The biological effects of double-dose Alpha 1 Antitrypsin augmentation therapy: A pilot study

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Supplementary methods

Procedure to switch the brand of Alpha 1 Antitrypsin (AAT) therapy to Zemaira®

All study phases were performed using Zemaira® (CLS Behring, King of Prussia, PA, USA) as the augmentation therapy brand. Subjects with Alpha 1 Antitrypsin Deficiency (AATD) receiving AAT therapy with a brand other than Zemaira® (Glassia®, Shire; Prolastin®, Grifols, Inc.; Aralast™, Alpha Therapeutic Corporation), who were interested in participating in the study, were required to switch to Zemaira at least one month prior to study enrollment. The switching procedure was as follows:

- All patients were required to provide informed consent.
- The switch required coordination and approval by the patient's insurance company; if denied, the patient was considered to have failed the screening process.
- Each patient was given regular Zemaira® infusions at 60 mg/kg/week for at least 4 weeks prior to starting study procedures (study day 1).
- After conclusion of the study, the brand of AAT therapy to be used was based on patient preference.

Bronchoscopy

Bronchoscopy was performed under conscious sedation in a monitored setting as for usual clinical care according to published guidelines (1, 2). Procedure overview:

Bronchoalveolar lavage

1. After airway inspection, the bronchoscope was wedged in the right middle lobe or lingula and the collection trap was placed directly onto the bronchoscope's suction pipe.
2. Warmed normal saline (20 mL) was infused and the contents were gently aspirated; this first aspirate was placed in the collection trap in an ice bucket (contents labelled "bronchial BAL").
3. A new specimen trap was placed next to the bronchoscope and two additional 20-mL infusions of normal saline were infused; the aspirates were placed in the collection trap in an ice bucket (label contents as "alveolar BAL").

4. The total volumes infused for each bronchial and alveolar specimen were recorded.

5. Samples were kept on ice and immediately centrifuged at 1200 rpm for 10 minutes; supernatants were stored at −80°C in 1.5 mL aliquots until processed.

Blood samples

1. Blood for cytokines and other specific research measures was collected in EDTA tubes and centrifuged at 2500 (RC) for 15 minutes at 4°C; isolated plasma was stored in 1 mL aliquots at −80°C.

2. Blood used for AAT, C-reactive protein and clinical laboratory measures was collected in citrate and EDTA tubes and sent to the local clinical laboratory for processing (Quest Diagnostics, Secaucus , NJ)

Table E1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria

– Males or females aged between 18 and 75 years
– Diagnosis of AATD
  – PiZZ, PiSZ or Znull
  – Baseline AAT level <11 µM[†]
– Evidence of COPD (emphysema or airflow obstruction) with FEV_1 <80% predicted
– Receiving therapy with Zemaira® for ≥1 month at the standard dose of 60 mg/kg/week
– At least one of the following criteria of disease severity:
  1. Two or more acute exacerbations in the past 12 months
    – Defined as the use of antibiotics and a course of steroids to treat a flare of pulmonary symptoms, regardless of whether or not the subject required ER care or hospital admission
  2. SGRQ total score >60
  3. Chronic bronchitis
    – Defined as daily or almost daily sputum expectoration at least 3 months of the year for at least two consecutive years
  4. Documented FEV_1 decline of ≥60 mL/year for two consecutive years while receiving AAT therapy
	– FEV_1 <40% predicted (bronchoscopy safety)
	– Patients participating in other clinical trials
	– Recent exacerbation (<4 weeks)
	– Use of chronic antibiotics or oral steroids
	– Continued smoking (per patient report)
	– Contraindications for bronchoscopy (i.e., coagulopathy or severe hypoxemia)
	– Inability to sign informed consent
	– Use of systemic steroids within the last month
	– Pregnant or wishing to become pregnant
	– Known IgA deficiency (only patients already receiving AAT therapy will be included; therefore, unlikely to encounter this exclusion criterion)

