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**A PHASE II STUDY OF THE USE OF ULTRA LOW-DOSE BILATERAL
WHOLE LUNG RADIATION THERAPY IN THE TREATMENT OF
CRITICALLY ILL PATIENTS WITH COVID-19 RESPIRATORY
COMPROMISE**

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PATIENTS WITH COVID-19 RESPIRATORY COMPROMISE**

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1. STUDY SUMMARY AND SCHEMA

STUDY SUMMARY

Title	A Phase II Study of the Use of Ultra Low-Dose Bilateral Whole Lung Radiation Therapy in the Treatment of Critically Ill Patients with COVID-19 Respiratory Compromise
Short Title	Ultra Low-Dose Radiation Therapy for Critically Ill COVID-19 Patients
Protocol Number	VENTED
Phase	2
Methodology/Study Design	Open-label, single-arm, phase II study to evaluate the efficacy of ultra-low dose bilateral whole lung radiation therapy (ULD-WLRT) in patients with COVID-19 pneumonia requiring mechanical ventilation. No effective therapies have yet been identified for this group of patients who experience a high mortality rate. Our goal is to effect improved survival, decreased dependence on mechanical ventilation and supplemental oxygen, decreased length of ICU and hospital stay, and decrease in lung infiltrates characteristic of COVID-19. Patients will be treated with a single dose of 80 cGy to the bilateral lungs in a manner that is simplified such that it can be designed and delivered quickly in one session to this critically ill population. For those who do not experience any respiratory toxicity attributable to ULD-WLRT, but who do not show signs of improvement, a second dose of 80 cGy 3-10 days later will be <i>optionally</i> available.
Study Duration	18 months for accrual of all patients
Study Center(s)	Single Center, OSUWMC
Objectives	<i>Primary Objectives:</i> ● To evaluate 30-day mortality rate after ULD-WLRT. <i>Secondary Objectives:</i> ● To evaluate overall survival after ULD-WLRT ● To evaluate total and post-ULD-WLRT ICU length of stay ● To evaluate total and post-ULD-WLRT length of hospital stay ● To evaluate total and post-ULD-WLRT requirement for mechanical ventilation (ventilator-free days) ● To evaluate total and post-ULD-WLRT requirement for supplemental oxygen therapy (days) ● To evaluate oxygenation index for 14 days post treatment or until

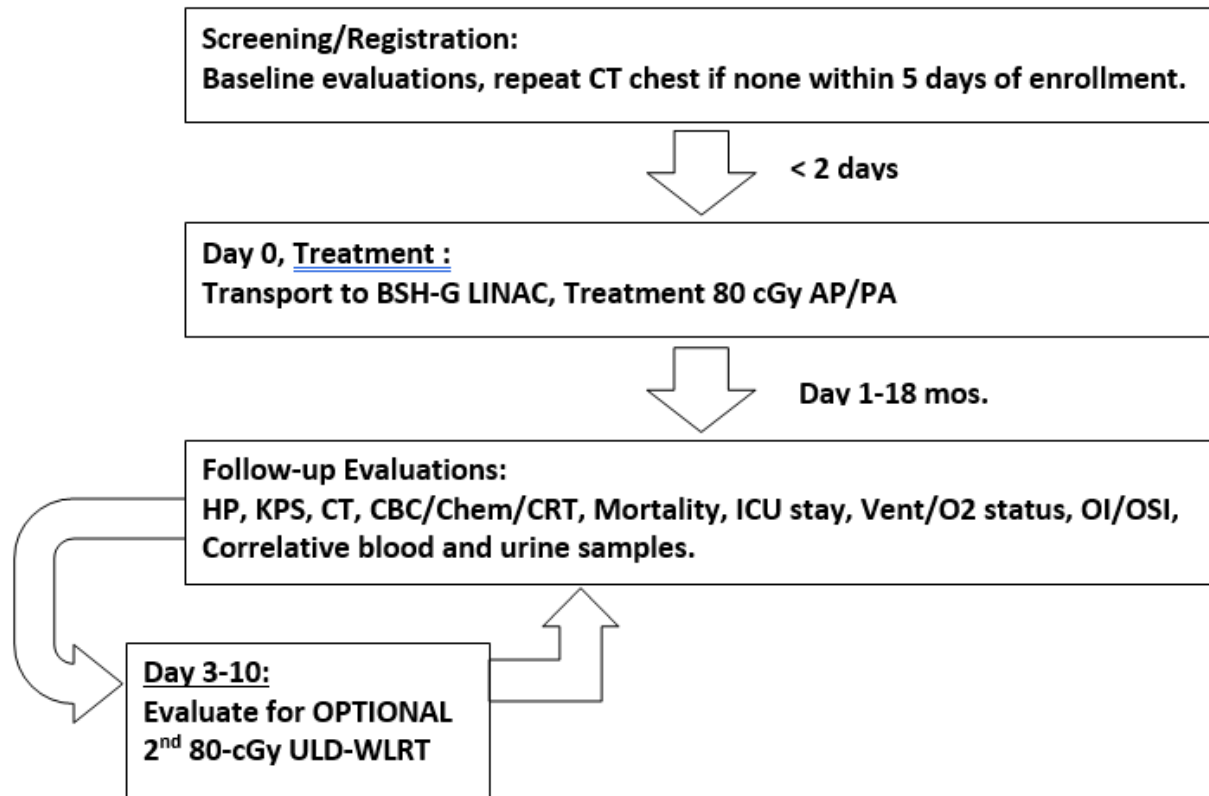
	extubated ● To quantitate post-ULD-WLRT differences between baseline and Day 7, 14, and 28 CT chest finding (number of involved lung segments, size of GGO, lung infiltrate/opacification percentage) ● To evaluate SARS-CoV2 viral titers at baseline and post-ULD-WLRT at Day 7, 14, and 28. ● To establish feasibility, safety, and tolerability of this regimen. <i>Exploratory Objectives:</i> ● To evaluate molecular biomarker levels in COVID-19 patients upfront as well as after treatment with ULD-WLRT (e.g. levels of inflammatory and DNA damage markers). ● To determine baseline molecular biomarkers that predict response to ULD-WLRT in COVID-19 patients. ● To determine molecular biomarkers that change over the treatment course, correlating with disease status and resolution. ● To retrospectively evaluate rapidly planned treatment using volumetric treatment plan evaluation based on CBCT ● To collect raw diagnostic and CBCT imaging data for machine-learning assessment of longitudinal progression in patients with COVID-19 ● To collect raw imaging data for further evaluation of the potential for identification of COVID-19 in diagnostic scans
Number of Subjects	24 ● 16 patients in initial phase, followed by interim analysis ● 8 additional patients, if criteria for continuation are met
Diagnosis and Main Inclusion Criteria	Male and female patients ≥ 18 years of age with documented COVID-19 respiratory compromise requiring mechanical ventilation. <i>Key Inclusion Criteria:</i> ● Patient age ≥ 18 years of age ● Positive COVID-19 test within 14 days of enrollment. ● CT or radiographic findings typical of COVID-19 pneumonia within 5 days of enrollment ● Receiving ICU-based mechanical ventilation ● Life expectancy ≥ 24 hours, as judged by investigator ● Hypoxemia defined as a Pa/FiO₂ ratio < 300 or SpO₂/FiO₂ < 315 ● Signed informed consent by patient or his or her legal/authorized representatives ● Concurrent, prior, or planned future therapy with passive convalescent immune serum administration is allowed.

Exclusion Criteria	● Moribund with survival expected < 24 hours ● Expected survival < 30 days due to chronic illness present prior to COVID infection ● Patient or legal representative not committed to full disease specific therapy i.e. comfort care (DNRCCA is allowed) ● Treatment with immune suppressing medications in last 30 days (steroids for ARDS or septic shock allowed) ● Presumed COVID-associated illness greater than 14-days ● Inpatient admission greater than 14-days ● Patient deemed unsafe for travel for radiation therapy ● Chronic hypoxemia requiring supplemental oxygen at baseline ● Documented active connective tissue disease (scleroderma) or idiopathic pulmonary fibrosis ● Active or history of prior radiation therapy resulting in $\geq$ grade 2 radiation pneumonitis within 365 days of enrollment. ● Active or history of prior radiation to the thorax completed within 180 days of enrollment (skin or surface only skin treatments are acceptable) ● Known active uncontrolled bacterial or fungal infections of the lung. ● Active cytotoxic chemotherapy ● Pregnancy ● Breast Feeding
Study Regimen	Patients will be registered on trial, baseline blood work and thoracic CT imaging obtained (if not already available). Within 2 days of enrollment, patients will be transported to an OSU Department of Radiation Oncology linear accelerator (LINAC) and aligned on the treatment table (with cameras trained on ICU monitoring equipment). After CBCT and digital AP and PA images are obtained at the LINAC, doses are calculated. Finally, 80 cGy is delivered from AP and PA open (non-blocked) radiation fields encompassing the bilateral whole lungs. A second, <i>OPTIONAL</i> single-visit treatment, 3-10 days after the first, may be administered for patients who have no toxicity greater than grade 2 definitely attributable to the first treatment, but who have not met criteria for extubation.
Duration of Treatment	This is a single-visit treatment, the duration of which is estimated to be less than 30 minutes (5-10 minutes transfer and positioning, 5 minutes AP/PA imaging, 2 minutes CBCT, 5 minutes calculation, 1 minute treatment delivery, 3 minutes transfer to hospital cart for transport back to ICU). A second <i>OPTIONAL</i> treatment for those eligible would be identical to the first.

Reference Therapy	Standard, supportive ICU care, with or without passive convalescent immune serum administration.
Statistical Methodology	<i>Analysis plan for Primary Objectives:</i> Patients will be stratified into treated/control through stratified randomization with permuted blocks (maximum size 8) to insure balance of demographic and temporal confounders. Stratification variables include age (decade of life), race, sex, and disease type. Kaplan-Meier curves with 95% confidence intervals will be generated. Cox proportional hazard models (including testing of the proportional hazard assumption) will be employed to identify differences in survival between the groups. <i>Analytic plan for Secondary Objectives:</i> Time to event secondary objectives (e.g. time to discharge) analyses will be performed with a competing risk model (leaving the study because of death). Pre/post measurements will be compared using generalized linear mixed models with a random effect for patients. Comparison between treatment groups for other secondary objectives (size of GGO/opacification, for example) will be accomplished with generalized linear models.

SCHEMA**A PHASE II STUDY OF THE USE OF ULTRA LOW-DOSE BILATERAL WHOLE LUNG RADIATION THERAPY IN THE TREATMENT OF CRITICALLY ILL PATIENTS WITH COVID-19 RESPIRATORY COMPROMISE**

Figure 1.



2. BACKGROUND & RATIONALE

2.1 The COVID-19 Pandemic

The city of Wuhan in Hubei province, People's Republic of China experienced the emergence of an outbreak of the coronavirus disease 2019 (COVID-19), caused by what is now known as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 virus spread quickly through China and other countries in late 2019 through early 2020 (1). On January 30, 2020 The World Health Organization (WHO) declared a Public Health Emergency of International Concern and by March 11, 2020 COVID-19 was declared a full pandemic. As of April 10, 2020, all countries have reported cases of COVID-19 (2).

Zhou et al. describes the clinical spectrum of SARS-CoV-2 infection to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness, and severe viral pneumonia with respiratory failure and even death, with many patients being hospitalized with pneumonia in Wuhan. COVID-19 has spread worldwide and has become a pandemic like nothing experienced in modern day history. Although the disease is relatively new there is some early data on COVID-19. Fatal cases of COVID-19 are characterized by acute respiratory distress syndrome (ARDS), sepsis, pneumonia and respiratory failure (2). The high transmission rate of the virus and the corresponding rapid escalation in the number of infections has resulted in unprecedented strains on healthcare systems worldwide, particularly as healthcare workers struggle to treat COVID-19 pneumonia (1).

