Circulating immunological transcriptomic profile identifies *DDX3Y* and *USP9Y* on the Y chromosome as promising biomarkers for predicting response to programmed death 1/programmed death ligand 1 blockade

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To the Editor: Immune checkpoint inhibitors (ICIs) have shown remarkable clinical responses; however, their efficacy remains limited to a small subset of patients. Peripheral blood mononuclear cells (PBMCs) offer an effective, accessible, and minimally invasive approach to assess tumor immune status and identify ICI responders. In this study, we aimed to elucidate the role of PBMC gene expression in ICI treatment response and prognosis.

This study received approval from the Biomedical Ethics Committee of West China Hospital, Sichuan University (No. 2019 [1045]) and the requirement to obtain informed consent was waived. We enrolled patients with histologically and clinically confirmed solid tumors who were scheduled to undergo anti-programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) immunotherapy at West China Hospital of Sichuan University between May 1, 2020, and May 31, 2021 for the discovery and validation cohort [Supplementary Materials and Methods, Supplementary Figure 1, http://links.lww.com/CM9/C240]. Of these, 55 were included in the discovery cohort and 98 in the validation cohort. According to Response Evaluation Criteria in Solid Tumours version 1.1 (RECIST version 1.1), patients with no disease progression (PD) or tumor-induced deaths within 6 months of anti-PD-1/PD-L1 treatment, including those with complete response (CR), partial response (PR), or stable disease (SD), were grouped as ICI responders. Patients experiencing PD within 6 months were classified as ICI non-responders. We identified 27 ICI responders and 28 non-responders in the discovery cohort and 47 responders and 51 non-responders in the validation cohort. No statistically significant differences in sex, age, cancer type, clinical stage, treatment line, or treatment regimen were observed between responders and non-responders in either the discovery or validation cohort (P >0.05, Supplementary Table 1, http://links.lww.com/CM9/C240).

Transcriptome sequencing of PBMCs in the discovery cohort (N = 55) identified a total of 206 differentially expressed genes (DEGs) (P <0.05, fold change [FC] >1.3 or <0.76), with 116 upregulated and 90 downregulated genes [Figure 1A, B]. Principal component analysis (PCA) based on these DEGs accurately distinguished ICI responders and non-responders as separate groups [Figure 1C]. Gene Ontology analyses revealed DEGs enrichment in the positive regulation of protein kinase B signaling, epithelial cell development, and external encapsulating structure organization [Supplementary Figure 2, http://links.lww.com/CM9/C240]. Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed significant DEGs enrichment in the nitrogen metabolism, calcium, and Ras-related protein 1 (Rap1) signaling pathways [Supplementary Figure 2, http://links.lww.com/CM9/C240]. Through reverse transcription-polymerase chain reaction (RT-PCR), we confirmed statistically significant differences in *DDX3Y, USP9Y, UTY, KDM5D*, and *RPS4Y1* expression, between responders and non-responders (P <0.05, Figure 1D, Supplementary Figure 3, http://links.lww.com/CM9/C240).

Further, in an independent external validation cohort (N = 98), we demonstrated that *DDX3Y* and *USP9Y*

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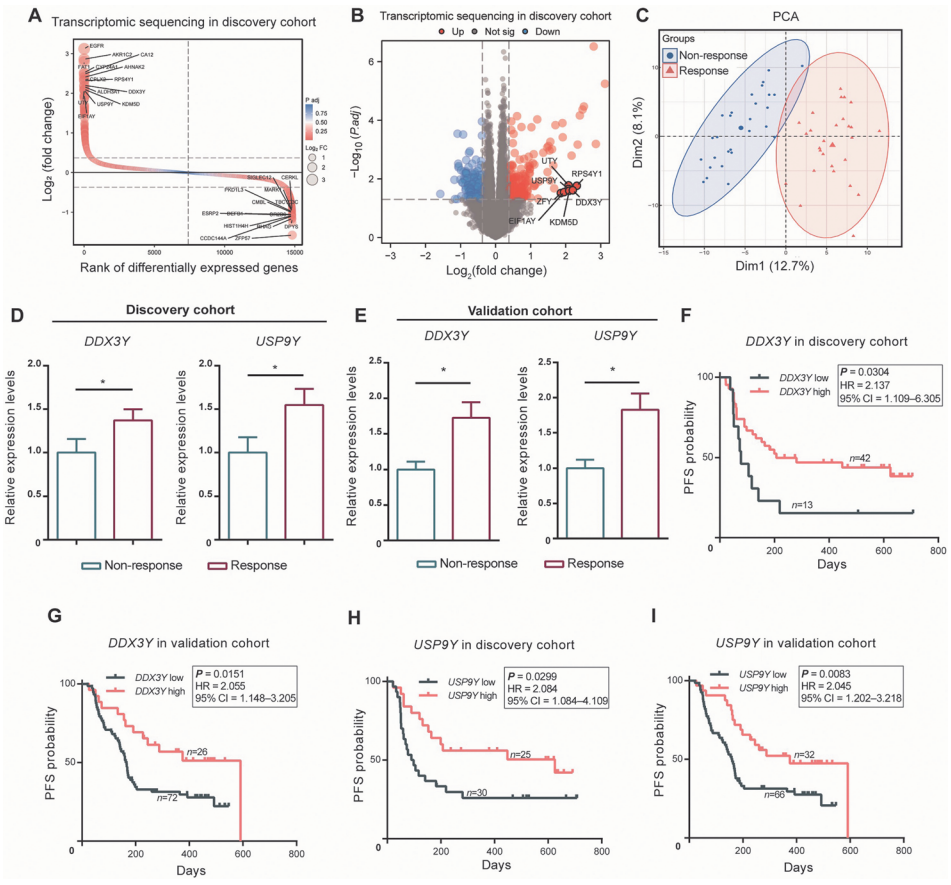

