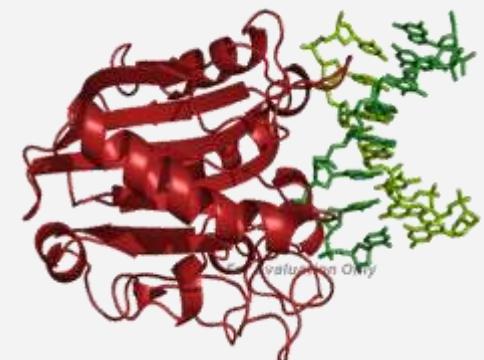


Alidornase alfa-
Actin Inhibition Resistance DNase:
In vitro & ex vivo Efficacy and Delivery

Dr. Yoseph Shaaltiel
Protalix

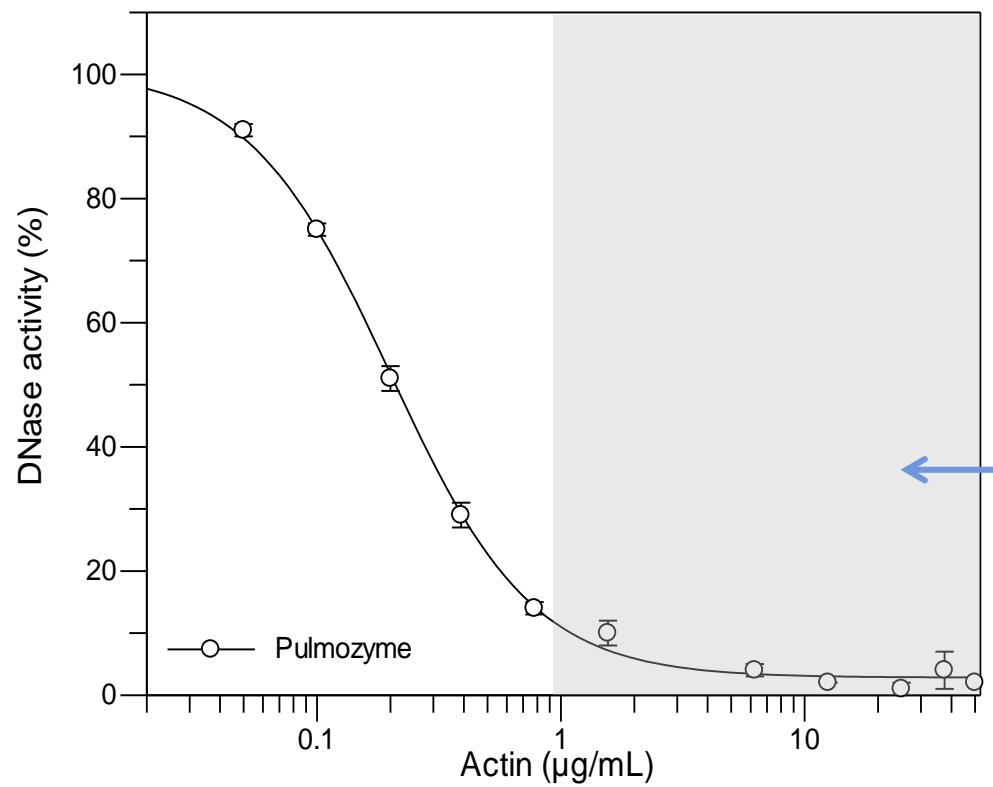


alidornase alfa - Product Rationale

Protalix aim:

- To develop a Plant Recombinant Human DNase I resistant to actin inhibition, while maintaining enzymatic activity
- Potentially, alidornase alfa will exhibit superior activity in breaking down extracellular DNA and lowering mucus viscosity
- Potential to improved lung function and lower recurrent incidences of infections in CF patients