A recent article by Kirkby and Mackenzie draws attention to the potential for using low doses (< 100 cGy) of low linear energy transfer (LET) radiation to treat viral pneumonia as a possible therapy for COVID-19 patients (3). X-ray therapy was standard treatment for patients with pneumonia in the early 20th century. A 2013 review by Calabrese EJ et al nicely summarized the historical use of x-rays therapy to resolve pneumonia caused from several sources including viruses (4-6). These studies used doses ranging from 20 to a few hundred Roentgen, which today would be translated to a 10- < 100 cGy, and reported a significant reduction in pneumonia related mortality, from 30 percent to 10 percent on average. After treatment, some patients experienced relief from symptoms within just a few hours (5). These significant effects were also observed and studied in animal models. Mouse studies showed a 50% reduction in the acute phase of pneumonia after ultra-low dose whole lung radiotherapy (7).

From radiobiology standpoint, it has been observed that low-dose radiotherapy with single fraction below 1.0 Gy exerted anti-inflammatory properties (8), which can be explained by the decreased levels of pro-inflammatory cytokines including IL-1b, or inhibiting leukocyte recruitment (9, 10). Therefore, an ULD-WLRT treatment (< 100 cGy) to the whole lungs of a patient with COVID-19 pneumonia could effectively reduce the inflammation, relieve the severe symptoms, and alter clinical outcomes.

In light of the current COVID-19 pandemic, we are in desperate need for an effective treatment. Ultra-low dose radiation therapy has historically been used to successfully treat viral pneumonia, and as ionizing radiation does not discriminate among viruses, could serve as a readily available and quick treatment for COVID-19-based lung infections.

2.2 COVID-19 Imaging Manifestations

Given the sometimes severe pulmonary manifestations, many groups have documented imaging findings in COVID-19 patients throughout the disease course -- from onset to recovery -- in both chest computed tomography (CT) and x-Ray. In general, chest x-ray is a poor diagnostic tool and is normal or only mildly abnormal in up to 89% of COVID-19 positive patients (11). The 3-dimensional visualization afforded by computed tomography (CT) imaging of the chest provides a much more nuanced picture of the effects of COVID-19 at any given time point and lends itself well to monitoring disease over time.

Several chest CT findings and patterns have been found to be more typical of COVID-19 infection (see Figure 2). The most common finding in patients with known COVID-19 are simple ground-glass opacities (GGO's), which are present in up to 96% of patients hospitalized for COVID-19 pneumonia (12, 13). GGOs may be present with separate areas of consolidation in up to 55% of COVID-19 pneumonias. Up to 70% of these patients' chest CT's also revealed readily discernible crazy-paving pattern, appearing as a GGO within which is intra- and inter-septal thickening, giving the appearance of stone paving.

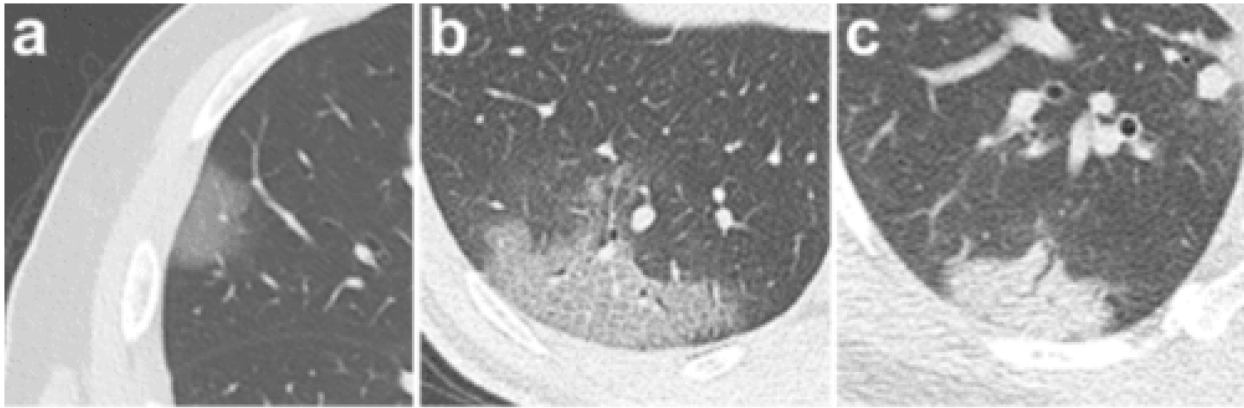


Figure 2: Chest CT findings visualized on lung windows that are typical of COVID-19. (a) Ground-glass opacity; (b) crazy-paving pattern, which is ground glass opacity with attendant interlobular and intralobular septal thickening revealing a pattern reminiscent of random stone paving; (c) consolidation. Adapted from Pan, et al, Radiology, 2020.

Within lungs, involvement is bilateral in 79% of patients, peripheral in 56% of patients. Right lower lobes tend to be involved first, but any unilateral involvement often progresses to bilateral involvement in the majority of patients, with GGOs progressing to consolidation and mixed consolidation/GGO patterns (14). A semi-quantitative method for scoring these findings on chest CT involves visually scoring each of the 5 lung lobes on a 0-5 scale (0, no involvement to 5, >75% involvement). In evaluating such scores over time, this CT score increases over the first 10 days from onset of symptoms, then generally decreases. Lower lobes are reported in multiple studies to show earlier and higher level of involvement and remained involved longer than the upper (or right middle) lobes (13).

2.3 Immune mechanisms of viral pneumonia

Lungs as the vital air-exchanging organ are constantly exposed to environmental insults including toxic molecules, bacteria, and viruses. Our bodies recognize these insults through inflammatory responses of several immune cells such as dendritic cells, macrophages, neutrophils, and lymphocytes. Normally, these cells can orchestrate the progression of inflammation; however, the excessive inflammation can lead to severe pneumonia and acute respiratory distress syndrome. The human immune systems typically fall under two categories--innate and adaptive immunity. Innate immunity refers to nonspecific defense mechanisms, which rapidly respond to an antigen's appearance in the body. Adaptive immunity refers to antigen-specific immune response.

The immune system is activated by sensing the viral component such as uncapped viral RNA, cellular stress and metabolic changes (15). Recognition of viral infection is initiated primarily by germline-encoded pattern recognition receptors (PRRs) (16). Until now, five types of PRRs have been discovered including C-type lectin receptors (CLRs), Toll-like receptors (TLRs), intracellular retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide oligomerization and binding domain (NOD)-like receptors (NLRs), and the Pyrin-HIN domain (PYHIN) receptors (17, 18).

The roles of TLRs and RLRs in virus invasion have been intensively studied. One of the critical roles of the RLRs activations by virus infection is the induction of Type I IFNs, which play an important role in antiviral activities. On the other hand, activations of TLRs modulate the transcription factor nuclear factor-kappaB (NF-kappaB), which induce the transcriptional expression of many inflammatory cytokine genes (19). Further, IFNs also induce the adaptive immune response through specific cell types including natural killer (NK) cells and dendritic cells (DCs) (20). For example, IFN γ production plays a pivotal role in Th1-type immune response, activating CD8⁺ cytotoxic T cells and NK cells to clear in viral infection and promptly responding to the second infection through the renewal of CD8⁺ Memory T Cells (21).

The activation of immune systems causes the productions of antiviral IFNs, pro-inflammatory cytokines, and chemokines including TNF- α , IL-1 β , IL-6, IL-8, and IL-12, participating in the acute inflammatory response during the viral infection and leading to the pneumonia symptom. Patients infected with COVID-19, SARS-CoV or MERS-CoV have shown unusually high mortality and pathogenicity. The hyperinnate inflammatory response (so called cytokine storm) in SARS-CoV is associated with the upregulation of IFN- γ , IL-1, IL-6, IL-12, IL-8, MCP-1 and IP-10 in serum, indicating the activation of Th1 cell-mediated immunity (22). Similarly, MERS-CoV infection induced a strong pro-inflammatory response by productions of IFN- γ , TNF- α , IL-15 and IL-17 (23). The cytokine storm was directly linked to disease severity and ICU admission.

However, COVID-19 infection can also produce Th2 cytokines (e.g., IL4 and IL10) and suppress inflammation response at the early stage, which can partially explain the incubation period of COVID-19 infection. It is necessary to characterize the Th1 and Th2 immune responses to understand pathogenesis in our study.

2.4 Available Treatments for COVID-19

There are currently no proven effective treatments beyond supportive care for COVID-19 at any stage of illness. Supportive care essentially centers around provision of antibiotics to prevent bacterial superinfection, oxygen therapy or mechanical ventilation to support oxygenation and ventilation, and vasopressors to support perfusion. On the heels of promising *in vitro* results, there is hope that antiviral drugs (e.g., lopinavir and remdesivir) may have activity against SARS-CoV2 infection in humans. However, given the recency of COVID-19 emergence, there has not

been adequate time to obtain sufficient clinical trial evidence in humans (24, 25). There is concern over using corticosteroids to reduce pulmonary inflammation in COVID-19 pneumonia given the risk of permissiveness to viral growth in the setting of immunosuppression (26, 27). Convalescent plasma therapy may be useful in the absence of specific antiviral therapies. This involves the administration of serum from individuals recovered from COVID-19 to patients with active disease. Use of this passive immunity approach in ten severely-ill patients was associated with decreased viremia and C-reactive protein as well as varying degrees of resolution of lung lesions in one study (28). This approach depends, however, on the availability of serum from convalescing patients. In the absence of a large banked supply, this would not be available during the early stages of any pandemic. This protocol does allow prior, concurrent, or post-protocol treatment administration of convalescent serum in light of the promising early results of this approach and the critical need for any effective therapy.

2.5 Effect of Ultra Low-Dose Radiation on Inflammation

The radiobiological effects of low-dose radiation greatly differ from that of the high-dose radiation that is used to treat cancer malignancies. While the mechanisms underlying low-radiation are still being investigated, several research groups have shed light on some of the biological processes affected by low-dose radiation. In general, low-dose radiation (< 1 Gy) exerts an anti-inflammatory effect, whereas high-dose radiation (> 1 Gy) promotes a proinflammatory response. Specifically, when low-dose radiation is administered, it inhibits the expression of inducible nitric oxide synthase (iNOS) which inhibits endothelial cell-leukocyte interactions and reduces vasodilatation. Additional anti-inflammatory cytokines are produced (IL-10, transforming growth factor-1 (TGF- β 1), thereby increasing apoptosis and diminishing NO production. Low-dose radiation also decreases expression of adhesion molecules (P-, L-, E-selectins, intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM)), and decreases secretion of CCL20 (C-C motif chemokine ligand 20) and neutrophils, which correlates to a lower adhesion to endothelial cells (29). Further, *in vivo* studies have identified high level expression of TGF- β 1 24 hours after low-dose radiation as well as decreased levels of pro-inflammatory cytokines (TNF- α , IL-1, iNOS) and concomitant increase of heme oxygenase-1 (HO-1) and heat shock protein 70 (HSP70) (Table 1) (29). A proposed mechanism by which low-dose radiation mitigates inflammation and facilitates healing is through the polarization of macrophages to an anti-inflammatory or M2 phenotype. Data suggests that macrophages are polarized more toward M2 and the anti-inflammatory state when doses descend below 1.0 Gy and, conversely, more toward M1 and the pro-inflammatory state when doses ascend above 1.0 Gy. The following events have been identified as being involved in mediating an M2 polarization response: decreases in NO levels and in iNOS, decreases in reactive oxygen species (ROS), increases in HO-1, suppression of TNF- α , increases in TGF- β 1, and the activation of several transcription factors, including nuclear factor- κ B (NF- κ B), and activating factor protein 1 (Figure 3) (30). As highlighted in Section 2.3 a large inflammatory response facilitates pneumonia and this is particularly true for COVID-19. There is substantial overlap

between the pro-inflammatory cytokines and chemokines upregulated in COVID-19 patients (aka cytokine storm) and those that are suppressed by low-dose radiation making this treatment particularly personalized to treating this virus (Table 2).

