Introduction

The role of the immune system in cancer development has long been considered and studied. One of the originators of the theory of immune system involvement in cancer was Paul Ehrlich who postulated that the immune system can suppress the growth of tumor cells (1). Decades of work trying to verify this hypothesis followed and in the 1950s Burnet and Thomas each contributed to the theory that is now referred to as immunosurveillance (2,3). Essentially, the immune system functions as a sentinel to monitor the body for tumor specific neo-antigens. Once detected, the host immune system then targets nascent cancer cells for destruction.

As the complexity of the relationship between the host immune system and tumors became more defined it became clear that immunosurveillance was only a single storyline in a complex narrative. With additional advances in our understanding of the multifaceted relationship between tumor cells, the tumor microenvironment and the immune system, immunosurveillance is now considered to be one component of immunoediting (4-6). The concept of cancer immunoediting was proposed in 2002 as an umbrella process to include findings discordant with the immunosurveillance hypothesis (4). For example, in certain scenarios the immune system may promote, rather than suppress, the development of tumors that are able to evade the immune system. Cancer immunoediting is divided into three phases: elimination (immunosurveillance), equilibrium (quiescent state) and escape (immune evasion). Discoveries in each of these phases have clarified the understanding of the complex relationship between the immune system and tumorigenesis. The ability of cancer cells to evade the immune system is now recognized as one of the hallmarks of cancer (7).

The escape phase of immunoediting is defined by settings in which the immune system is unable to effectively block tumor cells growth. Due to the complexity of this pathway there are numerous ways this phenotype can manifest including: avoiding recognition by the immune system (via decreased antigen presentation), expression of receptors that suppress the immune system such as PD-1, CTLA-4, LAG-3 and others, production of proteins that increase resistance and survival of tumor cell as well as cytokines that can function by enhancing angiogenesis, by suppression of the immune system (8).

The potential benefit of therapeutically targeting these mechanisms of immune escape is illustrated by the development of anti-CTLA-4 agents like Ipilimumab and anti PD-1 agents such as nivolumab, pembrolizumab and atezolizumab which have transformed the treatment paradigms of metastatic melanoma, non-small cell lung cancer (NSCLC) and other cancers (9-16). An even more compelling reflection is to consider that these two categories of drugs, that only represent a small proportion of the
possible immune related therapeutic targets, have made such a dramatic difference in patient outcomes. Numerous drugs are in clinical testing to target other mechanisms of immune escape.

**Rationale**

In cells, major histocompatibility complex (MHC) class I proteins present short intracellular peptides on the tumor cell surface. These peptides are one of the ways that the immune system detects cells that have become mutated or infected with a foreign microorganism. When a T-cell recognizes a compromised cell, it can be targeted for destruction. This mechanism of detection requires functionality of multiple different pathway components including the MHC class I genes [human leukocyte antigen (HLA)-A, HLA-B, HLA-C]. Therefore, downregulation, silencing, or loss of HLA alleles results in decreased peptide antigen presentation, and an increased likelihood of tumor cell escape from T cell mediated attack. The magnitude and significance of loss of the HLA haplotype has not been systematically evaluated in human cancer cells due to bioinformatics challenges as well as our nascent understanding of the complex interplay between the immune system and tumor cells.

In their recently published work, McGranahan et al. focus on the escape phase of immunoediting and the contribution of allele-specific HLA loss to tumor development (17). Specifically, they focused on characterizing HLA loss of heterozygosity (LOH) in lung cancer. LOH has been evaluated previously in the study of tumor suppressor genes and in association with clinical outcomes in NSCLC, however, these studies were often focused on large genomic regions or used cell lines (18-20). Interestingly, though these studies all reported frequencies of LOH in different genomic regions in the 30–60% range. HLA-1 loss has been previously evaluated as a mechanism used by cancer cells in immune escape and can dictate the success of some immunotherapies (21-27). This new work is unique in its single gene allele focus in tumor tissue and the characterization and implications of LOH on immune evasion.

**Findings**

The first and critical step in the authors’ analysis was to overcome the challenge of the polymorphic HLA loci. This is one of the reasons that HLA heterozygosity has been difficult to characterize with existing bioinformatics approaches. This challenge was overcome by utilizing the patient’s own germline sequence as the reference instead of the standard human reference. Once validated, this new methodology was used to characterize the prevalence and timeline of HLA LOH and allelic imbalance in 90 patients from the TRACERx cohort (28). This was an informative and compelling analysis given that it demonstrated that 40% of 90 NSCLC samples had HLA LOH. They also found that LOH was significantly more frequent in squamous cell carcinoma compared with adenocarcinoma. They further used the multi-region TRACERx dataset to demonstrate that the LOH is more frequent in subclonal populations (65%). The authors answered the natural follow-up question of applicability to metastatic disease sites with an analysis from a study of paired metastatic and primary tumors and found that 46% of 37 patient samples demonstrated HLA LOH in the primary tumor only, metastasis only, or was present in both. Similar to early stage disease, they found increased HLA LOH in subclonal branches of the tumors. They took this analysis further by comparing the primary tumor and metastatic samples and determined that in the majority of cases the LOH occurs in the metastatic site and not in the primary site of disease.

