Advanced Glycation Endproducts and Bone Material Strength In T2D Treated With Pyridoxamine

AAAR5451

October 10, 2018

Columbia University Human Subjects Protocol Data Sheet

General Information

Protocol:	AAAR5451(M00Y02)	**Protocol Status:**	Approved
Effective Date:	10/10/2018	**Expiration Date:**	10/02/2019
Originating Department Code:		MED Endocrinology (751850X)	
Principal Investigator:		Rubin, Mishaela (mrr6)	
From what Columbia campus does this research originate:		Medical Center	
Title:	Advanced Glycation Endproducts and bone material strength in T2D treated with pyridoxamine		
Protocol Version #:	1	**Abbreviated Title:**	B6 for Diabetic Bone
Was this protocol previously assigned a number by an IRB:		No	

Is the purpose of this submission to obtain a "Not Human Subjects Research" determination?

No

Renewal Information

Enrollment status:

Open to enrollment or ongoing review of records/specimens

Provide any additional information necessary to explain the study status:

Protocol renewal

Since the last renewal:

Have there been any changes in the relevant literature that would affect the study design or procedures?

No

Have there been any interim findings associated with this study?

No

Have there been any publications resulting from this study?

No

Have any participants been enrolled using the Short Form process?

No

Is there a Data Monitoring Committee (DMC), Data Safety Monitoring Board (DSMB), or other monitoring entity for this study?

Yes

Is the most recent report attached as part of this submission?

No

Provide an explanation of why the report is not attached and either the attachment date of the most recently submitted report or when the next report will be available.

The meeting will take place on October 11th, 2018.

Is an annual Progress Report required by the funding organization or coordinating center for this study?

No

Does this submission include a modification?

Yes

Provide a description of, and explanation for, all changes being proposed in this submission:

please find attached the tracked changes on ICF and protocol. On the other hand, you may also find the attachment of table 1 where changes were also made.

Indicate which sections of the Rascal submission are affected by the proposed modification. Each marked section must be revised as part of this submission:

[ ]General Information
[ ]Attributes
[ ]Funding
[ ]Research Aims and Abstracts
[ ]Locations
[x]Data Security and Privacy
[x]Informed Consent/Recruitment
[ ]No revisions to submission content required
[ ]Exempt and Expedited
[ ]Personnel
[x]Background
[x]Procedures
[ ]Subjects
[x]Risks/Benefits/Monitoring
[x]Attachments (including Rascal-generated attachments)

Has the consent form been revised in this submission?

Yes

Does this submission include a report of a protocol violation?

No

Attributes

Special review type: Check all that apply or check "None of the Above" box.

[ ]Review for 45 CFR 46.118 Determination (involvement of human subjects is anticipated but is not yet defined)
[ ]Funding review for Administrative IRB approval (such as for Center or Training Grants)
[x]None of the above

IRB of record information: Will a Columbia IRB be the IRB that is responsible for providing review, approval, and oversight for this study?

Yes

Select the most appropriate response:

Columbia will be the IRB of record for the study procedures conducted by Columbia researchers (Note: this response will apply to most submissions).

Is this research part of a multicenter study?

No

Please indicate if any of the following University resources are utilized:

[ ] Cancer Center Clinical Protocol Data Management Compliance Core (CPDM)
[ ] CTSA-Irving Institute Clinical Research Resource (CRR)
[ ] CTSA- Irving Institute Columbia Community Partnership for Health (CCPH)
[x] None of the above

Background

Abbreviated Submission:

The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding local implementation of the study. However, entering study information into all of the relevant Rascal fields is not

required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the overall objectives.
If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Study Purpose and Rationale:
Provide pertinent background description with references that are related to the need to conduct this study. If this is a clinical trial, the background should include both preclinical and clinical data. Be brief and to the point.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

At least 25% of Americans over the age of 65 have type 2 diabetes (T2D), which, in addition to its well-recognized complications, is accompanied by a high fracture risk, particularly in elderly patients. Importantly, these fractures are associated with worse outcomes than fractures in the general population. Standard explanations, namely low bone mass and high bone turnover, do not explain how T2D adversely impacts the aging skeleton. Rather, low bone turnover, specifically reduced formation of new bone, likely predisposes to a high fracture risk. This is compounded by altered material properties of bone that together diminish bone strength and increase its propensity to fracture. Both etiologies – reduced bone formation and altered bone material properties – are increasingly thought to arise from the accumulation of advanced glycation endproducts (AGEs). Hence, preventing the accumulation of AGEs in bone tissue in T2D patients is a potential therapeutic strategy with likely effects in reversing bone fragility in T2D. In fact, there is currently no therapeutic agent that addresses the specific skeletal abnormalities in T2D. And, while anti-resorptive agents, such as bisphosphonates, have been utilized widely, there is no prospective clinical trial data for fracture efficacy. Furthermore, these latter drugs suppress bone turnover, and would seem contrary to the requirement for increased bone formation. There is thus a critical need for an effective therapeutic intervention that could lengthen the healthy, active years of life in older T2D individuals. One novel intervention directed to the etiologic culprit, AGE, is pyridoxamine, a naturally occurring and structurally distinct metabolite of vitamin B6 pyridoxal phosphate. Preclinical data show that pyridoxamine has specific molecular features which provide a unique multi-pronged effect to inhibit glycation reactions and the formation of AGEs. Pyridoxamine is also a potent inhibitor of AGE accumulation and improves bone material properties in diabetic animals. Notably, while it also reduces AGEs in patients with T2D with nephropathy, there are no clinical data on possible *skeletal* benefits in T2D. Our **overarching goal** is to reduce fracture risk in older patients with T2D. The **specific objective** of this application is to determine whether, in a short intervention study, pyridoxamine reverses bone fragility in patients with T2D, and to use this pilot data set as a foundation for larger and longer fracture studies. Our preliminary data show strong correlations between AGE accumulation and both bone formation and bone material properties (Furst *et al*. *JCEM*, 2016: PMCID: 4891790). Namely, we have shown that skin autofluorescence (SAF), a marker for tissue AGE accumulation, correlates strongly with decreased bone formation. Likewise, using a novel impact microindentation device that provides an *in vivo* index of cortical bone material properties, namely bone material strength index (BMSi), we show that older T2D women have increased AGE accumulation and decreased BMSi. Furthermore, T2D women with the lowest pyridoxamine stores had the highest SAFs and lowest bone formation and BMSi. Together, these data underscore our central **hypothesis: that pyridoxamine treatment in older T2D patients will increase bone formation and bone material strength by inhibiting AGE accumulation. If the resulting findings of pyridoxamine's skeletal benefits in T2D are translated clinically, a novel and much-needed therapeutic approach is likely to emerge that should reduce fractures in aging T2D patients.** Towards examining the effect of pyridoxime on bone formation and material properties, we will conduct a randomized, double-bind clinical study, comprising two specific aims. In **Specific Aim 1**, we will study the effect of pyridoxamine on the formation of new bone in older T2D patients. For this, we will treat 52 women over 65 years of age with T2D with pyridoxime or placebo, and measure bone turnover markers, namely P1NP (bone formation) and TRAP-5b (bone resorption), and skin autofluorescence (SAF), a marker for AGE

accumulation through 5 visits over 1 year. In **Specific Aim 2**, we will study the effect of pyridoxamine on bone material strength in the same cohort of older T2D patients. For this, we will perform impact microindentation to calculate BMSi at baseline and year 1. We expect that the proposed studies will identify positive effects of pyridoxamine on bone formation and bone material strength in older T2D patients and that the data will support an AGE-mediated mechanism for bone fragility in T2D. The findings will also define a new role for over-the-counter vitamin B6 in skeletal homeostasis. If it is confirmed that pyridoxine does improve bone formation and/or bone material properties, this data set will form the framework for large scale testing to advance the development of pyridoxamine as a therapeutic agent specifically for diabetic bone fragility. In this way, this project could have a positive impact on the large and ever growing population of elderly type 2 diabetic patients.

