a potential participant already be prescribed an MR antagonist (such as spironolactone or eplerenone) or an ENaC inhibitor (such as amiloride or triamterene); however, in these rare instances participants may still participate after a 2 week washout of these medications. Due to the potassium sparing effects of the possible study medications eplerenone/amiloride, subjects taking potassium supplements at the time of enrollment may also be asked to stop their potassium supplement (their potassium will be measured throughout our protocol, every 1-2 weeks, as part of our elaborate safety monitoring). For subjects undergoing a washout of an anti-hypertensive medication, during this medication washout, they will be prescribed doxazosin, an alpha-antagonist that does not interact with the RAAS or PTH, ***if needed***, for BP control to 105-150/50-90 mmHg, as the PI has safely performed in multiple prior

research protocols (see details below). Once the study interventions are initiated (below), doxazosin will be lowered or stopped if target BP is maintained. Women of child-bearing age (age 18-50) must have a negative pregnancy test.

EXCLUSION CRITERIA: Subjects will be excluded if they have an estimated glomerular filtration rate < 60mL/min/1,73m^2, serum potassium > upper limit of reference range, age <18 or >80 years, diabetes that is not well controlled (HbA1c>8%), liver or heart failure, history of myocardial infarction or stroke, use of atypical antipsychotic medications or lithium, active chronic inflammatory conditions (such as inflammatory bowel disease, rheumatoid arthiritis, sarcoidosis), initiation within 3 months of bisphosphonates or cinacalcet, need for imminent parathyroidectomy (within the next 6-8 weeks) as determined by their endocrinologist or surgeon, absolute serum calcium >13.0 mg/dL, or a positive pregnancy test on any of the study visits for women ages 18-50.

STUDY PROCEDURES:

- *Screening Visit:* Subjects will be contacted vis telephone or Doximity Dailer to confirm eligibility and schedule screenings visits. Doximity Dialer will be used to minimize in-person contact if study staff are unable to go into research facilities due to the COVID-19 pandemic. An in-person screening visit will involve a physical examination, blood pressure measurement, detailed history intake by the study PI, and if needed, screening labs to determine eligibility. Subjects who have had the necessary screening laboratories for clinical purposes in the 6 weeks preceding this screening visit (including serum calcium, PTH, potassium, creatinine, HbA1c) will not be required to repeat this testing if it is available for the PI to verify. Pregnancy testing for women aged 18-50 will be a part of the screening visit, as well as Study Visits 1, 3 and 5. Menopausal woman who have not had a period for the past 12 months or more, will not need to have a pregnancy test. Also, participants who have had any well-documented method of surgical sterilization will not need to have a pregnancy test. Methods of surgical sterilization include having had a hysterectomy, bilateral oophorectomy, a tubal ligation, or transvaginal occlusion. All other female participants must have a negative pregnancy test before starting the study drug.

Females who are sexually active and able to become pregnant, must agree to use an approved method of birth control for the entire study and for at least 1 month after their last dose of study drug. Abstinence, condoms, diaphragms, birth control pills, birth control patches and birth control injections will be considered acceptable methods. If the participant plans to use a birth control pill, patch or injection, they will be instructed to start using it at least one month before the washout period.

The screening visit will also provide time for the PI to describe the study procedures and risks in detail, review the consent form (which the subject will have had for >24 hrs prior to the visit), and provide informed consent.

In light of the COVID-19 pandemic, a remote screening visit procedure has been developed to minimize in-person contact if potential participants and study staff are unable to go into research facilities. This visit will be done by phone and televisit. We will schedule a time to complete the first portion of the screening visit over the phone at a time that is most convenient for the participant. The first portion of this visit will entail at-home monitoring of blood pressure. We will provide an at-home blood pressure monitor for the participant to measure blood pressure. Participants will be asked to remain seated with feet flat on the ground and to rest for 5-minutes prior to beginning these measurements. After 5-minutes, participants will be instructed to measure and record blood pressure and pulse three times, 2-minutes apart. At the time of the scheduled phone call, these blood pressure measurements and current height and weight are to be reported to us. In addition, personal medical history will be reviewed. If the participant meets the preliminary requirements of the study, a televisit will be scheduled with the study investigator/physician for further evaluation of eligibility.

