CHEST Online Supplement

Circulating Biomarkers From the Phase 1 Trial of Sirolimus and Autophagy Inhibition for Patients With Lymphangioleiomyomatosis

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e-Appendix 1.

Material and Methods

Study population

We recruited 14 women, one of whom was not included in this analysis due to failing to meet eligibility during the screening process (Supplemental Table 1). Subjects who were at least 18 years old, were eligible to enroll in the study if they had received a definite LAM diagnosis per the European Respiratory Society definition or were diagnosed with cystic lung disease featuring a VEGF-D level > 800 pg/mL. Women with FEV_1 or diffusion coefficient for carbon monoxide (DLCO) less than 80% or 70% predicted, respectively, were not eligible.

Sample processing

Serum was processed by allowing collected peripheral blood to clot for approximately 1 hour at room temperature, followed by centrifugation at 2,000 RPM for 15 minutes. Supernatants were then stored at -80°C for later analysis.

Analytes measurements

Serum was collected from every enrolled subject at each study visit. Analytes were measured using the DiscoveryMAP® platform which includes molecules associated with inflammation, vasculogenesis, matrix biology, metabolism, and cancer amongst other pathways. A full list of analytes is available at: https://myriadrbm.com/products-services/humanmap-services/discoverymap/ (DiscoveryMAP; Myriad RBM, Austin, TX, USA).

Sample Imputation

One hundred eighty-three analytes were measurable in all samples. The remaining 96 proteins featured at least one sample that was either above or below the assay limit of quantification. Fifty-six analytes were outside the quantification limit in greater than 50% of samples and were excluded from the analysis. One analyte was not measurable in any of the samples and was therefore removed from the analysis. Imputations were performed with the 39 proteins that had 50% or less sample missingness, with sample values that were above or below the quantification limit being replaced with the upper or lower limit of quantification for that given analyte, respectively. Therefore, a total of 222 proteins were included in the analysis.

Analyte association with baseline characteristics

All analyte levels were log10-transformed prior to analyses to address any potential data skewness. To determine if any analytes were associated with baseline subject characteristics, we ran separate Student's T tests for baseline levels of each of the 222 proteins included in the analysis and compared subjects with or without supplemental oxygen use and post-menopausal status at baseline. Analytes significantly associated with each characteristic were identified as proteins that had a P value < 0.05.

Repeated-measures ANOVA

To determine which proteins had significantly changed over the course of the study, repeated-measure analysis of variance (RM ANOVA) calculations using the Kenward-Roger approximation were implemented in separate linear mixed effects models with random intercepts for each of the 222 analytes (R package lmerTest v2.0.33). These analyses were performed over the entire study duration (baseline to week 48) and then over just the treatment period (baseline to week 24). For the analytes found to have significantly changed over the entire study period as well as for those changed over just the treatment period, RM ANOVA analyses were performed over the observation period, and those with non-significant outcomes were identified as analytes that remained stable after the treatment period, while those with significant outcomes were assessed as having returned to baseline analyte levels. A heat map displaying the log10-transformed levels at baseline, end of treatment, and end of study for the analytes changed over the treatment period was graphed using the R package gplots (version 3.0.1) [1] , while analytes and subjects were clustered using the built-in R function hclust and analyte levels were scaled per subject using the R package mousetrap (version 3.1.0) [2,3]. *P*-values were adjusted for multiple hypotheses with Benjamini-Hochberg false discovery rate (FDR) corrections [4], and significantly changed analytes were identified as those for which the *q* value was < 0.05.

Analyte association with lung function

To assess if the changes in any analytes were associated with changes in lung function, we first investigated the predictive capabilities of changes in analyte level. Using only the analytes that had significantly changed during the treatment period by RM ANOVA, separate longitudinal linear mixed effects models with random intercepts were run for each protein (R package lmerTest v2.0.33). These models included FEV_1 percentage of predicted post-bronchodilator measurements recorded over the course of the study as the outcome, the respective analyte level as the covariate, and a time variable (weeks) as a means of controlling for the time-varying aspect of the analyte. The analyses were run over the entire study duration (baseline to week 48) and then just over the treatment period (baseline to week 24). After Benjamini-Hochberg FDR correction, analytes that were significantly associated with changes in lung function were identified as those for which the *q* value of the analyte covariate was < 0.05.

We also assessed if analyte changes from visit 1 (baseline) to visit 2 (week 3) could predict changes in lung function. First, of the 222 analytes in the analysis, we identified those that had significantly changed from visit 1 to visit 2 by paired T test after Benjamini-Hochberg FDR correction ($q < 0.05$). Using these analytes, we ran separate longitudinal linear mixed effects models with random intercepts. The models again included the FEV_1

percentage of predicted post-bronchodilator measurements recorded throughout the study as the outcome, main effects for the log difference between visit 2 and visit 1 concentrations for the respective analyte and a time variable (weeks), and the interaction between the log difference and the time variable. Again, two separate sets of analyses were run: one investigating the entire study duration and another examining only the treatment period. Significant predictors of lung function by visit 2 were identified as analytes that featured a significant log difference-time interaction term after Benjamini-Hochberg FDR correction ($q < 0.05$).