In the next set of experiments, the authors demonstrated that first, HLA loss occurred more frequently than expected by chance (indicating positive selection) and second, that there was a significant increase in the number of non-synonymous mutations and neoantigens present in tumor samples containing HLA LOH in their combined dataset; though this difference was no longer significant when considering individual histologic subtypes (squamous cell versus adenocarcinoma). They found that among tumors with HLA LOH there was a significant increase in the number of subclonal but not clonal non-synonymous mutations. Additionally, they found in divergent subclonal populations derived from the same clone, there was an increase in non-synonymous mutations in the HLA LOH positive subclone. Further, they identified a significant relationship between HLA LOH and non-synonymous mutations in lung adenocarcinoma but not in squamous cell carcinoma. In another analysis, they demonstrated a significant enrichment of neoantigens that are computationally predicted to bind to the lost HLA allele compared to the remaining HLA allele. They also evaluated whether identifiable mutational signatures contributed to the mutational burden seen in the HLA LOH and did find an increase in mutations signatures.
associated with apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC), but not others known mutagens.

Finally, the authors demonstrated the clinical relevance of their findings. First, they evaluated the association between HLA loss and immune phenotype by demonstrating that tumors with HLA LOH had significantly higher PD-L1 expression on their immune cells and a trend toward higher PD-L1 expression on the tumor cells. They additionally used RNA sequencing to evaluate a larger The Cancer Genome Atlas (TCGA) dataset and found again that HLA LOH was highly prevalent and more frequent in lung squamous cell carcinoma compared to lung adenocarcinoma; however, somewhat paradoxically lung adenocarcinoma had a significantly higher non-synonymous mutation burden. They also evaluated a surrogate marker of CD8 cell activation and activity of natural killer (NK) cells and found an increase in both surrogate markers in lung adenocarcinoma, a finding that was verified by differential expression profiling.

**Relevance and unanswered questions**

This study adds significant structure to our understanding of the importance of immunoediting and immune evasion in cancer. The ability of the authors to determine the specific HLA allele that has been lost is a unique feature of their analysis and allows more accurate determination of HLA copy number as well as determination of which parental allele has been lost. With this in place the authors demonstrated these findings are applicable to both early and later stage disease, which is interesting from an evolutionary standpoint. They also demonstrated that in metastatic sites the LOH was more prevalent. We can infer that as cancer progresses to additional sites the level of immune evasion also increases. It would be extremely valuable to investigate this pattern in patients longitudinally to determine whether the pattern remains intact given that this has implications for treatment. Moreover, it will be important in the future to investigate whether distinct metastatic sites of disease show differential propensity for HLA copy number alteration; if so, the findings might be suggestive of context-specificity in the role of HLA LOH in metastatic site tropism or local outgrowth.

Furthermore, the authors demonstrate that there is a significant increase in HLA LOH and the number of non-synonymous mutations and neoantigens in the subclonal branches present in lung adenocarcinoma. The next step would be to characterize the molecular subpopulations of lung adenocarcinoma (EGFR-mutation positive, ALK gene rearrangement positive, TP53 mutated, etc.) to determine the distribution of HLA LOH in these genetic subtypes of the NSCLC. EGFR-mutated and ALK gene rearrangement positive NSCLCs typically have a lower mutational burden and have been demonstrated to not respond as robustly to immunotherapy (29-31). Further investigating these NSCLC subtypes may shed light on how to improve the clinical responses to treatment or the molecular underpinning for the lack of response to current immunotherapy agents. Additionally, further work exploring the relationship between the HLA LOH and the correlation to increased mutational signatures associated with APOBEC mediated mutagenesis is needed to determine whether these processes are functionally related or simply co-occurring in subclonal populations.

Clinical response data will also be an essential next step to understand how to utilize the information from this study. For example, what is the difference in survival in patients whose tumors have HLA LOH? Is there a difference in outcomes depending on whether the HLA LOH is clonal vs subclonal? Moreover, is there a differential response to systemic treatment (chemotherapy, immunotherapy, targeted therapy) in these patients with HLA LOH?

Another area of future inquiry stems from the finding of selective pressure for HLA LOH and the demonstration that there are more subclonal HLA LOH events than would be expected by chance, indicating selective pressure. As most of this analysis in this study was conducted in early stage NSCLCs, it will be enlightening to conduct a more extensive investigation in a larger cohort of patients with metastatic disease to demonstrate parallel findings. Investigations into how to leverage the residual subclonal population of neoantigens from the lost HLA allele(s) to improve our ability to target cancer cells are of particular interest.

Finally, the authors report the paradoxical finding of increased HLA LOH in squamous cell lung cancer yet increase non-synonymous mutational burden in adenocarcinoma and propose the presence of a more predatory microenvironment as the evolutionary driver to HLA LOH. Determining which of these events drove the other, if either, will be valuable to understand this complex relationship.

In summary McGranahan et al. have provided a valuable methodology to accurately determine allele specific HLA LOH and further characterize the TRACERx data set.
with preliminary data that provides a vista to visualize additional analyses critical to understand how to utilize these intriguing new data. Answering these next questions may ultimately allow us to more effectively develop immunotherapy and thereby provide additional effective treatment options for larger numbers of NSCLC patients.

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Footnote

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