Figure 1: Circulating immunological transcriptomic profile identifies Y chromosome genes as promising biomarkers for efficacy of PD-1/PD-L1 blockade. (A–C) DEGs determined by transcriptome sequencing between ICI-responsive and ICI-non-responsive patients in the discovery cohort. (A) Difference ranking chart of DEGs. (B) Volcano plot of DEGs. The dashed line indicates the screening threshold ($P = 0.05$, FC = 1.3). (C) PCA plot based on DEGs between ICI-responsive and ICI-non-responsive patients. (D, E) Expression levels of *DDX3Y* and *USP9Y* between responsive and non-responsive patients in the discovery (D) and validation cohorts (E). Differences in survival outcomes based on different *DDX3Y* (F, G) and *USP9Y* (H, I) expression levels in the discovery and validation cohorts. Data are presented as mean ± standard deviation. Statistical significance in (D, E) was determined by a two-sided unpaired *t*-test. Survival data in (F, G, H, I) were analyzed by log-rank test. $^{*}P < 0.05$. CI: Confidence interval; DEGs: Differentially expressed genes; FC: Fold change; HR: Hazard ratio; ICI: Immune checkpoint inhibitor; PCA: Principal component analysis; PD-1: Programmed death 1; PD-L1: Programmed death ligand 1; PFS: Progression-free survival.

could predict the therapeutic efficacy and survival outcomes of patients receiving PD-1/PD-L1 blockade. ICI responders exhibited higher expression levels of *DDX3Y* and *USP9Y* than non-responders [Figure 1D, E]. Both *DDX3Y* and *USP9Y* were associated with progression-free survival (PFS) in patients receiving PD-1/PD-L1 blockade. For *DDX3Y*, the median PFS was shorter in the *DDX3Y*-low group than in the *DDX3Y*-high group (*DDX3Y* low *vs.* *DDX3Y* high: 77.0 days *vs.* 207.0 days, log-rank $P = 0.0304$, HR [95% CI]: 2.137 [1.109–6.305] in the discovery cohort; *DDX3Y* low *vs.* *DDX3Y* high: 164.5 days *vs.* 591.0 days, log-rank $P = 0.0151$, HR [95% CI]: 2.055 [1.148–3.205] in the validation cohort) [Figure 1F, G]. For *USP9Y*, the median PFS was shorter in the *USP9Y*-low group than in the *USP9Y*-high group (*USP9Y* low *vs.* *USP9Y* high: 94.0 days *vs.* 625.0 days, log-rank $P = 0.0299$, HR [95% CI]: 2.084 [1.084–4.109] in the discovery cohort; *USP9Y* low *vs.* *USP9Y* high: 160.0 days *vs.* 375.0 days, log-rank $P = 0.0083$, HR [95% CI]: 2.045 [1.202–3.218] in the validation cohort) [Figure 1H, I].

Then, we conducted subgroup analyses to assess the potential of *DDX3Y* and *USP9Y* in predicting responses within specific subgroups. We identified *DDX3Y* as a predictor of ICI efficacy in various subgroups (log-rank $P < 0.05$), with a shorter median PFS in the *DDX3Y*-low group than in the *DDX3Y*-high group in both the discovery and validation cohorts; however, this difference was significant, except for the NSCLC subgroup [Supplementary Figure 4, http://links.lww.com/CM9/C240]. For *USP9Y*, patients in the *USP9Y*-high group demonstrated improved survival outcomes after PD-1/PD-L1 blockade treatment compared to those in the *USP9Y*-low group across both the discovery and validation cohorts; however, this finding was significant, except in the NSCLC subgroup of the validation cohort [Supplementary Figure 4, http://links.lww.com/CM9/C240]. Overall, our findings strengthen the evidence supporting *DDX3Y* and *USP9Y* as predictors of PD-1/PD-L1 blockade efficacy.

