Phase IIa Clinical Trial Results with alidornase alfa for the Treatment of CF

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Phase IIa - Study Objective and Design

- **Objective:** Open Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Exploratory Efficacy Parameters of alidornase alfa in Patients with Cystic Fibrosis previously treated with dornase alfa
- **Administration:**
  Once daily inhalation of 2.5 mg alidornase alfa for 28 days

Main Inclusion Criteria:

- Age ≥ 12 years
- At least 4 months on dornase alfa & stable inhaled regimen
- Medically stable for at least one month prior to screening
- FEV1 of >40% and <90% ; FVC ≥ 40% at screening

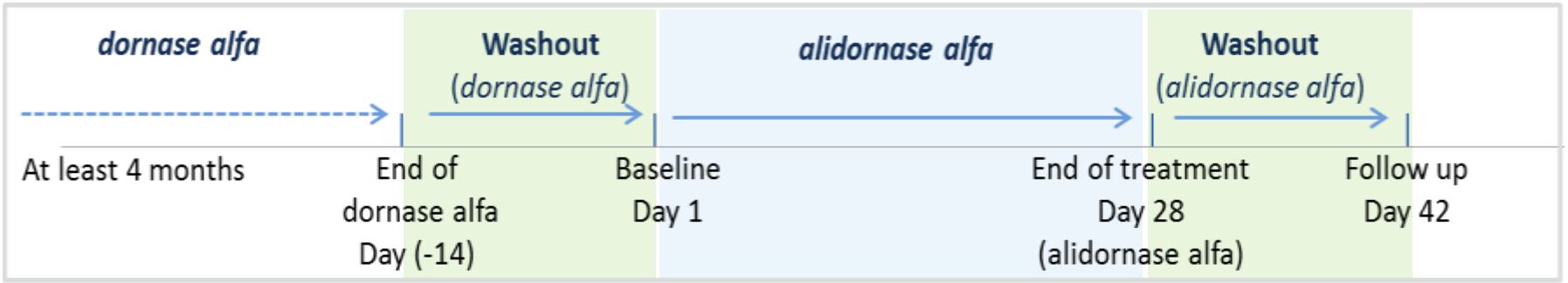
Main Exclusion Criteria:

- History of lung transplantation
- History of adverse reactions during aerosol delivery
- History of hypersensitivity to inhaled proteins

Phase IIa – Study Endpoints and Overall Design

- Safety and immunogenicity
- Pharmacokinetics
- Exploratory efficacy:
  - Effects on FEV1
  - Effect on sputum DNA parameters
  - Effect on rheology parameters

Study Design:



Phase IIa –Study Population and Status

Demographics & baseline CF patient characteristics

Total enrolled	16 CF patients
Age, yrs (Mean ± SD) (range)	25.8±9.1 (13-49)
Male : Female	10:6
Mean ppFEV1 ± SD (range) median ppFEV1	70.5% ± 10.8 (52-89) 70.5%
Pseudomonas aeruginosa , # positive subjects	8
Hypertonic saline, n (%)	11 (68%)

- All patients continued inhaled regimen of their respective CF medications throughout the study (steroids, hypertonic saline, antibiotics, etc.)
- All enrolled patients completed the 28 day treatment period

Phase IIa – Safety

24AEs (100%)* Total AEs in 11/16 subjects		
AEs mild and moderate	23 (96%)	
Severe AEs	1 (4%)	Anemia – not treatment related Medical history of peptic disease and previous GI hemorrhage
Serious AEs	0 (0%)	
Related, possibly related	8 (33%)	Vomiting, Throat irritation, Cough, Diarrhea, Dysphonia, Nausea
Not related, unlikely related	16 (67%)	Anemia, Gastrointestinal haemorrhage, Infective pulmonary exacerbation, Upper respiratory tract infection, Viral upper respiratory tract infection, Hypokalaemia, Myalgia, Cough, Dyspnoea, Haemoptysis, Rales, Sputum increased

* as reported by Investigators

Phase IIa – Pharmacokinetics & Immunogenicity

Plasma Pharmacokinetics (PK)

- alidornase alfa was found not to be absorbed to the circulation as part of GLP PK study using ELISA

