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SP-101 activity increases in a dose-dependent manner to vector and doxorubicin dose

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Background: SP-101 (AAV2.5T-SP183-hCFTRΔR) is a novel recombinant adeno-associated virus (AAV) gene therapy vector being investigated as an inhalation treatment, followed by inhaled doxorubicin, for people with cystic fibrosis (CF) in a mutation-agnostic manner. SP-101 is composed of a novel capsid optimized for efficient apical transduction of human airway epithelial (HAE) cultures and encodes a promoter/enhancer that drives expression of a human CF transmembrane conductance regulator (CFTR) minigene (hCFTRΔR). Doxorubicin is a small molecule able to increase expression of hCFTRΔR messenger ribonucleic acid (mRNA) when used in combination with SP-101. To understand the dose-response relationship between SP-101 and doxorubicin and the amount of mRNA expression required for functional correction, we investigated vector copy number (VCN), hCFTRΔR mRNA expression and vector tropism in relation to functional correction of human CF-HAE epithelia from donors with class I, II, or III mutations.
Methods: SP-101 was applied to the apical surfaces of CF-HAE cells cultured at the air-liquid interface with or without doxorubicin added to the basal media. Forskolin-induced CFTR chloride conductance, measured via Ussing chamber assay, was compared with cellular VCN, measured via droplet digital polymerase chain reaction, and mRNA expression, measured by quantitative polymerase chain reaction, 7 days after transduction. Cellular integrity was measured by evaluating transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) levels in culture media. Tropism to CF-HAE cells was determined by five-color wholemount immunostaining and confocal microscopy for mCherry-positive cells and various cell-type markers after transduction with an AAV2.5T-mCherry reporter vector.
Results: In the presence of doxorubicin, SP-101 restored forskolin-induced CFTR-mediated chloride conductance in all mutation classes tested to levels comparable with those of non-CF controls, with a class I mutation donor showing the largest chloride response at the highest multiplicity of infection (MOI; 1e5 vector genomes [vg]/cell). Functional chloride correction increased with increasing MOI, correlating with increasing VCN and hCFTRΔR mRNA expression. Similarly, increasing doxorubicin concentrations increased chloride conductance and hCFTRΔR mRNA expression in CF-HAE cells without significantly affecting VCN. Low doxorubicin concentrations (as low as 0.5 μM) were able to restore CFTR-mediated chloride conductance with as little MOI as 5e3 vg/cell, whereas SP-101 alone at the highest MOI (1e5 vg/cell) was not sufficient to restore chloride conductance. TEER and LDH levels were not significantly different from control epithelia, indicating no obvious toxicity as a result of treatment. Using an AAV2.5T-mCherry reporter vector, we were able to show that approximately 30% to 40% of CF-HAE cells, including ciliated, secretory, and basal-like cells, expressed the reporter gene under the same experimental conditions, providing insight into which and how many cells contribute to the correction of CF observed in vitro.
Conclusions: These data demonstrate the importance of doxorubicin co-administration with SP-101 to achieve hCFTRΔR mRNA expression levels resulting in correction of CF-HAE, supporting co-development of inhaled SP-101 followed by inhaled doxorubicin for the treatment of CF.

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Elexacaftor/tezacaftor/ivacaftor corrects function of H1085R-, N1303K-, and R334W-cystic fibrosis transmembrane conductance regulator and improves clinical status of patients

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Background: The next-generation cystic fibrosis (CF) transmembrane conductance regulator (CFTR) modulator elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) is the combination of correctors (ELX, TEZ) with a potentiator (IVA). ELX/TEZ/IVA has been approved in Europe for patients carrying at least one F508del-CFTR allele (p.Phe508del). In France, patients with severe respiratory impairment can receive ELX/TEZ/IVA treatment as part of a temporary authorization for use. We aimed to study the efficacy of ELX/TEZ/IVA in correcting activity of non-F508del-CFTR variants with processing defects in a human nasal epithelial (HNE) primary cell model.
Methods: People with CF with severe respiratory insufficiency were recruited for HNE cell sampling with the following genotypes (N1303 K/N1303 K, M1 T/R334W N1303 K/H1085R [n = 2]). HNE cells were amplified with conditional reprogramming and differentiated in air-liquid interface (ALI) conditions. To assess CFTR function, short-circuit current measures were made on ALI HNE cultures treated with dimethyl sulfoxide or ELX/TEZ/IVA (ELX and TEZ at 3 μM and IVA at 100 nM) for 48 hours.
Results: ELX/TEZ/IVA significantly corrected N1303K-CFTR and R334W-CFTR chloride secretion activity up to 5.2% (range 4.7–5.5%) and 10% (range 8.5–11.8%) of wild-type (WT)-CFTR function. The most spectacular, significant increase in CFTR-dependent chloride secretion was measured in N1303 K/H1085R cells, which reached a mean greater than 63% (range 57–69%) of WT-CFTR activity. Bicarbonate transport in the M1 T/R334W patient's HNE cells was improved up to 13% and in N1303 K/H1085R cells up to 60% (range 40%–70%) of WT-CFTR level. All these patients improved at short time FEV_1 by a mean of 40% (range 20–67%) and body weight by a mean of 4.3 kg (range 1.5–7.4 kg). Sweat chloride significantly decreased, by a mean of 65 mmol/L (range 56–74 mmol/L), except in the N1303K-homozygous patient.
Conclusions: ELX/TEZ/IVA significantly improves CFTR activity of N1303 K, H1085R, and R334W variants. Clinical data supported by in vitro results strongly suggest that use of ELX/TEZ/IVA could be therapeutically beneficial for patients with N1303 K, H1085R, and R334W mutations. A compassionate therapy program could confirm the predictive character of the HNE model and will allow new therapeutic molecules to be tested.
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