Loss of Heterozygosity is Low for Specific HLA Alleles in Cancer Patients with Driver Mutations

Roy C.,[1] Sewastianik T.,[1] Halvey P.,[1] Montesion M.,[2] Jindal A.,[1] Vallaster M.,[1] Lam H.,[1] Garrity S.,[1] Shapiro G.,[1] Klebanoff C.,[3] Vincent L.,[1] Opiteck G.J.,[1] Nagorsen D.[1]

[1]Affini-T Therapeutics, Inc.; [2] Foundation Medicine, Inc.; [3] Memorial Sloan Kettering Cancer Center

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Introduction

- While immunotherapies have revolutionized cancer treatment, the limited predictability of response and resistance mechanisms present challenges to the development of novel therapeutics for solid tumors.
- Novel therapeutic modalities use a sensitive and specific T cell receptor (TCR)-HLA:peptide interaction but could theoretically be impacted by allele specific HLA loss of heterozygosity (LOH).
- **Allele-agnostic HLA LOH analyses** assessing loss of any MHC class I allele **are relevant for HLA-agnostic approaches** such as immune checkpoint inhibitors; however, context-specific studies remain limited.
- For example, adoptive TCR T cell therapies and bi-specific TCR T cell engagers target neoantigen peptides derived from oncogenic driver mutations, such as those of KRAS and TP53, presented by a specific HLA allele.
- Thus, **allele-agnostic analyses may overestimate the frequency and relevance of HLA LOH for allele-specific modalities**.

Methods

- **Data source**: a real-world comprehensive genomic profiling dataset consisting of 78,418 cases (Montesion *et al.*) [1], from solid tumor biopsies germline heterozygous for one or more HLA class I locus.
- **Approach**: HLA LOH was analyzed in the context of frequent driver mutations, the corresponding HLA alleles (**Table 1**), and within tumor subtypes frequently harboring these mutations (including breast (BRCA), colorectal (CRC), non-small cell lung (NSCLC), and pancreatic (PDAC) cancers). Combinations with small sample size (N<20) we excluded from analysis. Two-tailed Fisher's exact or Chi-square tests were used for allele-specific comparisons within specific indications, depending on the sample size.

Table 1. HLAs presenting common oncogenic driver mutations

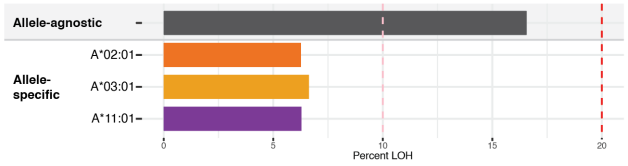
HLA	Gene	Mutations	References
HLA-A*02:01	KRAS	G12V	Wu *et al.*
	TP53	R175H	Lo *et al.*
HLA-A*03:01	KRAS	G12C	Choi *et al.*, Gurung *et al.*
		G12D	Choi *et al.*, Wang *et al.*, Gurung *et al.*, Yang *et al.*
		G12V	Choi *et al.*, Douglass *et al.*, Gurung *et al.*, Yang *et al.*
HLA-A*11:01	KRAS	G12C	Choi *et al.*, Gurung *et al.*
		G12D	Choi *et al.*, Wang *et al.*, Gurung *et al.*
		G12V	Choi *et al.*, Wang *et al.*, Gurung *et al.*

Results

Table 2. Number of analyzed patients and HLA LOH frequencies across all indications

		Total Patients	Patients with LOH	Percent of patients with LOH
Allele-agnostic		78,418	12,990	**16.6%**
Allele-specific	A*02:01	32,090	2,008	**6.3%**
	A*03:01	18,594	1,232	**6.6%**
	A*11:01	9,793	615	**6.3%**

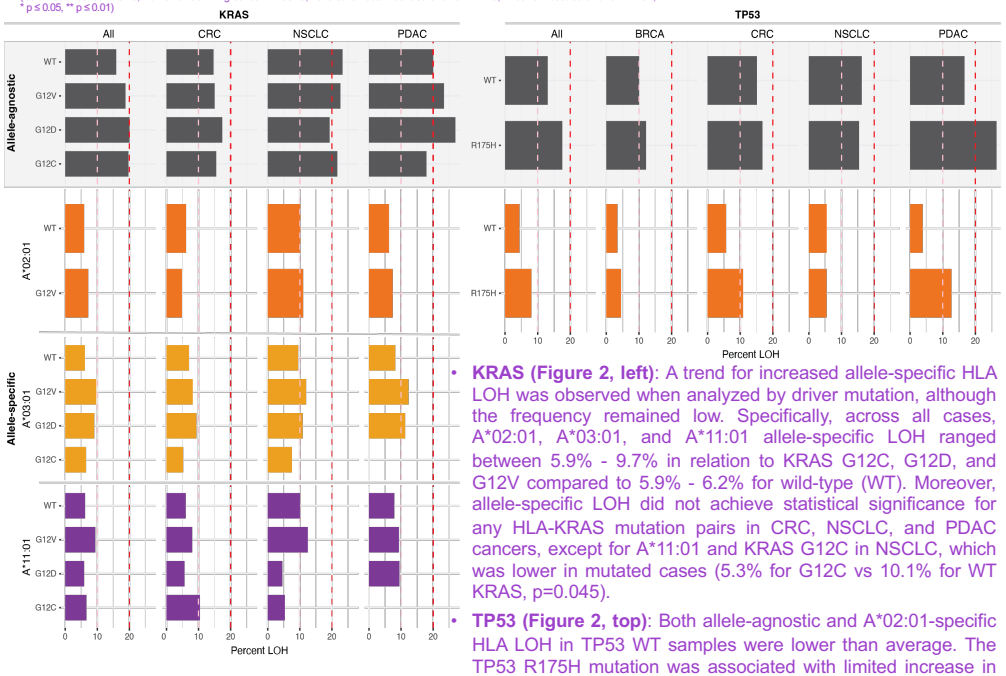
Figure 1. HLA LOH frequencies across all indications



- Allele-agnostic HLA LOH was observed in 16.6% of all samples and varied between indications, which is consistent with previously published literature.
- Notably, allele-specific loss of HLAs A*02:01, A*03:01, and A*11:01 across all samples was much lower than allele-agnostic loss (6.3%, 6.6%, and 6.3% cases, respectively; **Table 2** and **Figure 1**), which is lower than half of general loss, possibly due to a fraction of losses involving only B and/or C loci, and lower frequency of LOH relative to other A alleles.

