The association of efficacy of immune checkpoint blockade with loss of heterozygosity in HLA

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The association of efficacy of immune checkpoint blockade with loss of heterozygosity in HLA

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ABSTRACT

The association of efficacy of immune checkpoint blockade with loss of heterozygosity in HLA

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(Directed by Professor Sangwoo Kim)

Immunotherapy is a next-generation personalized treatment method that has recently attracted attention as a cancer treatment method. Immunotherapy is a method in which immune cells recognize neoantigens derived from cancer cells and increase reactivity with the patient's specific HLA allele to induce the death of cancer cells. In other study, it is supposed that the loss of heterozygosity in HLA is related to the case where the treatment effect does not appear even if the neoantigen known to be associated with immunotherapy is sufficient. It is assumed that loss of heterozygosity and allelic copy loss in HLA gene is associated with poor response rates in immune checkpoint inhibitor treatment. However, the accurate extents or consistency in cancer types have not been explored. Therefore, a total of 281 whole exome sequencing data from three cohorts of patients who received immune checkpoint blockade immunotherapy were analyzed, including Anti-PDL1 treated in metastatic urothelial cancer (N=216), anti-PD1 treated metastatic melanoma (N=26), and anti-CTLA4 treated metastatic melanoma (N=39). Although the loss of heterozygosity in HLA was generally expected to be an inhibitory factor in the immune treatment response by causing T-cell

immune evasion, our analysis demonstrates no explicit relationships.

Key words : immune checkpoint blockade, loss of heterozygosity, human leukocyte antigen

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I. INTRODUCTION

Cancer is always attempting to evade the immune system. Immune regulatory cells[1], immunosuppressive mediators[2, 3], and defective antigen presentation[4] are some of the factors that cause immune evasion . Immunotherapy is one of the most widely used cancer treatments. Immunotherapy is a treatment strategy that improves the immune environment and elicits an immune response, rather than a previous treatment method that directly targets[5]. Immune checkpoint blockade (ICB) is an immunotherapy drug that blocks inhibitory molecules that suppress cytotoxic T cells and can lead to an immune evasion mechanism. Programmed death protein 1 (PD1), Programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) are the main targets of this treatment[6]. Because ICB improves the immune system for cancer, this treatment has the potential for a positive response and a long overall survival[7]. However, the number of people who responded to this treatment was small. Immune phenotype, target gene expression, and tumor mutation burden have all been associated to this cause[8-10].

Understanding the tumor immune microenvironment is important for

immunotherapy to be an effective cancer treatment. Among them, HLA-I (Human Leukocyte Antigen Class I) is associated with a patient's immune response[11]. HLA-A, -B, and -C genes make up the majority of HLA-I genes. These genes present self-antigen, which can be used to detect pathogens. These molecules are found on the surface of cells and contain the 8-11mer peptide known as self-antigen[12]. The cytotoxic immune cells that kill pathogens or abnormal cells benefit from this complex. Neoantigens are non-self-antigens that abnormal cells present, and cytotoxic T cells that recognize them have a killing ability[13].

There are over 25,000 alleles in the HLA system. Alleles are constantly being discovered, and the number of them is increasing[14]. Because HLA has so many alleles, it has a lot of different structures. Because of this various structures, the self-antigen displayed on the HLA may differ for each allele. Furthermore, the expression of neoantigens will appear differently[15, 16].

The number of neoantigens that can be present will increase if the HLA allele is diverse. Additionally, the likelihood of the cancer being detected will increase. As a result, the immune system finds it easier to detect neoantigens when HLA types diverge in patients[17].

Loss of heterozygosity(LOH) in HLA is a representative immune evasion mechanism[18, 19]. As previously mentioned, the immune cells detect neoantigen presenting on HLA molecules. If HLA is a loss of allele, however, it has a disadvantage in antigen presentation[20]. As a result, this condition promotes immune evasion and immunotherapy has a poor response.

In this study, we examined the clinical response of patients with LOH in three ICB treatment cohorts to investigate how it relates to the tumor immune environment. We observed LOH in HLA and used immune profiling to try to figure out what the tumor microenvironment was like.

II. MATERIALS AND METHODS

1. Dataset collection for ICB-treated patient cohort and response evaluation

Datasets from three previous studies on patients who had ICB treatment were used for the analysis. The three patient cohorts composed of a metastatic urothelial cancer patient cohort which had anti-PD-L1 treatment (EGAS00001002556)[21], two metastatic melanoma patient cohorts which had anti-PD1 treatment (GSE78220)[22], and anti-CTLA-4 treatment (phs000452.v2)[23], respectively. Data were selected for patients whose matching normal DNA, tumor DNA, and tumor RNA were available. Based on the response evaluation criteria in solid tumors (RECIST) version 1.1 guideline[24], we defined complete response (CR) and partial response (PR) as a responder group, and stable disease (SD) and progressive disease (PD) as a non-responder group. Patients who were not evaluable (NE) or who have duplicated sequencing data were excluded were excluded. Finally, data of 216 patients from EGAS00001002556, 26 patients from GSE78220, and 39 patients from phs000452.v2 were enrolled for the analysis.

2. Data processing

We used bwa-mem (v0.7.10) algorithm to align whole-exome DNA sequence data in FASTQ format to the human reference genome (hg38). Genome Analysis ToolKit (GATK, v4.0.9.0)[25] package was used to mark and fix duplicate reads in the alignment data.

RNA sequence data were aligned using STAR aligner with 2-pass method (v2.7.3a)[26], to the human reference genome (hg38) and annotated transcripts in gene transfer format from Ensembl (release 103)[27].