Identifying hydroxychloroquine-dependent analytes

To differentiate analytes that were primarily regulated by rapamycin alone from those that may have been impacted by the combination of rapamycin and hydroxychloroquine treatments, we ran separate longitudinal linear mixed effects models with random intercepts to compare the changes in each of the 222 analytes over the treatment period between the subjects treated with low dose hydroxychloroquine (LD) and those treated with high dose hydroxychloroquine (HD). These models contained the respective analytes as the outcome, the main effects of hydroxychloroquine group assignment (HD = 1, LD = 0) and time in weeks, and the interaction between hydroxychloroquine group and time. Analytes with rates of change significantly different between LD and HD groups were identified as those for which the group-time interaction was significant ($P < 0.05$). Furthermore, paired Student's T tests were implemented comparing levels of these identified analytes at each visit to baseline levels for all subjects and separately for LD and HD subjects to determine which analytes had significantly changed from the start of the study ($P < 0.05$). In addition, to more directly assess the effects of hydroxychloroquine on these analytes, Student's T tests were performed comparing LD and HD levels at each study visit, with significant results indicating hydroxychloroquine-dependent regulation of a given analyte ($P < 0.05$).

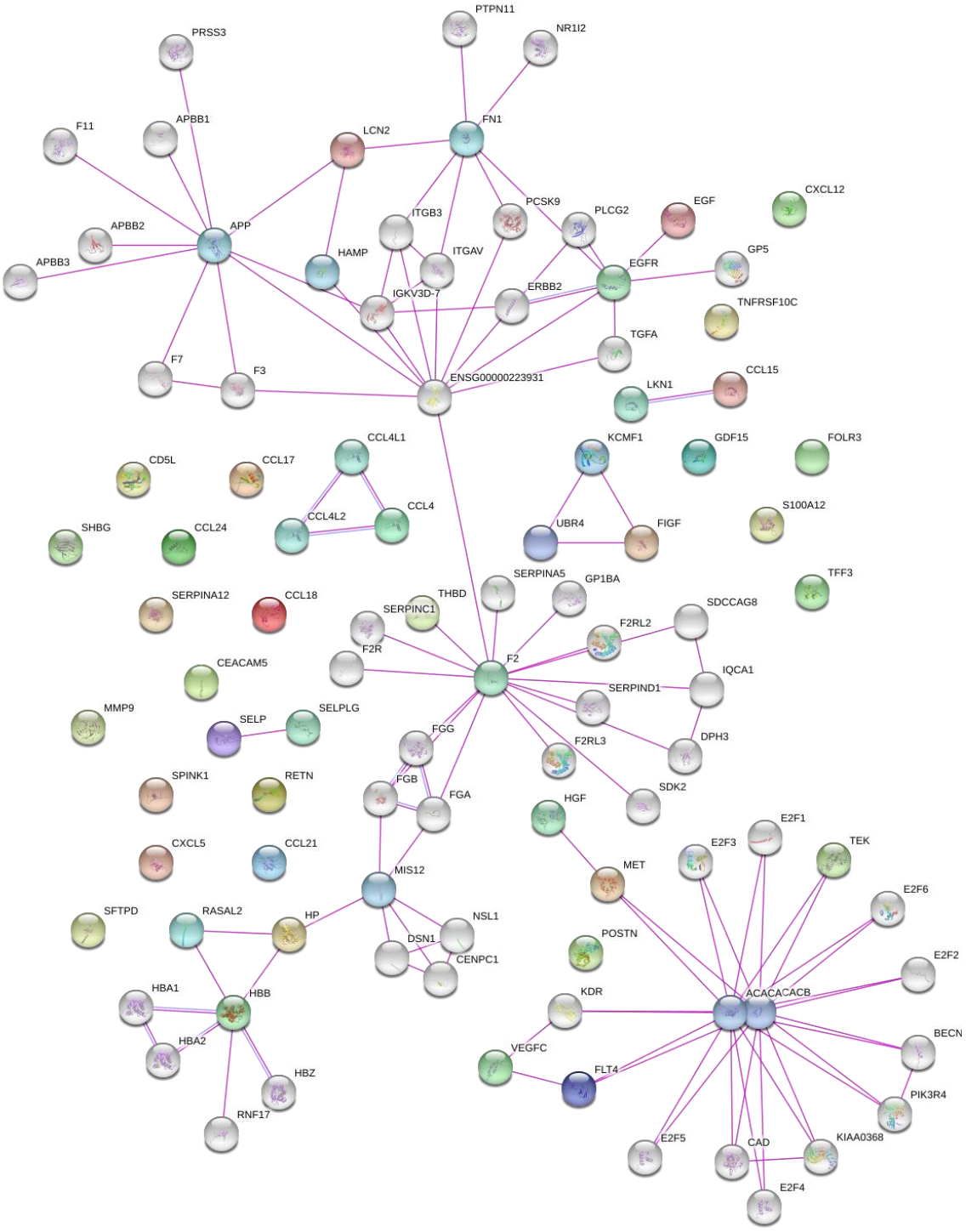
Determining indicators of autophagy inhibition

To assess the effect of hydroxychloroquine on autophagy, we cross-referenced all measurable analytes with a human autophagy gene database[5] and identified common analytes. As with the identified hydroxychloroquine-dependent analytes, for each autophagy-associated analyte we implemented paired Student's T tests to assess differences from baseline at each study visit for all subjects and for LD and HD subjects separately and used Student's T tests to directly compare analyte levels between LD and HD subjects at each study visit.

