(Figure 1). This injurious inflation pattern is known to cause overstretch and tidal recruitment in dependent, atelectatic lung, and thereby worsen dependent lung injury (7–9). Reverse triggering increased dependent lung stretch equivalent to that applied by V_T of up to 15 ml/kg during muscle paralysis, despite a constant V_T (Figure 2).

Second, the magnitude of negative swing in P_{es} during reverse triggering is proportional to the local lung stretch in dependent lung, reflecting greater propensity to injury. Monitoring P_{es} or electrical activity of diaphragm could contribute in two ways. Each of these modalities increases the recognition of reverse triggering, and they quantify the magnitude of inspiratory muscle force during reverse triggering. Although reverse triggering associated with breath stacking is readily detected, in the absence of breath stacking it is likely to be unnoticed by clinicians relying on standard waveforms (e.g., airway pressure and flow). In addition, it will be underestimated when V_T is controlled (4).

Reverse triggering occurs in deeply sedated patients, a scenario in which clinicians consider the risk for asynchrony to be minimal (4), in contrast to easily detected vigorous effort, which is widely recognized to be harmful (2, 10). Thus, sedation may reduce harm from vigorous effort but increase the incidence of subtle and unsuspected reverse triggering.

Finally, estimation of inspiratory muscle force during reverse triggering may be important to decrease dependent lung stretch and potentially lessen injury. It is uncertain whether the inflation pattern associated with reverse triggering is also observed with other ventilator modes.

In conclusion, during reverse triggering, injurious inflation occurred in dependent lung despite the constant overall V_T , and dependent lung stretch was proportional to the negative deflection in P_{es} . Early recognition of reverse triggering may be beneficial. ■

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References

1. Mauri T, Yoshida T, Bellani G, Goligher EC, Carteaux G, Rittayamai N, et al.; PLeUral pressure working Group (PLUG—Acute Respiratory

- Failure section of the European Society of Intensive Care Medicine). Esophageal and transpulmonary pressure in the clinical setting: meaning, usefulness and perspectives. *Intensive Care Med* 2016;42:1360–1373.
2. Thille AW, Rodriguez P, Cabello B, Lellouche F, Brochard L. Patient-ventilator asynchrony during assisted mechanical ventilation. *Intensive Care Med* 2006;32:1515–1522.
3. Blanch L, Villagra A, Sales B, Montanya J, Lucangelo U, Luján M, et al. Asynchronies during mechanical ventilation are associated with mortality. *Intensive Care Med* 2015;41:633–641.
4. Akoumianaki E, Lyazidi A, Rey N, Matamis D, Perez-Martinez N, Giraud R, et al. Mechanical ventilation-induced reverse-triggered breaths: a frequently unrecognized form of neuromechanical coupling. *Chest* 2013;143:927–938.
5. Colombo D, Cammarota G, Alemani M, Carena L, Barra FL, Vaschetto R, et al. Efficacy of ventilator waveforms observation in detecting patient-ventilator asynchrony. *Crit Care Med* 2011;39:2452–2457.
6. Chanques G, Kress JP, Pohlman A, Patel S, Poston J, Jaber S, et al. Impact of ventilator adjustment and sedation-analgesia practices on severe asynchrony in patients ventilated in assist-control mode. *Crit Care Med* 2013;41:2177–2187.
7. Yoshida T, Torsani V, Gomes S, De Santis RR, Beraldo MA, Costa EL, et al. Spontaneous effort causes occult pendelluft during mechanical ventilation. *Am J Respir Crit Care Med* 2013;188:1420–1427.
8. Yoshida T, Nakahashi S, Nakamura MAM, Koyama Y, Roldan R, Torsani V, et al. Volume-controlled ventilation does not prevent injurious inflation during spontaneous effort. *Am J Respir Crit Care Med* 2017;196:590–601.
9. Morais CCA, Koyama Y, Yoshida T, Plens GM, Gomes S, Lima CAS, et al. High positive end-expiratory pressure renders spontaneous effort noninjurious. *Am J Respir Crit Care Med* 2018;197:1285–1296.
10. Yoshida T, Fujino Y, Amato MB, Kavanagh BP. Fifty years of research in ARDS: spontaneous breathing during mechanical ventilation: risks, mechanisms, and management. *Am J Respir Crit Care Med* 2017;195:985–992.