[†]Historical baseline AAT levels were obtained from medical records and correspond to levels recorded before the initiation of AAT therapy

AAT, alpha-1 antitrypsin; AATD, alpha-1 antitrypsin Deficiency; COPD, chronic obstructive pulmonary disease; ER, emergency room; FEV_1, forced expiratory volume in one second; SGRQ, St. George's Respiratory Questionnaire

Table E2: Patient pulmonary function following AAT therapy for patients who completed all study procedures (n=8)

Variables Mean ± StD	SD AAT therapy (60 mg/kg/week)	DD AAT therapy (120 mg/kg/week)
FVC, L	3.8 ± 0.4	3.8 ± 0.3
FVC, % predicted	88.3 ± 5.3	87.9 ± 5.1
FEV_1, L	1.7 ± 2.3	1.8 ± 2.4
FEV, % predicted	50.9 ± 4.1	53.9 ± 4.4
FEV_1/FVC	44.8 ± 2.2	47.7 ± 2.5
TLC, L	7.9 ± 0.4	–
TLC, % predicted	123.3 ± 7.5	–
IC, L	2.7 ± 0.3	–
IC, % predicted	88.3 ± 6.9	–
DL_{CO}, % predicted	57.0 ± 5.4	–

AAT, alpha 1 antitrypsin; DD, double dose; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; FEV, forced expiratory volume; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IC, inspiratory capacity; StD, standard deviation; TLC, total lung capacity

Table E3: Pathway analysis of cytokines studied

Pathway	Genes involved
Cytokine-cytokine receptor interaction	CCL11, CCL3, CCL7, CSF1, CSF2, IFNγ, IL-10, IL-15, IL12p40, IL-17, IL-2, IL-3, IL-4, IL-9, LIF, TNFα
Jak-STAT signaling pathway	CSF2, IFNγ, IL-10, IL12p40, 1L-15, 1L-2, IL-3, 1L-4, 1L-9, LIF
Rheumatoid arthritis	CCL3, CSF1, CSF2, IFNγ, IL-15, IL-17, MMP-1, TNFα
Inflammatory bowel disease	IFNγ, IL-10, IL12p40, 1L-17, IL-2, IL-4, TNFα
Asthma	CCL11, IL-10, IL-3, IL-4, IL-9, TNFα
T cell receptor signaling pathway	CSF2, IFNγ, IL-10, IL-2, IL-4, TNFα
TNF signaling	CSF1, CSF2, IL-15, LIF, MMP-9, TNFα
Allograft rejection	IFNγ, IL-10, IL12p40, 1L-2, IL-4, TNFα

CCL, chemokine (C-C motif) ligand; CSF, Colony Stimulating Factor; IFN, interferon; IL, interleukin;

LIF, leukemia inhibitory factor; MMP, matrix metalloproteinase; TNF, tumor necrosis factor

Table E4: BALF concentrations of multiple cytokines, chemokines, and growth factors following the three phases of AAT therapy

