

# THE INDUCED IMMUNE RESPONSE IN CF PATIENTS

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## INTRODUCTION AND AIM

CF patients experience recurrent and persisting lung infections characterized by widespread lung inflammation leading to increased morbidity and mortality.

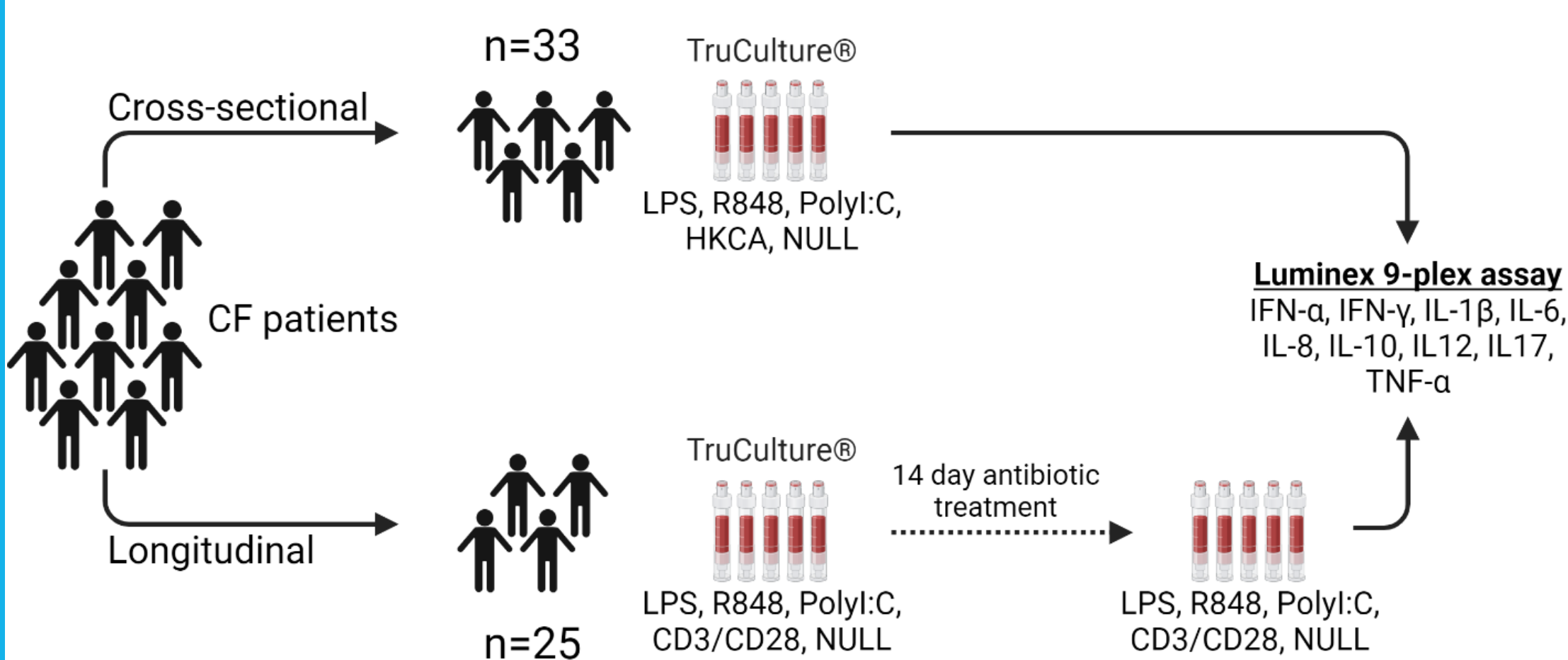
The hyperinflammatory responses and the repeated lung infections highlight the dysfunctional phenotype of the CF immune response. It is still not fully understood which immunological parameters and networks contribute to disease progression in CF patients. Recent studies have shown that CF immune cells have impaired innate inflammatory responses to bacterial antigen stimulation and that strong innate responses in circulating cells are positively associated with increased lung function (1,2).

In this study, we wanted to extend these observations by analyzing the systemic induced immune response in CF patients persistently infected with *Pseudomonas aeruginosa* using the whole blood *ex vivo* culture system TruCulture® coupled with a functionally diverse 9-plex cytokine analysis panel.

## METHODS

Blood samples from CF patients were collected at the Cystic Fibrosis Center at Rigshospitalet in Copenhagen, Denmark.