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Ataluren, a New Therapeutic for Alpha-1 Antitrypsin-Deficient Individuals with Nonsense Mutations

To the Editor:

AAT (alpha-1 antitrypsin) deficiency (AATD) is a hereditary disorder characterized by low circulating levels of AAT and development of chronic obstructive pulmonary disease (1). The decreased levels of AAT in the lung lead to a protease–antiprotease imbalance and unopposed NE (neutrophil elastase) activity. Infusion of plasma-purified AAT protein (“augmentation therapy”) has proven therapeutic benefit in AATD (2, 3). However, plasma-purified AAT is not a limitless resource, and alternative treatments for AATD are necessary. Null mutations of AAT include nonsense and frameshift mutations, resulting in a premature termination codon (PTC) in the

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mRNA coding region. Various read-through compounds including ataluren (PTC124) have been shown to suppress disease-causing PTCs in mammalian cells (4), and clinical studies in patients with Duchenne muscular dystrophy (5) and cystic fibrosis showed initial promise (6). Here, we extend these concepts to AATD. Some of the results of this study have been previously reported in the form of an abstract (7).

Methodology

Patient demographics and AAT studies. The $Q0_{bolton}$ mutation was diagnosed by sequencing all coding exons (II–V) of the AAT gene (*SERPINA1*, RefSeq: NG_008290). Healthy donor (MM) and patient-specific induced pluripotent stem cell (iPSC)-derived hepatic cells were generated as previously described (8). AAT was isolated from plasma by use of Alpha-1 Antitrypsin Select Resin and chromatographed by fast protein liquid chromatography (GE Healthcare Life Sciences).

Statistical analysis. Data sets were analyzed for statistical significance using GraphPad Prism 5.0 software package (GraphPad Software), with significance determined at $P < 0.05$. For data sets with three or more paired groups, a repeated measures ANOVA was employed, followed by a post hoc Bonferroni multiple comparison test. Results are expressed as mean $\pm$ SEM.

Results

A 46-year-old never-smoker presented with dyspnea, obstructive lung disease (FEV₁, 59% predicted; FEV₁/FVC, 55%; DL_{CO}, 56% predicted), and chest computed tomography scan showing bi-basal pan-acinar emphysema. Plasma AAT was undetectable by routine nephelometry. After sequencing of all coding exons (II–V) of the *SERPINA1* gene, the patient was identified as homozygous for the $Q0_{bolton}$ mutation, with two PTCs at amino acid 373 and 374 on

exon V. Molecular dynamics simulations of $Q0_{bolton}$ -AAT suggested sufficient interactions to stabilize native-like protein structure, should it form during the early steps of protein folding. As a consequence, we investigated the potential presence of $Q0_{bolton}$ -AAT protein in the patient's plasma by chromatography and two-dimensional fluorescence difference gel electrophoresis and successfully identified $Q0_{bolton}$ -AAT as a truncated protein of a molecular mass of 49 kD (Figure 1A).

The main function of AAT is to act as an antiprotease; thus, ensuing experiments investigated the ability of patient-purified $Q0_{bolton}$ -AAT to inhibit NE. The Western blot in Figure 1B (top) depicts the AAT complex that occurs when M-AAT (52 kD) inactivates NE (29 kD) at a 1:1 ratio, with no free AAT remaining. Although less effective than M-AAT, an increase in $Q0_{bolton}$ -AAT-NE binding, indicated by an increase in the intensity of an immunoband of approximately 78 kD, during the 20-minute time course was observed (Figure 1B, lower).

In subsequent experiments, we evaluated read-through compounds for their ability to overcome the PTC in the *SERPINA1* gene in the $Q0_{bolton}$ -AAT, leading to translation of the full-length AAT protein. iPSC-derived hepatic cells from this individual were treated with ataluren (62.5 μ g/ml) for 48 hours. For $Q0_{bolton}$ -iPSC-hepatic cells, a significant 10-fold increase in AAT mRNA expression levels was observed compared with untreated cells ($P < 0.05$). Moreover, a significant 2-fold increase in the concentration of secreted $Q0_{bolton}$ -AAT protein was recorded with ataluren treatment ($P < 0.001$; Figure 2A). By Western blot analyses, it was demonstrated that the reduced molecular mass of $Q0_{bolton}$ -AAT (49 kD) compared with M-AAT (52 kD) persisted (Figure 2B).