BALF concentration, pg/mL	AAT therapy phase			p value
	SD	DD	SD	SD vs. DD* / DD vs. SD**
G-CSF	58.4 (11–111)	35.3 (8–66)	61.4 (23–95)	0.146 / 0.175
IFNα2	335.7 (97–627)	266.3 (120–547)	217.2 (56–513)	0.330 / 0.163
IL-1α	405.2 (89–1686)	185 (55–550)	227.5 (91–658)	0.148 / 0.250
LIF	810.5 (154–1788)	353.8 (212–987)	366.5 (180–968)	0.148 / 0.547
IL-15	1360 (342–4692)	681.7 (363–2247)	694.0 (294–1832)	0.074 / 0.062
MCP3	395.2 (65–816)	198.5 (88–355)	170.1 (82–462)	0.078 / 0.077
IL-1β	94 (12–334)	55.4 (13–254)	16.0 (8–101)	0.919 / 0.110
IL-1RA	3229 (2043–63378)	3913 (2342–25161)	2447 (434–45566)	0.688 / 0.461
IL-2RA	288.8 (87–691)	136.0 (55–612)	177.8 (57–501)	0.250 / 0.742
IL-5	99.1 (3–231)	66.9 (32–148)	53.5 (10–132)	0.651 / 0.337
IL-6	618.9 (198–1606)	650.2 (141–959)	328.4 (142–739)	0.396 / 0.187
IL-7	1085 (408–3503)	1118 (594–2064)	837.6 (418–1826)	0.547 / 0.641
IL-8	5467 (1235–24522)	4277 (642–11208)	3115 (1243–9061)	0.844 / 0.547
IL-12p70	924.2 (319–1919)	655.1(286–1034)	547.1 (182–1388)	0.184 / 0.687
IL-13	112.4(47–234)	132.7 (27–232)	73.1 (16–160)	0.102 / 0.082
IL-16	5542 (2225–20664)	5175 (2259–20253)	5428 (1172–15891)	0.844 / 0.383
IL-18	124.2 (56–222)	65.2 (32–188)	52.6 (26–180)	0.109 / 0.383
CXCL1/GROα	41773 (8418–97319)	19792 (8164–55949)	23117 (13633–60566)	0.109 / 0.945
CXCL9	5058 (2395–8382)	3760 (1532–34187)	3716 (516–8598)	0.844 / 0.195
CXCL-10/IP-10	37121 (23474–97909)	34164 (11682–143990)	31762 (9518–50069)	0.742 / 0.109
CXCL12/SDF1α	9267 (2439–31167)	7166 (4084–18199)	6340 (2443–19806)	0.461 / 0.641
CCL2/MCP-1	2029 (697–4200)	1132 (341–7744)	1052 (285–6651)	0.641 / 0.195

CCL4/MIP1β	1563 (572–5476)	1298 (638–3319)	829.0 (413–2683)	0.547 / 0.555
PDGF-BB	761.4 (324–2072)	773.3 (289–968)	610.2 (252–1643)	0.233 / 0.962
HGF	1294 (498–3752)	1209 (449–2452)	1174 (169–2798)	0.445 / 0.767
βNGF	82.4 (25–157)	76.8 (27–118)	62.1 (14–123)	0.404 / 0.148
VEGF	14577 (5397–51697)	11050 (7419–23795)	10309 (2970–24458)	0.461 / 0.641
CCL5/RANTES	14577 (5397–51697)	11050 (7419–23795)	10309 (2970–24458)	0.461 / 0.641
CCL27/CTAK	845.1 (219–2052)	786.9 (181–1674)	546.9 (110–2039)	0.250 / 0.383
SCF	788.9 (355–1492)	594.5 (212–901)	479.9 (92–953)	0.127 / 0.423
SCGF-β	647.2 (43–2895)	1332 (311–1867)	894.5 (264–1732)	0.881 / 0.296
TNFβ	5.2 (4–19)	4.9 (3–11)	5.2 (4–9)	0.250 / 0.461
TRAIL	7087 (2530–32589)	7644 (1750–12137)	4443 (2240–9644)	0.742 / 0.461

Data presented as median (range); *p value comparing SD (starting doses) to DD; **p value comparing DD to SD (final doses)

AAT, Alpha 1 antitrypsin; CCL, chemokine (C-C motif) ligand; CTACK, Cutaneous T-cell-attracting chemokine; CXC, chemokine (C-X-C motif) ligand;
DD, double dose; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; IP, Interferon gamma-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NGF, nerve growth factor; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; SCF, stem cell factor; SCGF, stem cell growth factor; SD, standard dose; SDF, stromal cell-derived factor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor

Table E5: Plasma concentrations of multiple cytokines, chemokines, and growth factors following the three phases of AAT therapy