Ontology and Network Analysis

To determine ontology enrichments of analyte lists of interest, the respective analytes were submitted to the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Version 6.8) [6,7] and any gene ontology (GO) terms pertaining to the category biological process 1 (GOTERM_BP_1) that were significantly enriched by Benjamini-Hochberg FDR were identified ($q < 0.05$). These lists of proteins were also submitted to STRING (version 10.5, [8]) to determine significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichments ($q < 0.05$). Within Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), 1st and 2nd shell interactors were added to the query analytes (Experimental only active interaction source; minimum required interaction score = 0.700) to build protein-protein interaction networks.

e-Figure 1:



STRING analysis of 32 analytes differentially changed over time and multiple visits between baseline and end of treatment.

e-Table 1: study subjects baseline characteristics (Additional details in reference 5)

Subjects enrolled and received study drug	13
Age	49 (40-65)
FEV1 (% predicted)	59 ± 21
FVC (% predicted)	81 ± 16
DLCO (% predicted)	43 ± 15

e-Table 2: Description of data

100% Within Limit of Quantification		
Analyte	**Percent of Samples Outside Detection Limit**	**Percent of Subjects with Samples Outside Limit of Detection**
A.disintegrin.and.metalloproteinase.with.thrombospondin.motifs.8..ADAMTS8.	0	0
Adiponectin	0	0
Adrenomedullin..ADM.	0	0
Aggrecan.core.protein..Aggrecan.	0	0
Alpha.1.acid.glycoprotein.1..AGP.1.	0	0
Alpha.1.Antitrypsin..AAT.	0	0
Alpha.1.Microglobulin..A1Micro.	0	0
Alpha.2.Macroglobulin..A2Macro.	0	0
Angiogenin	0	0
Angiopoietin.1..ANG.1.	0	0
Angiopoietin.2..ANG.2.	0	0
Angiopoietin.related.protein.4..ANGPTL4.	0	0
Angiotensin.Converting.Enzyme..ACE.	0	0
Antileukoproteinase..ALP.	0	0
Antithrombin.III..AT.III.	0	0
Apolipoprotein.a...Lp.a..	0	0
Apolipoprotein.A.I..Apo.A.I.	0	0
Apolipoprotein.A.II..Apo.A.II.	0	0
Apolipoprotein.B..Apo.B.	0	0
Apolipoprotein.C.I..Apo.C.I.	0	0
Apolipoprotein.C.III..Apo.C.III.	0	0
Apolipoprotein.D..Apo.D.	0	0
Apolipoprotein.E..Apo.E.	0	0
Apolipoprotein.H..Apo.H.	0	0
AXL.Receptor.Tyrosine.Kinase..AXL.	0	0
B.cell.activating.factor..BAFF.	0	0

Beta.2.Microglobulin..B2M.	0	0
Beta.Amyloid.1.40..AB.40.	0	0
Brain.Derived.Neurotrophic.Factor..BDNF.	0	0
C.C.motif.chemokine.15..CCL15.	0	0
C.Peptide	0	0
C.Reactive.Protein..CRP.	0	0
Cadherin.1..E.Cad.	0	0
Cancer.Antigen.15.3..CA.15.3.	0	0
Carcinoembryonic.Antigen..CEA.	0	0
Carcinoembryonic.antigen.related.cell.adhesion.molecule.1..CEACAM1.	0	0
Cathepsin.D	0	0
CD163	0	0
CD27.antigen..CD27.	0	0
CD40.Ligand..CD40.L.	0	0
CD5.Antigen.like..CD5L.	0	0
Chemokine.CC.4..HCC.4.	0	0
Chromogranin.A..CgA.	0	0
Clusterin..CLU.	0	0
Complement.C3..C3.	0	0
Complement.Factor.H...Related.Protein.1..CFHR1.	0	0
Complement.Factor.H..CFH.	0	0
Cystatin.B	0	0
Cystatin.C	0	0
Decorin	0	0
Dickkopf.related.protein.1..DKK.1.	0	0
E.Selectin	0	0
Endoglin	0	0
Endostatin	0	0
Eotaxin.2	0	0
Epidermal.Growth.Factor..EGF.	0	0
Epidermal.Growth.Factor.Receptor..EGFR.	0	0
Epithelial.Derived.Neutrophil.Activating.Protein.78..ENA.78.	0	0
Factor.VII	0	0
Fatty.Acid.Binding.Protein..adipocyte..FABP..adipocyte.	0	0
Ferritin..FRTN.	0	0