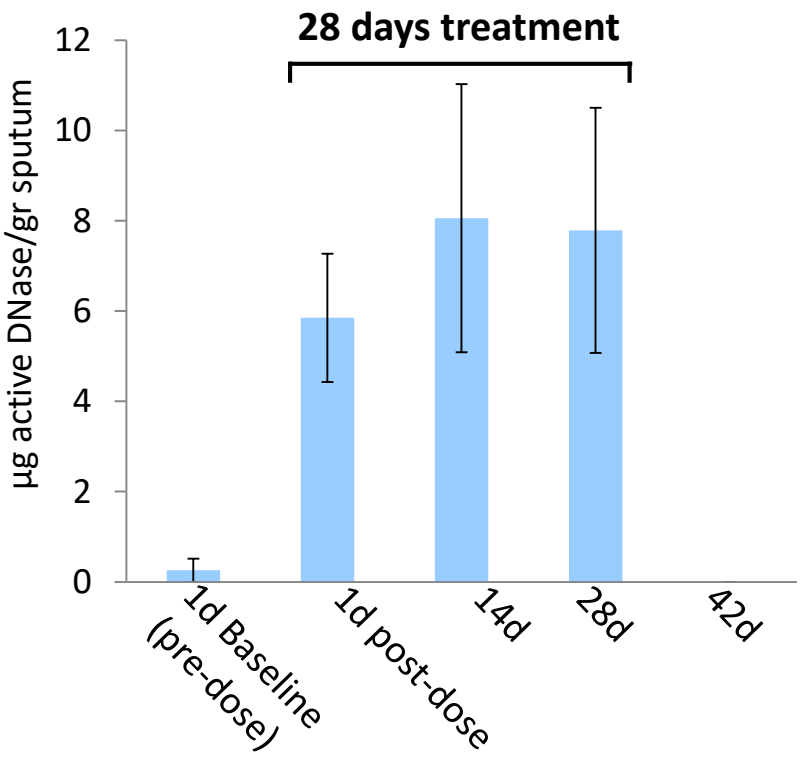
Activity in Sputum

- Active alidornase alfa was detected in the patients' sputa during the entire treatment period

Immunogenicity

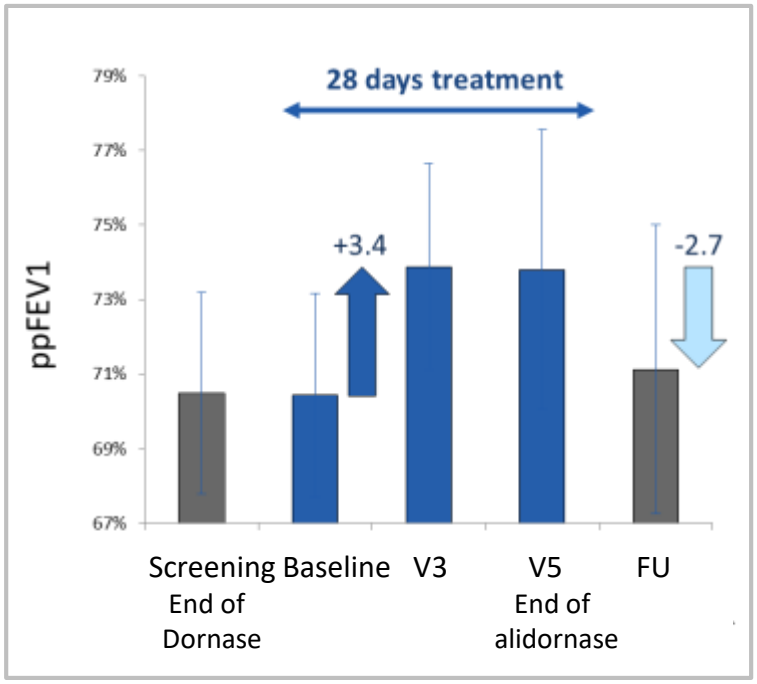
- Only 1 /16 patients was found to be treatment induced positive for anti-alidornase alfa (maximal low titer of 298 at visit 5)

