A Prospective, Non-Randomized, Multi–Center Observational Study to Establish a Physical Baseline Profile for Individual Study Subjects Using Various Modalities and Identify Deviations via Longitudinal Monitoring that May Develop Over Time and May be Relevant to Human Health and Longevity

Protocol Short Title:	PUER ("Previously Unrecognized Emerging Risks") Life Clinical Study
Identifying Number:	PLI001
Protocol Version and Date:	Version v.2.0 06 October 2022
Sponsor/Manufacturer:	PUER Research, LLC 8601 Dunwoody Place, Suite 304 Atlanta, GA 30350 United States
Sponsor/Manufacturer Contact:	Karl Blass PUER Research, LLC karl@puerlife.health 404-277-7597
Principal Investigator:	Adam Friedlander, MD 8601 Dunwoody Place, Suite 304 Atlanta, GA 30350 adam@puerlife.health 678-792-8442

PROTOCOL APPROVAL

Protocol Number: PLI001

A Prospective, Non-Randomized, Multi–Center Observational Study to Establish a Physical Baseline Profile for Individual Study Subjects Using Various Modalities and Identify Deviations via Longitudinal Monitoring that May Develop Over Time and May be Relevant to Human Health and Longevity.

Protocol Version: Version 2.0

Protocol Date: 06 October 2022

This protocol has been read and approved by:
Sponsor:

_____

Name: Karl Blass Date: dd-mm-yyyy

REVISION HISTORY

Version	Date	Significant Revisions
1.0	08 August 2022	Initial Version
2.0	06 October 2022	Updated miscellaneous language and included language to exclude women with positive pregnancy test at any visit.

STATEMENT OF INVESTIGATOR COMPLIANCE

This protocol is a prospectively designed clinical study of the PUER Research, LLC
I agree to:

- Implement and conduct this study diligently and in strict compliance with the protocol, Good Clinical Practices (GCP) and all applicable laws and regulations.
- Maintain all information supplied by PUER Research, LLC in confidence and, when this information is submitted to an Institutional Review Board (IRB), it will be submitted with a designation that the material is confidential.
- Ensure that all persons assisting with the trial are adequately informed about the protocol and their trial-related duties and functions.

This document contains confidential information belonging to the Sponsor (PUER Research, LLC) and therefore, may not be disclosed to any other person or entity without the prior written permission of the Sponsor unless such disclosure is required by law or regulation.

Investigator Signature

I have read and understand the contents of the clinical protocol including this Statement of Investigator Compliance. I agree to follow and abide by the guidelines set forth in this document.

Adam Friedlander, MD
Principal Investigator Name

_____ _____
Principal Investigator Signature Date (dd-mmm-yyyy)

Protocol Synopsis

Study Title: A Prospective, Non-Randomized, Multi–Center Observational Study to Establish a Physical Baseline Profile for Individual Study Subjects Using Various Modalities and Identify Deviations via Longitudinal Monitoring that May Develop Over Time and May be Relevant to Human Health and Longevity.	
Short Title	PUER Research Clinical Study
Study Type	This is a minimal-risk, prospective, non-randomized, multi-center population based observational study to establish a physical baseline profile for individual study subjects using various modalities and identify deviations through longitudinal monitoring that may develop over time and may be relevant to human health and healthy longevity.
Study Description	The three key elements of the PUER Research protocol include (1) Medical history and physical exam (PE), (2) molecular and laboratory profiling and (3) non-invasive imaging and wearables/ "quantified self" measurements. The medical history and PE will consist of a comprehensive medical history and physical examination, administered by a trained Research Associate. Laboratory assessments will be conducted using peripheral blood samples, and potentially urine, stool or saliva samples. Non-invasive imaging assessment will consist of magnetic resonance imaging (MRI) and computerized tomography (CT) scans. Other functional assessments, including respiratory, cardiac and other functions, as well as self-quantifiable assessments via wearables will also be conducted. The study will result in longitudinal real world data collection to inform if any modalities employed here can inform of baseline deviations for individuals. Researchers at study sites will not be blinded to the data being collected during this study.
Study Sites	This study will be conducted in up to 20 study sites in the United States.
Study Population	The study will be conducted in up to 10,000 participants.
Study Duration	72 months
Enrollment Duration	36 months
Objectives	• The primary objective is to establish a baseline profile for individual healthy study subjects using various modalities and monitor them longitudinally to identify deviations that develop over time and may be relevant to human health and longevity The secondary objective is to identify the modality that has the highest impact on the identification of deviations from baseline profile.
Adverse Event (AE) Monitoring	Adverse events associated with the study procedures, of venipuncture, non-invasive imaging and functional tests will be reported, although they are expected to be limited due to the nature of these common procedures.

Inclusion Criteria

To participate in the study, patients must meet the following criteria;

1. Male or non-pregnant female; age 18 to 90.
2. Absence or presence of any medical history or any signs or symptoms of any disease.
3. Women of childbearing potential (WOCBP) must have a negative urine pregnancy test (UPT) at all visits.