Table 1: Differences in the effect of low and high-dose radiation (adapted from Torres Royo et al. 2020)(29). *While most studies define high dose radiation as > 1 Gy, others use > 2 Gy.

Low Irradiation Doses (< 1 Gy)	High Irradiation Doses (> 2 Gy)*
Anti-inflammatory properties	Pro-inflammatory cytokine production
IL-10, TGF-1, IL-4, NF-B, HO-1, HSP70, L-Selectin, E-Selectin, ICAM, VCAM, CCL20, AP-1, ROS, iNOS Apoptosis	IL-1, TNF- α , IL-6, IL-8, IL-12, ROS, iNOS

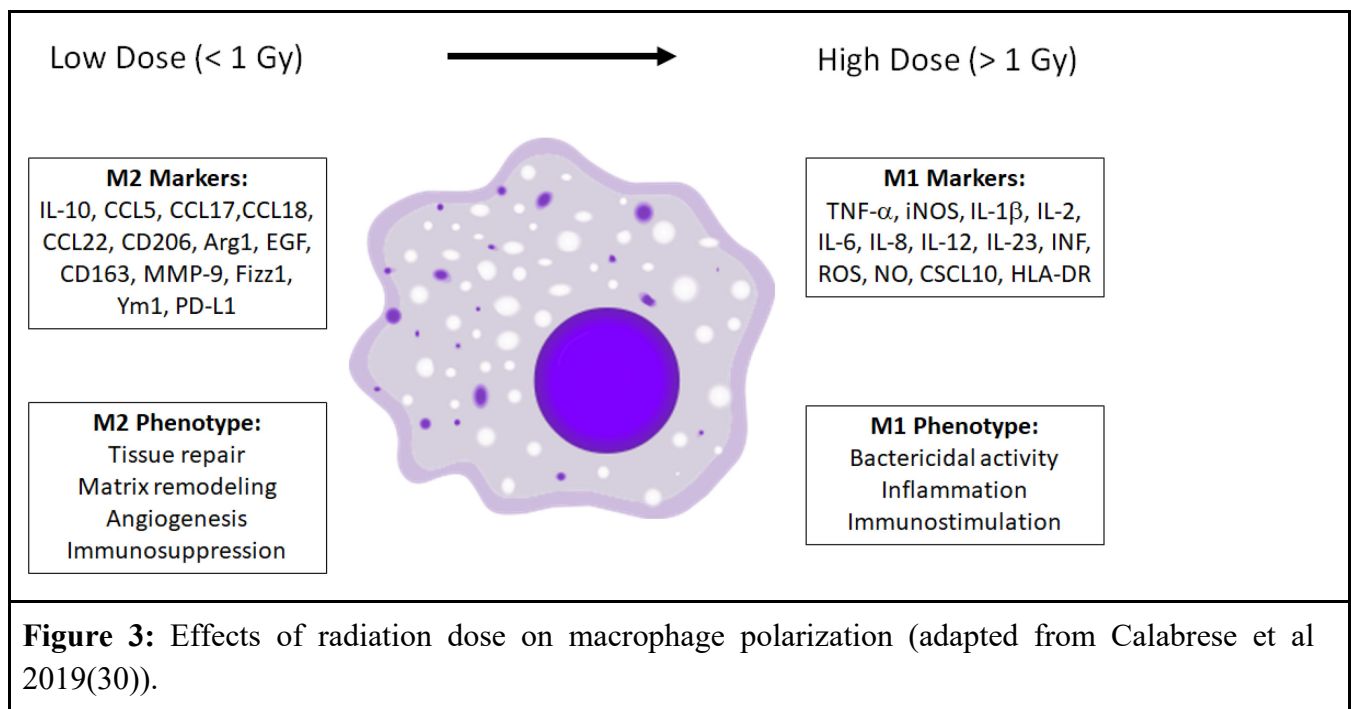


Table 2. Pro-inflammatory cytokines and chemokines upregulated in COVID-9 and suppressed by LD-RT

<u>Pro-inflammatory Cytokines/Chemokines</u>		
Up in COVID-19	Down by LD-RT	Reference
TNF- α	TNF- α	Arenas M. et al 2012;Ye Q et al. 2020
IL-1 β	IL-1 β	Arenas M. et al 2012;Ye Q et al. 2020
IL-2/R	IL-2	Calabrese et al 2019;Ye Q et al. 2020
IL-6	IL-6	Calabrese et al 2019;Ye Q et al. 2020
CXCL10	CXCL10	Calabrese et al 2019;Ye Q et al. 2020
INF- γ	INF- γ	Ye Q et al. 2020;Pandey R et al. 2009
IL-7		Ye Q et al. 2020
CXCL1		Ye Q et al. 2020
CXCL5		Ye Q et al. 2020
	IL-8	Calabrese et al 2019;Ye Q et al. 2020
	IL-12	Calabrese et al 2019;Ye Q et al. 2020
	CCL20	Rodel F. et al 2008;Ye Q et al. 2020

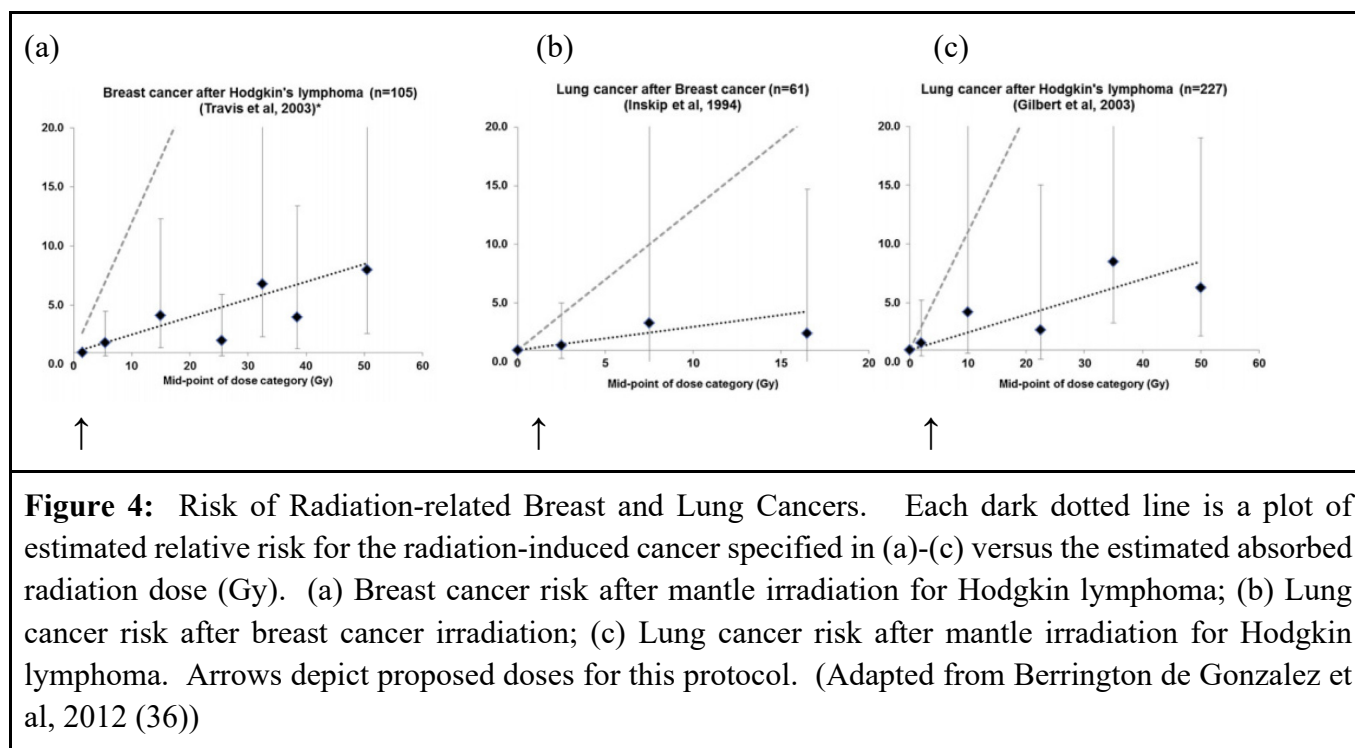
2.6 Whole lung irradiation and potential toxicities

Whole lung irradiation has been used as a standard-of-care regimen in childhood tumors for over 50 years, and continues to be used in standardly in the treatment of Ewing's sarcoma and Wilms tumor (31). The doses typically used in these treatments are nearly 19 times on average the dose of 80 cGy proposed in the population included in this protocol. Doses in children over the decades have ranged from 1,000-2,100 cGy (32). The current Children's' Oncology Group (COG) standard of care for whole lung irradiation in children with Ewing Sarcoma is 1500 cGy in 10 fractions daily. To date, there are no reports of death attributable to whole lung irradiation to these doses, and toxicities are minimal (33). In adults with Ewing sarcoma, similar treatment to 1,200-1,500 cGy has also been shown to be tolerable with low rates of acute toxicity. Time-limited acute toxicities occur in fewer than 50% overall, but are typically grade 1 nausea, likely due to some gastric mucosa being irradiated. No patients in a recent analysis of 26 adult patients were found to experience pneumonitis or cardiac toxicity (34). Structures potentially contacted by radiation as proposed in this protocol are lung, heart, spinal cord, shoulder joints, chest, skin, breast, partial liver, partial stomach, and potentially a fraction of kidneys. Recent publications documenting "Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC)" have shown the minimum tolerance of each of these structures to radiation before development of dysfunction to be far greater than the 80 cGy proposed in this trial (35). Therefore, acute toxicities to these structures are extremely unlikely. Anecdotal experience suggests that an episode of nausea may occur after a single low-dose treatment contacting stomach mucosa, but this is limited to a single episode and easily prevented.

Risk of radiation-induced malignancy is always a primary concern in children and in those receiving any radiation therapy for benign conditions. Before radiation is undertaken, the risk of morbidity due to untreated illness must be balanced against the risk of radiation-induced malignancy, and treatment should only be delivered for those with the most favorable therapeutic

ratio. Radiation-related malignancies take years to decades to develop; therefore, children and young adults are most at risk. Much has been studied regarding radiation risk in typical therapeutic dose (>5000 cGy total) *focused* radiation, and very low dose *whole body radiation*, such as that received by survivors of the Hiroshima and Nagasaki atomic bombings (36). For example, in patients who received less than 700 cGy of therapeutic radiation, the excess relative risk per Gray unit of radiation was 0.1 or less (36). There is no clear data regarding risk of radiation-related malignancy in focused low-dose treatment in doses as low as that proposed herein, but fitted relative risks for lung cancer and breast cancer are shown in **Figure 4**.

In summary, the high likelihood of risk of death (>30-90%) for patients to be included on this protocol outweighs the low risk of acute toxicity (mainly easily controlled rare nausea) and risk of late term radiation-induced malignancy.



2.7 Historical Use of Low-Dose Radiation for Infectious Pneumonia

Pneumonia has long been a serious risk and burden on our health care system. Treatment for pneumonia has changed over the years to improve outcomes. For example, in the early 1900's, leaders at the Rockefeller Institute treated with equine serum therapy, but this proved to be expensive and not useful to patients with allergic reactions. Thus, the use of serum therapy would be eliminated and replaced with sulfonamides in 1939. During the same time, a potential alternative to this therapeutic monopoly was emerging in the form of radiotherapy. Radiotherapy

had been broadly accepted by the radiological community starting in the second decade of the 20th century, with notable successes in the treatment of a wide range of inflammatory and infectious diseases (37), such as gas gangrene (38), carbuncles (39), sinusitis (40), arthritis (41, 42), and inner ear infections (43). Based on clinical successes in these and other areas (44, 45), it was not unexpected to see exploratory attempts to treat pneumonia patients with X-rays using similar protocols.