Participants will then undergo a brief physical examination and confirmatory review of past medical history via televisit with the study investigator/physician. If the remote screening visit shows result consistent with inclusion criteria, and does not meet any exclusion criteria, we will inform the participant of their eligibility to come in for screening labs. This entails a blood draw and a urine pregnancy test (if applicable), to ensure there are no abnormalities that would

prevent participation. If the results of the screening labs meet the requirements of the study, we will inform participants within a few days that they are eligible to participate.

If participants are unable to physically come in to have screening labs drawn, they will be asked to inform us. Labs drawn within the last 3 months of signing the consent form that provide results required of the study may be used in place of screening labs to determine eligibility.

- *Study Visits:* Following screening, this study involves three main study visits (**Figure**).
    - **Visit A:** to establish baseline measurements.
    - **Visit B:** 4 weeks +/- 3 days later to assess study outcomes following blinded, randomized, intervention.
    - **Visit C:** 2 weeks +/- 3 days later to assess study outcomes following cinacalcet add-on therapy. Additional safety visits are involved (black diamonds in **Figure**).

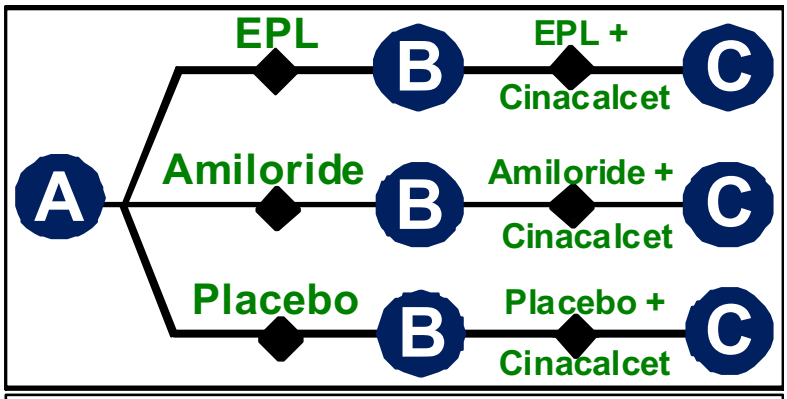

Figure 8: Aim 1 Study Design. Subjects with P-HPTH will be randomized to EPL, amiloride, or placebo for 4 weeks +/- 3 days, and then receive add-on therapy with CT for an additional 2 weeks +/- 3 days.

• *Study Drug Interventions:* Enrolled subjects will undergo detailed measurements of outcome variables (see detailed outcome variables below) to establish a baseline (**Figure, visit A**). All study visits will be conducted in the morning, following at least 1 hour of monitored supine rest in the Clinical Research Center. Following study visit A, subjects will be randomized to the blinded study drug by the BWH Research Pharmacies. The initial dose of the blinded study drug will be dictated by the subject's blood pressure and the PI's clinical judgment as it relates to reasonable safety. Subjects with blood pressures near the lower range of our safety margin of 105/50 mmHg will receive the lowest dose, and subjects with higher blood pressures (or known hypertension) will receive a higher dose. The initial doses will range from: eplerenone (EPL) 12.5 mg/d to 25 mg/BID, amiloride 1.25 mg/d to 2.5 mg/BID, or placebo. The EPL arm represents the active intervention: a direct MR antagonist. The placebo arm represents a negative control. Since EPL is expected to influence more than just PTH (EPL will also decrease blood pressure and urinary excretion of potassium and calcium) when compared to placebo, the amiloride intervention represents a *second control* to provide insights into the *mechanism* by which EPL may influence PTH. Like EPL, amiloride is a potassium-sparing diuretic, with mild anti-hypertensive and hypocalciuric effects, and will therefore induce similar changes in blood pressure and urinary potassium/calcium excretion. Unlike EPL, amiloride does not directly block the MR. Rather, it blocks epithelial sodium transport channels (ENaC) in the renal distal tubule (and vascular epithelium).