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study participation;

1. Unwillingness or inability to participate in the study
2. Unwillingness or inability to provide written Informed Consent Form
3. WOCBP with positive pregnancy test at enrollment or at any visit

Study Schedule

Event	Baseline Visit	6-Month Visit ± 1month	12-Month Visit ± 1month	18-Month Visit ± 1month	24-Month Visit ± 1month	36-Month Visit ± 1month
Informed Consent	X					
Review of Inclusion/Exclusion Criteria	X					
Medical history and physical exam	X	X	X	X	X	X
UPT[1] for WOCBP[2]	X	X	X	X	X	X
Whole Genome Sequencing Analysis	X					
Biochemical/Omics Analysis	X	X	X	X	X	X
Non-Invasive Imaging[3]	X	X	X	X	X	X
Functional Assessments (as needed)		X	X	X	X	X
Wearables and Quantified Self Assessments (as needed)		X	X	X	X	X
Adverse Event Monitoring	X	X	X	X	X	X

1. UPTs must have a minimum sensitivity of 25 mIU ß-hCG/mL.
2. WOCBP include any female who has experienced menarche or is 10 years of age or older and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea >12 consecutive months in women 50 years of age and older).
3. See Section 3.3.2 for detailed schedule of imaging

ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
EDC	Electronic Data Capture
EEG	Electroencephalogram
EMG	Electromyography
FDA	Food and Drug Administration
GBCM	Gadolinium-Based Contrast Media
GCP	Good Clinical Practice
GINA	Genetic Information Nondiscrimination Act
HR	Heart Rate
HRV	Heart Rate Variability
IRB	Institutional Review Board
miR	Micro-RNA
mL	Milliliter
mRNA	Messenger RNA
MRI	Magnetic Resonance Imaging
NNT	Number Needed to Treat
PE	Physical Examination
PHI	Protected Health Information
PMI	Precision Medicine Initiative
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
UPT	Urine Pregnancy Test
US	United States
WGS	Whole Genome Sequencing
WOCBP	Women of Childbearing Potential

Table of Contents

PROTOCOL SYNOPSIS **4**

ABBREVIATIONS **6**

TABLE OF CONTENTS **7**

1. INTRODUCTION **9**

1.1. BACKGROUND 9
1.2. RATIONALE 9

2. OBJECTIVES AND ENDPOINTS **12**

2.1. MAIN OBJECTIVES: 12
2.2. MAIN ENDPOINTS: 12
2.3. EXPLORATORY OBJECTIVES: 12

3. STUDY DESIGN **14**

3.1. STUDY SCHEMA 14
3.2. GENERAL SELECTION CRITERIA 14
3.2.1. Inclusion Criteria *14*
3.2.2. Exclusion Criteria *14*
3.3. STUDY PROCEDURES 15
3.3.1. Study Design *15*
3.3.2. Methodology *15*

4. BENEFIT/RISK ASSESSMENT **18**

4.1. POTENTIAL RISKS 18
4.2. POTENTIAL BENEFITS 19
4.3. OVERALL BENEFIT/RISK RATIO 19

5. ADVERSE EVENTS **20**

5.1. DEFINITIONS 20
5.2. PREGNANCY 20
5.3. REPORTING 20
5.4. SAFETY 21

6. DATA MANAGEMENT **22**

6.1. CASE REPORT FORMS 22
6.2. DATABASE 22
6.3. SAFEGUARDING OF GENOMIC DATA 22
6.4. QUALITY CONTROL 23

7. STATISTICAL CONSIDERATIONS **24**

8. ETHICAL, REGULATORY, AND ADMINISTRATIVE CONSIDERATIONS **25**

8.1. INFORMED CONSENT 25
8.2. CONFIDENTIALITY 25
8.3. INSTITUTIONAL REVIEW 25
8.4. PROTOCOL INTERPRETATION AND COMPLIANCE 25
8.5. COMPLETION OF CASE REPORT FORMS (CRFS) 25
8.6. MAINTENANCE OF STUDY DOCUMENTATION 26
8.7. PUBLICATION POLICY 26
8.8. FINANCING AND INSURANCE 26
8.9. FINAL STUDY REPORT 26
8.10. RECORD RETENTION 26
9. REFERENCES 27

1. Introduction

1.1. Background

Scientific and medical progress has been exponential over the past decades, yet the implementation of new technologies into every-day clinical practice can lag by 5-10 years, thus preventing a large portion of the population from benefiting from those advances during their meaningful lifetime (Glasziou & Haynes, 2005). Earlier detection leads to earlier intervention where needed and accordingly, deploying novel technologies is the cornerstone of avoiding undesirable outcomes. However, the path and timeline of discovery, development, regulatory approval, and commercialization can delay implementation of potentially life-saving technologies by 5-10 years.

There have been tremendous advances in the understanding of health, and longevity over the past decades. The early 2000s marked the ushering in of the genomic era, with the sequencing of the human genome (Guttmacher & Collins, 2003). Since that time, the required time and cost of genome sequencing has dropped dramatically, and the actionability of sequencing the entire human genome has increased exponentially (Hayden, 2014) (Scaria, 2014) (Dorschner, et al., 2013) (Amendola, et al., 2015) (Goldstein, 2013) (Bromberg, 2013). Beyond DNA sequencing, other "omics" analyses such as DNA methylation, RNA sequencing, proteomics, metabolomics and lipidomics technologies have matured to the point of significant reproducibility, providing important biological insights. Conventional biochemical laboratory technologies have improved and matured and there are a large number of diagnostics laboratory tests available in the commercial setting.

Similarly, non-invasive imaging techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT) modalities have continued to evolve, using artificial intelligence- and computer-aided design-based analytical methods to improve the quality of diagnostic images and enhance clinical care (Uppot, et al.,, 2018) (Onal, et al., 2014) (Zhu, et al., 2018) (Anthimopoulos, et al., 2016) (Li, et al., 2008) (Arnoldi, et al., 2010) (Lemaitre, et al., 2015) (Yang, et al., 2014)

The above techniques, as well as others, can now be applied both in healthy individuals, as well as in individuals exhibiting certain deviations from a healthy baseline profile. However, the impact of deploying a large suite of genomic, biochemical, and non-invasive imaging modalities on human health and longevity is unknown. Understanding such impact of the combination of such modalities, as well as the relative contribution of individual modalities, represents a leap forward in the understanding of human health and healthy longevity.