BACKGROUND, SIGNIFICANCE AND INNOVATION Data from two well-conducted meta-analyses involving 1.3 million individuals show that aging patients with T2D have a 40-50% increase in the risk of hip fracture. In the general population, 20% of hip fracture patients die within one year and more than half never regain functional independence. Post-fracture complications are also worse in T2D. Notably, a cohort study with >1 million patients found an increased complication risk in hip fracture patients with T2D, with a 27% increase in mortality (95% CI 1.02–1.60). Thus, it is our premise that healthy active years of life can be extended by reducing fracture risk in T2D. **Studies with extended follow-up and rigorous fracture adjudication show that skeletal fragility in diabetes occurs despite normal areal bone mineral density (aBMD) and high body mass index (BMI), and is not explained by increased falls**. In contrast to the increased levels of bone turnover markers noted in postmenopausal osteoporosis, we and others find reduced bone turnover in T2D. That fracture risk is increased in T2D, despite increased bone mass and low bone turnover, implicates other etiologies, such as abnormal bone material properties and/or bone microarchitecture. Although increased cortical porosity has been reported using high resolution peripheral quantitative computed tomography, we do not find evidence of this deficit. Likewise, a large (n=1057) bone microarchitecture study has failed to document increased cortical porosity in T2D. Nonetheless, a rigorously designed study by Farr *et al.* of T2D postmenopausal women using impact microindentation (IMI) has provided evidence for compromised bone matrix properties, measured as bone material strength index (BMSi). Although most IMI studies are limited by cross-sectional designs, they show that BMSi discriminates between those with or without osteoporosis-related fractures, and importantly, independent of a BMD. As noted below, we posit that increased advanced glycation endproducts (AGEs) contribute, at least in part, to the noted decreases in BMSi, and that skin AGE levels serve as a valid surrogate for bone AGE accumulation. We have therefore extended the work of Farr *et al.* to show that longer duration of T2D and higher skin AGEs are associated with reduced bone material strength and bone formation (Furst *et al*. *JCEM*, 2016: PMCID: 4891790). **We hypothesize that reducing AGEs will improve bone formation and bone material strength in older T2D patients and, in doing so, reduce fracture risk.** Free-floating sugars in prolonged hyperglycemia interact with exposed amino acid residues on collagen resulting in a reversible Amadori intermediate that undergoes oxidation to form irreversible AGEs. Excess AGEs compromise bone formation by interfering with osteoblast precursor proliferation and differentiation, cell attachment to the collagen matrix, and collagen and mineral deposition. Low bone formation, in turn, further increases AGEs, perpetuating a vicious cycle. Our data confirm that bone formation is reduced in T2D. Notably, histomorphometric indices of bone formation, namely bone formation rate and osteoblast numbers, as well as serum levels of the bone formation marker procollagen type 1 amino-terminal propeptide (P1NP) are lower in postmenopausal T2D women (Manavalan *et al*. *JCEM,* 2012; PMCID: 3431571). Bone resorption, likely due to coupling with formation, is also reduced. Relevant to our hypothesis, there is a strong correlation (r=-0.63, *P*=0.01) between reduced bone formation (low P1NP) and increased skin AGEs (skin autofluorescence, SAF) in T2D, whereas no associations are seen in controls.

Fig. 1. Pyridoxamine. The functional groups inhibit AGE accumulation.

To study whether lowering AGE accumulation in bone will improve bone formation and reduce bone fragility in T2D, we propose to utilize a metabolite of vitamin B6, pyridoxamine. Prospective cohort studies, *albeit* limited in scope, support this notion. The Rotterdam Study showed that lower B6 intakes were associated with a higher fracture risk[25]. Likewise, the Framingham Osteoporosis Study reported that lower B6 levels were associated with higher bone loss and hip fractures. In preclinical studies, pyridoxamine is a potent AGE inhibitor possessing specific molecular features that are not present in other B6 isoforms (pyridoxal and pyridoxol). Based on a pyridine ring structure, with OH, CH_3, NH_2 and CH_2OH substituents, pyridoxamine is unique in inhibiting AGE formation (Fig. 1). Voziyan and others showed that the OH at position 3 and NH_2 group at position 4 of its ring endow pyridoxamine with a unique triple mechanism of action: (i) inhibition of AGE formation by binding of catalytic redox metal ions, thus blocking oxidative degradation of the Amadori intermediate of the Maillard reaction; (ii) direct scavenging of toxic carbonyl products of glucose and lipid degradation; and (iii) trapping of reactive oxygen species. While rodent models show that pyridoxamine inhibits AGE-mediated diabetes complications, including diabetic nephropathy and retinopathy, clinical data are scant. In a phase II study in patients with diabetic nephropathy, pyridoxamine reduced plasma AGE levels over 6 months, suggesting not only the inhibition of new AGE formation but also removal of existing AGEs. *Albeit* limited by a *post hoc* analysis, serum creatinine levels also decreased by ~48% (P =0.03). A prospective randomized study of pyridoxamine for 1 year in proteinuric T2D nephropathy patients showed that only those with less advanced disease responded to pyridoxamine. Of note, plasma pyridoxamine is only detectable in subjects given pyridoxamine, but not in untreated subjects whose plasma levels are below the detection limit. As pyridoxamine easily reacts with carbonyls in the circulation and is converted into pyridoxal, the latter is the dominant measurable form of vitamin B6 in plasma. We have therefore used serum pyridoxal levels as a surrogate for circulating pyridoxamine, and find, in 6 patients with T2D, a strong correlation between serum pyridoxal and the bone formation marker P1NP (r=0.79, P=0.06). These data further support our working hypothesis that pyridoxamine increases bone formation in older T2D patients by preventing new AGE formation (Fig. 2).

Fig. 2. Hypothesis of Pyridoxamine Effect on Bone in Older T2D Patients. Inhibition of AGEs will lead to improved bone formation (SA1) and/or bone material strength (SA2).

In addition to a direct action on osteoblastic bone formation, AGEs impair bone biomaterial properties with an eventual negative effect on bone strength. The sharp increase in bone pentosidine, a key AGE, in insulin-deficient diabetic rats is associated with a lower femoral bending stiffness *versus* wild type littermates, despite no differences in BMD. Likewise, in our mouse model of type 1 diabetes (T1D), the increase in bone AGE content was correlated inversely with bone toughness (Rubin *et al. PLoS One,* 2016; PMCID: 4854398). A relationship between AGEs and bone strength has also been established in a small clinical study in which trabecular bone from fracturing T1D patients exhibited higher pentosidine levels than non-fracturing T1D or controls. These correlations have not been observed in T2D, apart from our preliminary studies. Notably, in older patients with T2D, we find a strong inverse correlation between AGEs and BMSi (r=-0.65, P=0.006). **Thus, we hypothesize that the inhibition of new AGE formation by pyridoxamine will improve bone material properties and reduce fracture risk in older diabetic patients.** Our preliminary data in 6 older T2D women show that serum pyridoxal (pyridoxamine) levels tend to be lower in patients with the lowest BMSis (r=0.71, P

=0.1) and highest skin AGEs (r =-0.67, *P*=0.1). Together, these data suggest that pyridoxamine correlates with bone material properties in diabetes, specifically *via* AGE accumulation.

Study Design:

Describe the methodology that will be used in this study, covering such factors as retrospective vs. prospective data collection, interventional vs. non-interventional, randomized vs. non-randomized, observational, experimental, ethnography, etc.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

In a small cohort of 6 patients with T2D, we find a strong correlation between serum pyridoxal levels (a surrogate for pyridoxamine) and the bone formation marker P1NP (r=0.79, *P*=0.06). We will therefore test the working **hypothesis** that pyridoxamine increases bone formation by preventing new glycation of collagen. For this, we will use an interventional protocol to determine whether pyridoxamine alters bone formation in older T2D patients. Our overall approach will be to compare the change in bone formation in response to pyridoxamine *vs.* placebo given for 12 months. We **expect** that pyridoxamine will increase serum P1NP levels in older T2D. Furthermore, we expect that those with milder T2D and lower AGEs will display a greater response magnitude. These results should provide preliminary data for future studies on the potential use of pyridoxamine as a specific therapeutic for diabetic bone fragility.**Study Protocol: We will compare the effect of pyridoxamine dihydrochloride (200 mg po bid) (n=26) *vs.* identical placebo (n=26) in a 1 year double-blind randomized study of older postmenopausal T2D. This dose is available over-the-counter and is safe and effective at lowering serum AGEs in T2D patients. Study drug (HPLC-certified content, see Appendix) and placebo will be supplied by LifeLink Pharmaceuticals. A neurological safety evaluation will be performed *per* visit for the unlikely onset of vitamin B6 neurotoxicity (see Human Subjects). Our Research Pharmacy will randomize patients with a block scheme stratifying for race. We will not stratify for baseline vitamin B6 levels because they are not expected to modify the response to pyridoxamine. In addition to evaluating P1NP, serum tartrate-resistant acid phosphatase 5b (TRAP-5b) levels will be measured as a surrogate for bone resorption. AGE levels will be determined using our skin autofluorescence (SAF) protocol, with authentication in a sub-set (n=10) by bone biopsy AGE measurements.Patient Recruitment:** Participants will be recruited from Medicine and Endocrinology clinics at Columbia, where >1,000 T2D outpatients are seen annually with 60% of local residents carrying a diagnosis of T2D. We will also recruit from 2 satellite locations in Washington Heights and Riverdale, where >2,400 T2D patients are seen annually. We have used these populations to recruit >100 T2D in prior studies (Furst *et al*. *JCEM*, 2016: PMCID: 4891790). Dr. Starr (Co-I) will support recruitment. Her clinic sees ~15 postmenopausal T2D patients every week; these patients are mostly hospital employees and interested in research participation. Based on these resources, we do not foresee any difficulty in recruiting 52 patients within 6 months. **d. Inclusion and Exclusion Criteria:** We will include postmenopausal women (n=52) 65 years with T2D who meet ADA criteria (HbA1c 6.5%). African Americans, in whom T2D is common, will be included (n=4/group). In addition to exclusions noted in Table 1 (which can be seen attached), certain specific considerations apply. First, and importantly, we will not screen for serum pyridoxamine as levels are very low (~10 nM) in those not on pyridoxamine supplementation and are thus unlikely to predict response to therapy. Of note is that, with supplementation, levels increase to ~10 µm. It is also unknown whether circulating pyridoxamine is reflective of bone levels. Second, we will not exclude patients based on serum pyridoxal values. While we have used these as a surrogate for pyridoxamine in our preliminary studies, they do not fully capture pyridoxamine because pyridoxal is also derived from pyridoxine. Third, low vitamin D (serum 25OHD <30 ng/dl) can be exclusionary as it is often low in T2D and can be a confounder. Although the IOM threshold is <20 ng/dl, our higher cut-off will avoid any possibility of deficiency. However, if the subject's vitamin D level results are less than 20ng/ml and they meet all other criteria, the investigator will prescribe 50,000 IU of vitamin D weekly for one month and then the subject will be re-