3. Gene expression quantification

Gene expression level was calculated from aligned RNA sequence data, through RSEM algorithm[28]. Transcripts per million (TPM) value was used in the analysis, and the median value of TPM of each gene was used to divide expression-high group and expression-low group within the study cohort.

4. Genotyping and LOH status determination of HLA genes

We used Polysolver[29] algorithm for the genotyping of HLA genes from

aligned normal DNA sequence data, to acquire genotypes of HLA genes in an 8-digit notation for downstream analysis. Genotyping step is then followed by LOHHLA[19] algorithm to assess and determine the LOH status of HLA genes. Briefly, LOHHLA determines whether the loss of allele-specific copy number is significantly different between the normal DNA data and the tumor DNA data. If p-value from the Student's t-test between two data is less than 0.01, LOHHLA determines that the HLA allele is lost.

5. Assessment of tumor immune microenvironment

Tumor infiltrating immune cell profiling was carried out using CIBERSORT algorithm with absolute mode[30] in order to identify immune cells and its proportion inside the tumor microenvironment. Gene expression data in TPM values from tumor RNA sequencing were used as input.

6. Statistical methods

Fisher's exact test was used to assess the relationship between LOH in each HLA genes and treatment response within the cohort. P-values less than 0.05 were considered as the difference between two groups is statistically significant.

Wilcoxon rank-sum test was used to determine whether the difference of estimated immune cell scores was significant, between allelic copy number loss group and allelic copy number intact group. P-values less than 0.05 were considered as showing statistically significant difference between the two groups.

Log-rank test was used to calculate the difference in survival rates between the two groups in terms of the patient's overall survival. P-values less than 0.05 were considered as showing statistically significant survival difference between two groups.

III. RESULTS

1. Clinical response and evaluation of loss of heterozygosity in HLA within cohort

We were able to identify alleles of three HLA genes(HLA-A, -B, and -C) to determine allelic loss from the patients with ICBs using whole-exome sequence data(N=281)(Figure 1). Initially, we excluded homozygosity in HLA-A, -B, and -C genes to identify allelic copy loss in each HLA gene. Patients with HLA-A heterozygosity are 239, HLA-B heterozygosity is 260 , and HLA-C heterozygosity is 244 in 3 cohorts(Figure 2a). Then, we divided each of the two groups into an allelic copy number loss group and an allelic intact group. We discovered that LOH in HLA was not associated with clinical response in melanoma patients treated with ICB (Figure 2b-d). Furthermore, clinical response to anti-PDL1 in metastatic urothelial cancer was associated with HLA-A LOH(Fisher's exact test).

This tendency can also be seen in the overall survival rate (Figure 3). In between HLA-A allelic copy number loss group and intact groups, loss group had significantly better overall survival rate. Although there was no correlation between survival and LOH in the two cohorts of metastatic melanoma, the survival of the allelic copy number loss group in metastatic urothelial cancer was significantly better

