



Immunogenicity In Ferrets After Inhalation Delivery of SP-101

(AAV2.5T-hCFTR Δ R) for the
Treatment of Cystic Fibrosis

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Presenter Disclosure

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Outline

 Introduction to SP-101 + Doxorubicin

 Ferret Study Overview

 Nonclinical Immunogenicity Evaluation: Methods and Data

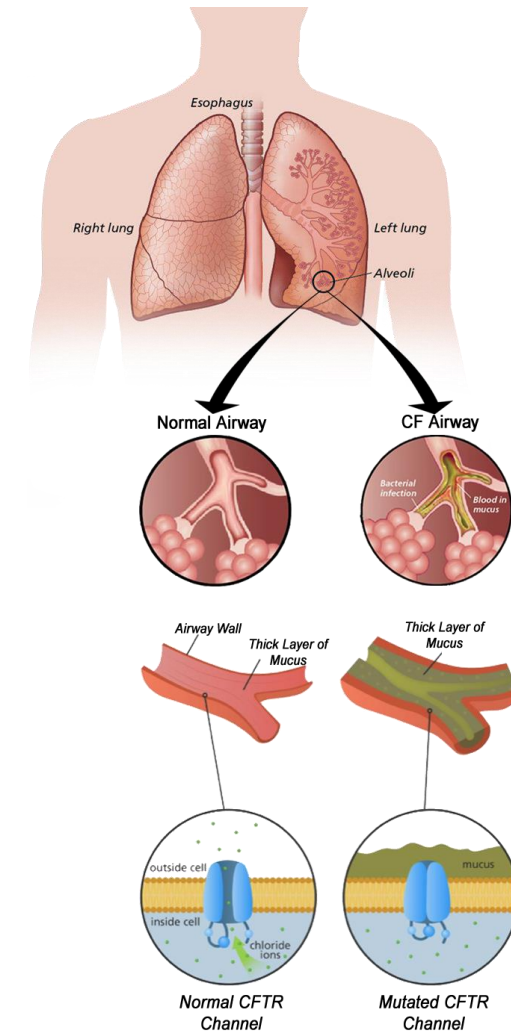
 Conclusions

 Questions?

Cystic Fibrosis is a monogenic, fatal respiratory disease

- Caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene
- CFTR is an anion channel: mediates chloride (Cl^-) and bicarbonate (HCO_3^-) transport
- The most serious manifestations are within the respiratory tract
- Characterized by thickened mucus, leading to infection, progressive inflammation, and respiratory failure
- Early mortality with high morbidity among all patients
- Small molecule modulators address specific mutations in (eg, ivacaftor, elexacaftor, lumacaftor)
- 10-20 % of people with CF have CFTR mutations that do not or only poorly respond to small molecule modulators

GENE THERAPY IS THE ANSWER FOR CURING CF



Ratjen 2015
Cystic Fibrosis
Foundation

SP-101 – a novel, inhaled gene therapy to treat CF

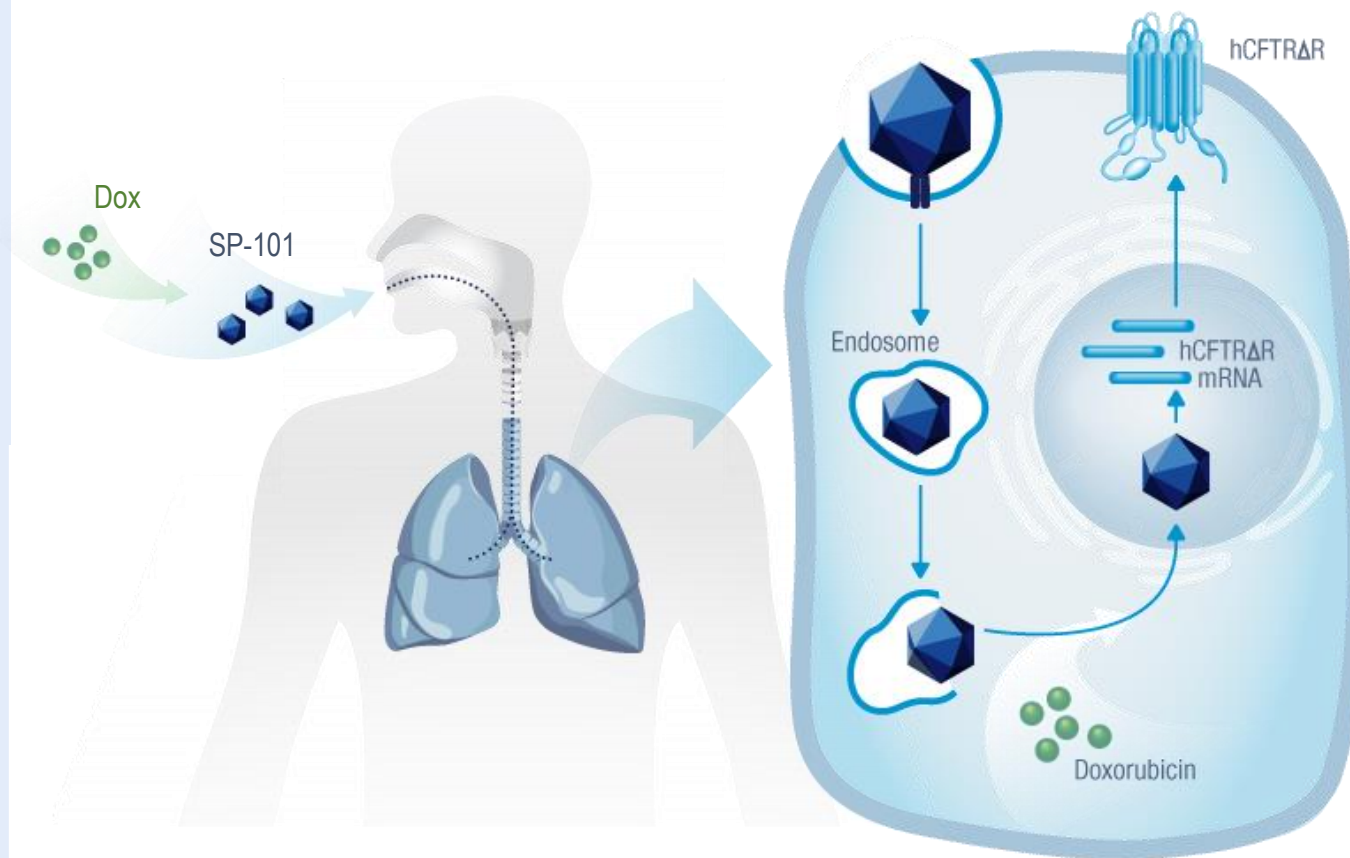
DESIGN FEATURES



- AAV 2.5T capsid selected for tropism to the apical surface of human airway epithelia (HAE)¹
- hCFTR Δ R contains partial deletion with regulatory elements^{2,3}