1.2. Rationale

The fundamental basis of the PUER ("Previously Unrecognized Evidence of Risk") study is based in the central dogma of biology (Crick, 1970). The central dogma of biology stipulates that human health and longevity are determined in the context of the interactions of the human genome (DNA) and the environment, and these interactions will determine the transcription of DNA to RNA, the translation of RNA to proteins, and such structural and functional proteins will determine the complex molecular milieu in an individual, leading to quantifiable phenotypes of health and disease. The human genome (DNA) contains approximately 6.4 billion nucleotides organized into ~25,000 genes, transcribed into approximately 100,000 different RNA transcripts and ~25,000-28,000 transcribed gene constructs that are translated into about 20,000 human proteins. These proteins make up human cells and organs and

regulate the intracellular and extracellular milieu of the human body, containing over 10,000 small molecules and lipid/phospholipid species.

Technologies exist today to measure the majority of these biochemical and "omics" components. Whole genome sequencing (WGS) technologies are now able to decipher all 6.4 billion nucleotides in each individual ("wet lab component"), and cutting-edge bioinformatics pipelines can tie individual genetic variants to disease susceptibility, pharmacogenomics, and disease carrier status ("dry lab component") (Netto & Kaul, 2019). Both messenger RNA (mRNA) and micro-RNA (miR) can be sequenced from blood-based, peripheral circulating cells and such gene expression signatures have already been translated to clinically approved diagnostic blood tests (Rosenberg, et al., 2010). A number of different techniques are available for proteomics profiling based on mass spectrometry (Han, et al., 2008) and other techniques, such as aptamers, somamers (Gold, 2010) and dual-antibody approaches (Assarsson, et al., 2014). Such proteomics signatures have also been turned into diagnostic signatures (Ganz, et al., 2016). Metabolomics profiling is now also a reality, using mass spectrometry (Yet, et al., 2016), NMR-technologies (Fest, et al., 2019) and other techniques (Bennuru, et al., 2017). Similar to metabolomics, lipidomics profiling is also available today (David, et al., 2022). Many commercial laboratories have developed Food and Drug Administration (FDA)-cleared diagnostic and screening tests that can be applied in everyday clinical practice.

While non-invasive imaging modalities, such as CT and MRI have been around for several decades, artificial intelligence-based post-processing and interpretation approaches are transforming the imaging field with the potential to radically impact clinical care (Uppot, et al., 2018).

Yet another important concept is the concept of the "N-of-1" clinical study (Schork, 2015). The traditional path in science and medicine has been a population-based approach, where large populations were compared across different timepoints. Accordingly, most nutraceutical, pharmaceutical and medical interventions have been developed for populations, with varying degree of efficacy in individuals. The most important and most impactful drugs in the history of medicine have been limited on an individual basis. The "number needed to treat" (NNT) is the number of people who need to receive the drug (or other intervention) in order for just one person to receive a benefit, can be an important number to consider. For reference, an NNT of 5 or less ($\leq$5) is probably associated with a meaningful health benefit for an individual, while an NNT of 15 or more ($\geq$15) is quite certain to be associated with, at most, a small net health benefit (Chong, et al., 2006). The NNT for the most successful drugs in history is quite high. For simvastatin, the NNT is approximately 81, which is a staggeringly high number ("Design and baseline" 1993) ("Randomized trial" 1994). Precision medicine and "N-of-1 studies" may provide an opportunity to significantly increase the efficacy of certain nutraceuticals, pharmaceuticals, and other medical interventions.

In the context of laboratory evaluation and the evaluation of certain biochemicals and laboratory measurements, the concept of the "N-of-1 study" stipulates that rather than using population-based cut-points for laboratory values and applying them to individuals, we should use an individual as their own control over time. If we are able to monitor a large suite of biochemicals and laboratory measurements in an individual over time, this will establish their own baseline, and any significant deviation from their own baseline may signal the need for further evaluation and testing by the study subject's health care provider. This concept leads to the approach of longitudinal monitoring and surveillance over time.

Fortunately, the concept of individualized, or "precision medicine" has been gaining traction even at the level of the United States (U.S.) federal government. The Precision Medicine Initiative (PMI) was unveiled at the State of the Union Address on January 30, 2015 and had an initial investment of $215M from the 2016 federal budget. The distilled goal of the program is "to accelerate biomedical discoveries

and provide clinicians with new tools, knowledge, and therapies to select which treatments will work best for which patients." Simply, the PMI seeks to shift the healthcare paradigm from a one-size-fits-all approach to one which individualizes treatment plans based on individual differences in genetics, lifestyle, and environment. The original language of the initiative specifically mentions metabolomics and the microbiome, signaling the acceptance and promotion of cutting-edge health technology and research, beyond what is currently considered standard of care. Success in this area exists, most recognizably in the field of oncology, but the next steps into this frontier require the translation and broadening of current concepts to include more patients and every field of medicine. To do this, public and private collaboration will be required to leverage advances in genomics, big data, and health information technology, specifically regarding health data portability, for collaboration between patients and multidisciplinary healthcare teams. The PMI calls on millions of Americans to contribute health data to "catalyze a new era of data-based and more precise medical treatment." Participants will have granular control of the privacy of the data they share and may withdraw consent for any component at any time, however the goal will be the creation of secure, trustworthy platforms which will engender confidence in sharing deidentified data on a longitudinal, ongoing basis, allowing physician scientists to collect and analyze that data over the course of millions of lifetimes. The PMI's All of US research program is already the largest longitudinal study in the history of the United States.