Figure 2. HLA LOH frequencies in the context of high frequency driver mutations and within tumor subtypes

(Colo-Rectal Cancer = CRC; Non-Small Cell Lung Cancer = NSCLC; Pancreatic Ductal AdenoCarcinoma = PDAC; Invasive Breast Carcinoma = BRCA; * p ≤ 0.05, ** p ≤ 0.01)



- **KRAS (Figure 2, left)**: A trend for increased allele-specific HLA LOH was observed when analyzed by driver mutation, although the frequency remained low. Specifically, across all cases, A*02:01, A*03:01, and A*11:01 allele-specific LOH ranged between 5.9% - 9.7% in relation to KRAS G12C, G12D, and G12V compared to 5.9% - 6.2% for wild-type (WT). Moreover, allele-specific LOH did not achieve statistical significance for any HLA-KRAS mutation pairs in CRC, NSCLC, and PDAC cancers, except for A*11:01 and KRAS G12C in NSCLC, which was lower in mutated cases (5.3% for G12C vs 10.1% for WT KRAS, p=0.045).
- **TP53 (Figure 2, top)**: Both allele-agnostic and A*02:01-specific HLA LOH in TP53 WT samples were lower than average. The TP53 R175H mutation was associated with limited increase in HLA-A*02:01 LOH (from 4.4% for WT to 8.1% for TP53 R175H) and varied between indications.

Considerations

- The analyzed database is enriched for advanced/metastatic cancer patients.
- Patients homozygous for all HLA class I loci were excluded from the database since these patients can't lose heterozygosity; thus, the frequency of HLA LOH in all patients might be even lower than reported herein.
- HLA allele losses are usually monoallelic (McGranahan *et al.*), and it is hypothesized that the second chromosome is preserved to prevent NK cell response (Moretta *et al.*). Thus, we further hypothesize that patients homozygous at the targeted HLA allele (2 copies of the same allele or 1 copy, but the other one lost) may have lower chance of HLA-related immune escape from TCR based therapies.
- HLA LOH is frequently sub-clonal and occurs late in the cancer pathogenesis (McGranahan *et al.*). Cases with HLA LOH in a metastatic lesion, may be retain both alleles in the truncal tumor and, thus, still benefit from the HLA/driver mutation-based therapy.

Conclusions

- Based on this real-world dataset, **over 90% of cases** retain the TCR-targeted allele and, therefore, **preserve the molecular complex necessary for HLA/driver mutation-based therapies.**
- Allele specific and pan-indication LOH ranged from **6.3–6.6%** (Table 2).
- In the assessed mutation/HLA pairs allele-specific **HLA LOH was found in only:**
  - **5.9% - 9.7% of tumors across all indications**
  - 4.4% - 12.7% of tumors within specific indications
- Further studies should test correlations with treatments and whether tumors in earlier lines of treatment have an even lower rate of HLA LOH.

References

1. Montesion, Meagan, Karthikeyan Murugesan, Dexter X. Jin, Radwa Sharaf, Nora Sanchez, Ameet Guria, Max Minker, et al. 2021. "Somatic HLA Class I Loss Is a Widespread Mechanism of Immune Evasion Which Refines the Use of Tumor Mutational Burden as a Biomarker of Checkpoint Inhibitor Response." *Cancer Discovery* 11 (2): 282–92. https://doi.org/10.1158/2159-8290.CD-20-0672.
2. Lo, Winifred, Maria Parkhurst, Paul F. Robbins, Eric Tran, Yong-Chen Lu, Li Jia, Jared J. Gartner, et al. 2019. "Immunologic Recognition of a Shared P53 Mutated Neoantigen in a Patient with Metastatic Colorectal Cancer." *Cancer Immunology Research* 7 (4): 534–43. https://doi.org/10.1158/2326-6066.CIR-18-0886.
3. Douglass, Jacqueline, Emily Han-Chung Hsiue, Brian J. Mog, Michael S. Hwang, Sarah R. DiNapoli, Alexander H. Pearlman, Michelle S. Miller, et al. 2021. "Bispecific Antibodies Targeting Mutant RAS Neoantigens." Science Immunology 6 (57): eabd5515. https://doi.org/10.1126/sciimmunol.abd5515.
4. Choi, Jaewon, Scott P. Goulding, Brandon P. Conn, Christopher D. McGann, Jared L. Dietze, Jessica Kohler, Divya Lenkala, et al. 2021. "Systematic Discovery and Validation of T Cell Targets Directed against Oncogenic KRAS Mutations." *Cell Reports Methods* 1 (5): 100084. https://doi.org/10.1016/j.crmeth.2021.100084.
5. Wu, Liqun, Herui Yao, Hui Chen, Aodi Wang, Kun Guo, Wenli Gou, Yanfei Yu, et al. 2022. "Landscape of Somatic Alterations in Large-Scale Solid Tumors from an Asian Population." *Nature Communications* 13 (1): 4264. https://doi.org/10.1038/s41467-022-31780-9.