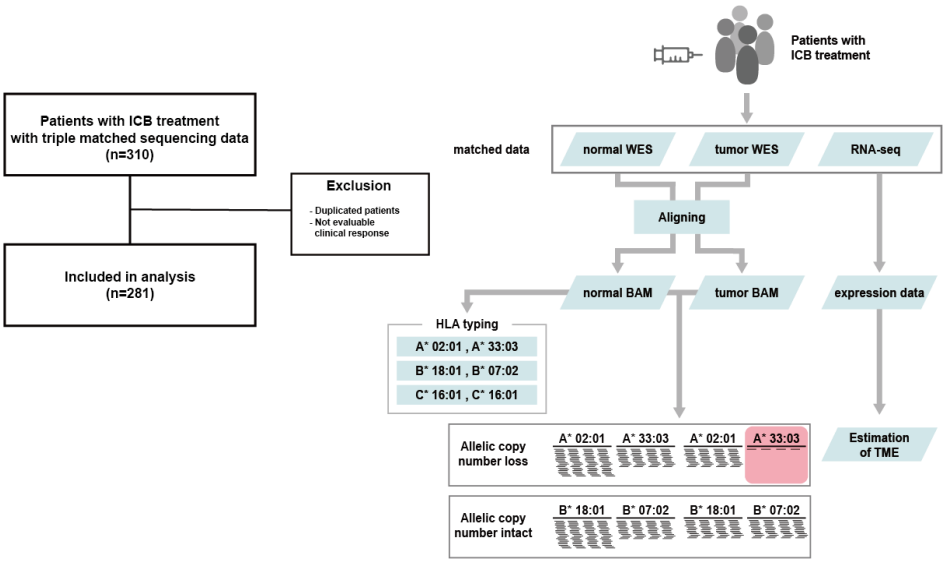

Figure 1. Overall workflow for analysis

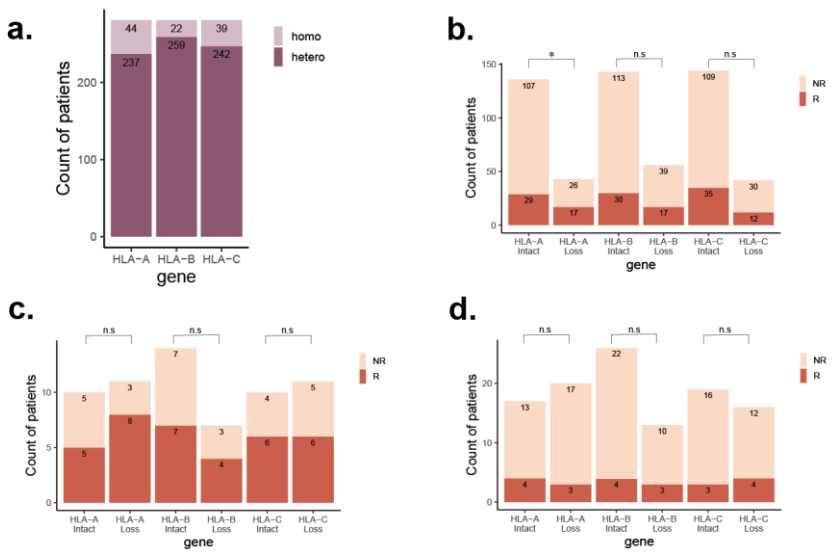

Figure 2. HLA genotyping and LOH status in 3 cohorts. (a) Total HLA genotype in three cohorts. The number of patients with allelic copy number loss and intact in each HLA gene, as well as clinical response information, are shown in (b)metastatic urothelial cancer with anti-PDL1, (c)metastatic melanoma with anti-PD1, and (d) metastatic melanoma with anti-CTLA4. The asterisk indicates that the p-value (Fisher's exact test) was less than 0.05

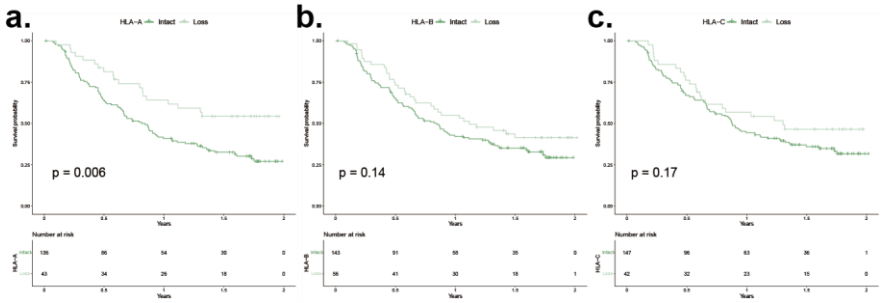

Figure 3. Overall survival between HLA allele loss and HLA allele intact group in metastatic urothelial cancer with anti-PDL1. Allelic copy number loss in HLA group had better overall survival rate in (a) HLA-A, (b) HLA-B, and (c) HLA-C

2. Tumor microenvironment analysis in HLA allelic copy loss

A. Immune-related gene expression with loss of heterozygosity in HLA

We identified tumor microenvironment in cohorts with ICB treatments to better understand immune related molecule components when a patient had LOH in HLA. Clinical response was related to the expression of ICB-targeted genes. Furthermore, we hypothesized that HLA allelic loss was correlated with decreased HLA expression. As a result of the significant relationship between responder and HLA-A LOH, we expected that the expression of the targeted gene was enriched while the expression of the HLA gene was decreased. We observed that an ICB-targeted gene is associated with overall survival in an expression-high group at each cohort without metastatic melanoma cohort with anti-PD1 treatment (Figure 4). However, there were no significant differences in the expression of immune-related genes between HLA allelic loss group and HLA allelic intact group(Figure 5-7). Extraordinarily, the differences in expression of ICB-targeted gene were statistically significant in anti-PD1 treatment data with LOH in HLA-B but that was not related to overall survival with ICB-target gene expression-high group (Figure 6d).

These findings show that HLA allelic loss in DNA has no effect on HLA gene expression or immune gene expression.

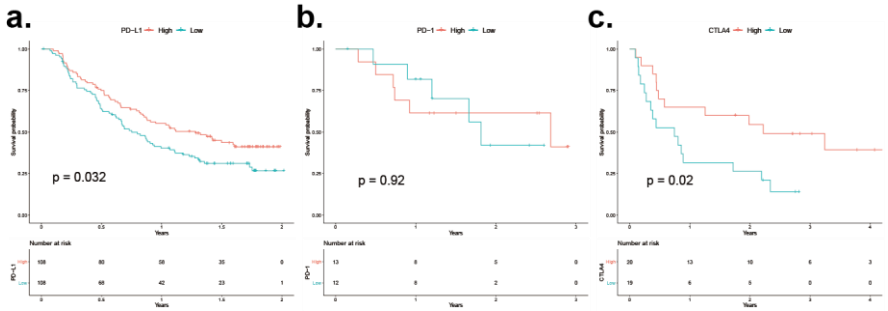

Figure 4. Overall survival between HLA allele loss and HLA allele intact group. Each cohort was (a) Metastatic urothelial cancer with anti-PDL1, (b) metastatic melanoma with anti-PD1, and (c) metastatic melanoma with anti-CTLA4