screened. Fourth, the blood thinners Clopidogrel, and Rivaroxaban will not be exclusionary because there has been no excessive bleeding with the use of these agents with microindentation according to Dr. Adolfo Diez-Perez, a leader in the technique of microindentation. Finally, insulin users will not be excluded because even though insulin has been implicated in increasing fracture risk, it is probably not due to insulin itself, but rather due to the disease or comorbidities. **Potential Sources of Biological Variation:** We will control for known age-associated bone changes (see Statistical Analysis). We will also restrict our study to postmenopausal women in view of our preliminary data, and because including men in a relatively small cohort will only increase heterogeneity. Moreover, postmenopausal women are at higher fracture risk and thus might be more likely to display a pyridoxamine treatment benefit on fractures numerically even in a small cohort. We will include T2D men in an extended cohort in a future R01 application. Little is known about the influence of race on the mechanisms of diabetic bone fragility. We will thus assess our outcomes separately and in combination with race to assess group differences (see Statistical Analysis). Variables related to fracture risk, such as nephropathy; years since menopause; nutrient intake; weight; weight bearing exercise; and sun exposure will be treated as confounders.**Patient Visits:** At the **screening visit**, we will assess demographics and covariates using a **questionnaire** [footnote[1]]. We will obtain an AM fasting metabolic panel (BMP) with liver function, HbA1c and 25-hydroxyvitamin D. BMP and HbA1c are measured by colorimetric assays (Cobas Integra 400 Plus); 25-hydroxyvitamin D is measured by LCMS on an Agilent 6430 (intra- and inter-assay variability <10%). Patients meeting criteria will undergo randomization, where we will measure bone mineral density (BMD) by a dual energy X-ray absorptiometer (DXA) using standard protocols within a month of the screening visit (Table 2).

At each **subsequent study visit** as seen in (Table 2):randomization/baseline (0^b), month 1 (M1), month 6 (M6), month 9 (M9) and month 12 (M12), we will measure serum *intact* P1NP by radioimmunoassay (IDS; intra- and inter-assay variability <10%). Note that considering that stage I-II CKD may be present, it would be important to measure intact P1NP, which represents the trimeric form that is not renally cleared. TRAP-5b will be quantified by ELISA (IDS; intra- and inter-assay variability of 10%). TRAP-5b is selected because it is an enzyme derived from osteoclasts and unlike serum C-telopeptide (derived from collagen) will not be affected by alterations in collagen degradation due to glycation, the screening,(0^b), M12 visits, we will measure **Skin Autofluorescence (SAF)**, which we and others have shown is highly reproducible. Notably, this new technique effectively measures tissue AGE accumulation and has been validated against direct AGE measurements including pentosidine levels in skin. The PI is experienced in SAF measurements and obtained relevant preliminary data (Furst *et al*. *JCEM*, 2016: PMCID: 4891790). SAF is measured with the AGE Reader (DiagnOptics Technologies BV).**Preliminary Validation of AGE Testing:** Extrapolating from skin to bone may be imprecise, as skin collagen has a longer half-life than bone collagen and glycated skin proteins might be absent in bone. Therefore, prior to randomization, we will perform a preliminary corroboration of our SAF technique with direct measurement of AGEs in bone biopsies in a randomly selected, *albeit* small, sub-set of Caucasian patients with high (2.6; n=5) or low (2.0; n=5) SAF levels. We have not proposed biopsies for all subjects because of invasiveness, time and cost. The biopsy AGE assay will precisely quantify the degree of collagen glycation with a fluorometric assay that is routinely used by our collaborator, Dr. Deepak Vashisth (Professor of Biomedical Engineering, RPI; see Letter). Cortical and cancellous compartments will be analyzed separately because of known compartment differences in glycation. The PI has long-standing experience in obtaining biopsies and our collaborator is a leading expert in measurement of bone glycation. **Power Calculations: Assuming our preliminary findings, mean ± SD P1NP of 51.2 ± 16.2 in healthy controls and 38.4 ± 9.8 in T2D, a standardized difference of 0.95-SD, and 80% power, a 1% and an analysis of covariance (ANCOVA) where covariates have a correlation with outcome of r = 0.50, normalization of P1NP at 1-year requires 20 subjects in each group**[49]. This allows us to have an adjusted 95% confidence interval surrounding the between group difference in means of ± 4.99 for a minimum detectable 13% increase in P1NP in the T2D. We propose to increase enrollment by 6 subjects *per* group to both enroll minority subjects (n=4/g) and for potential attrition (n=2/g). Between treatment group differences in minority enrollees will be an exploratory analysis to provide estimates for future

study design parameters.

---

[1] **Covariates for Questionnaire**: age; race; duration of T2D; co-morbidities including hypertension, nephropathy, neuropathy, retinopathy; weight; diabetes medication(s); medical history; medications; menopause age; nutrient intake; weight bearing exercise; and sun exposure.

Statistical Procedures:

Provide sufficient details so that the adequacy of the statistical procedures can be evaluated including power calculations to justify the number of participants to be enrolled into the study. Definitions of subject terms such as enrolled and accrued as used for Rascal submissions can be found in the Subjects section.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Briefly, data will be reviewed for accuracy, completeness and assumptions held for categorical variables and distributional characteristics of continuous variables. Transformations will be applied prior to inferential testing as necessary to achieve normal distributions. The pattern of missing data will be examined and, if found to be missing completely at random or missing at random, the fully conditional specification multiple imputation method of Van Burren[50]. The primary outcome is the 1-year change in the P1NP marker of bone formation and the primary intent-to-treat analysis is a one-way ANCOVA with fixed effect of treatment (treated *vs.* placebo), and baseline P1NP as a continuous covariate. Secondary outcomes of TRAP-5b and SAF will be analyzed in similar ANCOVA models with *P*-value adjustment for multiple endpoint comparisons. Exploratory analyses will employ linear mixed models for repeated measures to estimate the between treatment group difference in the temporal course of change in P1NP, TRAP-5b and SAF with fixed effect of treatment, time (baseline, 1, 6, 9 and 12-months), random effects for subject and the baseline value of the outcome, and a covariance structure chosen by empirical fit prior to inferential testing. The influence of additional theoretical confounders will be explored in this model: age, duration of T2D, diabetes complications and baseline DXA. Predictors of the change in P1NP in the pyridoxamine-treated group will be estimated using multiple linear regression to identify variables that track or lead changes in P1NP. Bone collagen glycation differences in the subset of randomly selected high and low SAF patients at baseline will be assessed by T-test and, although underpowered, an intraclass correlation coefficient calculated to quantify agreement between bone and skin AGE content. **Expected Outcomes and Data Interpretation:** We expect that P1NP levels will increase with pyridoxamine at 1, 6, 9, and 12 months *vs.* placebo. We anticipate that P1NP will increase to a greater extent in those with milder T2D. This expectation is based on published pyridoxamine data in T2D nephropathy patients, where those with milder disease responded best to pyridoxamine[33]. Our preliminary data that P1NP levels are lowest with the highest AGEs[6] leads to a similar expectation. It is thus anticipated that lower baseline SAF levels (known to be associated with shorter T2D duration, fewer T2D complications and higher baseline P1NP levels) will predict a greater increase in P1NP with pyridoxamine. Also, as noted with other agents, such as teriparatide[51], serum TRAP-5b levels are also expected to increase because of the coupling between bone formation and bone resorption. We do not anticipate baseline BMD to predict change in P1NP, as it does not relate to bone formation levels or SAF in our preliminary data (Furst *et al*. *JCEM*, 2016: PMCID: 4891790)[6]. A decline in SAF is also expected with pyridoxamine; however, this may not be detectable by 12 months, especially because skin turnover might underestimate bone turnover. We also expect that SAF levels in the biopsy sub-set of patients will correlate with direct bone AGE levels, as a preliminary authentication our skin test. Overall, we expect these outcomes to identify an effect of pyridoxamine to increase bone formation *via* an AGE-inhibiting mechanism. This sets the stage for reversing diabetes-specific skeletal deficits, which until now has not been possible with approved osteoporosis therapies.

Exempt and Expedited

Is the purpose of this submission to obtain an exemption determination, in accordance with 45CFR46.101(b):
No

Is the purpose of this submission to seek expedited review , as per the federal categories referenced in 45CFR46.110?
No

Funding

Is there any external funding or support that is applied for or awarded, or are you the recipient of a gift, for this project?
Yes

Award Type	Funding Source Name	Name of awarding agency	Status	Award # or Application Date	Federal/State /Local Government Direct or Subcontract	What is the award covering?	Rascal PT Number
Federal/State/ Local Government	NIH	National Institute on Aging/NIH/DHHS	Applied for/Proposed	02/14/2017	Direct Recipient: No Subcontract Sites	Entire Protocol	PT-AABN4168

Locations

Location Type	Facility Name	Domestic or International	Geographic Location	Local IRB Ethics Approval	Local Site Approval
Offsite	Columbia Doctors Riverdale 3050 Corlear Avenue, Suite 204, Bronx, NY 10463	Domestic	Bronx, NY	Unsure if approval is needed	Unsure if approval is needed
Columbia/CUMC	180th Fort Washington, Harkness Pavilion, 9th Floor Rm 945, New York, NY 10032				

Personnel

UNI/Phone	Name	Role	Department	Edit/View	Obtaining Informed Consent
mrr6	Rubin, Mishaela	Principal	MED	Edit	Y

UNI/Phone	Name	Role	Department	Edit/View	Obtaining Informed Consent
212-305-7859		Investigator	Endocrinology (751850X)		
	Roles and Experience: principal leader of the study including recruitment, eligibility criteria, data				
bo2248 212-342-3687	Omeragic, Beatriz	Coordinator	MED Endocrinology (751850X)	Edit	Y
gt2397 212-305-6257	Tabacco, Gaia	Other Engaged Personnel	MED Endocrinology (751850X)	Edit	Y
	Roles and Experience: Recruiter				
jrf2147 516-242-0170	Starr, Jessica	Investigator	MED Endocrinology (751850X)	Edit	Y
	Roles and Experience: help recruit, perform study, analyze data				
mps2205 212-304-5536	Siu, Monica	Coordinator	MED Endocrinology (751850X)	View	Y
rm3324 212-305-3231	Majeed, Rukshana	Coordinator	MED Endocrinology (751850X)	Edit	Y
xij2000 646-577-2343	Javier-Espinal, Xiomara	Coordinator	MED Endocrinology (751850X)	Edit	Y

Training and COI

The PI must ensure that each individual that is added as personnel has met the training requirements for this study (http://www.cumc.columbia.edu/dept/irb/education/index.html). For help identifying which research compliance trainings you may be required to take, visit the Research Compliance Training Finder.

