ACQUIRED RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE BY PHENOTYPIC PLASTICITY OF MELANOMA

Emily Robitschek*, Arnav Mehta, Jia-Ren Lin, Dennie Frederick, Alvin Shi, Ana Larque, Benchun Miao, Tatyana Sharova, John Shin, Manolis Kellis, Nir Hacohen, Keith Flaherty, Genevieve Boland, Ivan Chebib, David Liu, Ryan Sullivan.

1Dana Farber Cancer Institute, Boston, MA, United States; 2Massachusetts General Hospital, Boston, MA, United States; 3Harvard Medical School, Boston, MA, United States; 4Broad Institute of MIT and Harvard, Cambridge, MA, United States; 5Massachusetts Institute of Technology, Boston, MA, United States; 6Hospital Clinic Barcelona, Barcelona, Spain; 7Broad Institute of Harvard and MIT, Cambridge, MA, United States

Background The vast majority of immunotherapy resistant tumors do not harbor obvious resistance mutations and little remains known about alternative mechanisms of adaptive resistance.

Methods We present a patient with Stage III melanoma treated with adjuvant Pembrolizumab (Pembro) who developed progressive disease three months after starting therapy. The patient was started on a trial of Pembrolizumab (Pembro) and Entinostat, a histone deacetylase inhibitor (HDACi), and had a mixed response. Progressive lesions were palliatively resected and found to have distinct histologies of either melanoma or pleomorphic rhabdomyosarcoma. Six tumors were analyzed with whole exome sequencing, bulk RNA-seq, and CyCIF (cyclic immunofluorescence). Quality-control, calling of somatic mutations and copy number alterations, and inference of phylogenetic relationships were performed.

Results Sequenced tumors shared 1007 SNV (single nucleotide variant) mutations and clonal loss of heterozygosity (LOH) of 1p, 4, 6q, 9, 18, 21q, and 22q confirming a common ancestor clone with homozygous deletion of CDKN2A and driver mutations in NRAS, NF1 (bi-allelic), CDK12, and SMARCA4. Genomic features unique to the melanoma phenotype included a clonal LOH of chromosome 11, while the rhabdomyosarcomas shared clonal LOH of chromosome 19. Phylogenetic analysis revealed an early split between histologic melanomas and rhabdomyosarcoma subtypes. We further uncovered enriched expression signatures for myogenesis, epithelial mesenchymal transition (EMT) and several immune signatures enriched in rhabdomyosarcoma samples relative to melanoma samples. CyCIF imaging confirmed the mutual exclusivity of the melanoma and rhabdomyosarcoma phenotypes, elevated levels of M2 macrophages in rhabdomyosarcoma samples, and high NGFR signaling in post combination treatment tumors.

Conclusions Our data suggest phenotypic switching as a form of immune evasion. The majority of lesions biopsied to be rhabdomyosarcomas initially emerged (radiographically) as progressive lesions on adjuvant Pembro. These lesions were resistant (stable/progressive disease) to Pembro/Entinostat, while other disease lesions responded, suggesting that the phenotype switch to rhabdomyosarcoma from an initial melanoma ancestral clone was associated with ICB resistance. Other resistant lesions had a melanoma phenotype, and our analysis revealed that the genomic divergence between the melanoma and rhabdomyosarcoma phenotypes occurred prior to Pembro/Entinostat treatment, suggesting alternative mechanisms of treatment resistance. While unlikely that a HDACi led to the phenotypic conversion of pre-existing tumor lesions, its role in selection or enrichment of phenotypically switched cells is undetermined. The higher NGFR signal post Pembro/Entinostat treatment could represent a concurrent therapeutic resistance mechanism. More broadly, detailed integrated clinical/genomic longitudinal studies within individual patients can shed light on the evolution and underlying mechanisms of clinical therapeutic resistance.

Ethics Approval The work described herein was approved by the Dana Farber Harvard Cancer Center IRB, protocol #11-181, to which the patient signed informed consent.

Consent The work described herein was approved by the Dana Farber Harvard Cancer Center IRB, protocol #11-181, to which the patient signed informed consent.