Supplemental Material for *Gene expression profiling in kidney transplants with immune checkpoint inhibitor-associated adverse events*

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Supplemental Table 1. 25 custom genes analyzed with NanoString PanCancer Immune Profiling Panel.

Gene	Accession	Target sequence
ADAMDEC1	NM_014479.2	TTGACAACTTCCTGAGATGGCACAGTTCTAACCTGGGGAAAAAGATCCACGACCATGCTCAGCTTCTCAGCGGGATTAGCTTCAACAATCGACGTGTGGG
AIM2	NM_004833.1	TAGCAAGATATTATCGGCACAGTGGTTTCTTAGAGGTAAATAGCGCCTCACGTGTGTTAGATGCTGAATCTGACCAAAAGGTTAATGTCCCGCTGAACAT
ANKRD22	NM_144590.2	ACTGGCAGAGCAAATATGACTCAGAAACCGGCTCCTCAGGGTTGTAACATTAGATGATACAGGCTTGGGTCGTTACACATGACACCAGTGCCTTTGTTTC
AOAH	NM_001637.3	CTCTGGTGCTACAGGATTTCTGGACTCCACTGTTGGAATTAAAGAAAAATCTATTTACCTTCGCTTATGGAAAAGAAACCACTGTAATCACAGGGACTAC
B2M	NM_004048.2	CGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTATCCA
BASP1	NM_006317.3	GATCCGCGTCTGAAAGTGCAGTACATCGTTTGTACCTGAAACTGCCGCCACATGCACTCCTCCACCGCTGAGAGTTGAATAGCTTTTCTTCTGCAATGGG
CD72	NM_001782.2	CCGCCATCTGCCTGGGAGTGCGCTATCTGCAGGTGTCTCAGCAGCTCCAGCAGACGAACAGGGTTCTGGAAGTCACTAACAGCAGCCTGAGGCAGCAGCT
GBP2	NM_004120.3	GAGCTTTTTTCCAGCATTTGTGTGGACTCTCAGAGATTTCACCCTGGAACTGGAAGTAGATGGAGAACCCATCACTGCTGATGACTACTTGGAGCTTTCG
IGH	AW575313.1	AGGAGTCCTCCCTGACGGCCGAGGCAGGCGCTGGCCGCACTCCCGCTCGAGTCTCCCTTCCTGTCTGCAGATTCTGCGTGACAGTCACGGAACGGCGTGA
IGHG1	M87789.1	GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC
IGHM	AW134608.1	TACAAAGGCATTGCTTTGTGAAGAACTGGAAATTGAGAAAGAAGCCGGATCCTCTGCCACAGATAATGTGAGACACGTGGACACAGCGTGTGTAGTCTGG
IGHMBP2	NM_002180.2	GTCAACAACCATGCATTTTTGAAGACCCTGGTGGAGTATTTCACACAGCATGGGGAAGTACGCACGGCCTTTGAGTATCTTGACGATATTGTCCCAGAAA
IGKC	J00241.1	GAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCC
IGKV1D-8	Z00008.1	CTGCTGCTCTGGCTCCCAGGTAAGGAAGGAGAACACTATGAATTTACTCAGCCAATGTGCTCAGTACAGCCTGGCCCTTCAGGGAAATTCTCTTACTACA
IGL	U96394.1	ACTCCTCATCTATAGGAATAATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTC
IGLJ3	HF541920.1	CTCCTTGGGGGCAAAGCTGCCCTGACCCTTTCGGGTGCGCAGCCTGAGGATGAGGCTGAATATTACTGCTTGCTCTCCTATAGTGGTATTCGGGTGTTCG
IGLL2	L02326.1	CGGGAATCTTGATGGTGACCTGGAAGGCAGATGGTACCCTCATCACCCAGAGCGTGGAGAAGACCACGCCCTCCAAACAGAGCAACAACAAGTACGTGGC
IL18BP	NM_001039659.1	CACAACTGGACACCAGACCTCAGCCCTTTGTGGGTCCTGCTCCTGTGTGCCCACGTCGTCACTCTCCTGGTCAGAGCCACACCTGTCTCGCAGACCACCA