UNI	Name	COI	HIPAA	HSP (CITI)	Research with Minors (CITI)	FDA-Regulated Research (CITI)	S-I	CRC	Good Clinical Practice (GCP)	GCP - Third-party tracking	Genetic Research Consent
mrr6	Rubin, Mishaela	10/09/2018	07/27/2014	06/22/2018	05/21/2015	05/21/2015	04/15/2018		09/29/2017		
bo2248	Omeragic, Beatriz	05/14/2018	11/06/2015	09/01/2016	09/01/2016	11/10/2015		11/12/2015	09/27/2016		
gt2397	Tabacco, Gaia	11/05/2017	11/05/2017	11/10/2017		11/10/2017			11/10/2017		
jrf2147	Starr, Jessica	05/03/2018	09/26/2013	09/26/2016		09/26/2016			09/26/2017		
mps2205	Siu, Monica	08/02/2018	07/11/2018	07/19/2018		07/19/2018		07/30/2018	07/26/2018		
rm3324	Majeed, Rukshana	03/15/2018	04/14/2015	09/21/2016	09/21/2016	09/21/2016	04/14/2015	04/14/2015	09/27/2016		
xij2000	Javier-Espinal, Xiomara	06/28/2018	06/07/2018	06/29/2018		06/25/2018			07/10/2018		

Departmental Approvers

Electronic Signature: Mishaela Rubin (751850X) - Principal Investigator Date: 10/08/2018

Privacy & Data Security

Indicate the methods by which data/research records will be maintained or stored (select all that apply):

[x]Hardcopy (i.e., paper)

Describe where and how the data will be stored:

Study documents will be labeled with a code and locked in the investigator's office. The code to the subjects will be kept in a separate file cabinet, also locked in a file cabinet in the investigator's office. Only members of the study team who must access the study data will have access to the files. Study data will also be collected in password protected computer files that are stored on the Department of Medicine MC Domain Server (IP Address: 10.171.8.150), a multi-user server that has been registered and certified by CUMC IT(System ID number:3924). The server will be accessed through password protected computers only (no laptops, no portable data drives).

[x]Electronic

Where will the data be stored?

Y

[x]On a System

[ ]On an Endpoint

Does this study involve the receipt or collection of Sensitive Data?

Yes

If any Sensitive Data is lost or stolen as part of your research protocol, you must inform both the IRB and the appropriate IT Security Office (CUMC IT Security if at CUMC; CUIT if at any other University campus).

What type of Sensitive Data will be obtained or collected? Select all that apply:

[ ]Personally Identifiable Information (PII), including Social Security Numbers (SSN)

Will Social Security Numbers (SSNs) be collected for any purpose?

[x]Protected Health Information (PHI), including a Limited Data Set (LDS)

If any PHI is lost or stolen, you must inform both the IRB and the Office of HIPAA Compliance.

Indicate plans for secure storage of electronic sensitive data: check all that apply

[x]Sensitive data will not be stored in electronic format

[ ]Sensitive data will be stored on a multi-user system

[ ]Sensitive data will be stored on an encrypted endpoint

Provide a description of how the confidentiality of study data will be ensured, addressing concerns or protections that specifically relate to the data storage elements identified above (e.g. hard copy, electronic, system, and/or endpoint):

Study documents will be labeled with a code and locked in the investigator's office. The code to the subjects will be kept in a separate file cabinet, also locked in a file cabinet in the investigator's office. Only members of the study team who must access the study data will have access to the files. Non-sensitive study data will also be collected in password protected computer files that are stored on the Department of Medicine MC Domain Server (IP Address: 10.171.8.150), a multi-user server that has been registered and certified by CUMC IT (System ID number:3924). The server will be accessed through

password protected computers only (no laptops, no portable data drives).

Is there or will there be a Certificate of Confidentiality (CoC) for this research?
No

Provide a description of the protections in place to safeguard participants' privacy while information is being collected:
Patients will only be interviewed by Drs. Starr and Rubin in a private room to ensure their PHI is not inappropriately revealed. Data will be collected and recorded on case report forms on which the only identifier is a research ID code, which will be a code. Only the PI and project coordinator has access to the link between the research ID code and PHI, which will be kept in a separate password protected file. No names or identifying information will accompany specimens.

Procedures

Is this project a clinical trial?
Yes

Is this project a clinical trial that requires registration with www.clinicaltrials.gov?
No

Is this project associated with, or an extension of, an existing Rascal protocol?
Yes

Existing Rascal protocol #:
AAAE1078

Do study procedures involve any of the following?

Analysis of existing data and/or prospective record review
No

Audio and/or video recording of research subjects
No

Behavioral Intervention?
No

Biological specimens (collection or use of)
Yes

Cancer-related research
No

Drugs or Biologics
Yes

Future use of data and/or specimens
Yes

Genetic research
No

Human embryos or human embryonic stem cells
No

Imaging procedures or radiation
Yes

Medical Devices
Yes

Surgical procedures that would not otherwise be conducted or are beyond standard of care

No

Will any of the following qualitative research methods be used?

Survey/interview/questionnaire

Yes

NOTE: You must attach a PDF version of the survey(s)/interview(s)/questionnaire(s) to this protocol prior to submission.

Systematic observation of public or group behavior

No

Program evaluation

No

Will any of the following tests or evaluations be used?

Cognitive testing

No

Educational testing

No

Non-invasive physical measurements

Yes

Taste testing

No

Is there an external protocol that describes ALL procedures in this study?

No

Please describe ALL study procedures in detail.

NOTE: Be sure to detail all of the procedures above to which a "yes" response was selected. Also detail any additional procedures that may or may not fall into the categories listed above.

1) DXA will be performed twice. This will be done at Baseline visit and M12. This is a testing of bone mineral density.
2) 6 tubes (about ten teaspoons) of fasting blood will be collected during the screening from subjects and controls to measure:
calcium, glucose, HgbA1c, creatinine, 25hydroxyvitamin D, and bone turnover marker
(TRAP-5b). At screening visit, baseline, M1, M6, M9, and M12, we will draw 3 tubes of blood (about five teaspoons) to measure P1NP (a marker of bone formation) and TRAP-5b (a bone turnover marker reflective of osteoclast activity).
3) 1 tube of serum blood samples will be collected from all African American subjects and all controls (random sample) to measure blood pentosidine levels.
Either during the screening, randomization, or M12 visits, the following will be studied:
4)A measure advanced glycation endproducts (AGEs) in the skin will be made using a noninvasive measurement of skin autofluorescence (SAF), a method based on the fluorescent properties of AGEs. We will perform this test at, screening, baseline and at M12. Participants will be asked not to use any sunscreen within 2 days before the measurement. All measurements will be performed at room temperature in a semi-dark environment while participants are at rest in a seated position. The forearm of a participant will be positioned on top of the device. SAF will be measured with the AGE Reader (DiagnOptics Technologies BV). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to estimate the level of AGE accumulation in the skin. The AGE Reader illuminates approximately 4cm2 of the skin (which is guarded against surrounding light) with an excitation light source of 300-420nm (peak excitation 350nm). Autofluorescence is then calculated by dividing the average light intensity emitted per nm over the 420- to 600-nm range by the average light intensity emitted per nm over the 300- to 420-nm range. Control measurements will first be obtained with the lights off, autofluorescence of the skin will then be measured six times (every 10 seconds) at the volar side of the arm, approximately 10mc below the elbow fold. This procedure involves no risk to the subject and is completely noninvasive.
5) Transiliac percutaneous bone biopsy will be performed in a total of 10 subjects for assessment of bone structure,

