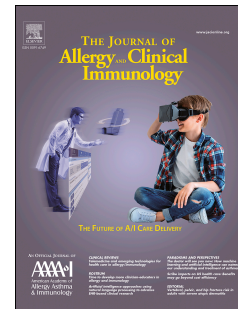


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Title page: letter to the editor

**Drug allergy to CFTR modulator therapy associated with lumacaftor-specific
CD4+ T lymphocytes**

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Capsule summary: Allergy to CFTR modulators will have a high impact on affected patients in the future. This work demonstrates the use of *in vitro* cell culture methods to detect the causative component of CFTR modulator combinations.

Keywords: T-cells, drug hypersensitivity, human

To the Editor: Patients with cystic fibrosis (CF) have been reported to have an increased risk to develop T-cell mediated drug allergies against antibiotics.^{1, 2} This clinical challenge is well known to cause restrictions to optimal anti-infective treatment and may limit individual patient outcomes.^{3, 4} However, the risk of allergy to small molecule cystic fibrosis transmembrane regulator (CFTR) modulator drugs (ivacaftor, lumacaftor, tezacaftor and elaxacaftor) that target the underlying cause of the disease and have become available for an increasing percentage of patients with CF remains unknown.⁵⁻⁷

Here, we report a T-cell mediated non-immediate allergy in a 20-year old female patient with CF homozygous for the Phe508del mutation following initiation of CFTR modulator therapy with lumacaftor/ivacaftor. The patient presented with advanced lung disease (FEV1 percent predicted of 40%), chronic pulmonary infection with *Pseudomonas aeruginosa* and *Achromobacter*, and a history of non-immediate drug allergy to piperacillin. Two weeks after starting lumacaftor/ivacaftor therapy, the patient developed malaise and a severe generalized skin reaction that featured progressive rash, facial swelling, and pruritus (Figure 1A). There was no clinical evidence for SJS or DRESS because there was no skin blistering, no involvement of the mucosa, no liver involvement and no eosinophilia in the peripheral blood. This allergic reaction led to hospitalization of the patient, treatment with high dose systemic corticosteroids and discontinuation of lumacaftor/ivacaftor therapy. The symptoms ceased rapidly thereafter. To determine if lumacaftor/ivacaftor could be restarted and given that no other CFTR modulator option was available at that time, an allergy work-up was performed. Medical history revealed stable concomitant medication, which was continued without change after the allergic reaction had occurred. Skin prick tests with ground commercial tablets containing ivacaftor, lumacaftor/ivacaftor and tezacaftor/ivacaftor (note,

the testing protocol with CFTR modulators is not validated) were negative for immediate and late readings. Patch and intradermal testing were not possible due to the absence of testing reagents.

In vitro diagnostics including the lymphocyte transformation test and IFN- γ peripheral blood mononuclear cells (PBMC) ELISpot yielded negative results with ivacaftor, lumacaftor and tezacaftor (pure compounds for testing were obtained from Selleck Chemicals, Houston, USA); however, a T-cell-mediated mechanism was identified through the detection of drug-responsive T-cell clones. T-cell lines were established by culturing PBMC from the patient with ivacaftor, lumacaftor and tezacaftor (each at a concentration of 50 μ M) for 14 days in IL-2-containing medium. Clones were then generated by serial dilution and repetitive mitogen stimulation, and characterized using established methods.² The proliferative response and cytokine release were measured using [³H]thymidine incorporation and ELISpot, respectively, after incubating clones (5×10^4 / 50 μ L) with autologous EBV transformed B-cells (1×10^4 / 50 μ L) in a U-bottomed 96 well plate in the presence and absence of ivacaftor, lumacaftor and tezacaftor (each at a concentration of 50 μ M) for 48 hours (37°C, 5% CO₂). Thirty three drug-responsive clones expressing different V β receptors were generated from the lumacaftor lines (a total of 96 clones were assessed; Figure 1B). In contrast, clones generated from ivacaftor and tezacaftor lines were not activated with either drug. Lumacaftor-responsive clones were stimulated to proliferate in a dose-dependent manner (Figure 1C). Figure 1D shows the structure of the test compounds with hydrogen bond acceptors/donors indicated. Cross-reactivity with ivacaftor and tezacaftor was not observed (Figure 1E). Lumacaftor-treatment resulted in the secretion of IFN- γ , IL-10, IL-13 and IL-22, alongside the cytolytic molecule granzyme B (Figure 1F).

All lumacaftor-responsive clones were CD4⁺ (as assessed by flow cytometry) and anti-HLA antibody blocking experiments revealed that T-cell activation was HLA class II-restricted (Figure 2A). Drugs activate T-cells through direct reversible HLA binding or through the covalent modification of proteins. Covalently-modified proteins are processed into peptides within antigen presenting cells, some of which associate with HLA molecules for presentation to T-cells.⁸ The pathway of drug presentation can be determined *in vitro* through exclusion of antigen presenting cells, antigen presenting cells pulsing assays (antigen presenting cell washing removes weakly bound drug), fixation of antigen presenting cells (fixation blocks protein processing) and addition of glutathione (glutathione blocks the binding of reactive drugs to thiol-containing proteins). Lumacaftor-responsive CD4⁺ clones required antigen presenting cells for effective drug presentation. Lumacaftor-pulsed antigen presenting cells did not stimulate the clones to proliferate, whereas fixation of antigen presenting cells and addition of glutathione had no effect on the lumacaftor-specific T-cell response (Figure 2B). These data indicate that lumacaftor binds directly to HLA class II molecules to activate the T-cell clones.

