MAGE-A3–Directed T-Cell Immunity in Lung Cancer: Convergence of Systematic Evidence, Patient-Level Single-Cell Context, and *in vitro* Validation

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Abstract

Background: MAGE-A3 is a cancer-testis antigen with restricted normal-tissue expression and recurrent up-regulation in epithelial tumours. Our recent systematic review synthesised clinical and preclinical evidence showing frequent immunogenicity but platform-dependent efficacy, underscoring the need for biomarker-guided translation. Complementing this, an *in silico* analysis highlighted MAGE-A3's association with antigen-presentation pathways.

Methods: We assessed MAGE-A3–directed responses in A549 models using bulk $CD3^+$ T cells. Functional readouts included proliferation (CFSE), tumour apoptosis against parental A549 and CSC-enriched fractions (Annexin V/PI), effector release (granzyme B ELISA), and checkpoint/exhaustion markers (PD-1, TIM-3, TIGIT, CTLA-4) by flow cytometry. To situate these findings clinically, we performed patient-level single-cell analysis in LUAD (TISCH2/GSE131907). Malignant/epithelial and immune lineages were taken from provided annotations; CD8 T cells were defined by minor-lineage or an expression-based fallback. We derived an exhaustion–memory (E–M) index (z-exhaustion minus z-memory markers) and modelled E–M ~ MAGEA3_mean × APM_high + CD274 (APM module = mean HLA-A/B/C, B2M, TAP1/2) using OLS with 95% CIs; stratified slopes were reported.

Results: MAGE-A3 stimulation increased $CD3^+$ T-cell proliferation, tumour apoptosis and granzyme B release versus unstimulated controls, with differential susceptibility of CSC-like targets. The single-cell module related epithelial MAGEA3_mean to the CD8 E–M balance in a manner contingent on antigen-presentation context (APM_high), providing patient-level alignment with the bench findings.

Conclusions: Systematic, computational and experimental data converge to support MAGE-A3 as a rational immune target in lung cancer, while highlighting antigen presentation and exhaustion as actionable levers for combination strategies.

Keywords

Mage-a3, lung cancer, $cd3^+$ t cells, single-cell rna-seq, antigen presentation.