BIOMARKERS FOR GRAFT-VERSUS-HOST DISEASE

Cross-Reference to Related Applications

This application claims priority to U.S. Provisional Appl. No. 62/657,193, filed April 13, 2018, and U.S. Provisional Appl. No. 62/773,308, filed November 30, 2018. The content of the prior applications are incorporated by reference herein in their entirety.

Technical Field

The present invention relates generally to biomarkers and Graft-Versus-Host Disease.

Background

Graft-Versus-Host Disease (GvHD) occurs when immunologically competent cells transferred to an allogeneic recipient attack tissues in the recipient. Tissues of the skin, gut epithelia, and liver are often targets and may be destroyed during the course of GvHD. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow transplantation. GvHD is the second leading cause of death following allogeneic hematopoietic stem cell transplant. GvHD can also occur following other transplants, such as heart and liver transplants.

Janus kinase (JAK) inhibitors have been developed as agents for the treatment of GvHD. However, as for any therapeutic, JAK inhibitors may not be equally effective in all subjects that have GvHD. There is a need for means of identifying those subjects having GvHD that could most benefit from treatment with a JAK inhibitor as well as

identifying those subjects that exhibit a therapeutic response to treatment with a JAK inhibitor.

Summary

The present application is based, at least in part, on the identification of biomarkers that are predictive of a GvHD subject's responsiveness to a therapy comprising a JAK inhibitor and biomarkers that identify a subject that has undergone a therapeutic response to a JAK inhibitor. The level of certain proteins (e.g., the proteins listed in Table 1 and Table 2) prior to treatment is identified as a useful predictor of responsiveness to a therapy comprising a JAK inhibitor. In addition, the change in level of certain proteins (e.g., the proteins listed in Table 13) during the course of treatment is identified as a useful identifier of responsiveness to a therapy comprising a JAK inhibitor. Thus, the biomarkers and compositions described herein are useful, for example, in identifying, stratifying, and/or selecting a patient or a subset of patients having, suspected of having, or at risk of developing GvHD that could benefit, or have benefitted, from treatment with a JAK inhibitor. In addition, the methods described herein are useful, for example, in selecting appropriate treatment modalities (e.g., therapy comprising a JAK inhibitor) for a subject suffering from, suspected of having, or at risk of developing GvHD.

The disclosure features a method of treating a human subject having, suspected of having, or at risk of developing GvHD by administering to the human subject a therapy comprising a JAK inhibitor, wherein the human subject has been previously determined to have (i) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the

group consisting of IL8, HAOX1, ENPP7, ACE2, SULT2A1, MCP-3, CES1, MFGE8, PLXNB1, TNFRSF10A, CCL15, SEMA4C, PREB, NFATC3, CCL19, DLL1, ENTPD2, IL-4RA, EPHA2, FOSB, CXCL10, VAMP5, ALDH3A1, MVK, IL12RB1, CALCA, AHCY, PRSS2, LILRB4, DDAH1, IL-1ra, NECTIN2, PDCD1, CD74, PD-L1, REG3A, CA5A, N2DL-2, CDCP1, U-PAR, SIGLEC7, ANGPTL4, ALDH1A1, SPINK1, HTRA2, PRDX6, IL-1RT2, IGFBP-1, HNMT, TRAIL-R2, CXADR, CTSL1, IFN-gamma-R1, IL-18R1, KRT19, KYNU, and TGM2 in a biological sample obtained from the human subject that is lower than a control, and/or (ii) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, CNTN1, IGFBP3, LEP, Notch 3, TN-R, HSD11B1, FAM19A5, NCAN, F11, GDF-8, CCL28, GALNT10, BCAN, TIMP4, CRISP2, CD207, WNT9A, MBL2, EN-RAGE, TWEAK, CR2, MFAP5, KIT, GH, PFKM, CDSN, CRH, GCP5, KLK6, and DRAXIN in a biological sample obtained from the human subject that is higher than a control.

In some embodiments, the human subject has been previously determined to have (i) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, ALDH1A1, CES1, REG3A, KYNU, IL-4RA, CDCP1, MVK, FOSB, NFATC3, N2DL-2, DDAH1, IGFBP-1, ALDH3A1, CXADR, PLXNB1, CD74, ENTPD2, PREB, CCL19, HNMT, HTRA2, IL-1RT2, and IL-18R1 in a biological sample obtained from the human subject that is lower than a control, and/or (ii) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8 proteins) selected from the group consisting of PON3, LEP,

MBL2, GH, GDF-8, EN-RAGE, CRISP2, and CR2 in a biological sample obtained from the human subject that is higher than a control.

In some embodiments, the human subject has been previously determined to have (i) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, and ALDH1A1 in a biological sample obtained from the human subject that is lower than a control, and/or (ii) a baseline concentration of at least one protein (e.g., at least 1, 2, or 3 proteins) selected from the group consisting of PON3, LEP, and MBL2 in a biological sample obtained from the human subject that is higher than a control.

In some embodiments, the human subject has been previously determined to have (i) a baseline concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, and SPINK1 in a biological sample obtained from the human subject that is lower than a control, and/or (ii) a baseline concentration of at least one protein (e.g., at least 1 or 2 proteins) selected from the group consisting of PON3 and LEP in a biological sample obtained from the human subject that is higher than a control.

The disclosure also features a method of treating a human subject having, suspected of having, or at risk of developing GvHD, by: providing a biological sample obtained from the human subject; measuring in the biological sample a reduced concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group

consisting of IL8, HAOX1, ENPP7, ACE2, SULT2A1, MCP-3, CES1, MFGE8, PLXNB1, TNFRSF10A, CCL15, SEMA4C, PREB, NFATC3, CCL19, DLL1, ENTPD2, IL-4RA, EPHA2, FOSB, CXCL10, VAMP5, ALDH3A1, MVK, IL12RB1, CALCA, AHCY, PRSS2, LILRB4, DDAH1, IL-1ra, NECTIN2, PDCD1, CD74, PD-L1, REG3A, CA5A, N2DL-2, CDCP1, U-PAR, SIGLEC7, ANGPTL4, ALDH1A1, SPINK1, HTRA2, PRDX6, IL-1RT2, IGFBP-1, HNMT, TRAIL-R2, CXADR, CTSL1, IFN-gamma-R1, IL-18R1, KRT19, KYNU, and TGM2, and/or an increased concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, CNTN1, IGFBP3, LEP, Notch 3, TN-R, HSD11B1, FAM19A5, NCAN, F11, GDF-8, CCL28, GALNT10, BCAN, TIMP4, CRISP2, CD207, WNT9A, MBL2, EN-RAGE, TWEAK, CR2, MFAP5, KIT, GH, PFKM, CDSN, CRH, GCP5, KLK6, and DRAXIN; and administering a therapy comprising a JAK inhibitor to the human subject.

In some embodiments, the method includes: measuring in the biological sample a reduced concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, ALDH1A1, CES1, REG3A, KYNU, IL-4RA, CDCP1, MVK, FOSB, NFATC3, N2DL-2, DDAH1, IGFBP-1, ALDH3A1, CXADR, PLXNB1, CD74, ENTPD2, PREB, CCL19, HNMT, HTRA2, IL-1RT2, and IL-18R1, and/or an increased concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8 proteins) selected from the group consisting of PON3, LEP, MBL2, GH, GDF-8, EN-RAGE, CRISP2,

and CR2; and administering the therapy comprising the JAK inhibitor to the human subject.

In some embodiments, the method includes: measuring in the biological sample a reduced concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, and ALDH1A1, and/or an increased concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, or 3 proteins) selected from the group consisting of PON3, LEP, and MBL2; and administering the therapy comprising the JAK inhibitor to the human subject.

In some embodiments, the method includes: measuring in the biological sample a reduced concentration, as compared to a control, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8 proteins) selected from the group consisting of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, and SPINK1, and/or an increased concentration, as compared to a control, of at least one protein (e.g., at least 1 or 2 proteins) selected from the group consisting of PON3 and LEP; and administering the therapy comprising the JAK inhibitor to the human subject.

The disclosure also features a method of predicting the response of a human subject having, suspected of having, or at risk of developing GvHD to a therapy comprising a JAK inhibitor, by: providing a biological sample obtained from the subject before the therapy comprising the JAK inhibitor; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, CNTN1, IGFBP3, LEP, Notch 3, TN-R, HSD11B1, FAM19A5, NCAN, F11, GDF-8,

CCL28, GALNT10, BCAN, TIMP4, CRISP2, CD207, WNT9A, MBL2, EN-RAGE, TWEAK, CR2, MFAP5, KIT, GH, PFKM, CDSN, CRH, GCP5, KLK6, DRAXIN, IL8, HAOX1, ENPP7, ACE2, SULT2A1, MCP-3, CES1, MFGE8, PLXNB1, TNFRSF10A, CCL15, SEMA4C, PREB, NFATC3, CCL19, DLL1, ENTPD2, IL-4RA, EPHA2, FOSB, CXCL10, VAMP5, ALDH3A1, MVK, IL12RB1, CALCA, AHCY, PRSS2, LILRB4, DDAH1, IL-1ra, NECTIN2, PDCD1, CD74, PD-L1, REG3A, CA5A, N2DL-2, CDCP1, U-PAR, SIGLEC7, ANGPTL4, ALDH1A1, SPINK1, HTRA2, PRDX6, IL-1RT2, IGFBP-1, HNMT, TRAIL-R2, CXADR, CTSL1, IFN-gamma-R1, IL-18R1, KRT19, KYNU, and TGM2 in the biological sample, wherein a reduced concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of IL8, HAOX1, ENPP7, ACE2, SULT2A1, MCP-3, CES1, MFGE8, PLXNB1, TNFRSF10A, CCL15, SEMA4C, PREB, NFATC3, CCL19, DLL1, ENTPD2, IL-4RA, EPHA2, FOSB, CXCL10, VAMP5, ALDH3A1, MVK, IL12RB1, CALCA, AHCY, PRSS2, LILRB4, DDAH1, IL-1ra, NECTIN2, PDCD1, CD74, PD-L1, REG3A, CA5A, N2DL-2, CDCP1, U-PAR, SIGLEC7, ANGPTL4, ALDH1A1, SPINK1, HTRA2, PRDX6, IL-1RT2, IGFBP-1, HNMT, TRAIL-R2, CXADR, CTSL1, IFN-gamma-R1, IL-18R1, KRT19, KYNU, or TGM2, and/or an increased concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of PON3, CNTN1, IGFBP3, LEP, Notch 3, TN-R, HSD11B1, FAM19A5, NCAN, F11, GDF-8, CCL28, GALNT10, BCAN, TIMP4, CRISP2, CD207, WNT9A, MBL2, EN-RAGE, TWEAK, CR2, MFAP5, KIT, GH, PFKM, CDSN, CRH, GCP5, KLK6, or DRAXIN is predictive that the subject will respond to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, LEP, MBL2, GH, GDF-8, EN-RAGE, CRISP2, CR2, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, ALDH1A1, CES1, REG3A, KYNU, IL-4RA, CDCP1, MVK, FOSB, NFATC3, N2DL-2, DDAH1, IGFBP-1, ALDH3A1, CXADR, PLXNB1, CD74, ENTPD2, PREB, CCL19, HNMT, HTRA2, IL-1RT2, and IL-18R1 in the biological sample, wherein a reduced concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, ALDH1A1, CES1, REG3A, KYNU, IL-4RA, CDCP1, MVK, FOSB, NFATC3, N2DL-2, DDAH1, IGFBP-1, ALDH3A1, CXADR, PLXNB1, CD74, ENTPD2, PREB, CCL19, HNMT, HTRA2, IL-1RT2, or IL-18R1, and/or an increased concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8) of PON3, LEP, MBL2, GH, GDF-8, EN-RAGE, CRISP2, or CR2 is predictive that the subject will respond to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 proteins) selected from the group consisting of PON3, LEP, MBL2, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, and ALDH1A1 in the biological sample, wherein a reduced concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16) of MCP-3, HAOX1, CA5A, CALCA, IL8,

SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, or ALDH1A1, and/or an increased concentration, as compared to a control, of at least one (e.g., at least 1, 2, or 3) of PON3, LEP, or MBL2 is predictive that the subject will respond to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 proteins) selected from the group consisting of PON3, LEP, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, and SPINK1 in the biological sample, wherein a reduced concentration, as compared to a control, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, or 8) of MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, or SPINK1, and/or an increased concentration, as compared to a control, of at least one (e.g., at least 1 or 2) of PON3 or LEP is predictive that the subject will respond to the therapy comprising the JAK inhibitor.

In some embodiments of the methods described herein, the control is a pre-established cut-off value.

In some embodiments of the methods described herein, the control is the concentration of the protein in a sample or samples obtained from one or more subjects that have not responded to treatment with the JAK inhibitor.

The disclosure also features a method for measuring the amount of a protein in a sample, by: providing a biological sample obtained from a human subject having, suspected of having, or at risk of developing GvHD; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, CNTN1, IGFBP3, LEP, Notch 3, TN-R, HSD11B1, FAM19A5, NCAN, F11, GDF-8, CCL28, GALNT10,

BCAN, TIMP4, CRISP2, CD207, WNT9A, MBL2, EN-RAGE, TWEAK, CR2, MFAP5, KIT, GH, PFKM, CDSN, CRH, GCP5, KLK6, DRAXIN, IL8, HAOX1, ENPP7, ACE2, SULT2A1, MCP-3, CES1, MFGE8, PLXNB1, TNFRSF10A, CCL15, SEMA4C, PREB, NFATC3, CCL19, DLL1, ENTPD2, IL-4RA, EPHA2, FOSB, CXCL10, VAMP5, ALDH3A1, MVK, IL12RB1, CALCA, AHCY, PRSS2, LILRB4, DDAH1, IL-1ra, NECTIN2, PDCD1, CD74, PD-L1, REG3A, CA5A, N2DL-2, CDCP1, U-PAR, SIGLEC7, ANGPTL4, ALDH1A1, SPINK1, HTRA2, PRDX6, IL-1RT2, IGFBP-1, HNMT, TRAIL-R2, CXADR, CTSL1, IFN-gamma-R1, IL-18R1, KRT19, KYNU, and TGM2 in the biological sample.

In some embodiments, the method includes measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of PON3, LEP, MBL2, GH, GDF-8, EN-RAGE, CRISP2, CR2, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, ALDH1A1, CES1, REG3A, KYNU, IL-4RA, CDCP1, MVK, FOSB, NFATC3, N2DL-2, DDAH1, IGFBP-1, ALDH3A1, CXADR, PLXNB1, CD74, ENTPD2, PREB, CCL19, HNMT, HTRA2, IL-1RT2, and IL-18R1 in the biological sample.

In some embodiments, the method includes measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 proteins) selected from the group consisting of PON3, LEP, MBL2, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, SPINK1, ENPP7, ACE2, CTSL1, PRSS2, CXCL10, MFGE8, KRT19, and ALDH1A1 in the biological sample.

In some embodiments, the method includes measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 proteins) selected from the

group consisting of PON3, LEP, MCP-3, HAOX1, CA5A, CALCA, IL8, SULT2A1, VAMP5, and SPINK1 in the biological sample.

In some embodiments of the methods described herein, the concentrations of no more than 50, 40, 30, 20, 15, 10, or 5 proteins are measured.

The disclosure also features a method of treating a human subject having, suspected of having, or at risk of developing GvHD, by: measuring, in a first biological sample obtained from the human subject prior to administering a therapy comprising a JAK inhibitor, the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1,

CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1, ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, MATN3, TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and SMOC2; administering the therapy comprising the JAK inhibitor to the human subject; and measuring, in a second biological sample obtained from the human subject after administering the

therapy comprising the JAK inhibitor, a reduced concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1, ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE,

ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, and MATN3, and/or an increased concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and SMOC2.