The initial objectives of the PMI focused on strengthening existing areas of success, while broadening federal support and soliciting private support for both existing and new initiatives, with definitive commitment to patient engagement, privacy, and data security. The language of the PMI discusses the creation of a "cancer knowledge network" via funding to the National Cancer Institute for the design and testing of tailored treatments for cancer based on the genetics of patients and specific cancers. Next, the initiative calls for multi-departmental collaboration on the creation of a voluntary national research project via the National Institutes of Health, The Department of Health and Human Services, The Department of Veterans Affairs, The Department of Defense, and other relevant stakeholders, with the goal of enrolling over a million diverse individuals. Through this project, participants will contribute a wide variety of data sources "including medical records; profiles of the patient's genes, metabolites (chemical makeup), and microorganisms in and on the body; environmental and lifestyle data; patient-generated information; and personal device and sensor data." Radiology studies are included in this complete digital health profile, as well, as is all other available historical data, and all new data collected via the participating programs. To date, radiology studies have played a key role in the phenotyping that goes hand-in-hand with advanced genomics exploration. The amount of information in a single individual's digital health profile can be massive, and for that reason, the big data component of the PMI is critical to the initiative's success. As to privacy and data security, the PMI definitively commits to soliciting input from federal, public, and private entities to identify and address legal and technical issues surrounding privacy and data security, while the FDA will focus on "regulatory modernization" with specific attention to genetic testing. Lastly, the PMI emphasizes the importance of forging strong public-private partnerships for infrastructure development and patient enrollment, with attention to patient engagement and empowerment throughout each individual's experience over their lifetimes.

Accordingly, the objective of the PUER study is to assess the impact of longitudinal, comprehensive evaluation based on certain currently available modalities in a large, unselected population of individuals.

If our study demonstrates that comprehensive baseline and longitudinal assessment employing these various modalities identifies previously unknown deviations from a study subject's baseline, this could inform lifestyle changes and other potential interventions to maintain a healthier self. Longitudinal testing at frequent intervals, such as every 6 months, is critical, as metabolomic and proteomic changes

occur on the order of hours and days, and diseases such as pancreatic cancer can progress rapidly within a 4 to 6 month interval.

2. Objectives and Endpoints

2.1. Main Objectives:

- To establish a baseline profile for individual study subjects and monitor them longitudinally to identify deviations that develop over time and may be relevant to human health and healthy longevity.
- To identify the modality that has the highest impact on the identification of deviations from baseline profile.

2.2. Main Endpoints:

- The number of new deviations from a study subject's baseline profile 12 months after enrollment that were previously not known at time of enrollment

- The number of new deviations not known at the time of enrollment, at the following timepoints after enrollment:
  - 6 months after enrollment
  - 18 months after enrollment
  - 24 months after enrollment

- The number of new deviations identified by each employed modality

2.3. Exploratory Objectives:

- To assess the impact of introducing leading-edge scientific methods on the number of medications and/or dietary supplements taken by individuals

- To assess the impact of leading-edge scientific methods on the number of *unscheduled* health care visits

2.4. Exploratory Endpoints

- The number and change in the number of medications taken by an individual at 6 months, 12 months, 18 months, and 24 months after enrollment

- The number and change in the number of dietary supplements taken by an individual at 6 months, 12 months, 18 months, and 24 months after enrollment

- Number of unscheduled, urgent care/emergency department visits within 24 months and 36 months after enrollment

- Number of unscheduled hospital admissions within 24 months and 36 months after enrollment

3. Study Design

This is a prospective, population-based observational study. Participants may be recruited from clinical sites that are actively participating in the PUER Research comprehensive evaluation model. Participants and all participating health care providers are unblinded.

3.1. Study Schema

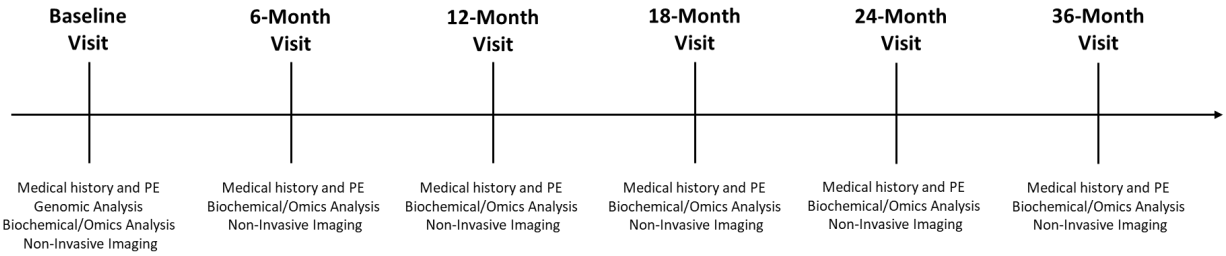

Figure 1. Overall Study Schema. *The Baseline Visit includes a Medical history and physical examination (PE) Genomic Analysis, Biochemical/"Omics" Analysis and Non-Invasive Imaging. Subsequent visits at 6 months, 12 months, 18 months, 24 months and 36 months include slightly modified and targeted Medical history and PE, Biochemical/"Omics" Analysis and Non-Invasive Imaging, based on prior findings. Functional Assessments and Wearables and Quantified Self Assessments are included at the 6 months, 12 months, 18 months, 24 months and 36 months visits as determined by the Medical history and PE at the baseline visit.*

3.2. General Selection Criteria

To be included in the study, participants must meet the following inclusion criteria and none of the exclusion criteria.

3.2.1. Inclusion Criteria

- Male or non-pregnant female; age 18 to 90
- Absence or presence of any medical history or any signs or symptoms of any disease
- Women of childbearing potential (WOCBP) must have a negative urine pregnancy test (UPT) at all visits.

3.2.2. Exclusion Criteria

- Unwillingness or inability to participate in the study
- Unwillingness or inability to provide written Informed Consent Form
- WOCBP with positive pregnancy test at enrollment or at any visit

3.2.3 Participant withdrawal criteria

A participant may be withdrawn from the study prior to completion for any of the following reasons:

- Adverse Event (AE)
- Death
- Physician decision
- Pregnancy at any visit
- Protocol deviation
- Other (e.g., any reason that may affect the outcome of the study or safety of participants)
- Participant no longer wants to participate (without penalty)

When a participant is withdrawn from the study for an AE (as defined in Section 5), when possible, the participant should be followed until resolution or stabilization of the AE.

