RAD5484-21: Mechanism and Predictors of Cardiotoxicity after Prostate Cancer Treatment: A Parallel Cohort and Randomized Trial Comparing Radiation Alone, Radiation plus Leuprolide, and Radiation plus Relugolix

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REVISION HISTORY

Revision #	Version Date	Summary of Changes
	4.0	Addition of Prostate Cancer Foundation Young Investigator Award funding source
	5.0	Removal of Dr. Brian Olson from study team, modification of accrual goal
	6.0	Modification of follow-up cardiac CT timeline to 6-12 months (previously 12 months) after baseline cardiac CT for control arm (i.e. Radiation alone)

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1. Study Summary

Project Title	**Biomarker-Based Approaches to Understand and Predict Cardiovascular Toxicity from Androgen Deprivation Therapy Targeting the Gonadotropin Releasing Hormone Pathway in Prostate Cancer Men**
Project Design	Open-label randomized clinical trial with parallel prospective cohort study
Primary Objective	Elucidating the pathophysiologic link between gonadotropin releasing hormone agonist hormone therapy (e.g. Lupron) and cardiovascular (CV) toxicity, and the mechanism by which CV risk-reduction is achieved by the novel oral GNRH-antagonist, relugolix. This objective will be studied using imaging and blood biomarkers.
Secondary Objective(s)	Identify genomic alterations that predispose an individual to enhanced CV toxicity following hormone therapy with GNRHa or relugolix
Research Intervention(s)/Interactions	A) GNRHa (Lupron, trelstar) versus relugolix [randomization] B) Parallel, non-interventional cohort of men receiving no hormone therapy
Study Population	Men >18 years with localized intact or recurrence prostate cancer undergoing radiation therapy (with or without prior prostatectomy), with or without hormone therapy
Sample Size	94 men (goal n=30 in each treatment arm; additional 4 patients for accrual to account for study drop-out)
Study Duration for individual participants	12 months
Study Specific Abbreviations/ Definitions	Radiation therapy (RT) Androgen deprivation therapy (ADT) Gonadotropin-releasing hormone agonist (GNRHa) Gonadotropin-releasing hormone antagonist (GNRHaa) Nonsteroidal anti-androgen (AA)

	Combined androgen blockade (CAB) Major adverse cardiac events (MACE)
Funding Source (if any)	Pfizer-Prostate Cancer Foundation-Myovant Challenge Award

2. Objectives

Androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone agonist (GNRHa) is a highly effective and commonly used treatment for prostate cancer (PCa) with over 500,000 men with PCa receiving ADT annually in the United States[3,4]. However, large observational studies suggest GNRHa is associated with increased risk of myocardial infarction (MI) and sudden cardiac death, raising concerns that ADT causes cardiovascular (CV) harm[4-6]. In fact, as more men survive PCa due to increasing ADT use, ***CV mortality, <u>not cancer</u>, is now the most common cause of death in PCa patients***[7,8]. However, this increased CV damage has been greatly diminished with the approval of GNRH antagonists such as relugolix, which demonstrated a decreased incidence of CV adverse events compared with GNRHa[2]. Yet, despite this increased risk of CV damage, GNRHa remain the most widely utilized form of ADT due to high cytoreductive efficacy, lower cost, convenience in delivery, and minimal injection-associated adverse effects compared with available injectable GNRH antagonists. ***It is critically important to identify both the mechanism as well as predictive biomarkers that drive ADT-induced CV damage*** to triage patients towards the method of ADT that will be most effective for a patient's overall health.

The immune system can play a central role in the development of CV injury, including promoting accelerated atherosclerosis via cholesterol deposition and immune cell aggregation in the arterial wall. Peripheral innate and adaptive immune cells (both pro- and anti-inflammatory) regulate subsequent atherosclerosis progression[9,10]. Additionally, baseline genomic aberrations (including clonal hematopoiesis of indeterminate potential (CHIP)) are also associated with enhanced inflammatory immunity and CV injury[11,12]. While the link between immune-mediated inflammation and acute coronary syndrome remains complex, accumulating evidence has demonstrated that vascular inflammation plays pivotal roles in the pathogenesis of atherosclerosis and plaque rupture, and thus coronary artery disease (CAD) is considered an inflammation-related disease.

To examine the role that the immune system plays in ADT-induced CV disease, we propose a mixed-methods clinical study using patient-level data from our Cardio-Oncology PROstate Cancer (CO-PRO) institutional, open-label prospective clinical trial. In this study, patients with stage I-III PCa will receive leuprolide or relugolix with radiation therapy, or radiation alone. Using imaging and peripheral blood samples from this trial, ***the main goal of this proposal is elucidating the pathophysiologic link between GNRHa and CV toxicity and the mechanism by which CV risk-reduction is achieved by the novel oral GNRH-antagonist, relugolix***. We hypothesize that ***early CV toxicity from GNRHa is mediated by a GNRHa-activated inflammatory cascade directly***

impacting vascular integrity and resulting in accelerated coronary atherosclerosis, and this effect will not be observed in men receiving relugolix. Aim 1 of this study will utilize non-invasive imaging to identify how leuprolide versus relugolix affect the development of coronary artery disease, and whether validated imaging biomarkers can predict these changes. As secondary laboratory-based correlative studies, Aim 2 will evaluate how pre-existing or augmented immune responses following ADT predict for development of CV toxicity. Finally, in Aim 3 we will examine how pre-existing genomic alterations that pre-dispose an individual to enhanced inflammatory immunity (and have been associated with accelerated atherosclerosis) correlate with the development of CV toxicity following ADT with leuprolide or relugolix. By identifying major contributors to CV risk from ADT, we will ultimately develop a precision medicine approach to optimize risk prediction and allow clinicians to appropriately counsel patients on risk-benefit ratio of ADT and utilize novel risk-reducing ADT agents such as relugolix.

3. Background

Association of ADT and Cardiovascular Morbidity

The role of ADT in CV harm was ignited with the publication of large observational studies in the past decade, which suggested that ADT use in the form of a GNRHa for PCa men was associated with a 20% increased risk of CAD, 10-30% increased risk of MI, and a 15% increased risk of sudden cardiac death[4-6]. With increasing ADT use and improving radiation/surgical technique, **CV mortality, not cancer, is now the most common cause of death in PCa patients**[7,8]. Additionally, while the addition of ADT with radiation therapy has been shown to improve survival for men with unfavorable risk PCa, multiple post-hoc studies have suggested that the addition of ADT may result in a survival *detriment* in men with CV comorbidities[13,14].

Traditionally, CV injury following ADT is thought to be driven by an indirect mechanism of prolonged hypogonadism precipitating metabolic syndrome, insulin resistance, and weight gain over several years[7,15-17]. However, **the effect of ADT, namely GNRHa, may be direct.** There are several observations from recent studies that support this premise. First, studies have shown that ADT, after only 3-6 months of therapy, is associated with an increased risk of atherothrombotic events[7,8]. Second, data from the recent HERO trial[2] evaluating GNRHa (leuprolide) versus GNRH-antagonist (relugolix) in PCa men showed an increased incidence of major adverse CV events with GNRHa compared with GNRH-antagonist *as soon as 4 weeks after initiation of treatment* (**Figure 1**). Further, testosterone suppression was *greater* with GNRH-antagonist than GNRHa, countering the testosterone-mediated CV effect. Finally, the excess CV event risk with ADT in the large observational studies mentioned above[4-6] was seen with GNRHa therapy, not orchiectomy/surgical castration. In fact, meta-analysis of several observational studies[5] have

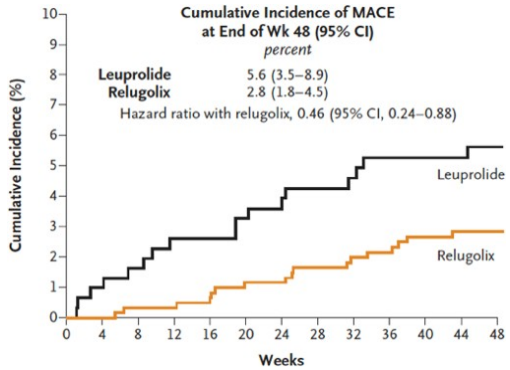


Figure 1. Incidence of major adverse cardiac events (MACE) in men with advanced PCa receiving leuprolide versus relugolix. Incidence curves separate immediately after onset of treatment, with GNRHa resulting in more MACE[2].

shown a hazard ratio for risk of MI with GNRHa (compared with other forms of ADT) to be 1.57 (95% CI 1.26-1.94). Nonetheless, GNRHa remains the most widely utilized form of ADT in the United States due to lower cost, convenience in delivery, and minimal injection-associated adverse effects compared with injectable GNRH-antagonist.

Association of Immune Response and Coronary Atherosclerosis

Most acute CV events, including MI, are caused by rupture of an atherosclerotic plaque. The molecular mechanisms of atherosclerosis involve cholesterol deposition and immune cell aggregation in the arterial wall. Innate and adaptive immune cells with both proinflammatory and anti-inflammatory effects regulate subsequent atherosclerosis progression[9,10]. The link between inflammation and acute coronary syndrome is complex, but accumulating evidence has shown that vascular inflammation plays a central role in the pathogenesis of atherosclerosis and plaque rupture, making acute coronary syndrome an inflammation-related disease.

Plaques prone to rupture are characterized by a large core of lipids and necrotic debris covered by a thin cap of smooth muscle cells and connective tissue. The rupture is caused by a degradation of the cap connective tissue by infiltrating macrophages releasing matrix-degrading proteases[18,19]. Following systemic or local inflammatory activation, endothelial cells enhance the migration and attachment of T cells and macrophages to the arterial wall via upregulated adhesion molecules. During this process, both proatherogenic and antiatherogenic immune networks are activated, and if sustained, can lead to plaque disruption, rupture, and subsequent coronary arterial occlusion. These mechanisms are supported by the CANTOS trial[20], which randomized >10,000 patients with prior myocardial infarction and hsCRP > 2.0 mg/L (i.e. marker of elevated baseline inflammation) to canakinumab, a monoclonal antibody targeting IL-1β, versus placebo. Patients receiving canakinumab had significantly decreased rates of major adverse CV events and inflammatory biomarkers, independent of aggressive cholesterol control, demonstrating the role of inflammatory immunity in mediating CV toxicity.

