Results: Characteristics were similar between the single (n=25) and the standard dose group (n=68), except more post-operative ECMO patients were in the standard dose group (p=.006, Table 1). There was no difference in grade or time to development of ACR. The single dose group achieved therapeutic tacrolimus levels sooner (p=0.009) but there was no correlation between days to therapeutic level and ACR (r=-0.042). Differences in DSA development and CMV viremia within one year were not statistically significant.

Conclusion: There was no difference in DSA development, ACR, or CMV viremia. Patients with PGD requiring ECMO or with post-operative renal dysfunction were given a second dose of basiliximab. Time to therapeutic tacrolimus level did not correlate with ACR, but this may be confounded by basiliximab administration in cases when tacrolimus was delayed. This suggests renal dysfunction as a possible indication for multiple dosing of basiliximab. Otherwise, a single dose could be sufficient for induction and greatly cost-effective. Further investigation is needed with increased sample size and long-term chronic rejection outcomes.

Table 1. Demographics and outcomes (*excludes patients deceased prior to data collection)

	Single dose	Standard dose	p-value
Gender (n, %)			0.149
Male	16 (64.0%)	32 (47.1%)	
Female	9 (36.0%)	36 (52.9%)	
Age at transplant (average years)	62.52	60.22	0.238
On ECMO after transplant (n, %)	1 (4.0%)	15 (22.0%)	0.006
PGD Grade (n, %)			0.069
1	0 (0.0%)	3 (4.4%)	
2	1 (4.0%)	4 (5.9%)	
3	2 (8.0%)	14 (20.6%)	
PRA Class I level (average %)	5.92	5.76	0.967
PRA Class II level (average %)	5.33	7.12	0.717
Time to therapeutic tacrolimus (average days)	5.5	7.0	0.009
Highest grade ACR (n, %*)			0.109
A1	3 (13.0%)	11 (18.6%)	
A2	1 (4.3%)	7 (11.9%)	
A3	3 (13.0%)	1 (1.7%)	
A4	1 (4.3%)	0 (0.0%)	
DSA positive (n, %*)	4 (17.4%)	18 (28.6%)	0.265
CMV viremia within 1 year (n, %*)	1 (4.3%)	6 (10.9%)	0.354

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Lung Transplantation and Extracorporeal Photopheresis as Induction Therapy in Cystic Fibrosis Patients: Immune System Profile Changes

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Purpose: Acute rejection (AR), common during the first year after lung transplantation (LuTx), can trigger chronic rejection (CR), the leading cause of late morbidity and mortality of LuTx. Extracorporeal photopheresis (ECP) has emerged as a promising treatment for chronic rejection. Only few studies are focus on ECP as prophylactic therapy of AR and CR. This study aims to verify, in recipients affected by cystic fibrosis (CF), whether the induction therapy with ECP can decrease the rate of AR, in order to impact positively on CR (primary end point: survival, AR). The expected results are the reduction of AR episodes in its clinical and histopathological manifestations.

Methods: This is a pilot clinical trial on 20 CF lung-transplanted patients, randomize, 2 parallel arms: standard immunosuppressive therapy and ECP (ECP) vs standard immunosuppressive therapy alone (CTR). We investigated the effect of ECP by the evaluation of lymphocyte immunophenotype by multiplex essay (CD4 + and CD25 +), the cytokine profile, the leukocytes subsets (by flow cytometry) in blood and BAL at different time points. AR episodes and infections were recorded, as far as ECP-related adverse events.

Results: No differences were detected in terms of AR episodes. Treg cells were significantly increased in the ECP group at 3 weeks post LuTx, and this difference was more evident 1 year post LuTx . Th17 cells were diminished in the ECP group. The anti-inflammatory IL10-producing NKs were significantly increased in the ECP group. Cytokine profile, both in BAL and plasma obtained at defined time points shows that in ECP group pro-inflammatory cytokines were early reduced and anti-inflammatory cytokines were upregulated.

Conclusion: In FC lung transplanted patients ECP was well tolerated without increasing in opportunistic infections. Its tolerogenic effect was not pointed out in terms of AR episodes, but has been confirmed in the immunological setting of ECP patients vs control: it prevents decline in Treg and NK observed with standard immunosuppressive drugs, with higher expression of anti-inflammatory cytokines (Il10, Il1RA) and less pro-inflammatory ones (Il1beta, Il6). Its effect is more evident months after the end of ECP treatment. The schedule of ECP prophylactic treatment has to be tested in an ampler cohort in order to reach its best immunomodulatory effect.

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Derepression of the IL-10 Gene by CRISPR-Cas Genome Editing as a Therapeutic Strategy for Persistent Immunomodulation of Donor Lungs for Transplantation

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Purpose: Anti-rejection immunosuppression is a persistent challenge in lung transplantation. We envisioned creating less immunogenic donor organs to obviate or reduce the need for immunosuppression. To induce persistent expression of the immunomodulatory cytokine IL-10, we aimed to harness CRISPR-Cas-mediated genome editing. We hypothesized that mutating the regulatory region of the IL-10 could induce persistent IL-10 upregulation with practicality in organ modification. To prove our concept, we investigate the efficacy and persistence of this approach in vitro.

Methods: Plasmids encoding Streptococcus pyogenes Cas9 (SpCas9), a puromycin cassette, and a single guide RNA (gRNA), designed to bind the promoter of the rat IL-10 gene, were delivered into a rat lung epithelial cell line. Transfected cells were enriched using puromycin and assessed for mutations and gene expressions after 14 and 28 days. The treatment group (targeting gRNA group) was compared to a no gRNA group and a non-targeting gRNA group.

Results: Insertions/deletions at the target site were detected in 77±20 % of alleles at day 14 and 87±11% at day 28 in the treatment group (Fig. 1A). The treatment group showed enhanced rat IL-10 expression while other groups showed mostly undetectable IL-10 expression (457±338- and 865 +573- fold increase compared to negative control at day 14 and 28 respectively, Fig.1B). Importantly, IL-10 upregulation lasted for 28 days despite a decline in SpCas9 expression (Fig. 1C) suggesting an effective and persistent expression after one round of genome editing.

Conclusion: We have demonstrated that CRISPR-Cas9-mediated genome editing at the promoter region induces stable IL-10 expression. Targeted editing outside of the coding region potentially leads to a safer application of whole organ gene editing. Our findings expand the potential of genome editing towards engineering optimized donor organs for transplantation.