In some embodiments, the method includes: measuring, in the first biological sample obtained from the human subject prior to administering the therapy comprising the JAK inhibitor, the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY,

PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, and RELT; administering the therapy comprising the JAK inhibitor to the human subject; and measuring, in the second biological sample obtained from the human subject after administering the therapy comprising the JAK inhibitor, a reduced concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1,

LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, and RELT, and/or an increased concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, and GPNMB.

In some embodiments, the method includes: measuring, in the first biological sample obtained from the human subject prior to administering the therapy comprising the JAK inhibitor, the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, and HAVCR2; administering the therapy comprising the JAK inhibitor to the human subject; and measuring, in the second biological sample obtained from the human subject after administering the therapy comprising the JAK inhibitor, a reduced concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, and HAVCR2, and/or an increased concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 proteins) selected from the group

consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, and KIM1.

In some embodiments, the method includes: measuring, in the first biological sample obtained from the human subject prior to administering the therapy comprising the JAK inhibitor, the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, and CNTNAP2; administering the therapy comprising the JAK inhibitor to the human subject; and measuring, in the second biological sample obtained from the human subject after administering the therapy comprising the JAK inhibitor, a reduced concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, and CNTNAP2, and/or an increased concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, or 7 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, and CCL25.

In some embodiments, the method includes: measuring, in the first biological sample obtained from the human subject prior to administering the therapy comprising the JAK inhibitor, the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, INPPL1, LAT2, CLEC7A, PPP1R9B,

NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, and ITGB2; administering the therapy comprising the JAK inhibitor to the human subject; and measuring, in the second biological sample obtained from the human subject after administering the therapy comprising the JAK inhibitor, a reduced concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, and ITGB2, and/or an increased concentration, as compared to the first biological sample, of at least one protein (e.g., at least 1, 2, 3, 4, or 5 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, and NCAM1.

The disclosure also features a method of identifying a therapeutic response of a human subject having, suspected of having, or at risk of developing GvHD to a therapy comprising a JAK inhibitor, by: providing a first biological sample obtained from the human subject before administering the therapy comprising the JAK inhibitor; measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1,

VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1, ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, MATN3, TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1,

TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and SMOC2 in the first biological sample; providing a second biological sample obtained from the subject after administering the therapy comprising the JAK inhibitor; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1,

AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1, ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, MATN3, TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and SMOC2 in the second biological sample, wherein a reduced concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 19, or 20) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1, ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET,

GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, and/or MATN3, and/or an increased concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and/or SMOC2 indicates that the human subject has undergone a therapeutic response to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5,

SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, and RELT in the first biological sample; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5,

REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, and RELT in the second biological sample, wherein a reduced concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2,

PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, and RELT, and/or an increased concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, and GPNMB indicates that the human subject has undergone a therapeutic response to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B,

MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, and HAVCR2 in the first biological sample; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, KIM1, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, and HAVCR2 in the second biological sample, wherein a reduced concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9,

and HAVCR2, and/or an increased concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, hK14, and KIM1 indicates that the human subject has undergone a therapeutic response to the therapy comprising the JAK inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, and CNTNAP2 in the first biological sample; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, and CNTNAP2 in the second biological sample, wherein a reduced concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, and CNTNAP2, and/or an increased concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, or 7) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, and CCL25 indicates that the human subject has undergone a therapeutic response to the therapy comprising the JAK

inhibitor.

In some embodiments, the method includes: measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, and ITGB2 in the first biological sample; and measuring the concentration of at least one protein (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 proteins) selected from the group consisting of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, and ITGB2 in the second biological sample, wherein a reduced concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, and ITGB2, and/or an increased concentration in the second biological sample, as compared to the first biological sample, of at least one (e.g., at least 1, 2, 3, 4, or 5) of TMPRSS15, CCL11, FAM3B, MMP7, and NCAM1 indicates that the human subject has undergone a therapeutic response to the therapy comprising the JAK inhibitor.

The disclosure also features a method of treating a human subject having, suspected of having, or at risk of developing GvHD, by administering to the human subject a therapy comprising a JAK inhibitor, wherein the human subject has been previously determined to have (i) a baseline concentration of at least one protein selected from the group consisting of MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and DDAH1 in a biological sample obtained from the human subject that is lower than a control, and/or

(ii) a baseline concentration of at least one protein selected from the group consisting of PON3, SCF, GH, SRC, and CR2 in a biological sample obtained from the human subject that is higher than a control.

The disclosure also features a method of treating a human subject having, suspected of having, or at risk of developing GvHD by: providing a biological sample obtained from the human subject; measuring in the biological sample a reduced concentration, as compared to a control, of at least one protein selected from the group consisting of MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and DDAH1, and/or an increased concentration, as compared to a control, of at least one protein selected from the group consisting of PON3, SCF, GH, SRC, and CR2; and administering a therapy comprising a JAK inhibitor to the human subject.

The disclosure also features a method of predicting the response of a human subject having, suspected of having, or at risk of developing GvHD to a therapy comprising a JAK inhibitor by: providing a biological sample obtained from the subject before the therapy comprising the JAK inhibitor; and measuring the concentration of at least one protein selected from the group consisting of PON3, SCF, GH, SRC, CR2, MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and DDAH1 in the biological sample, wherein a reduced concentration, as compared to a control, of MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and/or DDAH1, and/or an increased concentration, as compared to a control, of PON3, SCF, GH, SRC, and/or CR2 is predictive that the subject will respond to the therapy comprising the JAK inhibitor.

In some embodiments of the methods described herein, the control is a pre-established cut-off value.

In some embodiments of the methods described herein, the control is the concentration of the protein in a sample or samples obtained from one or more subjects that have not responded to treatment with the JAK inhibitor.

The disclosure also features a method for measuring the amount of a protein in a sample by: providing a biological sample obtained from a human subject having, suspected of having, or at risk of developing GvHD; and measuring the concentration of at least one protein selected from the group consisting of PON3, SCF, GH, SRC, CR2, MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and DDAH1 in the biological sample.

In some embodiments of any of the methods described herein, the concentrations of no more than 20 proteins are measured.

In some embodiments of any of the methods described herein, the concentrations of no more than 10 proteins are measured.

In some embodiments of any of the methods described herein, the biological sample is blood, serum, plasma, urine, spinal fluid, saliva, lacrimal fluid, or sweat. In some embodiments, the biological sample is blood, serum, or plasma.

In some embodiments of any of the methods described herein, the concentration of the protein is measured by an immunological method. The immunological method can be, for example, an enzyme-linked immunosorbent assay, enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, or western blotting.

In some embodiments of any of the methods described herein, the concentration of the protein is measured by mass spectrometry.

In some embodiments of any of the methods described herein, the JAK inhibitor is itacitinib.

In some embodiments of any of the methods described herein, the JAK inhibitor is 4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide or a pharmaceutically acceptable salt thereof or ((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile or a pharmaceutically acceptable salt thereof.

In some embodiments of any of the methods described herein, a second therapeutic agent is administered to the human subject in combination with the JAK inhibitor. The second therapeutic agent can be, for example, a corticosteroid (e.g., methylprednisolone or prednisone), methotrexate, cyclosporine, mycophenolate mofetil, tacrolimus, sirolimus, everolimus, antithymocyte globulin, alemtuzumab, cyclophosphamide, ibrutinib, imatinib, infliximab, etanercept, tocilizumab, alemtuzumab, basiliximab, daclizumab, rituximab, denileukin diftitox, pentostatin, ciclosporin, thalidomide, halofuginone, hydroxychloroquine, or mesenchymal stem cells. The JAK inhibitor and the second therapeutic agent can be administered simultaneously or sequentially.

In some embodiments of any of the methods described herein, the GvHD is acute GvHD.

In some embodiments of any of the methods described herein, the GvHD is chronic GvHD.

The term "baseline concentration" of protein refers to the concentration of a protein in a subject prior to initiation of treatment with a JAK inhibitor.

The term "reduced concentration" means a concentration of the protein being analyzed that is lower than the concentration of that protein in a control or in a previous sample. For example, the concentration of the protein being analyzed can be at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 25, 50, 75, or 100 times lower, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1,000%, 1,500%, 2,000%, 2,500%, 3,000%, 3,500%, 4,000%, 4,500%, or 5,000% lower, than the concentration of that protein in a control.

The term "increased concentration" means a concentration of the protein being analyzed that is higher than the concentration of that protein in a control or in a previous sample. For example, the concentration of the protein being analyzed can be at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 25, 50, 75, or 100 times higher, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1,000%, 1,500%, 2,000%, 2,500%, 3,000%, 3,500%, 4,000%, 4,500%, or 5,000% higher, than the concentration of that protein in a control.

The term "respond to a therapy" means that the subject administered with the therapy shows a positive response to the JAK inhibitor therapy provided.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the exemplary methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by

reference in their entirety. In case of conflict, the present application, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DETAILED DESCRIPTION

This disclosure provides methods and compositions for treating a subject having, suspected of having, or at risk of developing GvHD with a JAK inhibitor. The disclosure provides predictive biomarkers (e.g., protein expression levels) to identify those subjects having, suspected of having, or at risk of developing GvHD for whom administering a therapy comprising a JAK inhibitor is likely to be effective.

Graft Versus Host Disease

GvHD occurs when donor T cells respond to genetically defined proteins (including but not limited to Human Leukocyte Antigens) on host cells. Acute GvHD is generally defined to occur prior to day 100 post-transplant, whereas chronic GvHD occurs after that time.

The clinical manifestations of acute GvHD occur in the skin, gastrointestinal tract, and liver. Skin is the most commonly affected organ in acute GvHD and is usually the first organ involved, often coinciding with engraftment of donor cells. The characteristic maculopapular rash is pruritic and can spread throughout the body. In severe cases, the skin may blister and ulcerate. Other features include dyskeratosis,

exocytosis of lymphocytes, satellite lymphocytes adjacent to dyskeratotic epidermal keratinocytes, and a perivascular lymphocytic infiltration in the dermis. Gastrointestinal tract involvement of acute GvHD usually presents as diarrhea but may also include vomiting, anorexia, and/or abdominal pain. The histologic features of liver disease caused by GvHD are endothelialitis, lymphocytic infiltration of the portal areas, pericholangitis, and bile duct destruction.

Chronic GvHD is the major cause of late non-relapse death following hematopoietic cell transplant. Its presentation may be progressive (e.g., acute GvHD merging into chronic GvHD), quiescent (acute GvHD that resolves completely but is later followed by chronic GvHD), or it may occur *de novo*. Older recipient age and a history of acute GvHD are the greatest risk factors for chronic GvHD. Clinical signs of chronic GvHD often first appear in the buccal mucosa.

Methods of Predicting Responsiveness to a Therapy Comprising a JAK Inhibitor

Several proteins have been identified in the Examples whose expression levels are useful in predicting responsiveness (e.g., improvement in disease scores and/or disease resolution) of a subject having GvHD to a therapy comprising a JAK inhibitor. These proteins are listed in Tables 1 and 2.

Table 1: Biomarkers Exhibiting Reduced Expression in GvHD Subjects that Respond to Treatment with a JAK inhibitor as Compared to Control Subjects that do not Respond

Protein
IL8
HAOX1
ENPP7
ACE2
SULT2A1
MCP-3
CES1
MFGE8
PLXNB1
TNFRSF10A
CCL15
TNFRSF10A
SEMA4C
PREB
NFATC3
CCL19
DLL1
ENTPD2
IL-4RA
EPHA2
FOSB
CXCL10
VAMP5
ALDH3A1
MVK
IL12RB1
CALCA
AHCY
PRSS2
LILRB4
DDAH1
IL-1ra
NECTIN2
PDCD1
CD74
PD-L1
REG3A
CA5A
N2DL-2
CDCP1
U-PAR

Protein
SIGLEC7
ANGPTL4
ALDH1A1
SPINK1
HTRA2
PRDX6
IL-1RT2
IGFBP-1
HNMT
TRAIL-R2
CXADR
CTSL1
IFN-gamma-R1
IL-18R1
KRT19
KYNU
TGM2

Table 2: Biomarkers Exhibiting Increased Expression in GvHD Subjects that Respond to Treatment with a JAK inhibitor as Compared to Control Subjects that do not Respond

Protein
PON3
CNTN1
IGFBP3
LEP
Notch 3
TN-R
HSD11B1
FAM19A5
NCAN
F11
GDF-8
CCL28
GALNT10
BCAN
TIMP4
CRISP2
CD207
WNT9A
MBL2
EN-RAGE
TWEAK
CR2
MFAP5
KIT
GH
PFKM
CDSN
CRH
GCP5
KLK6
DRAXIN

A reduced protein concentration compared to a control of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14) proteins listed in Table 1 is indicative/predictive that a subject that has, is suspected of having, or is at risk of

developing GvHD will respond to a therapy comprising a JAK inhibitor. For example, low concentrations (compared to a control) of CXCL10 protein in a biological sample obtained from a subject prior to treatment with the therapy comprising a JAK inhibitor are predictive that the subject will respond to the therapy comprising a JAK inhibitor.

An increased protein concentration compared to a control of one or more (e.g., at least 1, 2, 3, 4, or 5) proteins listed in Table 2 is indicative/predictive that a subject that has, is suspected of having, or is at risk of developing GvHD will respond to a therapy comprising a JAK inhibitor. For example, increased concentrations (compared to a control) of PON3 protein in a biological sample obtained from a subject prior to treatment with the therapy comprising a JAK inhibitor are predictive that the subject will respond to the therapy comprising a JAK inhibitor.

A reduced protein concentration compared to a control of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14) proteins listed in Table 1 combined with an increased protein concentration compared to a control of one or more (e.g., at least 1, 2, 3, 4, or 5) proteins listed in Table 2 is indicative/predictive that a subject that has, is suspected of having, or is at risk of developing GvHD will respond to a therapy comprising a JAK inhibitor. For example, low concentrations (compared to a control) of CXCL10 protein and increased concentrations (compared to a control) of PON3 protein in a biological sample obtained from a subject prior to treatment with the therapy comprising a JAK inhibitor are predictive that the subject will respond to the therapy comprising a JAK inhibitor. In another example, low concentrations (compared to a control) of MCP-3, CA5A, IL8, CXCL10, IL6, CCL19, CTSL1, ACE2, ALDH1A1, TNFRSF6B, KYNU, FOSB, ALDH3A1, and DDAH1 proteins and increased concentrations (compared to a control) of PON3, SCF, GH, SRC, and CR2 proteins in a

biological sample obtained from a subject prior to treatment with the therapy comprising a JAK inhibitor are predictive that the subject will respond to the therapy comprising a JAK inhibitor.

In some embodiments, the GvHD is acute GvHD. In other embodiments, the GvHD is chronic GvHD.

Controls

As described above, the methods of the present invention can involve, measuring the concentration of one or more proteins (e.g., one or more proteins depicted in Table 1 and/or Table 2) in a biological sample from a subject having, suspected of having or at risk of developing GvHD, wherein the concentration of one or more proteins, compared to a control, predicts the response of a subject to treatment comprising a JAK inhibitor. In certain embodiments, when the concentration of a protein in Table 1 in a biological sample from a subject having, suspected of having or at risk of developing GvHD is lower than the control, the subject is identified as likely to respond to a therapy comprising a JAK inhibitor. In other embodiments, when the concentration of a protein in Table 2 in a biological sample from a subject having, suspected of having or at risk of developing GvHD is higher than the control, the subject is identified as likely to respond to a therapy comprising a JAK inhibitor. In this context, the term "control" includes a sample (from the same tissue type) obtained from a subject who is known to not respond to a therapy comprising a JAK inhibitor. The term "control" also includes a sample (from the same tissue type) obtained in the past from a subject who is known to not respond to a therapy comprising a JAK inhibitor and used as a reference for future comparisons to test samples taken from subjects for

which therapeutic responsiveness is to be predicted. The "control" expression level/concentration for a particular protein in a particular cell type or tissue may be pre-established by an analysis of protein expression in one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 or more) subjects, of the same species, that have not responded to treatment with a JAK inhibitor. This pre-established reference value (which may be an average or median expression level/concentration taken from multiple subjects that have not responded to the therapy) may then be used for the "control" concentration / expression level of the protein in the comparison with the test sample. In such a comparison, the subject is predicted to respond to a therapy comprising a JAK inhibitor if the expression level of the protein being analyzed is lower (Table 1) or higher (Table 2) than the pre-established reference.