Many other inflammatory cytokines/mediators have been implicated in the development of accelerated atherosclerosis and/or coronary plaque rupture[9]. For example, proinflammatory T-helper 1 (Th1) T cells are important macrophage activators and are the dominant T-cell type in atherosclerotic plaques[21]. T cells express GNRH receptors, and activation of these receptors via GNRHa has been shown to stimulate T-cell expansion and differentiation into Th1 phenotype, suggesting that GNRHa may promote

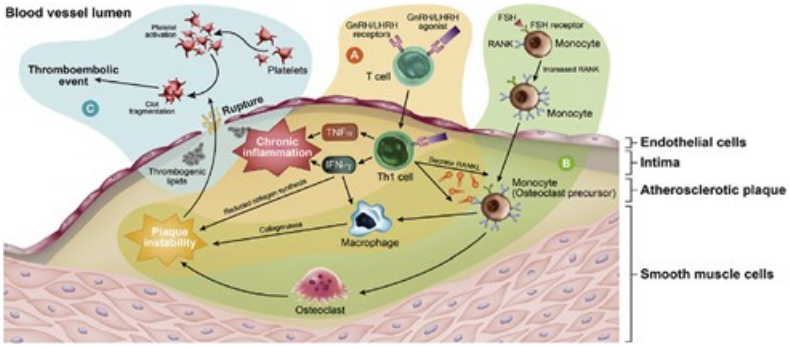


Figure 2. Potential interaction between GNRH, immune effector response, and coronary atherosclerosis and plaque rupture resulting in major adverse cardiovascular events, such as myocardial infarction[1]

destabilization of atherosclerotic plaques[22] (**Figure 2**). Additionally, follicle-stimulating hormone (FSH) is upregulated following GNRHa (e.g. leuprolide) but not GNRH-antagonists (e.g. relugolix), and has been implicated in excess adiposity, enhanced proinflammatory response secondary to adipocytes, and upregulation of RANK/osteoclast differentiation involved in calcium resorption (such as that found on stable coronary plaques)[23-25]. As such, the risk of atherosclerotic plaque destabilization and rupture may be mitigated with a GNRH-antagonist, such as relugolix.

An underlying factor that can contribute to the development of this increased CV risk are somatic mutations that impact immune frequency and function. In particular, somatic alterations associated with clonal hematopoiesis of indeterminate potential (CHIP) have been shown to lead to dysregulated inflammatory immune responses and poor CV outcomes[12]. CHIP, present in 10-20% of the population over age 70, arises from somatic mutations in hematopoietic stem cells that produce abnormal circulating immune cells. These CHIP mutations are associated with twice the risk of CAD and ischemic stroke, as well as increased CV events[11,26,27]. This is thought to occur via immune-mediated pathways, as CHIP-engineered mice have increased atherosclerosis mediated by inflammatory cytokines like IL-1β, TNFα, and IL-6[11,28,29]. However, how CHIP mutations impact CV outcomes following ADT in PCa men remains to be explored.

The role of inflammatory immune responses in potentiating CV disease ties directly in with the increased risk of CV disease following ADT in that ADT has long been shown to modulate systemic and prostate-infiltrating inflammatory immune responses. These include the induction of thymic regrowth and release of naïve T cells into circulation, an increase in various inflammatory immune populations (both myeloid and lymphocytes, particularly CD4+ Th1 cells), and decreased numbers of regulatory T cells[30-35]. While these enhanced responses following GNRHa treatment has been well-established, the immunomodulatory effects of the GNRH-antagonist relugolix remain to be explored, as do the relationship between these enhanced inflammatory responses and increased CV disease.

Understanding biological mechanisms of ADT-associated CV toxicity, especially the difference between that of GNRHa and GNRH-antagonists, will enhance our ability to decide on appropriate treatment strategies for PCa men receiving ADT, depending on an individual's risk for CV injury. Additionally, we will explore novel associations between patient-level clinical and genomic features and baseline imaging biomarkers with ADT-associated CV toxicity. By identifying major contributors to CV risk from ADT, we ultimately plan to develop a precision medicine approach to optimize risk prediction and allow clinicians to appropriately counsel patients on risk-benefit ratio of ADT and utilize novel risk-reducing ADT agents such as relugolix

4. Study Endpoints

Aim 1: To identify and compare the association of GNRH-agonist leuprolide versus GNRH-antagonist relugolix with accelerated coronary plaque development in men with prostate cancer

Rationale: Coronary atherosclerosis and plaque rupture is an immune-mediated disease, and GNRH has been implicated in its pathogenesis. In addition to circulating T cells, GNRH receptors have been identified on cardiac endothelium[38]. We hypothesize that there is a direct GNRHa-mediated effect in susceptible vessels leading to plaque propagation and CV injury in PCa

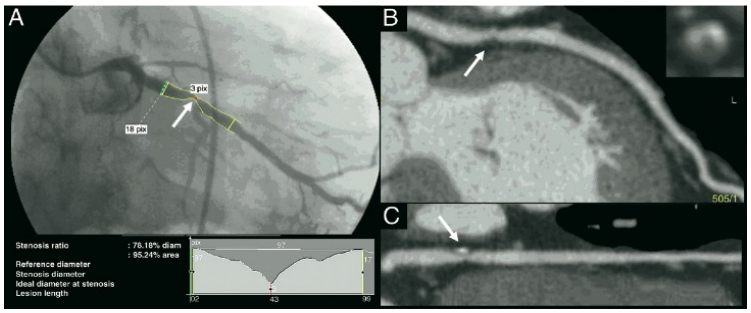


Figure 4. Coronary catheterization (left) compared to coronary CT angiogram (CCTA, right), in the same patient. This image demonstrates the high spatial resolution of coronary plaque/stenosis on CCTA. Early coronary changes can be detected with this non-invasive technique.

patients treated with ADT. This premise has been informed by preliminary data in a study that Dr. Sagar Patel led. This study was a multi-institutional retrospective analysis of coronary CT angiogram (CCTA) findings in PCa patients who did (n=60) and did not (n=42) receive ADT. CCTA is a non-invasive imaging tool with >95% sensitivity and >90 specificity for coronary atherosclerosis compared with the gold standard coronary catheterization, which is highly invasive[39,40] (**Figure 4**).

At a median 9.6 months between PCa diagnosis and CCTA, we found that men who received ADT had a 27% higher rate (48.3% versus 21.4% of controls – p=0.07) of left anterior descending, left circumflex artery, and or right coronary artery moderate stenosis (i.e. plaque volume of >50% of the vessel lumen). However, no differences in incidence of electrocardiogram or echocardiogram changes based on receipt of ADT treatment were found. These findings suggest that ADT-associated CV toxicity may be driven by a direct impact of ADT on coronary vasculature leading to accelerated coronary atherosclerosis.

Value of CCTA as Primary Endpoint: Coronary computed tomographic angiography (CCTA) has emerged as an accurate non-invasive method for the detection of obstructive coronary artery disease (CAD). Further, CCTA permits evaluation of numerous other coronary artery plaque characteristics, including plaque compositions, which are generally graded as non-calcified, calcified, and mixed[41]. Classification of plaques by CCTA based upon composition has important clinical implications: calcified plaque is generally considered more stable, while non-calcified or mixed plaques possess a thin cap fibroatheroma that is more likely to rupture and result in acute cardiac events such as a MI. Using CCTA, the evolution of these plaques after starting ADT will be evaluated in this study, providing mechanistic insight into the pathophysiology underlying GNRHa-mediated CV toxicity (i.e. immune- and FSH-mediated responses involving cytokines and biomarkers that will be studied in Aims 2 and 3). For example, if we observe an increase in coronary occlusion involving non-calcified lesions with GNRHa (but not relugolix), this finding would provide insight that GNRHa leads to downstream pathways involving plaque instability and rupture. If we also observe an increase in coronary occlusion involving calcified lesions with GNRHa (but not relugolix), this finding would provide insight that GNRHa may lead to FSH

upregulation leading to osteoclast-mediated breakdown of calcified caps and plaque disruption.

Thus, this imaging technique will both characterize and validate a novel imaging biomarker to predict men are at high risk of CV events after initiating ADT, as well as provide further validation of hypothesized mechanisms of GNRHa-mediated CV disease.

Aim 2: Determine the relationship between GNRH-agonist versus relugolix with downstream immune effector response that is implicated in atherosclerosis

While the increased risk of CV disease and morbidity associated with ADT has been established by large observational studies, the mechanism behind this risk remains unclear. However, increasing evidence has demonstrated that inflammatory immunity can play a central role in mediating cardiac damage, especially at the coronary vessel endothelium. Aim 1 will provide insight into anatomic/pathophysiologic mechanism of ADT-associated CV toxicity, testing our hypothesis that CV damage following ADT is due to accelerated coronary artery atherosclerosis and/or plaque instability associated with inflammatory immune responses. In Aim 2, we will evaluate peripheral blood samples (drawn at 3-, 6-, and 12-months and stored) from patients enrolled on study to determine how pre-existing or augmented inflammatory immunity impacts the risk of CV injury following ADT, testing the underlying hypothesis that ***pre-existing inflammatory immunity increases the risk of CV injury following ADT due to an immune-mediated effect of GNRHa but not antagonists***. These studies provide important mechanistic insight into how inflammatory immunity contributes to ADT-related CV toxicity, as well as identifying biomarkers that may predict individuals at heightened risk of adverse CV events from ADT, and thereby benefit from cardiac risk-reducing relugolix.

Subaim 2.1: To determine how proteomic biomarkers associated with inflammatory immunity correspond with CV toxicity following ADT.