bone metabolism and quantification of advanced glycation end products in collagen. An additional separate consent form will be used for the bone biopsy. Participants who undergo the bone biopsy will be compensated $250.
6) Reference Point Indentation via a microindentation at the midshaft of the anterior tibia will be performed for assessment of bone material strength (BMS) in a minimally invasive approach that uses a hand-held probe (OsteoProbe®) to obtain indentation measurements of bone. We will perform this procedure at baseline and at M12. The procedure will be performed in an exam room under sterile conditions. 2% lidocaine will be used as a local anesthetic. The lidocaine will be administered by 2 study physicians, Drs. Mishaela Rubin and Jessica Starr who are also going to be the only two study physicians doing the microindentation procedure. Both Drs. Starr and Rubin have learned how to use lidocaine injections as part of their general medical training (medical school and internal medicine residency). Additionally, Dr. Starr, one of the study physicians, met with Dr. Adolfo Diez-Perez, the investigator who has pioneered the technique and has performed microindentation in hundreds of subjects. He reviewed the procedure in detail with Dr. Starr and she observed the actual procedures with him. Secondly, we have both successfully used the tool in research protocol AAAE1078. The probe will be held perpendicular to the tibia as the leg is flat against the exam table and will be inserted through the soft tissue and periosteum until it reaches the bone surface. The indentation occurs via trigger mechanism and takes 0.25ms. The size of the indentation is quite small, about 187m, which is approximately the width of 1-2 human hairs. The measurement obtained from this procedure is known as the IDI (Indentation Distance Increase) and will be converted by a computer to BMS. In order to obtain accurate results, there will be 10 measurements made per subject but the probe will only be adjusted 0.2mm from the initial indentation site for each additional measurement. Also, the probe will not be lifted out of the periosteum, just out of the bone for the additional measurements. This procedure has been well-studied in humans with no complications observed to date in 400 people who have undergone the microindentation procedure. The site of microindentation will be covered with a bandage and subjects should be able to resume their regular daily activities immediately. The Principal Investigator will be responsible for receiving the device and will have proper accountability, handling, and storage of the OsteoProbe®. We will get one shipment which will include the device and the probes once the study is approved. The probes will be discarded in sharps disposal bins (red bins). The OsteoProbe® handheld device and the individual probes will be received by the Principal Investigator. There will only be one OsteoProbe® device from the company that will be sent to CUMC one time in the beginning of the study. The probes themselves will come individually packaged with a lot number that we will record for each subject. Each subject will also be assigned a subject number consisting of first initial-last initial and three digit study number (ie 001). The probes will be single use and will be discarded in a sharps disposal bin (red bin) located at the site of the procedure. Devices will be handled only by individuals listed on the protocol and the microindentation will only be performed by the study physicians, Drs. Starr and Rubin. We will receive one probe per subject so the probes will be discarded after each use. While the probes are clean, they are not sterile so we will sterilize them with steam autoclaving, which will be performed through New York Presbyterian Hospital in accordance with hospital standards for sterilizing medical equipment. The OsteoProbe® itself will be cleaned before and after each use with alcohol prep pads.
The OsteoProbe® will be stored at room temperature in a carrying case that has a built-in soft pouch to protect the device from damage. The probes will be stored at room temperature. The carrying case and the OsteoProbe® will be kept in a locked office that only the study personnel will have access to. The probes will be used one time per subjects and will be placed into sharps disposal bins available in the exam rooms where the microindentation is to be performed.

Biological Specimens

Add an individual entry for each human specimen type that will be collected or utilized for the proposed study. For each specimen type, indicate the source or sources from which you will obtain the specimens.

The use of specimens for research purposes may require that informed consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) be obtained from subjects.

Type:
Other
Bone biopsy

Pathology approval may be necessary if the project includes the collection of specimens that will be collected from a procedure that is conducted for, or will potentially involve, diagnostic information. The Pathology request form can be found at the following link: https://research.columbia.edu/sites/default/files/content/HRPO/BlankPathologyIRBApprovalForm.doc

Source:
[x] From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia
Select all that apply to this specimen type:
[x] Specimens will be prospectively collected specifically for this research.
[ ] Residual specimens from clinical care that would otherwise be discarded have been or will be collected.
[ ] Specimens to be analyzed will be (or have been) collected from a commercial source.
[ ] Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.
[ ] From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia
Description of Specimen and Method of Obtaining:
Percutaneous transiliac crest bone biopsy will be obtained.
Indicate the manner in which the specimens will be labeled:
[ ] Specimens will be labeled with direct identifiers
[x] Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier
[ ] The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain
[ ] Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Type:
Blood
Source:
[x] From Columbia and/or NYP Subjects/Patients or Repositories managed by Columbia
Select all that apply to this specimen type:
[x] Specimens will be prospectively collected specifically for this research.
[ ] Residual specimens from clinical care that would otherwise be discarded have been or will be collected.
[ ] Specimens to be analyzed will be (or have been) collected from a commercial source.
[ ] Specimens to be analyzed will be (or have been) collected under a separate CU IRB-approved protocol; this could be an approved research repository protocol.
[ ] From Non-Columbia/NYP Subjects/Patients or Repositories not managed by Columbia
Description of Specimen and Method of Obtaining:
Blood will be collected by research coordinators using vacutainers and butterfly needles.

Indicate the manner in which the specimens will be labeled:

[ ] Specimens will be labeled with direct identifiers

[x] Specimens will be labeled with a code and the research team has the key and can link specimens to direct identifiers. This code would be considered an indirect identifier

[ ] The identifiers will be removed prior to the receipt of the specimens by Columbia researchers and no link will remain

[ ] Specimens were originally collected without identifiers

If specimens are collected or received at any point in time as with direct or indirect identifiers by the current researchers, then the specimens are considered to be identifiable, and the requirements for Informed Consent (or a waiver, if applicable) and HIPAA Authorization (or a waiver, if applicable) apply. The necessary information will need to be included in the respective sections of this Rascal submission.

Devices

On the General Information page you have indicated that the protocol version associated with the use of this medical device is as follows: 1

Please note that a Protocol Version # is required for protocols using a medical device, and you will not be allowed to submit this protocol until the Protocol Version # field is complete. Please ensure that the Protocol Version # is completely and accurately reported on the General Information page.

Please enter the requested information for each device that is the object of the study or is being used because it is relevant to the aims of the protocol, whether the medical device is not yet FDA-approved [i.e., is investigational] or is an approved device that is being used in an investigational manner (i.e., off-label use is being studied).

Note that the questions apply only to devices used in clinical investigations or protocols that involve a Humanitarian Use Device. Emergency use of a device that is not yet FDA-approved is not a clinical investigation, and a submission in Rascal may not be required. Please contact the IRB for assistance if emergency use of a device that is not yet FDA-approved is being considered: (212)305-5883.

Device name:

AGE Reader

Device description:

The AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to estimate the level of AGE accumulation in the skin by measuring autofluorescence of the skin at the forearm.

Device Model/Version #:

not applicable

Phase of Study:

Feasibility

Manufacturer Information

Name:	DiagnOptics Technologies BV
Address:	abc
Contact information:	abc

Is the device a Humanitarian Use Device (HUD)?

No

Is the device FDA-approved and used in accordance with its labeling?

No

An Investigational Device Exemption (IDE) may be required.

Select Category:

Not FDA-approved

Provide plans for storage, control and accounting of the device:

Device is stored in a locked cabinet in a locked office in the HP building, room 945. Only Drs. Starr and Rubin have access to the device as well as coordinators.

Is an FDA-issued Investigational Device Exemption (IDE) required?

No. This is a Nonsignificant Risk device (21 CFR 812.2(b)).

Will a representative of the Sponsor/Manufacturer be involved with the use of the device at Columbia/ NYPH, e.g., for training purposes?

No

Device name:

OsteoProbe®

Device description:

The osteoProbe® is a stainless steel handheld device slightly larger than a highlighter with three component parts: an impact generation mechanism, a displacement transducer and a probe. The probe has a tip diameter of approximately 375m and a tip sharpness radius of less than 10m. The operator uses one hand to press the device through the skin at the anterior tibia until it sinks slightly into the bone. At this point, the instrument is full compressed and this causes the impact.

Device Model/Version #:

not applicable

Phase of Study:

Feasibility

Manufacturer Information

Name:	Active Life Scientific, Inc.
Address:	32 Anacapa St, suite D, Santa Barbara, USA, 93101, 805-770-2600, Davis@activelifescientific.com
Contact information:	Davis Brimmer

Is the device a Humanitarian Use Device (HUD)?

No

Is the device FDA-approved and used in accordance with its labeling?

No

An Investigational Device Exemption (IDE) may be required.

Select Category:

Not FDA-approved

Provide plans for storage, control and accounting of the device:

Device is stored in a locked cabinet in a locked office in the HP building, room 945. Only Drs. Starr and Rubin have access to the device as well as coordinators.

Is an FDA-issued Investigational Device Exemption (IDE) required?

No. This is a Nonsignificant Risk device (21 CFR 812.2(b)).

Will a representative of the Sponsor/Manufacturer be involved with the use of the device at Columbia/ NYPH, e.g., for training purposes?

No

Drugs/Biologics

On the General Information page you have indicated that the protocol version associated with the use of this drug/biologic is as follows: 1

Please note that a Protocol Version # is required for protocols using a drug or biologic, and you will not be allowed to submit this protocol until the Protocol Version # field is complete. Please ensure that the Protocol Version # is completely and accurately reported on the General Information page.

List each drug or biologic that will be administered as the object of the protocol or is being used because it is relevant to the aims of the research protocol. This applies whether the drug/biologic is not yet FDA-approved (i.e., is investigational), is FDA approved and used in accordance with its labeling, or is an approved product that is being used in an investigational manner (i.e., off-label use is being studied).

Note that the questions apply only to drugs used in clinical investigations. Emergency use of a drug that is not yet FDA-approved is not a clinical investigation, and a submission in Rascal may not be required. Please contact the IRB for assistance if emergency use of a drug or biologic that is not yet FDA-approved is being considered: (212) 305-5883.