Fetuin.A	0	0
Ficolin.3	0	0
Follicle.Stimulating.Hormone..FSH.	0	0
Galectin.3	0	0
Gelsolin	0	0
Glucose.6.phosphate.Isomerase..G6PI.	0	0
Growth.differentiation.factor.15..GDF.15.	0	0
Growth.Regulated.alpha.protein..GRO.alpha.	0	0
Haptoglobin	0	0
HE4	0	0
Heat.Shock.protein.70..HSP.70.	0	0
Hemopexin	0	0
Heparin.Binding.EGF.Like.Growth.Factor..HB.EGF.	0	0
Hepatocyte.Growth.Factor..HGF.	0	0
Hepatocyte.Growth.Factor.receptor..HGF.receptor.	0	0
Hepsin	0	0
Human.Epidermal.Growth.Factor.Receptor.2..HER.2.	0	0
Immunoglobulin.A..IgA.	0	0
Insulin.like.Growth.Factor.Binding.Protein.2..IGFBP.2.	0	0
Insulin.like.Growth.Factor.Binding.Protein.7..IGFBP.7.	0	0
Intercellular.Adhesion.Molecule.1..ICAM.1.	0	0
Interferon.gamma.Induced.Protein.10..IP.10.	0	0
Interleukin.1.receptor.antagonist..IL.1ra.	0	0
Interleukin.1.receptor.type.1..IL.1RI.	0	0
Interleukin.1.receptor.type.2..IL.1RII.	0	0
Interleukin.16..IL.16.	0	0
Interleukin.18..IL.18.	0	0
Interleukin.2.receptor.alpha..IL.2.receptor.alpha.	0	0
Interleukin.6.receptor..IL.6r.	0	0
Interleukin.6.receptor.subunit.beta..IL.6R.beta.	0	0
Kallikrein.5	0	0
Kallikrein.7..KLK.7.	0	0
Lactoferrin..LTF.	0	0
Latency.Associated.Peptide.of.Transforming.Growth.Factor.beta.1..LAP.TGF.b1.	0	0
Leptin	0	0

Leptin.Receptor..Leptin.R.	0	0
Leucine.rich.alpha.2.glycoprotein..LRG1.	0	0
Luteinizing.Hormone..LH.	0	0
Macrophage.Derived.Chemokine..MDC.	0	0
Macrophage.inflammatory.protein.3.beta..MIP.3.beta.	0	0
Macrophage.Stimulating.Protein..MSP.	0	0
Mast.stem.cell.growth.factor.receptor..SCFR.	0	0
Matrix.Metalloproteinase.1..MMP.1.	0	0
Matrix.Metalloproteinase.2..MMP.2.	0	0
Matrix.Metalloproteinase.3..MMP.3.	0	0
Matrix.Metalloproteinase.7..MMP.7.	0	0
Matrix.Metalloproteinase.9..total..MMP.9..total.	0	0
Monocyte.Chemotactic.Protein.2..MCP.2.	0	0
Monocyte.Chemotactic.Protein.4..MCP.4.	0	0
Myeloid.Progenitor.Inhibitory.Factor.1..MPIF.1.	0	0
Myeloperoxidase..MPO.	0	0
Myoglobin	0	0
N.terminal.prohormone.of.brain.natriuretic.peptide..NT.proBNP.	0	0
Neuropilin.1	0	0
Neutrophil.Activating.Peptide.2..NAP.2.	0	0
Neutrophil.Gelatinase.Associated.Lipocalin..NGAL.	0	0
Omentin	0	0
Osteoprotegerin..OPG.	0	0
P.Selectin	0	0
Pancreatic.Polypeptide..PPP.	0	0
Pancreatic.secretory.trypsin.inhibitor..TATI.	0	0
Paraoxonase.1..PON.1.	0	0
Pepsinogen.I..PGI.	0	0
Periostin	0	0
Pigment.Epithelium.Derived.Factor..PEDF.	0	0
Plasminogen.Activator.Inhibitor.1..PAI.1.	0	0
Platelet.Derived.Growth.Factor.BB..PDGF.BB.	0	0
Platelet.endothelial.cell.adhesion.molecule..PECAM.1.	0	0
Prolactin..PRL.	0	0
Prostasin	0	0