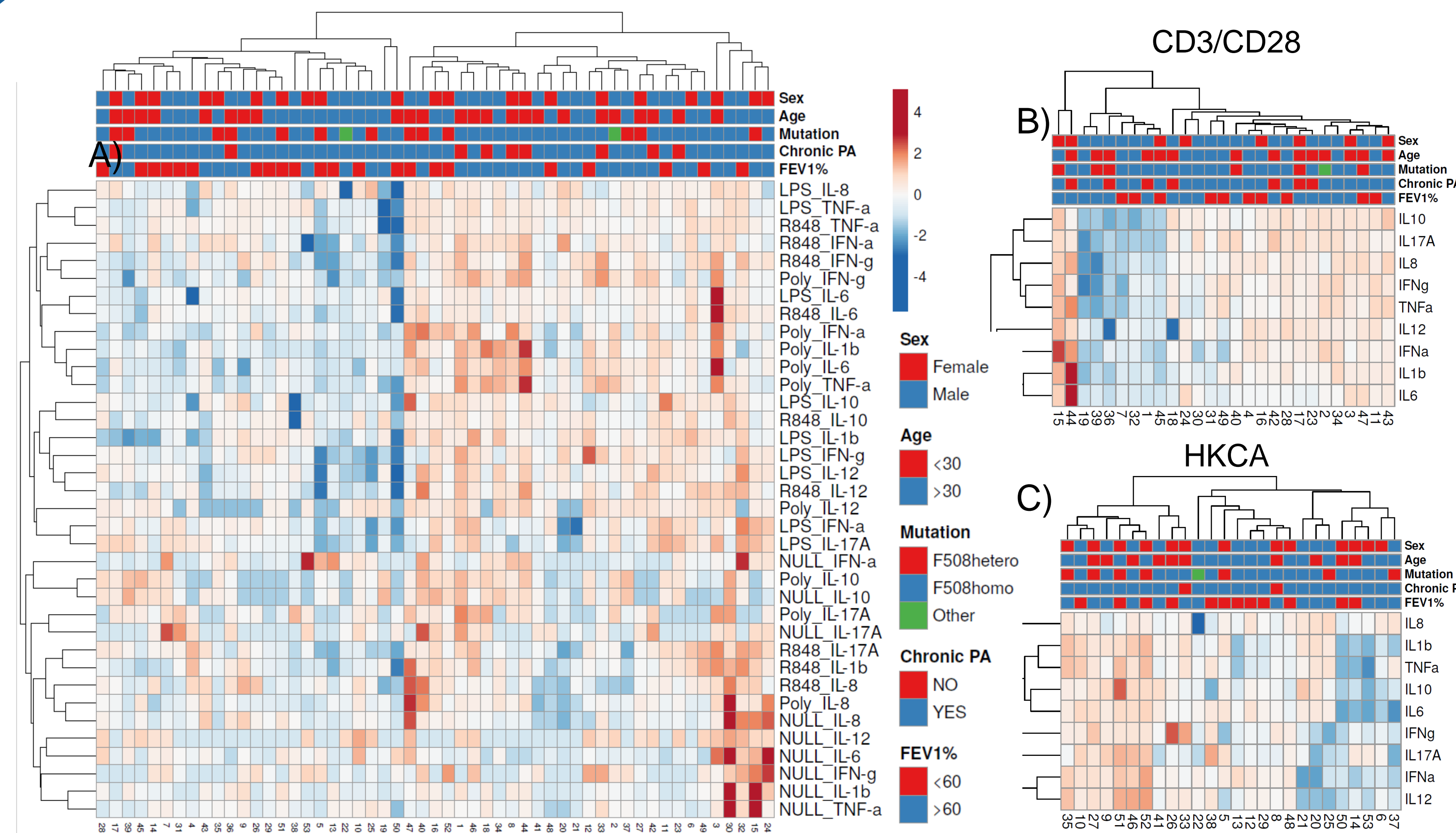
Peripheral blood was drawn directly into five different TruCulture blood collection tubes pre-coated with the immune stimulants LPS, resiquimod (R848), Poly I:C, Heat-killed candida albicans (HKCA), CD3/CD28 antibodies and into a non-coated control tube (NULL). The tubes were incubated for 22 hours at 37°C and the cell pellets and the supernatants were separated. The concentration of the cytokines IFN-α, IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-17A and TNF-α were quantified in the supernatants using a 9-plex Luminex-based assay.