Following a safety visit at t=2 weeks +/-3 days (see details of safety visits below), the blinded study drug may increase from the initial dose if the PI perceives the risk for hypotension to be low and as long as all safety parameters are fulfilled. The maximum dose any subject may receive is (EPL 50mg BID, amiloride 5 mg BID, placebo 1 dose BID). If the safety parameters are not within normal limits, the safety check will be repeated within 5 days and the dose may be reduced. At t=4 weeks +/- 3 days, subjects will return to the Clinical Research Center to assess outcome variables (**Figure, visit B**). Following visit B, all subjects will receive open-label cinacalcet ***in addition*** to continuing their blinded study drug. The initial dose of cinacalcet will be dictated by the subject's serum calcium levels and the PI's clinical judgment as it relates to reasonable safety. The initial doses of cinacalcet will range from 15 mg/d to 15 mg BID. Generally, if the subject is hypercalcemic, the initial dose will be 15mg BID, and if they are normocalcemic the dose will be 15 mg/d; however, the PI may alter this if other clinical factors indicate a more judicious approach for safety (i.e. preventing hypocalcemia). Following another safety visit 1 week +/- 3 days after cinacalcet initiation, cinacalcet may be increased to a maximum dose of 30 mg BID. If the serum calcium levels have normalized to within reference

range, it will be at the PI's discretion to increase the dose of cinacalcet, or maintain the same dose, based on the perceived risk of hypocalcemia. If the safety parameters are not within normal limits, the safety check will be repeated within 5 days and the cinacalcet dose may be reduced. Cinacalcet acts as a calcimimetic by allosteric activation of the calcium-sensing receptor and thereby decreases PTH. Cinacalcet administration to subjects with P-HPTH will lower PTH and calcium, and will serve to evaluate whether the combination of EPL+cinacalcet can induce *additive or synergistic* reductions in PTH, when compared to those induced by placebo+cinacalcet (see *Anticipated Results*). Subjects who are instructed to increase their dose following the safety check will be told to take the blinded study drug and cinacalcet everyday at 8AM and 8PM. Subjects will return after 2 weeks +/- 3 days of cinacalcet therapy for the final study visit (**Figure, visit C**).

- *Outcome assessment:* The following measurements will be obtained while fasting at every study visit (**A**, **B**, **C**): serum PTH, iCa and total serum calcium, serum phosphate and fibroblast growth factor-23 (FGF-23), 25(OH)D, markers of bone resorption (serum C-telopeptide, urinary N-telopeptide, tartrate-resistant acid phosphatase 5b), markers of bone formation (osteocalcin, bone-specific alkaline phosphatase, P1NP), basic metabolic panel with creatinine, plasma renin activity, serum angiotensin II and ALDO. In addition, 24h urine collections at every study visit, as described above, will be used to measure urinary sodium, potassium, calcium, magnesium, and aldosterone.

Measures of PTH and calcium will be obtained in a standardized fashion after at least 1h of supine rest, at 1h of supine rest, 2h of supine rest, and 3h of supine rest. Subjects will have the option of receiving separate venipunctures at each hour or having an IV placed in their arm for the duration of the study visit which will be used to draw aliquots every hour. The lowest PTH value will be used in analyses.

At study visit A, peripheral blood will be collected for DNA isolation. Although no genetic studies are planned at this time, this DNA will be stored to test future research hypotheses.

Parathyroid tissue will be collected from any subjects that undergo parathyroidectomy (for clinical indications determined by their providers) in the 5 years following their study completion. It is estimated that ~50% of participating subjects may have a surgical parathyroidectomy in the 5 years following their study completion. If the surgery occurs at BWH or MGH, tissue will be collected and flash frozen in liquid nitrogen in the operating room and stored in the BWH pathology

repository, and also formalin fixed in paraffin. Samples will be analyzed for mRNA and protein expression of MR to determine whether there is a correlation of eplerenone-induced ΔPTH with MR expression from adenomatous/ hyperplastic parathyroid glands of subjects that undergo parathyroidectomy following study completion. If the surgery occurs at a non-Partners institution, formalin fixed paraffin embedded blocks of tissue will be requested to core a sample and return the block.