We will conduct in-depth proteomic profiling, which has previously been utilized and validated to identify signatures associated with inflammatory immune responses and cardiovascular risk[50,51]. To evaluate whether these signatures are associated with CV toxicity following ADT using leuprolide but not relugolix, pre-treatment and 12-month post-ADT PBMC will be subjected to proteomic profiling in conjunction with the Emory Integrated Proteomic Core as published by our colleagues at the Winship Cancer Institute[52].

Aim 3: To determine how pre-existing genomic alterations promoting inflammatory immunity impact development of CV toxicity following GNRHa versus relugolix.

Numerous genetic alterations have been shown to result in either enhanced or diminished ability to mount inflammatory immune responses, including genes associated with 'clonal hematopoiesis of indeterminate potential' (CHIP) such as DNMT3A, TET2, ASXL1, PPM1D, KDM6A, and BCOR[53-55], as well as several others that alter inflammatory immune function[56-62]. The impact of these immune-related genomic alterations on the development of inflammatory immunity and CV toxicity following ADT will be examined in Aim 3, testing the underlying hypothesis that ***somatic mutations associated with enhanced effector responses lead to increased CV injury in men receiving GNRH agonists but not relugolix.***

5. Study Intervention/Investigational Agent

Hormone Therapy Intervention

For men who will be receiving ADT with radiation therapy, there will be a 1:1 randomization between GNRHa (i.e. leuprolide or triptorelin) versus oral relugolix. Dosing for these agents are per standard FDA-approved guidelines, and the study has no impact on dose or duration of therapy.

For men receiving GNRHa, standard FDA-approved intramuscular or subcutaneous injectable doses will apply (i.e. leuprolide 7.5 mg monthly, 22.5 mg q3month, 45 mg q6month; triptorelin 11.25 mg q3month, 22.5 mg q6month). Each patient may receive 21-30 days of oral bicalutamide 50 mg daily beginning at the time of first injection (i.e. to hinder the initial testosterone flare associated with GNRHa) at the discretion of the treating provider. Duration of therapy will depend on the risk category of prostate cancer, as detailed in section 6.

For men receiving oral relugolix, standard FDA-approved oral doses will apply (i.e. 360 mg on day 1, followed by 120 mg daily for the duration of therapy). The tablet is in doses of 120 mg, and it can be taken by mouth with or without food. If the subject misses > 7 days of medication, then a loading dose of 360 mg will be initiated followed by re-initiation of 120 mg daily thereafter. Participants will be asked to complete a drug diary and bring the diary in addition to medication for reconciliation at each follow up visit. Duration of therapy will depend on the risk category of prostate cancer, as detailed in section 6.

Relugolix will be provided at no cost by the study sponsor (i.e. Myovant Sciences). All medication supply will be stored and managed by Emory Investigational Drug Services Core. Medication delivery to enrolled subjects will be mediated by clinical research coordinators. Additionally, drug diaries will be reconciled by clinical research coordinators with oversite by the principal investigator Dr. Sagar Patel.

Coronary Computed Tomography Angiogram (CCTA) Endpoint

As discussed in Aim 1 above, this study will utilize a state-of-the-art, validated non-invasive cardiac imaging, coronary CT angiogram, as the primary endpoint in this study. This imaging technique offers remarkable spatial resolution of coronary vasculature to test the primary hypothesis that ADT-mediated CV risk, namely from GNRHa, is driven by accelerated atherosclerosis and coronary disease. With modern imaging techniques, such as prospective triggering, radiation exposure to the patient is minimized, as imaging is gated to be performed in only one phase of the cardiac cycle (rather than continuous during the entire cardiac cycle). Additionally, other measures, including limited field of view, limited scan length, and minimal tube voltage, will ensure that the additional radiation exposure sustained by CCTA in this study will remain around 3.2 mSv per patient (which is comparable or lower than the 3-7 mSv the average person receives annually from natural sources). Other cardiac imaging techniques, including catheter-based angiography or nuclear medicine studies, result in a substantially

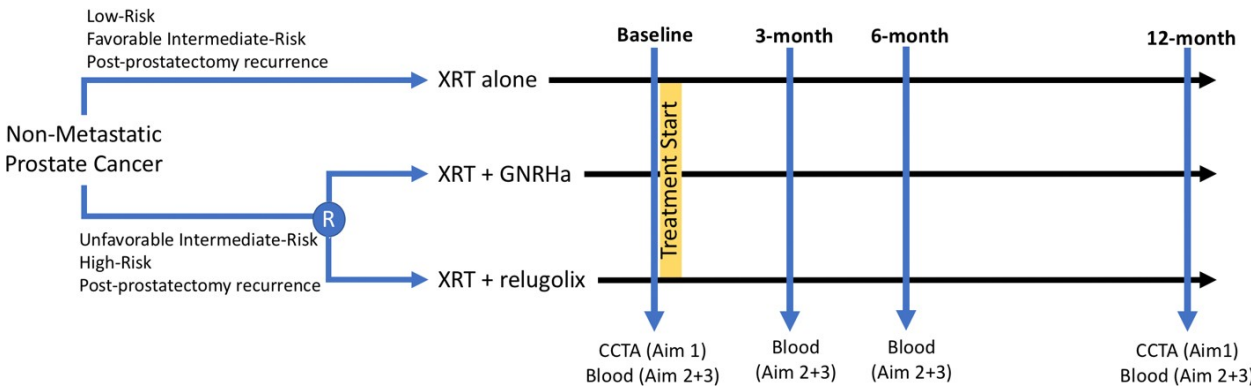
larger amount of radiation exposure to the patient (e.g. 21-41 mSv), and are therefore, less optimal to use as an endpoint in this study. Additionally, CCTA endpoints will allow to test both mechanism (e.g. involving accelerated coronary atherosclerosis and/or plaque instability), as well as help identify a novel imaging biomarker that may be used in clinical practice to identify those men at highest risk of an adverse CV outcome from ADT.

6. Procedures Involved

Men with localized intact PCa, or non-metastatic recurrence following prostatectomy, and prescribed radiation therapy (XRT) with or without concomitant ADT will be consented and enrolled on this prospective trial. Eligible subjects are men > 18 years old diagnosed with non-metastatic PCa planning to undergo curative-intent therapy. Men with prior cardiac stent or bypass surgery will be excluded. Men with prior exposure to ADT will be excluded.

Each patient will undergo external beam XRT with or without concomitant ADT of 6-24 months duration. **For those men with National Comprehensive Cancer Network (NCCN)-defined unfavorable intermediate- or high-risk disease, or with biochemically recurrent PCa requiring salvage XRT plus ADT, each will be randomized 1:1 between injectable intramuscular or subcutaneous GNRHa depot (*leuprolide 22.5 mg every 3 months, leuprolide 45 mg every six months, triptorelin 11.25 mg every 3 months, triptorelin 22.5 mg every 6 months*) versus GNRH-antagonist (oral relugolix 360 mg loading dose followed by 120 mg daily)**. Men with NCCN-defined low- or favorable intermediate-risk intact disease, or those with biochemically recurrent PCa requiring salvage XRT without ADT, will be treated with XRT alone. Each patient will provide consent for (1) pre-treatment and 12-month post-ADT prospective ECG-gated, contrast-enhanced CCTA and (2) peripheral blood banking at baseline and at 3-, 6-, and 12-months post-treatment (**Figure 5**). Men receiving XRT alone will undergo CCTA 6-12 months after baseline CCTA is complete.

Figure 5. Study Schema



ADT randomization will be created using preconstructed allocation sequences using maximal randomization (https://ctrandomization.cancer.gov/tool/). All principal investigators and co-investigators will be blinded to this randomization block. Lead statistician (Dr. Yuan Liu) and clinical research coordinators will have access to this allocation sequence.

Methods: As seen in above study schema, each patient in each cohort will undergo pre-treatment and 6-12-month post-ADT initiation coronary CT. Each patient will undergo a non-contrast CT scan using standard coronary artery calcium (CAC) imaging protocol for coronary calcium quantification prior to the acquisition of the CCTA. CAC images will be obtained using prospective ECG-triggering at 70% of the R-R interval, using tube voltage of 120 kV and an automated tube current modulation and minimum slice collimation (0.6 mm). Images will be reconstructed in 3 mm slice thickness at 3 mm intervals. Following CAC scan, contrast-enhanced CCTA will be performed using an ECG-gated, single-beat acquisition scan to include 30-80% of the R-R interval. Scanning will extend from the tracheal bifurcation to 2 cm below the left ventricular apex; 0.5 mm detector collimation will be used.

Additionally, peripheral blood will be drawn and stored in our established biorepository (under protocol WINSHIP5179-20) at the following intervals: pre-treatmnet, and 3-, 6-, and 12-months post-ADT initiation. Further details regarding analysis plan of peripheral blood below in Aims 2 and 3.

Aim 1.1: To determine the relationship between ADT (i.e. GNRHa versus relugolix) and accelerated coronary plaque development and/or rupture in PCa men, all contrast enhanced CCTA images will be analyzed by a blinded, level 3 boarded cardiac imaging expert (Dr. Stephanie Cantu) in a core laboratory setting. Multiplanar reconstructed images will be assessed for the presence and qualitative composition (e.g. calcified, non-calcified, mixed) of atherosclerotic plaque for each coronary artery segment as defined by the Society of Cardiac Computed Tomography. Luminal stenosis will be graded on an ordinal scale 0-100%. Each coronary segment will be assessed for high-risk plaque features defined as positive remodeling, low attenuation plaque, and spotty calcium. CCTA scans pre- and 12 months post-treatment will be compared. We hypothesize that ***men receiving GNRHa will be at increased risk of accelerated atherosclerosis (i.e. increase in occlusive non-calcified plaque volume or disruption of calcified plaque volume) compared with men receiving relugolix or no ADT.***

Analysis Plan: We will measure coronary plaque burden using CCTA images at both baseline and 12-months post-treatment initiation. The primary endpoint will be (1) coronary plaque volume (continuous), (2) high-risk plaque features (binary), and (3) coronary vessel stenosis (ordinal). The change in endpoint metrics between baseline and 12-month will be estimated and tested using paired tests (Wilcoxon signed rank test or McNemar test). The difference at 12- month endpoints for the two sets of comparisons (GNRHa group vs. relugolix group) will be tested using Fisher's exact test for binary endpoints or Wilcoxon Rank-Sum test for continuous. Data transformation to fit statistical assumptions will be done as needed. In addition, we will implement multivariable models that control for potential confounders (e.g. anti-platelet, anti-coagulation, statin, or other confounding cardiac mediators) using general linear regression and/or logistic regression.