3.3. Study Procedures

3.3.1. Study Design

- This is a prospective, population-based observational study. Participants may be recruited from clinical sites that are actively participating in the PUER Research™ Comprehensive Evaluation protocol. Participants and all participating health care providers are unblinded.

- The three key elements of the PUER Research protocol include (1) Medical history and PE, (2) molecular and laboratory profiling and (3) non-invasive imaging, with the option of adding functional assessments and wearables/quantified self-assessments, as determined by the Medical history and PE.

3.3.2. Methodology

Each of the visits include a combination of Medical history and PE, biochemical and "omics" assessments, non-invasive imaging assessments and optional functional assessments and wearables and quantified self-assessments.

*Medical history and PE*The Medical history and PE consists of a comprehensive "History and Physical Examination", administered by a trained Research Associate. The elements of the history and physical examination are recorded in the Case Report Form (CRF).

- *Pregnancy Testing*

Pregnancy testing consists of a UPT for all WOCBP[1]. The results must be negative for the subject to be able to participate in each visit.

- *Genomic Analysis*
  - Whole genome sequencing (WGS) analysis ("DNA")

- *Biochemical and "Omics" Assessments*

  Biochemical and "omics" assessments consist of the following elements, using peripheral blood samples, and potentially urine, stool or saliva samples.

  - Whole genome methylation analysis ("DNA methylation")
  - Whole transcriptome sequencing from circulating mononuclear cells (mRNA and miR)
  - Proteomics profiling
  - Metabolomics profiling
  - Conventional biomarker analysis
  - Urine analysis and profiling

- *Non-Invasive Imaging Assessments*

  Non-invasive imaging assessment consists of the following elements, as directed, and determined by the Medical history and PE and biochemical and "omics" assessment, as described above. Imaging will follow the schedule in the table below, with an "x" indicating all participants and an "[x]" indicating imaging needed for risk-specific participants. Any deviation from the average population may trigger a dedicated organ specific MRI. Imaging may also be done at 18-, 24- and/or 36-month visits, depending on Medical history and PE at each visit, following the schedule at 6 or 12 months.

Imaging Procedure	**Baseline**	**6 mos**	**12 mos**
CT of head	x		
CT Angiogram of head/neck/face/sinus/orbits	x		
MRI of brain with and without contrast	x		x
MR Angiogram of head/neck time of flight			x
MRI of brain without contrast		[x]	
CT of chest, abdomen, and pelvis - mixed A/V	x	[x]	[x]
CT of chest, abdomen, and pelvis - venous only		[x]	x
MRI of spine without contrast (cervical/thoracic/lumbar)	x		x

[1] WOCBP include any female who has experienced menarche or is 10 years of age or older and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea >12 consecutive months in women 50 years of age and older).

X-rays of extremities	x	[x]	x
Ultrasound (reproductive organs/bladder)	x	[x]	x

- *Functional Assessments (determined by the Medical history and PE, described above)*
  - VO2-max functional testing (maximum oxygen consumption)
  - Non-invasive endothelial function testing
  - Electroencephalogram (EEG)
  - Electromyography (EMG)
  - Ophthalmologic examination for vision preservation and other pathology
  - Optional: sleep study

- *Wearables and Quantified Self Assessments (determined by the Medical history and PE, described above)*
  - Ambulatory Electrocardiogram (ECG) monitoring (Apple Watch, ePatch or similar)
  - Continuous glucose monitoring
  - Oura ring (may obviate the need for formal sleep study), or similar product(s) measuring heart rate (HR), heart rate variation (HRV), sleep duration, sleep stage

4. Benefit/Risk Assessment

4.1. Potential Risks

The potential risks of the PUER study are minimal. The PUER study does not prescribe any pharmaceutical or medical device-based interventions. Information collected from the genomic analysis, comprehensive "omics" analysis, non-invasive imaging and functional assessment will be shared with the individual and, if desired, with the health care provider of choice of the individual. Any potential treatment decision of further diagnostic evaluation-related decision will be made by the individual and their chosen health care provider.

Accordingly, any potential risks of the PUER study are only related to the comprehensive evaluation. For comprehensive biochemical and "omics" profiling, individuals will undergo blood draw through venipuncture. The risks of blood draw and venipuncture are minimal and can be mitigated. AEs that may be associated with venipuncture and that must be included in the informed consent include:

- Pain
- Bruising
- Bleeding at the puncture site
- Fainting
- Inflammation of the vein

Individuals will also undergo MRI. MRI is a safe, non-radiation-based modality and when contraindication to MRI are adequately assessed, MRI examination does not pose any risk to the participants. Standard screening for contraindications to MRI examination will be performed at the imaging center, using the centers' policies and procedures for screening to identify contraindications to MRI examinations. Gadolinium-based contrast agents may be administered in connection with the MRI examination. Gadolinium-based contrast agents have been associated with potential risks, including rare anaphylactic reactions and necrotizing systemic fibrosis (American College of Radiology, 2021).

The use of gadolinium contrast agents may be associated with anaphylactic reaction in 0.001% to 0.01% of cases, and gadolinium contrast agents have also been associated with nephrogenic systemic fibrosis (NSF) in patients with compromised renal function. Based on the 2021 ACR Manual on Contrast Media, any adverse event rate with gadolinium-based contrast media ("GBCM") ranges from 0.07% to 2.4%. Most reactions are mild and physiologic. Allergic-like reactions are uncommon and vary in frequency from 0.004% to 0.7%. Severe life-threatening anaphylactic reactions are exceedingly rare (0.001% to 0.01%).

Individuals will also undergo CT. CT is a safe imaging method that includes exposure to ionizing radiation with or without contrast agents. The contrast is either given orally, rectally or intravenously. This contrast material can rarely cause medical problems or allergic reactions. Most of these reactions are mild and result in a rash or itchiness. In rare instances, an allergic reaction can be serious, even life-threatening.