Risks will be minimized in this study by using sound inclusion and exclusion criteria that will permit a study population at very low risk of adverse events; use of study interventions that are relatively very low risk; and a very detailed and meticulous monitoring plan. The risk with the highest potential of developing involves that related to study medications, including cinacalcet, eplerenone, and amiloride. These potential side effects are uncommon, but mainly involve laboratory abnormalities that can be monitored. All subjects will have frequent blood tests during the course of the study to ensure safety. Detailed algorithms to manage these risks are also described in the rare case they may occur (below).

Safety Plans and Visits

We have extensive experience conducting patient-oriented studies and implementing detailed safety plans. To address safety concerns associated with the study drugs, all subjects will have safety blood work performed at and in between every study visit (**Figure; visits A,B,C and black diamonds**) for serum calcium, potassium and creatinine (every 1-2 weeks +/- 3 days throughout the study). The development of hyperkalemia (considered rare since eGFR>60mL/min is an inclusion criteria), renal dysfunction, hypocalcemia, symptomatic hypercalcemia (new symptoms suggestive of memory loss or intense fatigue, or new onset constipation) or an absolute serum calcium>13.0mg/dL, will either result in disempanelment from the study to receive appropriate clinical care, or the decision to not escalate the active medical therapy. All abnormal lab values will be repeated within 1-5 days to ensure they are truly abnormal. The PI will determine, based on the severity of the abnormality, whether it can be managed with a medication dose change or will require discontinuation of medications and disempanelment. If hyperkalemia >6.5 is confirmed, the medications will be stopped and labs repeated the following day, and every 1-2 days until normalization is encountered. If milder hyperkalemia (5.4-6.5) and a substantial decline in renal function (eGFR<60 mL/min AND >25% decline in eGFR) are confirmed, the PI will lower the dose of the study drug if possible and

repeat labs in the next 1-5 days. If this occurs on the lowest dose (EPL12.5 g/d, amiloride 1.25 mg/d, placebo) and is confirmed on repeat testing, then medications will be discontinued, subject disempaneled, and labs followed to normalization as aforementioned. The use of cinacalcet can cause hypocalcemia, although the doses and duration in this study are unlikely to cause this. If calcium lower than the lower limit of reference range is encountered, the dose of cinacalcet will be lowered; if already on the lowest dose of 15mg/d, then the medication discontinued and the subject disempaneled.

If abnormal lab values (including, but not limited, to serum calcium, ionized calcium, serum potassium and serum creatinine) are encountered during the final visit 5, we will ask the participant to return for repeat labs within 1-5 days. Participants will be asked to discontinue all study medications at the end of the final visit 5, therefore the repeat labs will be performed 1-5 days after completion of the study, and it is expected that any abnormalities should resolve. If the results do not resolve, the PI will use his discretion in referring the patient to his or her provider.

All subjects will be taught how to use a home blood pressure monitor, and will be provided with a home blood pressure monitor (automated Omron cuff), since two of the study medications are mild anti-hypertensives (EPL and amiloride). Subjects using an ACEi, ARB, MR antagonist (such as spironolactone or eplerenone), or ENaC inhibitor (such as triamterene or amiloride) will undergo a washout of this medication for 2 weeks prior to study initiation, and will receive the blood pressure monitor at that time (automated Omron cuff). We anticipate the use of MR antagonists or ENaC inhibitors to be extremely infrequent or rare in this study population; however, ACEi and ARB use may be relatively common. Due to the potassium sparing effects of the possible study medications eplerenone/amiloride, subjects taking potassium supplements at the time of enrollment may also be asked to stop their potassium supplement (their potassium will be measured throughout our protocol, every 1-2 weeks, as part of our elaborate safety monitoring). Subjects will be instructed to measure their blood pressure twice daily throughout the study and report it to study staff once weekly. The PI has conducted multiple IRB approved studies with similar anti-hypertensive washout procedures safely. The target blood pressure for the study is 105-150/50-90 mmHg.