The "control" concentration for a particular protein in a particular cell type or tissue may alternatively be pre-established by an analysis of protein expression in one or more subjects that have responded to treatment with a JAK inhibitor. This pre-established reference value (which may be an average or median expression level taken from multiple subjects that have responded to the therapy) may then be used as the "control" expression level in the comparison with the test sample. In such a comparison, the subject is predicted to respond to a therapy comprising a JAK inhibitor if the concentration of the protein being analyzed is the same as, or comparable to (e.g., at least 85% but less than 100% of), the pre-established reference.

In certain embodiments, the "control" is a pre-established cut-off value. A cut-off value is typically a concentration of a protein above or below which is considered predictive of responsiveness of a subject to a therapy of interest. Thus, in accordance with the methods and compositions described herein, a reference protein concentration

(e.g., of a protein of Table 1 or Table 2) is identified as a cut-off value, above or below of which is predictive of responsiveness to a therapy comprising a JAK inhibitor. Cut-off values determined for use in the methods described herein can be compared with, e.g., published ranges of concentrations but can be individualized to the methodology used and patient population.

In some embodiments, the concentration of the protein being analyzed is reduced as compared to the concentration of that protein in a control. For example, the concentration of the protein being analyzed can be at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 25, 50, 75, or 100 times lower, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1,000%, 1,500%, 2,000%, 2,500%, 3,000%, 3,500%, 4,000%, 4,500%, or 5,000% lower, than the concentration of that protein in a control.

In some embodiments, the concentration of the protein being analyzed is increased as compared to the concentration of that protein in a control. For example, the concentration of the protein being analyzed can be at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 25, 50, 75, or 100 times higher, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1,000%, 1,500%, 2,000%, 2,500%, 3,000%, 3,500%, 4,000%, 4,500%, or 5,000% higher, than the concentration of that protein in a control.

Methods of Identifying Therapeutic Responsiveness to a Therapy Comprising a JAK Inhibitor

Several proteins have been identified in the Examples whose expression levels, in subjects who respond to treatment with a JAK inhibitor, change during the course of

treatment and are therefore useful in identifying therapeutic responsiveness (e.g., improvement in disease scores and/or disease resolution) of a subject having GvHD to a therapy comprising a JAK inhibitor. These proteins are identified in Table 13.

A reduced protein concentration in a biological sample obtained from a subject after treatment with a JAK inhibitor, as compared to the baseline expression level in a biological sample obtained from the subject before treatment with a JAK inhibitor, of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1,

ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, and/or MATN3 is indicative that the subject has undergone a therapeutic response to the JAK inhibitor.

An increased protein concentration in a biological sample obtained from a subject after treatment with a JAK inhibitor, as compared to the baseline expression level in a biological sample obtained from the subject before treatment with a JAK inhibitor, of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, CCL11, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10,

FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR, PROC, FURIN, PIgR, and/or SMOC2 is indicative that the subject has undergone a therapeutic response to the JAK inhibitor.

A reduced protein concentration in a biological sample obtained from a subject after treatment with a JAK inhibitor, as compared to the baseline expression level in a biological sample obtained from the subject before treatment with a JAK inhibitor, of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of INPPL1, LAT2, CLEC7A, PPP1R9B, NEMO, SH2B3, BCR, CD5, DNAJB1, CCL17, ITGB2, BANK1, TPSAB1, YES1, LAMP3, GM-CSF-R-alpha, CNTNAP2, ZBTB16, CD163, TXLNA, MEPE, BACH1, MAX, NFKBIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIM5, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, HAVCR2, CD84, STX8, LY9, ZBTB16, CD200R1, TOP2B, THY 1, PRKRA, ITGB1BP2, CD48, CD244, HCLS1, MPO, SIT1, ICAM3, SOST, DDX58, TNF-R2, TRAF2, SMAD1, LAIR-2, PIK3AP1, VSIG4, SIGLEC10, CD6, SKAP1, FCRL5, CD177, KLRD1, ERBB2IP, MILR1, MIF, SNAP23, NUB1, TIGAR, STAMPB, DSC2, LAIR1, FKBP1B, RASSF2, FATC1, CBL, IgG Fc receptor II-b, GLO1, PVALB, SCAMP3, SLAMF8, STX16, TNF-R1, DFFA, PPP1R2, ANG-1, CCL5, MAP2K6, CRKL, CD38, CXCL5, PILRA, IRAK1, CA13, STX6, PRTN3, IL-5R-alpha, ESM-1, EGLN1, CLEC1B, TYMP, SNAP29, PDGF subunit A, TNFRSF11A, gal-8, GCNT1, STK4, TNC, THBS4, CLEC4D, SIGLEC6, WASF1, WAS, COMT, RETN, SH2D1A, RNASE3, PAR-1, CD69, SIGLEC1, FR-gamma, ADAM 8, AZU1, AREG, SDC4, DCTN2, BID, RELT, CLEC5A, APEX1, PSP-D, FGR, SELE, SELL, MESDC2, IQGAP2, CRTAM, LILRB2, TANK, CPXM1,

ARSB, SLAMF1, PEBP1, STIP1, PDGF subunit B, SCARF1, DEFA1, EPHB4, ARHGAP1, CLM-1, DAB2, LYN, CASP-8, APBB1IP, ANXA11, ICAM1, PRKCQ, VCAM1, HDGF, CD2AP, TNFRSF6B, CLEC1A, TNFRSF14, TACC3, MMP-1, NRP1, ZBTB17, NADK, PLXNA4, MMP-9, NCR1, AMIGO2, FES, CD79B, TNXB, TXNDC5, TRANCE, ARG1, PCDH17, LRMP, C1QTNF1, CLM-6, CKAP4, APP, PGLYRP1, LILRA5, CLEC10A, NMNAT1, IL-6RA, ATG4A, TIMP1, COCH, DKN1A, CD1C, DECR1, DAG1, IGFBP-2, RET, GSAP, PILRB, CLEC6A, PECAM-1, PXN, ADGRG1, DPP7, TDRKH, Siglec-9, CD40-L, VEGFC, LYVE1, FADD, FCRL1, EGF, HGF, GZMH, CLEC4G, LY75, PRDX3, COL4A1, CEACAM8, SEMA7A, NUDT5, FCRL6, PAPPA, FASLG, GRN, and/or MATN3 combined with an increased protein concentration in a biological sample obtained from the subject after treatment with a JAK inhibitor, as compared to the baseline expression level in a biological sample obtained from the subject before treatment with a JAK inhibitor, of one or more (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) of TMPRSS15, CCL11, FAM3B, MMP7, NCAM1, Gal-3, CCL25, THPO, CCL11, hK14, KIM1, Flt3L, PLIN1, SPON2, Gal-4, FABP4, DNER, GAL, CPM, VWC2, PPY, PAM, PVR, SERPINA5, ST3GAL1, CST5, CES2, CNDP1, CX3CL1, HO-1, PRELP, ADM, VSIG2, FABP2, CEACAM5, SLITRK2, MCP-1, NTRK3, CLUL1, CXCL16, SCF, TMPRSS5, REG4, hK11, SCGB3A1, DKKL1, NEP, CPA2, Ep-CAM, THBS2, GPNMB, ITGB5, GT, APLP1, TACSTD2, NINJ1, REN, GCG, SERPINA9, KAZALD1, SERPINA12, PODXL, AMN, IGF1R, LTBP2, ANGPTL3, SCARA5, B4GAT1, ROBO2, PDGFC, CA12, DDC, EDIL3, XPNPEP2, PRTG, NQO2, AMBP, ERBB2, IL6, MCP-1, VEGFD, GDF-2, MUC-16, KLK10, FAM3C, uPA, AGR2, METRNL, RTN4R, IGF2R, NTRK2, ITGB6, SCARF2, SCGB3A2, RGMB, EZR,

PROC, FURIN, PIgR, and/or SMOC2 is indicative that the subject has undergone a therapeutic response to the JAK inhibitor.

In some embodiments, the GvHD is acute GvHD. In other embodiments, the GvHD is chronic GvHD.

Biological Samples

Suitable biological samples for the methods described herein include any biological fluid, cell, tissue, or fraction thereof, which includes proteins of interest. A biological sample can be, for example, a specimen obtained from a human subject or can be derived from such a subject. For example, a biological sample can be a biological fluid such as blood, serum, plasma, urine, spinal fluid, saliva, lacrimal fluid, or sweat, or such a sample absorbed onto a substrate (e.g., glass, polymer, or paper).

A biological sample can be obtained from a subject having, suspected of having, or at risk of developing, GvHD. In certain embodiments, the subject has acute GvHD. In some embodiments, the subject has chronic GvHD.

Methods for obtaining and/or storing samples that preserve the activity or integrity of molecules (e.g., proteins) in the sample are well known to those skilled in the art. For example, a biological sample can be further contacted with one or more additional agents such as buffers and/or inhibitors, including one or more of nuclease, protease, and phosphatase inhibitors, which preserve or minimize changes in the molecules in the sample.

Determining Expression Levels/Concentrations of Biomarkers

The presence or expression level (amount) of a gene can be determined by detecting and/or measuring the level of protein expression of the gene.

In one embodiment, the expression of a gene can be determined by detecting and/or measuring expression or concentration of a protein encoded by the gene. Methods of determining protein expression/concentration are well known in the art. A generally used method involves the use of antibodies specific for the target protein of interest. For example, methods of determining protein expression include, but are not limited to, western blot or dot blot analysis, immunohistochemistry (e.g., quantitative immunohistochemistry), immunocytochemistry, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunosorbent spot (ELISPOT; Coligan, J. E., et al., eds. (1995) Current Protocols in Immunology. Wiley, New York), radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immuno-chromatographic assay, and antibody array analysis (see, e.g., U.S. Publication Nos. 20030013208 and 2004171068, the disclosures of each of which are incorporated herein by reference in their entirety).

In one example, the presence or amount of protein expression of a gene (e.g., a gene depicted in Table 1, Table 2 , or Table 13) can be determined using a western blotting technique. For example, a lysate can be prepared from a biological sample, or the biological sample itself, can be contacted with Laemmli buffer and subjected to sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE-resolved proteins, separated by size, can then be transferred to a filter membrane (e.g., nitrocellulose) and subjected to immunoblotting techniques using a detectably-labeled

antibody specific to the protein of interest. The presence or amount of bound detectably-labeled antibody indicates the presence or amount of protein in the biological sample.

In another example, an immunoassay can be used for detecting and/or measuring the protein expression of a gene (e.g., a gene depicted in Table 1, Table 2 , or Table 13). As above, for the purposes of detection, an immunoassay can be performed with an antibody that bears a detection moiety (e.g., a fluorescent agent or enzyme). Proteins from a biological sample can be conjugated directly to a solid-phase matrix (e.g., a multi-well assay plate, nitrocellulose, agarose, sepharose, encoded particles, or magnetic beads) or it can be conjugated to a first member of a specific binding pair (e.g., biotin or streptavidin) that attaches to a solid-phase matrix upon binding to a second member of the specific binding pair (e.g., streptavidin or biotin). Such attachment to a solid-phase matrix allows the proteins to be purified away from other interfering or irrelevant components of the biological sample prior to contact with the detection antibody and also allows for subsequent washing of unbound antibody. Here as above, the presence or amount of bound detectably-labeled antibody indicates the presence or amount of protein in the biological sample.

There is no particular restriction as to the form of the antibody and the present disclosure includes polyclonal antibodies, as well as monoclonal antibodies. The antiserum obtained by immunizing animals, such as rabbits with a protein or fragment thereof (i.e., a protein or an immunological fragment thereof from Table 1, Table 2 , or Table 13) , as well polyclonal and monoclonal antibodies of all classes, human antibodies, and humanized antibodies produced by genetic recombination, are also included. Antibodies or antibody fragments specific for a protein encoded by one or

more biomarkers can also be generated by *in vitro* methods such as phage display. Moreover, the antibody may be an antibody fragment or modified-antibody, so long as it binds to a protein encoded by a biomarker of the invention. For instance, Fab, F (ab') 2, Fv, or single chain Fv (scFv) in which the H chain Fv and the L chain Fv are suitably linked by a linker (Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883, (1988)) can be given as antibody fragments.

The antibodies may be conjugated to various molecules, such as fluorescent substances, radioactive substances, and luminescent substances. Methods to attach such moieties to an antibody are already established and conventional in the field (see, e.g., US 5,057,313 and 5,156,840).

Examples of methods that assay the antigen-binding activity of the antibodies include, for example, measurement of absorbance, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), and/or immunofluorescence. For example, when using ELISA, a protein encoded by a biomarker of the invention is added to a plate coated with the antibodies of the present disclosure, and then, the antibody sample, for example, culture supernatants of antibody-producing cells, or purified antibodies are added. Then, secondary antibody recognizing the primary antibody, which is labeled by alkaline phosphatase and such enzymes, is added, the plate is incubated and washed, and the absorbance is measured to evaluate the antigen-binding activity after adding an enzyme substrate such as p-nitrophenyl phosphate. As the protein, a protein fragment, for example, a fragment comprising a C-terminus, or a fragment comprising an N-terminus may be used. To evaluate the activity of the antibody of the invention, BIAcore (GE Healthcare) may be used.

By using these methods, the antibody and a sample presumed to contain a protein of interest are contacted, and the protein encoded by a biomarker of the invention is detected or assayed by detecting or assaying the immune complex formed between the above-mentioned antibody and the protein.

Mass spectrometry based quantitation assay methods, for example, but not limited to, multiple reaction monitoring (MRM)-based approaches in combination with stable-isotope labeled internal standards, are an alternative to immunoassays for quantitative measurement of proteins. These approaches do not require the use of antibodies (see, for example, Addona et al., *Nat. Biotechnol.*, 27:633–641, 2009; Kuzyk et al., *Mol. Cell Proteomics*, 8:1860–1877, 2009; Paulovich et al., *Proteomics Clin. Appl.*, 2:1386-1402, 2008). In addition, MRM offers superior multiplexing capabilities, allowing for the simultaneous quantification of numerous proteins in parallel. The basic theory of these methods has been well-established and widely utilized for drug metabolism and pharmacokinetics analysis of small molecules.

In some embodiments, the concentration of two proteins, three proteins, four proteins, five proteins, six proteins, seven proteins, eight proteins, nine proteins, 10 proteins, 11 proteins, 12 proteins, 13 proteins, or 14 proteins, or at least two proteins, at least three proteins, at least four proteins, at least five proteins, at least six proteins, at least seven proteins, at least eight proteins, at least nine proteins, at least 10 proteins, at least 11 proteins, at least 12 proteins, at least 13 proteins, or at least 14 proteins from Table 1 can be assessed and/or measured.

In some embodiments, the concentration of two proteins, three proteins, four proteins, or five proteins, or at least two proteins, at least three proteins, at least four proteins, or at least five proteins from Table 2 can be assessed and/or measured.

In some embodiments, the concentration of two proteins, three proteins, four proteins, five proteins, six proteins, seven proteins, eight proteins, nine proteins, 10 proteins, 11 proteins, 12 proteins, 13 proteins, 14 proteins, 15 proteins, 16 proteins, 17 proteins, 18 proteins, 19 proteins, or 20 proteins, or at least two proteins, at least three proteins, at least four proteins, at least five proteins, at least six proteins, at least seven proteins, at least eight proteins, at least nine proteins, at least 10 proteins, at least 11 proteins, at least 12 proteins, at least 13 proteins, at least 14 proteins, at least 15 proteins, at least 16 proteins, at least 17 proteins, at least 18 proteins, at least 19 proteins, or at least 20 proteins from Table 13 can be assessed and/or measured.