Protein.S100.A6..S100.A6.	0	0
Pulmonary.and.Activation.Regulated.Chemokine..PARC.	0	0
Pulmonary.surfactant.associated.protein.D..SP.D.	0	0
Receptor.for.advanced.glycosylation.end.products..RAGE.	0	0
Receptor.tyrosine.protein.kinase.erbB.3..ErbB3.	0	0
Resistin	0	0
Retinol.binding.protein.4..RBP.4.	0	0
Sclerostin	0	0
Serum.Amyloid.A.Protein..SAA.	0	0
Serum.Amyloid.P.Component..SAP.	0	0
Sex.Hormone.Binding.Globulin..SHBG.	0	0
ST2	0	0
Stromal.cell.derived.factor.1..SDF.1.	0	0
Superoxide.Dismutase.1..soluble..SOD.1.	0	0
T.Cell.Specific.Protein.RANTES..RANTES.	0	0
Tamm.Horsfall.Urinary.Glycoprotein..THP.	0	0
Tenascin.C..TN.C.	0	0
Tetranectin	0	0
Thrombin.Activatable.Fibrinolysis..TAFI.	0	0
Thrombomodulin..TM.	0	0
Thrombospondin.1	0	0
Thymus.and.activation.regulated.chemokine..TARC.	0	0
Thyroid.Stimulating.Hormone..TSH.	0	0
Thyroxine.Binding.Globulin..TBG.	0	0
Tissue.Inhibitor.of.Metalloproteinases.1..TIMP.1.	0	0
Tissue.Inhibitor.of.Metalloproteinases.2..TIMP.2.	0	0
Tissue.Inhibitor.of.Metalloproteinases.3..TIMP.3.	0	0
Tissue.type.Plasminogen.activator..tPA.	0	0
TNF.Related.Apoptosis.Inducing.Ligand.Receptor.3..TRAIL.R3.	0	0
Transferrin.receptor.protein.1..TFR1.	0	0
Transthyretin..TTR.	0	0
Trefoil.Factor.3..TFF3.	0	0
Tumor.necrosis.factor.ligand.superfamily.member.12..Tweak.	0	0
Tumor.necrosis.factor.ligand.superfamily.member.13..APRIL.	0	0
Tumor.necrosis.factor.receptor.2..TNFR2.	0	0

Tumor.Necrosis.Factor.Receptor.I..TNF.RI.	0	0
Tyrosine.kinase.with.Ig.and.EGF.homology.domains.2..TIE.2.	0	0
Urokinase.type.plasminogen.activator.receptor..uPAR.	0	0
Uteroglobin	0	0
Vascular.Cell.Adhesion.Molecule.1..VCAM.1.	0	0
Vascular.Endothelial.Growth.Factor..VEGF.	0	0
Vascular.endothelial.growth.factor.D..VEGF.D.	0	0
Vascular.Endothelial.Growth.Factor.Receptor.2..VEGFR.2.	0	0
Vascular.endothelial.growth.factor.receptor.3..VEGFR.3.	0	0
Visceral.adipose.tissue...derived.serpin.A12..Vaspin.	0	0
Visfatin	0	0
Vitamin.D.Binding.Protein..VDBP.	0	0
Vitamin.K.Dependent.Protein.S..VKDPS.	0	0
Vitronectin	0	0
von.Willebrand.Factor..vWF.	0	0
X6Ckine	0	0
YKL.40	0	0
Above Limit of Quantification		
Analyte	**Percent of Samples Above Detection Limit**	**Percent of Subjects with Samples Above the Limit of Detection**
Folate.receptor.gamma..FOLR3.	11.54	15.38
Interleukin.18.binding.protein..IL.18bp.	3.85	7.69
EN.RAGE	1.28	7.69
Fibulin.1C..Fib.1C.	1.28	7.69
Below Limit of Quantification		
Analyte	**Percent of Samples Below**	**Percent of Subjects with Samples Below the**

	Detection Limit	Limit of Detection
Ciliary.Neurotrophic.Factor..CNTF.	100	100
Eotaxin.3	100	100
Granulocyte.Macrophage.Colony.Stimulating.Factor..GM.CSF.	100	100
Interferon.alpha..IFN.alpha.	100	100
Interferon.gamma..IFN.gamma.	100	100
Interleukin.12.Subunit.p70..IL.12p70.	100	100
Interleukin.13..IL.13.	100	100
Interleukin.17..IL.17.	100	100
Interleukin.2..IL.2.	100	100
Interleukin.3..IL.3.	100	100
Interleukin.4..IL.4.	100	100
Interleukin.5..IL.5.	100	100
Interleukin.6..IL.6.	100	100
Interleukin.7..IL.7.	100	100
Maspin	100	100
Monocyte.Chemotactic.Protein.3..MCP.3.	100	100
Nerve.Growth.Factor.beta..NGF.beta.	100	100
Prostate.Specific.Antigen..Free..PSA.f.	100	100
Transforming.Growth.Factor.beta.3..TGF.beta.3.	100	100
Tumor.Necrosis.Factor.beta..TNF.beta.	100	100
Vascular.Endothelial.Growth.Factor.Receptor.1..VEGFR.1.	100	100
Betacellulin..BTC.	98.72	100
Interleukin.10..IL.10.	98.72	100
Neuronal.Cell.Adhesion.Molecule..Nr.CAM.	98.72	100
S100.calcium.binding.protein.B..S100.B.	98.72	100
Tumor.Necrosis.Factor.alpha..TNF.alpha.	98.72	100
Alpha.Fetoprotein..AFP.	97.44	100
Amphiregulin..AR.	97.44	100
Neurofilament.heavy.polypeptide..NF.H.	97.44	100
Macrophage.Inflammatory.Protein.1.alpha..MIP.1.alpha.	94.87	92.31
Epiregulin..EPR.	93.59	100
Fatty.Acid.Binding.Protein..heart..FABP..heart.	93.59	100
Fibrinogen	93.59	100