***Power*:** Based on preliminary data discussed above, we hypothesize that there is an approximate 30% decreased rate of developing new moderately severe coronary atherosclerosis (defined as

>50% luminal stenosis) in a major coronary vessel in the XRT+relugolix or XRT alone arms compared with XRT+GNRHa arm. With n=30 patients in each cohort, we have 80% power to detect an effect size of 0.71 using a one-sided Fischer's exact test and an alpha of 0.025.

Aim 1.2: Whether there are predictive imaging biomarkers that may identify men at highest risk of CV injury from GNRHa (and thus may benefit from relugolix) remains unknown. To determine the relationship between baseline cardiac imaging biomarkers traditionally associated with CV disease (i.e. calcium score and epicardial adipose tissue) with ADT-associated coronary plaque development, all non-contrast enhanced CAC images will be analyzed by a blinded, level 3 boarded cardiac imaging expert (Dr. Stephanie Cantu) in a core laboratory setting. Total Agatston CAC scores will be calculated for each patient using cardiac post-processing software (Vitrea, Vital Images, Canon; Syngo.via, Siemens)[42]. Epicardial adipose tissue (EAT) volume and attenuation will be measured via validated techniques[43]. We hypothesize that ***elevated baseline CAC (i.e. indicative of higher burden of coronary calcified plaque) will predict for increased risk of occlusive coronary disease following initiation of leuprolide, but not relugolix, due to GNRHa/FSH-mediated activation of osteoclasts leading to calcium resorption and coronary plaque instability[23,24]***. Additionally, we hypothesize that ***elevated baseline EAT predicts for increased risk of occlusive coronary disease following initiation of leuprolide, but not relugolix, due to elevated FSH-mediated plaque instability and adipokine proinflammatory activation[25,44]***.

***Analysis Plan*:** Change in coronary plaque burden (i.e. volume, high-risk features, and luminal stenosis) will be compared in multiple subgroups based on CAC and EAT, respectively. Treating CAC score and EAT volume/attenuation as continuous variable, their association with coronary plaque development (e.g. plaque volume of >50% of the vessel lumen) at 12-months will be assessed using logistic regression (for binary endpoints) or general linear regression (for continuous endpoints). In addition, their predictive performance to binary endpoints will be explored using ROC analysis with estimated Area Under Curve (AUC) and Youden's optimal cut-off value. Their association with continuous endpoints will be described using Spearman correlation coefficient. As we expect the prediction or association relationship could be different by treatment arms, the same analyses as above will be repeated by treatment arms separately, or all data will be utilized in a multivariable model that contains an interaction effect between predictive biomarker and treatment arms.

Power: Due to the exploratory nature of this subaim, the power calculation is intended to describe the magnitude of effect size we can detect given the sample size as defined in Aim 1. With a total of n=30 in each of 3 arms with complete follow-up data, we have 80% power to detect an odds ratio of 0.54 for one-standard deviation increase above mean in a biomarker with endpoint of plaque volume of >50% of the vessel lumen (binary) using logistic regression. The calculation is based on significance level of 0.05 and an assumption of an overall 30% incidence rate in plaque volume of > 50% of vessel lumen.

***Aim 2.1**: To determine how pre-existing and ADT-augmented inflammatory immune responses impact CV toxicity,* we will evaluate pre-treatment, 3-, 6-, and 12-month peripheral blood mononuclear cells (PBMC) samples from all patients enrolled on trial. We will first evaluate the frequency and function of inflammatory immune populations by flow cytometry. While we will include a comprehensive panel evaluating lymphocyte and myeloid populations, we will specifically conduct an in-depth analysis quantifying the frequency, phenotype, and activation of T cells and macrophages. In particular, we will evaluate the frequency of naïve (CD44-CD62L+), effector memory ($CD62L^{lo}$CCR7-$CD27^{lo/int}$), central memory ($CD62L^{hi}$ $CCR7^{hi}$ $CD44^{hi}$), CD4 T-helper subsets (staining for the transcription factors Tbet, EOMES, RORγ, and GATA), and CD4+ Tregs cells (CD25+GITR+CD127-Foxp3+)[47]. Circulating macrophages will be evaluated for inflammatory M1 (CD80, CD86) and immunosuppressive M2 (CD163, CD206) sub-populations. Immune cells will also be evaluated for expression of GNRH receptors, as well as function by expression of intracellular cytokines associated with T cell activity (including IFNγ, TNFα, IL-2, IL-4, IL-6, IL-10, and Granzyme B as we have published[48,49]) or macrophage activity (Arg1, TNFσ, IL-6, IL-12).

In addition to evaluating peripheral immune populations, plasma will be isolated at the same timepoints and evaluated for chemokine and cytokine production by multiplex assay (Human Cytokine/Chemokine/Growth Factor 71-Plex Clinical Assay, Eve Technologies). This assay measures both inflammatory and suppressive chemokines and cytokines, as well as growth factors involved in cardiac activity. Factors found to be altered via multiplex (either following ADT, between treatment groups, or between patients who develop CV toxicity) will be validated by ELISA. Finally, to evaluate the mechanistic significance of these alterations, human cardiac myocytes (the AC16 cell line and primary cardiomyocytes (Sigma Aldrich)) grown under wild-type or androgen-deprived conditions will be evaluated for altered expression of these factors by ELISA. Supernatants from these cells will be used in transwell migration assays to evaluate how androgen deprivation-induced alteration of these factors impacts the migration of T cells, macrophage, or other relevant immune populations across a transwell. Follow-up experiments will be conducted in which altered factors are either blocked or restored (recombinant protein) to determine if these factors are sufficient to alter immune migration towards cardiac cells.

***Aim 2.2**: To determine how proteomic biomarkers associated with inflammatory immunity correspond with CV toxicity following ADT,* we will conduct in-depth proteomic profiling, which has previously been utilized and validated to identify signatures associated with inflammatory immune responses and cardiovascular risk[50,51]. To evaluate whether these signatures are associated with CV toxicity following ADT using leuprolide but not relugolix, pre-treatment and 12-month post-ADT PBMC will be subjected to proteomic profiling in conjunction with the Emory Integrated Proteomic Core as published by our colleagues at the Winship Cancer Institute[52]. Using nanocapillary liquid chromatography coupled with tandem mass spectrometry, this technology will be used to analyze patient PBMC samples for highly sensitive protein identification, posttranslational site mapping, and protein quantification. Briefly, PBMCs will be thawed under controlled conditions, sonicated in the presence of lysis buffer, protease inhibitors and phosphatase inhibitors. Protein samples will be sequentially digested, desalted, and dried under vacuum. Following reconstitution, samples will be loaded and eluted from a silica column driven by a UPLC system, monitoring elution spectra on a Fusion Mass Spectrometer. The mass spectrometry scans will be collected at a resolution of 120,000 at m/ 200 in profile mode while

in a higher-energy collision dissociation fragmentation MS/MS spectra. Following sequencing, the combined *Homo sapiens* UniProt subsets will be used as references for proteomics searches. Data will be normalized to account for systematic differences in protein signal distributions, by aligning the medians of the *log*2 protein signal distributions. Pathway enrichment will be conducted using gene sets obtained from the KEGG Pathway and MSigDB databases.

***Analysis Plan*:** Immune responses (e.g., frequency or function of immune populations) and circulating proteomic biomarkers will first be summarized and plotted by the follow-up time points to illustrate the change pattern over time for all patients and then separated by treatment arms. We will first test whether these biomarker change pattern would be altered by treatment groups (e.g., GNRH-agonist versus GNRH-antagonist) using Kruskal-Wallis test, and then we will link such pattern (e.g., percentage change from baseline for biomarkers) with related CV injury metrics as in Aim 1 using the similar statistical strategy as in Aim 1.2. P-value will be adjusted for multiple testing using Holm-Bonferroni method to control familywise error rate.

***Aim 3.1*: To determine how pre-existing genomic alterations promoting inflammatory immunity impact development of CV toxicity following GNRHa versus relugolix**, pre-treatment PBMC samples will be subjected to whole exome sequencing to determine alterations to the protein-coding regions of the genome. DNA will be extracted from PBMC and analyzed for quality. Following this analysis, DNA will be fragmented using restriction enzymes and undergo a second round of quality control. Fragmented DNA will be used to generate next-generation sequencing libraries and will undergo capture for exomic DNA sequences (Illumina Nextera Rapid Capture Expanded Exome), followed by amplification and repeated QA/QC. DNA sequencing will be conducted using the Illumina HiSeq platform. The FASTQ data files will be aligned using BWA-MEM, and variants from the human genome will be determined using VarScan and annotated using ANNOVAR. To be considered a valid mutation it will need to comprise at least 2% of the reads, as has been utilized to identify CHIP-related mutations[27,55].

***Analysis Plan*:** The correlation among baseline genomic biomarkers, inflammatory biomarkers, and proteomic biomarkers will be described using Spearman Correlation Coefficient for all patients and by treatment group separately. The association of genomic alterations with CV injury metrics (as defined in Aim 1) will be handled by similar statistical strategy as in Subaim 1.2. Additionally, the association of genetic aberrations with peripheral immune responses (as in Aim 2) will be carried out by similar statistical strategy as in Aim 2.

