Background The NRAS driver oncogene is frequently mutated in diverse cancer types of high unmet medical need, including colorectal cancer, thyroid cancer, and ~25% of cutaneous melanomas. Most colorectal and thyroid cancers fail to respond to immune checkpoint inhibitors. Further, unlike other genomic variants of melanoma, those that harbor mutant NRAS respond poorly to current immunotherapies. Public neoantigens (NeoAgs) represent an elite class of clonally conserved, cancer-specific epitopes derived from recurrently mutated driver genes. Because public NeoAgs are restricted by prevalent HLA alleles they are shared among cancer patients and can be therapeutically targeted using off-the-shelf reagents. Here, we report on the immunogenic landscape and T cell responses to public NeoAgs resulting from recurrent NRAS (Q61) hotspot mutations in cancer patients.

Methods We combined a mass spectrometry screen, dextramer-based T cell detection, single-cells RNA sequencing, and functional immune validation assays to determine the immunogenicity of NRAS(Q61) epitopes. As part of these efforts, we assembled and performed comprehensive immune monitoring of peripheral blood and tumor samples from \( n = 26 \) HLA-A*01+ patients with an NRAS(61) mutated cancer.

Results We discovered that neoepitopes derived from the three most common NRAS(Q61) hotspot substitutions (R, K, and L) are naturally processed and presented in the context of HLA-A*01, an allele expressed by ~25% of North Americans. Immune monitoring demonstrated that NRAS public NeoAgs-specific T cell responses occur in ~50% of patients, including subjects with melanoma and non-melanoma cancers. Responses occurred at comparable frequencies whether the mutated NRAS residue was basic or aliphatic. Using single-cell sequencing, we retrieved and functionally validated a panel of \( n = 18 \) TCRs from patient samples that confer specific recognition to cancer cells that express an NRAS Q61 public neoepitope. All of the patient-derived TCRs are high-affinity and function in a CD8 co-receptor-independent manner. Moreover, a subset TCR candidates demonstrate “cross-protection” towards multiple NRAS Q61 mutated variants, allowing a single receptor to provide therapeutic coverage for >90% of NRAS mutations. Mechanistically, we demonstrate that NRAS public NeoAg TCR cross-protection is tuned by expression of the CD8αβ co-receptor and requires recruitment of LCK.

Conclusions Collectively, our results demonstrate that multiple NRAS(Q61) hotspot mutations give rise to immunogenic public NeoAgs that can be studied across patients and therapeutically targeted using a TCR-based approach. These findings establish the foundation for an innovative new class of precision immune-genomic treatments for mutant NRAS cancers.

Ethics Approval All patients provided written informed consent for tumor and white blood cell sequencing and review of patient medical records for demographic, pathological and treatment information under a Memorial Sloan Kettering Cancer Center (MSKCC) institutional review board-approved biospecimen umbrella protocol (protocol 12-245; ClinicalTrials.gov ID: NCT01775072).