In some embodiments of the methods described herein, the method includes measuring a concentration of MCP-3 that is below 15 pg/ml, below 10 pg/ml, below 9 pg/ml, below 8 pg/ml, below 7 pg/ml, below 6 pg/ml, below 5 pg/ml, below 4 pg/ml, or below 3 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of Reg3A that is below 45,000 pg/ml, below 40,000 pg/ml, below 35,000 pg/ml, below 30,000 pg/ml, below 25,000 pg/ml, below 20,000 pg/ml, below 15,000 pg/ml, or below 10,000 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of TNFRSF6B that is below 400 pg/ml, below 350 pg/ml, below 300 pg/ml, below 250 pg/ml, or below 200 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of SCF that is above 350 pg/ml, above 400 pg/ml, above 450 pg/ml, above 500 pg/ml, above 600 pg/ml, or above 650 pg/ml.

In some embodiments of the methods described herein, the method includes

measuring a concentration of CXCL10 that is below 900 pg/ml, below 800 pg/ml, below 700 pg/ml, below 600 pg/ml, below 500 pg/ml, or below 400 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of IL-8 that is below 40 pg/ml, below 35 pg/ml, below 30 pg/ml, below 25 pg/ml, below 20 pg/ml, below 15 pg/ml, or below 10 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of ST2 that is below 140,000 pg/ml, below 130,000 pg/ml, below 120,000 pg/ml, below 110,000 pg/ml, below 100,000 pg/ml, below 90,000 pg/ml, below 80,000 pg/ml, or below 70,000 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of CALCA that is below 3,000 pg/ml, below 2,900 pg/ml, below 2,800 pg/ml, below 2,700 pg/ml, below 2,600 pg/ml, below 2,500 pg/ml, below 2,400 pg/ml, below 2,300 pg/ml, below 2,200 pg/ml, below 2,100 pg/ml, or below 2,000 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of TNF-R1 that is below 12,000 pg/ml, below 11,500 pg/ml, below 11,000 pg/ml, below 10,500 pg/ml, below 10,000 pg/ml, or below 9,500 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of IL-6 that is below 3.5 pg/ml, below 3 pg/ml, below 2.5 pg/ml, below 2 pg/ml, or below 1.5 pg/ml.

In some embodiments of the methods described herein, the method includes measuring a concentration of CCL19 that is below 1,000 pg/ml, below 900 pg/ml, below 800 pg/ml, below 700 pg/ml, below 600 pg/ml, or below 500 pg/ml.

In some embodiments of the methods described herein, the method includes

measuring a concentration of PON3 that is above 150,000 pg/ml, above 200,000 pg/ml, above 250,000 pg/ml, above 300,000 pg/ml, above 350,000 pg/ml, or above 400,000 pg/ml.

JAK inhibitors

In some embodiments, the JAK inhibitor is a compound that inhibits JAK1, JAK2, JAK3, and/or TYK2. In some embodiments, the JAK inhibitor is selective for JAK1 and JAK2 over JAK3 and TYK2. In some embodiments, the JAK inhibitor is selective for JAK1 over JAK2, JAK3, and TYK2. For example, some of the compounds described herein, or a pharmaceutically acceptable salt thereof, preferentially inhibit JAK1 over one or more of JAK2, JAK3, and TYK2. In some embodiments, the compounds or salts inhibit JAK1 preferentially over JAK2 (e.g., have a JAK2/JAK1 IC_{50} ratio >1). In some embodiments, the compounds or salts are about 10-fold more selective for JAK1 over JAK2. In some embodiments, the compounds or salts are about 3-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold more selective for JAK1 over JAK2 as calculated by measuring IC_{50} at 1 mM ATP.

In some embodiments, the JAK inhibitor is 3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile.

In some embodiments, the JAK inhibitor is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile (ruxolitinib; also known as INCB018424).

3-Cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile and ruxolitinib can be made by the procedure described in US 7,598,257 (Example 67), filed December 12, 2006, which is incorporated herein by

reference in its entirety.

In some embodiments, the JAK inhibitor is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile phosphoric acid salt.

In some embodiments, the JAK inhibitor is baricitinib, tofacitinib, oclacitinib, filgotinib, gandotinib, lestaurtinib, momelotinib, bacritinib, PF-04965842, upadacitinib, peficitinib, fedratinib, cucurbitacin I, ATI-501 (Aclaris), ATI-502 (Aclaris), JTE052 (Leo Pharma and Japan Tobacco), or CHZ868.

In some embodiments, the JAK inhibitor can be an isotopically-labeled compound, or a pharmaceutically acceptable salt thereof. An "isotopically" or "radio-labeled" compound is a compound of the disclosure where one or more atoms are replaced or substituted by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature (i.e., naturally occurring). Suitable radionuclides that may be incorporated in compounds of the present disclosure include but are not limited to ^{2}H (also written as D for deuterium), ^{3}H (also written as T for tritium), ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{18}F, ^{35}S, ^{36}Cl, ^{82}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{123}I, ^{124}I, ^{125}I and ^{131}I. For example, one or more hydrogen atoms in a compound of the present disclosure can be replaced by deuterium atoms (e.g., one or more hydrogen atoms of a C_{1-6} alkyl group of Formula (I) can be optionally substituted with deuterium atoms, such as $-CD_3$ being substituted for $-CH_3$).

One or more constituent atoms of the compounds described herein can be replaced or substituted with isotopes of the atoms in natural or non-natural abundance. In some embodiments, the compound includes at least one deuterium atom. In some embodiments, the compound includes two or more deuterium atoms. In some embodiments, the compound includes 1-2, 1-3, 1-4, 1-5, or 1-6 deuterium atoms. In

some embodiments, all of the hydrogen atoms in a compound can be replaced or substituted by deuterium atoms.

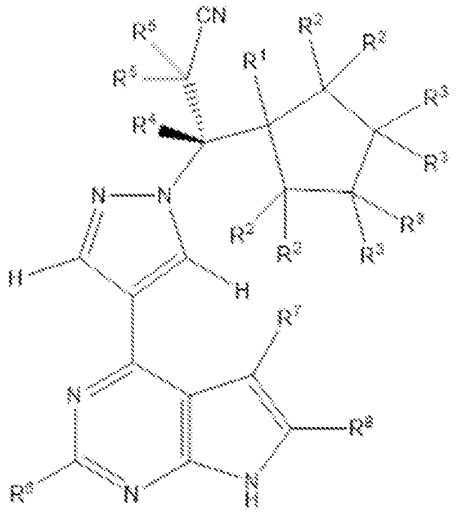
Synthetic methods for including isotopes into organic compounds are known in the art (Deuterium Labeling in Organic Chemistry by Alan F. Thomas (New York, N.Y., Appleton-Century-Crofts, 1971; The Renaissance of H/D Exchange by Jens Atzrodt, Volker Derdau, Thorsten Fey and Jochen Zimmermann, Angew. Chem. Int. Ed. 2007, 7744-7765; The Organic Chemistry of Isotopic Labelling by James R. Hanson, Royal Society of Chemistry, 2011). Isotopically labeled compounds can be used in various studies such as NMR spectroscopy, metabolism experiments, and/or assays.

Substitution with heavier isotopes, such as deuterium, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances. (see *e.g.*, A. Kerekes et. al. J. Med. Chem. 2011, 54, 201-210; R. Xu et. al. J. Label Compd. Radiopharm. 2015, 58, 308-312). In particular, substitution at one or more metabolism sites may afford one or more of the therapeutic advantages.

Accordingly, in some embodiments, the JAK inhibitor is a compound, wherein one or more hydrogen atoms in the compound are replaced by deuterium atoms, or a pharmaceutically acceptable salt thereof.

In some embodiments, the JAK inhibitor is ruxolitinib, wherein one or more hydrogen atoms are replaced by deuterium atoms, or a pharmaceutically acceptable salt thereof. In some embodiments, the JAK inhibitor is any of the compounds in US Patent 9,249,149 (which is incorporated herein by reference in its entirety), or a pharmaceutically acceptable salt thereof. In some embodiments, the JAK inhibitor is CTP-543, or a pharmaceutically acceptable salt thereof. In some embodiments, the

compound is a compound of Formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is selected from H and D;

each R^2 is independently selected from H and D, provided that each R^2 attached to a common carbon is the same;

each R^3 is independently selected from H and D, provided that each R^3 attached to a common carbon is the same;

R^4 is selected from H and D;

each R^5 is the same and is selected from H and D; and

R^6, R^7, and R^8 are each independently selected from H and D; provided that when R^1 is H, each R^2 and each R^3 are H, R^4 is H, and each of R^6, R^7, and R^8 is H, then each R^5 is D.

In some embodiments, the JAK inhibitor is a compound of Formula I selected from the following compounds 100-130 in the table below (wherein R^6, R^7, and R^8 are each H), or a pharmaceutically acceptable salt thereof. In some embodiments, the JAK

inhibitor is a compound of Formula I selected from the following compounds 200-231 in the table below (wherein R^6, R^7, and R^8 are each D), or a pharmaceutically acceptable salt thereof.

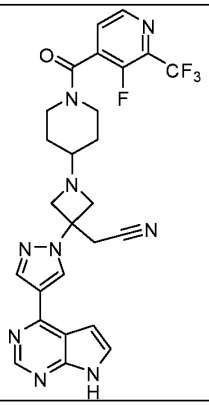
Compound	R^1	Each R^2	Each R^3	R^4	Each R^5
100	H	H	H	D	H
101	H	H	H	H	D
102	H	H	H	D	D
103	H	H	D	H	H
104	H	H	D	D	H
105	H	H	D	H	D
106	H	H	D	D	D
107	H	D	H	H	H
108	H	D	H	D	H
109	H	D	H	H	D
110	H	D	H	D	D
111	H	D	D	H	H
112	H	D	D	D	H
113	H	D	D	H	D
114	H	D	D	D	D
115	D	H	H	H	H
116	D	H	H	D	H
117	D	H	H	H	D
118	D	H	H	D	D
119	D	H	D	H	H
120	D	H	D	D	H
121	D	H	D	H	D
122	D	H	D	D	D
123	D	D	H	H	H
124	D	D	H	D	H
125	D	D	H	H	D
126	D	D	H	D	D
127	D	D	D	H	H
128	D	D	D	D	H
129	D	D	D	H	D
130	D	D	D	D	D

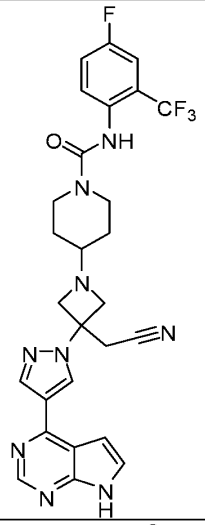
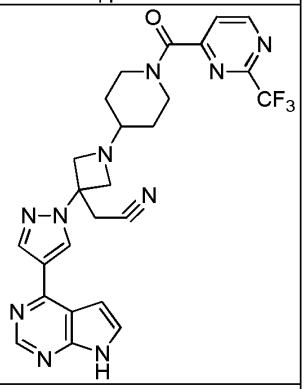
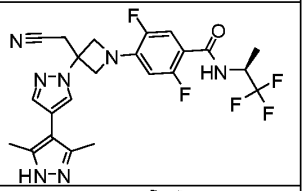
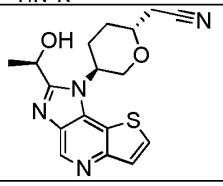
200	H	H	H	D	H
201	H	H	H	H	D
202	H	H	H	D	D
203	H	H	D	H	H
204	H	H	D	D	H
205	H	H	D	H	D
206	H	H	D	D	D
207	H	D	H	H	H
208	H	D	H	D	H
209	H	D	H	H	D
210	H	D	H	D	D
211	H	D	D	H	H
212	H	D	D	D	H
213	H	D	D	H	D
214	H	D	D	D	D
215	D	H	H	H	H
216	D	H	H	D	H
217	D	H	H	H	D
218	D	H	H	D	D
219	D	H	D	H	H
220	D	H	D	D	H
221	D	H	D	H	D
222	D	H	D	D	D
223	D	D	H	H	H
224	D	D	H	D	H
225	D	D	H	H	D
226	D	D	H	D	D
227	D	D	D	H	H
228	D	D	D	D	H
229	D	D	D	H	D
230	D	D	D	D	D
231	H	H	H	H	H

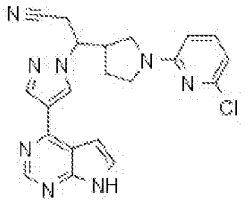
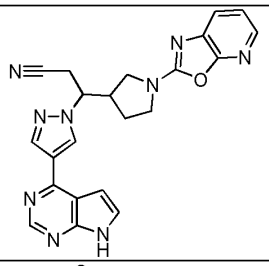
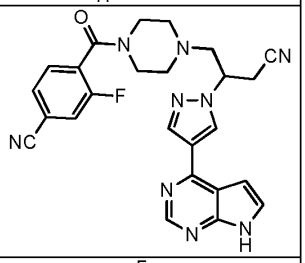
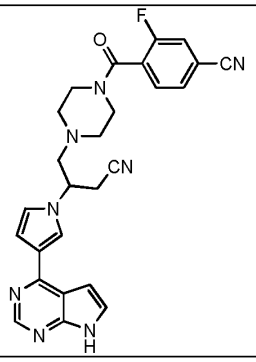
In some embodiments, the JAK inhibitor is baricitinib, wherein one or more hydrogen atoms are replaced by deuterium atoms, or a pharmaceutically acceptable salt thereof. In some embodiments, the JAK inhibitor is any of the compounds in US Patent 9,540,367 (which is incorporated herein by reference in its entirety), or a pharmaceutically acceptable salt thereof.

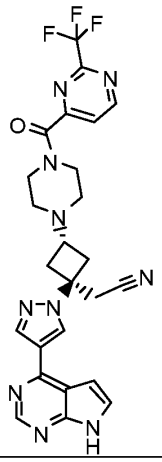
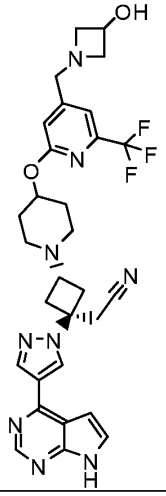
In some embodiments, the JAK inhibitor is a compound of Table 3, or a pharmaceutically acceptable salt thereof. The compounds in Table 3 are selective JAK1 inhibitors (selective over JAK2, JAK3, and TYK2).