The initial report using X-rays to treat patients with pneumonia was in 1905 by Musser and Edsall (46) at the University of Pennsylvania. They believed that X-rays may be useful in the treatment of unresolved pneumonia, that is, when the disease resolution process fails to proceed to a cure, with lung exudate material showing consolidation, enhanced bacterial infestation, and risk of prolonged serious illness. Quimby and Quimby later stated “no pathological process in the body responds quicker to an X-ray exposure than the non-resolved following pneumonia” (47). However, it was not until 1924 that Heidenhain and Fried (48, 49) stimulated research on the effects of X-rays on the clinical course of pneumonia. They reported on 243 cases of acute and subacute pyogenic infections of numerous types treated with X-rays. Heidenhain and Fried (48, 49) showed that not only did X-ray treatment block/reduce superficial inflammations such as those seen with carbuncles and furuncles, but inflammation of all types, regardless of location in the body and whatever the cause, was also reduced. Another study completed by Powell (50) evaluated the effects of 231 patients, about 70 percent of whom had lobar pneumonia and the remainder with bronchopneumonia. The mortality rates were about 5 percent and 13 percent, respectively, rates that were considerably improved over his past mortality experience without the X-ray treatment. Krost subsequently reported that X-ray treatment accelerated recovery of unresolved pneumonia in children (51). Unfortunately, the treatment was not widely adopted within the public health system, and despite these studies, the therapeutic utility of X-rays for the treatment of pneumonias would quickly disappear upon the arrival of other drugs -- including penicillin -- as treatment effective treatment options (30).

While there are substantial and consistent findings supporting a therapeutic role for X-rays in the treatment of pneumonia, the question remains as to how to reactivate a well-established, yet 65-year-old hypothesis, with contemporary research questions, methods, and technologies that still may hold public health potential. This concept may not be as unusual as it seems (30). Due to recent events of a pandemic causing worldwide deaths from pneumonia, this may just be the time to investigate low dose radiation oncology can help us treat our patients dying from pneumonia.

2.8 Technical Review of Treatment of Pneumonia using Radiation Therapy

Radiotherapy has evolved tremendously since the use of X-rays in the 1920's for treatment of pneumonia. The x-ray generation technology used in early treatments included X-ray tubes operated in the kilovolt energies. Today, radiotherapy is delivered using Linear Accelerators (LINAC) that generate megavoltage x-rays that allow for skin sparing.

Skin erythema dose (SED) was originally used for determining the amount of radiation dose to deliver to a patient based on the toxicity of skin reaction. In order to quantify the delivered radiation, the roentgen was adopted by the International Commission of Radiation Units and Measurements (ICRU) in 1928 to help provide standardization for treatment doses. The roentgen can be converted to absorbed dose to a medium which is energy dependent. Assuming the use of 100 keV x-rays, we can use a conversion factor, or f-factor, of 0.956 (rad/R) for dose to water (52).

While there is some documentation on the technical parameters of patient treatment, the x-ray tubes at the time had outputs that were not standardized even with the same nominal settings of voltage and current for treatment (53). The treatments recorded used voltages of 100-150 kV with the use of beam filtration using materials such as aluminum or copper to provide beam hardening including a variety of treatment times from 5-20 minutes (5, 50, 54). Prescriptions were cited as exposures ranging from 35-350 R which translate approximately to 30-300 cGy in water or muscle at 100 keV with estimates of converted dose in Table 3 (5, 50, 51, 54). Oppenheimer states that doses corresponding to $\frac{1}{3}$ to $\frac{1}{5}$ the dose used to treat inflammations were used for the treatment of non-neoplastic lesions including bronchopneumonia (5). In some cases the patient was treated more than once if symptoms persisted (5).

Table 3: Estimates of historical doses prescribed to pneumonia patients at two x-ray tube energies (100 and 150 keV).

Date	Author	Published Dose (r)	For x-ray tube at 100 keV			For x-ray tube at 150 keV			Context
			Dose to water (cGy)	Dose to bone (cGy)	Dose to Muscle (cGy)	Dose to water (cGy)	Dose to bone (cGy)	Dose to Muscle (cGy)	
1924	Fried & Heidenhain	35	33	51	33	34	37	34	lower bound of prescribed dose for pediatrics
1924	Fried & Heidenhain	45	43	65	43	44	48	43	upper bound of prescribed dose for pediatrics
1924	Fried & Heidenhain	50	48	73	48	48	53	48	lower bound of prescribed dose for adults for first 2-3 days
1924	Fried & Heidenhain	60	57	87	57	58	64	58	upper bound of prescribed dose for adults for first 2-3 days
1924	Fried & Heidenhain	70	67	102	67	68	74	67	lower bound of prescribed dose for adults after a week
1924	Fried & Heidenhain	90	86	131	86	87	95	86	upper bound of prescribed dose for adults after a week
1937	McIntire and Smith	70	67	102	67	68	74	67	lower bound of prescribed dose compared to carbuncle treatment
1937	McIntire and Smith	125	120	181	120	121	133	120	upper bound of prescribed dose compared to carbuncle treatment
1938	Powell	250	239	363	239	242	265	240	lower bound of prescribed dose
1938	Powell	350	335	508	335	338	371	336	upper bound of prescribed dose
1943	Correll & Cowan	112	107	162	107	108	119	108	military hospital
1943	Oppenheimer	22	21	32	21	21	23	21	lower bound of prescribed dose for the Journal of Pediatrics
1943	Oppenheimer	180	172	261	172	174	191	173	upper bound of prescribed dose for the Journal of Pediatrics
1943	Oppenheimer	50	48	73	48	48	53	48	average dose used after ten years of treatment

X-ray tubes providing voltages up to 300 kVp were the source of external beam radiation treatments until about 1950 when higher energy treatment machines were produced (Khan, 38). Advantages of modern radiation therapy include the advent of treatment with linear accelerators enabling treatment using energies in the megavoltage range. Unlike the x-ray tubes of the early 20th century, LINACs can provide reliable beam production and uniformity of the treatment field. In addition, beam characteristics are well controlled and characterized to provide standardized treatments. The higher energies provide a buildup region of dose at the surface reducing skin toxicity.

With state-of-the-art technology for dose measurement and advanced algorithms for treatment planning, radiation therapy has advanced considerably since the original studies. Present-day

LINACs also have on-board imaging including planar x-ray and volumetric cone-beam CT (CBCT) providing anatomical and positioning information at the time of treatment.

2.9 Summary

In summary, the most serious risk accompanying infection by SARS-CoV-2 is viral pneumonia. For those whose symptoms progress to the extent that mechanical ventilation is required, the prognosis is grim with a high rate of mortality. Without any currently proven therapies beyond supportive care for such patients, we propose the use of a low-risk, historically successful therapy. Ultra low-dose radiation therapy has the advantage over corticosteroids that it has the potential to both reduce inflammation in the lung caused by viral infection, as well as to damage the viral nucleic acids themselves. Corticosteroids used in SARS-CoV-2 are currently employed at very high doses complicated by severe hyperglycemia necessitating high-dose insulin infusions; in addition, corticosteroids have numerous adverse effects including risk of secondary bacterial infections. The use of 80 cGy *total dose* to the bilateral whole lungs represents just over 50% of the 150-cGy dose of whole lung irradiation given to Ewing sarcoma patients on a *daily* basis -- a dose well tolerated daily by these patients. Moreover, this total dose represents only 5% of the *total dose* administered per Children's Oncology Group Standard. With this treatment, there has been shown to be a low risk of acute toxicity. From broader analyses, there also appears to be a low risk of radiation related malignancy.

In the high-mortality risk group included in this proposal, who have no other clear therapeutic option to date, we will examine the efficacy and safety of a historically successful treatment using modern technology. We propose delivery of ULD-WLRT under modern conditions with a rapid delivery technique. The rapid delivery technique will minimize risk to critically-ill patients by minimizing the time that they are away from the intensive care environment.

More broadly, we propose this protocol as a modern revival of a useful historical paradigm to evaluate future utility of low-dose radiation for other viral or pathogenic pneumonias. COVID-19 is the first deadly pandemic illness in many decades. But we must not underestimate the broader challenge; widespread regional outbreaks of viral pneumonias without known therapeutics occur relatively frequently in our world. SARS (Sudden acute respiratory syndrome), a relative of COVID-19, occurred primarily in China 20 years ago with a striking mortality rate of approximately 10%. MERS (Middle East Respiratory Syndrome) -- another coronavirus -- killed nearly 35% of people who were infected during its outbreak about 10 years after the SARS outbreak [Mahase, BMJ 2020]. This protocol evaluates not only a potential therapeutic option for those suffering from COVID-19 now, but also evaluates -- in the broader sense -- a potential therapeutic option for those who will inevitably be exposed to future epidemic pulmonary viral illnesses that outpace antiviral development.

3. OBJECTIVES

3.1 Hypothesis

In this proposal we hypothesize that ULD-WLRT by conventional linear accelerators will result in decreased mortality in patients who are critically ill requiring ventilatory support for COVID-19 pulmonary disease (compared to historical controls).

3.2 Primary endpoint

- To evaluate 30-day mortality rate after ULD-WLRT.

3.3 Secondary endpoints

- To evaluate overall survival after ULD-WLRT
- To evaluate total and post-ULD-WLRT ICU length of stay
- To evaluate total and post-ULD-WLRT length of hospital stay
- To evaluate total and post-ULD-WLRT requirement [10] for mechanical ventilation (ventilator free days)
- To evaluate total and post-ULD-WLRT requirement [11] for supplemental oxygen therapy (days)
- To evaluate oxygenation index/ oxygen saturation index for 14 days post treatment or [12] until extubated
- To quantitate post-ULD-WLRT differences at baseline and between Day 7, 14, and 28 CT chest finding (number of involved lung segments, size of GGO/opacification, lung opacification percentage)
- To evaluate SARS-CoV2 viral titers post-ULD-WLRT at baseline and Day 7, 14, and 28.
- To establish feasibility, safety, and tolerability of this regimen.
- To establish KPS changes post-ULD-WLRT at baseline and Day 7, 14, and 28.

3.4 Exploratory Endpoints

- To evaluate molecular biomarker levels in COVID-19 patients upfront as well as after treatment with ULD-WLRT (e.g. levels of inflammatory and DNA damage markers).
- To determine baseline molecular biomarkers that predict response to ULD-WLRT in COVID-19 patients.
- To determine molecular biomarkers that change over the treatment course, correlating with disease status and resolution.
- To retrospectively evaluate rapidly planned treatment using volumetric treatment plan evaluation based on CBCT
- To collect raw diagnostic and CBCT imaging data for machine-learning assessment of longitudinal progression in patients with COVID-19
- To collect raw imaging data for further evaluation of the potential for identification of COVID-19 in diagnostic scans

4. PATIENT ELIGIBILITY

In general, eligibility and exclusion criteria for this trial are designed to capture patients with a confirmed laboratory diagnosis of COVID-19 and CT or radiographic findings consistent with COVID-19 pneumonia, who are critically ill with a requirement for mechanical ventilation, but who do not have major contraindications to thoracic radiation therapy or suspected other major causes of ventilatory dysfunction. Contraindications to radiation therapy in this protocol are more permissive than other circumstances given the ultra-low dose to be used.