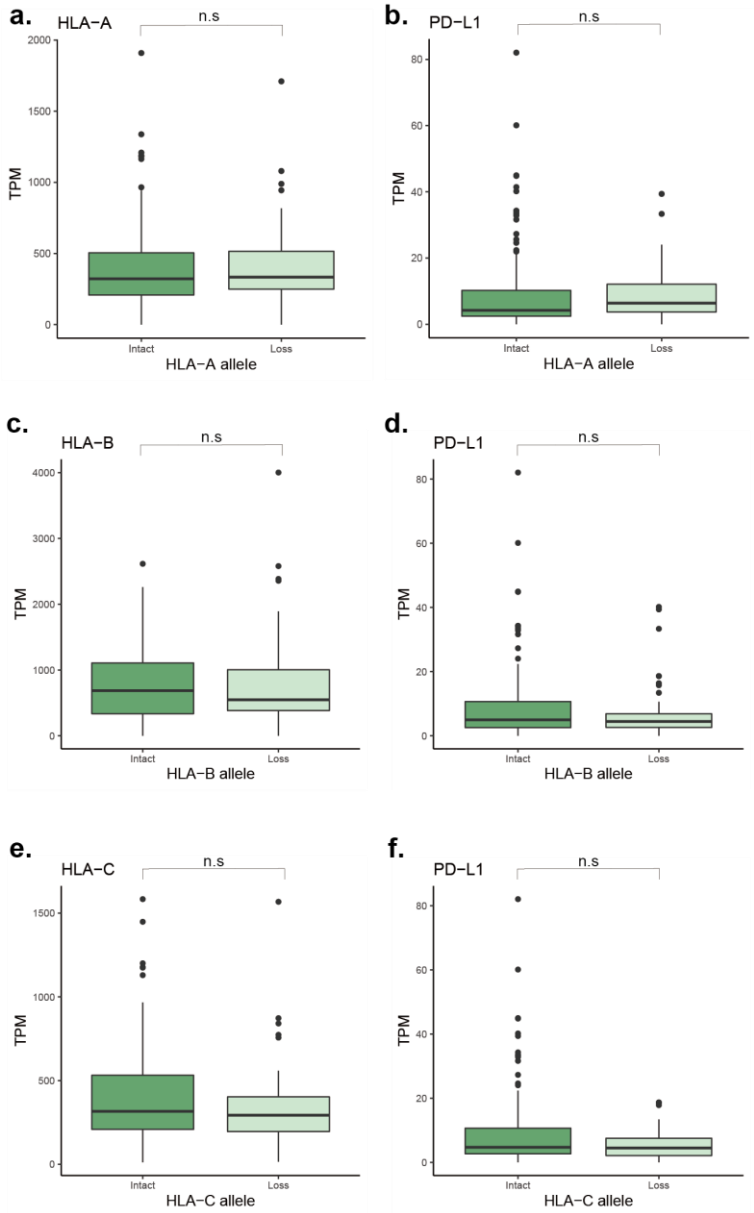

Figure 5. The expression of immune-related gene between HLA allele loss and HLA allele intact group in metastatic urothelial cancer with anti-PDL1.

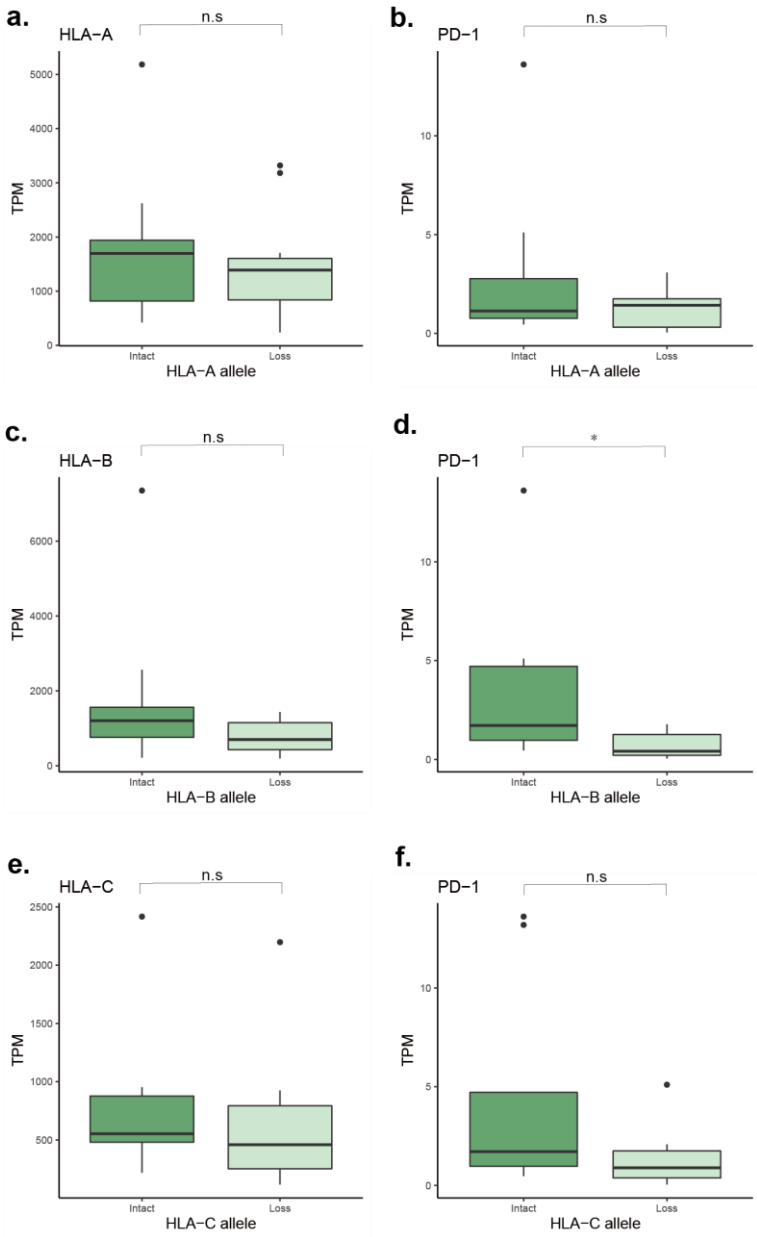

Figure 6. The expression of immune-related gene between HLA allele loss and HLA allele intact group in metastatic melanoma with anti-PD1.