***Other statistical considerations*:**
Safety Population: All patients receiving at least one dose of GNRHa or Relugolix (for parallel randomized arms) or radiation alone (for parallel single cohort arm) will be considered evaluable for safety analyses.
Intent-to-Treat Population: Patients who are evaluable for safety and who are furthermore evaluable at 6 or 12-month CCTA assessment will be considered evaluable for correlative biomarker analyses.
Safety Analysis: All AEs experienced data will be described by summary statistics. Adverse events will be assessed according to CTCAE version 5.0 and will be evaluated by grade, attribution, and organ class. Adverse event listings and tabulated summaries of categorized AEs

will be generated for all patients collectively and will be summarized by arms. Vital signs and laboratory data will be summarized for changes over time on study.
Stopping Rule: The two ADT test agents are FDA-approved drugs that are used in clinical practice, and hence no stopping rule for safety is in place officially.

7. Data Specimen Banking

To achieve Aims 2 and 3 discussed above, peripheral blood specimens will be collected longitudinally four times for each enrolled patient (i.e. pre-treatment, 3 months, 6 months, 12 months).

Draw 1 (pre)	Draw 2 (3mo)	Draw 3 (6mo)	Draw 4 (12mo)
Pre-ADT	2-4 months post-ADT initiation	6-8 months post-ADT initiation	12-15 months post-ADT initiation

The blood is collected and processed according to the standard operating practices and regulatory parameters of the Winship biorepository and under the guidance of a board-certified pathologist.

At each collection, approximately 20-30 cc of venous blood will be collected in three purple-top tubes with EDTA anticoagulant, and about 6-8 cc of venous blood will be collected in one PAXgene tube. The blood will be procured for mononuclear cells and plasma, and it will be snap-frozen or cryopreserved. Blood samples will be processed via ficoll-hypaque density gradient centrifugation to obtain cellular material.

Winship tissue procurement staff and other authorized personnel will provide the blood samples upon request to investigators on this protocol or their designees. Blood will be distributed according to the prioritization decided by the PIs and study team and utilizing the standard operating procedures of the Winship research biorepository core.

This protocol and a detailed biorepository request will be submitted to the Winship Discovery prostate working groups for review and approval. If approved, deidentified blood and coded, limited datasets will be obtained from the Winship biorepository with oversight from the Winship informatics team under the Emory Honest Broker protocol. Blood will be distributed to Dr. Brian Olson Lab according to the standard practices and regulatory parameters of the Winship biorepository, WRISR, and the Winship Cancer Tissue and Pathology Shared Resource (CTPSR). Data will be transmitted through password protected systems within the Emory University/Healthcare Firewall.

8. Sharing of Results with Participants

Each enrolled participant on trial will be eligible to received results of their coronary CT scans (pretreatment #1, and 6-12-month #2) after completion of the 12-month study period. All CT scans will be blinded until completion of scan #2. After that period, patients are eligible to receive report of the image findings (i.e. scan #1 and #2). These scans are guideline-concordant cardiac

screening tests, and findings may be used to appropriate manage patients with regards to his cardiovascular health. As all scans are blinded before completion of #2 scan (i.e. 12 months after enrollment), no scan #1 image findings will be disclosed to patients until after the 12 month study period. Of note, all CT images will be intereprted regarding coronary artery (e.g. stenosis, calcficiation) and epicardial adipose tissue findings only; any pulmonary, mediastinal, or osseous findings will not be interpreted by the cardiac radiologist.

Blood bank analyses (for Aims 2 and 3) are for research purposes only and will not be disclosed to patients during or after completion of the study period.

9. Study Timelines

Each patient will be enrolled on study for a total of ~12 months, which includes time from pre-treatment coronary CT and blood draw, to 6-12-month coronary CT and final blood draw. Each patient will undergo XRT +/- ADT for management of localized prostate cancer in the interim.

Based on current volume of newly diagnosed localized prostate cancer eligible for definitive XRT across all Winship Cancer Institute sites, we anticipate to complete accrual of the study (i.e. 3 treatment cohorts, n=30 in each arm; see Figure 5 Study Schema) within 12 months after study opening. Study endpoints for Aim 1-3 (i.e. imaging findings, blood analyses) will be completed on a rolling basis, and thus, we anticipate completion of data acquisition within 24 months after study opening.

Completion of this mixed-methods research will involve a robust multidisciplinary team across Winship, including Radiation Oncology (Drs. Sagar Patel and Ashesh Jani; leading the accrual of patients to trial), Cardiology/Cardiac Imaging (Drs. Stephanie Cantu, Arthur Stillman, Anant Mandawat; leading the coronary CT interpretation on study for Aim 1), Hematology and Medical Oncology (Dr. Brian Olson; leading the laboratory analyses of peripheral blood for Aims 2-3), and Biostatistics (Dr. Yuan Liu; leading all statistical analyses for Aims 1-3).

Projected funding start date: 12/1/2021

Projected funding end date: 11/30/2023

Timeline for Completion of Proposed Research Months	3	6	9	12	15	18	21	24
Aim 1. Effect of leuprolide versus relugolix on coronary plaque development using non-invasive cardiac imaging								
Subaim 1.1. Relationship between ADT and accelerated coronary plaque development/rupture								
Subaim 1.2.How baseline cardiac imaging biomarkers correlate with ADT-associated plaque development								
Aim 2. Relationship between leuprolide versus relugolix on immune effector responses implicated in atherosclerosis								
Subaim 2.1 How pre-existing and ADT-augmented inflammatory immune responses impact CV toxicity								
Subaim 2.2. How circulating proteomic biomarkers predict for cardiac toxicity								
Aim 3. How pre-existing genomic alterations impact development of cardiac toxicity following leuprolide versus relugolix								
Subaim 3.1: Identification of how pre-existing genomic alterations impact immune responses and CV toxicity								

10. Inclusion and Exclusion Criteria

Inclusion:

- Men ≥18 years old
- Non-metastatic prostate cancer
- Non-metastatic, biochemically recurrent prostate cancer
- Plan to undergo curative-intent pelvic radiation therapy (photons or protons) with or without brachytherapy

Exclusion:

- Metastatic prostate cancer requiring >24 months of ADT
- Prior exposure to androgen deprivation therapy
- Prior exposure to chemotherapy, immunotherapy, or radiation therapy
- History of cardiac bypass surgery or percutaneous coronary intervention

11. Vulnerable Populations

Not applicable. This study does not involve individuals who are vulnerable to coercion or undue influence, such as pregnant women, children/minors, cognitively-impaired, prisoners, etc.

12. Local Number of Participants

Our accrual goal for this protocol is 90 patients (n=30 in each arm).

Based on current prostate cancer volume at Winship Cancer Institute, accrual is expected to be complete in 12 months. Specifically, approximately 40 new patients with localized intact or recurrent PCa eligible for XRT (with or without ADT) are seen at all Winship sites monthly. We predict an enrollment rate of 20% with a low dropout rate, given that the final blood draw and coronary CT will occur 6-12 months after treatment initiation, which corresponds to the time of first or second standard post-treatment follow up appointment after completion of therapy.

Clinical research coordinators will screen all prostate cancer radiation oncology providers (directed by PI Dr. Sagar Patel) for all eligible patients to enroll. Co-investigators will be responsible for consenting patients in radiation oncology clinic.

13. Recruitment Methods

Patients will be recruited from those seen at the Department of Radiation Oncology at Winship Cancer Institute (EUH, EUHM, ESJH, EPTC). A brief description of the study will be given verbally to the patients, followed by written informed consent, and any relevant supplemental material as needed. The patients will be given ample time to review the consent and a time for questions and answer will be provided.

Study Enrollment Procedures

A copy of the institution's IRB-approved informed consent document and written justification for any changes made to the informed consent for this protocol must be on file at the Online Collaborative Research Environment (OnCore, https://oncore.emory.edu) and available to the Emory University Office for Clinical Research) before any patients may enter. The Winship Cancer Center institution consent form must be reviewed and approved and all documents must be received (i.e., IRB approved documentation, IRB approved consent form, etc.).

Patient Registration

All patients entering this study will be registered with the Clinical Trials Office (CTO) at the Winship Cancer Institute, Atlanta, GA. The CTO is open Monday through Friday from 8am-5pm (EST). OnCore will be used to record information for all registered patients including their assigned patient ID.

Study personnel will notify Winship Central Subject Registration (WCSR) by email at winshipcsr@emory.edu, once subject has been consented for a trial.
Email notification must be done within 24 hours after consent has been obtained and it will include scanned copies of:

- Signed patient consent form
- HIPAA authorization form
- Emory Research Management System (ERMS; *https://erms.emory.edu*) Enrollment Fax Cover

The WCSR will enter the subject into the OnCore Research Management System, which is the system of record for Winship Cancer Institute Clinical Trials.

Eligible patients will be enrolled on study centrally at Winship Cancer Institute by the Study Coordinator. When all required test results are available, complete the eligibility checklist and provide the checklist and the supporting documentation to the IRB approved investigator for review and sign-off. Once the investigator (sub-investigator, Co-Investigator) has signed the eligibility checklist, enrollment may proceed. Oncore and ERMS must be updated to reflect eligibility and on treatment status.

14. Withdrawal of Participants

Participation in the study should continue until one of the following criteria applies:

- Intercurrent illness that prevents delivery of planned radiation therapy or ADT
- Unacceptable treatment-related adverse event(s), including patient death
- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Noncompliance with treatment plan, including delays to treatment or acquisition of additional diagnostic imaging that significantly exceeds the proposed timeline of study events
- General or specific changes in the patient's condition that render the patient unacceptable to receive further treatment in the judgment of the investigator
- Lost to follow up. In the event of a patient's withdrawal, the Investigator will make every effort to complete the End of Treatment/follow-up procedures specified in the Schedule of Events.

15. Risk to Participants

Radiation therapy +/- hormone therapy (either GNRHa or relugolix) are FDA-approved, guideline-supported standard treatments for prostate cancer. There is no additional risk associated with treatment on study, including the randomized portion of treatment (i.e. GNRHa with Lupron/Trelstar and Relugolix are acceptable ADT options for men with prostate cancer undergoing radiation therapy). There is no additional risk associated with blood draws (which will correspond to blood draws taken at standard clinical intervals to minimize additional appointment or peripheral venous sticks).