4.1 Inclusion Criteria

- Age ≥ 18 years
- Patients with a laboratory diagnosis of COVID-19 within 14 days of enrollment.
- Patients with CT findings or radiographic findings typical of COVID-19 pneumonia within 5 days of enrollment
- Patients receiving ICU-based mechanical ventilation
- Patients with life expectancy ≥ 24 hours, as judged by investigator
- Hypoxemia defined as a Pa/FiO₂ ratio < 300 or SpO₂/FiO₂ < 315
- Signed informed consent by patient or his or her legal/authorized representatives
- All standard of care COVID treatment will be allowed as deemed safe by the investigator

4.2 Exclusion Criteria:

- Moribund with survival expected < 24 hours, as judged by investigator and treating team, irrespective of treatment
- Expected survival < 30 days, as judged by investigator and treating team, due to chronic illness present prior to COVID infection
- Patient or legal representative not committed to full disease specific therapy i.e. comfort care (DNRCCA is allowed)
- Treatment with immune suppressing medications that is determined unsafe for the patient to receive radiation treatment for this trial
- Presumed COVID-associated illness greater than 14-days
- Inpatient admission greater than 14-days
- Patients deemed unsafe for travel for radiation therapy
- Chronic hypoxemia requiring supplemental oxygen at baseline
- Documented active connective tissue disease (scleroderma) or idiopathic pulmonary fibrosis
- History of prior radiation therapy resulting in $\geq$ grade 2 radiation pneumonitis within 365 days of enrollment.
- Active or history of prior radiation to the thorax completed within 180 days of enrollment (skin or surface only skin treatments are acceptable).

- Known active uncontrolled bacterial or fungal infections of the lung.
- Active cytotoxic chemotherapy
- Females who are pregnant or have a positive pregnancy test
- Breast feeding
- All standard of care treatments for COVID including convalescent immune plasma therapy, clinical trials, or any standard therapy is not an exclusion criterion, but will be noted.

5. STUDY PROCEDURES

5.1 Enrollment, patient selection, and informed consent

Appropriate patients for screening will be identified by the co-investigators. Pulmonary and Critical Care co-investigators will have first knowledge of appropriate patients and will be first contacts for patients.

Due to the pandemic, the consent discussion may be completed by phone or video conference to limit direct contact with patient or legally authorized representative (LAR). When the consent discussion is completed via telephone or video conference, a signed informed consent form must be obtained prior to any study procedures being completed. Prior to the phone/video visit the consent may be provided via an email, fax, or mail. After the discussion by phone/video with the designated study team member and the subject agrees to participation, the consent form will be signed. A copy/photo of the signed consent form will be sent to the team member via email, fax or mail. The study team member will sign the consent form upon receipt and will send a copy of the fully signed consent to the participant or LAR. The consent discussion will be documented and no research study procedures will occur until fully executed consent form is signed by both parties. A reasonable attempt to obtain the original signature from the consent document will be employed.

5.2 Screening and Baseline Studies

At the time of screening:

Investigators will determine appropriateness of patient based on age ≥ 18 years; life expectancy ≥ 24 hours, as judged by investigator; the presence of Hypoxemia defined as a Pa/FiO₂ ratio < 300 or SpO₂/FiO₂ < 315 .

In addition to evaluating other inclusion and exclusion criteria (APPENDIX II), investigators will also make assessments in concert with critical care physicians to ensure that the patient is not moribund with survival expected < 24 hours; does not have expected survival < 30 days due to chronic illness present prior to COVID-19 infection, that the patient or legal representative is not committed to full disease specific therapy (i.e., comfort care; DNRCCA is allowed); and that the patient is not unsafe for travel for radiation therapy.

Patient will not be receiving active cytotoxic chemotherapy

Female patients are not pregnant and have a negative pregnancy test, and are not breastfeeding

Within 5 days of enrollment:

Patients will have had a CT of the thorax with or without contrast or other radiographic imaging with findings typical of COVID-19 (bilateral GGOs, consolidations, crazy paving). This baseline study will be analyzed as described in section 6.3.

Within 14 days of enrollment,

Patients will have laboratory confirmed COVID-19 infection.

Patient has not been admitted greater than 14 days or had presumed COVID-associated illness for > 14 days.

Patient will have serum D-Dimer and fibrinogen.

Within 30 days of enrollment:

Patient has not had treatment with immune suppressing medications, with the exception of steroids for ARDS or septic shock)

Within 180 days of enrollment:

Patients have not had radiation therapy to the thorax completed within 180 days of enrollment (with the exception of skin or surface-only treatments that avoid lung parenchyma, including typical breast cancer treatments).

Within 365 days of enrollment:

Patient has not had radiation therapy that resulted in $\geq$ grade 2 radiation pneumonitis.

5.3 Ultra Low-Dose Whole Lung Radiation Therapy

- **Patient positioning and simulation**

Patients will be transported directly to the LINAC room and mechanical ventilation established, with visibility of monitoring devices. Patients unable to be safely moved from a hospital bed to the treatment table may use an air transport system from the radiation therapy department to facilitate the transition to the treatment machine without lifting the patient. These critical patients can be positioned on an air transport table in the intensive care unit such that all necessary equipment can be set up before traveling for treatment to minimize changes for the patient when transitioning to the treatment table. No CT simulation is used for this treatment. Simulation will be performed using typical “clinical

setup” procedures on the LINAC on which treatment will be delivered, using on-board imaging capabilities to design treatment ports. Patients will be positioned with arms at sides, preferably with sufficient distance to keep the arms out of the primary beam. Arms can be positioned akimbo with hand pegs or positioning devices can be used to create separation between the arms and the body if possible. Patients should be optimally positioned with a treatment table minimizing skin toxicity. Additional immobilization devices such as head holders are recommended for patient positioning and comfort.

- **Radiation Targets**

In this protocol, no standard radiation targets (i.e., GTV, CTV, PTV) are used, and no contouring is required. The borders are set on the treatment machine just prior to treatment

In bilateral whole-lung radiation therapy in this protocol, both lungs are irradiated with open fields that include both the entire lung volume and the pleural recesses. The inferior extent of the anterior and posterior costo-diaphragmatic recesses of the pleural cavity may be determined by a lateral radiograph of the chest with the patient’s arms at sides. Borders with open fields will be set by the radiation oncologist. The superior and inferior borders will be set to include all lung parenchyma with approximately 2-cm margin; typical borders are C6/7 interspace to T12/L1 interspace. Lateral borders will be set to encompass the entire lungs with at approximately 2 cm margin. Multileaf collimator (MLC) leaves will not be used, nor will any method of blocking other than LINAC jaws.

- **Bilateral Whole-Lung Irradiation**

Bilateral lung irradiation will be performed using an AP-PA technique following institutional standard techniques developed. The patient will receive 1 fraction of radiation therapy. Patients must be treated on an image-guided (IGRT)-enabled machine. Orthogonal kV/kV projection imaging will be used to verify the location of lungs prior to delivery of the first treatment beam. A secondary shift based on the location of anatomy should be utilized, as indicated by the position of the patient.

- **Radiation treatment planning**

A parallel opposed plan using 2D dose calculations based on separation at the level of the carina. Two open fields consisting of an AP and PA field will be used to encompass the entire lung.

Fields will be defined based on the anterior image at the treatment console. Rectangular fields will be used with superior, inferior, and lateral borders of the treatment fields placed at least 2 cm beyond the definition of the lungs. Separation and depth for the dose calculation will be defined from the CBCT at the level of the carina. The prescription point is at or near the carina on the central axis of the radiation fields.

The use of IMRT is not permitted in this study.

- **Tissue Heterogeneity**

No corrections for attenuation will be made for treatment calculation to facilitate a quick treatment for this critically ill population. However, the tissue heterogeneities will be calculated retrospectively to evaluate the rapidly planned treatment using volumetric treatment plan evaluation based on CBCT.

- **Equipment and Calibration**

Linear accelerators with a minimum photon energy of 6MV are required for photon treatment. 2D radiation therapy with opposed fields for the whole lung will be used.

- **Dose Prescription and Normal tissue Constraints**

Patients will be treated with a single dose of 80 cGy to the bilateral lungs in a manner that is simplified such that it can be designed and delivered quickly in one session to this critically ill population. No specific normal tissue constraints are employed in this protocol.

- **Optional second treatment**

For those who do not experience any CTC-AE toxicity greater than grade 2 that is definitely attributable to the first dose of ULD-WLRT, *AND* who have not met criteria for extubation, a second dose of 80 cGy 3-10 days after the initial dose will be **OPTIONALLY** available.

5.4 Risks and Toxicities associated with treatment regimen

- **Transport from ICU**

Of note, the LINAC will have a ventilator on site dedicated to the support of patients treated on this protocol.

- **Radiation therapy**

Toxicity associated with low-dose radiation therapy is generally absent or mild. There is a reasonable expectation that ULD-WLRT will generate no acute toxicity. This is based on the fact that ULD-WLRT complete course of 80-cGy is significantly lower than a single daily fraction of radiation delivered for malignancies (typically, 150-200 cGy), and acute toxicities are not typically observed at that dose. Acute radiation toxicities with multi-fraction standard regimens generally include fatigue and skin redness. Some individuals may experience mild nausea after a single fraction if a portion of the stomach receives some radiation dose, which is possible in this protocol. The likelihood of this cannot be quantified at this rarely used low dose of radiation therapy. In the event that nausea is experienced, suitable antiemetics in a single, low dose is standardly highly

effective (e.g., ondansetron, prochlorperazine, metoclopramide).

In addition, one aim of the execution of this study is to limit toxicities and morbidity in general by minimizing the time that a critically ill patient is in transport and away from the intensive care unit environment and by using a dose of radiation therapy that is far below standard daily doses when used for malignancy.

6. STUDY MONITORING AND PATIENT ASSESSMENTS

6.1 Details of follow-up Clinical Studies/ Follow-up Schedule

The timing of follow-up visits and assessments for the study outlined in Tables 4 and 5.

Table 4: Follow-up Schedule

Study Visit	Target Day
1 day	24 hrs. post RT
1 week	± 2 days
2 weeks	± 2 days
1 month	± 2 days
6 months	± 5 days
12 months	± 10 days

Table 5: Follow-up Schedule by Clinical Research Coordinator

Study Visit	Target Day
1 day	24 hrs. post RT
1 week	± 2 days
2 weeks	± 2 days
1 month	± 2 days
3 months	± 5 days
6 months	± 5 days
9 months	± 5 days
12 months	± 10 days
18 months	± 10 days

Table 6 summarizes the patient clinical assessment schedule over the course of the study.

6.2 Pretreatment evaluations

The following observations are evaluations for eligibility and appropriateness for low-dose radiation therapy and should be determined < 5 days before initiation of radiation therapy.

- History, physical examination.

- Karnofsky performance score (Appendix IV).
- CBC with differential and platelet count, lymphocyte count, serum creatinine, bilirubin, alkaline phosphatase, ALT, and AST.
- Correlative research blood collection
- Correlative research urine collection
- Baseline levels of molecular biomarkers in biofluids (e.g. inflammatory/DNA damage markers)
- Cytokine and chemokine measurement-IL1B, IL1RA, IL2, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12p70, IL13, IL15, IL17A, CCL11, basic FGF2, GCSF (CSF3), GMCSF (CSF2), IFN γ , IP10 (CXCL10), MCP1 (CCL2), MIP1A (CCL3), MIP1B (CCL4), PDGFB, RANTES (CCL5), TNF α , and VEGFA using Human Cytokine Standard 27-Plex Assays panel on the Bio-Plex 200 system.
- D-dimer, Fibrinogen
- Viral load
- Urine Biomarkers
- Previous CT imaging to treatment if available to estimate field size prior to treatment

6.3 Post-radiation therapy evaluations

The following observations are evaluations for after completion of low-dose whole lung radiation therapy. The schedule of studies is listed in Table 6:

Intermittent assessments:

The following will be assessed or obtained at intervals as described in Table 6:

- History and physical exam
- Karnofsky Performance Status
- Follow-up x-ray or chest CT if clinically indicated. Chest CTs will be analyzed using the method described by Pan et al [Radiology, 2020]. This semi-quantitative method for scoring chest CT findings will be performed by 2 radiologists independently. Each of the 5 lung lobes are screened visually and scored on a 0-5 scale for involvement with typical COVID-19 findings such as GGOs and consolidation. The scoring system is as follows: 0, no involvement; 1, <5% involvement; 2, 25% involvement, 3, 26-49% involvement; 4, 50-75% involvement; and 5, >75% involvement). Characteristics of each lobar score and a total score for all 5 lobes (ranging from 0-5) will be recorded [Pan et al Radiology, 2020].
- Serum chemistries (Na, K, Cl, CO₂), organ function studies (BUN, Creatinine, LFTs [AST, ALT, Alkaline Phosphatase])
- C-reactive protein, as a measure of inflammation.
- Cytokine and chemokine measurement
- IL1B, IL1RA, IL2, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12p70, IL13, IL15, IL17A, CCL11, basic FGF2, GCSF (CSF3), GMCSF (CSF2), IFN γ , IP10 (CXCL10), MCP1 (CCL2), MIP1A (CCL3), MIP1B (CCL4), PDGFB, RANTES (CCL5), TNF α , and VEGFA using Human Cytokine Standard 27-Plex Assays panel on the Bio-Plex 200 system.