Inhibition of DNase I Activity by Actin

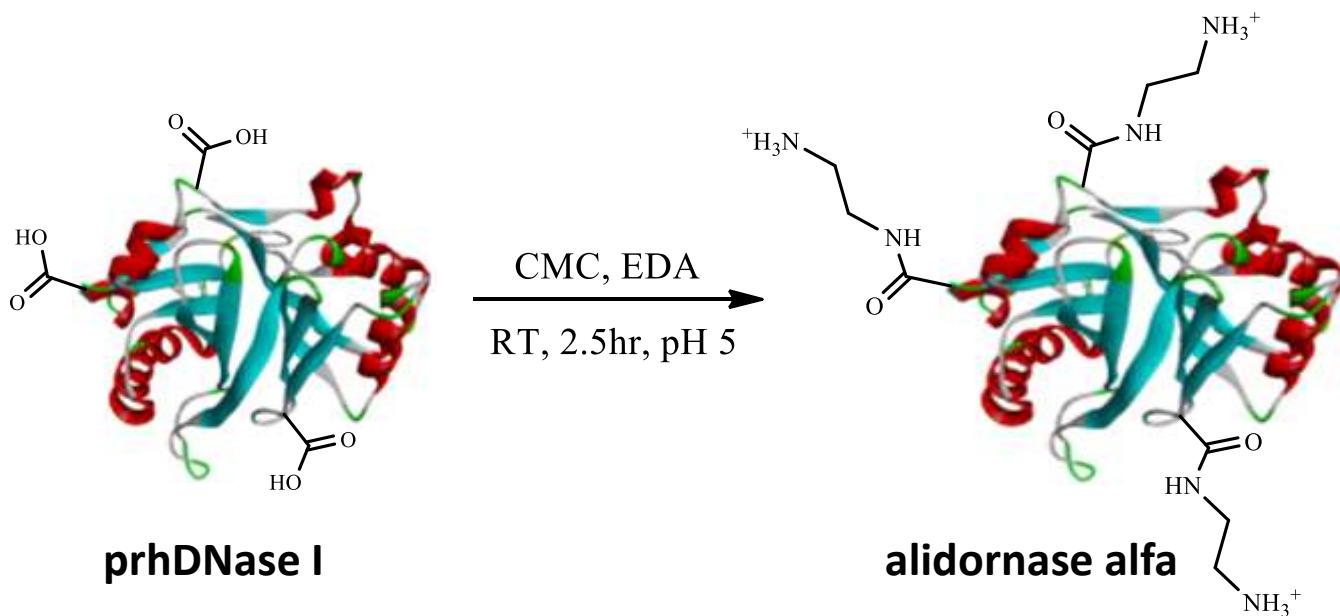


Relevant concentrations
of actin and DNase in
human CF patients' sputa
following treatment



Actin may influence the effectiveness of inhaled DNase I in CF lungs

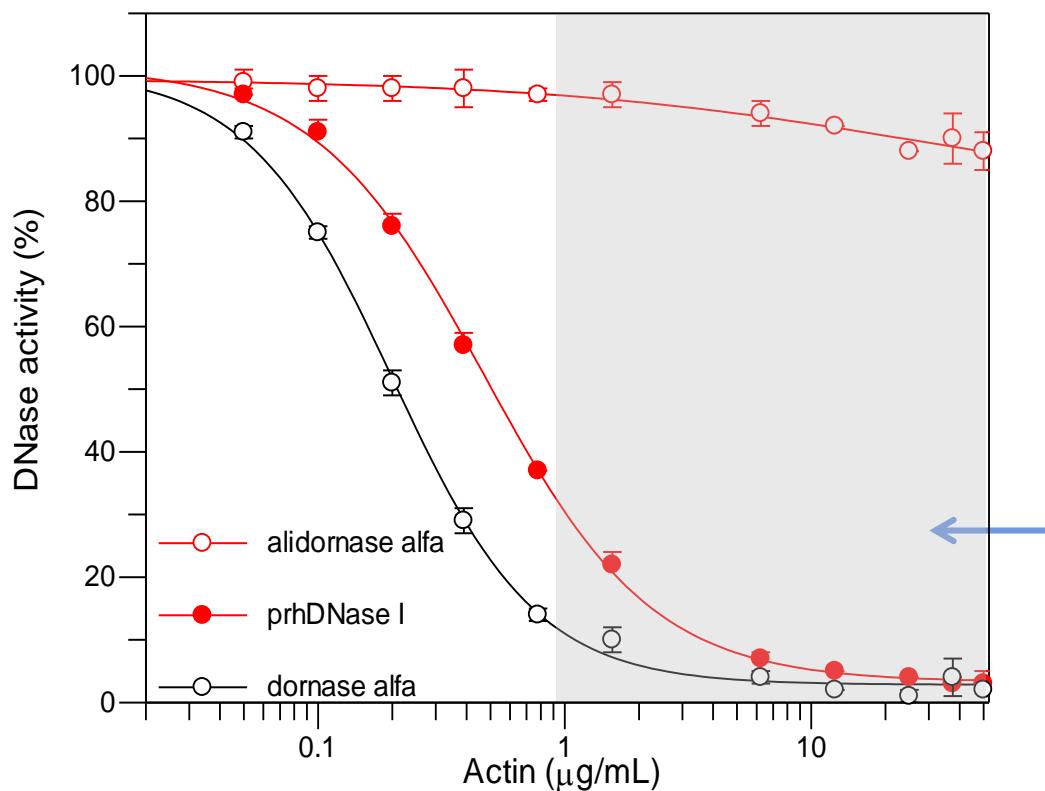
Actin Inhibition Resistance is Achieved by Chemical Modification