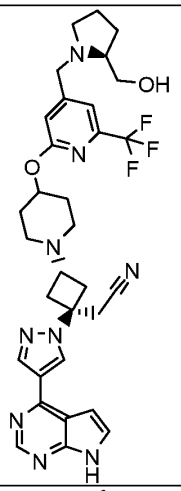
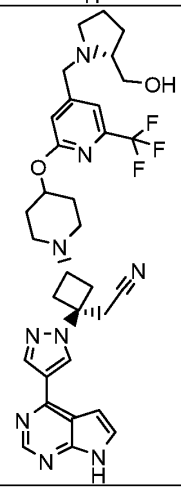
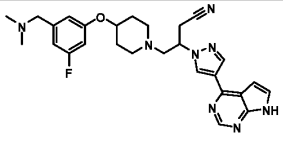
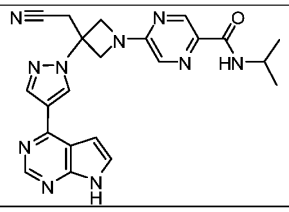
Table 3: Examples of JAK inhibitors

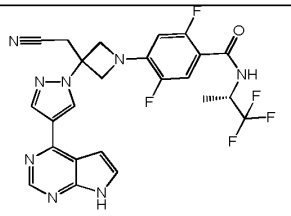
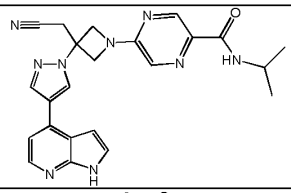
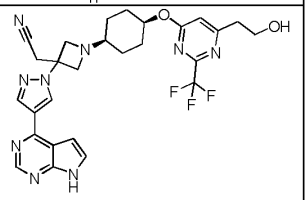
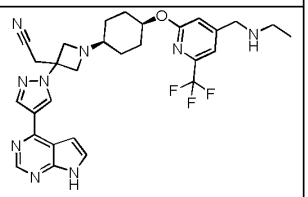
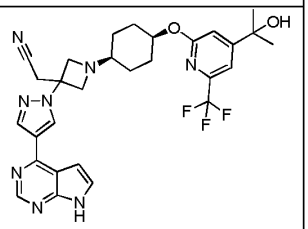
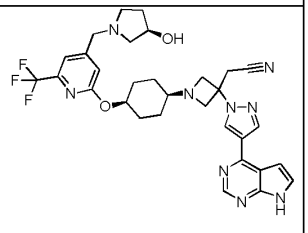
Comp. No.	Prep.	Name	Structure
1	US 2011/ 0224190 (Example 1)	{1-{1-[3-Fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile (itacitinib; ; also known as INCB039110)	

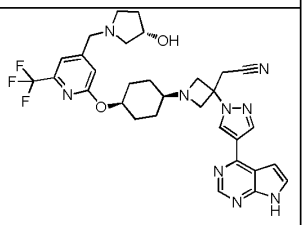
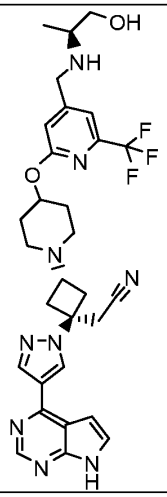
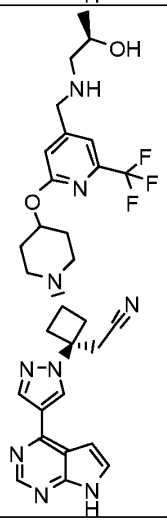
Comp. No.	Prep.	Name	Structure
2	US 2011/0224190 (Example 154)	4-{3-(Cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-[4-fluoro-2-(trifluoromethyl)phenyl]piperidine-1-carboxamide	
3	US 2011/0224190 (Example 85)	[3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-1-(1-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperidin-4-yl)azetidin-3-yl]acetonitrile	
4	US 2014/0343030 (Example 7)	4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide	
5	US 2014/0121198 (Example 20)	((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile	

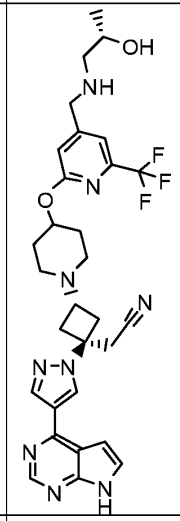
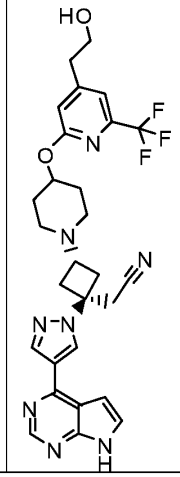
Comp. No.	Prep.	Name	Structure
6	US 2010/0298334 (Example 2)	3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile	
7	US 2010/0298334 (Example 13c)	3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile	
8	US 2011/0059951 (Example 12)	4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile	
9	US 2011/0059951 (Example 13)	4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile	

Comp. No.	Prep.	Name	Structure
10	US 2012/0149681 (Example 7b)	[*trans*-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-(4-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperazin-1-yl)cyclobutyl]acetonitrile	
11	US 2012/0149681 (Example 157)	{*trans*-3-(4-{[4-[(3-hydroxyazetidin-1-yl)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	

Comp. No.	Prep.	Name	Structure
12	US 2012/0149681 (Example 161)	{*trans*-3-(4-{[4-{[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	
13	US 2012/0149681 (Example 162)	{*trans*-3-(4-{[4-{[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	
14	US 2012/0149682 (Example 20)	4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile	
15	US 2013/0018034 (Example 18)	5-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide	

Comp. No.	Prep.	Name	Structure
16	US 2013/0018034 (Example 28)	4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide	
17	US 2013/0018034 (Example 34)	5-{3-(cyanomethyl)-3-[4-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide	
18	US 2013/0045963 (Example 45)	{1-(*cis*-4-{[6-(2-hydroxyethyl)-2-(trifluoromethyl)pyrimidin-4-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
19	US 2013/0045963 (Example 65)	{1-(*cis*-4-{[4-[(ethylamino)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
20	US 2013/0045963 (Example 69)	{1-(*cis*-4-{[4-(1-hydroxy-1-methylethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
21	US 2013/0045963 (Example 95)	{1-(*cis*-4-{[4-{[(3R)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-	

Comp. No.	Prep.	Name	Structure
		yl}acetonitrile	
22	US 2013/0045963 (Example 95)	{1-(*cis*-4-{[4-{[(3S)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
23	US 2014/0005166 (Example 1)	{*trans*-3-(4-{[4-({[(1S)-2-hydroxy-1-methylethyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	
24	US 2014/0005166 (Example 14)	{*trans*-3-(4-{[4-({[(2R)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	

Comp. No.	Prep.	Name	Structure
25	US 2014/0005166 (Example 15)	{*trans*-3-(4-{[4-({[(2S)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	
26	US 2014/0005166 (Example 20)	{*trans*-3-(4-{[4-(2-hydroxyethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	

In some embodiments, the JAK inhibitor is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or a pharmaceutically acceptable salt thereof.

In some embodiments, the JAK inhibitor is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile adipic acid salt.

The synthesis and preparation of {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile and the adipic acid salt of the same can be found, e.g., in US Patent Publ. No. 2011/0224190, filed March 9, 2011, US Patent Publ. No. 2013/0060026, filed September 6, 2012, and US Patent Publ. No. 2014/0256941, filed March 5, 2014, each of which is incorporated herein by reference in its entirety.

In some embodiments, the JAK inhibitor is 4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the JAK inhibitor is 4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide phosphoric acid salt.

The synthesis and preparation of 4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide and the phosphoric acid salt of the same can be found, e.g., in US Patent Publ. No. US 2014/0343030, filed May 16, 2014, which is incorporated herein by reference in its entirety.

In some embodiments, the JAK inhibitor is ((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile, or a pharmaceutically acceptable salt thereof.

In some embodiments, the JAK inhibitor is ((2R,5S)-5-{2-[(1R)-1-

hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile monohydrate.

Synthesis of ((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile and characterization of the anhydrous and monohydrate forms of the same are described in US Patent Publ. No. 2014/0121198, filed October 31, 2013 and US Patent Publ. No. 2015/0344497, filed April 29, 2015, each of which is incorporated herein by reference in its entirety.

In some embodiments, the compounds of Table 3 are prepared by the synthetic procedures described in US Patent Publ. No. 2011/0224190, filed March 9, 2011, US Patent Publ. No. 2014/0343030, filed May 16, 2014, US Patent Publ. No. 2014/0121198, filed October 31, 2013, US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed August 31, 2010, US Patent Publ. No. 2012/0149681, filed November 18, 2011, US Patent Publ. No. 2012/0149682, filed November 18, 2011, US Patent Publ. 2013/0018034, filed June 19, 2012, US Patent Publ. No. 2013/0045963, filed August 17, 2012, and US Patent Publ. No. 2014/0005166, filed May 17, 2013, each of which is incorporated herein by reference in its entirety.

In some embodiments, JAK inhibitor is selected from the compounds, or pharmaceutically acceptable salts thereof, of US Patent Publ. No. 2011/0224190, filed March 9, 2011, US Patent Publ. No. 2014/0343030, filed May 16, 2014, US Patent Publ. No. 2014/0121198, filed October 31, 2013, US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed August 31, 2010, US Patent Publ. No. 2012/0149681, filed November 18, 2011, US Patent Publ. No. 2012/0149682, filed November 18, 2011, US Patent Publ. 2013/0018034, filed June 19,

2012, US Patent Publ. No. 2013/0045963, filed August 17, 2012, and US Patent Publ. No. 2014/0005166, filed May 17, 2013, each of which is incorporated herein by reference in its entirety.

Methods of Treatment

The methods disclosed herein enable the assessment of whether or not a subject having, suspected of having or at risk of developing GvHD is likely to respond (e.g., likely to have greater improvement in disease as evidenced by reduced disease severity and/or disease remission/resolution) to a therapy comprising a JAK inhibitor. A subject having, suspected of having or at risk of developing GvHD who is likely to respond to a JAK inhibitor can be administered a JAK inhibitor (e.g., itacitinib). Conversely, a subject having, suspected of having or at risk of developing GvHD who is less likely to respond to a JAK inhibitor (e.g., itacitinib) can be administered an additional therapy that is suitable for treatment of GvHD.

The methods of this disclosure also enable the stratification of subjects having, suspected of having or at risk of developing GvHD into groups of subjects that are more likely to benefit, and groups of subjects that are less likely to benefit, from treatment comprising a JAK inhibitor. The ability to select such subjects from a pool of GvHD subjects who are being considered for treatment with a JAK inhibitor is beneficial for administering an effective treatment to the subject.

In one embodiment, the subject to be treated with a JAK inhibitor (e.g., itacitinib) has, is suspected of having, or is likely to develop GvHD. In certain embodiments, the subject to be treated with a therapy comprising a JAK inhibitor (e.g., itacitinib) has, is suspected of having, or is likely to develop acute GvHD. In other

embodiments, the subject to be treated with a therapy comprising a JAK inhibitor (e.g., itacitinib) has, is suspected of having, or is likely to develop chronic GvHD.

If the subject having GvHD is more likely to respond to a therapy comprising a JAK inhibitor (based on concentrations of one or more of the biomarkers described above (see Tables 1 and 2)), the subject can then be administered an effective amount of the JAK inhibitor (e.g., itacitinib). An effective amount of the JAK inhibitor can suitably be determined by a health care practitioner taking into account, for example, the characteristics of the patient (age, sex, weight, race, etc.), the progression of the disease, and prior exposure to the drug. If the subject is less likely to respond to a therapy comprising a JAK inhibitor, the subject can then be optionally administered a therapy that does not comprise a JAK inhibitor.

The methods can also be applied to individuals at risk of developing GvHD. Such individuals include those who (i) have undergone a transplant (e.g., a hematopoietic stem cell transplant) but have not developed GvHD, or (ii) are preparing for receipt of a transplant (e.g., a hematopoietic stem cell transplant).

After stratifying or selecting a subject based on whether the subject will be more likely or less likely to respond to a JAK inhibitor, a medical practitioner (e.g., a doctor) can administer the appropriate therapeutic modality to the subject. Methods of administering a JAK inhibitor are well known in the art.

In cases where the subject having GvHD and predicted to respond to a JAK inhibitor has been previously administered one or more non-JAK inhibitor therapies, the therapy comprising a JAK inhibitor can replace or augment a previously or currently administered therapy. For example, upon treating with the therapy comprising a JAK inhibitor, administration of the one or more non-JAK inhibitor therapies can cease or

diminish, e.g., be administered at lower levels. Administration of the previous therapy can be maintained while the therapy comprising a JAK inhibitor is administered. In some embodiments, a previous therapy can be maintained until the level of the therapy comprising a JAK inhibitor reaches a level sufficient to provide a therapeutic effect.

A subject treated with a JAK inhibitor (e.g., itacitinib) according to the methods described herein can be treated in combination with one or more additional compositions that are effective for treatment of GvHD. Examples of compositions that can be used in such combination treatment include corticosteroids (e.g., methylprednisolone or prednisone), methotrexate, cyclosporine, mycophenolate mofetil, tacrolimus, sirolimus, everolimus, antithymocyte globulin, alemtuzumab, cyclophosphamide, ibrutinib, imatinib, infliximab, etanercept, tocilizumab, alemtuzumab, basiliximab, daclizumab, rituximab, denileukin diftitox, pentostatin, ciclosporin, thalidomide, halofuginone, hydroxychloroquine, and mesenchymal stem cells.

The following are examples of the practice of the invention. They are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

Example 1: Identification of Proteins Differentially Expressed in Patients with Acute Graft-Versus-Host Disease that are Complete Responders to Treatment with Itacitinib

Plasma samples were collected from individuals enrolled in a study of itacitinib in combination with corticosteroids for the treatment of Acute Graft-Versus-Host Disease (GvHD). All subjects underwent a first allogeneic hematopoietic stem cell

transplantation from any donor source (matched unrelated donor, sibling, haploidentical) using bone marrow, peripheral blood stem cells, or cord blood for hematologic malignancies. The subjects exhibited clinically suspected Grades IIB to IVD acute GvHD, occurring after the allogeneic hematopoietic stem cell transplant. All subjects consented to the blood collection.

Once collected, plasma samples underwent broad proteomic profiling using OLINK™, which allows analysis of >1000 proteins. Samples were separated into the following groups based on the clinical response to treatment with itacitinib (INCB039110). Specifically, samples were classified as "complete responder" (CR), "partial/mixed responder", or "progressive disease/death" (PD/Death) based on their therapeutic response at day 28 of treatment.

Broad proteomic analysis of plasma identified a total of 118 differentially expressed proteins between the CR and PD/Death groups of subjects. Differentially expressed proteins were those that showed a statistically significant difference ($p < 0.05$) and at least a 1.2 fold change between baselines of complete responders and progressive disease/death cohorts. Fold change in this example represents the change of a baseline protein expression level between complete responders and progressive disease/death groups of subjects. Fifty-three proteins were increased and 65 proteins were decreased in CR compared to PD/Death (Table 4). Down-regulated proteins are proteins whose expression decreased over time, while up-regulated proteins are proteins whose expression increased over time. Fold change in expression is shown for each protein, which is a ratio of protein expression level post-treatment to expression level pre-treatment (baseline). Values greater than 1 indicate an increase from baseline, whereas values less than 1 indicate a decrease from baseline.