Plasma concentration, pg/mL	AAT therapy phase			p value SD vs. DD* / DD vs. SD**
	SD	DD	SD	
IL-1β	6.8 (1–33)	7.7 (1–25)	5.4 (1–35)	0.844 / 0.578
IL-2	130.0 (9–320)	124 (6–227)	39.6 (3–292)	0.844 / 0.109
IL-4	13.0 (3–32)	11.7 (2–32)	6.8 (2–38)	0.945 / 0.109
IL-5	29.3 (2–190)	28.1 (1–183)	2.9 (1–254)	0.742 / 0.313
IL6	202.4 (9–554)	196.9 (11–672)	77.9 (5–502)	0.945/ 0.063
IL-10	66.8 (3–232)	69.9 (3–178)	32.2 (4–229)	0.997 / 0.195
IL-12p70	11.1 (1–34)	9.5 (1–35)	4.1 (2–52)	0.945 / 0.297
IFNγ	43.7 (7–178)	67.9 (6–154)	28.0 (5–192)	0.945 / 0.383
TNFα	4.7 (1–27)	8.2 (1–20)	4.1 (1–38)	0.688 / 0.578
α-2 macroglobulin	114356 (50275–633741)	138640 (60392–636758)	192000 (60773–307520)	0.742 / 0.195
CRP	364.3 (118–1037)	262.0 (142–5870)	286.7 (94–1337)	0.641 / 0.640
Ferritin	16462 (3996–29630)	11380 (2878–45935)	14874 (2909–44483)	0.996 / 0.641
Fibrinogen	65999 (32876–141400)	87763 (16339–206335)	117385 (36066–250824)	0.365 / 0.354
Haptoglobin	98859 (30485–3960000)	117740 (38432–3440000)	268488 (11679–4440007)	0.945 / 0.078
Procalcitonin	11986 (267–21045)	9626 (21–23136)	9920 (202–21045)	0.931 / 0.978
Amyloid A	1772 (376–2865)	1512 (213–7525)	1368 (381–3245)	0.742 / 0.945
Plasminogen	12843 (4381–66937)	8287 (70–69998)	13231 (446–55870)	0.641 / 0.813
Amyloid P	9938 (2387–20544)	8610 (1111–23130)	9047 (930–19910)	0.887 / 0.763

Data presented as median (range); *p value comparing SD (starting doses) to DD; **p value comparing DD to SD (final doses)

AAT, Alpha 1 antitrypsin; CRP, C-reactive protein; DD, double dose; IL, interleukin; IFN, interferon; SD, standard dose; TNF, tumor necrosis factor

Table E6: Summary of reported AEs and SAEs

Subject Number	Study Phase	Description	Causality/Relation to DD AAT therapy	Causality/Relation to study procedures	Severity, Seriousness[†]
3	1	Mild stridor post-bronchoscopy; found to have oropharyngeal candidiasis (removed from study)	Not related	Likely	Mild, Not serious
7	1	Mild COPD exacerbation	Not related	Not related	Mild, Not serious
9	1	Mild COPD exacerbation	Not related	Not related	Mild, Not serious
10	1	Mild COPD exacerbation	Not related	Not related	Mild, Not serious
1	1	Dyspnea, hemoptysis requiring hospitalization 3 days after bronchoscopy – probable exacerbation (removed from study)	Not related	Probable	Severe, Serious
3	1	Severe COPD exacerbation requiring hospitalization, influenza	Not related	Not related	Severe, Serious
5	2	Arm bruise	Not related	Likely	Mild, Not serious
6	2	Nausea and anxiety 1 day post-bronchoscopy	Not related	Probable	Mild, Not serious
		Rash on forearm	Not related	Not related	Mild, Not serious
8	3	Severe COPD exacerbation requiring hospitalization, hemoptysis, pneumonia days after third bronchoscopy	Not related	Probable	Severe, Serious

[†]Defined based on treatment usage

AAT, alpha-1 antitrypsin; AE, adverse event; COPD, chronic obstructive pulmonary disease; DD, double dose; SAE, serious adverse event.

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