Interleukin.31..IL.31.	93.59	100
Fatty.Acid.Binding.Protein..liver..FABP..liver.	92.31	100
Granulocyte.Colony.Stimulating.Factor..G.CSF.	92.31	100
Glycogen.phosphorylase.isoenzyme.BB..GPBB.	89.74	92.31
MHC.class.I.chain.related.protein.A..MICA.	87.18	100
Beta.Amyloid.1.42..AB.42.	80.77	92.31
Fibroblast.growth.factor.23..FGF.23.	78.21	100
Interleukin.1.beta..IL.1.beta.	76.92	100
Calbindin	75.64	100
Cellular.Fibronectin..cFib.	75.64	84.62
Glucagon.like.Peptide.1..total..GLP.1.total.	75.64	84.62
Interleukin.15..IL.15.	73.08	100
Placenta.Growth.Factor..PLGF.	73.08	92.31
Macrophage.Inflammatory.Protein.3.alpha..MIP.3.alpha.	70.51	100
Kidney.Injury.Molecule.1..KIM.1.	69.23	84.62
Human.Chorionic.Gonadotropin.beta..hCG.	67.95	69.23
Fas.Ligand..FasL.	65.38	100
T.Lymphocyte.Secreted.Protein.I.309..I.309.	65.38	92.31
Thyroglobulin..TG.	62.82	76.92
Interleukin.1.alpha..IL.1.alpha.	60.26	92.31
Cancer.Antigen.125..CA.125.	60.26	69.23
Interleukin.8..IL.8.	56.41	92.31
Matrix.Metalloproteinase.9..MMP.9.	52.56	100
B.Lymphocyte.Chemoattractant..BLC.	50	92.31
Insulin	48.72	76.92
Interleukin.22..IL.22.	43.59	92.31
Gastric.inhibitory.polypeptide..GIP.	42.31	76.92
Osteopontin	34.62	69.23
Growth.Hormone..GH.	28.21	69.23
Dopamine.beta.hydroxylase..DBH.	21.79	38.46
Fibroblast.Growth.Factor.21..FGF.21.	20.51	38.46
Macrophage.Inflammatory.Protein.1.beta..MIP.1.beta.	17.95	46.15
Cancer.Antigen.19.9..CA.19.9.	16.67	23.08
Macrophage.Colony.Stimulating.Factor.1..M.CSF.	15.38	30.77
Interferon.inducible.T.cell.alpha.chemoattractant..ITAC.	12.82	46.15

Carbonic.anhydrase.9..CA.9.	11.54	30.77
Epithelial.cell.adhesion.molecule..EpCam.	11.54	30.77
Protein.DJ.1..DJ.1.	10.26	15.38
Erythropoietin..EPO.	8.97	23.08
Macrophage.Migration.Inhibitory.Factor..MIF.	8.97	23.08
Interleukin.12.Subunit.p40..IL.12p40.	7.69	38.46
Lectin.Like.Oxidized.LDL.Receptor.1..LOX.1.	7.69	30.77
Eotaxin.1	7.69	15.38
Immunoglobulin.M..IgM.	7.69	7.69
Thymus.Expressed.Chemokine..TECK.	6.41	23.08
Stem.Cell.Factor..SCF.	5.13	15.38
Monokine.Induced.by.Gamma.Interferon..MIG.	3.85	15.38
Neuron.Specific.Enolase..NSE.	2.56	15.38
Interleukin.23..IL.23.	2.56	7.69
Sortilin	2.56	7.69
Urokinase.type.Plasminogen.Activator..uPA.	2.56	7.69
Bone.morphogenetic.protein.9..BMP.9.	1.28	7.69
Collagen.IV	1.28	7.69
FASLG.Receptor..FAS.	1.28	7.69
Insulin.like.Growth.Factor.Binding.Protein.1..IGFBP.1.	1.28	7.69
Matrix.Metalloproteinase.10..MMP.10.	1.28	7.69
Monocyte.Chemotactic.Protein.1..MCP.1.	1.28	7.69
Progranulin	1.28	7.69
Non-readable		
Analyte	**Percent of Non-readable Samples**	**Percent of Subjects with Non-readable Samples**
Immunoglobulin.E..IgE.	100	100

e-Table 3: GO terms associated with baseline supplemental oxygen use.