Table 4. Differentially Expressed Proteins at Baseline in the Plasma of Complete Responders Compared to the Progressive Disease/Death Groups

Up-Regulated in CR Compared to PD/Death				**Down-Regulated in CR Compared to PD/Death**		
Protein	Fold Change	Raw P Value		Protein	Fold Change	Raw P Value
PON3	3.9728	0.0005		MCP-3	-6.0399	0.0004
GCG	3.481	0.0022		CA5A	-4.5712	0.0085
SCF	3.0746	0.003		CALCA	-4.4035	0.0349
PDGF subunit B	3.0721	0.0188		HAOX1	-4.3448	0.0251
LEP	3.0194	0.0441		IL8	-4.0642	0.0216
FKBP1B	2.9644	0.0357		IL6	-3.8938	0.0207
MBL2	2.9639	0.0094		SPINK1	-3.8821	0.012
SCF	2.9526	0.0026		CXCL10	-3.8124	0.0065
GAL	2.936	0.0119		SULT2A1	-3.5143	0.0149
SCF	2.9112	0.003		IL6	-3.4705	0.0258
ITGB1BP2	2.7985	0.0306		ENPP7	-3.3791	0.0388
PVALB	2.6979	0.0084		PLXNB1	-2.99	0.0105
THPO	2.6532	0.0056		VAMP5	-2.9842	0.0315
CD40-L	2.645	0.041		CCL19	-2.9578	0.0041
ANG-1	2.6308	0.0116		CTSL1	-2.9056	0.0018
SCGB3A1	2.5332	0.0093		ACE2	-2.8742	0.0054
CD69	2.4963	0.0119		IL6	-2.8377	0.0121
FAM3B	2.481	0.0235		CRTAM	-2.6741	0.0312
GH	2.4121	0.0155		ALDH1A1	-2.6667	0.0141
CCL5	2.3402	0.0064		SIGLEC10	-2.5993	0.0148
MANF	2.3026	0.0174		KRT19	-2.5964	0.0312
SRC	2.1146	0.036		SLAMF8	-2.5417	0.0189
CRISP2	2.0487	0.0148		IL6	-2.5189	0.0141
SAA4	2.0164	0.0348		CDCP1	-2.4173	0.0113
CR2	2.0089	0.0197		N2DL-2	-2.3079	0.0075
SERPINA5	2.0067	0.0044		GZMB	-2.307	0.0289
PFKM	1.9756	8.93E-05		TNFRSF6B	-2.3053	0.0037
APOM	1.9231	0.0255		KYNU	-2.2368	0.0217
DCTN2	1.8684	0.0304		FOSB	-2.2367	0.0169
HSD11B1	1.8131	0.0173		ALDH3A1	-2.1808	0.0104
PDGF subunit A	1.7697	0.0424		IGFBP-1	-2.1383	0.0472
IGFBP3	1.7304	0.0287		CLM-1	-2.1323	0.012

HS3ST3B1	1.7175	0.0355		NFATC3	-2.0978	0.0189
CDSN	1.7055	0.0227		HAVCR2	-2.0933	0.0196
APP	1.7003	0.0484		TNF-R2	-2.0374	0.0091
TWEAK	1.6972	0.0338		DDAH1	-2.036	0.0289
TN-R	1.653	0.0321		CD74	-1.9824	0.0051
AMBP	1.6302	0.0127		CKAP4	-1.9573	0.0069
CNTN1	1.5831	0.0275		NINJ1	-1.9125	0.0043
GCP5	1.5583	0.0383		ENTPD2	-1.8643	0.0227
CNDP1	1.555	0.0034		TNFRSF9	-1.8239	0.0276
NCAM1	1.5413	0.0011		SIGLEC1	-1.8208	0.0229
PROC	1.514	0.0289		PREB	-1.8178	0.0327
F11	1.489	0.008		AHCY	-1.7883	0.043
NCAN	1.4147	0.0259		IL12RB1	-1.7236	0.0186
NTRK3	1.3885	0.0255		TNFRSF10A	-1.7188	0.0133
TIMP4	1.3827	0.044		SIRPB1	-1.7175	0.0467
VEGFD	1.3649	0.0365		DSC2	-1.6964	0.0261
HSP 27	1.3522	0.0446		U-PAR	-1.687	0.0422
GALNT10	1.3452	0.0207		TNFRSF4	-1.677	0.0423
CCL11	1.3225	0.0111		TNFRSF10A	-1.6687	0.0145
LY75	1.2722	0.0376		IL-18R1	-1.668	0.0121
DKKL1	1.2291	0.0499		IL-1ra	-1.6559	0.0334
				CLEC7A	-1.6558	0.0316
				SIGLEC7	-1.5857	0.0148
				COL4A1	-1.5794	0.0156
				TLR3	-1.5753	0.0412
				PD-L1	-1.5245	0.0139
				IL-18BP	-1.4932	0.0342
				PILRA	-1.4815	0.0341
				CCL15	-1.4614	0.0279
				uPA	-1.4223	0.0314
				DLL1	-1.2888	0.0194
				THBS2	-1.2178	0.004
				SPON2	-1.1748	0.0105

Example 2: Characterization of Protein Expression During the Course of Treatment

Plasma samples were collected from individuals enrolled in the clinical study of Example 1 at baseline and at day 28. Table 5 lists proteins that were significantly modulated by treatment between baseline and day 28. Table 6 lists proteins that were stably expressed throughout the study and were not significantly modulated by treatment between baseline and day 28.

Table 5 identifies proteins that changed in complete responders between day 1 (baseline) to day 28. Fold change in this example represents the change of a protein level between day 1 (baseline) versus day 28. A paired t test was used to compare the relative change between level of a protein between day 1 and day 28. Table 5 identifies biomarkers of therapeutic response.

Table 5. Proteins Significantly Modulated in Complete Responders Between Baseline and Day 28

Increased Expression from Baseline to Day 28				**Decreased Expression from Baseline to Day 28**		
Protein	Fold Change	Raw P Value		Protein	Fold Change	Raw P Value
GCG	2.3961	0.0168		PDGF subunit B	-2.7388	0.001
GAL	2.0603	0.0052		FKBP1B	-2.5654	0.0036
THPO	1.8262	0.0094		ITGB1BP2	-2.1159	0.0047
FAM3B	1.7937	0.0011		CD69	-2.1102	0.0016
CNDP1	1.5692	0.0134		ANG-1	-2.0923	0.0062
CCL11	1.401	0.0013		PVALB	-2.0632	0.0145
SERPINA5	1.3313	0.0089		CD40-L	-1.9741	0.0256
DKKL1	1.2705	0.0014		CCL5	-1.9502	0.0065
NCAM1	1.2481	0.0284		HS3ST3B1	-1.9303	0.0193
SPON2	1.1003	0.0106		MBL2	-1.9284	0.0215
THBS2	1.0834	0.0051		CLEC7A	-1.8985	0.0006
GCG	2.3961	0.0168		APP	-1.8356	0.0053
GAL	2.0603	0.0052		PDGF subunit	-1.7507	0.0068

				A		
THPO	1.8262	0.0094		DCTN2	-1.6367	0.0274
FAM3B	1.7937	0.0011		SLAMF8	-1.5899	0.0244
CNDP1	1.5692	0.0134		VAMP5	-1.5851	0.0053
CCL11	1.401	0.0013		SIGLEC10	-1.4859	0.0226
SERPINA5	1.3313	0.0089		CLM-1	-1.4729	0.0319
DKKL1	1.2705	0.0014		DSC2	-1.4423	0.0017
NCAM1	1.2481	0.0284		HAVCR2	-1.3876	0.0126
SPON2	1.1003	0.0106		SIRPB1	-1.3861	0.0072
THBS2	1.0834	0.0051		COL4A1	-1.3412	0.0369
				PILRA	-1.2552	0.0282
				LY75	-1.2217	0.024

Table 6 identifies proteins that did not modulate in complete responders between baseline (day 1) and day 28. Therefore, these proteins are designated as baseline predictive biomarkers.

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Table 6. Proteins Stably Expressed in Complete Responders Between Day 1 and Day 28

Proteins Increased But Not Significant				**Proteins Decreased But Not Significant**		
Protein	**Fold Change**	**Raw P Value**		**Protein**	**Fold Change**	**Raw P Value**
IL6	1.7545	0.1382		MANF	-1.6753	0.0584
HAOX1	1.7169	0.0995		SRC	-1.6394	0.0535
IGFBP-1	1.6812	0.1999		TWEAK	-1.4741	0.1325
IL6	1.6701	0.1579		CR2	-1.4025	0.0966
IL6	1.5995	0.1953		SIGLEC1	-1.3628	0.0991
ENPP7	1.5432	0.1385		TNF-R2	-1.3591	0.0931
IL6	1.5407	0.2577		TNFRSF6B	-1.3442	0.1025
SCF	1.5147	0.0799		AHCY	-1.3275	0.0854
SCGB3A1	1.5084	0.0863		CRTAM	-1.3192	0.3574
SCF	1.4346	0.0961		CKAP4	-1.2761	0.1239
SCF	1.3988	0.1184		CA5A	-1.267	0.3739
PON3	1.3708	0.0631		CALCA	-1.2236	0.6545
MCP-3	1.3171	0.2406		IL-1ra	-1.2159	0.3242
IL8	1.286	0.2913		TNFRSF9	-1.2128	0.2866
NINJ1	1.239	0.0994		DDAH1	-1.1849	0.2464
NTRK3	1.2351	0.0846		HSP 27	-1.1778	0.1094
CNTN1	1.2158	0.1831		SAA4	-1.1593	0.5157

Proteins Increased But Not Significant				Proteins Decreased But Not Significant		
Protein	**Fold Change**	**Raw P Value**		**Protein**	**Fold Change**	**Raw P Value**
ACE2	1.2118	0.4374		PFKM	-1.1575	0.2464
IGFBP3	1.1814	0.3669		TIMP4	-1.1371	0.2962
GH	1.1727	0.676		LEP	-1.1316	0.6995
CCL15	1.1726	0.2308		IL-18BP	-1.1268	0.5792
SULT2A1	1.169	0.6268		CDSN	-1.1137	0.2888
uPA	1.1545	0.1665		PD-L1	-1.0969	0.4905
FOSB	1.1542	0.1953		IL-18R1	-1.0806	0.2996
PLXNB1	1.1495	0.1726		N2DL-2	-1.0683	0.695
PREB	1.1356	0.1311		CCL19	-1.061	0.8598
CXCL10	1.133	0.805		ALDH3A1	-1.0553	0.5588
PROC	1.1313	0.4097		U-PAR	-1.054	0.6793
TNFRSF4	1.1251	0.4331		SPINK1	-1.038	0.8215
KYNU	1.1183	0.5652		TNFRSF10A	-1.0214	0.7961
AMBP	1.1176	0.3079		CTSL1	-1.0209	0.9115
VEGFD	1.1116	0.1912		F11	-1.0199	0.8362
DLL1	1.1055	0.2106		SIGLEC7	-1.015	0.9064
NCAN	1.0964	0.0631		KRT19	-1.0136	0.9496
TN-R	1.0934	0.5608		IL12RB1	-1.0129	0.9484
GALNT10	1.064	0.5285		CRISP2	-1.0113	0.8654
NFATC3	1.0637	0.7243		TNFRSF10A	-1.0093	0.9187
CDCP1	1.0579	0.7832		ALDH1A1	-1.0018	0.9927
HSD11B1	1.0468	0.7981				
APOM	1.0434	0.8235				
ENTPD2	1.0429	0.3761				
TLR3	1.035	0.5615				
GCP5	1.0249	0.7675				
GZMB	1.0134	0.9703				
CD74	1.0118	0.8996				

Example 3: Identification of Proteins that do or do not Correlate with REG3α, TNFR1, and ST2

Several inflammatory mediators have been identified and associated with increased risk of acute GvHD in steroid treated subjects that have received a hematopoietic stem cell transplant. These inflammatory mediators include REG3α,

TNFR1, and ST2 (Hartwell et al., "An early-biomarker algorithm predicts lethal graft-versus-host disease and survival, JCI Insight, 2(3):e89798). Using plasma samples from subjects enrolled in the clinical study of Example 1, proteins were evaluated for their correlation with REG3α, TNFR1, and ST2 levels at baseline. Correlation refers to potential biomarkers showing similar change or distribution as REG3α, TNFR1, and ST2. Table 7 identifies proteins that significantly ($p<0.1$) correlate with REG3α, TNFR1, and ST2 at baseline. Table 8 identifies proteins that do not significantly ($p>0.1$) correlate with REG3α, TNFR1, and ST2 at baseline.

Table 7. Proteins that Significantly Correlate with REG3α, TNFR1, and ST2

Proteins that correlate with REG3A			**Proteins that correlate with TNFR1**			**Proteins that correlate with ST2**		
Protein	Correlation	P Value	Protein	Correlation	P Value	Protein	Correlation	P Value
CD74	0.9158	0.0002	FAM3B	0.7439	0.0136	HAOX1	0.7127	0.0207
CALCA	0.8675	0.0011	TIMP4	0.7076	0.0221	VAMP5	0.5498	0.0996
N2DL-2	0.8435	0.0022	AMBP	0.6938	0.0261	TLR3	-0.7273	0.0171
SPINK1	0.8116	0.0044	GCG	0.6566	0.0392	HS3ST3B1	-0.6144	0.0588
CKAP4	0.7651	0.0099	MANF	0.621	0.0553	NCAM1	-0.6032	0.0648
NFATC3	0.7568	0.0113	VAMP5	0.6094	0.0614	SCGB3A1	-0.5909	0.072
SLAMF8	0.7158	0.0199	LEP	0.5986	0.0675			
DSC2	0.7037	0.0231	IGFBP-1	0.5712	0.0846			
PLXNB1	0.701	0.0239	F11	0.5683	0.0865			
VEGFD	0.6982	0.0247	PD-L1	0.5512	0.0987			
HAVCR2	0.6898	0.0273	ENPP7	-0.5962	0.0689			
KRT19	0.6864	0.0284						
PILRA	0.6774	0.0314						
IL-18BP	0.659	0.0382						
TNF-R2	0.6456	0.0438						
CDCP1	0.6439	0.0445						
U-PAR	0.6349	0.0486						
CLEC7A	0.6315	0.0502						
CCL15	0.6276	0.0521						
IL-1ra	0.6237	0.054						
SAA4	0.607	0.0627						
IL-18R1	0.6039	0.0645						
TNFRSF9	0.5953	0.0694						
CLM-1	0.5886	0.0734						
ITGB1BP2	0.5683	0.0865						
APOM	0.5667	0.0876						
IL12RB1	0.5528	0.0975						
CRISP2	-0.6654	0.0357						

Table 8. Proteins that do not Significantly Correlate with REG3α, TNFR1, and ST2

Proteins that do not correlate with REG3A			Proteins that do not correlate with TNFR1			Proteins that do not correlate with ST2		
Protein	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**
CDSN	0.5421	0.1055	SCF	0.5327	0.1129	SRC	0.5154	0.1273
SIRPB1	0.5295	0.1155	SCF	0.5302	0.1149	FOSB	0.4886	0.1519
TNFRSF4	0.5048	0.1368	CCL15	0.5161	0.1267	SULT2A1	0.4758	0.1645
CCL5	0.5026	0.1387	DCTN2	0.5131	0.1293	KYNU	0.4712	0.1692
SIGLEC10	0.5024	0.1389	DDAH1	0.5114	0.1309	SIGLEC10	0.4656	0.1751
SIGLEC7	0.4969	0.144	DLL1	0.5068	0.1349	CTSL1	0.4628	0.1781
MANF	0.4955	0.1453	SIRPB1	0.5053	0.1363	CA5A	0.4588	0.1823
SIGLEC1	0.4867	0.1537	COL4A1	0.5019	0.1393	COL4A1	0.4535	0.188
APP	0.4833	0.1571	SRC	0.4937	0.147	MANF	0.4374	0.2062
CTSL1	0.4797	0.1606	TNFRSF4	0.4851	0.1553	MCP-3	0.4227	0.2236
PDGF subunit A	0.4733	0.1671	ALDH3A1	0.4831	0.1572	FAM3B	0.4091	0.2405
IGFBP-1	0.4368	0.2069	SULT2A1	0.4796	0.1607	GCG	0.4017	0.2499
SPON2	0.4365	0.2072	SERPINA5	0.4683	0.1723	CCL15	0.3789	0.2802
SRC	0.4349	0.209	CLEC7A	0.4674	0.1731	DDAH1	0.3768	0.2832
GCP5	0.4261	0.2196	TNF-R2	0.4671	0.1734	AHCY	0.3758	0.2845
CRTAM	0.42	0.2269	CNTN1	0.4106	0.2386	PD-L1	0.3545	0.3149
TLR3	0.4143	0.2339	FKBP1B	0.404	0.247	SPON2	0.3342	0.3452
PD-L1	0.4068	0.2434	CLM-1	0.39	0.2652	KRT19	0.3166	0.3727
MCP-3	0.4	0.252	SIGLEC1	0.3765	0.2835	CRTAM	0.3123	0.3797
CXCL10	0.3944	0.2594	SCF	0.3748	0.2858	ALDH1A1	0.3079	0.3868
IL8	0.3778	0.2818	MBL2	0.3728	0.2887	ITGB1BP2	0.2858	0.4233
CNTN1	0.3588	0.3086	GALNT10	0.3583	0.3093	NFATC3	0.2854	0.4242
PDGF subunit B	0.3581	0.3096	TNFRSF9	0.351	0.3201	IL6	0.2821	0.4298
FKBP1B	0.3529	0.3172	PILRA	0.3496	0.322	ALDH3A1	0.2716	0.4477
CD69	0.3487	0.3235	CD40-L	0.3423	0.3329	ACE2	0.2692	0.452
ENPP7	0.3255	0.3587	IL-18BP	0.3311	0.3501	IL8	0.2651	0.4591
LY75	0.3251	0.3594	GCP5	0.3298	0.352	IL6	0.2604	0.4675
uPA	0.3224	0.3636	CD69	0.3237	0.3616	DCTN2	0.2494	0.4871
DCTN2	0.3165	0.373	U-PAR	0.3234	0.362	SPINK1	0.2493	0.4872
VAMP5	0.3128	0.3788	TWEAK	0.3205	0.3666	IL6	0.2411	0.5022
ANG-1	0.308	0.3866	DSC2	0.3192	0.3687	CD69	0.2244	0.533
CD40-L	0.3077	0.3871	HSD11B1	0.3159	0.374	VEGFD	0.2227	0.5363
TIMP4	0.302	0.3964	HSP 27	0.2988	0.4018	PLXNB1	0.222	0.5377
CA5A	0.2969	0.4049	NTRK3	0.2821	0.4297	SIRPB1	0.2111	0.5582
TNFRSF6B	0.2891	0.4179	ITGB1BP2	0.2648	0.4597	MBL2	0.2088	0.5627
GCG	0.2604	0.4675	CA5A	0.2477	0.4901	TIMP4	0.2085	0.5633
DDAH1	0.2571	0.4733	SPINK1	0.2403	0.5037	IL6	0.2063	0.5675
KYNU	0.2506	0.485	FOSB	0.2371	0.5096	SIGLEC7	0.2015	0.5767