MECHANISM OF ACTION



- Efficient apical entry
- Enhanced SP-101 translocation to the nucleus provided by doxorubicin^{4,5}
- Increased CFTR expression

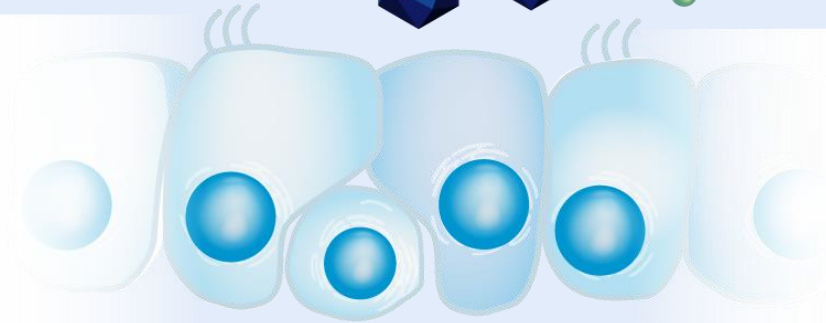


¹Excoffon et al., PNAS 2009; ²Ostedgaard et al., PNAS 2002; ³Yan et al., Hum Gene Ther. 2015; ⁴Yan et al. J Virol. 2004; ⁵Zhang et al., Mol Ther. 2004

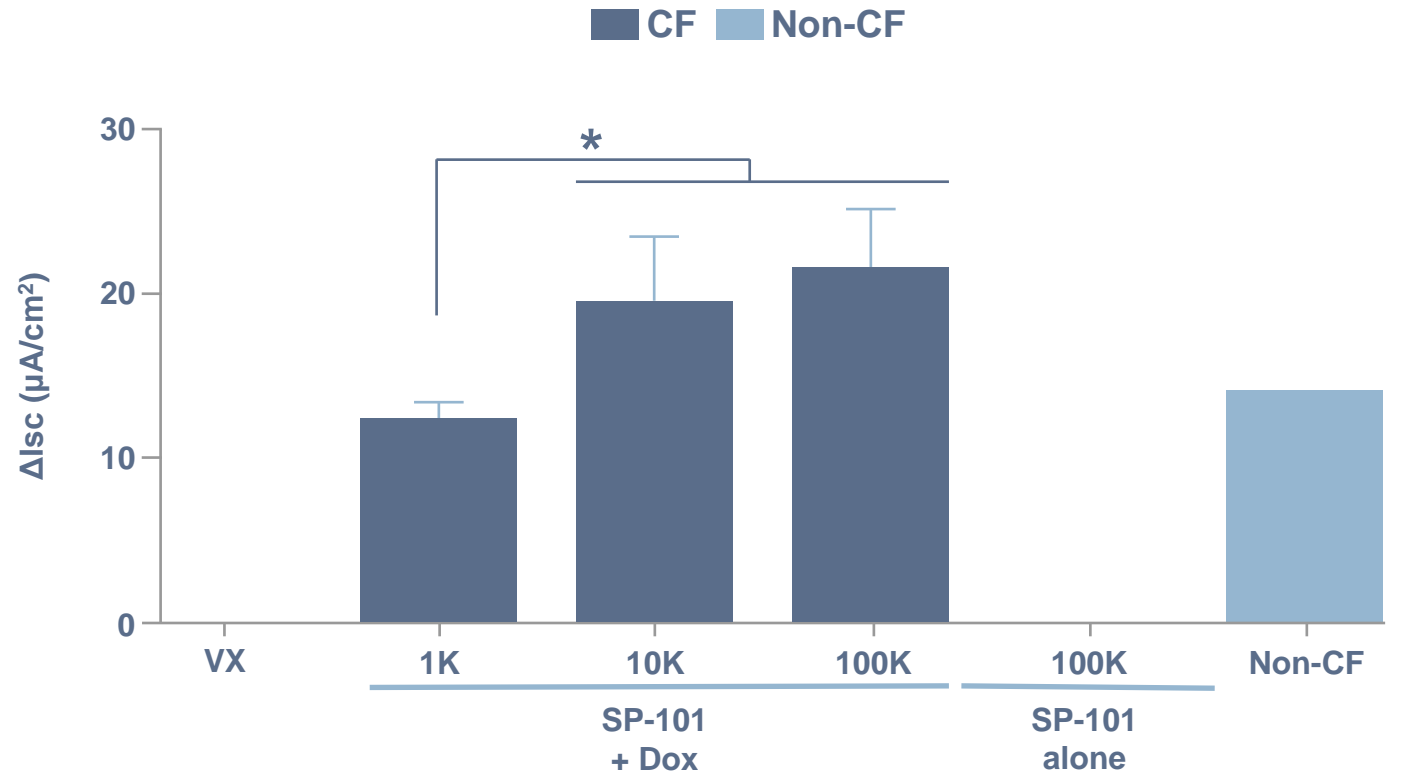
SP-101 demonstrates dose-dependent functional correction

Polarized primary human
CF airway epithelia with
class I mutations
(W1282X/R1162X, N=3)

