Supplementary Data

Dual Hybridization ELISA

To liquify the CF sputum samples, samples were thawed on ice and 1×sputum liquifier (Dalynn Biologicals) was added in a 1:1 (v/v) ratio. After a 15-minute incubation at 37°C, a serial dilution using phosphate-buffered saline (PBS) was made in duplicate for every sample. A standard curve of eluforsen in PBS was prepared as well.

A volume of 40 μL 10% sodium dodecyl sulfate (SDS) (Sigma Aldrich) was pipetted to all wells of a 96-well polymerase chain reaction (PCR) plate (Greiner), followed by adding 40 μL of the standard curve or sample dilutions in duplicate. A standard curve (duplicate) and a blank sample (duplicate) as negative control were also prepared for each plate. Next, 160 μL of a capture and detection probe (Midland) in 6×SSPE mixture was added to each well, and plates were sealed (Axygen Scientific, Inc.) and placed in a thermocycler. A program consisting of a 5-minute cycle at 80°C followed by a 1-hour cycle at 42°C was followed to allow sample and probe hybridization.

Next, a NeutrAvidin-coated 96-well plate (Pierce) was washed once with wash buffer (50 nM Trizma-HCl, 0.6M NaCl, 0.2@ Tween-20). Afterward, the plate was tapped on paper towel to remove access wash buffer. After completing the thermocycler program, a volume of 100 μL per sample was pipetted from the PCR plate into the NeutrAvidin plate. The NeutrAvidin plate was then closed with a plate sealer and incubated at room temperature (RT) for 1 hour on an orbital plate shaker to allow the hybridized sample to attach to the plate.

Next, the NeutrAvidin plate was washed twice with wash buffer, and a volume of 100 μL of anti-digoxigenin-AP (Abcam) working solution was added to each well. The plate was covered with a plate sealer and incubated at room temperature for 30 minutes on an orbital plate shaker. After the anti-digoxigenin-AP incubation was complete, the plate was washed twice with wash buffer, and 100 μL of Atto-Phos® (Promega) working solution was added to each well. The plate was incubated in the dark at RT for 30 minutes, and 50 μL of 20% ethylenediamine tetraacetic acid (EDTA) (Sigma Aldrich) stop solution was added to every well. The wells were analyzed by a Wallac Victor2 plate reader.

Hybridization High-Performance Liquid Chromatography (HPLC)

Frozen organs were crushed using a mortar, and a weight of 40–60 mg of powder per tissue was placed in a 1.5 mL eppendorf vial. Subsequently, tissue samples were stored at −80°C on dry ice before further use. A volume of 1 mL lysis solution was added to each tissue sample, and samples were treated with an ultrasonic stick until the mixture appeared homogenous, or for a maximum of 1 minute. For serum samples, the lysis solution was added in a 1:3 (v/v) ratio to thawed samples and was subsequently mixed. Subsequently, tissue and serum lysis samples were incubated for 30 minutes at 65°C to allow lysis to take place.

In a new PCR plate, 100 μL of the hybridization master mix was added to each well. Also, the required volume of the lysis solution was added (volume dependent on expected eluforsen concentration). Water was added to a final volume of 200 μL. Subsequently, the plate was sealed and centrifuged for 1 minute at 4000 rpm, after which it was incubated at 95°C for 15 minutes. After incubation, the plate was placed on ice for 5 minutes followed by 1-minute centrifuging at 4000 rpm and placing it into an high-performance liquid chromatography (HPLC) autosampler. Samples were analyzed with a Dionex HPLC-System Ultimate 3000 consisting of a Shimadzu Fluorescence Detector 20Axs, Ultimate pump DGP-3600A, Ultimate column heater TCC-3100 using a DNA-Pac PA200 column, precolumn-filter holder, and peek frit UHMW 2 μm. A flow rate of 1 mL/min and eluent buffers A (25 mM Tris-buffer pH 8.0, 30% acetonitrile, 1.5 M urea, and 1 mM EDTA), B (800 mM NaClO in A), and C (2.5 M NaClO44 in A) were used. The detection was performed at an excitation @ 436 nm and emission @ 484 nm. For all samples, an injection of 100 μL volume was used.

Ion-Pairing Reversed-Phase HPLC

Sample preparation was performed by diluting the samples down to ~0.2 mg/mL with mobile phase A, which consisted of 100 mM HFIP and 15 mM HEA. A volume of 10 μL of the diluted sample was injected into an HPLC-System Ultimate 3000 RS consisting of Ultimate 3000 RS LPG-Pump, Ultimate 3000 RS Autosampler, Ultimate 3000 RS Column Compartment, and Ultimate 3000 RS Variable Wavelength Detector with a Waters Acquity UPLC BEH130 C18 column. A flow rate of 250 μL/min and mobile phases A and B (100 mM HFIP, 15 mM HEA, and 80% MeOH) were used. Detection was performed at 260/280 nm. This method is stability indicating, allowing the (quantitative) detection of impurities and breakdown products.

Ion Exchange-High Performance Liquid Chromatography (IEX-HPLC)

Samples were pretreated with proteinase K before IEX-HPLC analysis. A sample volume of 20 μL was injected into an HPLC-System Ultimate 3000 consisting of Ultimate Pump DGP-3600, Ultimate Autosampler WPS-3000SD Analytical, Ultimate Column Heater TCC-3000SD, and Ultimate UV Detector MWD-3000 with a DNAPac PA-200RS column. A flow rate of 1.5 mL/min and mobile phases A [25 mM TRIS/HCl (pH 8), 1 mM EDTA, and 25% can] and B [25 mM TRIS/HCl (pH 8), 1 mM EDTA, 1500 mM LiBr, and 25% can] were used. Detection was performed at 260/280 nm. This method is stability indicating, allowing the (quantitative) detection of impurities and breakdown products.