

CLAIMS

1. A method of predicting the risk of developing, monitoring the risk of developing, and/or diagnosing immune-related adverse events (irAE) associated with immune checkpoint inhibitor (ICI) treatment in a subject comprising:
 - a. assessing the level of one or more transcript in a sample obtained from the subject; and
 - b. predicting risk for developing, or monitoring the risk of developing and/or diagnosing irAE in the subject wherein, the subject has a high risk of developing or diagnosed with having irAE if:
 - i. the transcript levels of one or more of leukocyte immunoglobulin like receptor B4 (LILRB4), cytokine inducible SH2 containing protein (CISH), poly(ADP-ribose) polymerase family member 9 (PARP9), ring finger protein 145 (RNF145), asialoglycoprotein receptor 2 (ASGR2), solute carrier family 16 member 13 (SLC16A13), lysophosphatidic acid receptor 6 (LPAR6), GTPase, IMAP family member 7 (GIMAP7), and/or C-X-C motif chemokine receptor 6 (CXCR6) are elevated prior to ICI treatment (baseline), compared to the transcript levels in a control sample; and/or
 - ii. if the transcript levels of one or more of Amphiregulin (AREG), epiregulin (EREG), Oncostatin M (OSM), cysteine and serine rich nuclear protein 1 (CSRNP1), DNA damage inducible transcript 4 (DDIT4), IL-10 (interleukin 10), Prostaglandin-endoperoxide synthase (PTGS2), Dual Specificity Phosphatase 1 (DUSP1), C-X-C chemokine receptor type 4 (CXCR4), Nuclear Factor, Interleukin 3 Regulated (NFIL3), Fos proto-oncogene, AP-1 transcription factor subunit (FOS), NFkB inhibitor alpha (NFKBIA), PPP1R15A (protein phosphatase 1 regulatory subunit 15A), CD79A, JunB proto-oncogene, AP-1 transcription factor subunit (JUNB), C-X-C motif chemokine ligand 8 (CXCL8), Early growth response 1 (EGR1), G0/G1 switch 2 (G0S2), paired box 8 (PAX8), activating transcription factor 6 beta (ATF6B), PAX8 antisense RNA1 (PAX8-AS1), RNA, variant U1 small nuclear 19 (RNVU1-19), vitelline membrane outer layer 1 homolog (VMO1), heparin binding EGF like growth factor (HBEGF), coiled-coil domain containing 144A (CCDC144A), shisa family member 8 (SHISA8),

nuclear receptor subfamily 4 group A member 2 (NR4A2), prostaglandin E synthase (PTGES), synapsin I (SYN1), C-X-C motif chemokine ligand 2 (CXCL2), Peripheral myelin protein 22 (PMP22), CD83, early growth response 3 (EGR3), NUA family kinase 1 (NUAK1), nocturnin (NOCT), atonal bHLH transcription factor 8 (ATOH8), polo like kinase (PLK2), inhibitor of DNA binding 1 (ID1), adrenoceptor beta 1 (ADRB1), snail family transcriptional repressor 1 (SNAI1), notch receptor 3 (NOTCH3), activating transcription factor 3 (ATF3), dual specificity phosphatase 2 (DUSP2), period circadian regulator 1 (PER1), TNF superfamily member 9 (TNFSF9), MAF bzip transcription factor F (MAFF), microRNA 4420 (MIR4420), glutathione peroxidase (GPX3), TNF alpha induced protein 3 (TNFAIP3), potassium voltage-gated channel modifier subfamily G member 1 (KCNG1), prostaglandin-endoperoxidase synthase 2 (PTGS2), A-kinase anchoring protein 5 (AKAP5), dual specificity phosphatase 1 (DUSP1), diacylglycerol kinase kappa (DGKK), beta-1,4,-N-acetyl-galactosaminyltransferase 3 (B4GALNT3), tribbles pseudokinase 1 (TRIB1), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), C-X-C motif chemokine receptor 4 (CXCR4), tumor protein p53 inducible nuclear protein 2 (TP53INP2), nuclear factor, interleukin 3 regulated (NFIL3), dual specificity phosphatase 4 (DUSP4), NFkB inhibitor alpha (NFKBIA), arginine vasopressin induced 1 (AVPI1), CD79a, ADP ribosylation factor like GTPase 4D (ARL4D), joining chain of multimeric IgA and IgM (JCHAIN), BTG anti-proliferation factor 2 (BTG2), TLE family member 1, transcriptional corepressor (TLE1), nuclear transport factor 2 like export factor 1 (NXT1), transducer of ERBB2, 1 (TOB1), phosphodiesterase 4D (PDE4D), DNAJ heat shock protein family member B1 (DNAJB1), AT-rich interaction domain 5B (ARID5B), G protein-coupled receptor 153 (GPR153), KLF transcription factor 9 (KLF9), SBDS ribosome maturation factor (SBDS), immediate early response 2 (IER2), TSC22 domain family member 3 (TSC22D3), GABA type A receptor associated protein like 1 (GABARAPL1), JunD proto-oncogene, AP-1 transcription factor subunit (JUND), RUNX family transcription factor 3 (RUNX3), BABAM2 antisense RNA 1 (BRE-AS1), putative salt inducible kinases 1B (LOC102724428), FAM46C (FAM46C), and/or general receptor

for phosphoinositides 1-associated scaffold protein (GRASP) are lower in the sample when compared to the transcript levels in a control sample.