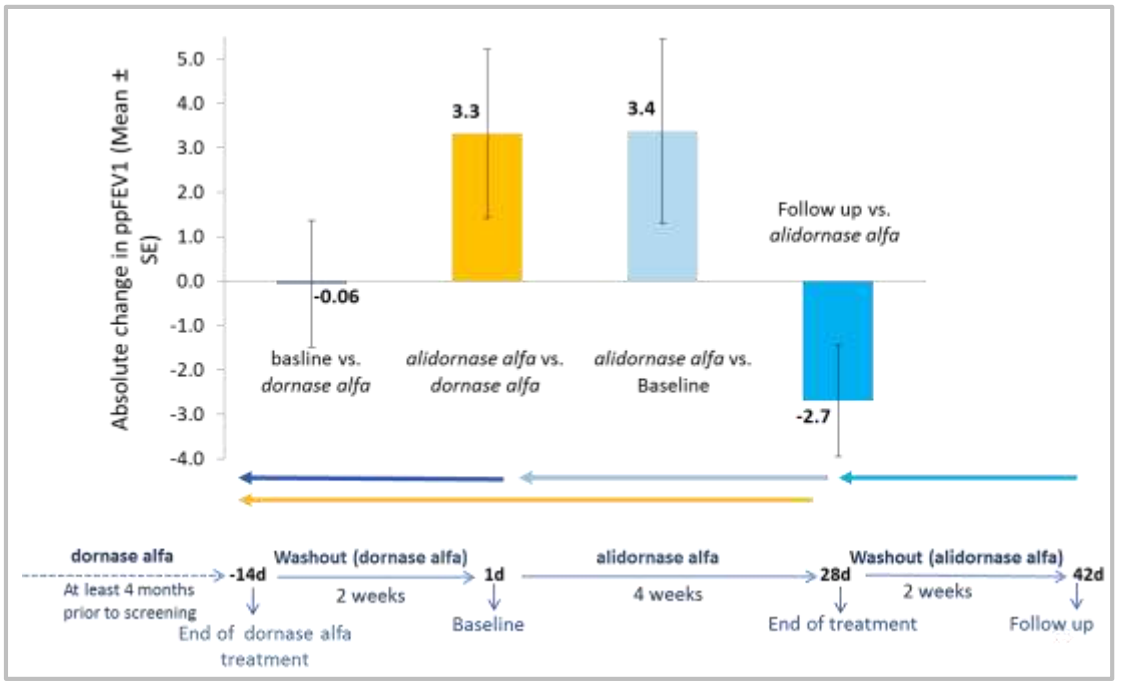
alidornase alfa activity in Sputa*



* By Methyl green-based enzymatic activity assay

alidornase alfa Effect on Lung Function

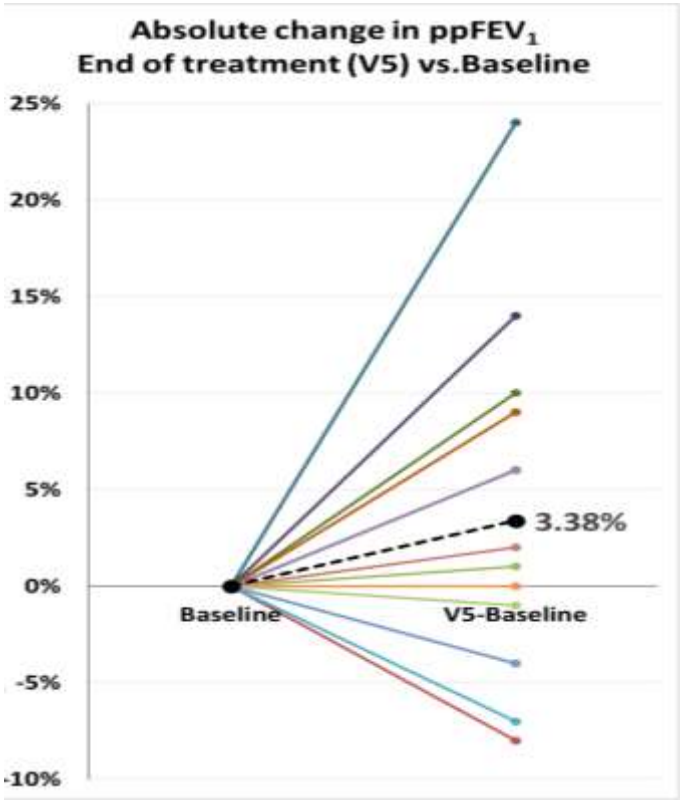


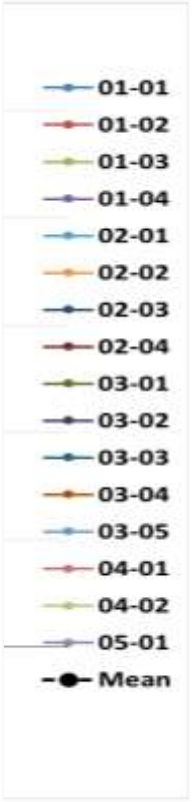


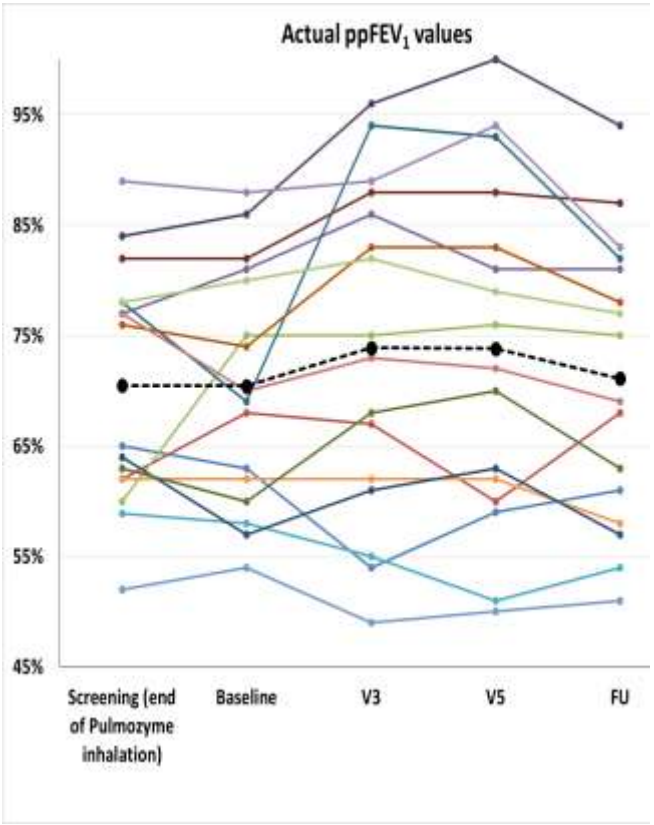
- No change in $ppFEV_1$ was observed during the washout period from dornase alfa
- Following 4 weeks of daily treatment with alidornase alfa, improvement in mean absolute $ppFEV_1$ was rapid and sustained
- The observed improvement shown with alidornase alfa was lost within 2 weeks of therapy discontinuation, causing a reduction in $ppFEV_1$

Absolute change and Individual ppFEV$_1$ – total 16 CF patients

Absolute change in ppFEV1 (%points)
Visit 5-Visit1 (baseline)