SP-101     Dox



CFTR Function (Ussing)
SP-101/Dox 16 h incubation, analysis at D7



*p < 0.05

VX - CFTR triple modulators VX-770/661/445

Ferret as a model to evaluate inhaled SP-101

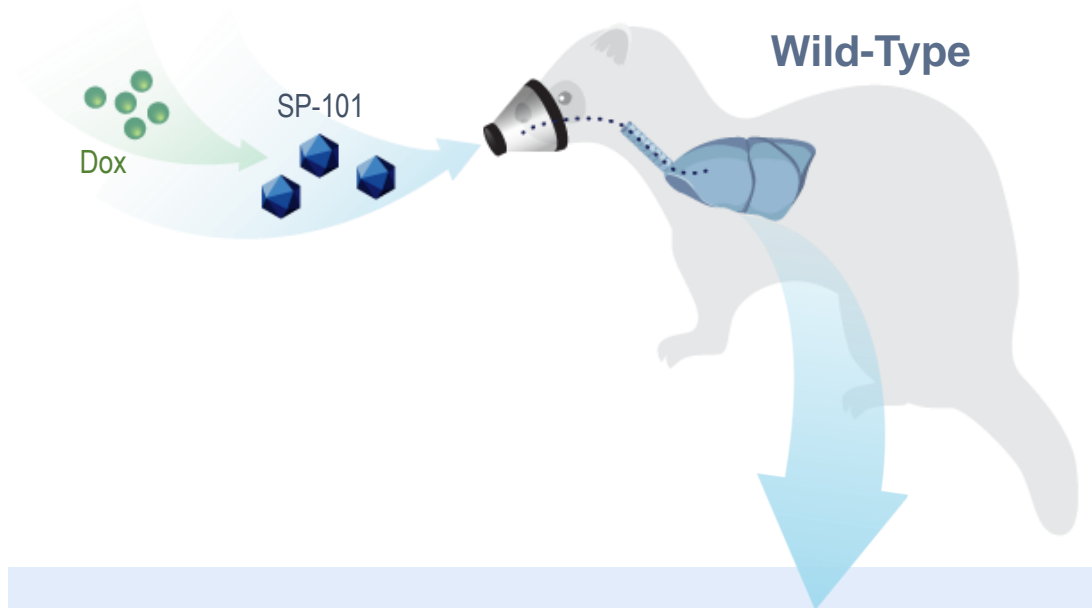


SP-101 capsid is tropic to ferret airway cells¹

CF ferret model recapitulates human CF lung pathology²

Ability to administer via inhalation

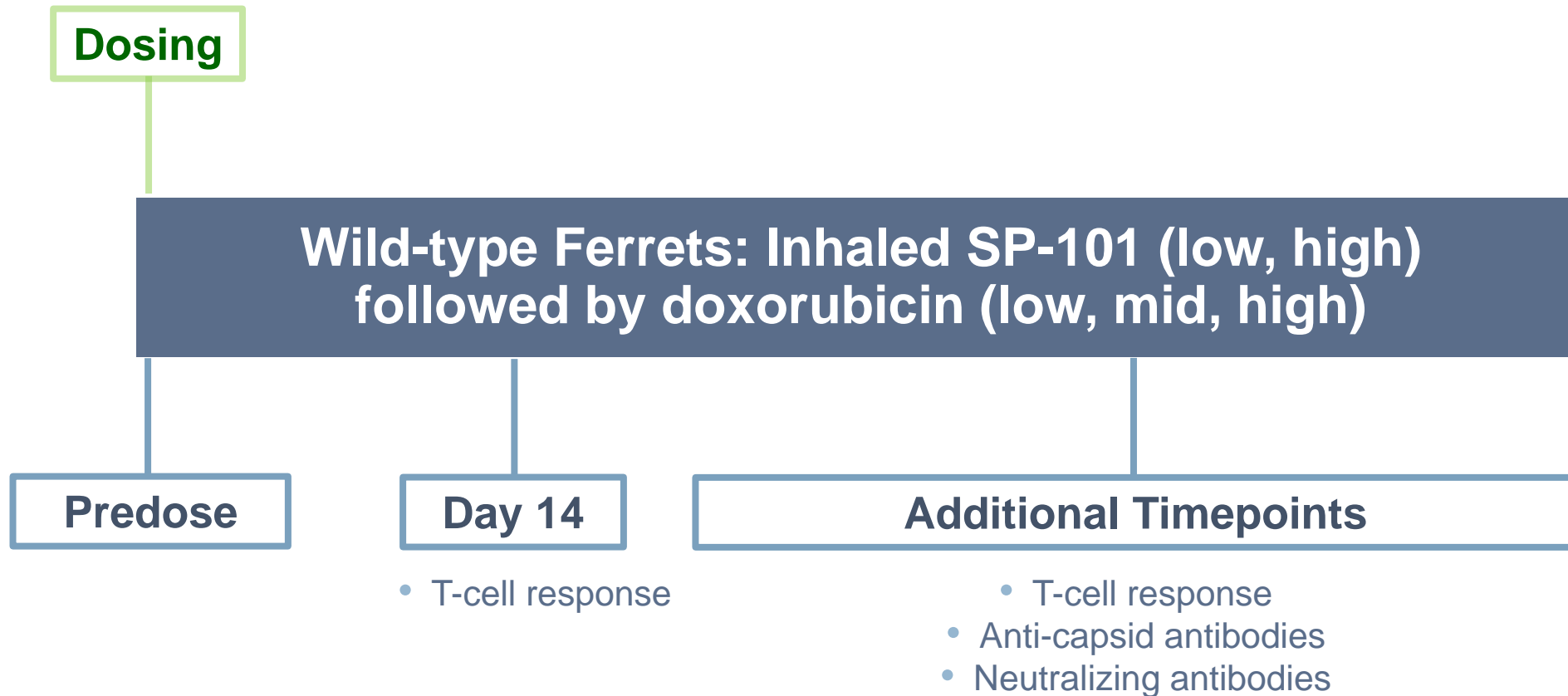
METHODS



- Total Antibodies Against AAV 2.5T
- Neutralizing Antibodies against AAV 2.5T
- T-Cell Response to AAV2.5T and hCFTR Δ R via IFN- γ ELISPOT