Name:
Pyridoxamine Dihydrochloride
Dose:
200mg Orally twice a day
Study phase:
Phase 2
Manufacturer Information

Name:	LifeLink Pharmaceuticals
Address:	LifeLink, P.O. Box 1299, Grover Beach, CA 93483-1299
Contact information:	+1(888)433-5266

Route of administration:
Oral
Is the drug/biologic FDA-approved and used in accordance with its labeling?
No

Select a category:
Not FDA-approved
Does the Use of the drug/biologic require an Investigational New Drug (IND) or Biological IND (BB-IND) application?
NO – this use is exempt.

Since you have indicated that the drug/biologic is either FDA approved but being used outside of its approved indication, or not FDA-approved, an IND is required unless the clinical investigation meets criteria to be exempt from the IND requirements. Please choose the regulatory category for exemption from the IND requirements that applies to your study.
21 CFR 312.2(b)(1) criteria met - This is a clinical investigation of a drug product that is lawfully marketed in the United States and all the following apply: (i)The investigation is not intended to be reported to FDA as a well-

controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug; (ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product; and (iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

This is a clinical investigation of a drug product that is lawfully marketed in the United States and all the following apply:

- The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;
- If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;
- The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

Will the drug/biologic be dispensed by the CUMC Research Pharmacy, which is responsible for the storage, handling, accountability, and dispensing of investigational drugs to research investigators? CUMC Research Pharmacy policy: https://researchpharmacy.cumc.columbia.edu/policies.html

Yes, I confirm the drug will be dispensed by the Research Pharmacy

Future Use

For what materials do you anticipate future research use? Select all that apply.

[x]Data

[x]Biological Specimens

For what materials do you anticipate future research use? Select all that apply.

[x]Some or all data and/or specimens, as applicable, will be retained by Columbia researchers for future use.

How are the materials intended to be used for research in the future?

Multiple researchers, which may include the current PI and research team, will be able to request use of the materials.

What is the intent for use of the materials? (Select all that apply.)

[x]The intent is to add the materials to an existing CU repository (e.g., HICCC Tumor Bank).

Provide the Columbia IRB protocol number, if known:

AAAE1078

[ ]The intent is to create a repository.

How will the data and/or specimens, as applicable, be labeled during storage for future uses.

[x]In the same manner as during collection (e.g., with direct identifiers, coded, de-identified, anonymous)

[ ]In a different manner than during collection. Select all that apply:

Describe the physical storage for the specimens/data, including location.

[x]In the same manner as during collection

[x]In a different manner than during collection

Describe the physical storage.

Specimen Samples will be stored on a CU Fridge (CU0247), REVCO Mod Ultima II ID#021766 at a -80 °C in a locked laboratory room located on Harkness Pavillion 180 Fort Washington Ave., Rm 925, New York, NY 10032. Data will be stored in the same manner as during collection. Bone specimens from the biopsy will be de-identify and sent outside of Columbia to Deepak Vashishth, Ph.D. in Rensselaer Polytechnic Institute 110 8th

Street, BT 2213 Troy NY 12180-3590 to be store and use for future research.

Describe who will have access to the stored data and/or specimens.

Drs. Mishaela Rubin and Jessica Starr.

[ ]Some or all data/specimens will be released to a non-Columbia entity for future use and Columbia researchers will not have direct control.

Imaging Procedures/Radiation Therapy

Will a contrast agent (e.g. gadolinium) be used in conjunction with radiation exposure that goes beyond the parameters established for the applicable standard of care (SOC), or will a contrast agent be administered for research purposes only?

No

For each type of radiation exposure (e.g., ionizing: CT, X-ray; non-ionizing: MRI), identify the procedure and whether the administration (e.g., radiation dosage, number or type of scans) is clinically indicated and in accordance with the parameters established for the applicable standard of care (SOC), or is "beyond" these parameters (i.e., includes procedures or exposure for research purposes only).

Procedure(s) Involving Ionizing Radiation

Procedure	The exposure to:
DEXA Scan	Beyond that established for the applicable SOC

Procedure(s) Involving Non-Ionizing Radiation

No data to display

Recruitment And Consent

Recruitment:

Describe how participants will be recruited:

Subjects will be recruited from Dr Starr's practice, which includes post-menopausal women with T2D, phone calls to the previous bone and diabetes study participants (protocol AAAE1078, see script attached) and through flyers that will be hung at CUMC (see attached flyer). Spanish participants will be enrolled the same way in their preferred language, as Dr Starr is fluent in Spanish and both the script and flyer will be translated by a certified Columbia University translator after their respective English versions are approved. In addition, we will be using social media. We will post on websites the identical text that is in the approved flyer. Finally, we will do mailings to women age 65 or older with HgbA1c 6.5% or higher who will be found in the electronic medical record (TRAC request at https://webapps.nyp.org/trac), using the

mailing letter and return postcard.

Select all methods by which participants will be recruited:

[ ] Study does not involve recruitment procedures
[x] Person to Person
[ ] Radio
[ ] Newspapers
[x] Direct Mail
[x] Website
URL: https://newyork.craigslist.org/
[ ] Email
[ ] Television
[x] Telephone
[x] Flyer/Handout
[ ] Newsletter/Magazine/Journal
[ ] ResearchMatch
[ ] CUMC RecruitMe

Additional Study Information: Please add a description of your study as you would like it to be displayed on the RecruitMe website.

Informed Consent Process:

Informed Consent Process, Waiver or Exemption: Select all that apply

[x] Informed consent with written documentation will be obtained from the research participant or appropriate representative.

Documentation of informed consent is applicable to:
The study in its entirety

Identify the portion of the study (e.g., prospective portion, focus groups, substudy 2) or subject population for which documentation of consent will be obtained::

Documentation of participation will be obtained from::
[x] Adult participants
[ ] Parent providing permission for a child's involvement
[ ] Legally Authorized Representatives (LARs)

Describe how participants' written consent will be obtained:
Participants will read and choose to sign the consent form if they are willing to participate. Investigators Drs. Starr and Rubin will be available to answer any study-related questions subjects may have. Dr. Starr is fluent in Spanish.

[ ] Informed consent will be obtained but a waiver of written documentation of consent (i.e., agreement to participate in the research without a signature on a consent document) is requested.

[ ] A waiver of some or all elements of informed consent (45 CFR 46.116) is requested.

[ ] Planned Emergency Research with an exception from informed consent as per 21 CFR 50.24.

[ ] Informed consent is not required; this is exempt research.

Subject Language

Enrollment of non-English speaking subjects is expected.

Languages anticipated:

Spanish

As you plan on enrolling non-English speaking subjects, administrative IRB approval of the translated documents (e.g., consent, recruitment materials, questionnaires) in the above selected languages are required. Please see the IRB's policy on the Enrollment of Non-English Speaking Subjects in Research for further details

(http://www.cumc.columbia.edu/dept/irb/policies/documents/Nonenglishspeakingsubjects.Revised.FINALDRAFT.111909.website.doc).

Capacity to Provide Consent:

Do you anticipate using surrogate consent or is research being done in a population where capacity to consent may be questionable?

No

Research Aims & Abstracts

Research Question(s)/Hypothesis(es):

Specific Aim 1: Study the effect of pyridoxamine on the formation of new bone in older T2D patients We will compare the change in bone formation in response to pyridoxamine *vs.* placebo given for 12 months. We **expect** that pyridoxamine will increase serum P1NP levels in older T2D. Furthermore, we expect that those with milder T2D and lower AGEs will display a greater response magnitude. These results should provide preliminary data for future studies on the potential use of pyridoxamine as a specific therapeutic for diabetic bone fragility.**Specific Aim 2: Identify the effect of pyridoxamine on bone material strength in older T2D patients.** We hypothesize that, by preventing new glycation of collagen, pyridoxamine as an intervention will reverse the deterioration of cortical bone material strength, a characteristic feature of diabetic bone fragility. To determine whether pyridoxamine given to older T2D patients positively affects bone material properties and strength, we will compare, as in Specific Aim 1, pyridoxamine *vs.* placebo given for 12 months, but with BMSi as the measured endpoint. We expect to identify a beneficial effect of pyridoxamine on BMSi, with a greater response when baseline AGEs are low. These results, together with those from Specific Aim 1, should provide preliminary data for a clinical trial of pyridoxamine in T2D and lead to its investigation as a mechanism-based therapeutic strategy for diabetic bone fragility.

Scientific Abstract:

At least 25% of Americans over the age of 65 have type 2 diabetes (T2D), which, in addition to its well-recognized complications, is accompanied by a high fracture risk, particularly in elderly patients. Standard explanations, namely low bone mass and high bone turnover, do not explain how T2D adversely impacts the aging skeleton. Rather, low bone turnover, specifically reduced formation of new bone, likely predisposes to a high fracture risk. This is compounded by altered material properties of bone that together diminish bone strength and increase its propensity to fracture. Both etiologies – reduced bone formation and altered bone material properties – are increasingly thought to arise from the accumulation of advanced glycation endproducts (AGEs). Hence, preventing the accumulation of AGEs in bone tissue in T2D patients is a potential therapeutic strategy with likely effects in reversing bone fragility in T2D. Our preliminary data show strong correlations between AGE accumulation and both bone formation and bone material properties (Furst *et al*. *JCEM*, 2016: PMCID: 4891790, IRB protocol AAAE1078).One novel intervention directed to the etiologic culprit, AGE, is pyridoxamine, a naturally occurring and structurally distinct metabolite of vitamin B6 pyridoxal phosphate. Preclinical data show that pyridoxamine has specific molecular features which provide a unique multi-pronged effect to inhibit glycation reactions and the formation of AGEs. Pyridoxamine is also a potent inhibitor of AGE accumulation and improves bone material properties in diabetic animals. Notably, while it also reduces AGEs in patients with T2D with nephropathy, there are no clinical data on possible *skeletal* benefits in T2D. Our study will explore the changes in bone formation, bone material strength and AGE accumulation over a one-year period during which a subset of postmenopausal women with T2D will be treated with pyridoxamine. This study has important ramifications for offering a novel treatment to preserve or improve skeletal health in postmenopausal women with T2D.