GO Term			**KEGG**	
GO Term	**P**	**Benjamini**	**Pathway**	**FDR**
immune system process	1.37E-04	0.00274183	None	
developmental process	2.25E-04	0.00224899		
multi-organism process	8.04E-04	0.00534707		
reproductive process	0.00267318	0.01329464		
reproduction	0.00269048	0.01071857		
regulation of biological process	0.00371973	0.01234539		
signaling	0.00429985	0.01223628		
biological adhesion	0.00687198	0.01709151		
biological regulation	0.00767962	0.01698578		
response to stimulus	0.01039723	0.02068635		
multicellular organismal process	0.0112848	0.02042304		
locomotion	0.02512563	0.04152434		
growth	0.03208992	0.04894045		
metabolic process	0.03440318	0.04878267		

e-Table 4: GO terms and KEGG pathways associated with baseline menopausal status.

GO Term			KEGG	
GO Term	**P**	**Benjamini**	**Pathway**	**FDR**
immune system process	4.59E-07	9.63E-06	Cytokine-cytokine receptor interaction	2.39E-09
multi-organism process	2.11E-06	2.22E-05	Rheumatoid arthritis	0.000397
signaling	1.10E-05	7.71E-05	Hematopoietic cell lineage	0.0126
response to stimulus	2.80E-05	1.47E-04	PI3K-Akt signaling pathway	0.042
reproductive process	4.52E-05	1.90E-04		
reproduction	4.58E-05	1.60E-04		
regulation of biological process	5.01E-05	1.50E-04		
locomotion	1.06E-04	2.78E-04		
biological regulation	1.81E-04	4.22E-04		
developmental process	4.45E-04	9.35E-04		
localization	0.00298831	0.00569721		
single-organism process	0.00533609	0.00931946		
biological adhesion	0.00623931	0.01005952		
multicellular organismal process	0.00671213	0.01005128		

e-Table 5: Analytes significantly changed over entire study period.

Analyte	p value	q value
Pulmonary.and.Activation.Regulated.Chemokine..PARC.	1.32E-13	2.92E-11
TNF.Related.Apoptosis.Inducing.Ligand.Receptor.3..TRAIL.R3.	1.21E-09	1.34E-07
Pulmonary.surfactant.associated.protein.D..SP.D.	3.17E-09	2.35E-07
Tyrosine.kinase.with.Ig.and.EGF.homology.domains.2..TIE.2.	5.07E-09	2.39E-07
Vascular.endothelial.growth.factor.D..VEGF.D.	5.39E-09	2.39E-07
Alpha.1.acid.glycoprotein.1..AGP.1.	1.33E-07	4.91E-06
Macrophage.Inflammatory.Protein.1.beta..MIP.1.beta.	5.44E-07	1.73E-05
Haptoglobin	1.23E-06	3.41E-05
Periostin	3.44E-06	8.49E-05
Resistin	4.05E-06	8.98E-05
Eotaxin.2	4.57E-06	9.23E-05
Matrix.Metalloproteinase.9..total..MMP.9..total.	5.99E-06	0.000110802
Trefoil.Factor.3..TFF3.	2.98E-05	0.000509089
Thrombomodulin..TM.	3.71E-05	0.000587842
P.Selectin	4.23E-05	0.000625318
Serum.Amyloid.P.Component..SAP.	5.15E-05	0.000714632
Fibulin.1C..Fib.1C.	5.79E-05	0.000756101
Stromal.cell.derived.factor.1..SDF.1.	9.41E-05	0.00116063
Clusterin..CLU.	0.000137	0.001522752
Sex.Hormone.Binding.Globulin..SHBG.	0.000143	0.001522752
CCL21	0.000147	0.001522752
Matrix.Metalloproteinase.3..MMP.3.	0.000151	0.001522752
Complement.Factor.H..CFH.	0.000182	0.001755165
Folate.receptor.gamma..FOLR3.	0.000218	0.002013258
Neutrophil.Gelatinase.Associated.Lipocalin..NGAL.	0.000244	0.002170751
Carcinoembryonic.Antigen..CEA.	0.000293	0.002437318
Thymus.and.activation.regulated.chemokine..TARC.	0.000296	0.002437318
Epithelial.cell.adhesion.molecule..EpCam.	0.000346	0.002739892
Epidermal.Growth.Factor..EGF.	0.000369	0.002826217
Insulin.like.Growth.Factor.Binding.Protein.2..IGFBP.2.	0.000498	0.003682711
Complement.Factor.H...Related.Protein.1..CFHR1.	0.000515	0.003685042
Growth.differentiation.factor.15..GDF.15.	0.000539	0.003741652
von.Willebrand.Factor..vWF.	0.000681	0.004582576
Adiponectin	0.000736	0.004717755