We have incorporated a state-of-the-art, validated non-invasive cardiac imaging, coronary CT angiogram, as the primary endpoint of Aim 1 in this study. This imaging technique offers remarkable spatial resolution of coronary vasculature to test the primary hypothesis that ADT-mediated CV risk, namely from GNRHa, is driven by accelerated atherosclerosis and coronary disease. With modern imaging techniques, such as prospective triggering, radiation exposure to the patient is minimized, as imaging is gated to be performed in only one phase of the cardiac cycle (rather than continuous during the entire cardiac cycle). Additionally, other measures, including limited field of view, limited scan length, and minimal tube voltage, will ensure that the additional radiation exposure sustained by coronary CT in this study will remain around 3.2 mSv per patient (which is comparable or lower than the 3-7 mSv the average person receives annually from natural sources). Other cardiac imaging techniques, including catheter-based angiography or nuclear medicine studies, result in a substantially larger amount of radiation exposure to the patient (e.g. 21-41 mSv), and are therefore, less optimal to use as an endpoint in this study. Additionally, the coronary CT endpoints will allow us to both test mechanism (which we hypothesize to be GNRHa-mediated immune effector response resulting in accelerated coronary atherosclerosis and/or

plaque instability), as well as help identify a novel imaging biomarker that may be used in clinical practice to identify those men at highest risk of an adverse CV outcome from ADT and therefore would be optimally treated with risk-reducing relugolix.

16. Potential Benefits to Participants

We plan to investigate early cardiac biomarkers and the comparative toxicity of ADT regimens delivered with radiation therapy for prostate cancer. The findings of our research could improve patient treatment and outcomes by improving our understanding of the pathophysiology behind CV toxicity from ADT. We also aim to identify men at especially high-risk of CV morbidity from ADT; these men may benefit from pre-treatment cardiac medical optimization or prostate cancer treatment modifications, such as truncation of concomitant ADT or utilization of alternative androgen signaling inhibitors, to minimize the risk of treatment-related morbidity and mortality.

17. Compensation to Participants

Financial incentive will be provided for each patient to account for additional travel time and transportation (e.g. vehicle gas) associated with non-standard coronary CT on study ($100 Visa gift card after completion of each scan; total $200 eligible per patient). Gift cards will be distributed to patients by mail after completion of each coronary CT scan (i.e. pretreatment, 12-month).

18. Data Management and Confidentiality

All blood and data are deidentified or coded under the existing IRB-approved protocol described above before they are used in this study. Data cannot be linked to an individual other than by a biorepository or Honest Broker delegate. Investigators on this protocol will be responsible for the management of study data and specimens and disposal upon completion of this study.

Clinical, imaging, blood analyses data will be stored in a password protected REDCap database. Access to stored data will be limited by personnel "roles" and comply with minimum necessary standards. Emory physicians on this study and their designees may access and use PHI to determine if patients are eligible to participate in this study and may also access PHI to assist with preparation of data for research. By limiting access to data through physical and cyber procedures, the risk of the improper release of PHI will be reduced. Appropriate administrative, technical, and physical safeguards to protect patient data will be implemented. Winship employees are trained in the protection of patient privacy; such training will be modified as needed to address privacy and security issues arising from new systems and processes created for this study.

19. Provisions to Monitor the Data to Ensure the Safety of Participants

Adverse Events (AEs)

From the time of treatment allocation through **30** days following cessation of treatment, all adverse events, that begin or worsen after informed consent, must be recorded by the investigator or designee at each examination on the Adverse Event case report forms/worksheets.

The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF/worksheet.

Adverse events will be assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Grade 1 to 5 will be used to characterize the severity of the Adverse Event. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, death related to the AE corresponding respectively to Grades 1 - 5, will be used. The occurrence of adverse events should be sought by non-directive questioning of the patient during the screening process after signing informed consent and at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-5)
2. Its duration (Start and end dates)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes) or Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes, investigational treatment, Yes, the study treatment (non-investigational), Yes, both and/or indistinguishable)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
5. Whether therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Whether it is serious,
7.Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).

If the event worsens the event should be reported a second time in the CRF noting the start date when the event worsens in toxicity. For grade 3 and 4 adverse events only, if improvement to a lower grade is determined a new entry for this event should be reported in the CRF noting the start date when the event improved from having been Grade 3 or Grade 4. All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded on the Adverse Event CRF.

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome. Progression of malignancy (including fatal outcomes), if documented by use of appropriate method (for example, as per RECIST criteria for solid tumors), should not be reported as a serious adverse event.

Laboratory abnormalities that constitute an Adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF.

Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. A dose hold or

medication for the lab abnormality may be required by the protocol in which case the lab abnormality would still, by definition, be an adverse event and must be reported as such.

Serious Adverse Events (SAEs)

Any adverse event that results with any of the following outcomes:

- Death
- Life-threatening experience
- Inpatient Hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other medically important events

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE, when, based upon medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. These events will also be recorded for each subject and is subject to review by the investigators and data safety monitoring committee (DSMC). In addition, these events will be reported to the responsible Institutional Review Board (IRB) and the study PI. All SAEs will be reported within 24 hours of discovery to Study Coordinator and the study PI, Dr. Sagar Patel (sagar.patel@emory.edu, 205-370-8119). SAEs will be reported using the SAE form in Appendix B. SAE should be emailed to both the study coordinator and Dr. Patel as well as inputted into OnCore.

Data and Safety Monitoring

The data safety monitoring plan will be implemented by Dr. Patel, the Principal Investigator (P.I.) of this study. The plan is based on self-monitoring, internal CTO real time monitoring using the quality assurance committee, and monitoring via Winship Cancer Institute ("WCI") Data Safety Monitoring Committee (DSMC) as per WCI CTO standard operating procedure. Dr. Patel and the investigators, the clinical research coordinator and the regulatory affairs coordinator will meet weekly to review and discuss study data to ensure subject safety. The research coordinators will maintain one spreadsheet that will summarize 1) all the patient data for patients actively being treated on the trial and 2) a roadmap detailing pending tests/treatments for each individual patient. The WCI DSMC is responsible for providing data safety-monitoring oversight for this protocol. Any comments that are generated by the WCI DSMC are forwarded to the IRB. The P.I. and the study investigators will discuss any required modifications to this study at the weekly meetings. No modifications to this study are implemented until they are submitted for review and approved by the Emory University IRB. The comments from the WCI DSMC are forwarded to the IRB at the time of the annual renewal of this study or sooner if warranted and requested by the WCI DSMC.

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will provide oversight for the conduct of this study. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high-quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Depending on the risk level of the protocol, the DSMC review may occur every 6 months or annually. For studies deemed High Risk, initial study monitoring will occur within 6 months from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. For studies deemed Moderate Risk, initial study monitoring will occur within 1 year from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed.

Subsequent monitoring will occur in routine intervals per the Winship Data and Safety Monitoring Plan (DSMP).

The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry, accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents. Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is required to address and respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor. Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data.

The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

20. Provisions to Protect the Privacy Interest of Participants

Participants will be assured of their voluntary participation in the study, their choice to answer or not any question, and the protocol for maintain confidentiality.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification

number. The study data entry and study management systems used by clinical sties and research staff will be secured and password-protected. At the end of the study, all study databases will be de-identified and archived.

21. Economic Burden to Participants

The costs of the patient's primary treatment, including pre-treatment consultation and necessary evaluations, simulation, XRT treatment course, and follow-up surveillance laboratory studies are expected to be covered by the patient's insurer/primary payor. For patients randomized to receive relugolix for ADT with XRT, drug will be provided by Myovant Sciences as part of the funding source (Pfizer-PCF-Myovant Challenge Award).

All blood draw appointments for the biorepository correspond to intervals of standard blood draws for cancer surveillance.

Patients will incur costs associated with travel and time (with non-routine coronary CT that will be taken pre-treatment and 12-months after initiating ADT (two imaging scans total per patient). Patients will be compensated $200 total (distributed at 2 intervals of $100 each, detailed above) to account for gas mileage usage for appointments/tests required on trial.

22. Informed Consent

This study will enroll patients with localized prostate cancer, or biochemically recurrent prostate cancer after prior prostatectomy, who are candidates for curative-intent radiation therapy (with or without ADT). Men who do not receive ADT will be enrolled on a prospective, single-arm cohort; men who are eligible for ADT will be randomized between two FDA-approved, guideline-supported ADT agents: (1) GNRHa (e.g. Lupron, Trelstar) or (2) Relugolix.

Informed consent is to be obtained prior to commencing any research procedures. Patients will be recruited from those seen at the Department of Radiation Oncology at Winship Cancer Institute (EUH, EUHM, ESJH, EPTC), either at the time of initial consultation or pre-treatment follow up appointment. A brief description of the study will be given verbally to the patients by the treating physician (and co-investigator of trial). This will be followed by written informed consent. The patients will be given ample time to review the consent and a time for questions and answer will be provided. The co-investigator may choose to consent the patient with or without presence of the clinical research coordinator. Once consent has been obtained, the treating physician will notify by email the contact principal investigator (Sagar Patel) and clinical research coordinators for final screening, enrollment, and (if needed) randomization.

A study investigator shall seek such consent only under such circumstances that provide the prospective patient opportunity to consider whether or not to participate and that minimizes the possibility of coercion or undue influence. The information given to the patient, or the representative, shall be in a language understandable to the subject or representative. The informed consent document may not include any exculpatory language through which the subject or representative is made to waive any of the subject's legal rights or releases, or appears to release the investigator, the sponsor or the institution from liability for negligence.

Non-English-Speaking Participants

A certified translator/interpreter will be present during the consenting process and all questions and concerns will be answered by the treating physician.

A Short Form in that specific language will be used. A certified translator/interpreter will be present during the consenting process and this will be documented. We will use what's available on Emory IRB website. For the languages that are not available, we will have the short form translated to that language and submit the IRB for review and approval prior to use. Process to Document Consent in Writing: Winship SOP 2.1:" Obtaining Informed consent for Interventional clinical trial" will be followed.