JCHAIN	NM_144646.3	GTGGAGCTGGATAATCAGATAGTTACTGCTACCCAGAGCAATATCTGTGATGAAGACAGTGCTACAGAGACCTGCTACACTTATGACAGAAACAAGTGCT
NKG7	NM_005601.3	CTGTGGCGGTCCCCGTCCTGGCTATGAAACCTTGTGAGCAGAAGGCAAGAGCGGCAAGATGAGTTTTGAGCGTTGTATTCCAAAGGCCTCATCTGGAGCC
PSME1	NM_006263.2	AAAGGGAATGATCTATTGAGAGCCCTCTCTCCCATTCTGTGATGAGTACAGCAGAGACCTTCCTGCTTTTTACTGGGGACTCCAGATTTTCCCCAAACTT
PTPN7	NM_002832.3	CACCAACTACTCTTAGGGAGGCTAAGCAGTCTCTGTTTTGCTTCCATGGCTCAAATAATACCCTGGGTATGCAGGACCCACTATACCTTGCATTTGCTGA
SLA	NM_001045556.2	CTTCTCATCACCACCTTACTTTGAGGACTAGCCAAGAACAGACACAATGGTTCATGCCCAAAAGGAACAGAAGTTCCAACTATTGCCTGGGATCTTGCGA
SLAMF8	NM_020125.2	AGCTGGCGACGGGAGACAACCATGGACTTTGGTATGGAACCACACAGCCTCTTCACAGACGGACAGGTGCTGAGCATTTCCCTGGGACCAGGAGACAGAG
SP140	NM_001005176.2	GGTTCTTCAGAGAAAACAAGGTGGAGATTGCAAGTGCAATAACAAGGCCATTTCCTTTCCTTATGGGCCTCCGAGACCGCTCCTTCATCTCCGAGCAGAT

Supplemental Table 2. Significantly differentially expressed genes in T-cell mediated rejection (TCMR; n=15) versus non-immune checkpoint inhibitor drug-associated acute interstitial nephritis (Drug-AIN; n=15).

Gene symbol	Fold change (TCMR vs. Drug-AIN)	Unadjusted *P*-value	Adjusted *P*-value (false discovery rate)
TNFRSF14	1.63	<0.001	0.008
IL32	3.73	<0.001	0.009
CX3CL1	1.51	<0.001	0.009
BCL2	1.34	0.001	0.010
HMGB1	1.59	0.001	0.010
STAT2	1.55	0.001	0.013
STAT5B	1.19	0.001	0.014
PSMB7	1.36	0.001	0.014
IFI27	1.71	0.002	0.016
HLA-E	1.87	0.002	0.017
IFIT1	1.49	0.002	0.017
CHUK	1.35	0.002	0.017
F2RL1	1.47	0.002	0.018
IRF1	2.94	0.002	0.018
CXCL9	3.20	0.003	0.018
MX1	1.59	0.003	0.020
MAPK3	1.26	0.003	0.020
ILF3	1.23	0.004	0.024
LAG3	4.42	0.004	0.026
SMAD3	1.31	0.005	0.026
LAMP2	1.46	0.005	0.028
PSMB8	2.05	0.006	0.031
IKBKB	1.37	0.007	0.034
HLA-C	1.93	0.007	0.034
STAT1	2.29	0.007	0.034
CXCL11	4.16	0.008	0.035
IL6R	1.45	0.008	0.035
NFKBIA	1.61	0.008	0.035
CXCL10	3.12	0.010	0.045
PSMB9	2.00	0.010	0.046

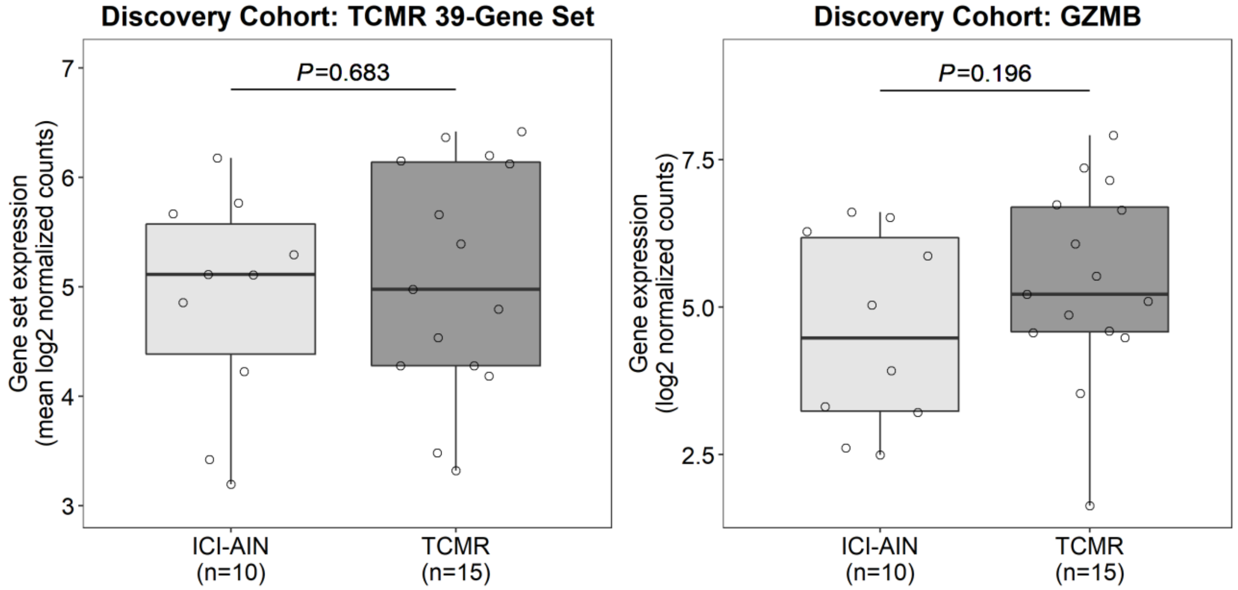
Supplemental Table 3. Significantly differentially expressed genes in non-immune checkpoint inhibitor drug-associated acute interstitial nephritis (Drug-AIN; n=15) versus T-cell mediated rejection (TCMR; n=15).