- The acidic side chains of prhDNase I are chemically modified with ethylenediamine (EDA).
- The chemical modification of alidornase alfa is non-selective (in terms of number and site of modifications per molecule). Therefore, the resulting alidornase alfa is a consistent mixture of isoforms of the EDA-modified DNase.

CMC = *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate; EDA = ethylenediamine;

DNase I Inhibition by Human Actin dornase alfa vs. prhDNase I vs. alidornase alfa



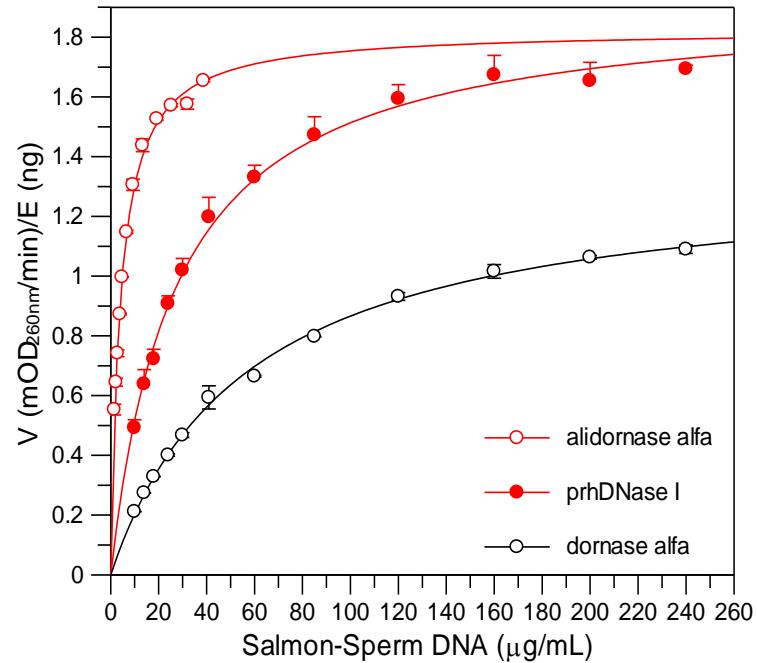
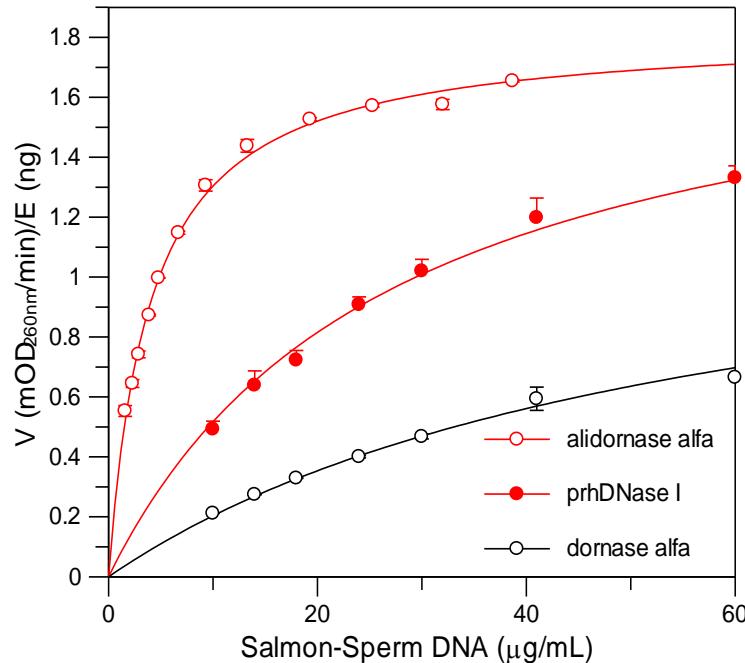
DNase I	IC ₅₀ ($\mu\text{g/mL}$)
alidornase alfa	NA
prhDNase I	0.47
dornase alfa	0.2