As *DDX3Y* and *USP9Y* are located on the Y chromosome, it is necessary to eliminate any sex-related bias. First, we confirmed no significant difference in sex-related distribution between responders and non-responders [Supplementary Table 1, http://links.lww.com/CM9/C240]. Subsequently, we conducted a subgroup analysis, specifically for male patients [Supplementary Figure 5, http://links.lww.com/CM9/C240], which revealed a significantly

higher expression of *DDX3Y* in ICI-responder males than in non-responder males ($P = 0.003$, Supplementary Figure 5A, http://links.lww.com/CM9/C240), and individuals with high *DDX3Y* expression exhibited longer PFS than those with low *DDX3Y* expression (log-rank $P = 0.0188$, HR [95% CI]: 1.630 [1.100–2.683], Supplementary Figure 5B, http://links.lww.com/CM9/C240). Similarly, the expression levels of *USP9Y* were higher in responder males ($P = 0.0009$, Supplementary Figure 5C, http://links.lww.com/CM9/C240) than in non-responder males, and individuals with low *USP9Y* expression had shorter PFS than those with high *USP9Y* expression (log rank $P = 0.0059$, HR [95% CI]: 1.812 [1.188–2.702], Supplementary Figure 5D, http://links.lww.com/CM9/C240). These findings further support the potential roles of *DDX3Y* and *USP9Y* as reliable predictors of the PD-1/PD-L1 blockade.

The RNA-sequencing results from the discovery cohort revealed a significant number of DEGs located on the Y chromosome, including *DDX3Y*, *USP9Y*, *UTY*, *KDM5D*, and *RPS4Y1*. These genes exhibited higher expression levels in ICI responders than in non-responders (as depicted in Figure 1A, B). Additionally, low expression of *UTY*, *KDM5D*, and *RPS4Y1* was closely associated with a shorter PFS following PD-1/PD-L1 blockade (*KDM5D*, HR [95% CI]: 2.677 [1.572–10.250], log-rank $P = 0.0044$; *RPS4Y1*, HR [95% CI]: 2.495 [1.430–7.706], log-rank $P = 0.0062$; *UTY*, HR [95% CI]: 3.257 [1.619–6.238], log-rank $P = 0.0010$; Supplementary Figures 3D–F, http://links.lww.com/CM9/C240) in the discovery cohort. In the independent external validation cohort, patients with higher *KDM5D* and *RPS4Y1* expression showed longer PFS (*KDM5D*, HR [95% CI]: 1.928 [1.222–3.289], log-rank $P = 0.0067$; *RPS4Y1*, HR [95% CI]: 2.061 [1.148–3.158], log-rank $P = 0.0135$), although we did not obtain results for the *UTY* gene because of insufficient samples [Supplementary Figure 3G, H, http://links.lww.com/CM9/C240]. Additionally, our study revealed that patients with a higher average expression of the aforementioned genes on the Y chromosome in PBMCs exhibited a higher percentage of whole-blood lymphocytes and a lower percentage of whole-blood neutrophils in both the discovery [Supplementary Figure 6A, http://links.lww.com/CM9/C240] and validation cohort [Supplementary Figure 6B, http://links.lww.com/CM9/C240]. Although the differences in the percentage of both lymphocytes and neutrophils based on different *DDX3Y* and *RPS4Y1* expression levels were not statistically significant in the validation cohort [Supplementary Figure 6B, http://links.lww.com/CM9/C240], their potential roles in immune cells should not be disregarded. Currently, the cause-and-effect relationship is not fully understood; however, our study proposes that the loss of chromosome Y (LOY) or extreme downregulation of chromosome Y (EDY) in immune cells may lead to a decrease in the proportion of lymphocytes, thereby resulting in primary resistance to PD-1/PD-L1 blockade immunotherapy. In summary, our findings suggest that LOY or EDY in PBMCs may serve as potential unfavorable predictors for PD-1/PD-L1 blockade response. This mechanism may function by inhibiting lymphocyte proliferation and activation, thereby weakening the immune response to ICI immunotherapy.

In summary, our data showed that the expression of a cluster of Y-linked genes, including *DDX3Y*, *USP9Y*, *KDM5D*, *UTY*, and *RPS4Y1*, was significantly lower in non-responders to PD-1/PD-L1 blockade than in responders. The higher expression of these genes located on the Y chromosome was closely associated with better survival outcomes. Sex-related differences in immune responses are well acknowledged.[1] It was also observed that ICIs are more effective in males than females.[2,3] We propose that LOY and EDY may serve as potential biomarkers and play a role in the immune response to PD-1/PD-L1 blockade. LOY and EDY are common in patients with solid tumors, occurring at varying frequencies.[4] A recent study reported that LOY in cancer cells can drive immune escape and tumor growth.[5] LOY and EDY also occur in immune cells, and their interactions with tumor cells are crucial for determining tumor progression, patient prognosis, and the efficacy of immunotherapy. Changes in Y chromosome in immune cells play an important role in tumor immune responses and ICI efficacy. Our findings suggest that gene expression on Y chromosome may regulate lymphocyte proliferation; however, further research is needed to understand these mechanisms.

In conclusion, *DDX3Y* and *USP9Y* emerge as useful biomarkers for predicting PD-1/PD-L1 blockade response. This study identified Y chromosome immunosurveillance as a key factor contributing to sex differences that affect the efficacy of PD-1/PD-L1 blockade.

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Conflicts of interest

None.

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