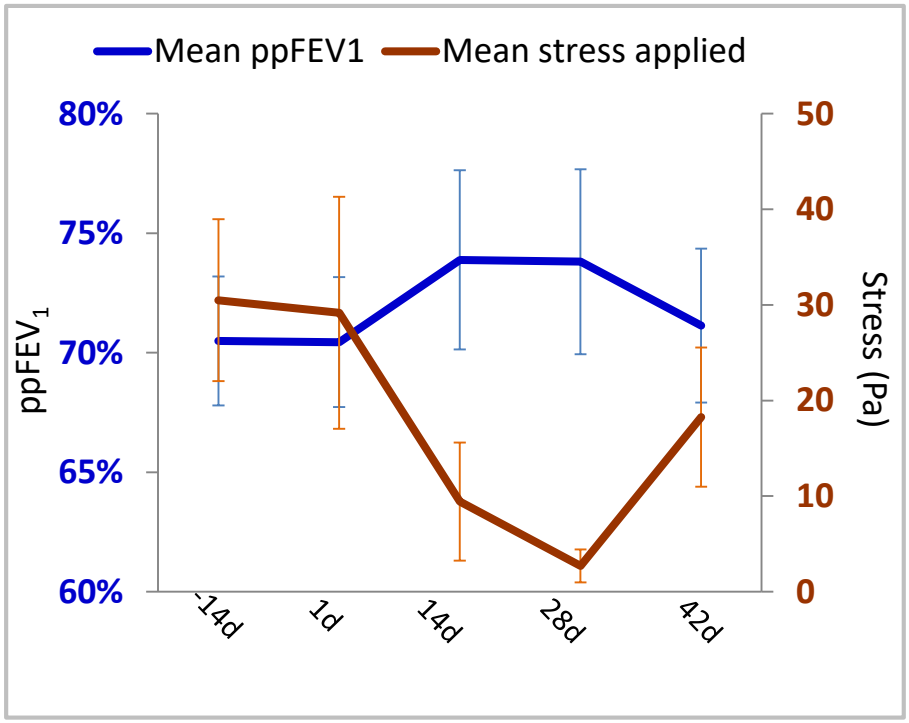




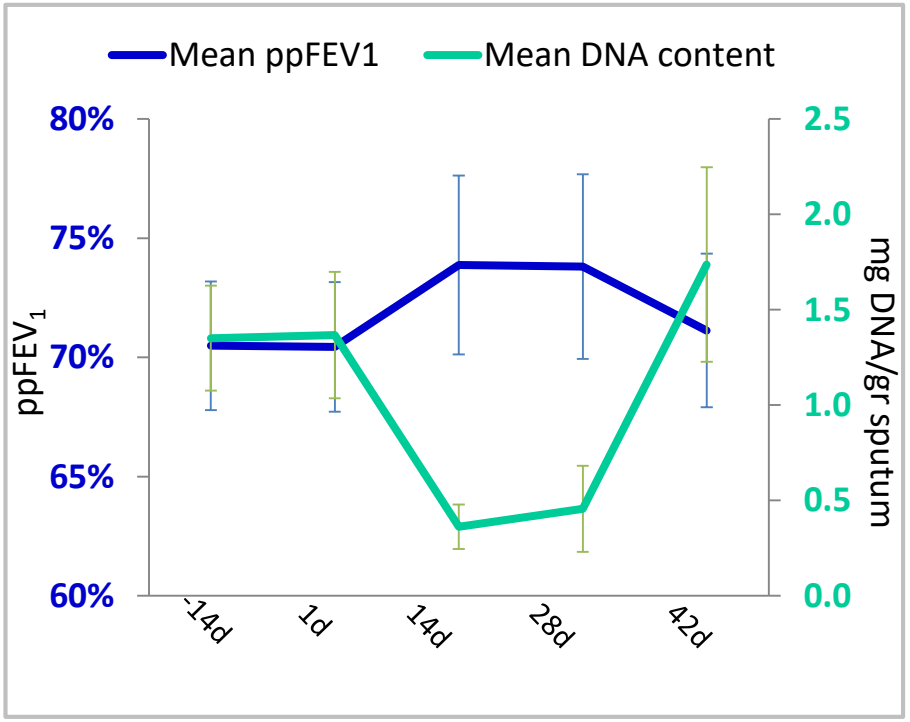
- Mean absolute increase in ppFEV1 of 3.4 points from baseline
- Mean absolute increase in ppFEV1 of 3.3 points over dornase alfa
- Mean absolute decrease in ppFEV1 of -2.7 points following

$ppFEV_1$ Correlation with DNA Content and Sputa Viscoelasticity

Sputum viscoelasticity vs ppFEV1



DNA content in sputum vs ppFEV1



Improvement in ppFEV1 following treatment with alidornase alfa correlates with:

- mean reduction of ~70 % in DNA content from baseline
- mean reduction of > 90% in sputa viscoelasticity from baseline

Alidornase alfa Potential in Lowering *P.aeruginosa* Respiratory Infections

Evaluation of Phase IIa patients sputa for P. aeruginosa presence			
Method	**Patients (total: 9 tested)**	**Results before and after alidornase treatment**	
		Baseline	**End of treatment**
qPCR	4	Negative	Negative
	5	Highly positive	> 70% reduction
Positive Patient #	**per Medical history**	**Baseline**	**End of treatment**
03-01	Chronic colonization	Positive	7% (93% reduction)
03-03	Chronic colonization	Positive	Negative (BLD)
03-04	Chronic colonization	Positive	Negative (BLD)
04-01	Chronic colonization	Positive	33% (77% reduction)
05-01	Chronic colonization	Positive	Negative (BLD)