Gene symbol	Fold change (Drug-AIN vs. TCMR)	Unadjusted *P*-value	Adjusted *P*-value (false discovery rate)
MPPED1	6.50	<0.001	0.002
CCL26	5.78	<0.001	0.002
CD1E	4.24	<0.001	0.004
CTSG	3.07	<0.001	0.004
OSM	5.51	<0.001	0.004
RRAD	5.07	<0.001	0.004
S100A7	5.98	<0.001	0.004
KIR3DL3	4.36	<0.001	0.006
CD1C	2.46	<0.001	0.006
IL13	4.41	<0.001	0.006
CCL22	3.67	<0.001	0.006
CCL17	2.82	<0.001	0.006
IGLL1	4.12	<0.001	0.006
FOXP3	3.56	<0.001	0.006
IL8	3.67	<0.001	0.006
CCR4	3.69	<0.001	0.006
C4BPA	3.40	<0.001	0.006
CCL20	2.93	<0.001	0.007
CD1A	4.36	<0.001	0.007
IFNL2	5.97	<0.001	0.007
PTGDR2	6.30	<0.001	0.007
IGH	7.03	<0.001	0.007
MS4A2	2.55	<0.001	0.008
NCAM1	3.18	<0.001	0.008
IL24	3.83	<0.001	0.008
IL25	4.51	<0.001	0.008
CMA1	2.04	<0.001	0.008
CEACAM6	5.48	<0.001	0.008
FUT5	5.09	<0.001	0.008
PRG2	3.96	<0.001	0.008
ULBP2	6.18	<0.001	0.009
C8A	3.65	<0.001	0.009
AIRE	5.50	<0.001	0.009
CCL16	4.62	<0.001	0.009
IL17F	3.65	<0.001	0.009
ITGA2B	6.31	<0.001	0.009
KIR3DL2	5.19	<0.001	0.009
FEZ1	2.22	<0.001	0.009
CCL1	4.94	<0.001	0.009
ICAM4	4.26	<0.001	0.009
IGKV1D-8	5.19	<0.001	0.009
CXCR1	3.05	<0.001	0.009
IL12RB2	2.09	<0.001	0.010

MNX1	5.14	<0.001	0.010
IFNB1	7.07	<0.001	0.010
IL22RA1	3.17	<0.001	0.010
CHIT1	4.98	<0.001	0.010
CLU	5.78	<0.001	0.010
CD55	4.29	<0.001	0.010
CXCL6	3.86	<0.001	0.010
TREM2	3.53	<0.001	0.011
MASP2	3.52	<0.001	0.011
TNFRSF8	3.06	<0.001	0.011
CXCL5	5.41	0.001	0.012
CD70	3.92	0.001	0.012
CSF2	2.92	0.001	0.013
IL7	3.36	0.001	0.013
CCL25	4.02	0.001	0.014
CCL23	2.83	0.001	0.014
POU2AF1	2.89	0.001	0.014
CCR3	5.78	0.001	0.014
F12	3.68	0.001	0.014
IL3RA	1.63	0.001	0.014
CXCR2	2.73	0.001	0.014
JAM3	1.47	0.001	0.014
CD207	5.08	0.001	0.014
IFNAR1	1.53	0.002	0.014
FUT7	3.92	0.002	0.014
TAL1	2.65	0.002	0.015
BST1	1.36	0.002	0.015
SPINK5	3.23	0.002	0.016
IFNA7	5.05	0.002	0.016
IL23R	4.04	0.002	0.016
TPSAB1	2.67	0.002	0.016
CAMP	5.10	0.002	0.016
C6	3.15	0.002	0.016
ELANE	2.53	0.002	0.016
TREM1	3.76	0.002	0.016
ARG1	4.88	0.002	0.016
IL5RA	4.12	0.002	0.016
NUP107	1.27	0.002	0.016
LTF	3.05	0.002	0.017
CD83	1.48	0.002	0.017
CLEC6A	3.88	0.002	0.017
IL26	4.81	0.002	0.017
LILRA4	1.99	0.002	0.018
KIR3DL1	5.03	0.002	0.018
SELE	2.36	0.003	0.018
PLA2G1B	3.83	0.003	0.018
TNFRSF18	2.35	0.003	0.018
IFNA8	4.52	0.003	0.019
C9	2.60	0.003	0.019