Proteins that do not correlate with REG3A			Proteins that do not correlate with TNFR1			Proteins that do not correlate with ST2		
Protein	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**
GH	0.2433	0.4982	IL12RB1	0.22	0.5414	TNF-R2	0.1932	0.5928
IL6	0.2424	0.4998	LY75	0.1997	0.5802	FKBP1B	0.1822	0.6143
MBL2	0.2384	0.5071	CCL19	0.195	0.5893	CNDP1	0.1782	0.6223
TNFRSF10A	0.2273	0.5277	ENTPD2	0.1913	0.5964	GAL	0.1763	0.6262
ACE2	0.222	0.5377	APOM	0.1908	0.5975	DLL1	0.1665	0.6458
IL6	0.2185	0.5442	SPON2	0.1893	0.6004	DSC2	0.1661	0.6464
TNFRSF10A	0.2081	0.564	CKAP4	0.1813	0.6162	SIGLEC1	0.1642	0.6503
IGFBP3	0.2074	0.5653	CCL5	0.1799	0.619	CLEC7A	0.161	0.6568
IL6	0.1987	0.5821	PROC	0.1767	0.6254	HAVCR2	0.1598	0.6592
HSP 27	0.191	0.5972	CTSL1	0.1592	0.6604	U-PAR	0.1579	0.6632
ALDH3A1	0.1869	0.6052	HAVCR2	0.1576	0.6636	CALCA	0.1474	0.6844
CNDP1	0.1755	0.6276	GAL	0.1471	0.6851	CRISP2	0.1461	0.6872
IL6	0.1694	0.64	PON3	0.1353	0.7094	PILRA	0.1439	0.6916
GZMB	0.1604	0.658	TNFRSF10A	0.1328	0.7146	PROC	0.1351	0.7098
SCGB3A1	0.1596	0.6597	CDSN	0.1261	0.7286	NCAN	0.1245	0.7318
CCL19	0.1423	0.6949	TNFRSF6B	0.125	0.7308	PDGF subunit A	0.124	0.7328
ALDH1A1	0.1224	0.7363	TNFRSF10A	0.1009	0.7815	IL-1ra	0.1166	0.7483
FOSB	0.1068	0.769	CRISP2	0.0895	0.8057	LEP	0.1156	0.7504
HAOX1	0.0985	0.7865	KYNU	0.0889	0.8071	GCP5	0.095	0.794
AHCY	0.0951	0.7938	PDGF subunit B	0.084	0.8176	SLAMF8	0.0876	0.8099
NTRK3	0.0842	0.8171	ANG-1	0.079	0.8284	CD74	0.0855	0.8142
FAM3B	0.0842	0.8172	CRTAM	0.0665	0.8552	ENPP7	0.0743	0.8384
COL4A1	0.0703	0.8469	MCP-3	0.0537	0.8828	IGFBP-1	0.0727	0.8419
THPO	0.0603	0.8685	THBS2	0.0536	0.8831	CD40-L	0.0692	0.8494
PROC	0.0564	0.877	SIGLEC7	0.0485	0.8942	TNFRSF10A	0.0606	0.8679
NCAM1	0.0319	0.9304	CR2	0.0378	0.9175	F11	0.0544	0.8813
NCAN	0.0227	0.9504	SAA4	0.0183	0.96	uPA	0.0506	0.8896
PON3	0.0187	0.9591	PREB	0	1	GALNT10	0.0387	0.9154
DLL1	0.0146	0.9681	NINJ1	0	1	THBS2	0.0387	0.9155
NINJ1	0	1	THPO	-0.0005	0.9989	CDCP1	0.0266	0.9418
PREB	0	1	PLXNB1	-0.0028	0.9938	PDGF subunit B	0.0201	0.956
SULT2A1	-0.0321	0.9298	NFATC3	-0.0033	0.9928	SCF	0.004	0.9913
TN-R	-0.0585	0.8724	PDGF subunit A	-0.01	0.9781	NTRK3	0.0026	0.9942
AMBP	-0.0823	0.8211	N2DL-2	-0.0164	0.9641	SCF	0.0018	0.9961
GALNT10	-0.0831	0.8194	HAOX1	-0.0276	0.9396	CCL5	0.0015	0.9968
TWEAK	-0.1065	0.7697	GZMB	-0.0298	0.9349	PREB	0	1
DKKL1	-0.1125	0.7569	uPA	-0.03	0.9343	NINJ1	0	1
PVALB	-0.118	0.7454	SLAMF8	-0.0371	0.919	TNFRSF10A	-0.0047	0.9896

Proteins that do not correlate with REG3A			Proteins that do not correlate with TNFR1			Proteins that do not correlate with ST2		
Protein	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**	**Protein**	**Correlation**	**P Value**
PFKM	-0.1796	0.6196	CDCP1	-0.0431	0.9058	TNFRSF9	-0.0047	0.9896
HSD11B1	-0.1829	0.6131	PVALB	-0.0674	0.8533	CLM-1	-0.0066	0.9855
ENTPD2	-0.1832	0.6124	CXCL10	-0.0675	0.853	CDSN	-0.0137	0.97
HS3ST3B1	-0.2478	0.49	SIGLEC10	-0.1138	0.7543	IL-18BP	-0.0184	0.9598
F11	-0.249	0.4879	IL-1ra	-0.1167	0.7481	CCL19	-0.0194	0.9576
CR2	-0.2631	0.4626	IGFBP3	-0.1316	0.7171	N2DL-2	-0.0203	0.9556
GAL	-0.2958	0.4067	CCL11	-0.1433	0.6928	TNFRSF4	-0.0231	0.9495
SCF	-0.3195	0.3681	CALCA	-0.1562	0.6666	SCF	-0.0345	0.9246
SCF	-0.3198	0.3677	NCAN	-0.1632	0.6523	HSD11B1	-0.0653	0.8578
SERPINA5	-0.3358	0.3427	TN-R	-0.1766	0.6254	APOM	-0.0674	0.8532
SCF	-0.3513	0.3196	PFKM	-0.1914	0.5963	CCL11	-0.0699	0.8479
THBS2	-0.3725	0.2892	DKKL1	-0.2137	0.5532	GZMB	-0.0739	0.8393
LEP	-0.3739	0.2872	CD74	-0.2144	0.5519	CKAP4	-0.0951	0.7938
CCL11	-0.4076	0.2423	ALDH1A1	-0.2245	0.5329	AMBP	-0.122	0.7371
			SCGB3A1	-0.2297	0.5233	THPO	-0.1323	0.7156
			NCAM1	-0.2349	0.5135	LY75	-0.1404	0.6989
			IL6	-0.249	0.4878	IL-18R1	-0.1526	0.6738
			CNDP1	-0.2571	0.4734	ENTPD2	-0.1561	0.6667
			IL6	-0.2641	0.4609	ANG-1	-0.1586	0.6618
			IL6	-0.2755	0.441	APP	-0.1633	0.6521
			KRT19	-0.278	0.4368	IL12RB1	-0.1745	0.6296
			ACE2	-0.2926	0.4119	SAA4	-0.1783	0.6221
			IL-18R1	-0.3026	0.3954	SERPINA5	-0.1971	0.5852
			APP	-0.3215	0.365	HSP 27	-0.2018	0.5762
			TLR3	-0.3408	0.3352	TN-R	-0.204	0.5719
			AHCY	-0.353	0.317	PFKM	-0.2317	0.5196
			IL6	-0.3819	0.2762	TNFRSF6B	-0.2566	0.4742
			VEGFD	-0.4069	0.2432	PON3	-0.2767	0.4389
			IL8	-0.4509	0.1909	IGFBP3	-0.2965	0.4054
			HS3ST3B1	-0.4519	0.1898	PVALB	-0.306	0.3899
			GH	-0.5459	0.1026	DKKL1	-0.3744	0.2865
						CNTN1	-0.3974	0.2555
						CXCL10	-0.4139	0.2344
						GH	-0.4141	0.2342
						TWEAK	-0.471	0.1695
						CR2	-0.5445	0.1036

Example 4: Selection of Proteins Capable of Predicting Positive Therapeutic Response with JAK Inhibition in GvHD

Data from the previous examples identified several proteins at baseline that predict a positive therapeutic response, as evidenced by classification as CR at day 28. Proteins listed in (but not limited to) Table 9 were found to be: (1) differentially expressed between the CR and PD/Death treatment groups; (2) stable between baseline and day 28; and (3) not correlated with REG3α, TNFR1, and ST2.

Table 9. Proteins Differentially Expressed Between the CR and PD/Death Treatment Groups, Stable Between Baseline and Day 28, and not Correlated with REG3α, TNFR1, and ST2

Proteins Increased in CR				**Proteins Decreased in CR**		
Protein	**Fold Change**	**Raw P Value**		**Protein**	**Fold Change**	**Raw P Value**
PON3	3.9728	0.0005		MCP-3	-6.0399	0.0004
SCF	3.0746	0.003		CA5A	-4.5712	0.0085
SCF	2.9526	0.0026		IL8	-4.0642	0.0216
SCF	2.9112	0.003		CXCL10	-3.8124	0.0065
GH	2.4121	0.0155		SULT2A1	-3.5143	0.0149
SRC	2.1146	0.036		IL6	-3.4705	0.0258
CR2	2.0089	0.0197		CCL19	-2.9578	0.0041
PFKM	1.9756	8.93E-05		CTSL1	-2.9056	0.0018
HSD11B1	1.8131	0.0173		ACE2	-2.8742	0.0054
IGFBP3	1.7304	0.0287		IL6	-2.8377	0.0121
CDSN	1.7055	0.0227		CRTAM	-2.6741	0.0312
TWEAK	1.6972	0.0338		ALDH1A1	-2.6667	0.0141
TN-R	1.653	0.0321		IL6	-2.5189	0.0141
CNTN1	1.5831	0.0275		GZMB	-2.307	0.0289
GCP5	1.5583	0.0383		TNFRSF6B	-2.3053	0.0037
PROC	1.514	0.0289		KYNU	-2.2368	0.0217
NCAN	1.4147	0.0259		FOSB	-2.2367	0.0169
NTRK3	1.3885	0.0255		ALDH3A1	-2.1808	0.0104
HSP 27	1.3522	0.0446		DDAH1	-2.036	0.0289
GALNT10	1.3452	0.0207		NINJ1	-1.9125	0.0043

Proteins Increased in CR				Proteins Decreased in CR		
Protein	**Fold Change**	**Raw P Value**		**Protein**	**Fold Change**	**Raw P Value**
				ENTPD2	-1.8643	0.0227
				SIGLEC1	-1.8208	0.0229
				PREB	-1.8178	0.0327
				AHCY	-1.7883	0.043
				TNFRSF10A	-1.7188	0.0133
				TNFRSF4	-1.677	0.0423
				SIGLEC7	-1.5857	0.0148
				uPA	-1.4223	0.0314
				DLL1	-1.2888	0.0194

Using a more stringent cutoff of 2 (absolute number), the number of predictive proteins was further reduced (Table 10).

Table 10. Selected Proteins Capable of Predicting Positive Therapeutic Response to JAK Inhibition in GvHD

Protein	**Fold Change CR vs PD/Death**	**Raw P Value**
PON3	3.9728	0.0005
SCF	3.0746	0.003
SCF	2.9526	0.0026
SCF	2.9112	0.003
GH	2.4121	0.0155
SRC	2.1146	0.036
CR2	2.0089	0.0197
MCP-3	-6.0399	0.0004
CA5A	-4.5712	0.0085
IL8	-4.0642	0.0216
CXCL10	-3.8124	0.0065
IL6	-3.4705	0.0258
CCL19	-2.9578	0.0041
CTSL1	-2.9056	0.0018
ACE2	-2.8742	0.0054
IL6	-2.8377	0.0121
ALDH1A1	-2.6667	0.0141
IL6	-2.5189	0.0141
TNFRSF6B	-2.3053	0.0037
KYNU	-2.2368	0.0217

Protein	Fold Change CR vs PD/Death	Raw P Value
FOSB	-2.2367	0.0169
ALDH3A1	-2.1808	0.0104
DDAH1	-2.036	0.0289

Example 5: Identification of Proteins Differentially Expressed in Patients with Acute Graft-Versus-Host Disease that are Complete Responders to Treatment with Itacitinib

The combination of itacitinib with corticosteroids was evaluated in a parallel-cohort phase 1 trial and improved overall responses for both steroid naïve and refractory aGvHD patients. A broad proteomic analysis identified predictive, prognostic, and pharmacodynamic biomarkers of response to the combination treatment.

Ten steroid-naive and eighteen steroid-refractory subjects with aGvHD were enrolled in the clinical trial. Plasma samples were collected from all 28 subjects prior to treatment (screening/baseline) and at day 28 following treatment. All subjects provided written consent prior to enrollment and sample collection. Based on the Center for International Blood and Marrow Transplant Research (CIBMTR) response criteria at day 28, subjects were separated into responders and non-responders. Responders included complete responders (CR; n=10), very good partial responders (VGPR; n=1), and partial responders (PR; n=8). Non-responders included mixed responders (n=2) and progressive disease/death (PD/Death; n=7).

Subjects were treated with corticosteroids in combination with either 200 mg (N=14) or 300 mg (N=14) of itacitinib once daily (QD). Clinical response was not significantly different between the two itacitinib doses; therefore, data from both cohorts were combined. Due to the limited sample size, steroid-naive (N = 10) and steroid-refractory (N = 18) patients were combined for further analysis. Broad proteomic analysis of over 1000 proteins was conducted by OLINK Proteomics

(Watertown, MA) using a proximity extension assay as described by the manufacturer. Data are presented as normalized protein expression (NPX) in log2 scale. Statistical differences were evaluated using unpaired and paired t tests, one-way analysis of covariance (ANOVA), and Pearson Correlation. Significance was conferred when $P < 0.05$.

Proteins were identified based on significant differences between the complete responder and progressive disease/death cohorts at baseline that achieved at least a 1.2 fold change between cohorts. See Table 11. Because some patients were re-classified based on their day 28 response, Table 11 represents an updated list of proteins originally shown in Example 1, Table 4.

A total of 146 differentially expressed proteins between the CR and PD/Death groups of subjects were identified. Fifty-seven proteins were increased and 89 proteins were decreased in CR compared to PD/Death. See Table 11.