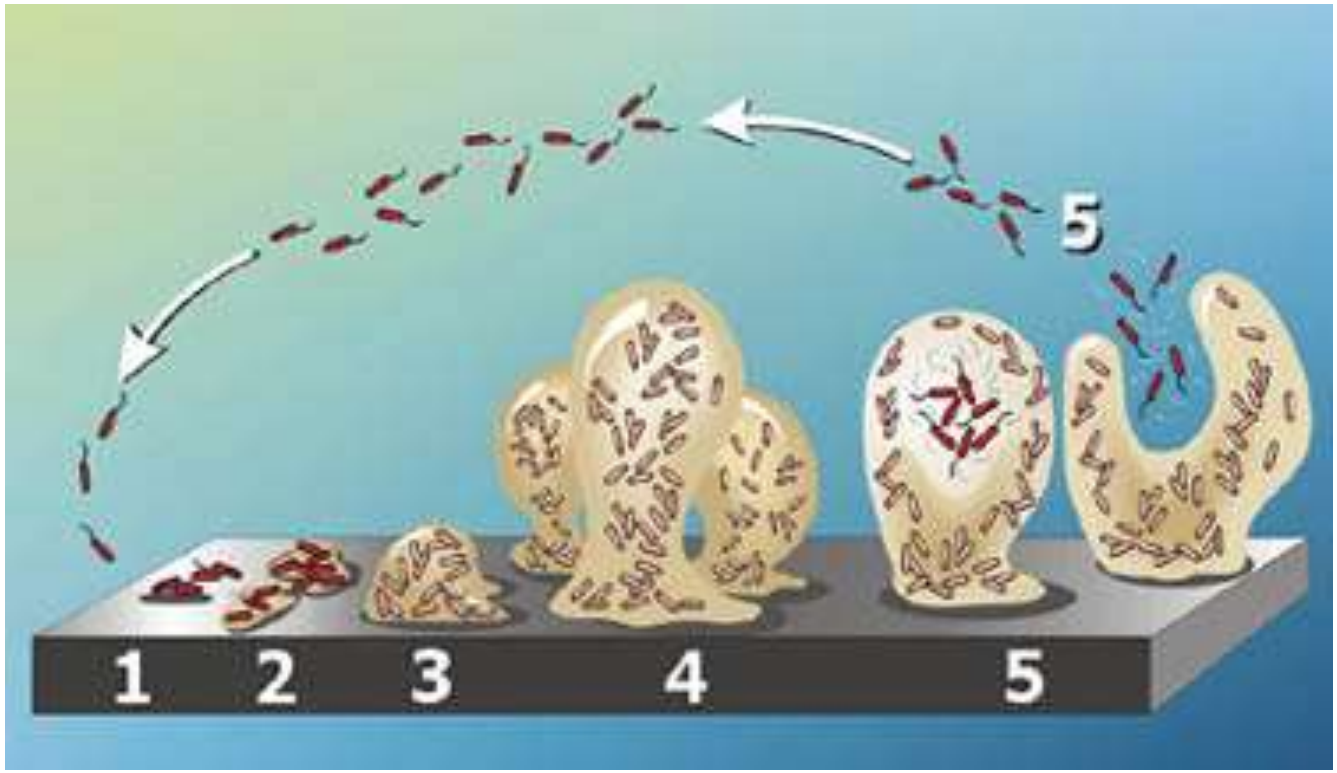
- Total DNA extracted from patients sputa
- the *gyrB* gene, for specific detection of P. aeruginosa by qPCR
- Data normalization with *N. Tabacum* Elongation Factor 1 (*EF1*) gene

Reduction of over 70% in the presence of Pseudomonas aeruginosa (qPCR) as a result of alidornase alfa treatment, reinforced by ex vivo observations

These results were further confirmed using a P. Aeruginosa specific DNA Chip using 15 replicates /sample

Extracellular DNA is an important biofilm component

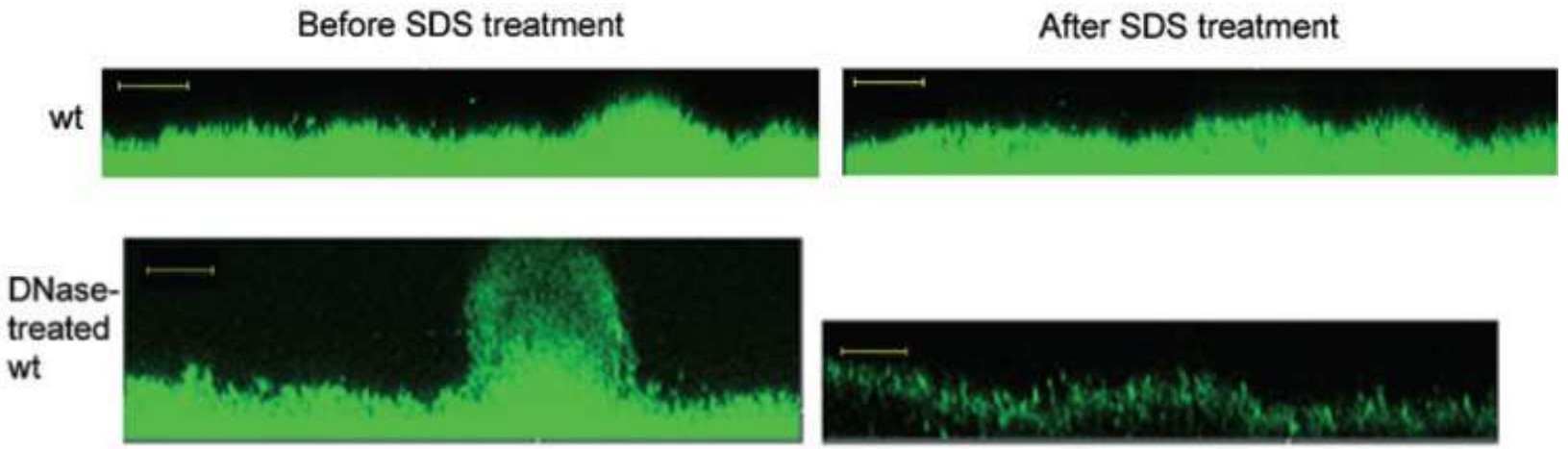
P. aeruginosa **produces extracellular DNA which functions as a matrix component in biofilms via lysis of a subpopulation of the bacteria**



Molecular Microbiology (2006) 59(4), 1114–1128
doi:10.1111/j.1365-2958.2005.05008.x
First published online 12 December 2005

A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms

DNase treatment of *P. aeruginosa biofilms* reduced the amounts of extracellular DNA, and made biofilms vulnerable to treatment with the detergent SDS



Allesen-Holm et al.

