

## **AAV delivery of PD-L1 with concomitant CTLA-4 immunoglobulin attenuates acute cellular rejection in a rat lung transplant model**

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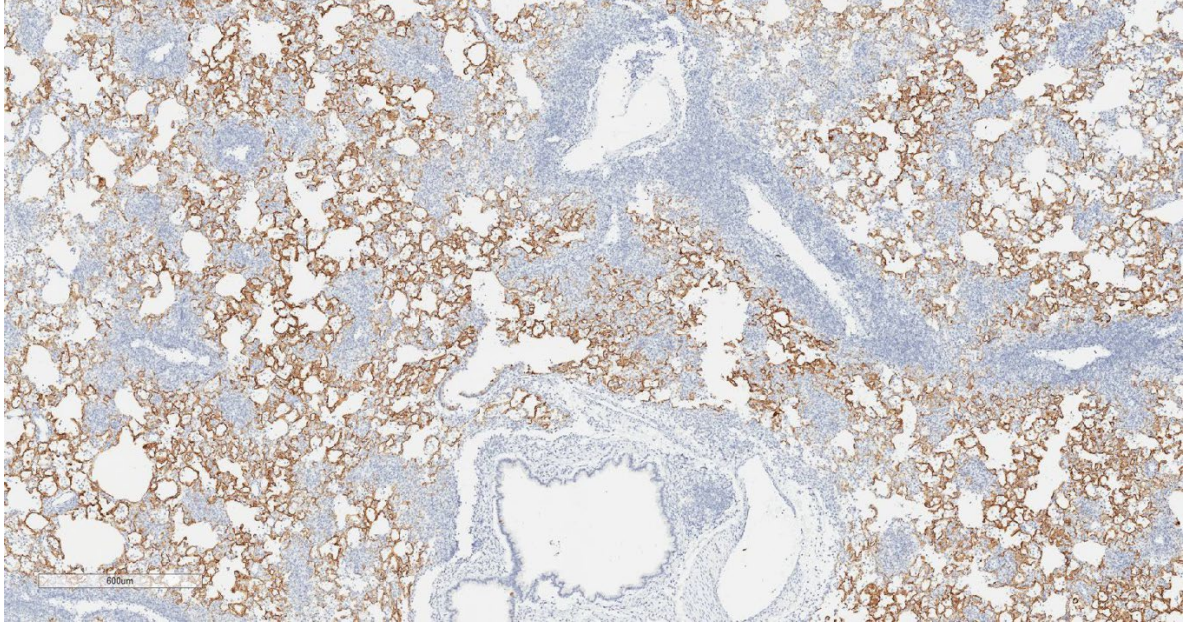
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**Objective:** T cell activation is central to the pathologic process of acute cellular rejection (ACR) and lymphocytic bronchiolitis following lung transplantation. Viral vector-mediated gene delivery serves as a potential strategy to suppress T cell activation to attenuate ACR, potentially reducing the need for high dose, toxic immunosuppression. Here, we evaluated the impact of AAV9-mediated PD-L1 expression in donor lungs in an allogeneic rat lung transplant (LTx) model.

**Methods:** Left lungs were procured from Brown Norway rats and transplanted into Fischer F344 recipients (n=11). In the experimental cohort, 100uL of 4e11 viral genomes (vg/mL) AAV9 vectors encoding PD-L1 gene were administered via left bronchus during static cold storage. The control group underwent transplantation without viral administration. Both cohorts were treated with a dose of CTLA-4 immunoglobulin (abatacept) on the first post-operative day (POD) and sacrificed on POD 14. Using H&E-stained slides, ACR was assessed by a blinded lung transplant pathologist following ISHLT lung rejection scoring guidelines. Immunohistochemistry was utilized to assess for PD-L1 transgene expression.

**Results:** All animals survived to the experimental endpoint. By POD 14, the control cohort (n=5) exhibited severe vascular rejection with a mean rejection score of 3.60, while the experimental cohort (n=6) had a lower mean rejection score of 1.83 (p=0.035). Importantly, significant expression of PD-L1 throughout the lung graft was observed on immunohistochemistry in all experimental animals.

**Conclusions:** Here, we demonstrate successful transgene expression using a novel AAV9 PD-L1 vector during static cold storage in an allogeneic rat lung transplant model. While graft PD-L1 expression with concomitant abatacept did not completely ameliorate ACR, this data suggests that this therapeutic combination can attenuate ACR and potentially improve post-transplant outcomes.



**Figure 1.** Immunohistochemistry demonstrating PD-L1 transgene expression following AAV9 delivery