## RESULTS

### Patient demographics

Patients included, n (%)	53 (100)
Female, n (%)	21 (33,3)
Male, n (%)	32 (66,7)
Age, median (range)	37 (12-61)
Mutation	
F508 homo, n (%)	38 (71,7)
F508 hetero, n (%)	13 (24,5)
Other, n (%)	2 (3,8)
Chronic PA, n (%)	44 (83,0)
FEV1%, median (range)	65,0 (21,0-100,1)



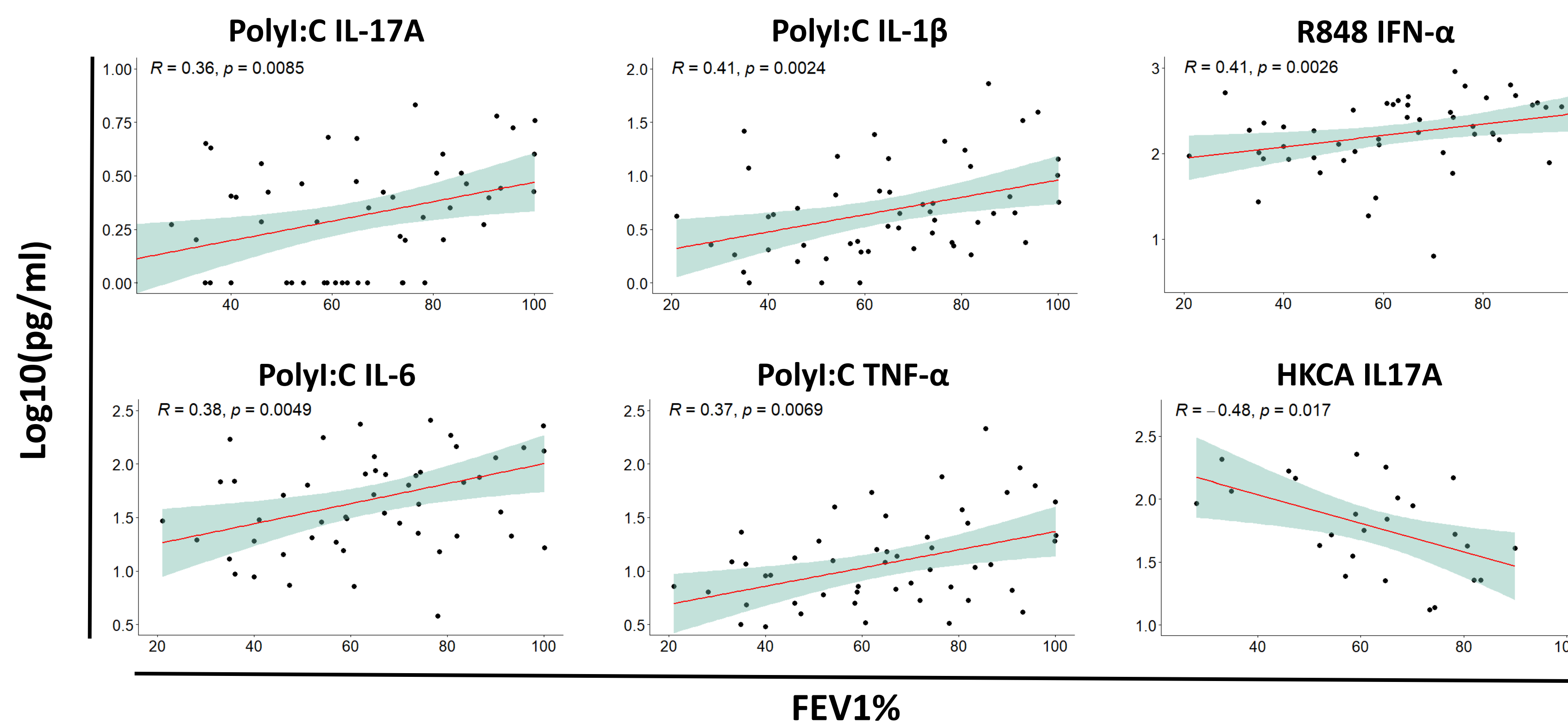
← CF patients cluster based on *ex vivo*-induced cytokine responses.

The patients cluster independently of sex, age, mutation, infection status and lung function. However, in A) patients with FEV1 < 60% are overrepresented in the "low response" cluster in the left part of the figure. Large response variations were observed in CD3/CD28 tubes clustering the patients in high, low and intermediate responders (B, from left to right).

Heatmaps were created based on centered and scaled log-transformed cytokine concentrations and dendrograms were drawn using complete-linkage clustering on Euclidean distances. LPS, R848, PolyI:C, NULL, n=53; CD3/CD28, n=27; HKCA, n=26.