2. The method of claim 1, wherein the irAE comprises ICI-related myositis, ICI-related myocarditis, or ICI-related myositis and myocarditis.

3. The method of any one of claim 1 or 2, wherein the assessment of transcript levels is performed on a sample obtained from the subject before ICI treatment.

4. The method of any one of claims 1-3, wherein the subject is predicted as high risk of developing irAE if the transcript levels of one or more of

a) LILRB4, CISH, PARP9, RNF145, ASGR2, SLC16A13, LPAR6, GIMAP7, DHRS9, FCGR1CP, ANKRD34B and/or CXCR6; or

b) LILRB4, CISH, and PAPP9; or

c) LILRB4, CISH, and GIMAP7; or

d) CXCR6, DHRS9, and FCGR1CP; or

e) ANKRD34B are elevated prior to ICI treatment (baseline), compared to the transcript levels in a control sample.

5. The method of any one of claims 1-3, wherein the subject is predicted as high risk of developing irAE if the transcript levels of one or more of

a) AREG, EREG, OSM, CSRNP1, DDIT4, IL-10, PTGS2, DUSP1, CXCR4, NFIL3, FOS, NFKBIA, PPP1 R15A, CD79A, JUNB, CXCL8, EGR1, G0S2, PAX8, ATF6B, PAX8-AS1, RNVU1-19, VMO1, HBEGF, CCDC144A, SHISA8, NR4A2, PTGES, SYN1, CXCL2, PMP22, CD83, EGR3, NUA1, NOCT, ATOH8, PLK2, ID1, ADRB1, SNAI1, NOTCH3, ATF3, DUSP2, PER1, TNFSF9, MAFF, MIR4420, GPX3, TNFAIP3, KCNG1, PTGS2, AKAP5, DUSP1, DGKK, B4GALNT3, TRIB1, PMAIP1, CXCR4, TP53INP2, NFIL3, DUSP4, NFKBIA, AVPI1, CD79a, ARL4D, JCHAIN, BTG2, TLE1, NXT1, TOB1, PDE4D, DNAJB1, ARID5B, GPR153, KLF9, SBDS, IER2, TSC22D3, JUND, GABARAPL1, RUNX3, BRE-AS1, LOC102724428, FAM46C and/or GRASP are lower prior to ICI treatment, compared to the transcript levels in a control sample; or

b) AREG, EREG, OSM, CSRNP1, DDIT4, IL-10, PTGS2, DUSP1, CXCR4, NFIL3, FOS, NFKBIA, PPP1R15A, CD79A, and/or JUNB are lower prior to ICI treatment, compared to the transcript levels control sample; or

c) AREG, EREG, CXCL8, EGR1, G0S2, PAX8, ATF6B, PAX8-AS1, RNVU1-19, VMO1, HBEGF, CCDC144A, and/or SHISA8 are lower prior to ICI treatment, compared to the transcript levels control sample; or

d) AREG, EREG, CXCL8, EGR1, PAX8, ATF6B, VMO1, HBEGF, BRE-AS1, NR4A2, OSM, NOCT, PLK2, LOC102724428, DUSP2, GRASP, PER1, TNFSF9, CSRNP1, MAFF, TNFAIP3, DUSP1, TRIB1, PMAIP1, FAM46C, NXT1, and/or KLF9, are lower prior to ICI treatment, compared to the transcript levels in a control sample.

6. The method of any one of claims 1 or 2, wherein the subject is predicted as high risk for irAE if

i. the transcript levels of one or more of PARP9, CISH, CXCR6, LPAR6, and/or ASGR2 are elevated prior to ICI treatment (baseline), compared to the transcript levels in a control sample; and/or

ii. the transcript levels of one or more of KLF9, CXCR4, ATF6B, CD79A, CD83, PLK2, GRASP, PPP1R15A, GPX3, PARP9, BTG2, PTGS2, NOTCH3, AKAP5, PDE4D, SBDS, TRIB1, SNAI1, PAX8, NFIL3, EREG, AREG, DGKK, PTGES, CSRNP1, DNAJB1, CXCL8, OSM, ARID5B, ID1, NR4A2, EGR3, JUNB, TLE1, CXCL2, DUSP1, GPR153, G0S2, ATF3, NFKBIA, TNFSF9, NUA1K1, ATOH8, TNFAIP3, ADRB1, HBEGF, IL-10, JUND, FOS, GABARAPL1, PMAIP1, DDIT4, and/or EGR1 are lower prior to ICI treatment (baseline), compared to the transcript levels in a control sample.

7. The method of any one of claims 1-6, wherein the sample is whole blood, serum, plasma, cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal fluid, bone marrow, tissue, urine, cerebrospinal fluid (CSF), or other body fluid, and optionally wherein assessing transcript levels (step b) comprises RNA-seq, nanopore sequencing, nanostring, multiplex RT-PCR, single-plex RT-PCR, NASBA, fluorescence measurements, or spectrophotometry.