Participants who are not yet adults (infants, children, teenagers): N/A
Cognitively Impaired Adults: N/A
Adults Unable to Consent: N/A
Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception): N/A

23. Setting

Winship Cancer Institute of Emory University School of Medicine (Atlanta, GA) is the only participating institution. Study/trial information will be made available to the Georgia Center for Oncology Research and Education (CORE) at https://www.georgiacancerinfo.org. Detailed trial information, including the protocol and all supplemental information will be submitted to, and made available to the Online Collaborative Research Environment (OnCore) at https://oncore.emory.edu in accordance with institutional regulations.

24. Resources Available

Emory University was founded in 1836 and is a national center for teaching, research, and service. Emory University has been named as one of the nation's top 25 universities for more than a decade by the U.S. News and World Report. Emory University research partners include the Georgia Institute of Technology, the University of Georgia, Morehouse School of Medicine, the US Centers for Disease Control and Prevention, Children's Healthcare of Atlanta, and the Georgia Clinical and Translational Science Alliance (GACTSA). Emory University researchers received $734 million from external funding agencies in fiscal year 2018, including approximately $441 million in funding from federal agencies, $359 million of this from the National Institutes of Health (NIH).

Facilities

Olson Laboratory: Dr. Olson has designated laboratory and office space located on the 3rd floor of the Winship Cancer Institute of Emory University, which includes over 450 square feet of recently renovated laboratory space, as well as more than 200 sq. ft of adjacent hallway space. The lab space is fully equipped for immunology research, including multiple laminar air-flow biosafety cabinets, chemical fume hoods, refrigerators/freezers, liquid nitrogen tank, etc. The laboratory space is also equipped for biochemistry and molecular biology work with electrophoresis apparatus, DNA thermal cyclers, etc. Common areas adjacent to this lab space contain dark rooms equipped with X-ray film processors, ultracentrifuge, incubator/shakers, spectrophotometers, cold rooms, and an autoclave room for dishwashing/water purification.

Clinical: The Winship Cancer Institute (a National Cancer Institute (NCI)-designed cancer center since 2009), serves as the coordinating center for cancer research, education and care throughout Emory

University Network, which includes Emory University Hospital (EUH), EUH-Midtown, Emory Saint Joseph's Hospital, and Emory Johns Creek Hospital. Winship investigators conduct more than 250 therapeutic clinical trials onto which they enroll nearly 1000 patients annually and is the largest unit in Georgia for phase I clinical trials and a Lead Academic Participating Site for the NCI National Clinical Trials Network.

Drs. Patel and Jani have clinical and office space in the Winship Cancer Institute, as well as at Emory Saint Joseph's Hospital and Emory Proton Therapy Center. **Dr. Mandawat** has dedicated Cardio-Oncology clinic and office space in the Winship Cancer Institute and at the Winship Cancer Institute of Emory University Midtown location. **Dr. Cantu** will oversee cardiac CT imaging completed on trial, which will be conducted using a Canon Aquillon One 320/640 multidetector CT scanner (Toshiba Medical Systems, Japan) at Emory University Hospital, located immediately adjacent to the Winship Cancer Institute where Dr. Olson's laboratory and the clinic and offices of Drs. Olson/Mandawat/Patel/Jani's are located. This physical proximity will be advantageous to ensure timely completion of imaging endpoints and frequent ad hoc meetings.

Shared Facilities: Emory University and Winship Cancer Institute provide independent investigators with a wide array of state of the art core facilities and resources (listed at https://winshipcancer.emory.edu/research/shared-resources and www.cores.emory.edu) that are all located in close proximity to Dr. Olson's laboratory space and which he has access to at a subsidized rate. In particular, shared facilities relevant to the proposal include:

Cancer Tissue and Pathology Shared Resource (CTP): The CTP shared resource is involved in the procurement and distribution of high-quality human cancer specimens. Within this core is also the Human Tissue Procurement Service, which is involved with collecting and preserving the peripheral blood collections on the CO-PRO study.

Pediatrics/Winship Flow Cytometry Shared Resource: The Pediatric/Winship Flow Cytometry Core (located in Winship) provides cytometry services for the analysis and sorting of cells as well as expert consultation for experimental design and planning. The Flow Cytometry Core offers access to several analytical flow cytometers (including four 28-45 color flow cytometers, and two 6-13 color flow cytometers), high-speed cell sorters (two 18-color BD AriaII sorters), and an Amins Imagestream X Mark II imaging cytometer.

Emory Integrated Genomics Core (EIGC): The EIGC uses state-of-the-art genomics platforms to help Emory investigators effectively pursue their research goals. Located at the Woodruff Memorial Research Building adjacent to Dr. Olson's laboratory, their services include whole genome single nucleotide polymorphism (SNP) genotyping, whole genome and exome sequencing, and structural variation detection. The EIGC also maintains CLIA certification, offering assay validation and nucleic acid extraction services from a wide variety of biological sources, including blood, serum, plasma, solid tissues, cell extracts, etc., to support both basic research and clinical efforts on campus. They also provide bioinformatic services in analyzing and preparing the results for subsequent publication.

Emory Integrated Proteomics Core (EIPC): The EPIC provides protein analytical services by cutting-edge mass spectrometry (MS). Located in the Whitehead Biomedical Research Building (less than 0.4 miles from Dr. Olson's laboratory), the EIPC houses several mass spectrometers—hybrid quadrupoleorbitrap including a Q-Exactive, and a Q-Exactive HF-X; tribrid mass spectrometers including two Orbitrap Fusion, and an Orbitrap Fusion Lumos; and a TSQ Altis triple-stage quadrupole. Each instrument is coupled with an autosampler and HPLC system. The system allows automated capillary LC-MS/MS runs for top-down,

middle-down and bottom-up analyses with high resolution. These mass spectrometers are capable of detecting peptides at subfemtomolar level, identifying hundreds to thousands of proteins in complex mixtures, mapping posttranslational modification sites, and quantifying proteins. They also provide bioinformatic services in analyzing and preparing the results for subsequent publication. The Olson Laboratory has full access on a subsidized fee for services and will utilize this core for the proteomic studies in Aim 2.

25. Multi-Site Research when Emory is the Lead Site

N/A.

26. References

1 Crawford, E. D. *et al.* The potential role of follicle-stimulating hormone in the cardiovascular, metabolic, skeletal, and cognitive effects associated with androgen deprivation therapy. *Urol Oncol* **35**, 183-191, doi:10.1016/j.urolonc.2017.01.025 (2017).

2 Shore, N. D. *et al.* Oral Relugolix for Androgen-Deprivation Therapy in Advanced Prostate Cancer. *N Engl J Med* **382**, 2187-2196, doi:10.1056/NEJMoa2004325 (2020).

3 Keating, N. L., O'Malley, A. J. & Smith, M. R. Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. *J Clin Oncol* **24**, 4448-4456, doi:10.1200/JCO.2006.06.2497 (2006).

4 Keating, N. L., O'Malley, A. J., Freedland, S. J. & Smith, M. R. Diabetes and cardiovascular disease during androgen deprivation therapy: observational study of veterans with prostate cancer. *J Natl Cancer Inst* **102**, 39-46, doi:10.1093/jnci/djp404 (2010).

5 Bosco, C. *et al.* Quantifying observational evidence for risk of fatal and nonfatal cardiovascular disease following androgen deprivation therapy for prostate cancer: a meta-analysis. *Eur Urol* **68**, 386-396, doi:10.1016/j.eururo.2014.11.039 (2015).

6 Jespersen, C. G., Norgaard, M. & Borre, M. Androgen-deprivation therapy in treatment of prostate cancer and risk of myocardial infarction and stroke: a nationwide Danish population-based cohort study. *Eur Urol* **65**, 704-709, doi:10.1016/j.eururo.2013.02.002 (2014).

7 O'Farrell, S. *et al.* Risk and timing of cardiovascular disease after androgen-deprivation therapy in men with prostate cancer. *J Clin Oncol* **33**, 1243-1251, doi:10.1200/JCO.2014.59.1792 (2015).

8 Sharifi, N., Gulley, J. L. & Dahut, W. L. Androgen deprivation therapy for prostate cancer. *JAMA* **294**, 238-244, doi:10.1001/jama.294.2.238 (2005).

9 Wang, H. *et al.* Immune and Inflammation in Acute Coronary Syndrome: Molecular Mechanisms and Therapeutic Implications. *J Immunol Res* **2020**, 4904217, doi:10.1155/2020/4904217 (2020).

10 Libby, P. & Theroux, P. Pathophysiology of coronary artery disease. *Circulation* **111**, 3481-3488, doi:10.1161/CIRCULATIONAHA.105.537878 (2005).

11 Jaiswal, S. *et al.* Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med* **377**, 111-121, doi:10.1056/NEJMoa1701719 (2017).

12 Yura, Y., Sano, S. & Walsh, K. Clonal Hematopoiesis: A New Step Linking Inflammation to Heart Failure. *JACC Basic Transl Sci* **5**, 196-207, doi:10.1016/j.jacbts.2019.08.006 (2020).

13 Nguyen, P. L. *et al.* Influence of androgen deprivation therapy on all-cause mortality in men with high-risk prostate cancer and a history of congestive heart failure or myocardial infarction. *Int J Radiat Oncol Biol Phys* **82**, 1411-1416, doi:10.1016/j.ijrobp.2011.04.067 (2012).

14 D'Amico, A. V., Chen, M. H., Renshaw, A., Loffredo, M. & Kantoff, P. W. Long-term Follow-up of a Randomized Trial of Radiation With or Without Androgen Deprivation Therapy for Localized Prostate Cancer. *JAMA* **314**, 1291-1293, doi:10.1001/jama.2015.8577 (2015).

15 Basaria, S. *et al.* Long-term effects of androgen deprivation therapy in prostate cancer patients. *Clin Endocrinol (Oxf)* **56**, 779-786 (2002).

16 Braga-Basaria, M. *et al.* Metabolic syndrome in men with prostate cancer undergoing long-term androgen-deprivation therapy. *J Clin Oncol* **24**, 3979-3983, doi:10.1200/JCO.2006.05.9741 (2006).