- D-dimer, Fibrinogen
- Viral load
- CBC with differential and platelet count, lymphocyte count, serum creatinine, bilirubin, alkaline phosphatase, ALT, and AST.
- Correlative research blood collection
- Correlative research urine collection
- Toxicity Assessment
- Evaluation for OPTIONAL 2nd dose of ULD-WLRT:
 - Patients who met inclusion criteria for an initial dose of ULD-WLRT, *AND* who have not experienced any CTC-AE toxicity greater than grade 2 that is definitely attributable to ULD-WLRT *AND* who have not met criteria for extubation may be offered a second *OPTIONAL* administration of 80 cGy exactly as described in section 5.3 above.

Continuous assessments:

The following will be assessed on an ongoing basis from the time of Baseline through the one-month (28-day) time point:

- Mortality
- ICU length of stay
- Requirement for mechanical ventilation (duration in days)
- Requirement for supplemental oxygen after mechanical ventilation no longer required (duration in days)
- Oxygenation Index, if arterial blood gas available: $[\text{FiO}_2(\%) \times \text{Mean Airway Pressure (MAP; mmHg)} \times 100] / \text{PaO}_2 \text{ (mmHg)}$
- Oxygen Saturation Index: $[\text{FiO}_2(\%) \times \text{Mean Airway Pressure (MAP; mmHg)} \times 100] / \text{SpO}_2 \text{ (\%)}$

6.4 Removal of Patients from Protocol

All patients enrolled in this protocol should be followed for the duration of 12 months. Patients found to have disease relapse should be encouraged to continue on protocol for sample collection and the remainder of protocol time points. Patients wishing to withdraw from this protocol should be asked to sign a consent withdraw form and this should be kept in the research chart.

Table 6: Summary of Patient Clinical and Research Assessments

Study Assessments/ Testing*	Screening	Baseline pre-RT	1 day after RT	1 wk*	2 wk*	1 mo*	3 mo †	6 mo †	9 mo †	12 mo ♦	18 mo ♦
CLINICAL STUDIES											
History, physical	X	X	X	X	X	X		X		X	
KPS	X	X	X	X	X	X	X	X	X	X	X
CT chest or x-ray	X [#]			X	X	X					
CHEM CRP & Clotting↔	X [#]	X	X	X	X	X					
Toxicity (CTC-AE)			X	X	X	X					
Continuous Assessments		● Mortality ● ICU Length of Stay ● Requirement of Mechanical Ventilation ● Requirement for Supplemental Oxygen ● Oxygenation Index/Ox Supplem Index (q2d)									
EVAL for/ Deliver OPTIONAL 2nd dose 80 cGy ULD-WLRT				X 3-10 days after initial dose							
CORRELATIVE SAMPLES											
Tracheal Aspirate:											
Viral load/titer		X	X	X	X	X					
Blood sample:											
Complete Blood Count	X	X	X	X	X	X	X	X	X	X	X
Immune Cell Count (i.e. lymphocyte count, etc.)		X	X	X	X	X	X	X	X	X	X
Plasma collection		X	X	X	X	X	X	X	X	X	X
Serum collection		X	X	X	X	X	X	X	X	X	X
Cytokine and Chemokine measurements		X	X	X	X	X	X	X	X	X	X
Biomarker evaluation		X	X	X	X	X	X	X	X	X	X
Urine sample:											

Biomarker evaluation		X	X	X	X	X	X	X	X	X	X
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Within 5 days of enrollment

* **+ 2 days**

† **+ 5 days**

♦ **+ 10 days**

☞ Serum chemistries (Na, K, Cl, CO₂), organ function studies (BUN, Creatinine, LFTs [AST, ALT, Alkaline Phosphatase]), and C-reactive protein, Clotting studies (D-Dimer and Fibrinogen)
All samples will be collected from participants regardless of disease status unless the patient withdraws consent for study participation. Samples will be sent to the regular blood processing unit and stored until batch analysis..

7. ADVERSE EVENTS, COMMON TOXICITY ASSESSMENT CRITERIA, AND SUPPORTIVE CARE

7.1 Adverse Events and Serious Adverse Events

- **Adverse Events (AEs)**

An adverse event (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the drug.

Toxicity will be scored using CTCAE Version 5.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 5.0 can be downloaded from the CTEP homepage:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.

All appropriate treatment areas have access to a copy of the CTCAE Version 5.0. All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug and for its seriousness. Toxicity and mortality will be monitored on a continuous basis.

All adverse events should be treated appropriately. Once an adverse event is detected, it should be followed until its resolution or stabilization of the event (to $\leq$ grade 2), whichever comes first. Assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

For both serious and non-serious adverse events, the investigator or sub-investigator must determine both the intensity of the event and the relationship of the event to drug administration. Relationship to drug administration will be determined by the investigator or sub-investigator responding yes or no to the question: Is there a reasonable possibility that the adverse event is associated with the drug? Deaths that occur during the patient's study participation will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

- **Serious Adverse Events (SAEs)**

Definition of an SAE: A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a persons' ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Clarification should be made between the terms "serious" and "severe" since they ARE NOT synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as "serious," which is based on patient/event outcome or action criteria described above and are usually associated with events that pose a threat to a patient's life or functioning. A severe adverse event does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be

considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild, but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

- **SAE Reporting Requirements**

The Ohio State University Office of Responsible Research Practices will serve as the IRB for this study. All SAE's should be forwarded to the IRB according to their policies and procedures.

The study specific guidelines may be found online at <http://orrrp.osu.edu/irb/>.

The duties of the DSMC are as follows:

1. Review all Serious Adverse Event (SAE) reports generated for patients who participate in this study.
2. Identify problems relating to safety over the course of the study. If any actionable issue is identified, the DSMC will inform the study Principal Investigator (PI) via written report, who in turn will ensure that all clinical site PI's receive this report.
3. The DSMC will meet on a regular schedule over the course of the study (i.e., not less than four times a year, with additional meetings as needed)
4. At each meeting, the DSMC will consider the rationale for continuation of the study, with respect to protocol adherence and compliance, safety issues, and outcome data, if relevant, and make a recommendation for or against continuation of the trial. If any actionable issue is identified with regards to the conduct of the study, a written report will be submitted to the Cancer Scientific Review Committee Executive Committee (CSRC EC) who will in turn review and direct the Principal Investigator (PI) on the required action in order to continue the study.

The investigator is required to submit all unexpected and serious adverse events to the OSU IRB within the institutional required timeframe. All AE/SAEs will be reported to the Data and Safety Monitoring Committee (DSMC) at the quarterly DSMC review meetings; however, the investigator determines corrective action is necessary, and "ad hoc" DSMC meeting will be called.

Fatal adverse events related to treatment which are unexpected must be reported within 10 days of the Investigators first awareness of the event to the OSU IRB and the DSMC. Fatalities not related to the study must be reported within the institutional required timeframe.

7.2 Supportive Care

All supportive care regarding COVID-19 respiratory distress and event associated with need for intensive care will be given in keeping with OSU standard policies and procedures, and

clinical care as deemed appropriate by ICU staff.

Nausea:

In the event that nausea is experienced, suitable antiemetics in a single, low dose is standardly effective (e.g., ondansetron, prochlorperazine, metoclopramide).

Skin erythema, dry desquamation:

Skin erythema or dry desquamation in the radiation treatment field, while unlikely, may be treated with application over the chest and back as required with topical non-alcohol-containing moisturizers, such as Remedy Olivamine Skin Repair or Aquaphor.

8. STATISTICAL CONSIDERATIONS

8.1 Study Design, Power and Sample Size Estimation

The primary outcome is the mortality rate 30 days after the ICU-based mechanical ventilation initiation. A Simon's minimax design with power of 90% and type I error of 20% was selected to detect an improvement of 20% mortality for the ventilated patients with the ULD-WLRT. Based on current data available, the mortality rate for ventilated patients is assumed to be 80% in the current design. An interim futility analysis will be conducted after 16 evaluable patients have received the ULD-WLRT. If at least 3 patients survive for at least 30 days, we will enroll additional 8 patients (total of 24 patients). Otherwise, the trial will stop for further evaluation. Due to the limited data currently available in local institutions about a 30 day mortality rate, we will retrospectively evaluate the mortality rate of the ventilated patients without the ULD-WLRT in our institution when data is available. The sample size and power depends on the scenario of the assumed P0 and P1. Here is a summary of the sample size and power based on the current design and other P0 and P1 with different decision scenarios:

P0	P1	N1	R1	PET	N	R	Actual Power	Actual Type I error	Design
0.8	0.6	16	2	0.352	24	6	0.901	0.187	*Minimax
0.7	0.5	17	4	0.389	28	10	0.902	0.187	Minimax
		16	2	0.099	28	10	0.907	0.191	**

0.75	0.55	12	2	0.391	27	8	0.901	0.199	Minimax
		16	2	0.197	29	9	0.908	0.165	**
0.6	0.4	20	7	0.416	30	14	0.9	0.173	Minimax
		16	2	0.018	30	14	0.903	0.175	**

P0: Assumed mortality rate for ventilated patients without ULD-WLRT

P1: Targeted mortality rate for ventilated patients with ULD-WLRT

N1: Number of patients to be enrolled in the first stage

N: Total patients will be enrolled

PET: The probability of early termination of the study

* Current selected minimax Simon's two-stage design

** Design meet both type-I error and power constrains of two-stage design, but not the optimal / minimax design

8.2 Interim Analysis

All patients enrolled in the study received ULD-WLRT will be included in the data analysis. An interim data analysis of the mortality rate will be conducted when 16 eligible patients received ULD-WLRT. If 3 or more patients survive for more than 30 days, the study will continue to enroll. After evaluating the mortality rate of the patients without ULD-WLRT and the data from this trial, additional modification will be considered at the interim analysis.

8.3 Analysis of Primary and Secondary Objectives

Mortality rate is defined as the proportion of patients (received ULD-WLRT) passed away within 30 days after the ICU-based mechanical ventilation initiation. The mortality rate along with the 95% exact confidence interval will be estimated using the Clopper–Pearson method.

Time to event secondary objectives (e.g. overall survival, time to discharge) analyses will be performed using Kaplan-Meier survival analysis, with a competing risk model (leaving the study because of death), including effects for demographic/clinical characteristics in the model. Proportional endpoints (such as % patients off ventilator) will be calculated along with the 95% Clopper–Pearson exact confidence interval. Pre/post measurements will be evaluated using linear mixed models for repeated measures (with proper data transformation as needed). Association between demographic/clinical characteristics and other secondary objectives (size of

GGO/opacification, for example) will be accomplished with generalized linear models.