¹ Tang et al, Mol Ther Methods Clin Dev. 2020 ² Sun et al, Sci Transl Med. 2019

Wild type ferret study design

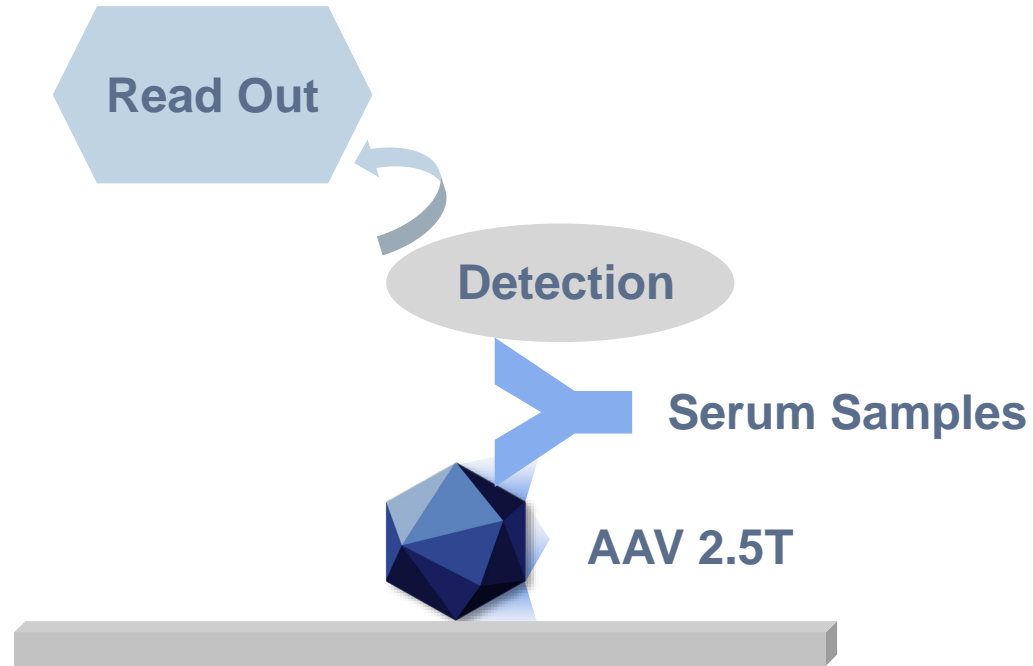


Immunogenicity of Inhaled SP-101 in Ferrets

SP-101 immunogenicity assays	Purpose
Total antibody: Capsid	<ul style="list-style-type: none">• Humoral immune response to SP-101 capsid• Potentially informative in case of safety findings
Neutralizing antibody: Capsid	<ul style="list-style-type: none">• Fraction of humoral immune response with functional neutralizing activity• Informative for potential re-administration
T-cell response via IFN-γ ELISPOT: Capsid*	<ul style="list-style-type: none">• Potentially informative in the case of activity loss
T-cell response via IFN-γ ELISPOT: hCFTRΔR*	<ul style="list-style-type: none">• Potentially informative in the case of activity loss

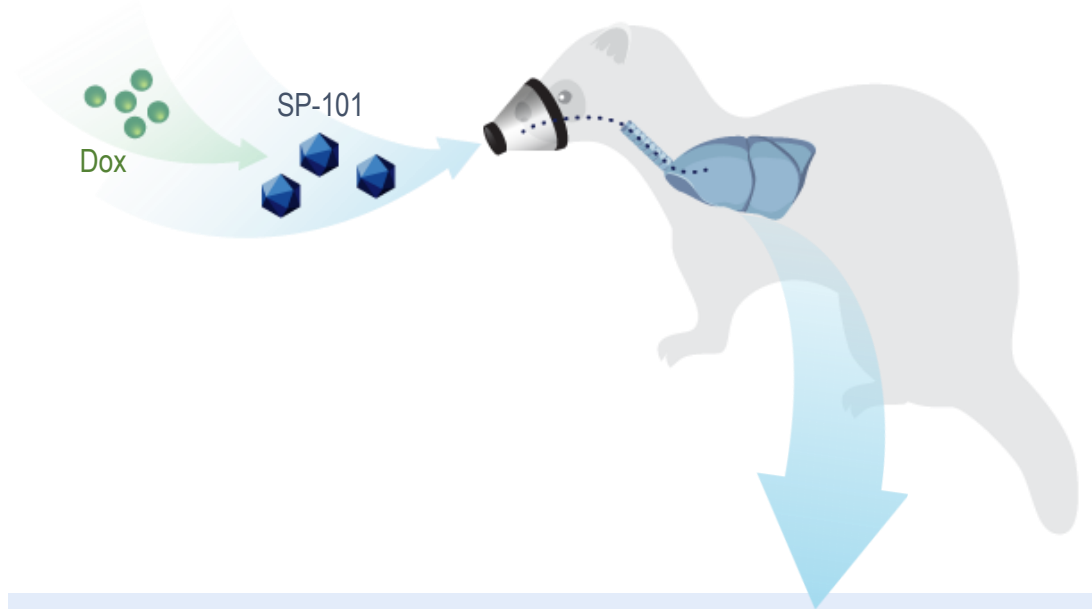
* T-cell response is to the peptide pools representative of the capsid and hCFTR Δ R proteins