If subjects undergoing a washout experience 5 days of consecutive blood pressure readings higher than 150/90mmHg during the washout period, they will be prescribed doxazosin 2mg daily (which may be titrated up to a maximal dose of 8mg daily) to achieve a blood pressure of 105-150/50-90 mmHg. If BP is >180/90 mmHg for more than 3 consecutive days (with our without doxazosin), subjects will be evaluated in person by the PI to confirm the blood pressure levels – if confirmed, the subject will be disempaneled from the study, advised to resume their original anti-hypertensive medication, and with their consent, their primary provider will be contact to relay this important health information.

Upon initiation of the study medications (EPL, amiloride, placebo), subjects with hypertension may experience improvement of their blood pressure since EPL and amiloride are mild anti-hypertensives; subjects will continue monitoring home blood pressure to monitor for low blood pressure. If blood pressure readings fall below 105/50 mmHg for more than 2 days (unlikely), and subjects experience symptoms suggestive of hypotension (lightheaded, dizzy, tired), they will be evaluated in person to confirm their blood pressure is low and that they have related symptoms. If symptomatic hypotension is confirmed, the study drug will either be lowered, if possible, or the subject disempaneled. If subjects experience blood pressure below 90/50 mmHg, even if they report feeling well and without symptoms, we will either lower their study drug or consider disempanelment for blood pressures that are too low. Subjects who are treated with doxazosin will continue to report their blood pressure – the PI may down-titrate the dose of doxazosin once the study medications have started to maintain a blood pressure window of 105-150-50-90 mmHg.

Counseling regarding abstinence and/or contraception, in addition to pregnancy testing at every study visit (**screening, A, B, C**), will be required for women aged 18- 50 (given the potential use of EPL).

RISKS AND DISCOMFORTS

<u>Blood drawing/IV Placement</u>

Phlebotomy does involve a mild stinging or painful sensation, and a very small risk for clot or infection. Standard sterile precautions will be taken to minimize this risk. On rare occasions, a hematoma or phlebitis may occur. In most circumstances, these complications resolve spontaneously or with local heat application.

Over the course of the entire 6-8 week study, we anticipate drawing approximately 450 mL of blood split over 5 study visits. By contrast, the Red Cross permits donation of 473 mL of blood (1 pint) in one session every 8 weeks.

Eplerenone

Common (But uncommon in healthy individuals with normal kidney function at doses used in this study):

Hyperkalemia
Hypotension

Uncommon/Rare:

Dizziness and fatigue
Hyponatremia
Breast pain
Gynecomastia
Hypercholesterolemia
Diarrhea
Abdominal pain
Abnormal vaginal bleeding
Elevated creatinine
Albuminuria
Cough
Flu-like syndrome
Angioneurotic edema
Elevated BUN
Elevated liver function tests
Rash
Elevated uric acid

Amiloride

Common (But uncommon in healthy individuals with normal kidney function at doses used in this study):

Hyperkalemia

Hypotension

Uncommon/Rare:

Gynecomastia

Hyperchloremic metabolic acidosis

Hyponatremia

Abdominal pain

Change in appetite

Constipation

Diarrhea

Gas pain

Nausea and vomiting

Impotence

Muscle cramps and weakness

Cough

Dyspnea

Cinacalcet

Common (But uncommon in individuals with P-HPTH at doses used in this study):

Hypocalcemia

Uncommon/Rare:

Hypotension

Headache

Fatigue

Depression

Nausea

Vomiting

Diarrhea
Anorexia
Constipation
Abdominal pain
Loss of appetite
Backache
Fracture of bone
Spasm
Asthenia
Tingling sensation

Wash-out period: Subjects may experience transient elevations in their BP if they need to stop an antihypertensive per the study protocol. The principal investigator and his group have an established protocol to safely monitor BP during these times and have used this in many prior similar studies (see detailed safety protocol above). In our experience, most patients undergoing the washout protocol will remain within the target BP range; for those who required doxazosin therapy, it is anticipated that with very low doses of doxazosin the target BP will be achieved.

