

P3.108: Development of an Orthotopic Mouse Model for Spread Through Air Space in *EGFR* E19del NSCLC With Gefitinib Resistance

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INTRODUCTION

- Tumor spread through air spaces (STAS) is an invasive growth pattern in non-small cell lung cancer (NSCLC) associated with poor clinical outcomes (1,2).
- Emerging evidence suggests that the prevalence and prognostic impact of STAS vary according to underlying genetic alterations (3).
- However, preclinical models that enable the study of genotype-dependent STAS and its interaction with the lung microenvironment remain limited.
- In this study, we aimed to develop a clinically relevant orthotopic mouse model using the human NSCLC cell line HCC827GR-Luc, harboring an *EGFR* exon 19 deletion (E19del) and acquired resistance to gefitinib, to enable the investigation of STAS in *EGFR*-mutant NSCLC.

METHODS

- Six-week-old male BALB/c nude mice were orthotopically injected with HCC827GR-Luc cells (5×10^5 cells in $40 \mu\text{L}$).
- Following in vivo bioluminescence imaging (IVIS), groups of three mice were sacrificed at predetermined time points (days 14, 21, 28, 35, and 42 after tumor cell inoculation), and the lungs were harvested.
- Lung tissues were formalin-fixed and paraffin-embedded (FFPE), and representative sections containing the largest tumor area were selected for histopathologic evaluation.
- The primary endpoint was the establishment of orthotopic tumors with biologically consistent growth characteristics and key histologic features, including STAS.

DISCUSSION AND CONCLUSION

- Tumor progression followed a **stepwise temporal course**, evolving from early microscopic foci to discrete nodules with central necrosis, and ultimately to extensive lung involvement at later time points.
- At the tumor margin, small clusters of tumor cells were frequently observed detaching from the main tumor mass, **resembling histologic features consistent with STAS**
- In some cases, tumor growth predominantly occurred within the pleural cavity rather than the lung parenchyma, which likely accounts for the relatively low bioluminescence signal observed in these cases.
- In conclusion, our results establish a genotype-specific orthotopic model of gefitinib-resistant *EGFR* E19del NSCLC that recapitulates STAS-like histological features, providing a **practical platform for future STAS-targeted therapeutic and mechanistic studies**.