Lay Abstract:

Type 2 Diabetes Mellitus (T2DM) has become one of the most important diseases of our time. Recent research shows that diabetes has negative effects on bones and that people with diabetes might be more likely to break a bone. We don't know the reasons for this, but we suspect that normal bone replacement is slowed down in diabetes and this could slow down the growth of new bone. It is possible that the normal bone material becomes weaker because sugar-related components ("Advanced Glycation Endproducts") are making the bone more brittle. We have shown in past research that people who have type 2 diabetes are more likely to have both weaker bone with lower "bone material strength" and also higher levels of sugar-related components ("Advanced Glycation Endproducts"). This study will focus on attempting to lower the sugar-related components ("Advanced Glycation Endproducts") by treating a group of patients with type 2 diabetes with a B vitamin, known as vitamin B6 or pyridoxamine for one year. We will compare post-menopausal women both before and after pyridoxamine use and study them in terms of different bone features based on blood tests, bone imaging, a bone indentation test and a measurement of sugar-related components in the skin. This study will help to clarify if using pyridoxamine helps improve bone strength in women with diabetes.

Risks, Benefits & Monitoring

Abbreviated Submission:

The IRB has an abbreviated submission process for multicenter studies supported by industry or NIH cooperative groups (e.g., ACTG, HVTN, NCI oncology group studies, etc.), and other studies that have a complete stand-alone protocol. The process requires completion of all Rascal fields that provide information regarding

local implementation of the study. However, entering study information into all of the relevant Rascal fields is not required, as the Columbia IRBs will rely on the attached stand-alone (e.g., sponsor's) protocol for review of the overall objectives. .
If you select the Abbreviated Submission checkbox and a section is not covered by the attached stand-alone protocol, you will need to go back and provide this information in your submission.

Potential Risks:
Provide information regarding all risks to participants that are directly related to participation in this protocol, including any potential for a breach of confidentiality. Risks associated with any of the items described in the Procedures section of this submission should be outlined here if they are not captured in a stand-alone protocol. Risks of procedures that individuals would be exposed to regardless of whether they choose to participate in this research need not be detailed in this section, unless evaluation of those risks is the focus of this research. When applicable, the likelihood of certain risks should be explained and data on risks that have been encountered in past studies should be provided.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

Potential risks of study participation include venipuncture, radiation exposure from DXA, impact microindentation and the bone biopsy. The skin autofluorescence protocol does not involve risk. **Venipuncture:** Risks include pain, bleeding, bruising, infection and inflammation at the site. The risks of venipuncture will be minimized by having trained, experienced professional staff to obtain all blood samples.**Radiation:** Each patient will have 2 DXA exam. The radiation exposure for DXA (spine, one hip and forearm) using the Hologic QDR4500 (fast scan) is 14.9 μSv. This compares to 2400 μSv natural background in a year, 8000 μSv for a standard chest CT, 450 μSv for a mammogram, and 60 μSv for a round-trip transcontinental plane flight. The total radiation exposure is less than the same relative risk as <1 month of natural background radiation and will be well below the maximal acceptable dose for normal subjects. All subjects will be counseled in detail about the amount of radiation that they will receive as a result of participation in the study as part of Informed Consent procedures.**Impact micoindentation:** The procedure causes minimal discomfort (only during the local anesthesia injection) and no complications have been observed to date. Patients who have a significant skin disorder, bruising, local edema, or infection, as well as those undergoing treatment for blood clots or severe coagulation defects will be excluded.**Bone biopsy:** In 10 randomly selected Caucasian patients with high (2.6; n=5) and low (2.0; n=5) skin autofluorescence levels, bone biopsies will be performed prior to randomization. The biopsy advanced glycation endproduct (AGE) assay will precisely quantify the degree of collagen glycation in the cancellous and cortical compartments with a fluorometric assay. The risks of bone biopsy includes the risks of conscious sedation and the risks of the biopsy itself. Conscious sedation will be offered to subjects who have a bone biopsy in order to minimize anxiety, provide pain relief and diminish recall. Patients receiving conscious sedation may have brief transient loss of protective reflexes. Risks will be minimized by complying with all procedures as required in the Columbia University Medical Center Policies and Procedures Manual. Specifically, all nurses and physicians involved in performing bone biopsies will have certification in Basic Life Support and Advanced Cardiac Life Support. The procedure will be conducted in an outpatient surgery suite, which has a stocked "crash cart"; vital signs (including O_2 saturation) will be monitored at regular intervals during and after the procedure. Complications from the biopsy itself are very unusual, but could include cutaneous nerve entrapment, bleeding, fracture of the iliac wing and prolonged

discomfort. Our experience has been that the aforementioned complications do not occur when bone biopsy is performed correctly. Infections at the biopsy site have occurred in only 2 of more than 1000 biopsy procedures done at Columbia. The sterile environment of the operating room or specialized surgical suite is very helpful in this regard. More commonly, there will be a bruise at the site or discomfort that persists for only a few days. Close follow-up by our research nurse identifies any problems after the procedure.**Administration of pyridoxamine dihydrochloride:** Pyridoxamine dihydrochloride will be administered at a dose of 200 mg PO BID. Randomization will be done by the Research Pharmacy with a block scheme stratifying for race. Study drug will be dispensed by the Research Pharmacy. The dose we are using is available as an over-the-counter supplement. We have considered the safety of our regimen. Excessive ingestion of vitamin B6 (pyridoxine) when used as a dietary supplement at doses up to 2 g/day has been reported to cause a neurotoxic syndrome in patients as characterized by paresthesias, muscle weakness and numbness [1]. In contrast, no neurological or behavioral signs of toxicity have been reported, to the best of our knowledge, following administration of pyridoxamine dihydrochloride. In a large (n=317) clinical trial of pyridoxamine, doses that are similar (150 mg po bid and 300 mg po bid, for 52 weeks) to our planned dose of 200 mg po bid for 52 weeks were used [2]. In that study, there was no association between pyridoxamine and serious adverse events. Recently, administration of up to 200 mg/kg/day in Sprague-Dawley rats of pyridoxamine dihydrochloride via IV infusion for seven consecutive days was not associated with any treatment-related findings including gross or microscopic findings in the nervous system (brain, spinal cord, spinal nerve roots/ganglia, and peripheral nerves), liver, kidneys, heart, spleen, thymus, pancreas, or the catheter emptying site. Based on those data, the no observed adverse effect level in Sprague-Dawley rats was determined to be 200 mg/kg/day [3]. To address the unlikely issue of vitamin B6 neurotoxicity, at each study visit neurological symptoms will be reviewed and a full neurologic exam will be performed. Any neurologic findings will be immediately reported to the Medical Monitor and will be reviewed with the Data Safety and Monitoring Board.1. Dalton K, Dalton MJ. Characteristics of pyridoxine overdose neuropathy syndrome. Acta Neurol Scand. 1987;76(1):8-11. PubMed PMID: 3630649.2. Lewis EJ, Greene T, Spitalewiz S, Blumenthal S, Berl T, Hunsicker LG, et al. Pyridorin in type 2 diabetic nephropathy. J Am Soc Nephrol. 2012;23(1):131-6. doi: 10.1681/ASN.2011030272. PubMed PMID: 22034637; PubMed Central PMCID: PMCPMC3269925.3. Sullivan DW, Jr., Peterson RC, Mujer CV, Gad SC. A 7-day intravenous toxicity study and neurotoxicity assessment of pyridorin in Sprague-Dawley rats. Hum Exp Toxicol. 2016. doi: 10.1177/0960327116661023. PubMed PMID: 27507076.

Potential Benefits:

Provide information regarding any anticipated benefits of participating in this research. There should be a rational description of why such benefits are expected based on current knowledge. If there is unlikely to be direct benefit to participants/subjects, describe benefits to society. Please note that elements of participation such as compensation, access to medical care, receiving study results, etc. are not considered benefits of research participation.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

There is no direct benefit to subjects. The benefit to society may be additional information about potential treatment for the deleterious effects of diabetes on bone.

Alternatives:

If this research involves an intervention that presents greater than minimal risk to participants, describe available alternative interventions and provide data to support their efficacy and/or availability. Note, participants always have the option not to participate in research.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

The alternative would be for the subject not to participate.

Data and Safety Monitoring:

Describe how data and safety will be monitored locally and, if this is a multi-center study, how data and safety will be monitored across sites as well.

[ ] Abbreviated Submission - This information is included in an attached stand-alone protocol. Proceed to the next question

We will be using a multi-server user that has been registered and certified by CUMC IT (System ID number 3924; IP Address: 10.171.8.150) to store and collect study data and SSN information. The server will be accessed through password protected computers only which are encrypted(no laptops, no portable data drives). We will have a Data and Safety Monitoring Board (DSMB) constitute of 2 endocrinologists and 1 statistician; we will meet once a year and have a conference call every 6 months or more often if necessary.