Total Antibodies Against AAV2.5T Capsid



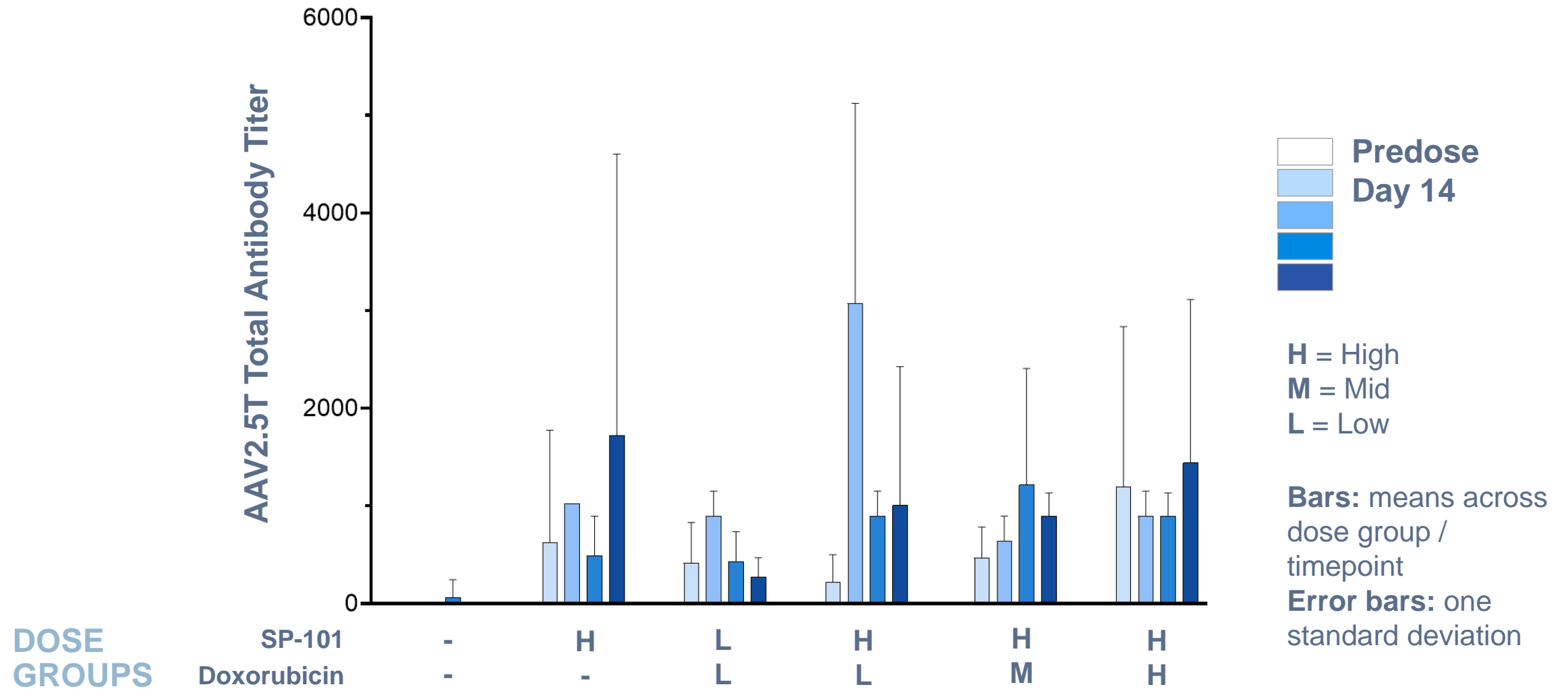
Tier 1 → Tier 2 → Tier 3

Positive cut-off: statistically calculated

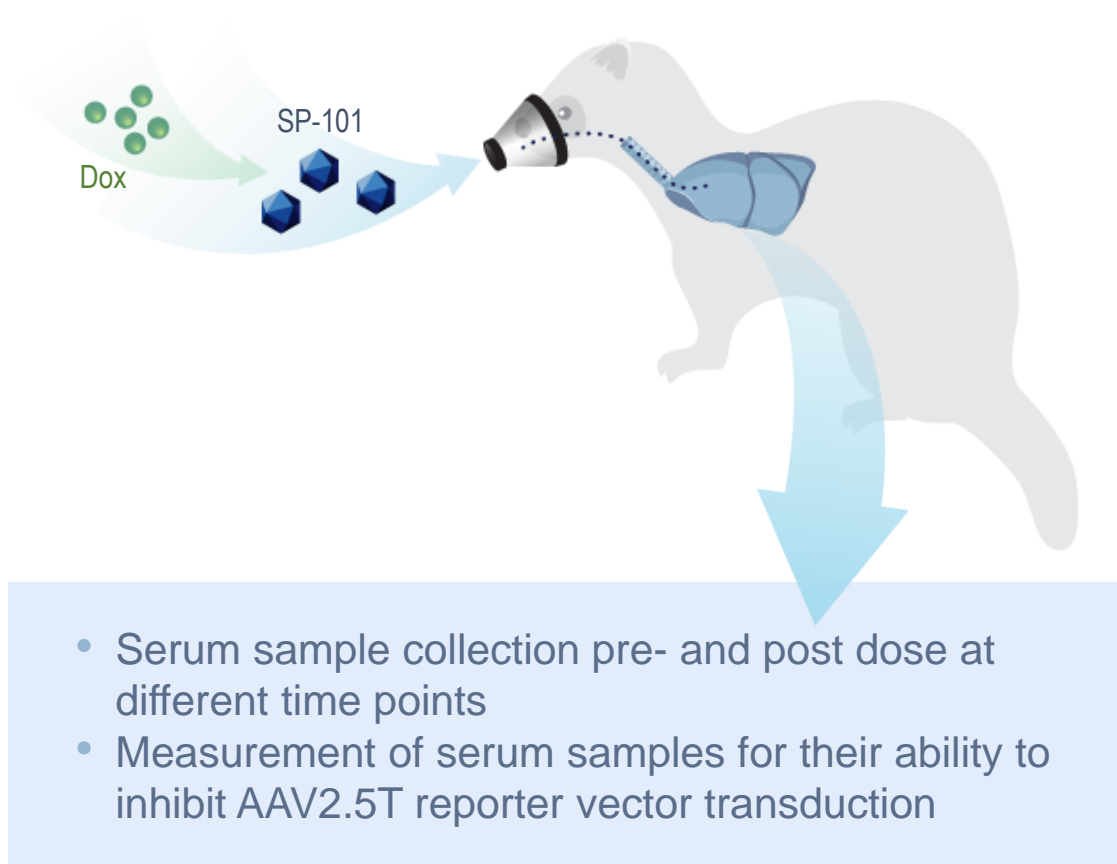
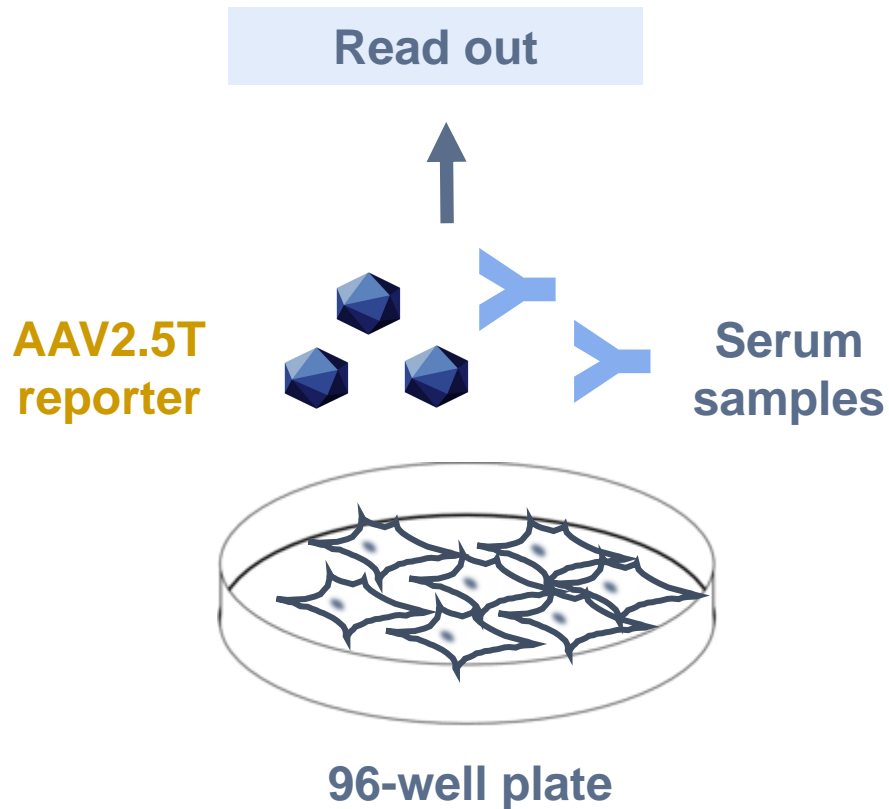