During an abdominal ultrasound exam, a trained technician will glide a small device over the participant's abdomen to take pictures of the tissue under the skin. There are no known side effects from taking part in an abdominal ultrasound exam. Ultrasound tests do not involve radiation and they do not penetrate the skin.

Due to the risk of imaging radiation exposure to a fetus, WOCBP must not be pregnant at any visit.

Individuals will also undergo x-ray imaging. X-ray is an imaging method that includes exposure to a low amount of electromagnetic radiation to take pictures of structures in the body, such as bones.

4.2. Potential Benefits

Participants may not derive any benefit at all from participating in the study. At the same time, the extensive genetic, biochemical, and non-invasive imaging evaluation may uncover unknown genetic susceptibilities and/or early identification of variance and deviations from a study subject's baseline profile that may be worthy of further evaluation and testing by the study subject's primary care physician, providing some potential benefits to participants.

4.3. Overall Benefit/Risk Ratio

Given that potential risks are minimal or negligible as described above, and given potential benefits as described above, the overall benefit/risk ratio favors participation in the study.

5. Adverse Events

5.1. Definitions

An *Adverse Event (AE)* is a noxious and unintended event observed in, or reported by, a participant who is participating in (or has participated in) a clinical study, and/or received study medication, an experimental device, or study-related procedure. The event may be related temporally either to immediate or long-term use of a drug, device, or procedure. It may not necessarily be caused by a drug, device, or procedure. Any event meeting these criteria shall be considered an adverse event, regardless of whether or not it is considered study related. A pre-existing condition that worsens during the trial is considered an AE.

The following events will be considered as adverse events:

- Event directly related to study procedure
- Unintended event as a result of non-standard of care study tests, medications or procedures

A *Serious Adverse Event (SAE)* is defined as one that is fatal or immediately life-threatening, severely or permanently disabling, requires or prolongs in patient hospitalization, a congenital anomaly, cancer or overdose (intentional or accidental); or suggests a significant hazard, contraindication, side effect, or precaution. All SAEs will be followed by the investigator through trial end until resolution or until reaching a clinically stable status per the investigator's discretion.

The following adverse events will be handled as serious even if they do not meet the above criteria for designation as serious:

- Death
- Congenital abnormality
- Hospitalization
- Life threatening illness
- Intervention to prevent permanent damage

5.2. Pregnancy

WOCBP (see Protocol Synopsis for definition of WOCBP) must have a negative UPT prior to study enrollment and at each study visit. Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy at each visit. The subject must sign an informed consent form documenting this discussion.

WOCBP should be instructed that if they are pregnant, they will not be permitted to be enrolled and are subject to withdrawal from the study with a positive UPT at any visit, as per Section 3.2.3.

5.3. Reporting

The PUER study does not prescribe any pharmaceutical or medical device-based intervention. Any AEs are associated with blood draw/venipuncture and MRI examinations that may or may not include Gadolinium-based contrast agents will be reported.

Each patient will be observed and queried in a nonspecific fashion at each contact during the study for any new or continuing symptoms since the last contact. All adverse events will be reported on the appropriate CRF. Details will include the type of event, date of onset, duration, intensity, causality relationship to the study procedure (if applicable), and outcome. Wherever possible, a diagnosis rather than symptom(s) will be reported.

If an AE should occur, every attempt will be made to obtain as much information as possible about event evaluation and outcome. Documents of this follow-up will be maintained with the patient's study records.

If a SAE occurs, the participation in the study will be interrupted or discontinued at the investigator's discretion.

All protocol deviations will be reported to the WCG IRB.

All SAEs will be reported immediately to the WCG IRB and the FDA (if applicable).

Endpoints will be adjudicated by the Principal Investigator. A written report detailing the endpoint adjudication will be provided by the Principal Investigator.

5.4. Safety

This study will be conducted in accordance with the principles of Good Clinical Practice (GCP) as outlined in the Declaration of Helsinki and the International Conference on Harmonization.

The principal investigator of the study will review study related events for adjudication.

All efforts will take place to ensure patient safety. Each patient will be monitored for safety throughout the trial utilizing clinical evaluations and laboratory markers. Laboratory markers and/or clinical evaluations that are out of normal range will be recorded as adverse events and reviewed with the investigator.

6. Data Management

Following completion of the consenting process (including informed consent and Authorization for Use/Disclosure of protected health information (PHI)) and it has been determined that the patient meets all of the inclusion and none of the exclusion criteria, enrollment will occur, and data collection will commence.

A secure Electronic Data Capture (EDC) and data management system will be used for entry, storage, review, and management of study data. The system will be compliant with applicable GCP and regulatory requirements. Investigators are responsible for accurate completion and timely submission of the data collected during the study. Sites will be trained in the use of the EDC system for entering study data and uploading supporting documents and will be given access for this purpose.

Quality assurance procedures will be established to ensure that complete, accurate and timely data are submitted, protocol requirements are followed, and that AEs are correctly reported. The management and retention of these data will be compliant with applicable regulatory requirements.

6.1. Case Report Forms

All required data for this study will be collected on standardized paper CRFs. The research staff will review completed CRFs to attest that all data entered on them are complete and accurate.

Medical history, demographics, hospital, office, and research records for any visit (including any notes, reports, test results, and lab reports from the clinical evaluation, biochemical and "omics" assessment, non-invasive imaging, functional assessments, and wearable/quantitative self-assessments) are considered source documentation and will be collected and reviewed to confirm clinical events and may be utilized for data analysis. Data will be collected on all patients and entered into a CRF.

The Investigator or designee will be responsible for completing, in a timely manner, the CRF for each participant related to previously reported or newly detected AEs. And end of study withdrawal form will also be included as part of the CRF.

6.2. Database

Data from the CRF will be transcribed to the EDC system. Data collected for omics and imaging will be retained with 3rd parties conducting these analyses.