ST2	0.000744	0.004717755
Complement.C3..C3.	0.000772	0.004759747
C.Reactive.Protein..CRP.	0.000890	0.005269279
EN.RAGE	0.000902	0.005269279
Tissue.type.Plasminogen.activator..tPA.	0.000966	0.005498643
Pancreatic.secretory.trypsin.inhibitor..TATI.	0.001083	0.006011956
Vascular.endothelial.growth.factor.receptor.3..VEGFR.3.	0.001180	0.006337519
Tumor.necrosis.factor.ligand.superfamily.member.12..Tweak.	0.001222	0.006337519
Leptin	0.001228	0.006337519
C.C.motif.chemokine.15..CCL15.	0.001302	0.006566766
Brain.Derived.Neurotrophic.Factor..BDNF.	0.001587	0.007752375
CD5.Antigen.like..CD5L.	0.001606	0.007752375
Interleukin.18..IL.18.	0.002068	0.00976851
Epithelial.Derived.Neutrophil.Activating.Protein.78..ENA.78.	0.002150	0.009944649
Ferritin..FRTN.	0.002415	0.010942207
Lactoferrin..LTF.	0.004077	0.01810089
Prostasin	0.004334	0.01886553
Vascular.Endothelial.Growth.Factor.Receptor.2..VEGFR.2.	0.005201	0.021734634
Lectin.Like.Oxidized.LDL.Receptor.1..LOX.1.	0.005334	0.021734634
Vitamin.D.Binding.Protein..VDBP.	0.005366	0.021734634
Myeloid.Progenitor.Inhibitory.Factor.1..MPIF.1.	0.005420	0.021734634
Visceral.adipose.tissue...derived.serpin.A12..Vaspin.	0.005483	0.021734634
Beta.2.Microglobulin..B2M.	0.007797	0.030368757
Urokinase.type.plasminogen.activator.receptor..uPAR.	0.008102	0.031010144
Macrophage.Derived.Chemokine..MDC.	0.008539	0.032129666
Latency.Associated.Peptide.of.Transforming.Growth.Factor.beta.1..LAP.TGF.b1.	0.008766	0.032185008
Leptin.Receptor..Leptin.R.	0.008844	0.032185008
Angiopoietin.2..ANG.2.	0.010437	0.037371988
Angiopoietin.1..ANG.1.	0.011363	0.040040439
Erythropoietin..EPO.	0.011779	0.040858688
Interleukin.23..IL.23.	0.012023	0.041062136
Monocyte.Chemotactic.Protein.4..MCP.4.	0.012920	0.043459164
Hepatocyte.Growth.Factor.receptor..HGF.receptor.	0.013344	0.044213997
Alpha.1.Antitrypsin..AAT.	0.014721	0.048060835

e-Table 6: Analytes that remained stable after the treatment period- Visits 5 to 7-

Analyte	**p value**	**q value**
EN-RAGE	0.031495427	0.051367539
Stromal cell derived factor 1 (SDF1)	0.032104712	0.051367539
CCL21	0.044538921	0.067868832
CC motif chemokine 15 (CCL15)	0.049521353	0.072031059
CD5 Antigen like (CD5L)	0.053357451	0.074236454
Eotaxin.2	0.061605605	0.082140806
Hepatocyte Growth Factor Receptor (HGFR)	0.113655563	0.145479121
Visceral adipose tissue derived serpin A12 (Vaspin)	0.119173961	0.146675644
Carcinoembryonic Antigen (CEA)	0.296735732	0.351686793
Pancreatic secretory trypsin inhibitor (TATI)	0.312686245	0.357355709
Vascular endothelial growth factor receptor-3 (VEGFR-3)	0.475284429	0.524451783
Growth differentiation factor 15 (GDF 15)	0.53569849	0.571411723
Platelet endothelial cell adhesion molecule (PECAM 1)	0.613357896	0.633143635
Thymus and activation regulated chemokine (TARC)	0.839664604	0.839664604

e-Table 7: Analytes that were differentially regulated by low and high dose hydroxycholoroquine.

Analyte	Additional weekly log Analyte change in HD Mean ± SEM	p value
Monokine.Induced.by.Gamma.Interferon..MIG.	-0.01774±0.006	0.002925
Protein.S100.A6..S100.A6.	-0.02102±0.007	0.004137
Angiopoietin.related.protein.4..ANGPTL4.	0.00970±0.003	0.005628
Transferrin.receptor.protein.1..TFR1.	-0.00637±0.002	0.008919
Interleukin.18..IL.18.	-0.00914±0.004	0.013817
Interleukin.2.receptor.alpha..IL.2.receptor.alpha.	0.00494±0.002	0.017641
Macrophage.Colony.Stimulating.Factor.1..M.CSF.	-0.00922±0.004	0.018207
Leptin	0.00918±0.004	0.018447
Sclerostin	0.00590±0.002	0.021584
Interleukin.18.binding.protein..IL.18bp.	-0.00651±0.003	0.021840
Protein.DJ.1..DJ.1.	-0.01750±0.008	0.028757
Macrophage.Migration.Inhibitory.Factor..MIF.	-0.02154±0.010	0.032137
Vitronectin	-0.00734±0.003	0.032619
Interferon.inducible.T.cell.alpha.chemoattractant..ITAC.	-0.01512±0.007	0.035007
Visfatin	-0.01361±0.006	0.038130
CD163	-0.00531±0.003	0.042382
Interferon.gamma.Induced.Protein.10..IP.10.	-0.01262±0.006	0.048156

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