- Serum sample collection pre- and post dose at different time points
- Sample evaluation in ELISA assay

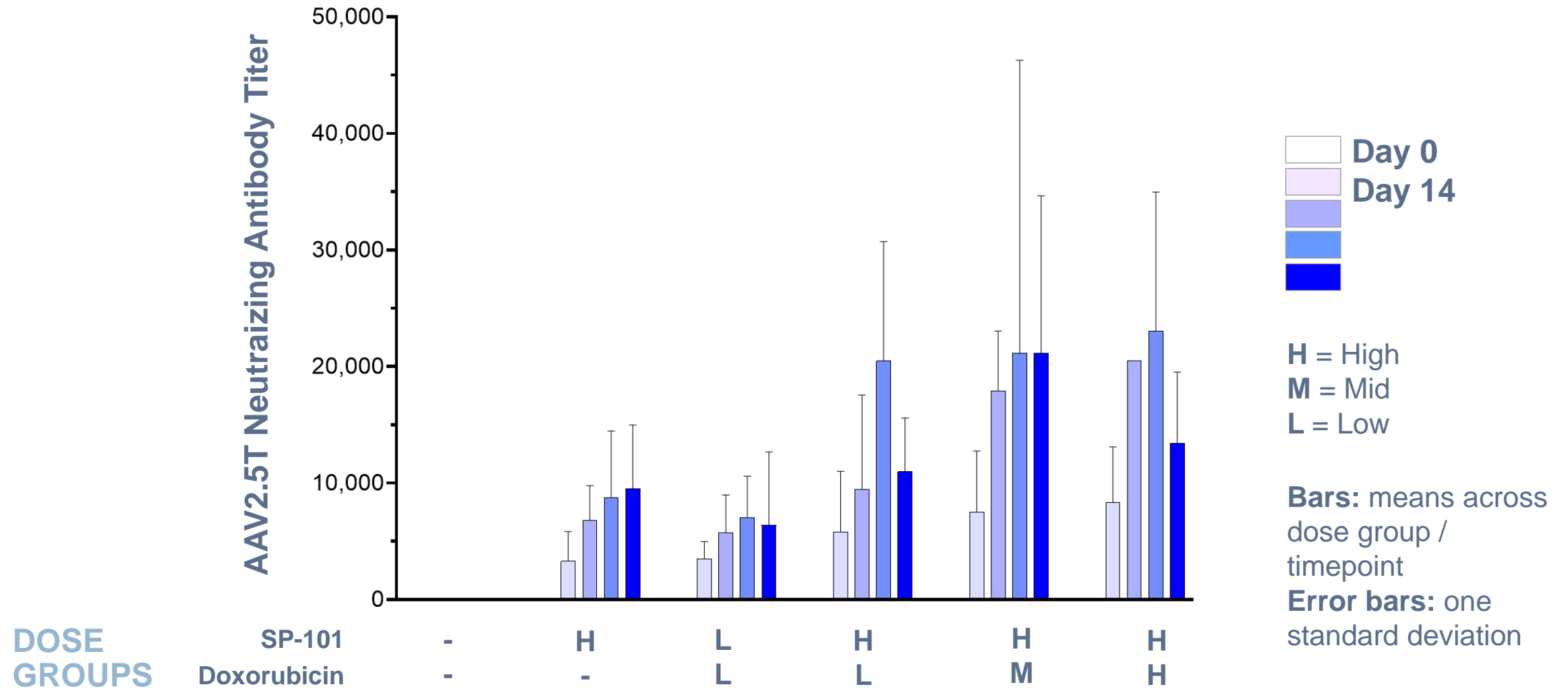
Exposure to SP-101 results in the development of Anti-AAV2.5T Antibodies