Collectively, these data show for the first time a strong patient-specific lymphocytic response to lumacaftor. At the same time, we could demonstrate that there was no response to ivacaftor or tezacaftor. Thus T-cell cloning enabled detection of the likely allergy-causing compound contained in the fixed CFTR modulator combination therapy lumacaftor/ivacaftor. These data suggest that the allergic reaction in our patient was potentially caused by lumacaftor-specific CD4⁺ lymphocytes, which led to discontinuation of lumacaftor/ivacaftor therapy and cessation of clinical symptoms in our patient. We

therefore speculate that the detection of T-lymphocytes reactive to specific CFTR modulators may be useful for future diagnosis, clinical management, therapeutic decision-making and for individualized therapeutic approaches in rare cases of T-cell-mediated allergy against CFTR modulators. This will be of great importance for patients who develop allergy to the components of highly effective triple therapy CFTR modulators.

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Figure legend

Figure 1: Characterization of lumacaftor-responsive clones. (A) Images showing the allergic skin reaction in a patient treated with CFTR modulator combination therapy. (B-E) Proliferation of lumacaftor-specific clones measured by [³H] thymidine incorporation. Clones (0.5×10^5) were incubated with irradiated EBV-transformed autologous B-cells (0.1×10^5) and study compounds for 48h before the addition of [³H] thymidine. (B) Initial specificity test identifying 33 lumacaftor-responsive clones. (C) Dose-response experiments (n=15 clones; dashed line shows average response). (D) Structure of the 3 study compounds showing similarities and differences (circle indicates structural similarity). (E) Cross-reactivity experiments (three representative clones shown). (F) Cytokine release from lumacaftor-responsive clones quantified by ELISpot (one representative clone shown).

Figure 2. Pathway of lumacaftor-specific T-cell activation. (A) Involvement of HLA molecules was investigated by omitting antigen presenting cells and adding antibodies against HLA class I and II (BD Biosciences, Oxford, United Kingdom) to the proliferation assay. (B) Clones were cultured with lumacaftor-pulsed antigen presenting cells, lumacaftor and fixed antigen presenting cells and lumacaftor and glutathione and proliferation was measured using [³H] thymidine.

The authors declare that they have no relevant conflicts of interest.

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Figure 1

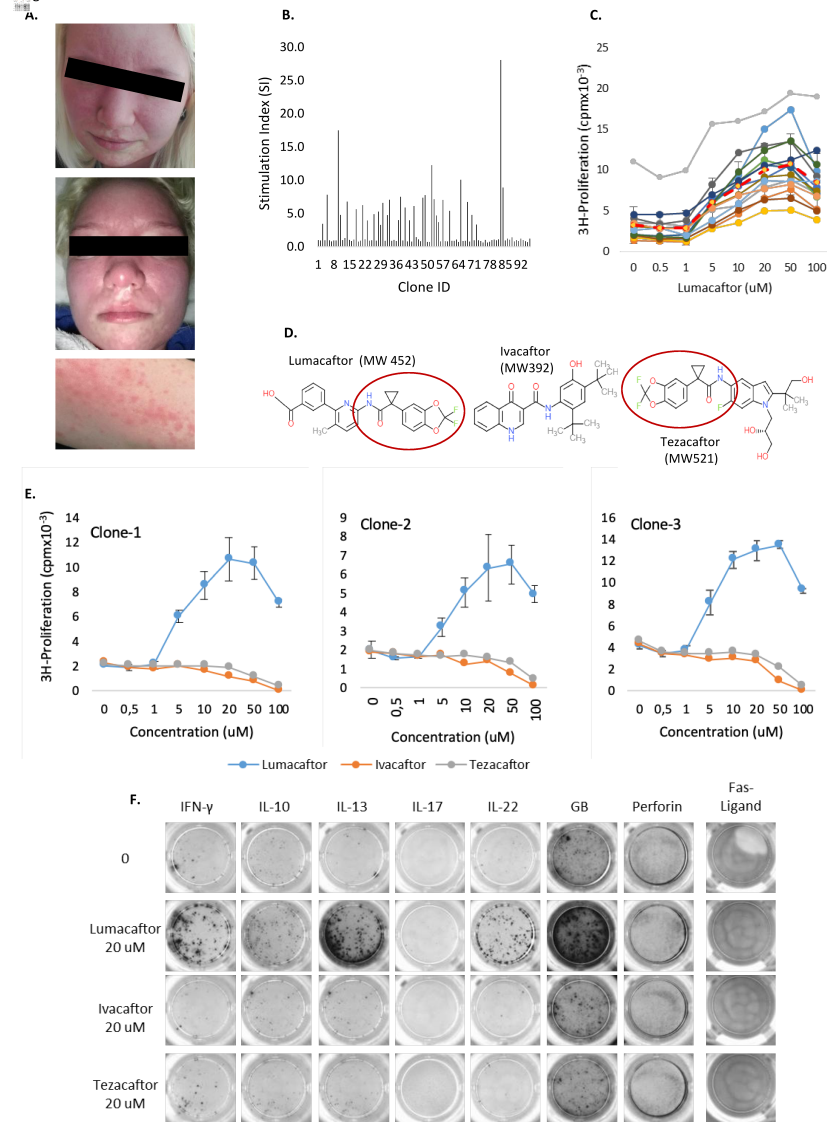


Figure 2