Relevant concentrations of actin and DNase in human CF patients' sputa following treatment

alidornase alfa exhibits resistance to actin inhibition compared to unmodified DNase I enzymes, in concentrations corresponding to those found in CF patients' sputa

Michaelis-Menten Kinetics

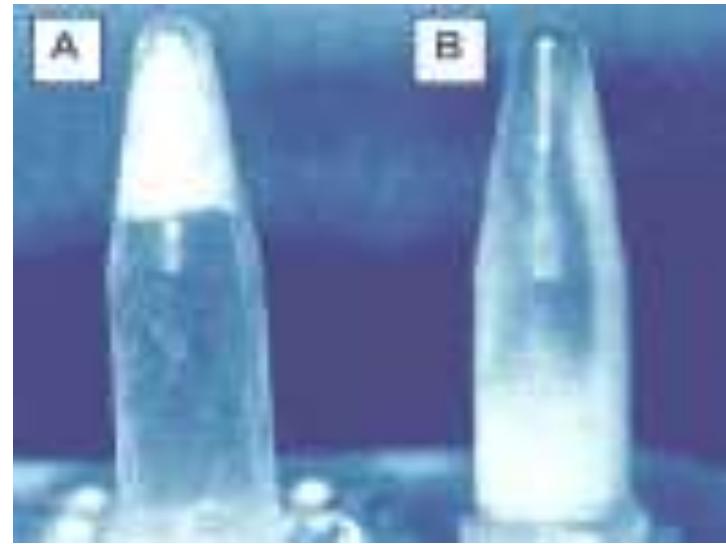
dornase alfa vs. prhDNase I vs. alidornase alfa



	V_{max} / E (mOD/min/ng DNase)	K_M (µg/mL)
alidornase alfa	1.8	4.0
prhDNase I	1.9	27.2
dornase alfa	1.4	56.8

alidornase alfa exhibits improved kinetic properties, compared to the unmodified DNase I enzymes