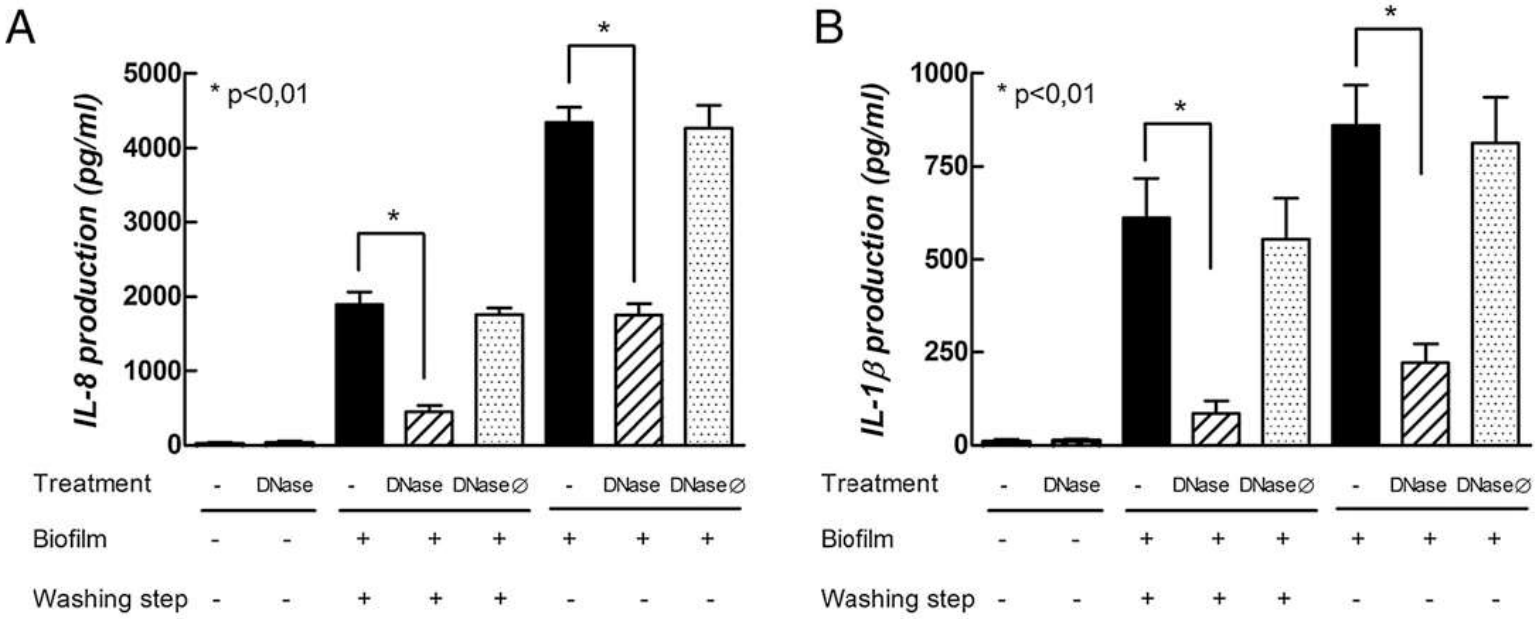
The Journal of Immunology

Extracellular DNA: A Major Proinflammatory Component of *Pseudomonas aeruginosa* Biofilms