REFERENCES & ACKNOWLEDGEMENTS

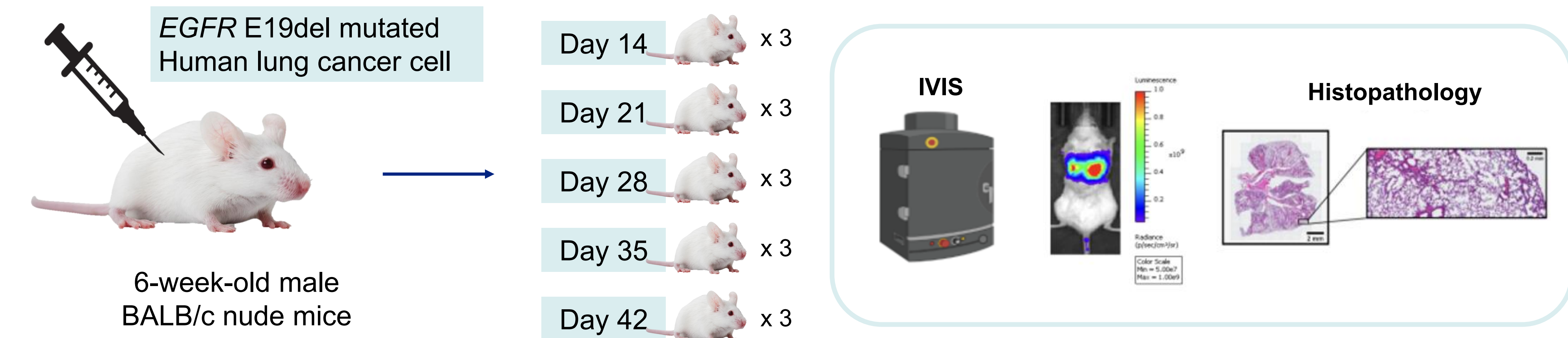
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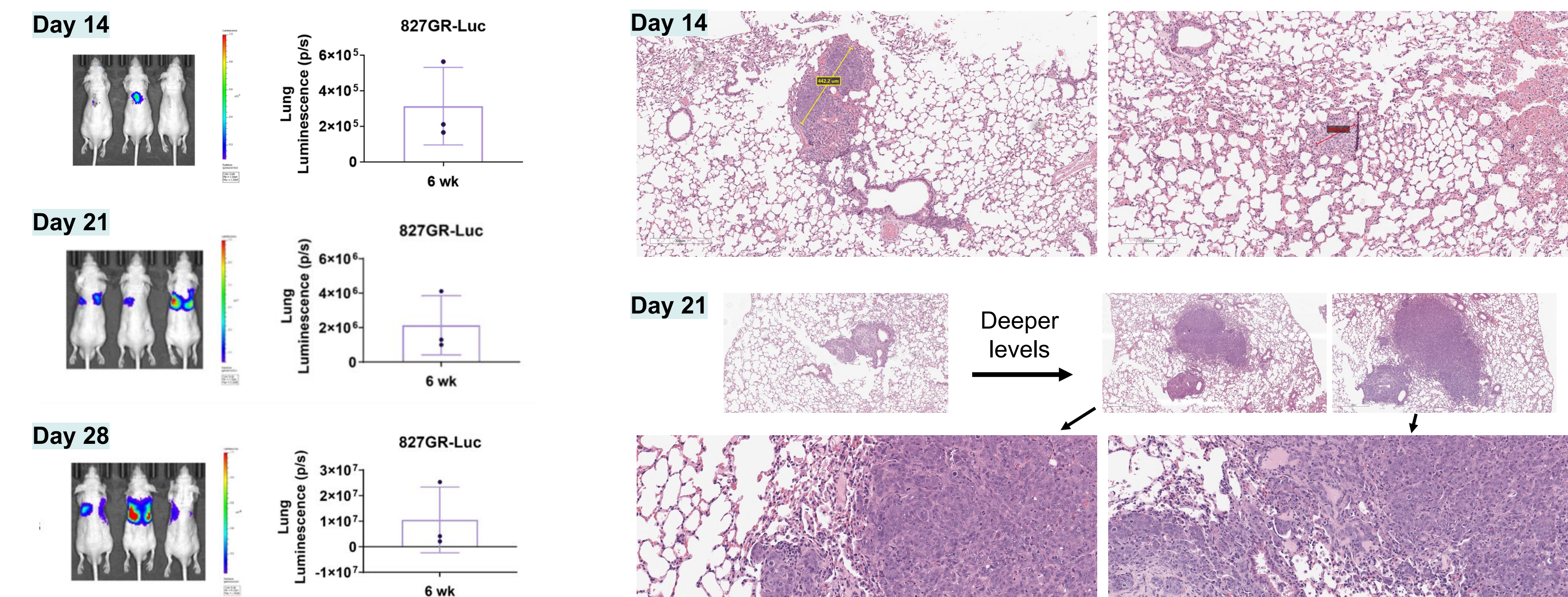
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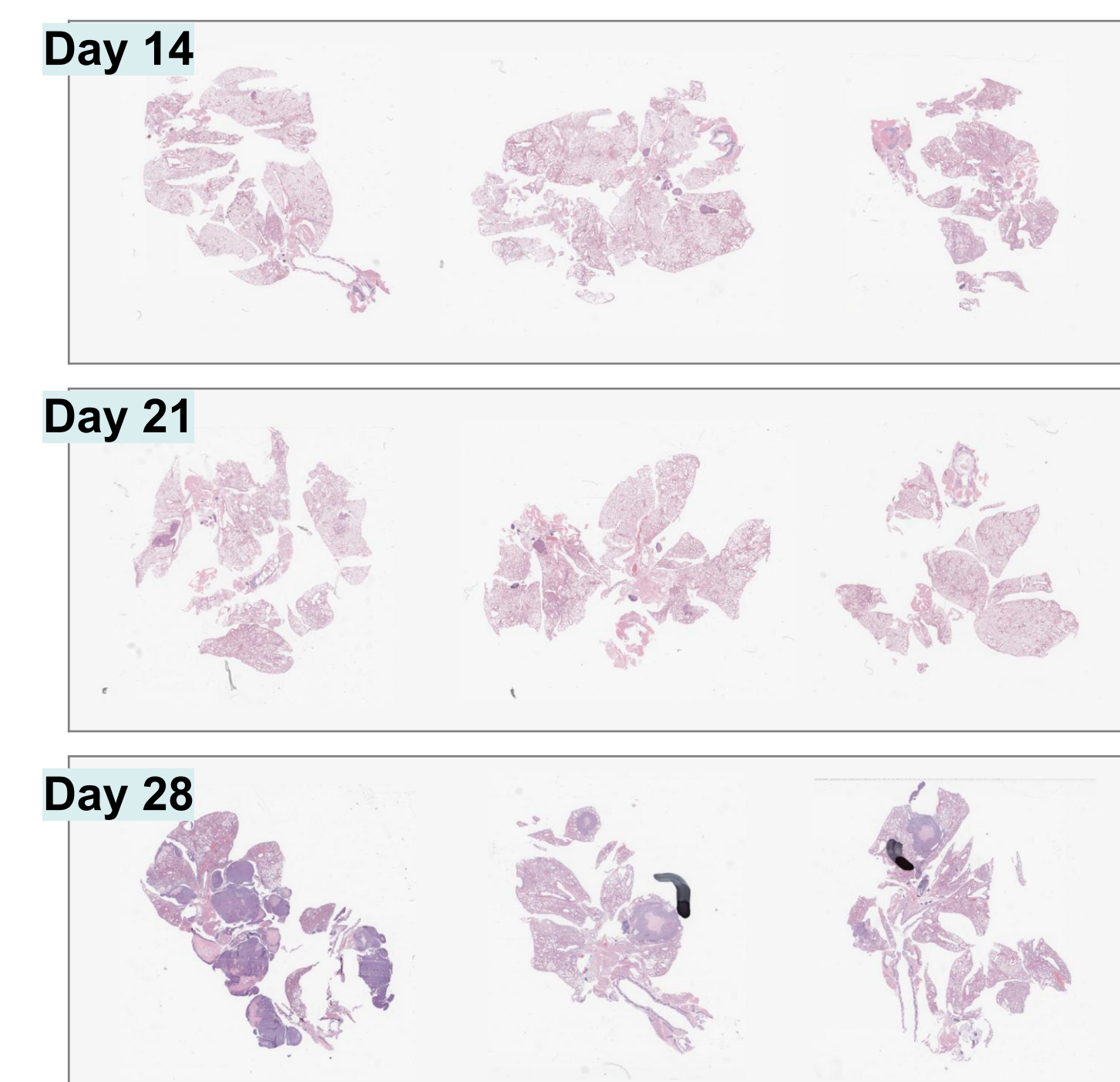
RESULTS



► **Figure 1. Overall experimental workflow of the orthotopic mouse model for STAS**



► **Figure 2. In vivo bioluminescence imaging for day 14, 21, and 28**



▼ **Figure 4. High-magnification histologic features of the tumor edge and adjacent alveolar spaces in orthotopic lung tumors**

Orthotopic tumors were successfully established and exhibited biologically consistent growth progression over time: small tumor foci at day 14; a single nodule at day 21; one to two nodules with central necrosis at day 28; and near-total lung involvement by days 35-42. At the tumor margins of samples from days 21 and 28, small clusters of tumor cells, detaching from the main tumor mass, were frequently identified - a pattern resembling STAS in human NSCLC.

► **Figure 3. Representative histologic sections of orthotopic lung tumors**

In one case at day 35 and one at day 42, tumor growth predominantly occurred within the pleural cavity rather than the lung parenchyma, which corresponded to relatively low bioluminescence signal intensity (not shown).