Subjects

Unless otherwise noted, the information entered in this section should reflect the number of subjects enrolled or accrued under the purview of Columbia researchers, whether at Columbia or elsewhere.

Target enrollment:

70

Number enrolled to date:

11

Number enrolled since the last renewal or, if this is the first renewal, since the initial approval:

11

Number anticipated to be enrolled in the next approval period:

52

Does this study involve screening/assessment procedures to determine subject eligibility?

Yes

Target accrual:

52

Number accrued to date:

0

Number accrued since the last renewal or, if this is the first renewal, since the initial approval:

11

Number anticipated to be accrued in the next approval period:

52

Of the number of subjects enrolled, or the number accrued for interventional studies with a screening process:

How many remain on the study?

11

How many are off study?

0

How many completed the study?

0

Have any withdrawn of their own initiative?

No

Have any been removed by PI?

No

Have any been lost to follow-up?

No

Have any died while on study?

No

Have any subject complaints been received?

No

Is this a multi-center study?

No

Does this study have one or more components that apply to a subset of the overall study population (e.g. Phase 1/2, sub-studies)?

Yes

Name/Procedure	Target enrollment	Enrolled to date	Enrollment Status
Bone Biopsy	10	3	Open to enrollment or ongoing review of records/specimens

Of the number enrolled, or the number accrued for interventional studies with a screening process, indicate:

Population Gender

Females	Males	Non Specific
100%	0%	0%

Population Age

0-7	8-17	18-65	>65	Non Specific
0%	0%	0%	100%	0%

Population Race

American Indian/Alaskan Native	Asian	Native Hawaiian or Other Pacific Islander	Black or African American	White	More than One Race	Non-Specific
0%	0%	0%	18%	82%	0%	0%

Population Ethnicity

Hispanic or Latino	Not Hispanic or Latino	Non-Specific
45%	0%	55%

Vulnerable Populations as per 45 CFR 46:

Will children/minors be enrolled

No

Will pregnant women/fetuses/neonates be targeted for enrollment?

No

Will prisoners be targeted for enrollment?

No

Other Vulnerable Populations:

[ ]Individuals lacking capacity to provide consent

[ ]CU/NYPH Employees/Residents/Fellows/Interns/Students

[ ]Economically disadvantaged

[ ]Educationally disadvantaged

[x]Non-English speaking

Please ensure that your plan to enroll subjects in their primary language is described on the Informed Consent page.

[ ]Other Vulnerable populations

[ ]None of the Populations listed above will be targeted for Enrollment

Subject Population Justification:

We are enrolling post-menopausal women with Type 2 diabetes to assess the effects of pyridoxamine on the interaction between bone material strength and AGEs accumulation. We are enrolling both English and Spanish-speaking women as the predominant minority group at CUMC are women from Latin America.

Does this study involve compensation or reimbursement to subjects?

Yes

Describe and justify reimbursement/compensation:

We are reimbursing $225 for completion of the one year study. We feel this amount compensates study subjects for their time and effort to be in the study.

If subjects chose to have the bone biopsy, they will be compensated an additional $250 as this is an invasive procedure and requires an additional study visit.

Are subjects eligible for compensation of $600 or more in a calendar year?

No

HazMats

Type	Number	Date Created	Principal Investigator
Radiation Safety (Appendix H)	APH-AAAW7271	08/23/2017	Maximo Gomez Almonte (meg2230)

Attached Attestation

Type	Principal Investigator	Date Created
C	Mishaela Rubin	09/13/2018

Attached HIPAA Forms

Number	Type	Title	Status
AAAO0049	A	B6 study - Spanish	Approve
AAAM7301	A	Form A Updated	Approve

Number	Type	Title	Status
AAAN9051	D	B6 Study	Approve

Attached Consent Forms

Number	Copied From	Form Type	Title	Active/InActive	Initiator
AABJ4500	AABJ4500	Consent	Consent for Bone Biopsy sub-study	Active	Xiomara Javier-Espinal (xij2000)
AABK9700	AABJ4550	Consent	B6 and microindentation consent	Active	Xiomara Javier-Espinal (xij2000)

Documents

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	Created By
No	AAAR5451 B6 ICF Spa	Consent Form/Addendum	AAAR5451 B6 ICF Spa.pdf	N		02/28/2018	Maximo Gomez Almonte (meg2230)
No	AAAR5451 B6-BBx ICF Spa	Consent Form/Addendum	AAAR5451 B6-BBx ICF Spa.pdf	Y		02/28/2018	Maximo Gomez Almonte (meg2230)
No	B6 ICF consent form tracket changes 9.4.2018	Consent Form/Addendum (tracked)	B6 ICF consent form tracket changes 9.4.2018.pdf	Y		09/12/2018	Xiomara Javier-Espinal (xij2000)
No	Rubin_applicationimage_ASSIST6	Funding/Grant Application/Subcontract	Rubin_applicationimage_ASSIST6.pdf	Y		09/29/2017	Maximo Gomez Almonte (meg2230)
No	Brochure AGE Reader	Investigator Brochure/Package Insert/Device Manual	Brochure AGE Reader mu English (3).pdf	Y		10/18/2017	Lisa Lotwin (ls432)
No	Brochure_AGE_Reader_mu	Investigator Brochure/Package Insert/Device Manual	Brochure_AGE_Reader_mu.pdf	Y		10/09/2017	Maximo Gomez Almonte (meg2230)
No	Diab-spot_brochure_Sept_2011_EN	Investigator Brochure/Package Insert/Device Manual	Diab-spot_brochure_Sept_2011_EN.pdf	Y		10/09/2017	Maximo Gomez Almonte (meg2230)
No	OsteoProbe RUO User's Guide	Investigator Brochure/Package Insert/Device Manual	OPG-001_A (OsteoProbe RUO User's Guide v1 0).pdf	Y		10/18/2017	Lisa Lotwin (ls432)
No	Osteoprobe Qualifications	Investigator Brochure/Package Insert/Device Manual	Osteoprobe Qualifications.pdf	Y		10/09/2017	Maximo Gomez Almonte (meg2230)
No	OsteoProbe RUO OnePager	Investigator Brochure/Package Insert/Device Manual	OsteoProbe RUO OnePager_Dr Mishaela Rubin of Columbia Univ.pdf	Y		10/18/2017	Lisa Lotwin (ls432)
No	OsteoProbe User Instructions	Investigator Brochure/Package Insert/Device Manual	OsteoProbe User Instructions.pdf	Y		10/09/2017	Maximo Gomez Almonte (meg2230)
No	PrimAGE-o	Investigator Brochure/Package Insert/Device Manual	PrimAGE-o.pdf	Y		10/09/2017	Maximo Gomez Almonte (meg2230)

Archived	Document Identifier	Document Type	File Name	Active	Stamped	Date Attached	Created By
No	Tetracycline_Label	Investigator Brochure/Package Insert/Device Manual	Tetracycline_Label.pdf	Y		10/23/2017	Maximo Gomez Almonte (meg2230)
No	Table 1 Protocol tracked changes 9.5.2018	Other	Table 1 Protocol tracked changes 9.5.2018.pdf	Y		09/12/2018	Xiomara Javier-Espinal (xij2000)
No	B6 Postcard-SPANISH	Recruitment Material	AAAR5451 B6 Postcard-SPA.pdf	Y		06/22/2018	Rukshana Majeed (rm3324)
No	Spanish Brochure	Recruitment Material	AAAR5451 Brochure B6-SPA.pdf	Y		06/22/2018	Rukshana Majeed (rm3324)
No	AAAR5451 Diabetes bone flyer Spa	Recruitment Material	AAAR5451 Diabetes bone flyer Spa.pdf	Y		02/28/2018	Maximo Gomez Almonte (meg2230)
No	Mailing Letter in Spanish	Recruitment Material	AAAR5451 Vit 6 Letter-SPANISH.pdf	Y		06/22/2018	Rukshana Majeed (rm3324)
No	B6 diabetes bone flyer	Recruitment Material	B6 diabetes bone flyer.pdf	Y		01/11/2018	Maximo Gomez Almonte (meg2230)
No	Brochure 5.16.2018	Recruitment Material	brochure 5-16-18.pdf	Y		05/16/2018	Rukshana Majeed (rm3324)
No	mailing about study 5-16-18-updated	Recruitment Material	mailing about study 5-16-18.pdf	Y		05/23/2018	Rukshana Majeed (rm3324)
No	Mailing letter 5.16.2018	Recruitment Material	mailing about study 5-16-18.pdf	N		05/16/2018	Rukshana Majeed (rm3324)
No	Postcard	Recruitment Material	postcard with mailing 5-16-18.pdf	Y		05/16/2018	Rukshana Majeed (rm3324)
No	Telephone script for recontacting subjects for new Diabetes	Recruitment Material	Telephone script for recontacting subjects for new Diabetes and MRR.pdf	Y		09/29/2017	Maximo Gomez Almonte (meg2230)
No	B6 ICF Protocol Tracket Changes 9.4.2018	Standalone/Sponsor's Protocol (tracked)	B6 ICF Protocol Tracket Changes 9.4.2018.pdf	Y		09/12/2018	Xiomara Javier-Espinal (xij2000)
No	Exercise Questions v10-1-17	Study Material/Instrument	Exercise Questions v10-1-17.pdf	Y		10/02/2017	Maximo Gomez Almonte (meg2230)