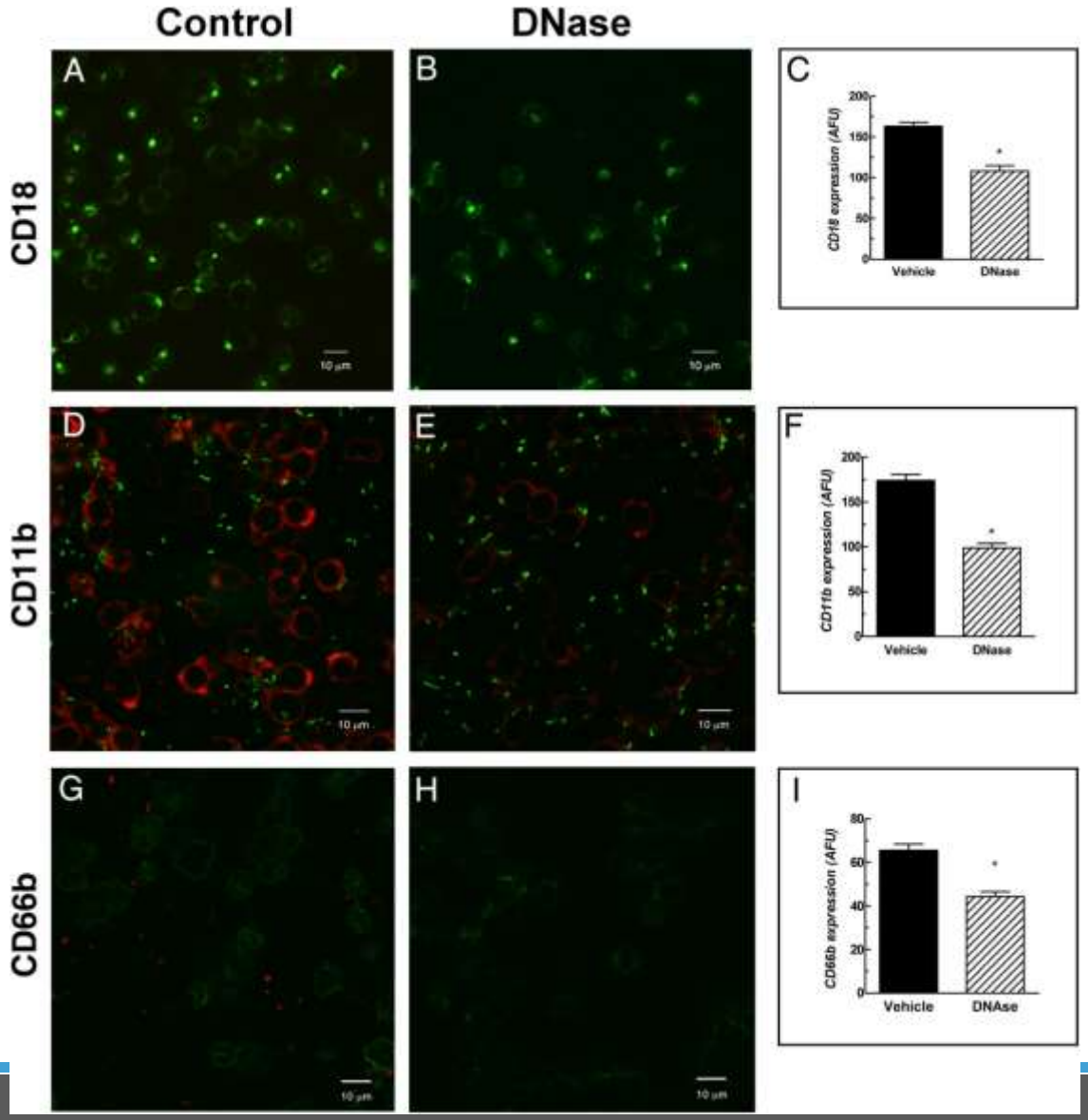
Juan I. Fuxman Bass, Daniela M. Russo, Maria L. Gabelloni, Jorge R. Geffner, Mirta Giordano, Mariana Catalano, Ángeles Zorreguieta and Analía S. Trevani

J Immunol 2010; 184:6386-6395; Prepublished online 26

Treatment of wild-type biofilms with DNase I markedly reduced neutrophil cytokine release



Treatment of the biofilms with DNase significantly reduced neutrophil upregulation of activation markers



Phase IIa: Conclusions

alidornase alfa - human rDNase I, Resistant to actin inhibition

Safety:

alidornase alfa was safe & well tolerated

- Low incidence of treatment related/possibly related AEs
- All AEs were resolved without sequelae
- No SAE
- Low incidence of treatment induced Anti Drug Antibodies

Pharmacokinetics:

- Alidornase alfa was not absorbed to the circulation
- Active alidornase alfa was measured in patient's sputa

Efficacy: alidornase alfa improved lung function:

- Mean absolute increase in ppFEV1 of 3.4 points from baseline
- Mean absolute increase in ppFEV1 of 3.3 points over dornase alfa
- Mean absolute decrease in ppFEV1 of -2.7 points following alidornase alfa 2 weeks washout period
- Sputa analyses form available samples indicates:
  - A mean reduction of ~70 % in DNA content from baseline
  - A mean reduction of > 90% in sputa viscoelasticity from baseline
  - Reduction of over 70% in the presence of pseudomonas (qPCR)

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Thank You