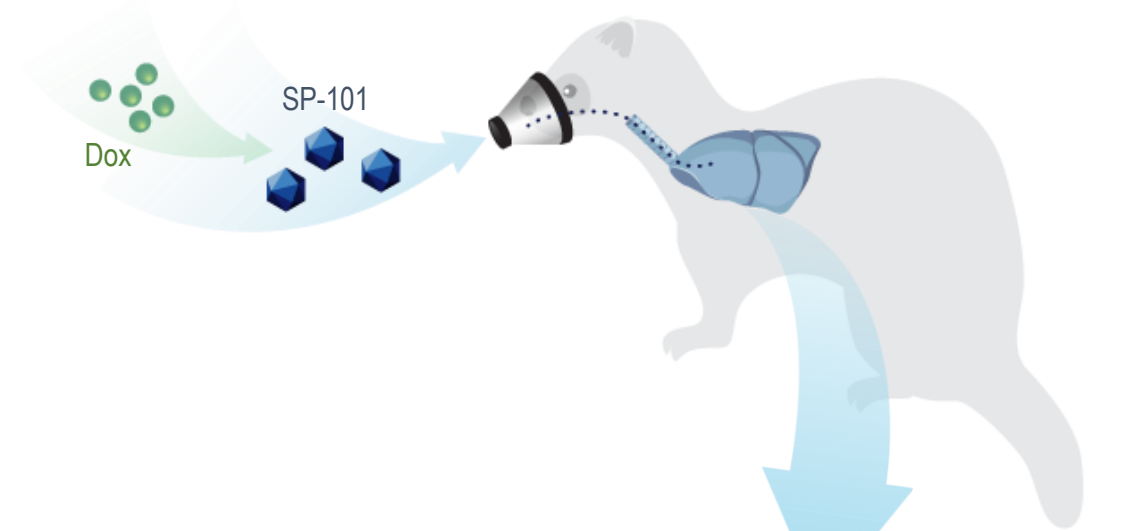
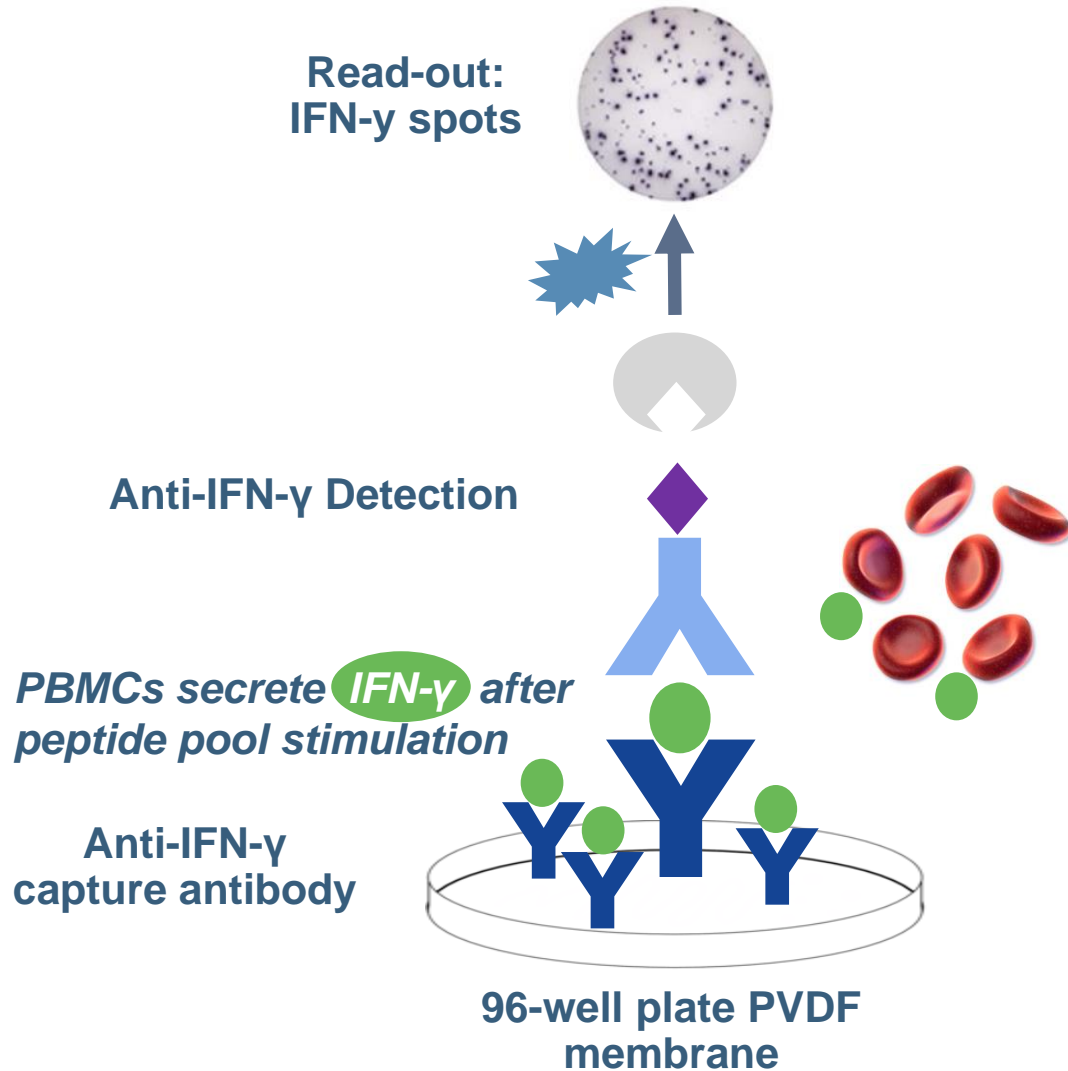
Neutralizing Antibodies Against AAV2.5T



Exposure to SP-101 results in the development of Anti-AAV2.5T Neutralizing Antibodies