8. The method of any one of claims 1 or 2, wherein said ICI treatment is to be administered as part of cancer treatment, and optionally,

wherein said ICI treatment comprises administration of an inhibitor of PD-1, PD-L1, TIM-3, LAG-3, CTLA-4, CSF-1R, or any combination thereof.

9. The method of any one of claims 1-8, wherein the method further comprises identifying if the expression of one or more autoantibodies are elevated in the sample from the subject compared to the expression in a control sample, optionally wherein the one or more autoantibodies are selected from Mi-2, GAD65, Myosin, Thyroglobulin, and/or TPO.

10. The method of any one of claims 1-9, wherein the method further comprises identifying if the expression of one or more cytokines are elevated in the sample from the subject compared to the expression in a control sample, optionally wherein the one or more cytokines are selected from CXCL2, CXCL5, CXCL6, CCL7, CCL19, IFN γ , IL-6, CXCL9, and/or CXCL10.

11. The method of any one of claims 1-10, wherein the method further comprises identifying if the abundance of one or more of PD-L+ naive B cells, and/or switched memory B cells are decreased, and/or if the abundance of CTLA-4+ monocyte is elevated, in the sample from the subject compared to the abundance in a control sample.

12. The method of claim 1, further comprising repeating steps (a)-(b) at a second time point, thereby permitting determination of a change in the subject's risk of developing irAE and/or diagnosis of irAE in the sample from the subject compared to a control sample.

13. The method of claim 1, further comprising predicting the subject as having low risk if:

i. the transcript levels of one or more of LILRB4, CISH, PARP9, RNF145, ASGR2, SLC16A13, LPAR6, GIMAP7, and/or CXCR6 prior to ICI treatment (baseline) are lower or equivalent compared to the transcript levels in a control sample; and/or

ii. the transcript levels of one or more of AREG, EREG, OSM, CSRN1, DDIT4, IL-10, PTGS2, DUSP1, CXCR4, NFIL3, FOS, NFKBIA, PPP1R15A, CD79A, JUNB, CXCL8, EGR1, G0S2, PAX8, ATF6B, PAX8-AS1, RNVU1-19, VMO1, HBEGF, CCDC144A, SHISA8, NR4A2, PTGES, SYN1, CXCL2, PMP22, CD83, EGR3, NUA1, NOCT, ATOH8, PLK2, ID1, ADRB1, SNAI1, NOTCH3, ATF3, DUSP2, PER1, TNFSF9, MAFF, MIR4420, GPX3, TNFAIP3, KCNG1, PTGS2, AKAP5, DUSP1, DGKK, B4GALNT3, TRIB1, PMAIP1, CXCR4, TP53INP2, NFIL3, DUSP4, NFKBIA, AVPI1, CD79a, ARL4D, JCHAIN, BTG2, TLE1, NXT1, TOB1, PDE4D, DNAJB1, ARID5B, GPR153, KLF9, SBDS, IER2, TSC22D3,

JUND, GABARAPL1, RUNX3, BRE-AS1, LOC102724428, FAM46C, and/or GRASP are elevated or equivalent during ICI treatment, compared to the transcript levels in a control sample.

14. A method of identifying the presence of at least one differentially expressed transcript associated with irAE in a biological sample obtained from a subject with cancer, the method comprising:

- a. assessing the levels of one or more transcripts in the sample, wherein the assessment comprises detecting if:
 - i. the transcript levels of one or more of LILRB4, CISH, PARP9, RNF145, ASGR2, SLC16A13, LPAR6, GIMAP7, and/or CXCR6 are elevated than the transcript levels in a control sample; and/or
 - ii. the transcript levels of one or more of AREG, EREG, OSM, CSRN1P1, DDIT4, IL-10, PTGS2, DUSP1, CXCR4, NFIL3, FOS, NFKBIA, PPP1R15A, CD79A, JUNB, CXCL8, EGR1, G0S2, PAX8, ATF6B, PAX8-AS1, RNVU1-19, VMO1, HBEGF, CCDC144A, SHISA8, NR4A2, PTGES, SYN1, CXCL2, PMP22, CD83, EGR3, NUA1K1, NOCT, ATOH8, PLK2, ID1, ADRB1, SNAI1, NOTCH3, ATF3, DUSP2, PER1, TNFSF9, MAFF, MIR4420, GPX3, TNFAIP3, KCNG1, PTGS2, AKAP5, DUSP1, DGKK, B4GALNT3, TRIB1, PMAIP1, CXCR4, TP53INP2, NFIL3, DUSP4, NFKBIA, AVPI1, CD79a, ARL4D, JCHAIN, BTG2, TLE1, NXT1, TOB1, PDE4D, DNAJB1, ARID5B, GPR153, KLF9, SBDS, IER2, TSC22D3, JUND, GABARAPL1, RUNX3, BRE-AS1, LOC102724428, FAM46C and/or GRASP are lower than the transcript levels in a control sample.