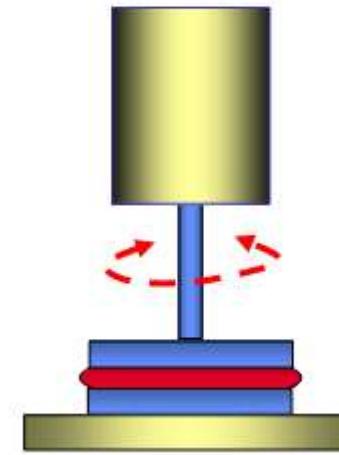
Efficacy Ex-vivo Study on CF Sputum



+
Pulmozyme

Measuring System - Rheometer

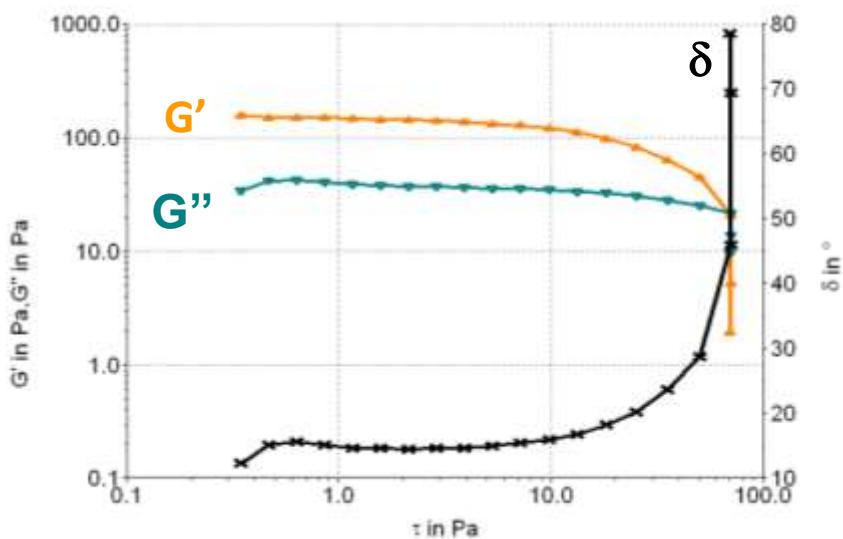
Parallel
Plates



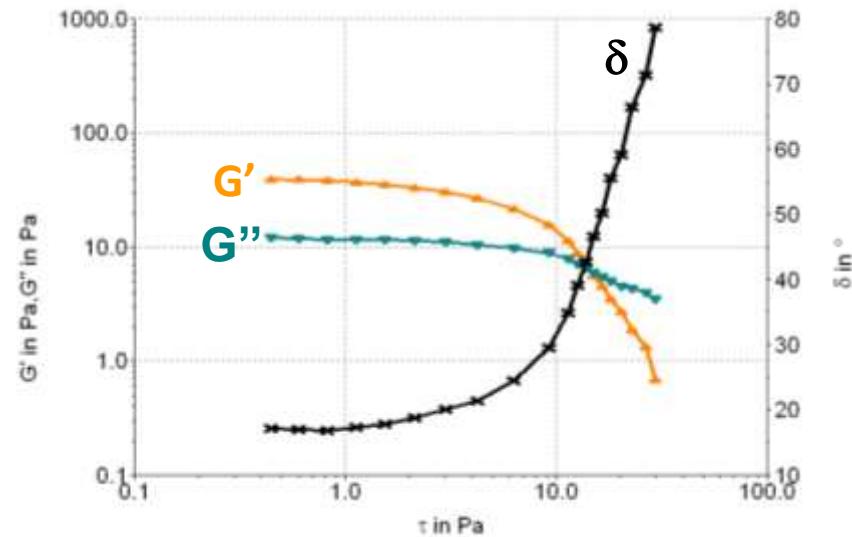
An oscillatory stress is applied to a sample → The material response is measured

Stress sweep:

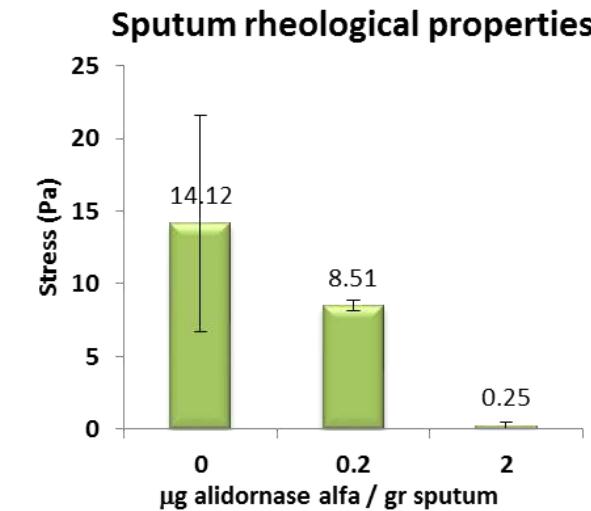
Formulation buffer - control



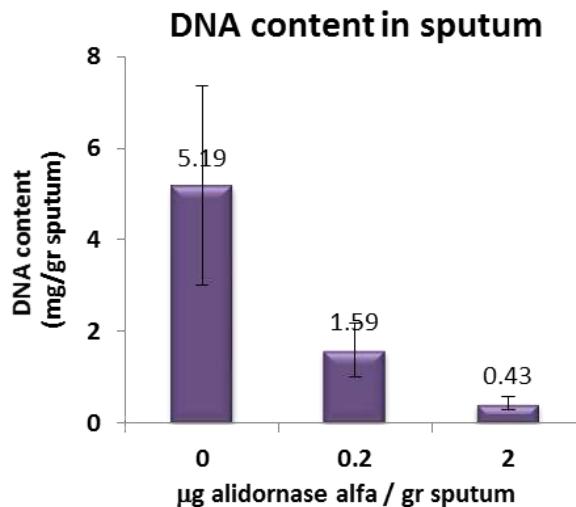
5ug alidornase alfa/ gr sputum



Dose Dependent Effect of alidornase alfa on Sputum Rheological Properties, DNA Content and DNA Fragmentation