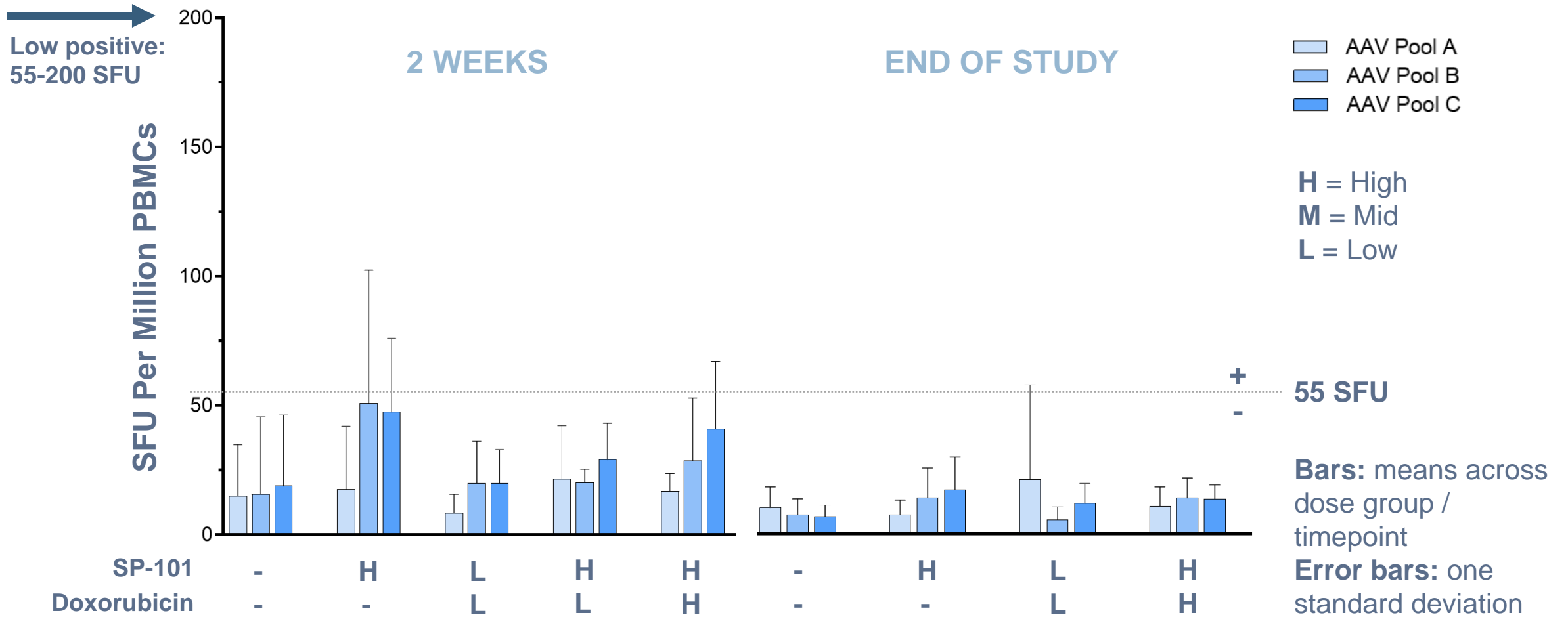


IFN- γ ELISPOT for T-cell responses to AAV2.5T and hCFTR Δ R in PBMCs

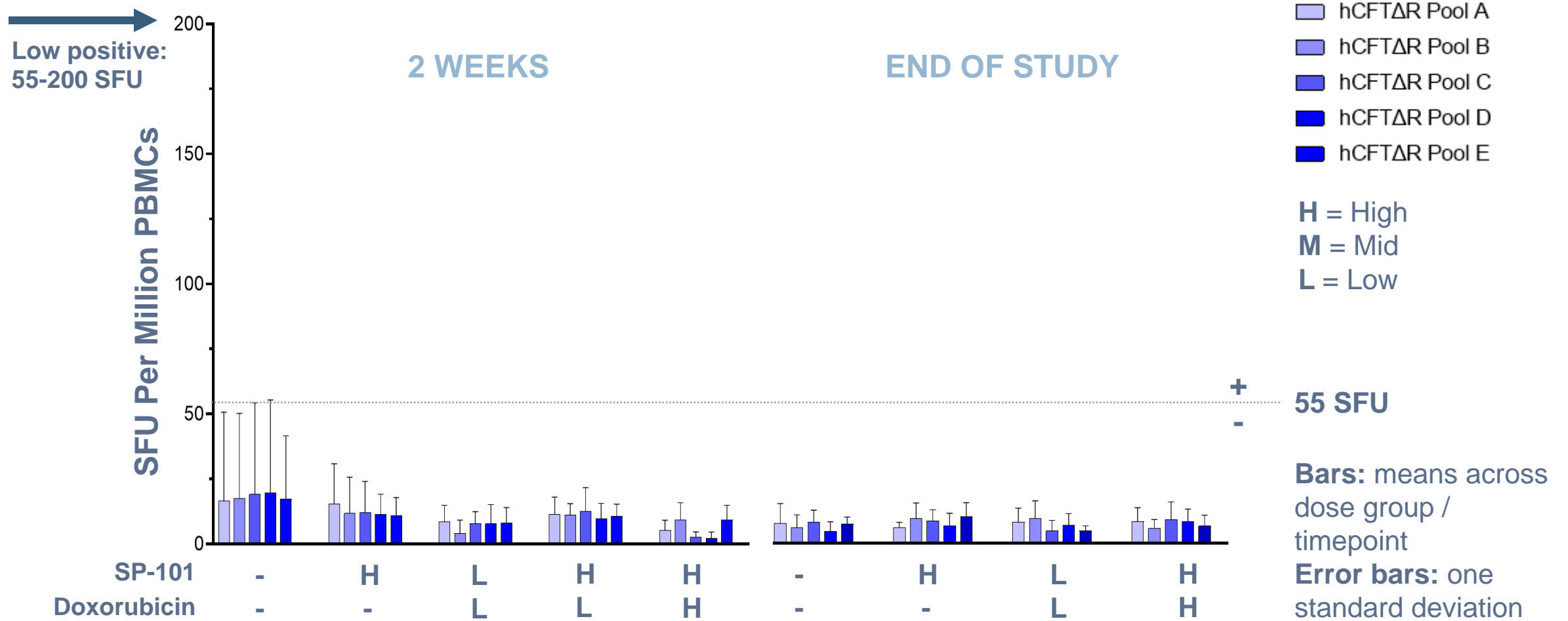


- PBMCs collected on day 14 post SP-101 administration and end of study
- Stimulation of cultured PBMCs with AAV2.5T and hCFTR Δ R peptide pools
- Measurement of IFN- γ spots on culture plate

Very weak T-cell response (via IFN- γ ELISPOT) against AAV2.5T peptide pools



No T-cell response (via IFN- γ ELISPOT) against hCFTR Δ R peptide pools



Conclusions



In exposed ferrets, serum samples were positive for Anti-SP-101 total antibodies at all post-exposure time points evaluated



In exposed ferrets, samples were positive for Anti-SP-101 neutralizing antibodies at all post-exposure time points evaluated



~20% of samples from SP-101 treated ferrets generated a low positive response in the IFN- γ ELISPOT assay predominantly against the AAV2.5T capsid peptide library



The low T-cell response against the AAV2.5T capsid and the human hCFTR Δ R transgene supports the durability of hCFTR Δ R mRNA expression in ferret lung

Remaining Questions

- How long lasting is the humoral immune response?
- What is the quality and duration of the local humoral immune response in the respiratory tract?