Background The recognition of cancer-cells by T-cells and the therapeutic efficacy of PD-1 axis blockers for cancer treatment are dependent on a fully functional HLA class-I antigen processing machinery (APM). The APM has been found to be defective in many types of human malignancies; however, there is limited information in non-small cell lung cancer (NSCLC). Here we studied cancer-cell APM defects in NSCLC focusing on transporter associated with antigen processing proteins TAP1 and TAP2.

Methods We utilized quantitative and spatially resolved multiplex quantitative immunofluorescence (mQIF) to simultaneously analyze the markers DAPI, cytokeratin (CK), CD8, TAP1 and TAP2 in >1,100 primary NSCLCs from 6 independent cohorts represented in tissue microarrays. Three cohorts (Cohorts #1–3) included 881 tumors from patients treated with standard of care non-immunotherapy and two cohorts included 139 cases treated with PD-1 axis blockers (Cohorts #4–5). One additional cohort included 130 primary lung adenocarcinomas clinically tested for oncogenic mutations in EGFR and KRAS (Cohort #6). The association between cancer-cell selective TAP1 and TAP2 protein downregulation, clinicopathologic variables and outcomes were studied. To investigate the functional consequences of TAP1 and/or TAP2 deficiency in lung cancer cells, we analyzed the surface peptide-HLA complex levels, functional phenotype/transcriptomic profile, and sensitivity to tumor-antigen specific T-cell killing of cultured tumor cells with targeted TAP1 and/or TAP2 silencing using siRNA or CRISPR-Cas9 based targeted gene deletion.

Results mQIF analysis revealed cancer-cell selective downregulation of TAP1 and TAP2 proteins in ~5% and ~45% of immunotherapy naïve primary NSCLCs, respectively. Cases with TAP2 downregulation showed lower CD8+ T-cell infiltration and shorter survival after PD-1 axis blockers. This effect was not seen in tumors with cancer-cell selective TAP1 downregulation or in cases treated without immunotherapy. Targeted silencing or genetic deletion of TAP2, but not TAP1 in human HER2+/HLA+ A549 lung cancer cells reduced the baseline and cytokine induced surface levels of the peptide-HLA complexes formed by the HER2<sub>369-377</sub> nonamer and HLA-A2 recognized using flow cytometry. In addition, TAP2 silencing was associated with a marked reduction in tumor cell killing after co-culture with human HLA-A2-positive CD8+ T-cells expressing the T-cell receptor recognizing the HER2<sub>369-377</sub> peptide. Targeted immune transcriptomic analysis of TAP2 deficient A549 cells revealed prominent alterations in IFNγ sensitivity and cytokine/chemokine expression.

Conclusions Cancer-cell selective TAP2 downregulation is common in NSCLC and mediates immune evasion and immunotherapy resistance via reduced antigen presentation and defective IFNγ responses. TAP2 deficiency in NSCLC has prominent biomarker and therapeutic potential.

Ethics Approval This study was carried out in accordance with the principles of the Declaration of Helsinki and all tissue and clinical information were used in a de-identified fashion after approval from the Yale Internal Review Board (Yale Human Investigation Committee) protocols #9505008219 and #1608018220 or local institutional protocols, which approved the patient consent forms or waiver of consent.