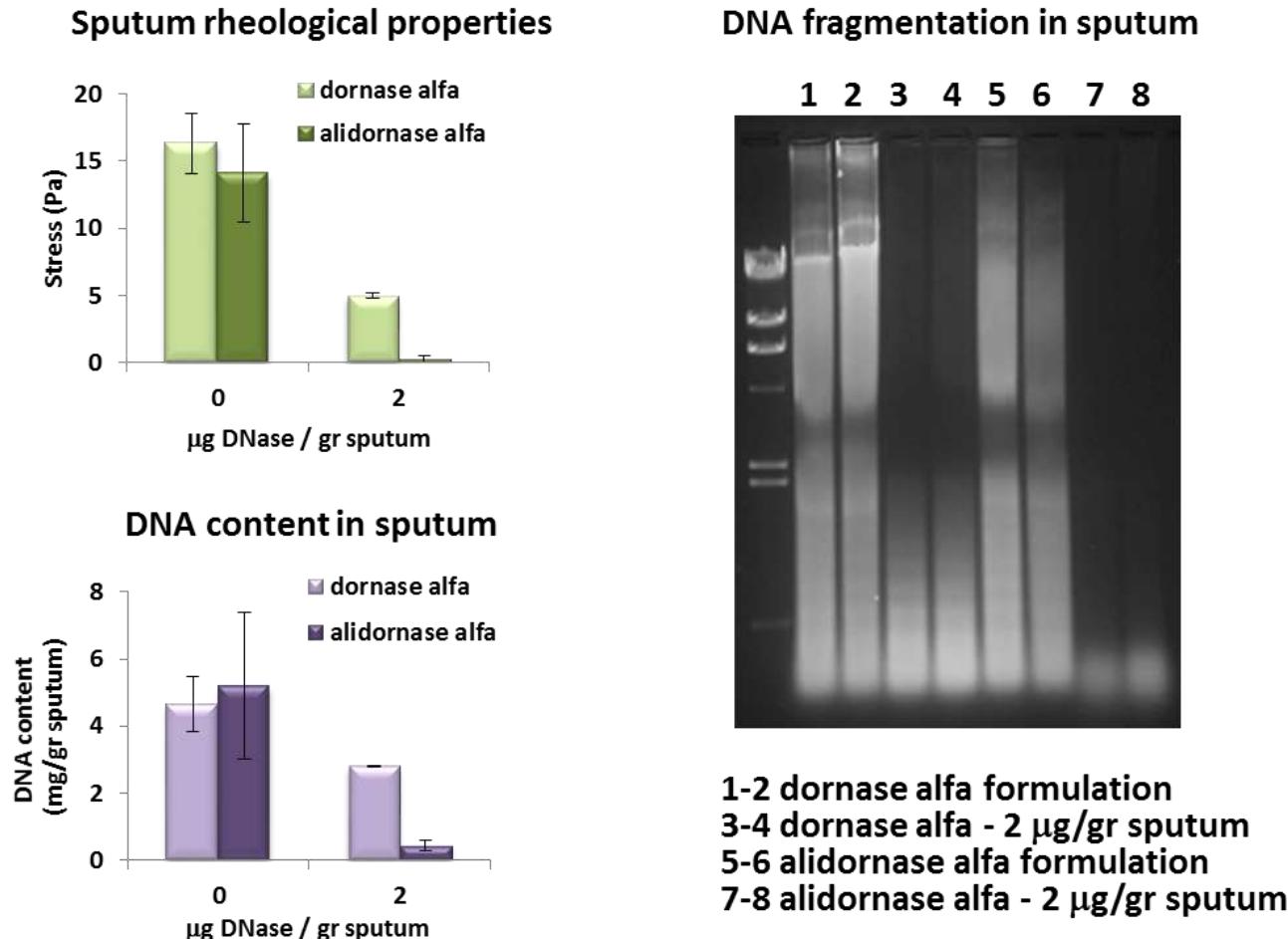


DNA fragmentation in sputum



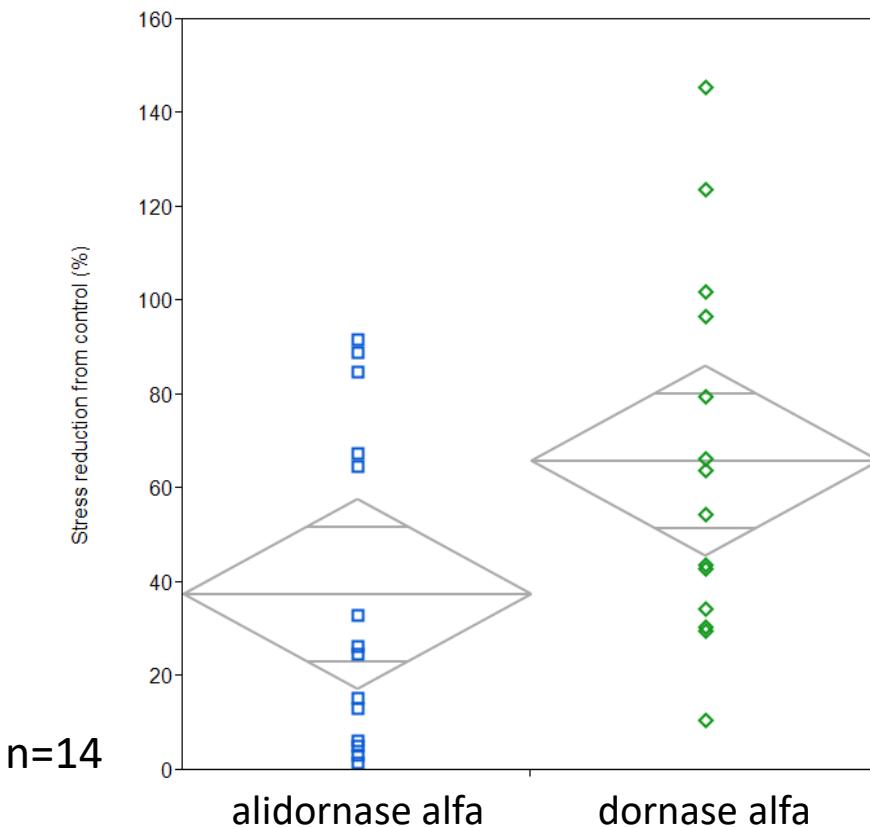
1-2 alidornase alfa formulation
3-4 alidornase alfa - 0.2 µg/gr sputum
5-6 alidornase alfa - 2 µg/gr sputum

Effect of DNase I on Sputum Rheological Properties, DNA Content and DNA Fragmentation alidornase alfa vs. dornase alfa