Discussion

In this study, the discovery of the truncated size of plasma-purified $Q0_{bolton}$ -AAT protein of 49 kD is expected because of the position

Figure 1. Premature stop codon in the *SERPINA1* gene encodes a truncated functional AAT (alpha-1 antitrypsin) protein. (A) Plasma-purified healthy donor (M)-AAT and patient $Q0_{bolton}$ -AAT protein were CyDye fluorescently labeled before two-dimensional gel analyses, using linear pH 4–7 immobilized pH gradient strips. Cy5-labeled $Q0_{bolton}$ -AAT (red and indicated by white bracket) demonstrated multiple glycoforms of a lower molecular mass and negative charge compared with Cy3-labeled M-AAT (green). (B) Formation of AAT:NE (neutrophil elastase) inhibitory complexes. M-AAT (top) was incubated with NE at a AAT:NE molar ratio of 1:1 for 10 minutes. Reactions were subjected to Western blot analysis employing a goat anti-human AAT antibody for AAT or AAT:NE complexes. M-AAT incubated with NE resulted in the formation of an 81-kD AAT:NE complex, with no free AAT (52 kD) remaining. $Q0_{bolton}$ -AAT incubated with NE for 0, 5, 10, or 20 minutes resulted in the formation of a 78-kD AAT:NE complex. Experiments illustrated are representative of three separate experiments.

Figure 2. Increased healthy donor (M)-AAT (alpha-1 antitrypsin) and Q0_{bolton}-AAT production by donor-derived induced pluripotent stem cell (iPSC)-hepatic cells in response to ataluren. (A) AAT gene and protein expression, by MM healthy control and Q0_{bolton} patient-derived iPSC-hepatic cells in response to ataluren (At; 62.5 μ g/ml) for 48 hours. Q0_{bolton}-AAT mRNA expression ($P < 0.05$), and levels of secreted M-AAT ($P < 0.01$) and Q0_{bolton}-AAT ($P < 0.001$) protein measured by ELISA, were significantly increased in the presence of At compared with untreated (Un) cells (ANOVA, three independent experiments). (B) Western blot analyses of extracellular secreted AAT from MM healthy control and Q0_{bolton} patient-derived iPSC-hepatic cells treated with ataluren. The electrophoretic mobility of 52-kD M-AAT and 49 kD truncated Q0_{bolton} AAT was observed. Whole-cell lysates were prepared, and Western blots for actin demonstrated equal protein loading supporting the use of equal cell numbers per reaction (representative blots of three separate experiments).

of the PTC on the mRNA sequence; however, the circulating retention time of Q0_{bolton}-AAT protein and the degree to which it is distributed in the body would need further investigation. Indeed, the lower abundance of the circulating protein may indicate an attempt to eradicate the aberrant protein at a cellular level. This is the case with other null AATD proteins, including Q0_{hongkong} (1), which is removed by endoplasmic reticulum-associated degradation. Moreover, for PTCs located more than 50 bp upstream of the most 3' exon-exon junction of the mRNA, nonsense-mediated decay is thought to eradicate the transcript (9). As a consequence, in contrast to Q0_{hongkong}, which is characterized by a complete absence of secreted protein, the Q0_{bolton}-AAT protein we have identified may either represent protein that has escaped nonsense-mediated decay or is not targeted for destruction because of the terminal location of the PTC.

In the current study, the Q0_{bolton}-AAT protein retained anti-NE capacity at a lower level than M-AAT but may provide lung tissue protection if present in sufficient quantity. We assessed the ability of ataluren (PTC124) to augment the levels of AAT protein. Ataluren has the ability to read through stop codons, with read-through highest in the genetic code UGA, followed by UAG and then UAA. The Q0_{bolton} mutation results in UAA in the coding region, suggesting that ataluren may potentially trigger read-through and production of full-length protein. Intriguingly, in this study, ataluren enhanced protein levels of both M-AAT and Q0_{bolton}-AAT in iPSC-derived hepatocytes, an effect most likely resulting from the stabilizing influence of ataluren on mRNA levels (10). Conversely, read-through was not apparent, as indicated by the persistence of truncated Q0_{bolton}-AAT protein of 49 kD. As ataluren increased the secretion of AAT regardless of the two genotypes, it would be interesting to study whether the same for the much more common Z variant could occur; however, the pros and cons of increased plasma Z-AAT would need to be carefully studied.