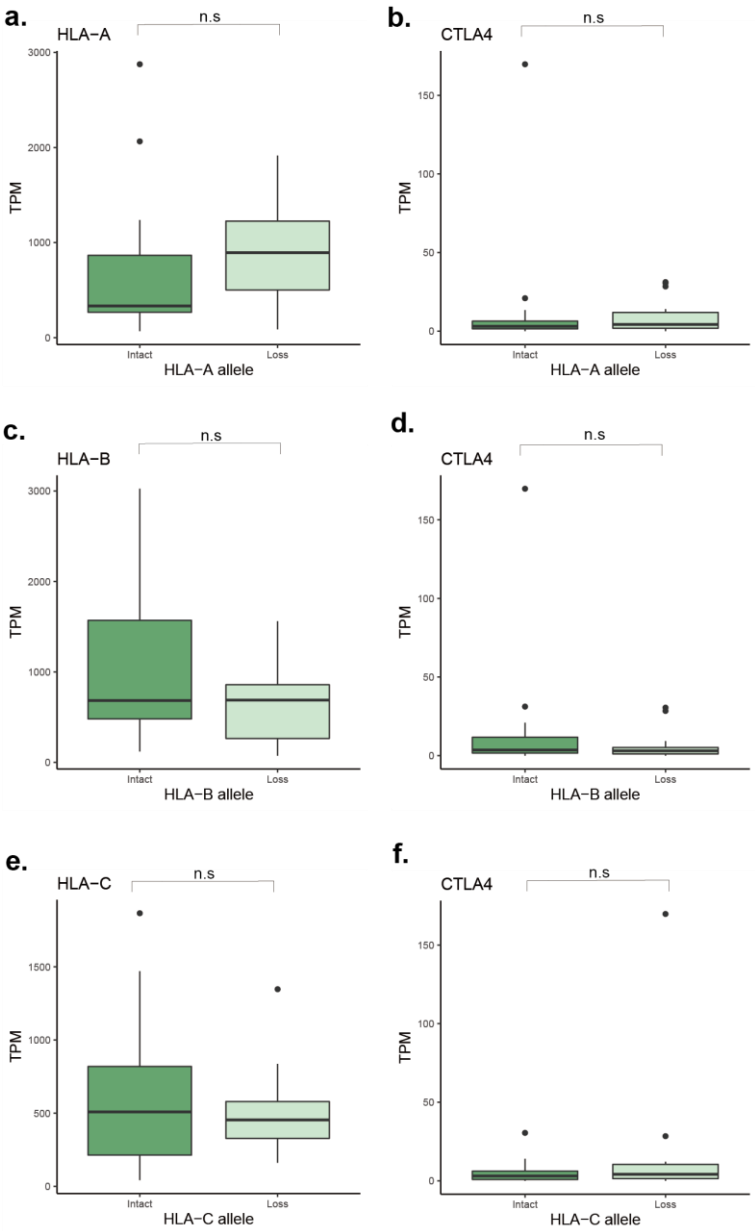

Figure 7. The expression of immune-related gene between HLA allele loss and HLA allele intact group in metastatic melanoma with anti-CTLA4.

B. Tumor microenvironment with loss of heterozygosity in HLA

We performed computational immune cell profiling on each patient to identify the immune cell composition that occurs when a patient has LOH in HLA. It was confirmed that among the immune cell scores, the cytotoxic immune cell score was significantly higher in patients with clinical response in metastatic urothelial cancer (Figure 8). This follows a similar pattern as previous research findings, and it appears to be beneficial to use ICB treatment if cytotoxic immune cells are present in large numbers in the cancer.

Next, we confirmed that only NK cells among the cytotoxic immune cell scores had a higher score in the allelic copy number loss in HLA-A group when comparing the immune cell score of patients in the allelic copy number loss group with patients in the allelic copy number intact group (Figure 9-11). These results show that cytotoxic immune cells do not show any significant difference in the allelic copy number loss group, but NK cells appearing in the tumor microenvironment of the patients appear more in the allelic copy number loss group.