IL22	3.39	0.003	0.019
CEACAM8	3.86	0.003	0.019
IL1RAPL2	2.42	0.003	0.020
CCR9	3.52	0.003	0.020
IFNA17	7.76	0.003	0.020
IFNA2	4.71	0.003	0.021
PPARG	1.85	0.003	0.021
CD40LG	2.07	0.003	0.021
CXCL1	1.90	0.004	0.023
SAA1	3.56	0.004	0.023
PTGS2	2.30	0.004	0.023
TLR9	4.03	0.004	0.024
TNFRSF12A	1.62	0.004	0.024
IL5	5.26	0.004	0.025
CD37	1.97	0.004	0.025
PMCH	3.43	0.005	0.026
LAMP3	1.86	0.005	0.026
LTK	3.17	0.005	0.026
TNFRSF4	2.01	0.005	0.026
ATM	3.52	0.005	0.028
IL12B	3.67	0.005	0.028
IL4	4.80	0.005	0.029
IL3	2.67	0.006	0.031
CASP10	3.36	0.006	0.031
LY9	1.98	0.006	0.031
CCR6	2.67	0.006	0.031
RAG1	2.39	0.006	0.032
TGFB2	1.72	0.007	0.033
KIR2DS1	4.66	0.007	0.033
ITGB4	1.64	0.007	0.034
FCER1A	3.86	0.007	0.034
IL1A	4.51	0.007	0.034
IL23A	3.14	0.007	0.034
IL27	4.49	0.007	0.035
CXCR5	2.19	0.008	0.035
LRRN3	2.21	0.008	0.035
TNFSF4	2.56	0.008	0.035
ADORA2A	2.29	0.008	0.036
LTA	3.32	0.008	0.037
PAX5	3.17	0.008	0.038
NFATC4	1.72	0.009	0.041
IL2	4.13	0.009	0.041
IL1B	2.21	0.010	0.046
MBL2	2.64	0.010	0.046
CLEC4C	1.76	0.011	0.046
FCGR2B	1.59	0.011	0.047
NCR1	2.06	0.011	0.047
IL17A	1.55	0.011	0.048