Example: alidornase alfa shows enhanced DNA fragmentation and enhanced disruption of sputum elastic structure, compared to dornase alfa

Summary of Ex-vivo Efficacy Study



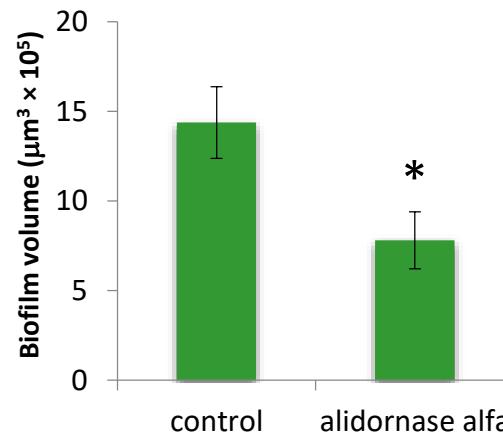
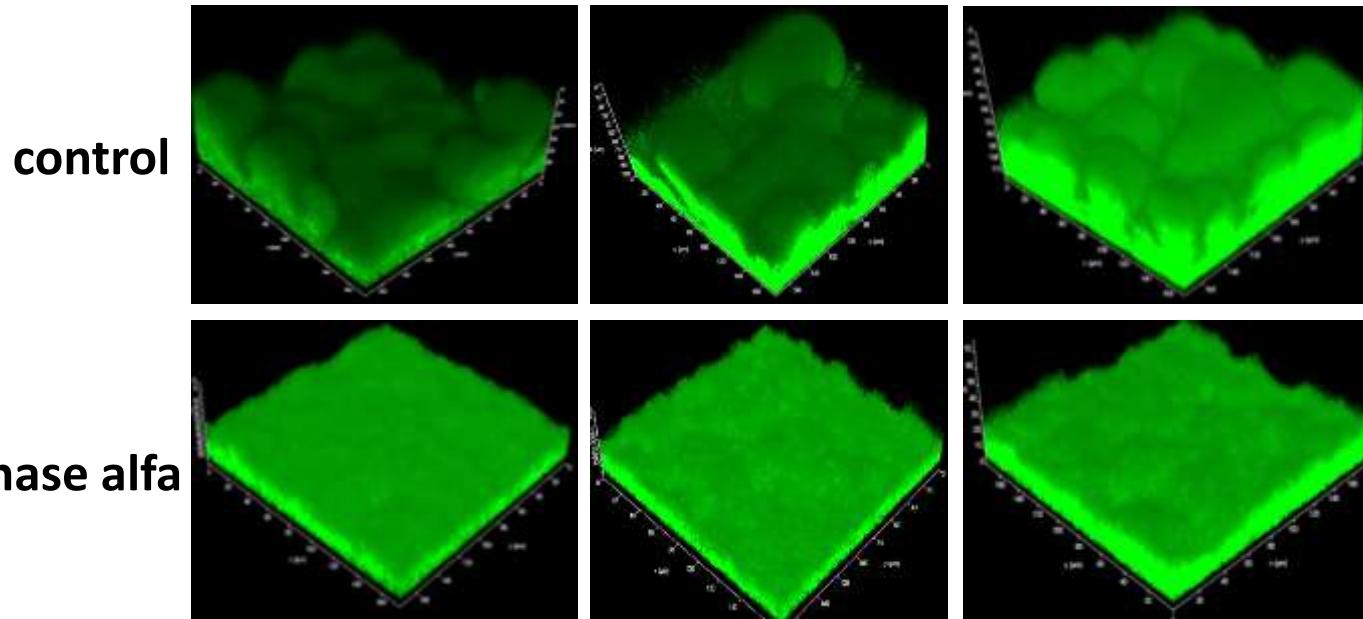
Response to treatment with		No. of CF patients sputa samples
alidornase alfa	dornase alfa	
+++	-	3
+++	+	3
+++	+++	3
-	-	4
+	+++	1