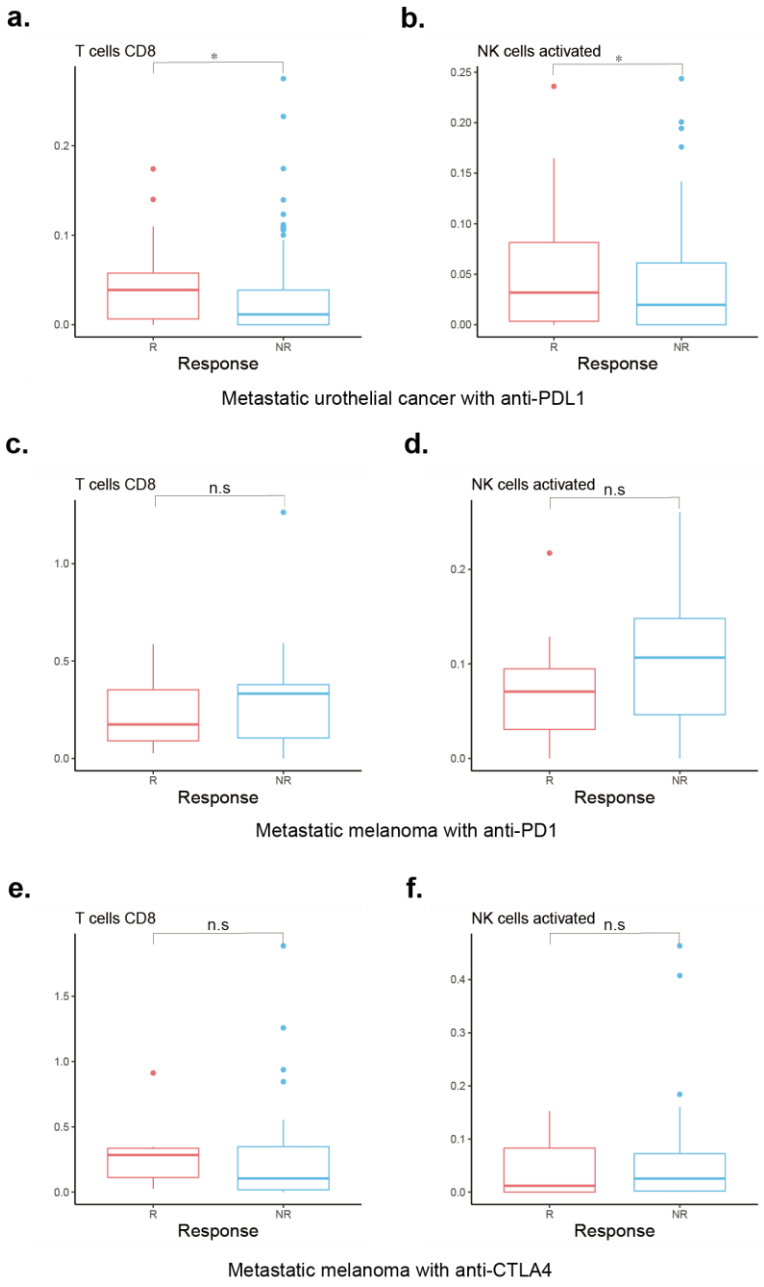

Figure 8. The cytotoxic immune cell score between clinical response.

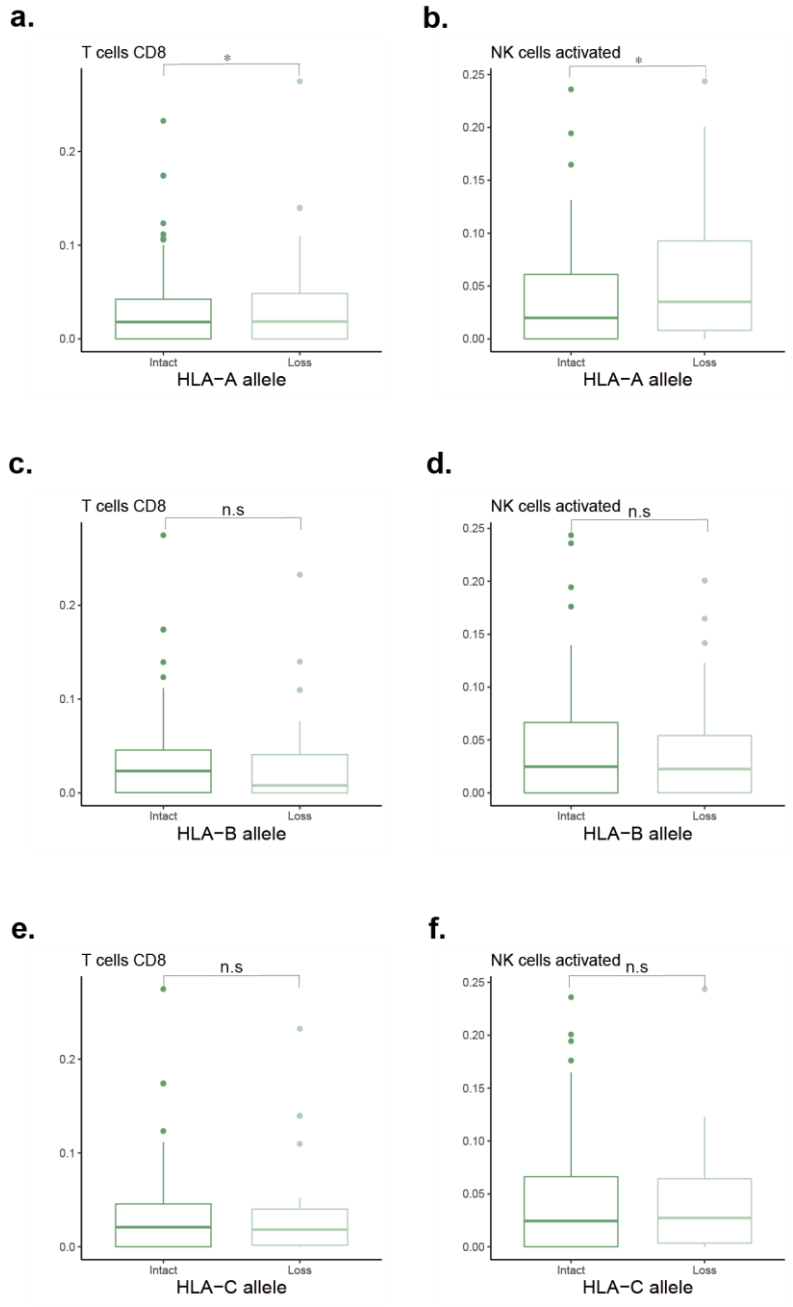

Figure 9. The cytotoxic immune cell score between HLA allele loss and HLA allele intact group in metastatic urothelial cancer with anti-PDL1.