Doxazosin

Common

Hypotension

Uncommon

Nasal congestion

Rash

Angioedema

Dizziness, headache, vertigo

Drowsiness

Fatigue

Pregnancy/Fetus: Pregnant women are at risk, and therefore excluded from this study, given the use of medication intervention. Women who are breast feeding are also excluded. None of the medications used in this study are recommended for pregnancy unless specifically necessary on a case by case basis. In particular, eplerenone can be teratogenic. All women or child-bearing age, age 18- 50, will therefore be counseled on the importance of abstinence and use of barrier or hormonal contraception during the study. Further, urinary pregnancy tests will be conducted at screening and at each study visit.

Menopausal woman who have not had a period for the past 12 months or more, will not need to have a pregnancy test. Also, participants who have had any well-documented method of surgical sterilization, will not need to have a pregnancy test. Methods of surgical sterilization include having had a hysterectomy, bilateral oophorectomy, a tubal ligation, or transvaginal occlusion. All other female participants must have a negative pregnancy test before starting the study drug.

Females who are sexually active and able to become pregnant, must agree to use an approved method of birth control for the entire study and for at least 1 month after their last dose of study drug. Abstinence, condoms, diaphragms, birth control pills, birth control patches and birth control injections will be considered acceptable methods. If the participant plans to use a birth control pill, patch or injection, they will be instructed to start using it at least one month before the washout period.

Privacy/Confidentiality: Subjects will be counseled regarding the importance of patient confidentiality and privacy of their health and research records. Subject information will be kept in de-identified databases and all laboratory tests will be identified through this code that will be stored on a secure Partners computer and accessible only to the PI and the main research coordinator of the study. Information regarding study outcomes can be shared with the subject's primary or specialty providers with their consent.

Tissue/DNA: For those subjects undergoing parathyroidectomy, discarded tissue will be collected, when possible, to conduct molecular and expression studies. These tissues will again be de-identified. Expression studies in the future may involve large quantities of sequencing, and in theory, there is a risk that information from studies involving genetic material may influence insurance companies and/or employers regarding patient health. To safeguard from this, no results of these molecular studies or sequencing analyses will be placed in patient charts.

Allergic Reactions

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or serious, and can even result in death in some cases. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing.

Unknown Risks

There may be other risks or side effects that are not known at this time.

BENEFITS

There is no direct benefit from participating in this study. Subjects who participate will be clearly informed that they are not being treated for any specific medical condition, but rather are volunteering for a study that is evaluating a potential novel medical treatment for P-HPTH. That being said, all participating subjects will in fact receive 2 weeks +/- 3 days of cinacalcet therapy, which is a proven and safe medical treatment to lower PTH and calcium in P-HPTH. Therefore, subjects will be informed that they may receive some transient benefit in their P-HPTH for 2 weeks +/- 3 days in this study, but the main objective of the study is not to be a “treatment study.”

To society, if our study aims were proven correct, the results would provide a novel medical therapy for primary hyperparathyroidism with new molecular insights into disease etiology. Because P-HPTH is such a common epidemiologic occurrence, evidence of a therapy to reduce PTH levels would be beneficial to society as a whole.

EQUITABLE SELECTION OF SUBJECTS

This study will exclude children below the age of 18. This exclusion is primarily for safety reasons – young children should not receive eplerenone during puberty. Further, the hormonal milieu in growing pubescent children is ever-changing, and therefore, from a scientific standpoint only grown adults are included.
We will not exclude any gender or racial/ethnic demographic. However, it is well established that P-HPTH affects women far more than men, and affects white women more than other racial/ethnic demographics, and generally occurs in older age (>40 years). Therefore, it is anticipated that the majority of enrollees will be older, white, women. We anticipate that the study population will reflect the greater metropolitan Boston area that is afflicted with P-HPTH as our recruitment will focus on patients at BWH and MGH.

Elderly individuals (>80 years of age) are being excluded to reduce the incidence of other co-morbid conditions that may increase the risk of adverse events.