In conclusion, this study demonstrates the potential use of ataluren to induce production and secretion of functional

Q0_{bolton}-AAT protein *in vivo*. By this approach, grouping AATD mutations according to theratype could provide new therapeutic options, although optimization to reach the necessary protective levels is required. ■

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References

1. Ferrarotti I, Carroll TP, Ottaviani S, Fra AM, O'Brien G, Molloy K, *et al.* Identification and characterisation of eight novel SERPINA1 null mutations. *Orphanet J Rare Dis* 2014;26:172.
2. Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, *et al.*; RAPID Trial Study Group. Intravenous augmentation treatment and lung density in severe α 1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; 386:360–368.
3. McElvaney NG, Burdon J, Holmes M, Glanville A, Wark PA, Thompson PJ, *et al.*; RAPID Extension Trial Group. Long-term efficacy and safety of α 1 proteinase inhibitor treatment for emphysema caused by severe α 1 antitrypsin deficiency: an open-label extension trial (RAPID-OLE). *Lancet Respir Med* 2017;5:51–60.
4. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, *et al.* PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007;447:87–91.
5. McDonald CM, Campbell C, Torricelli RE, Finkel RS, Flanigan KM, Goemans N, *et al.*; Clinical Evaluator Training Group; ACT DMD Study Group. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;390:1489–1498.
6. Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, *et al.*; Cystic Fibrosis Ataluren Study Group. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med* 2014;2:539–547.
7. O'Dwyer CA, McCarthy C, Saldova R, Carroll TP, Bergin DA, Henry M, *et al.* Targeting nonsense mutations for treatment of alpha-1 antitrypsin deficiency: implications for chronic obstructive pulmonary disease [abstract]. *Am J Respir Crit Care Med* 2015;191:A3639.
8. Wilson AA, Ying L, Liesa M, Segeritz CP, Mills JA, Shen SS, *et al.* Emergence of a stage-dependent human liver disease signature with directed differentiation of alpha-1 antitrypsin-deficient iPS cells. *Stem Cell Reports* 2015;4:873–885.
9. Mort M, Ivanov D, Cooper DN, Chuzhanova NA. A meta-analysis of nonsense mutations causing human genetic disease. *Hum Mutat* 2008;29:1037–1047.
10. Allamand V, Bidou L, Arakawa M, Floquet C, Shiozuka M, Paturneau-Jouas M, *et al.* Drug-induced readthrough of premature stop codons leads to the stabilization of laminin alpha2 chain mRNA in CMD myotubes. *J Gene Med* 2008;10:217–224.

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Does Dose Matter?

To the Editor:

Stolz and colleagues (1) have presented some exciting results showing the potential for intensified long-acting β -agonist (LABA) and inhaled corticosteroid (ICS) therapy to reduce severe exacerbations, particularly in those patients with severe chronic

obstructive pulmonary disease (COPD). We note that low-dose maintenance therapy with budesonide (200 μ g)/formoterol (6 μ g) twice daily was used as standard therapy.

However, studies recommending the use of budesonide/formoterol in COPD have used maintenance therapy at twice this dose (2, 3). It would seem that patients in this study may therefore have been receiving subtherapeutic doses throughout the trial and then received the recommended dose only when they developed upper respiratory tract symptoms. We are interested to understand why the authors chose this low-dose protocol. Is it possible that if patients simply used the recommended dose at all times, they would get even greater benefits? There is no analysis of patients who did not develop symptoms of an upper respiratory tract infection, and it is unclear what effect the low-dose ICS/LABA had on these patients.

Although the authors state that 54.7% of participants were using triple therapy (LABA, LAMA [long-acting muscarinic antagonist], and ICS) and that the use of LAMA therapy was equivalent between placebo and intervention groups, it is unclear what dose of LABA and ICS therapy patients were receiving before the trial. If patients had been taking higher doses they may have had their doses decreased when initiated into the trial. This may have resulted in an increased exacerbation rate. If there had been a difference between groups we wonder if this may have affected the results. Was there a run-in period for patients to acclimatize to their new inhalers?

We note there was quite a high death rate (11%) when compared with previous COPD trials—the FLAME (Effect of Indacaterol Glycopyrronium vs. Fluticasone Salmeterol on COPD Exacerbations) trial in 2016 reported a 1.4% death rate (4). The authors have not commented on this in their results. It is unclear whether mortality differed between groups. Is it possible that some of the excess mortality could be attributed to undertreatment with budesonide/formoterol? Although the data show that the intervention prevented severe exacerbations, it is unclear whether it had any effect on overall mortality.

Clarification of these points would enhance the usefulness of this interesting trial in guiding future practice. ■

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References

1. Stolz D, Hirsch HH, Schilter D, Louis R, Rakic J, Boeck L, *et al.* Intensified therapy with inhaled corticosteroids and long-acting β_2 -agonists at the onset of upper respiratory tract infection to prevent chronic obstructive pulmonary disease exacerbations: a multicenter, randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2018;197:1136–1146.
2. Make BJ, Eriksson G, Calverley PM, Jenkins CR, Postma DS, Peterson S, *et al.* A score to predict short-term risk of COPD exacerbations (SCOPEX). *Int J Chron Obstruct Pulmon Dis* 2015;10:201–209.

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