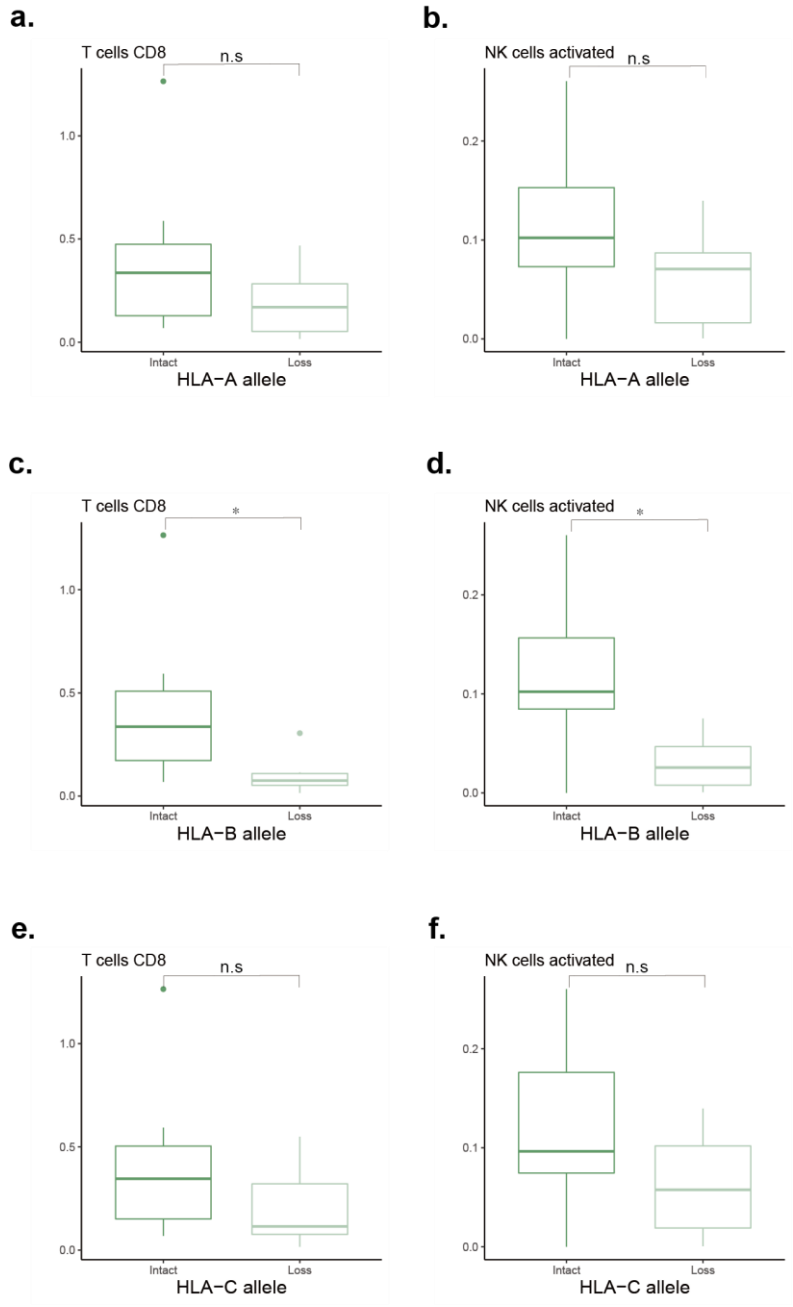

Figure 10. The cytotoxic immune cell score between HLA allele loss and HLA allele intact group in metastatic melanoma with anti-PD1.