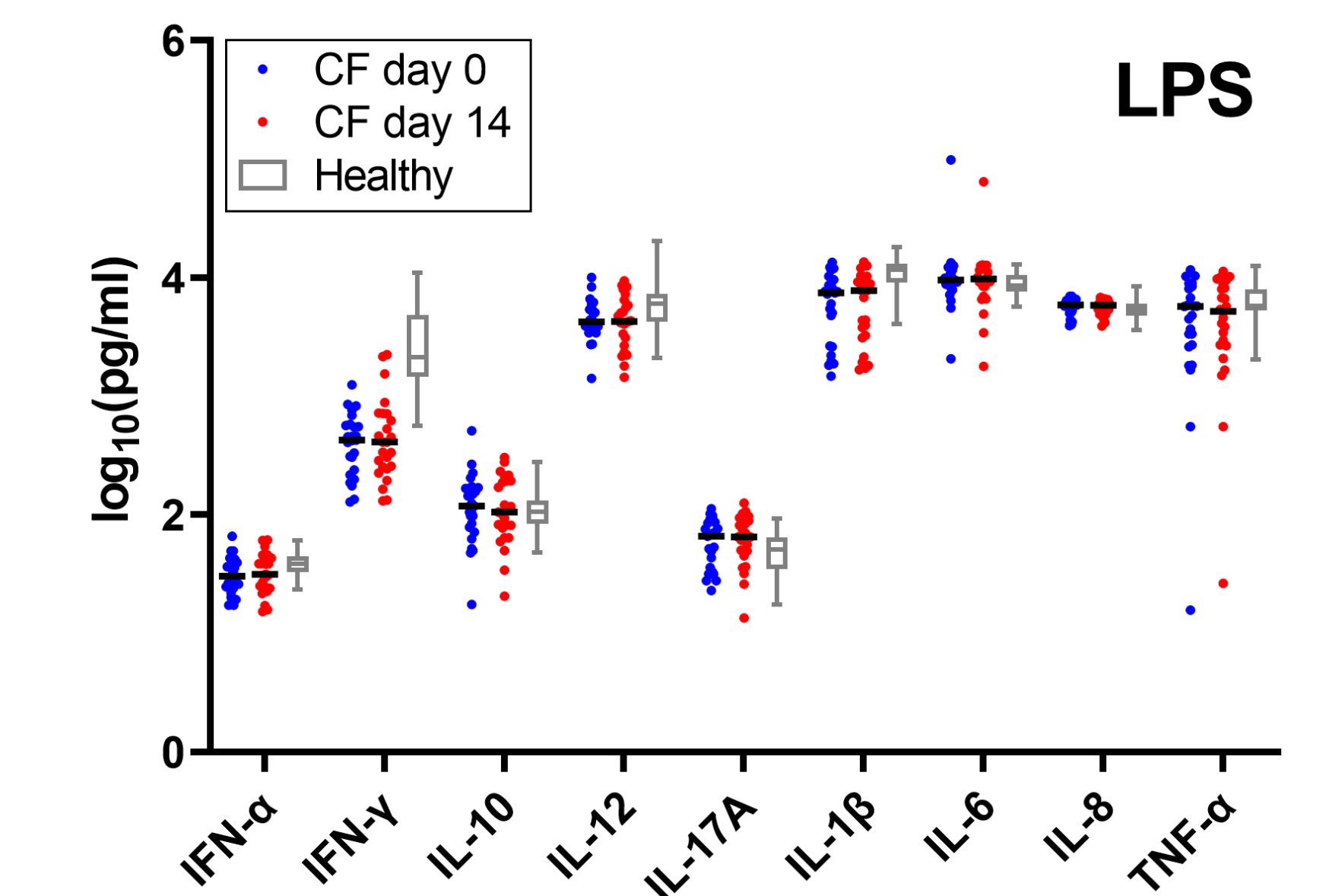
14-day i.v. antibiotic treatment does not affect the induced immune response in CF patients.

Fourteen days of intravenous antibiotic treatment did not alter the induced responses in any condition tested (see data from LPS tube below, n=25). A boxplot with 2.5 and 97.5 percentiles are plotted from a healthy reference cohort (n=32) shows that CF cytokine responses are within the healthy reference range except for IFN-γ, which is markedly below the reference range.



← Cytokine responses downstream of intracellular toll-like receptor (TLR) activation correlate positively with lung function in CF patients.

Log-transformed cytokine concentrations were plotted against the predicted % of FEV1 and correlations were analyzed by Spearman's rank correlation test. Six significant correlations were found: Five TruCulture variables correlated positively with lung function whereas HKCA-induced IL-17A correlated negatively.



## CONCLUSIONS

1. The concentrations of proinflammatory cytokines released after intracellular TLR activation are positively associated with lung function in CF patients.
2. Intensive antibiotic treatment does not alter the induced immune response in circulating immune cells
3. CF IFN-γ levels induced by several stimuli are low compared to reference levels from healthy blood donors

## REFERENCES

1. Leung ST et al. Immune and Cytokine Dysfunction in Cystic Fibrosis. *Am J Respir Cell Mol Biol.* 2019;61(5):656–8.
2. Kosamo S et al. Strong toll-like receptor responses in cystic fibrosis patients are associated with higher lung function. *J Cyst Fibros.* 2020;19(4):608–13.

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## CONTACT INFORMATION

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