RECRUITMENT PROCEDURES

The study will take place at BWH. Our research team has extensive experience and an established infrastructure for recruiting subjects with P-HPTH. Participants will be recruited from 4 sources:

1. *Brigham and Women's Hospital (BWH) Endocrinology Division:* The PI has developed a system to identify and potential subjects with P-HPTH in conjunction with clinical colleagues in his division. Clinicians in the division will be made aware of the study so that they may inform their potentially eligible patients that there is a research study that may be of interest to them. Flyers and advertisements will be provided to division clinicians to give to potentially interested patients. Research coordinators for our study will be present and available to talk to any patients that have indicated to their physician that they are interested in learning more about the study. If a physician in the division discusses the study with their potentially eligible patient, and the patient expresses interest in learning more, they will be contacted by the study team via telephone. If a physician has a potentially eligible patient but has not had the opportunity to discuss the study with them in person, we will send a letter to the patient, cosigned by their

physician and the PI of this study, informing them of their potential eligibility. In the letter, participants will be given our contact information if they are interested, and will be notified that we will call them to provide information about two weeks after receipt of the letter. Patients may contact us with questions or to opt out of receiving further information about the study. Dr. Vaidya will inform his own patients that he is conducting a study for which they might qualify. He will offer his patients the opportunity to take home the Consent Form, and call back if they wish to participate. If the patient expresses interest, study co-investigators (Dr. Mannstadt) will be available to discuss the study in more detail and answer any questions the patient might have. This will reduce the possibility of patients feeling obligated to participate because their own physician is asking.

.

2. *BWH Department of Endocrine Surgery:* The PI works closely with the endocrine surgeons at BWH where a large number of patients with P-HPTH exist. Similarly to #1, parathyroid surgeons will may inform their potential patients about our research study and provide them with our contact information and flyers. Our research coordinators will similarly be available to talk with potentially interested patients once they have discussed with their surgeon that they are interested in learning more. Also, with the permission of the surgeons, our study advertisement may be sent to patients in a patient-packet that is currently sent out by the Endocrine Surgery department prior to surgery consults. This advertisement will include the study coordinator's contact information and will give participants the opportunity to contact us directly to hear more about the study or to opt out.

3. *Massachusetts General Hospital (MGH) Endocrinology Division:* The PI's co-investigator, Dr. Mannstadt, is a parathyroid specialist at MGH who sees a large volume of patients with P-HPTH. A similar recruiting effort to the one at the BWH Division of Endocrinology will be implemented at the MGH division of endocrinology. Dr. Mannstadt will inform his own patients that he is conducting a study for which they might qualify. He will offer his patients the opportunity to take home the Consent Form, and call back if they wish to participate. If the patient expresses interest, study co-investigators (Dr. Vaidya) will be available to discuss the study in more detail and answer any questions the patient might have. This will reduce the possibility of patients feeling obligated to participate because their own physician is asking.

Similar to the strategy outlined at the BWH endocrine division, physicians may discuss the study with potentially eligible patients and those patients will be contacted with details about the study if they give permission to do so. Cosigned letters from the PI and physician may be sent to patients describing the study as outlined above.

4. *MGH Department of Surgery:* A similar recruiting effort to the one at the BWH Surgery Department will be implemented at the MGH surgery department.

Potentially eligible subjects may be pre-screened by telephone or Doximity Dialer to further determine eligibility. A member of the study staff will conduct this phone screen according to the approved telephone script and screening form.

The PI, Co-I, or research coordinator (with EPIC and HIPAA training) will also use the Research Patient Data Registry (RPDR) to identify potential research participants (between the ages of 18-80 with a diagnosis of primary hyperparathyroidism). This database would include the Brigham & Women's Hospital and Partners affiliates i.e. Faulkner Hospital, MGH, Newton Wellesley, etc. Once we have identified potentially eligible patients, we will identify whether they have indicated a "RODY YES" or a "RODY NO" response through the Research Opportunities Direct to You (RODY) program, which allows a patient to agree to be contacted directly by researchers without involving their clinician. For patients that have indicated a "RODY YES" response, we will send them a recruitment letter inviting them to participate in our study. For the "RODY NO" patients, we will identify their primary care physician, or endocrinologist, or other internist with close ties to the patient in our system, and request permission to contact their patient with our IRB approved letter (co-signed by the PI and physician) including information regarding the study. See consent procedures for other details. The letter is used to ask their permission to send their patients a co-signed letter inviting them to learn more about and potentially participate in our study. In the letter, participants will be notified that we will be calling about two weeks after receipt of the letter. For physicians that do not wish to co-sign our recruitment letter, we will give them the option to sign a separate letter notifying their patient of our study. If they agree, they will sign this letter which will be sent to their patient along with our recruitment letter as two separate letters. Our phone number will be provided so

individuals may contact us with questions or to opt out of receiving further information about the study.