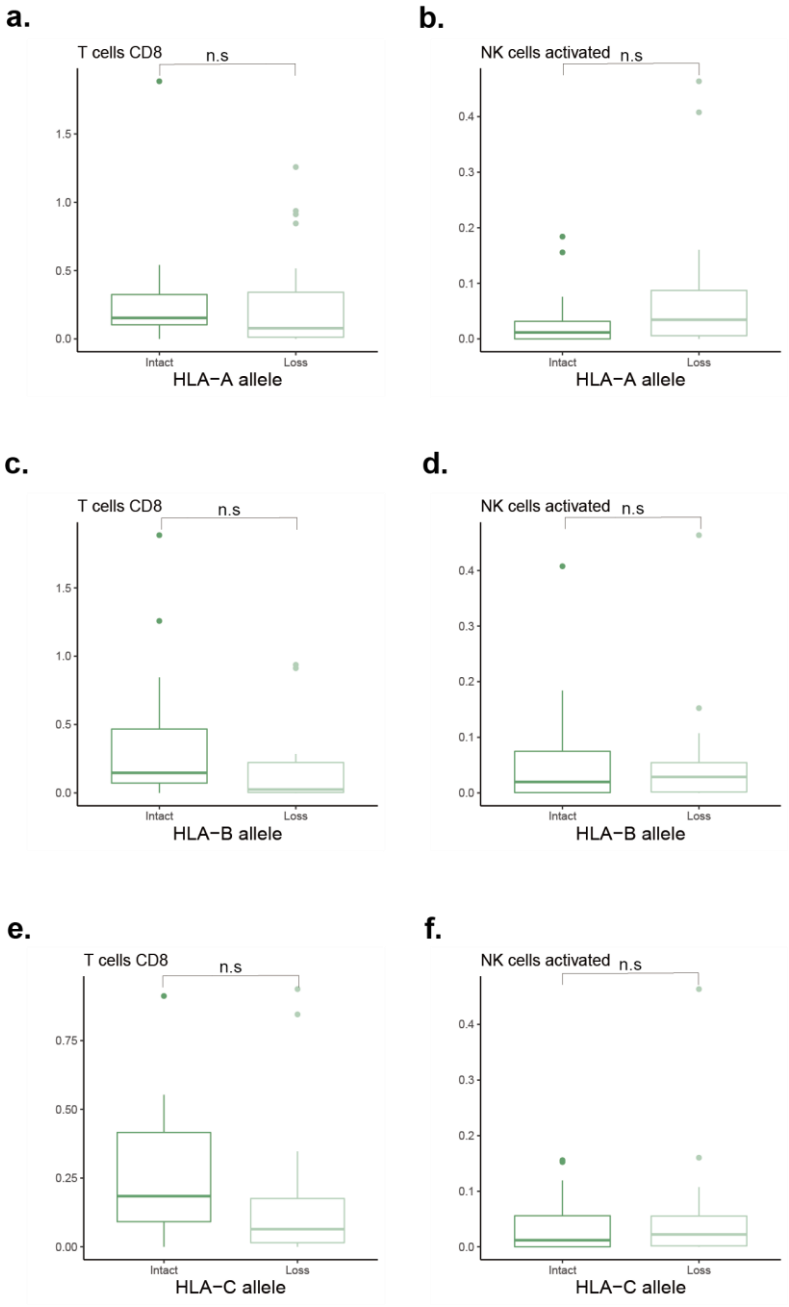

Figure 11. The cytotoxic immune cell score between HLA allele loss and HLA allele intact group in metastatic melanoma with anti-CTLA4.

IV. DISCUSSION

If loss of heterozygosity in HLA does not cause problems in the immune system, immune cell profiling was used to understand what kind of immune system it is. The LOH in HLA was expected to have low immune-mediated impact on NK cells and cytotoxic T cells, which are directly affected by HLA. As a result, cytotoxic immune cells were found in higher numbers in the responder group in metastatic urothelial cancer. After that, we looked at how immune cells correlated with LOH in HLA and discovered that patients with HLA-A allele loss had more NK cells. From this perspective, LOH in HLA not only reduces immunotherapy response (ref), but also causes cancer to evolve in a way that does not present neoantigen as a result of immune pressure, and also expresses an immune inhibitor. As a result, it is thought that when ICB treatment is used on cancer with such a tumor microenvironment, the immune response will be better because the immunogenicity environment has already been established.

We expect loss allele expression to be low in cancer cells if HLA allele copy number loss occurs[31], but it is difficult to indicate that it's always correlated with expression. Despite the development of a tool for predicting HLA allele expression[32], it is difficult to accurately predict allele-specific expression due to HLA polymorphism. As a result, it's thought that the precise allelic imbalance can only be determined once the correlation with allele-specific expression is established.

LOH in HLA has long been thought to be an important predictor of ICB treatment response, but this study shows that it is both significant and unrelated. Instead, in the immunogenic tumor microenvironment, LOH in HLA acts as an immune evolutionary factor, so some cancer types benefit from ICB treatment. Because cancer is caused by a complex set of factors, future research should consider not only immune factors but also other factors when assessing cancer and determining treatment options.

V. CONCLUSION

The goal of this study was to investigate if HLA allele loss in patients receiving ICB treatment was associated with their clinical response. As a result, we chose three ICB-treated cohorts to investigate allelic copy number loss in HLA using whole exome sequencing. Cancer was not removed because detection of the cytotoxic immune cell was difficult in patients with loss of heterozygosity in HLA. As a result, even if ICB treatment improves the tumor microenvironment, clinical response was not thought to be improved. However, our findings revealed that HLA allele loss did not differ significantly between responders and non-responders, and that HLA-A allele loss was more beneficial for clinical response in metastatic urothelial cancer.

To investigate this tendency, we proceeded with computational immune profiling, and we observed that there was association between the cytotoxic immune cells and loss of heterozygosity in HLA. It can be an immune evasion mechanism for cancer with hot immune environment, and it seems that the ICB response is better because these cytotoxic immune cells are many cancers.

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ABSTRACT(IN KOREAN)

인간 백혈구 항원의 이형 접합의 소실과 면역 관문 차단제의 효능과의 연관성

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양요한

면역 치료는 최근 암 치료 방법으로 주목받고 있는 차세대 맞춤형 치료 방법이다. 면역 치료는 면역세포가 암세포에서 유래한 신생 항원을 인지하고 환자의 특정 HLA 대립유전자와 반응성을 증가시켜 암세포의 사멸을 유도하는 방법이다. 지금까지 보고된 다른 연구에서는 HLA에서 이형 접합의 소실이 면역 치료와 관련이 있는 것으로 알려진 신생 항원이 충분하더라도 치료의 효과가 나타나지 않는 경우와 관련이 있다고 추정하고 있다. HLA 유전자의 이형 접합의 소실 및 대립유전자 복제 수의 소실은 면역 관문 차단제를 이용한 치료에서, 낮은 반응성을 보이는 것과 관련이 있는 것으로 추정되고 있다. 그러나 암 유형의 정확한 범위 또는 일관성이 조사되지는 않았다. 따라서, 면역 관문 억제제로 치료를 받은 3개의 코호트로 분석을 진행하였고, 이 코호트는 anti-PD1을 이용하여 치료를 받은 전이성 요로 상피암 환자(N=216), anti-PD1 치료를 받은 전이성 흑색종 환자(N=26) 그리고 anti-CTLA4로

치료를 받은 전이성 흑색종 환자(N=39)에서 총 281개의 엑솜 시퀀싱 데이터를 분석했다. HLA에서 이형접합체의 손실은 일반적으로 T 세포 면역 회피를 유발함으로써 면역 치료 반응의 억제 인자로 예상되었지만, 우리의 분석은 명확한 관계를 보여주지 않았다.

핵심되는 말 : 면역 관문 억제제, 이형 접합 소실, 인간 백혈구 항원