17 Shahani, S., Braga-Basaria, M. & Basaria, S. Androgen deprivation therapy in prostate cancer and metabolic risk for atherosclerosis. *J Clin Endocrinol Metab* **93**, 2042-2049, doi:10.1210/jc.2007-2595 (2008).

18 Naghavi, M. *et al.* From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation* **108**, 1772-1778, doi:10.1161/01.CIR.0000087481.55887.C9 (2003).

19 Kolodgie, F. D. *et al.* Pathologic assessment of the vulnerable human coronary plaque. *Heart* **90**, 1385-1391, doi:10.1136/hrt.2004.041798 (2004).

20 Ridker, P. M. *et al.* Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* **377**, 1119-1131, doi:10.1056/NEJMoa1707914 (2017).

21 Frostegard, J. *et al.* Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis* **145**, 33-43, doi:10.1016/s0021-9150(99)00011-8 (1999).

22 Chen, H. F., Jeung, E. B., Stephenson, M. & Leung, P. C. Human peripheral blood mononuclear cells express gonadotropin-releasing hormone (GnRH), GnRH receptor, and interleukin-2 receptor gamma-chain messenger ribonucleic acids that are regulated by GnRH in vitro. *J Clin Endocrinol Metab* **84**, 743-750, doi:10.1210/jcem.84.2.5440 (1999).

23 Iqbal, J., Sun, L., Kumar, T. R., Blair, H. C. & Zaidi, M. Follicle-stimulating hormone stimulates TNF production from immune cells to enhance osteoblast and osteoclast formation. *Proc Natl Acad Sci U S A* **103**, 14925-14930, doi:10.1073/pnas.0606805103 (2006).

24 Cannon, J. G., Kraj, B. & Sloan, G. Follicle-stimulating hormone promotes RANK expression on human monocytes. *Cytokine* **53**, 141-144, doi:10.1016/j.cyto.2010.11.011 (2011).

25 Liu, X. M. *et al.* FSH regulates fat accumulation and redistribution in aging through the Galphai/Ca(2+)/CREB pathway. *Aging Cell* **14**, 409-420, doi:10.1111/acel.12331 (2015).

26 Heuser, M., Thol, F. & Ganser, A. Clonal Hematopoiesis of Indeterminate Potential. *Dtsch Arztebl Int* **113**, 317-322, doi:10.3238/arztebl.2016.0317 (2016).

27 Papa, V. *et al.* Translating Evidence from Clonal Hematopoiesis to Cardiovascular Disease: A Systematic Review. *J Clin Med* **9**, doi:10.3390/jcm9082480 (2020).

28 Libby, P. *et al.* Clonal Hematopoiesis: Crossroads of Aging, Cardiovascular Disease, and Cancer: JACC Review Topic of the Week. *J Am Coll Cardiol* **74**, 567-577, doi:10.1016/j.jacc.2019.06.007 (2019).

29 Fuster, J. J. *et al.* Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* **355**, 842-847, doi:10.1126/science.aag1381 (2017).

30 Mercader, M. *et al.* T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc Natl Acad Sci U S A* **98**, 14565-14570. (2001).

31 Roden, A. C. *et al.* Augmentation of T cell levels and responses induced by androgen deprivation. *J Immunol* **173**, 6098-6108 (2004).

32 Mercader, M. *et al.* Early effects of pharmacological androgen deprivation in human prostate cancer. *BJU Int* **99**, 60-67, doi:10.1111/j.1464-410X.2007.06538.x (2007).

33 Morse, M. D. & McNeel, D. G. Prostate Cancer Patients Treated with Androgen Deprivation Therapy Develop Persistent Changes in Adaptive Immune Responses. *Hum Immunol* **71**, 496-504 (2010).

34 Morse, M. D. & McNeel, D. G. T cells localized to the androgen-deprived prostate are TH1 and TH17 biased. *Prostate* **72**, 1239-1247, doi:10.1002/pros.22476 (2012).

35 Gannon, P. O. *et al.* Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. *J Immunol Methods* **348**, 9-17, doi:10.1016/j.jim.2009.06.004 (2009).

36 Dwyer-Lindgren, L. *et al.* US County-Level Trends in Mortality Rates for Major Causes of Death, 1980-2014. *JAMA* **316**, 2385-2401, doi:10.1001/jama.2016.13645 (2016).

37 Bureau, U. S. C. *2010 Census.U.S. Census Bureau*, <http://www.census.gov/2010census/data/> (2010).

38 Skinner, D. C. *et al.* Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. *J Neuroendocrinol* **21**, 282-292, doi:10.1111/j.1365-2826.2009.01842.x (2009).

39 Budoff, M. J. *et al.* Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. *J Am Coll Cardiol* **52**, 1724-1732, doi:10.1016/j.jacc.2008.07.031 (2008).

40 von Ballmoos, M. W., Haring, B., Juillerat, P. & Alkadhi, H. Meta-analysis: diagnostic performance of low-radiation-dose coronary computed tomography angiography. *Ann Intern Med* **154**, 413-420, doi:10.7326/0003-4819-154-6-201103150-00007 (2011).

41 Foster, G., Shah, H., Sarraf, G., Ahmadi, N. & Budoff, M. Detection of noncalcified and mixed plaque by multirow detector computed tomography. *Expert Rev Cardiovasc Ther* **7**, 57-64, doi:10.1586/14779072.7.1.57 (2009).

42 Detrano, R. *et al.* Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med* **358**, 1336-1345, doi:10.1056/NEJMoa072100 (2008).

43 Mahabadi, A. A. *et al.* Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study. *J Am Coll Cardiol* **61**, 1388-1395, doi:10.1016/j.jacc.2012.11.062 (2013).

44 Choi, S. H., Hong, E. S. & Lim, S. Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health. *Front Endocrinol (Lausanne)* **4**, 97, doi:10.3389/fendo.2013.00097 (2013).

45 Arnett, D. K. *et al.* 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* **74**, 1376-1414, doi:10.1016/j.jacc.2019.03.009 (2019).

46 Ganz, P. *et al.* Development and Validation of a Protein-Based Risk Score for Cardiovascular Outcomes Among Patients With Stable Coronary Heart Disease. *JAMA* **315**, 2532-2541, doi:10.1001/jama.2016.5951 (2016).

47 Wherry, E. J. *et al.* Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* **4**, 225-234, doi:10.1038/ni889 (2003).

48 Olson, B. M. & McNeel, D. G. CD8+ T cells specific for the androgen receptor are common in patients with prostate cancer and are able to lyse prostate tumor cells. *Cancer Immunol Immunother* **60**, 781-792 (2011).

49 McNeel, D. G. *et al.* Real-time immune monitoring to guide plasmid DNA vaccination schedule targeting prostatic acid phosphatase in patients with castration-resistant prostate cancer. *Clin Cancer Res* **20**, 3692-3704, doi:10.1158/1078-0432.CCR-14-0169 (2014).

50 Haudek-Prinz, V. J. *et al.* Proteome signatures of inflammatory activated primary human peripheral blood mononuclear cells. *J Proteomics* **76 Spec No.**, 150-162, doi:10.1016/j.jprot.2012.07.012 (2012).

51 Cubedo, J., Padro, T. & Badimon, L. Coordinated proteomic signature changes in immune response and complement proteins in acute myocardial infarction: the implication of serum amyloid P-component. *Int J Cardiol* **168**, 5196-5204, doi:10.1016/j.ijcard.2013.07.181 (2013).

52 Koyen, A. E. *et al.* EZH2 has a non-catalytic and PRC2-independent role in stabilizing DDB2 to promote nucleotide excision repair. *Oncogene* **39**, 4798-4813, doi:10.1038/s41388-020-1332-2 (2020).

53 Dorsheimer, L. *et al.* Association of Mutations Contributing to Clonal Hematopoiesis With Prognosis in Chronic Ischemic Heart Failure. *JAMA Cardiol* **4**, 25-33, doi:10.1001/jamacardio.2018.3965 (2019).

54 Feusier, J. E. *et al.* Large-Scale Identification of Clonal Hematopoiesis and Mutations Recurrent in Blood Cancers. *Blood Cancer Discov* **2**, 226-237, doi:10.1158/2643-3230.bcd-20-0094 (2021).

55 Zink, F. *et al.* Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* **130**, 742-752, doi:10.1182/blood-2017-02-769869 (2017).

56 Sirker, A., Zhang, M. & Shah, A. M. NADPH oxidases in cardiovascular disease: insights from in vivo models and clinical studies. *Basic Res Cardiol* **106**, 735-747, doi:10.1007/s00395-011-0190-z (2011).

57 Tse, H. M. *et al.* NADPH oxidase deficiency regulates Th lineage commitment and modulates autoimmunity. *J Immunol* **185**, 5247-5258, doi:10.4049/jimmunol.1001472 (2010).

58 Sinha, S. *et al.* An autoimmune disease risk SNP, rs2281808, in SIRPG is associated with reduced expression of SIRPgamma and heightened effector state in human CD8 T-cells. *Sci Rep* **8**, 15440, doi:10.1038/s41598-018-33901-1 (2018).

59 Song, Z. *et al.* Essential role for UVRAG in autophagy and maintenance of cardiac function. *Cardiovasc Res* **101**, 48-56, doi:10.1093/cvr/cvt223 (2014).

60 Quach, C. *et al.* A truncating mutation in the autophagy gene UVRAG drives inflammation and tumorigenesis in mice. *Nat Commun* **10**, 5681, doi:10.1038/s41467-019-13475-w (2019).

61 Adamson, A. *et al.* Tissue inhibitor of metalloproteinase 1 is preferentially expressed in Th1 and Th17 T-helper cell subsets and is a direct STAT target gene. *PLoS One* **8**, e59367, doi:10.1371/journal.pone.0059367 (2013).

62 Erman, H. *et al.* The association of vascular endothelial growth factor, metalloproteinases and their tissue inhibitors with cardiovascular risk factors in the metabolic syndrome. *Eur Rev Med Pharmacol Sci* **20**, 1015-1022 (2016).