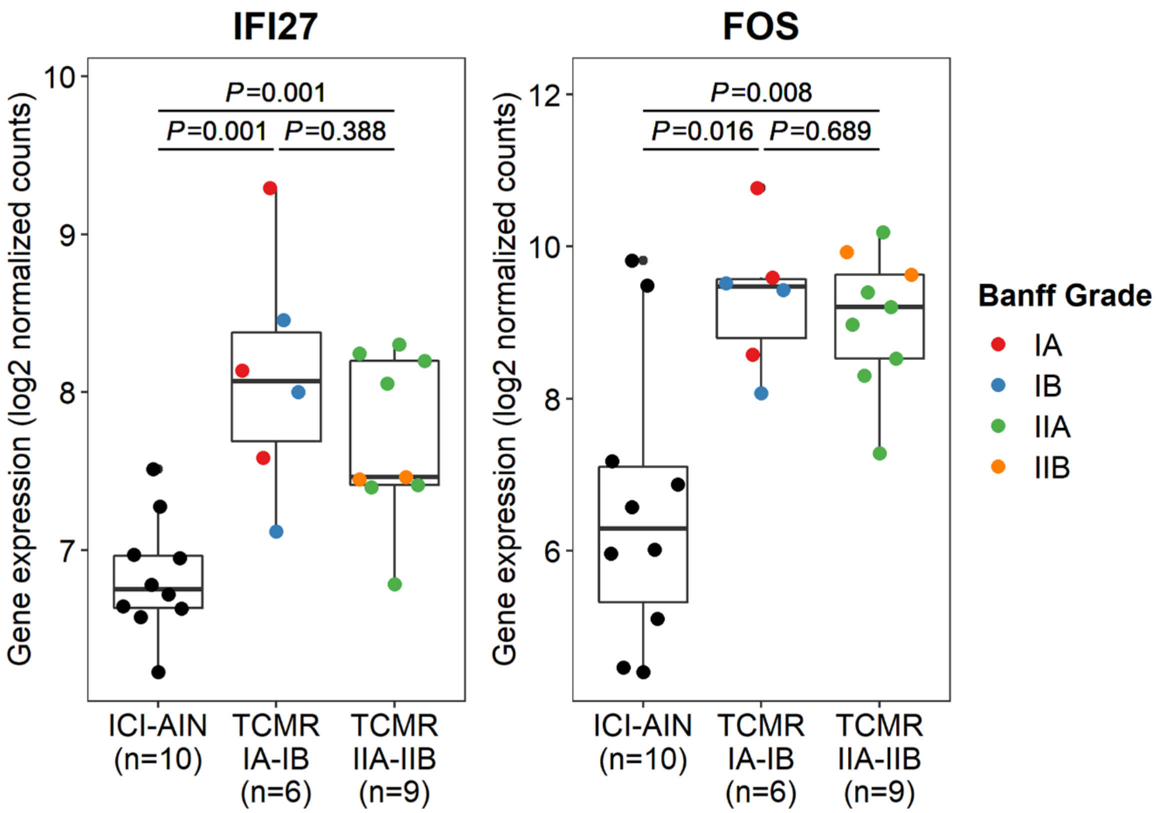
Supplemental Figure 1. Expression of T-cell mediated rejection 39-gene set and GZMB in discovery cohort. Aggregate gene set including the following 39 genes previously reported to be associated with T-cell mediated rejection demonstrates no significant differential expression between TCMR and ICI-AIN groups in the discovery cohort (*P*=0.683): ADAMDEC1, AIM2, ANKRD22, AOAH, BCL2, BTLA, CD274, CD28, CD3D, CD4, CD72, CD84, CD86, CD8A, CD8B, CD96, CRP, CTLA4, CXCL10, CXCL13, CXCL9, CXCR6, GZMB, ICOS, IFNG, IL12RB1, IL18BP, IL1RL1, IL21R, IL4, LAG3, PTPN7, SH2D1A, SLA, SLAMF8, SP140, TAP1, TIGIT, TNFSF8. Representative single gene analysis of GZMB demonstrates relatively higher mean expression in TCMR vs. ICI-AIN, but this difference does not reach statistical significance (*P*=0.196). ICI-AIN, immune checkpoint inhibitor-associated acute interstitial nephritis; TCMR, T-cell mediated rejection.



Discovery Cohort: TCMR 39-Gene Set

Discovery Cohort: GZMB

Supplemental Figure 2. *IFI27* and *FOS* gene expression in discovery cohort T-cell mediated rejection according to Banff grade. For *IFI27*, gene expression is higher in both Banff grades I and II TCMR versus ICI-AIN (*P*=0.001 for both), but there is no significant difference between Banff grades (*P*=0.388). Similarly, for *FOS*, gene expression is higher in both Banff grades I and II TCMR compared with ICI-AIN (*P*=0.016 and *P*=0.008, respectively), but there is no difference between Banff grades (*P*=0.689). These results suggest that increased *IFI27* and *FOS* gene expression in TCMR represents underlying pathophysiological mechanisms rather than immune response severity. ICI-AIN, immune checkpoint inhibitor-associated acute interstitial nephritis; TCMR, T-cell mediated rejection.



Supplemental Figure 3. Differential gene expression in validation cohort. Volcano plot shows fold change (y-axis) vs. linear regression p-value (x-axis) between immune checkpoint inhibitor-associated T-cell mediated rejection (ICI-TCMR) and immune checkpoint inhibitor-associated acute interstitial nephritis (ICI-AIN) for 725 immune-related genes. Blue, red and green points represent genes previously associated with T-cell mediated rejection, antibody-mediated rejection, and immune checkpoint pathway, respectively. After correction for multiple comparisons, no genes demonstrated statistically significant differential expression between ICI-TCMR and ICI-AIN (false discovery rate/FDR <0.05). However, *IFI27* was the second most relatively differentially expressed gene in ICI-TCMR vs. ICI-AIN (uncorrected *P*<0.001, FDR=0.134), providing support for the identification of this gene in the discovery cohort and confirmation of its diagnostic performance in the validation cohort. In contrast, *FOS* demonstrated minimal differential expression in the validation cohort (uncorrected *P*=0.368, FDR=0.544).