We will also utilize the RSVP for Health Database.

Subjects will be offered a total of $400.00 for participating in the study. This quantity is expected to compensate them for their time and transportation expenses. They will receive $100 for completion of each study visit (A, B, C) and an additional $100 for completion of the entire study. In addition, they will receive compensation for any parking expenses. We will reimburse the cost for bus, MBTA, commuter rail, or other public transportation expenses based on receipts that are provided by subjects. Subjects may receive additional compensation of $50/visit for any additional visits that are triggered due to abnormal lab values.

CONSENT PROCEDURES

As subjects are primarily recruited from the MGH and BWH Endocrinology and/or Surgery divisions, the medical records of potential subjects are readily available. The IRB Committee has waved the requirement to obtain informed consent and HIPPAA Authorization for review of medical records, Research Patient Data Registry (RPDR) and other documents containing PHI for the purpose of identifying prospective subjects for recruitment. Medical records of potential participants will be reviewed by the PI/study physicians to determine eligibility (for example, review medical chart to evaluate for inclusion and exclusion criteria) and decide whether arranging a formal screening visit is indicated. This will ensure that only those subjects who are likely to meet all eligibility criteria can be approached about the study.

Potentially eligible patients will be contacted to schedule a formal in-person or remote screening visit, and will be mailed the full study consent form at least 24 hours prior to this visit. At this time, the study physician or PI will conduct screening procedures and review the entire consent form, including study procedures, risks, and benefits.

DATA AND SAFETY MONITORING

The Principal Investigator will be responsible for reviewing the safety and/or efficacy data and determining whether the research should be altered or stopped.

Safety data will be reviewed on every case studied in the PI's monthly research group meeting. The PI's research group, the Cardiovascular Endocrinology Research Group (CERG), meets once a month on a Tuesday. The CERG includes more than 5 patient-oriented clinical investigators who are all endocrinologists with detailed knowledge of human subjects research and hyperparathyroidism and the medications used in this study. At each CERG meeting, each PI presents recruitment status, adverse events/safety issues, minor deviations, and other pertinent study-related issues to the group of PI's. Research coordinators also attend this meeting. Discussion of issues and a plan are formulated for each adverse event. The PI will be responsible for executing any plans agreed upon during these meetings.

In addition, the PI will meet his is co-investigator Dr. Mannstadt at MGH every 3 months to review study progress and safety.

MONITORING AND QUALITY ASSURANCE

The Principal Investigator will be responsible for reviewing the safety and/or efficacy of the data and determining whether the research should be altered or stopped. The study staff will be responsible for monitoring and assuring the validity and integrity of the data and adherence to the IRB-approved protocol. Data collected by the Research Coordinators are independently evaluated by the principal and co-investigators. The original recording of an observation will be retained as the source document. The Principle Investigator will review all data collected on a subject with the study staff once a month. The Principal Investigator will review the accuracy and completeness of the study data and informed consent of each subject as well as adherence to the IRB-approved protocol.

All consent forms are signed by the participant and a study physician. The principal investigators, Anand Vaidya MD, MMSc and Michael Mannstadt, MD, are responsible for monitoring and will meet weekly with study staff to discuss all subject recruitment, novel issues, study procedures and guidelines, adherence to IRB approved protocols, and any adverse events.

PRIVACY

Data, in the form of pertinent history (prior medical illnesses/conditions), unique identifying personal information (name, address), clinical and anthropometric measures (height, weight, waist circumference, blood pressure), and blood samples will be collected during the study. All of the collected material will be used solely for research purposes. Data will be collected by trained staff and will be stored in locked cabinets and freezers or password-protected computer files to protect confidentiality. All identifying information on blood samples and saved data will be de-identified into a study code with the identifying link saved on a network drive on the Partners network accessible only to the PI and his research coordinator(s).