6.3. Safeguarding of Genomic Data

Some blood samples will be used for genetic testing. There are laws that protect participants from discrimination based on their genetic information. A Federal law called the Genetic Information Nondiscrimination Act (GINA) makes it illegal for health insurance companies, group health plans, and most employers to discriminate against individuals based on their genetic information. GINA does not protect against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect against discrimination based on an already-diagnosed genetic condition or disease. In some cases, employers could use genetic information to decide whether to hire or fire an individual. There is a risk that someone could get access to participant

genetic information and identify them by name. PUER Research will protect these data in the study and will not share or sell these data to outside parties without participants' written consent.

6.4. Quality Control

PUER Research will fulfill the responsibilities identified in their standard operating procedures. These responsibilities include collecting and tracking data forms and instituting quality control measures for data entry verification and study compliance. PUER Research or its designee will be responsible for auditing the database and confirming the overall integrity of the data. PUER Research or its designee will ensure that all information pertaining to significant new developments and unanticipated AEs are provided to the appropriate regulatory authorities, the Investigators, and to the WCG IRB.

Monitoring of the study will be conducted at regular intervals in order to monitor study conduct and maintain GCP by periodic review of the data in the EDC. These inspections are conducted in order to verify adherence to the protocol and the completeness and accuracy of the data being entered into the CRF.

7. Statistical Considerations

By design, the study will not employ formal hypothesis testing. Study endpoints will be described using descriptive statistics, using means and standard deviations, medians with inter-quartile ranges, and other descriptive statistical methods, as appropriate.

Data from participants who discontinue the study prior to completion will only be considered through the date of withdrawal and will not be followed in the remaining longitudinal study endpoints.

8. Ethical, Regulatory, and Administrative Considerations

8.1. Informed Consent

The principles of informed consent are described in the Code of Federal Regulations 21 CFR, part 50 and 45 CFR, part 46. Once the Investigator has determined the patient's eligibility for the study, the background of the proposed study and the benefits and risks of the procedures and study must be explained to the patient. The patient must be able to comprehend the informed consent form and must sign it prior to performing any study specific procedures or prior to receiving medication. The patient and the Investigator will receive a copy of the informed consent. The original signed informed consent and Authorization for Use/Disclosure of PHI will be maintained in the patient's research chart. Only those patients who sign the IRB approved informed consent prior to participation are eligible to be in the study. Failure to provide written informed consent renders the patient ineligible for the study.

8.2. Confidentiality

All information and data collected and/or sent to PUER Research concerning patients or their participation in this study will be considered confidential. Only authorized PUER Research personnel will have access to these confidential files. Authorized personnel have the right to inspect and copy all records pertinent to this trial. All data used in the analysis and reporting of this evaluation will be without identifiable reference to any patient.

8.3. Institutional Review

The Principal Investigator will obtain approval for the study from the WCG IRB. All changes to the protocol must be reviewed and approved prior to implementation. The Principal Investigator will be responsible for obtaining annual renewal through the duration of the study, or more frequently if required by the WCG IRB. PUER Research will maintain all regulatory documents.

8.4. Protocol Interpretation and Compliance

The procedures defined in the protocol will be carefully reviewed by the Investigator and research staff prior to the time of study initiation to ensure appropriate interpretation and implementation. No deviations from the protocol will be acceptable. Any changes to the protocol in the form of an amendment must be submitted to PUER Research.

8.5. Completion of Case Report Forms (CRFs)

The Principal Investigator or his designee will be responsible for completing, in a timely manner, a CRF for each patient who is registered to participate in this study. The Principal Investigator will sign and date the indicated places on the CRF. This signature will indicate that a thorough inspection of the data therein has been made and will thereby certify the contents of the form.

8.6. Maintenance of Study Documentation

It is the responsibility of the Principal Investigator and PUER Research staff to maintain a comprehensive and centralized filing system of all study-related documentation, which is suitable for inspection at any time by the FDA. Elements should include:

- Participant files – containing the completed CRFs, supporting source documentation, and the Informed Consent.
- Regulatory Files – containing the protocol with all amendments and accountability records.

8.7. Publication Policy

The results of this study will help our understanding of human health, disease, and healthy longevity via individualized and longitudinal use of clinical evaluation, biochemical and "omics" assessments, non-invasive imaging assessments. PUER Research may publish the results of this research. However, names and other identifying information will be kept confidential. This study and its results will not be posted to www.clinicaltrials.gov or submitted to the FDA.

8.8. Financing and Insurance

Clinical trial insurance will be provided for by PUER Research although it is not expected a participant will suffer any study-related injury. Insurance will be obtained and maintained for this type of observational study.

8.9. Final Study Report

Upon completion of the study, the Principal Investigator is required to submit a final study summary report for the patients enrolled in the study.

8.10. Record Retention

All records, which are part of this study, will be retained for a period of two years following discontinuation/termination of the study.

9. References

Amendola, L. M., Dorschner, M. O., Robertson, P. D., Salama, J., Hart, R., Shirts, B. H., . . . Jarvik, G. P. (2015). Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Research, 25*(3), 305-315.

American College of Radiology. (2021). *ACR Manual on contrast media.* ACR.

Anthimopoulos, M., Christodoulidis, S., Ebner, L., Christe, A., & Mougiakakou, S. (2016). Lung Pattern Classification for Interstitial Lung Diseases Using a Deep Convolutional Neural Network. *IEEE Transactions on Medical Imaging*, 1207-1216.

Arnoldi, E., Arnoldi, E., Gebregziabher, M., Schoepf, U. J., Goldenberg, R., Ramos-Duran, L., . . . Thilo, C. (2010). Automated computer-aided stenosis detection at coronary CT angiography: initial experience. *European Radiology, 20*(5), 1160-1167.

Assarsson, E., Lundberg, M., Holmquist, G., Björkesten, J., Bucht Thorsen, S., & al., e. (2014). Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. *PLoS One, 9*(4), e95192.