Response to treatment:

9/10: alidornase alfa \geq dornase alfa

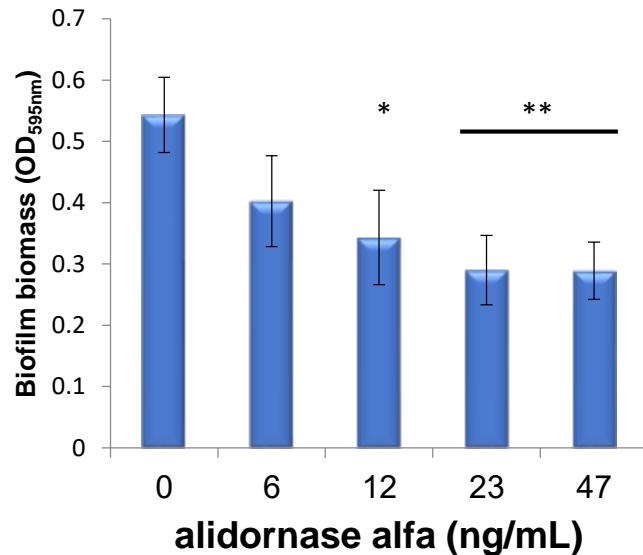
6/10: alidornase alfa > dornase alfa

Inhibition of *P. aeruginosa* Biofilm Formation (Flow Cell Model)

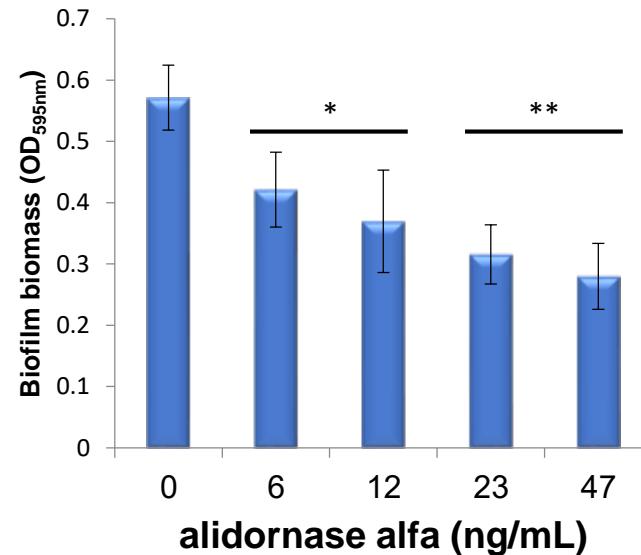


Biofilm Inhibition of High Biofilm Forming Clinical Isolates (Static Model)

2192 chronic CF isolate



39016 keratitis eye isolate



alidornase alfa is able to inhibit the biofilm formation of *P. aeruginosa* high biofilm forming clinical isolates