8.4 Patient Volume and Availability of Subjects

In mid-April, 2020, there were 93 COVID-19 positive patients that had been hospitalized at The Ohio State University Wesner Medical Center. This does not include the additional 10-15 patients waiting on their COVID-19 test results. This number continues to increase at approximately 10-12 people per day. Additionally, it is estimated that 37% of these patients ultimately require mechanical ventilation as part of their hospital course and treatment.

Currently, we are aware of no competing trials; however, given rapid evolution of pandemic and response, others may open. This trial will be prioritized above other non-OSU investigator initiated trials.

8.5 Planned accrual

Based on the number of patients estimated to meet inclusion criteria in coming months, we anticipate accrual to be completed to this trial within 18 months of activation.

Control group participants are not actively sought in this single-arm phase II trial. One intention of this trial is compassionate use due to lack of known effective treatment.

9. CORRELATIVE STUDIES

9.1 Background:

Molecular biomarkers in biofluids such as blood and urine have the ability to evaluate a patient's disease severity as well as monitor the biological responses that occur during and after a treatment. ULD-WLRT as a treatment to resolve pneumonia is well established and the major known mechanism of action is by inducing an anti-inflammatory biological response (29). As highlighted in Section 1.5, there are a number of immune-response genes and proteins that are induced or inhibited after ULD-WLRT. In addition to monitoring the inflammatory response in these patients it will be equally important to evaluate markers of radiation toxicity. Recent research indicates that circulating microRNAs as well as genes and proteins within DNA damage and repair pathways are most well-correlated with radiation-induced toxicities (55, 56). Additionally, a patient's inherent biological makeup can predict response to therapeutic interventions contributing greatly to survivorship. It is incredibly important to understand the biology behind treatment response and disease resolution in order to improve upon future clinical trial design and can aid in the optimization of future therapeutic strategies. This is particularly important for COVID-19 patients since the underlying biological mechanisms are poorly understood.

9.2 Hypotheses

- We hypothesize that specific baseline molecular biomarkers will predict a patient's response to ULD-WLRT.
- We hypothesize that molecular biomarkers in patient biofluids will change after treatment with ULD-WLRT and will correlate with disease status (i.e. viral load/pneumonia symptoms/lymphocyte count) and resolution (i.e. overall survival/time to recover).

9.3 Collection Methods

• Peripheral Venous Blood Sample Collection

Blood samples will be obtained at different time points, before, during and after radiation and during the follow-up. Peripheral blood will be procured for serum, and plasma and whole blood (for the collection of PBMC). The specimens will be labeled with the patient's initials, patient study ID or MRN, date drawn, time drawn, and time point and accompanied by the sample collection form. In some cases, a blood draw may be required solely for the purpose of this protocol. Patients will be informed that a draw is performed solely for the purposes of the protocol and would not directly benefit his/her care. Peripheral blood will be collected in one lavender top (EDTA) tube (10 ml blood), one serum separator (SST) tube (10 ml) and two green-top (heparin) tubes (2x10 ml, for preparation of PBMC). Blood samples will be obtained using standard phlebotomy technique. Processing will take place in room the Leukemia Tissue Bank (LTB) Hall using techniques that have been established for other clinical protocols following established blood-borne pathogen safety guidelines. Serum and plasma from patient blood samples will be centrifuged at 1000 RCF for 10 minutes. The serum will be collected and aliquoted in 5 labeled cryovials at 1 mL/vial and immediately stored at -70°C. The plasma will be collected and aliquoted in 5 labeled cryovials at 1mL/vial and immediately stored at -70C. The tubes should not be transported on ice and the time between collection and storage can be as long as 2-3 hours. Deidentified samples (serum/plasma/PBMCs or purified biological derivatives) will be shared for research purpose with external collaborators as needed, with appropriate Material Transfer Agreement.

• Urine Collection and Processing

Urine will also be collected at the same time points used for blood draw. Urine will be collected in 50 ml urine collection containers. Urine will be transferred to 50 ml falcon tubes, centrifuged for 5 min at 5000 g and transferred to 8 ml falcon tubes and frozen at -70°C for RNA analysis.

All blood and urine will be processed in a COVID-19 approved laboratory at The Ohio

State University.

- **Tracheal Aspirates**

Tracheal aspirates will be collected at the same time points used for blood draw through 30 days to evaluate viral load/titers.

9.4 Biological Derivatives of Biospecimens

Total RNA/DNA/protein/metabolites will be isolated from research specimens (serum/plasma/blood cellular components [i.e., WBCs, etc.]) and stored at -70-80°C in the Chakravarti lab (Wiseman 351). Thereafter, RNA/DNA/protein/metabolite quality will be measured. To preserve patient and donor confidentiality, samples are given a specimen ID number. Initially, we will perform validation of published molecular biomarkers using the techniques outlined in Section 1.5. In brief, inflammatory and DNA damage and repair pathway gene expression and proteomic profiles are of the plasma/serum and blood cellular components are of highest priority. The Genomics Shared Resources here at OSU will provide us with the expertise to conduct most of these experiments. We will outsource the remaining experiments to our collaborators at USC Epigenome Center (methylation), Case Western (proteomics), and Affymetrix (Oncoscan).

9.5 Planned Testing

Research biospecimen material may be subjected to but are not limited to the following tests: We intend to perform all tests primarily on biofluids and blood cellular components. Our priority in testing is evaluating the gene expression and proteomic profiles of patients.

1. Gene expression profiling (Affymetrix microarray, NanoString, RNA-sequencing, Taqman assay)
2. MicroRNA profiling (NanoString, small-RNA sequencing)
3. Sequencing (Sanger sequencing, next generation sequencing-targeted panel, whole-exome and whole-genome sequencing)
4. Methylation profiling (Infinium Methylation EPIC array)
5. Copy-number analysis (Oncoscan/Illumina-based sequencing panel)
6. Metabolomics (Mass spectrometry)
7. Proteomics/Phospho-Proteomics (Mass spectrometry/Immunohistochemistry)
8. Cytokine/chemokine measurement (Human Cytokine Standard 27-Plex Assays panel on the Bio-Plex 200 system)

The goal of this work is to have comprehensive analysis of the molecular variables including DNA, RNA, metabolite, and protein. The comprehensive analysis is necessary in order to get a true understanding of the molecular alterations and mechanisms underlying COVID-19 response and resolution in patients.

REFERENCES

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-62. Epub 2020/03/11. doi: 10.1016/S0140-6736(20)30566-3. PubMed PMID: 32171076.
2. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-13. Epub 2020/01/30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143; PubMed Central PMCID: PMC7135076.
3. Kirkby C, Mackenzie M. Is low dose radiation therapy a potential treatment for COVID-19 pneumonia? *Radiotherapy and Oncology*. 2020. Epub April 4, 2020.
4. Calabrese EJ, Dhawan G. How radiotherapy was historically used to treat pneumonia: could it be useful today? *Yale J Biol Med*. 2013;86(4):555-70. Epub 2013/12/13. PubMed PMID: 24348219; PubMed Central PMCID: PMC3848110.
5. Oppenheimer A. Roentgen therapy of "virus" pneumonia. *Am J Roentgenol & Rad Therapy*. 1943;49:635-8.
6. Rousseau JP, Johnson WM, Harrell GT. The value of roentgen therapy in pneumonia which fails to respond to the sulfonamides. *Radiology*. 1942;38:281-9.
7. Dubin IN, Baylin GJ, Gobble WG. The effect of roentgen therapy on experimental virus pneumonia; on pneumonia produced in white mice by swine influenza virus. *Am J Roentgenol Radium Ther* 1946;55:478-81.
8. Rödel F, Keilholz L, Herrmann M, Sauer R, Hildebrandt G. Radiobiological mechanisms in inflammatory diseases of low-dose radiation therapy. *Int J Radiat Biol*. 2007;83(6):357-66. doi: 10.1080/09553000701317358. PubMed PMID: 17487675.
9. Schae D, Jahns J, Hildebrandt G, Trott KR. Radiation treatment of acute inflammation in mice. *Int J Radiat Biol*. 2005;81(9):657-67. doi: 10.1080/09553000500385556. PubMed PMID: 16368644.
10. Arenas M, Gil F, Gironella M, Hernández V, Biete A, Piqué JM, et al. Time course of anti-inflammatory effect of low-dose radiotherapy: correlation with TGF-beta(1) expression. *Radiother Oncol*. 2008;86(3):399-406. Epub 2007/11/26. doi: 10.1016/j.radonc.2007.10.032. PubMed PMID: 18031855.
11. Weinstock MB, Echenique A, Russell JW. Chest x-ray findings in 636 ambulatory patients with COVID-19 presenting to an urgent care center: a normal chest x-ray is no guarantee. *J Urgent Care Med* 2020;14(7):13-8.
12. Li Y, Xia L. Coronavirus Disease 2019 (COVID-19): Role of Chest CT in Diagnosis and Management. *AJR Am J Roentgenol*. 2020:1-7. Epub 2020/03/04. doi: 10.2214/AJR.20.22954. PubMed PMID: 32130038.
13. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, et al. Time Course of Lung Changes On Chest CT During Recovery From 2019 Novel Coronavirus (COVID-19) Pneumonia. *Radiology*. 2020:200370. Epub 2020/02/13. doi: 10.1148/radiol.20200370. PubMed PMID: 32053470.
14. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis*. 2020;20(4):425-34. Epub 2020/02/24. doi: 10.1016/S1473-3099(20)30086-4. PubMed PMID:

32105637; PubMed Central PMCID: PMC7159053.

15. Wilkins C, Gale M. Recognition of viruses by cytoplasmic sensors. *Curr Opin Immunol.* 2010;22(1):41-7. Epub 2010/01/12. doi: 10.1016/j.coi.2009.12.003. PubMed PMID: 20061127; PubMed Central PMCID: PMC7159053.
16. Janeway CA, Medzhitov R. Innate immune recognition. *Annu Rev Immunol.* 2002;20:197-216. Epub 2001/10/04. doi: 10.1146/annurev.immunol.20.083001.084359. PubMed PMID: 11861602.
17. Sato S, St-Pierre C, Bhaumik P, Nieminen J. Galectins in innate immunity: dual functions of host soluble beta-galactoside-binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs). *Immunol Rev.* 2009;230(1):172-87. doi: 10.1111/j.1600-065X.2009.00790.x. PubMed PMID: 19594636.
18. Aoshi T, Koyama S, Kobiyama K, Akira S, Ishii KJ. Innate and adaptive immune responses to viral infection and vaccination. *Curr Opin Virol.* 2011;1(4):226-32. Epub 2011/07/30. doi: 10.1016/j.coviro.2011.07.002. PubMed PMID: 22440781.
19. Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med.* 2007;13(11):460-9. Epub 2007/10/29. doi: 10.1016/j.molmed.2007.09.002. PubMed PMID: 18029230.
20. Ito T, Amakawa R, Inaba M, Ikehara S, Inaba K, Fukuhara S. Differential regulation of human blood dendritic cell subsets by IFNs. *J Immunol.* 2001;166(5):2961-9. doi: 10.4049/jimmunol.166.5.2961. PubMed PMID: 11207245.
21. Cauley LS, Cookenham T, Hogan RJ, Crowe SR, Woodland DL. Renewal of peripheral CD8+ memory T cells during secondary viral infection of antibody-sufficient mice. *J Immunol.* 2003;170(11):5597-606. doi: 10.4049/jimmunol.170.11.5597. PubMed PMID: 12759439.
22. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol.* 2004;136(1):95-103. doi: 10.1111/j.1365-2249.2004.02415.x. PubMed PMID: 15030519; PubMed Central PMCID: PMC7159053.
23. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine.* 2018;104:8-13. Epub 2018/02/02. doi: 10.1016/j.cyto.2018.01.025. PubMed PMID: 29414327; PubMed Central PMCID: PMC7159053.
24. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020;30(3):269-71. Epub 2020/02/04. doi: 10.1038/s41422-020-0282-0. PubMed PMID: 32020029; PubMed Central PMCID: PMC7159053.
25. Lu H. Drug treatment options for the 2019-new coronavirus (2019-nCoV). *Biosci Trends.* 2020;14(1):69-71. Epub 2020/01/28. doi: 10.5582/bst.2020.01020. PubMed PMID: 31996494.
26. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet.* 2020;395(10223):473-5. Epub 2020/02/07. doi: 10.1016/S0140-6736(20)30317-2. PubMed PMID: 32043983; PubMed Central PMCID: PMC7159053.
27. Shang L, Zhao J, Hu Y, Du R, Cao B. On the use of corticosteroids for 2019-nCoV pneumonia. *Lancet.* 2020;395(10225):683-4. Epub 2020/02/12. doi: 10.1016/S0140-6736(20)30361-5. PubMed PMID: 32122468; PubMed Central PMCID: PMC7159053.
28. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma

- therapy in severe COVID-19 patients. *Proc Natl Acad Sci U S A*. 2020. Epub 2020/04/06. doi: 10.1073/pnas.2004168117. PubMed PMID: 32253318.
29. Torres Royo L, Antelo Redondo G, Áquez Pianetta M, Arenas Prat M. Low-Dose radiation therapy for benign pathologies. *Rep Pract Oncol Radiother*. 2020;25(2):250-4. Epub 2020/02/22. doi: 10.1016/j.rpor.2020.02.004. PubMed PMID: 32140081; PubMed Central PMCID: PMC7049618.
30. Calabrese EJ, Dhawan G, Kapoor R, Kozumbo WJ. Radiotherapy treatment of human inflammatory diseases and conditions: Optimal dose. *Hum Exp Toxicol*. 2019;38(8):888-98. Epub 2019/05/06. doi: 10.1177/0960327119846925. PubMed PMID: 31060383.
31. Whelan JS, Burcombe RJ, Janinis J, Baldelli AM, Cassoni AM. A systematic review of the role of pulmonary irradiation in the management of primary bone tumours. *Ann Oncol*. 2002;13(1):23-30. doi: 10.1093/annonc/mdf047. PubMed PMID: 11863105.
32. Marcus KJ, Yock TI, Tarbell NJ. Ewing Sarcoma. In: Constine LS, Tarbell NJ, Halperin EC, editors. *Pediatric Radiation Oncology*. 6th ed. Philadelphia: Wolters Kluwer; 2016.
33. Bölling T, Schuck A, Paulussen M, Dirksen U, Ranft A, Könnemann S, et al. Whole lung irradiation in patients with exclusively pulmonary metastases of Ewing tumors. Toxicity analysis and treatment results of the EICESS-92 trial. *Strahlenther Onkol*. 2008;184(4):193-7. doi: 10.1007/s00066-008-1810-x. PubMed PMID: 18398583.
34. Casey DL, Meyers PA, Alektiar KM, Magnan H, Healey JH, Boland PJ, et al. Ewing sarcoma in adults treated with modern radiotherapy techniques. *Radiother Oncol*. 2014;113(2):248-53. Epub 2014/11/26. doi: 10.1016/j.radonc.2014.11.023. PubMed PMID: 25613397.
35. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constine LS, Eisbruch A, et al. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys*. 2010;76(3 Suppl):S10-9. doi: 10.1016/j.ijrobp.2009.07.1754. PubMed PMID: 20171502; PubMed Central PMCID: PMC4041542.
36. Berrington de Gonzalez A, Gilbert E, Curtis R, Inskip P, Kleinerman R, Morton L, et al. Second solid cancers after radiation therapy: a systematic review of the epidemiologic studies of the radiation dose-response relationship. *Int J Radiat Oncol Biol Phys*. 2013;86(2):224-33. Epub 2012/10/24. doi: 10.1016/j.ijrobp.2012.09.001. PubMed PMID: 23102695; PubMed Central PMCID: PMC3816386.
37. Desjardins A. Radiotherapy of inflammatory conditions. *Journal of the American Medical Association*. 1931;96:401-8.
38. Calabrese EJ, Dhawan G. The role of x-rays in the treatment of gas gangrene: a historical assessment. *Dose Response*. 2012;10(4):626-43. Epub 2012/04/24. doi: 10.2203/dose-response.12-016.Calabrese. PubMed PMID: 23304109; PubMed Central PMCID: PMC3526332.
39. Calabrese EJ. X-Ray treatment of carbuncles and furuncles (boils): a historical assessment. *Hum Exp Toxicol*. 2013;32(8):817-27. doi: 10.1177/0960327112467046. PubMed PMID: 23821639.
40. Calabrese EJ, Dhawan G. The historical use of radiotherapy in the treatment of sinus infections. *Dose Response*. 2013;11:469-79. Epub 2013/04/11. doi: 10.2203/dose-response.13-004.Calabrese. PubMed PMID: 24298225; PubMed Central PMCID: PMC3834741.
41. Calabrese EJ, Calabrese V. Reduction of arthritic symptoms by low dose radiation therapy (LD-RT) is associated with an anti-inflammatory phenotype. *Int J Radiat Biol*.

2013;89(4):278-86. Epub 2012/12/19. doi: 10.3109/09553002.2013.752594. PubMed PMID: 23176159.

42. Calabrese EJ, Calabrese V. Low dose radiation therapy (LD-RT) is effective in the treatment of arthritis: animal model findings. *Int J Radiat Biol.* 2013;89(4):287-94. Epub 2012/12/19. doi: 10.3109/09553002.2013.752595. PubMed PMID: 23176184.

43. Calabrese EJ, Dhawan G. Historical use of x-rays: treatment of inner ear infections and prevention of deafness. *Hum Exp Toxicol.* 2014;33(5):542-53. Epub 2013/06/25. doi: 10.1177/0960327113493303. PubMed PMID: 23800998.

44. Trostler IS. Roentgenotherapy of conditions other than malignancy. *Illinois Medical Journal.* 1931:113-20.

45. Pendergrass EP, Hodes PJ. Roentgen irradiation in the treatment of inflammations. *Am J Roentgenol.* 1941;45(1):74-106.

46. Musser JH, Edsall DL. A study of metabolism in leukaemia, under the influence of the x-ray. *Tr Assn Am Physicians.* 1905;20:294-323.

47. Quimby AJ, Quimby WA. Unresolved pneumonia: Successful treatment by roentgen ray. *New York Medical Journal.* 1916;103:681-3.

48. Heidenhain L, Fried C. Rontgenstrahlen und Entzündung (Roentgen irradiation in inflammation). *Archiv fur Klinische Chirurgie.* 1924;133:624-65.

49. Heidenhain L, Fried C. Rontgenstrahlen und Entzündung (Roentgen irradiation in inflammation). *Klinische Wochenschrift.* 1924;3:1121-2.

50. Powell EV. Radiation therapy of lobar pneumonia. *Texas State Journal of Medicine.* 1936;32:237-40.

51. Krost GN. Unresolved pneumonia in children. Treatment with roentgen ray. *American Journal of Diseases of Children.* 1925;30(1):57-71.

52. Khan FM. *The Physics of Radiation Therapy.* Philadelphia: Lippincott Williams & Wilkins; 2003.

53. Jorgensen TJ. *Strange Glow: The Story of Radiation.* Princeton, New Jersey: Princeton University Press; 2016.

54. Correll HL, Cowan II. Primary Atypical Pneumonia, Analysis of Therapeutic Results in 155 Cases. *United States Naval Medical Bulletin.* 1943;41(4):980-7.

55. Marchetti F, Coleman MA, Jones IM, Wyrobek AJ. Candidate protein biodosimeters of human exposure to ionizing radiation. *Int J Radiat Biol.* 2006;82(9):605-39. doi: 10.1080/09553000600930103. PubMed PMID: 17050475.

56. Jacob NK, Cooley JV, Yee TN, Jacob J, Alder H, Wickramasinghe P, et al. Identification of sensitive serum microRNA biomarkers for radiation biodosimetry. *PLoS One.* 2013;8(2):e57603. Epub 2013/02/25. doi: 10.1371/journal.pone.0057603. PubMed PMID: 23451251; PubMed Central PMCID: PMC3581493.

APPENDIX I ABBREVIATIONS

ALT: alanine transaminase

AST: aspartate transaminase

COVID-19: coronavirus disease 2019

CT: computed tomography

CXR: chest X-ray

GGO: Ground glass opacities

OI: Oxygenation Index

OSI: Oxygen Saturation Index

SARS-C0V-2: severe acute respiratory syndrome coronavirus 2

ULD: Ultra Low-Dose

ULD-WLRT: Ultra Low-Dose Whole Lung Radiation Therapy

WLRT: Whole Lung Radiation Therapy

APPENDIX II ELIGIBILITY CHECKLIST

- ☐ (Y) Patient age ≥ 18 years of age
☐ (Y) Laboratory Covid-19 positive test within 14 days of enrollment.
☐ (Y) CT or radiographic findings typical of COVID-19 pneumonia within 5 days of enrollment
☐ (Y) Receiving ICU-based mechanical ventilation
☐ (Y) Life expectancy ≥ 24 hours, as judged by investigator
☐ (Y) Hypoxemia defined as a Pa/FiO₂ ratio < 300 or SpO₂/FiO₂ < 315
☐ (Y) Signed informed consent by patient or his or her legal/authorized representatives
- ☐ (Y / N) Allowed: Concurrent convalescent immune plasma therapy administered
- ☐ (N) Life expectancy less than 24 hours, as judged by investigator and treating team, irrespective of treatment
☐ (N) Expected survival < 30 days, as judged by investigatory and treating team, due to chronic illness present prior to COVID-19 infection.
☐ (N) Patient or legal representative not committed to full disease specific therapy i.e. comfort care (DNRCCA is allowed)
☐ (N) Treatment with immune suppressing medications that is determined unsafe for the patient to receive radiation treatment for this trial
☐ (N) Presumed COVID-associated illness greater than 14-days
☐ (N) Inpatient admission greater than 14-days
☐ (N) Patients deemed unsafe for travel for radiation therapy
☐ (N) Chronic hypoxemia requiring supplemental oxygen at baseline
☐ (N) Documented active connective tissue disease (scleroderma) or idiopathic pulmonary fibrosis
☐ (N) History of prior radiation therapy resulting in $\geq$ grade 2 radiation pneumonitis within 365 days of enrollment.
☐ (N) History of prior radiation to the thorax completed within 180 days of enrollment (skin or surface only skin treatments are acceptable).
☐ (N) Known active uncontrolled bacterial or fungal infections of the lung.
☐ (N) Active cytotoxic chemotherapy
☐ (N) Females who are pregnant or have a positive pregnancy test.
☐ (N) Females who are breast feeding
- All standard of care COVID treatment will be allowed as deemed safe by the investigator

The Eligibility Checklist must be completed, signed, and dated by the registering agent.

This document should be maintained in the patient's file.

Registering Agent Name: _____

Date: _____

Registering Agent Signature: _____

APPENDIX III TOXICITY**Table 7. Toxicity Grading**

CTC-AE version 5 is used in this protocol and is available at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf).

APPENDIX IV KARNOFSKY PERFORMANCE SCALE**Table 8. Karnofsky Performance Scale and Criteria.**

Score	Criteria
100%	Normal, no complaints, no signs of disease
90%	Capable of normal activity, few symptoms or signs of disease
80%	Normal activity with some difficulty, some symptoms or signs
70%	Caring for self, not capable of normal activity or work
60%	Requiring some help, can take care of most personal requirements
50%	Requires help often, requires frequent medical care
40%	Disabled, requires special care and help
30%	Severely disabled, hospital admission indicated but no risk of death
20%	Very ill, urgently requiring admission, requires supportive measures or treatment
10%	Moribund, rapidly progressive fatal disease processes
0%	Death