Bennuru, S., Lustigman, S., Abraham, D., & Nutman, T. B. (2017). Metabolite profiling of infection-associated metabolic markers of onchocerciasis. *Molecular and Biochemical Parasitology, 215*, 58-69.

Bromberg, Y. (2013). Building a genome analysis pipeline to predict disease risk and prevent disease. *Journal of Molecular Biology, 425*(21), 3993-4005.

Chong, C.A.K.Y., Tomlinson, G., Chodirker, L., Figdor, N., Uster, M., Naglie, G., Krahn, M.D. (2006). An unadjusted NNT was a moderately good predictor of health benefit. *Journal of Clinical Epidemiology*, 59(3):224-33.

Crick, F. (1970). Central dogma of molecular biology. *Nature, 227*(5258).

David C.E.B., Lucas A.M.B., Cunha P.L.O., Viana Y.I.P., Yoshinaga M.Y., Miyamoto S., … Facundo H.T. (2022) Calorie restriction changes lipidomic profiles and maintains mitochondrial function and redox balance during isoproterenol-induced cardiac hypertrophy. *Journal of Physiology and Biochemistry*. Epub ahead of print.

"Design and baseline results of the Scandinavian Simvastatin Survival Study of patients with stable angina and/or previous myocardial infarction". (1993). The American Journal of Cardiology. 71(5):393–400.

Dorschner, M. O., Amendola, L. M., Turner, E. H., Robertson, P. D., Shirts, B. H., Gallego, C. J., . . . Jarvik, G. P. (2013). Actionable, Pathogenic Incidental Findings in 1,000 Participants' Exomes. *American Journal of Human Genetics, 93*(4), 631-640.

Fest, J., Vijfhuizen, L. S., Goeman, J. J., Veth, O., Joensuu, A., Perola, M., . . . Hoen, P. A. (2019). Search for early pancreatic cancer blood biomarkers in five European prospective population biobanks using metabolomics. *bioRxiv*, 543686.

Ganz, P., Heidecker, B., Hveem, K., Jonasson, C., Kato, S., Segal, M. R., . . . Williams, S. A. (2016). Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients With Stable Coronary Heart Disease. *JAMA, 315*(23), 2532–41.

Glasziou, P., & Haynes, B. (2005). The paths from research to improved health outcomes. *BMJ Evidence-Based Medicine*(10), 4-7.

Gold, L. e. (2010). Aptamer-based multiplexed proteomic technology for biomarker discovery. *PPLoS One, 5*(12), e15004.

Goldstein, T. C. (2013). *Tools for Extracting Actionable Medical Knowledge from Genomic Big Data*. Retrieved 9 27, 2021, from https://escholarship.org/uc/item/53k1x0f5

Guttmacher, A. E., & Collins, F. S. (2003). Welcome to the Genomic Era. *The New England Journal of Medicine, 349*(10), 996-998.

Han, X., Aslanian, A., Aslanian, A., & Yates, J. R. (2008). Mass Spectrometry for Proteomics. *Current Opinion in Chemical Biology, 12*(5), 483-490.

Hayden, E. C. (2014). Technology: The $1,000 genome. *Nature, 507*(7492), 294-295.

Lemaitre, G., Martí, R., Freixenet, J., Vilanova, J. C., Walker, P., & Meriaudeau, F. (2015). Computer-Aided Detection and diagnosis for prostate cancer based on mono and multi-parametric MRI. *Computers in Biology and Medicine, 60*, 8-31.

Li, J., Huang, A., Yao, J., Liu, J., Uitert, R. L., Petrick, N., & Summers, R. M. (2008). Optimizing computer-aided colonic polyp detection for CT colonography by evolving the Pareto fronta. *Medical Physics, 36*(1), 201-212.

Netto, G., & Kaul, K. e. (2019). *Genomic Applications in Pathology.* Springer International Publishing.

Onal, S., Lai-Yuen, S. K., Bao, P., Weitzenfeld, A., & Hart, S. (2014). *Fully automated localization of multiple pelvic bone structures on MRI*. Retrieved 9 27, 2021, from https://ncbi.nlm.nih.gov/pubmed/25570709

"Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S"). Lancet. 1994 Nov 19;344(8934):1383-9. PMID: 7968073.

Rosenberg, S., Elashoff, M., Beineke, P., Daniels, S., Wingrove, J., Tingley, W., . . . Topol, E. (2010). Multicenter validation of the diagnostic accuracy of a blood-based gene expression test for assessing obstructive coronary artery disease in nondiabetic patients. *Annals of internal medicine, 153*(7), 425-434.

Scaria, V. (2014). Personal genomes to precision medicine. *Molecular Cytogenetics, 7*(1), 1-1.

Schork, N. (2015). Personalized medicine: Time for one-person trials. *Nature*(520), 609-611.

Uppot, R. N., Boas, G., & Flores, E. (2018). *Current Developments in Artificial Intelligence and Diagnostic Imaging*. Retrieved 9 27, 2021, from https://www.massgeneral.org/assets/MGH/pdf/imaging/radiology-rounds/radiology-rounds-may-2018.pdf

Yang, Q., Li, L., Zhang, J., Shao, G., Zhang, C., Zheng, B., & Zheng, B. (2014). Computer-Aided Diagnosis of Breast DCE-MRI Images Using Bilateral Asymmetry of Contrast Enhancement Between Two Breasts. *Journal of Digital Imaging, 27*(1), 152-160.

Yet, I., Menni, C., Shin, S. Y., Mangino, M., Soranzo, N., Adamski, J., . . . Bell, J. T. (2016). Genetic Influences on Metabolite Levels: A Comparison across Metabolomic Platforms. *PLoS One, 11*(4), e0153672.

Zhu, B., Liu, J. Z., Cauley, S. F., Rosen, B. R., & Rosen, M. S. (2018). Image reconstruction by domain-transform manifold learning. *Nature, 555*(7697), 487-492.