Table 11. Differentially Expressed Proteins at Baseline in the Plasma of Complete Responders Compared to the Progressive Disease/Death Groups

Up-Regulated in CR Compared to PD/Death				**Down-Regulated in CR Compared to PD/Death**		
Protein	Fold Change	Raw P Value		Protein	Fold Change	Raw P Value
CCL17	6.4545	0.0014		MCP-3	-7.865	0.000012941
PON3	4.8594	0.0005		HAOX1	-7.846	0.0027
LEP	4.402	0.0191		CA5A	-7.4693	0.001
GCG	3.904	0.0031		CALCA	-5.4107	0.0339
MBL2	3.7419	0.0014		IL8	-5.0031	0.0006
SCF	3.5978	0.0006		AREG	-4.8443	0.0377
SCF	3.5041	0.0008		SULT2A1	-4.7009	0.0062
SCF	3.2594	0.0024		VAMP5	-4.3186	0.0064
SCGB3A1	3.1352	0.0051		SPINK1	-4.2632	0.0171

Up-Regulated in CR Compared to PD/Death				Down-Regulated in CR Compared to PD/Death		
GAL	3.0034	0.0179		SIGLEC10	-3.8434	0.0002
FAM3B	2.8141	0.0165		ENPP7	-3.804	0.032
THPO	2.7391	0.007		ACE2	-3.7433	0.0021
GH	2.6866	0.0085		CTSL1	-3.7378	0.0004
PVALB	2.6266	0.0194		PRSS2	-3.6787	0.0454
ANG-1	2.3398	0.0462		CXCL10	-3.5343	0.0192
GDF-8	2.2804	0.0377		NEP	-3.4607	0.033
EN-RAGE	2.2642	0.0432		MFGE8	-3.4384	0.014
CRISP2	2.2475	0.0149		KRT19	-3.2722	0.0153
CR2	2.1595	0.0321		SLAMF8	-3.2642	0.0053
CCL5	2.1066	0.0342		CRTAM	-3.2086	0.0232
SERPINA5	2.0891	0.0079		IL6	-3.1755	0.0065
IGFBP3	1.9044	0.0219		ALDH1A1	-3.168	0.0096
PFKM	1.8788	0.0007		CES1	-2.9707	0.0286
TN-R	1.8732	0.0227		REG3A	-2.9432	0.0499
KLK6	1.8647	0.0002		KYNU	-2.9203	0.0061
AMBP	1.8381	0.003		IL-4RA	-2.8507	0.0018
SCGB3A2	1.8338	0.029		CDCP1	-2.792	0.0097
TWEAK	1.8183	0.0426		IL6	-2.781	0.0086
FAM19A5	1.813	0.0186		IL6	-2.7351	0.0088
CNTN1	1.791	0.0131		MVK	-2.6813	0.0346
VWC2	1.7842	0.0316		FOSB	-2.6284	0.0075
CD207	1.7751	0.0153		NFATC3	-2.5865	0.0042
HSD11B1	1.7507	0.0446		N2DL-2	-2.5399	0.0032
KIT	1.7369	0.0439		IL6	-2.5099	0.0151
Notch 3	1.7306	0.0281		DDAH1	-2.5062	0.0089
GCP5	1.7126	0.0062		IGFBP-1	-2.4965	0.0315
BCAN	1.6911	0.0092		ALDH3A1	-2.477	0.003
CDSN	1.6718	0.0496		CXADR	-2.4672	0.0111
hK14	1.6607	0.0429		HAVCR2	-2.4468	0.0022
DRAXIN	1.6547	0.0226		CKAP4	-2.3793	0.0008
NCAM1	1.6306	0.0012		PLXNB1	-2.3572	0.0015
F11	1.5723	0.0109		NINJ1	-2.3018	0.0004
CNDP1	1.5685	0.0117		TNFRSF6B	-2.24	0.0093
TIMP4	1.5269	0.0151		CLM-1	-2.2069	0.0188
NCAN	1.5261	0.0216		CD74	-2.2017	0.0012
WNT9A	1.5125	0.0206		ENTPD2	-2.1935	0.0084
MFAP5	1.4911	0.0342		PREB	-2.1563	0.0115
CCL28	1.4765	0.0365		CCL19	-2.1313	0.0252
GALNT10	1.4483	0.0079		SIGLEC1	-2.0772	0.0067

Up-Regulated in CR Compared to PD/Death				Down-Regulated in CR Compared to PD/Death		
VEGFD	1.4176	0.0439		Gal-4	-2.0631	0.0322
DNER	1.4054	0.0481		HNMT	-2.0614	0.0081
CRH	1.4003	0.0285		HTRA2	-2.0308	0.002
CCL11	1.3644	0.0126		VSIG4	-2.0226	0.0258
PAM	1.3347	0.0078		IL-1RT2	-2.0162	0.013
LY75	1.3276	0.0287		TNF-R2	-2.0135	0.0104
CCL11	1.3163	0.0439		IL-18R1	-2.0003	0.0005
KLK10	1.2844	0.0448		SIRPB1	-1.9684	0.02
				TNFRSF10A	-1.9427	0.0055
				AHCY	-1.8767	0.038
				DSC2	-1.8695	0.0132
				IL12RB1	-1.8353	0.0184
				TNFRSF10A	-1.8332	0.0075
				LILRB4	-1.832	0.0435
				TRAIL-R2	-1.8307	0.0191
				EPHA2	-1.7981	0.0105
				U-PAR	-1.7842	0.0224
				LAIR1	-1.7352	0.0179
				SEMA4C	-1.7209	0.0178
				CLEC7A	-1.7189	0.0411
				ANGPTL4	-1.7097	0.0494
				RTN4R	-1.6966	0.0441
				CD163	-1.6749	0.013
				VCAM1	-1.6662	0.0161
				COL4A1	-1.663	0.0168
				PDCD1	-1.6537	0.0108
				IFN-gamma-R1	-1.6515	0.0152
				IL-1ra	-1.6115	0.021
				CCL15	-1.6016	0.0159
				TGM2	-1.5838	0.0286
				DAG1	-1.58	0.0345
				NECTIN2	-1.5594	0.049
				PILRA	-1.5248	0.0281
				PD-L1	-1.5023	0.0147
				SIGLEC7	-1.4749	0.0364
				PRDX6	-1.4644	0.0461
				DLL1	-1.4438	0.0003
				EDIL3	-1.2737	0.0246
				THBS2	-1.2257	0.0104

Up-Regulated in CR Compared to PD/Death				Down-Regulated in CR Compared to PD/Death		
				SPON2	-1.212	0.0024

A total of 89 proteins from Table 11 were identified that did not modulate in complete responders between baseline (Day 1) and Day 28. Table 12 represents an updated list of proteins originally shown in Example 2, Table 6. These proteins are designated as baseline predictive biomarkers.

Table 12. Proteins from Table 11 that were Stably Expressed in Complete Responders Between Days 1 and 28

Up-Regulated in CR Compared to PD/Death				Down-Regulated in CR Compared to PD/Death		
Protein	Fold Change (D1 vs D28)	Raw P Value (D1 vs D28		Protein	Fold Change (D1 vs D28)	Raw P Value (D1 vs D28
PON3	1.3014	0.0529		IL8	1.6324	0.0814
CNTN1	1.1978	0.0872		HAOX1	1.5747	0.09
IGFBP3	1.1493	0.1918		ENPP7	1.4178	0.1392
LEP	1.133	0.5306		ACE2	1.301	0.072
Notch 3	1.1259	0.1626		SULT2A1	1.2251	0.42
TN-R	1.1234	0.2461		MCP-3	1.1945	0.3631
HSD11B1	1.1146	0.3215		CES1	1.1821	0.2881
FAM19A5	1.0841	0.4593		MFGE8	1.1591	0.3239
NCAN	1.0838	0.1333		PLXNB1	1.1347	0.069
F11	1.0834	0.2439		TNFRSF10A	1.1052	0.2863
GDF-8	1.0733	0.6406		CCL15	1.1003	0.3286
CCL28	1.0625	0.2987		TNFRSF10A	1.0972	0.2337
GALNT10	1.0608	0.3706		SEMA4C	1.079	0.4031
BCAN	1.0439	0.6251		PREB	1.0663	0.2
TIMP4	1.0265	0.8344		NFATC3	1.0623	0.7203
CRISP2	1.0244	0.7055		CCL19	1.0575	0.9296
CD207	1.0177	0.9042		DLL1	1.0479	0.5388
WNT9A	1.0058	0.9334		ENTPD2	1.0197	0.7027
MBL2	-1.439	0.0693		IL-4RA	1.0166	0.8889
EN-RAGE	-1.3322	0.1832		EPHA2	1.0139	0.8348

Up-Regulated in CR Compared to PD/Death				Down-Regulated in CR Compared to PD/Death		
TWEAK	-1.2551	0.1314		FOSB	1.0051	0.9581
CR2	-1.1488	0.312		CXCL10	-1.6949	0.2429
MFAP5	-1.1215	0.1239		VAMP5	-1.3344	0.0888
KIT	-1.0943	0.3754		ALDH3A1	-1.292	0.1504
GH	-1.0614	0.7936		MVK	-1.2699	0.0932
PFKM	-1.052	0.5545		IL12RB1	-1.2335	0.1735
CDSN	-1.05	0.6182		CALCA	-1.2293	0.4153
CRH	-1.0445	0.6559		AHCY	-1.1994	0.1462
GCP5	-1.0404	0.6364		PRSS2	-1.1946	0.2577
KLK6	-1.0386	0.6672		LILRB4	-1.1845	0.114
DRAXIN	-1.0356	0.6586		DDAH1	-1.1714	0.1479
				IL-1ra	-1.1696	0.2023
				NECTIN2	-1.1579	0.1167
				PDCD1	-1.1485	0.0783
				CD74	-1.1483	0.1266
				PD-L1	-1.1361	0.181
				REG3A	-1.1314	0.1677
				CA5A	-1.1295	0.5551
				N2DL-2	-1.1284	0.2413
				CDCP1	-1.1249	0.5025
				U-PAR	-1.0962	0.2869
				SIGLEC7	-1.0923	0.3111
				ANGPTL4	-1.088	0.618
				ALDH1A1	-1.0791	0.6027
				SPINK1	-1.0709	0.7767
				HTRA2	-1.0707	0.4567
				PRDX6	-1.061	0.5849
				IL-1RT2	-1.0504	0.6889
				IGFBP-1	-1.0455	0.8818
				HNMT	-1.0338	0.5282
				TRAIL-R2	-1.0337	0.6738
				CXADR	-1.0305	0.484
				CTSL1	-1.0288	0.8442
				IFN-gamma-R1	-1.0268	0.6494
				IL-18R1	-1.0192	0.5615
				KRT19	-1.0142	0.9196
				KYNU	-1.0138	0.9268
				TGM2	-1.0122	0.9074

Example 6: Characterization of Protein Expression During the Course of Treatment

Longitudinal differences in protein expression were analyzed by evaluating plasma samples from baseline/screening and day 28. Proteins were identified that were significantly modulated by treatment between screening/baseline and day 28 in responders, including CR, VGPR, and PR patients (N = 19). A total of 353 proteins were identified, and are shown in Table 13. From this list, 105 proteins were significantly elevated, and 248 proteins were significantly reduced between baseline and day 28. The list of proteins in Table 13 includes proteins included in Example 2, Table 5, and includes proteins modulated by treatment in CR, VGPR, and PR patients. Table 13 identifies biomarkers of therapeutic response.

Table 13. Proteins Significantly Modulated in Responders (CR, VGPR, PR; n = 19) Between Baseline and Day 28

Increased Expression from Baseline to Day 28				**Decreased Expression from Baseline to Day 28**		
Protein	Fold Change	Raw P Value		Protein	Fold Change	Raw P Value
TMPRSS15	3.0633	7.04E-07		INPPL1	-1.7131	8.25E-06
CCL11	1.3146	4.74E-06		LAT2	-1.7755	1.01E-05
FAM3B	1.7951	1.23E-05		CLEC7A	-1.8103	1.39E-05
MMP7	1.3986	2.15E-05		PPP1R9B	-1.6445	2.13E-05
NCAM1	1.3216	9.44E-05		NEMO	-1.7984	2.56E-05
Gal-3	1.3504	0.0001		SH2B3	-1.7273	3.46E-05
CCL25	2.106	0.0001		BCR	-1.8219	5.51E-05
THPO	1.867	0.0002		CD5	-1.9942	6.02E-05
CCL11	1.2818	0.0003		DNAJB1	-1.7332	8.26E-05
hK14	1.5574	0.0004		CCL17	-3.059	8.32E-05
KIM1	1.7741	0.0004		ITGB2	-1.6877	8.97E-05
Flt3L	2.3423	0.0004		BANK1	-2.0084	0.0001
PLIN1	2.4338	0.0005		TPSAB1	-1.9835	0.0001
SPON2	1.095	0.0006		YES1	-1.9824	0.0001

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
Gal-4	1.4274	0.0006		LAMP3	-1.7705	0.0001
FABP4	1.6926	0.0006		GM-CSF-R-alpha	-1.4489	0.0001
DNER	1.2957	0.0007		CNTNAP2	-1.3091	0.0001
GAL	1.6888	0.0008		ZBTB16	-1.9166	0.0002
KIM1	1.757	0.0009		CD163	-1.7206	0.0002
CPM	1.1479	0.0011		TXLNA	-1.5277	0.0002
VWC2	1.3331	0.0011		MEPE	-1.4941	0.0002
PPY	1.9703	0.0012		BACH1	-1.4794	0.0002
PAM	1.1653	0.0014		MAX	-1.449	0.0002
PVR	1.1964	0.0015		NFKBIE	-1.2885	0.0002
SERPINA5	1.2683	0.0015		hOSCAR	-1.2603	0.0002
ST3GAL1	1.3415	0.0016		LAT	-2.049	0.0003
CST5	1.3521	0.002		PTPRJ	-1.7421	0.0003
CES2	1.4212	0.0022		SIRT2	-1.6014	0.0003
CNDP1	1.3856	0.0024		SIRPB1	-1.3991	0.0003
CX3CL1	1.5454	0.0024		AXIN1	-1.8046	0.0004
HO-1	1.3293	0.0028		EIF4G1	-1.5969	0.0004
PRELP	1.1833	0.0029		PTX3	-1.5567	0.0004
ADM	1.2035	0.003		TRIM5	-1.4743	0.0004
VSIG2	1.2163	0.0031		IDUA	-1.3887	0.0004
FABP2	3.5893	0.0031		NCF2	-2.4951	0.0005
CEACAM5	1.6422	0.0039		SELP	-1.9244	0.0005
SLITRK2	1.2986	0.004		ARHGEF12	-1.8347	0.0005
MCP-1	1.6302	0.0044		CASP-3	-1.787	0.0005
NTRK3	1.2526	0.0045		CD27	-1.6873	0.0005
CLUL1	1.3108	0.0046		MAP4K5	-1.658	0.0005
CXCL16	1.2028	0.0053		DAPP1	-1.3805	0.0005
SCF	1.4714	0.0056		PRDX5	-2.0131	0.0006
TMPRSS5	1.3307	0.0057		TLT-2	-1.8372	0.0006
REG4	2.1118	0.0059		PARK7	-1.4104	0.0006
hK11	1.2975	0.0061		IL2-RA	-2.2452	0.0007
SCF	1.3943	0.0061		FOXO1	-1.3557	0.0007
SCGB3A1	1.5691	0.0061		ST1A1	-1.9523	0.0008
DKKL1	1.1495	0.0071		GRAP2	-1.6468	0.0008
NEP	1.8376	0.0077		NBN	-1.5879	0.0008
CPA2	1.7185	0.0088		CD93	-1.3029	0.0008
Ep-CAM	1.403	0.0089		FCGR2A	-1.5278	0.0009
THBS2	1.0963	0.0091		DCTN1	-1.4726	0.0009
GPNMB	1.2117	0.0092		IRF9	-1.4384	0.0009

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
ITGB5	1.229	0.0104		HAVCR2	-1.312	0.0009
GT	1.8699	0.0104		CD84	-1.7208	0.001
APLP1	1.4933	0.0117		STX8	-1.4796	0.001
TACSTD2	1.1931	0.0119		LY9	-1.4447	0.001
NINJ1	1.2723	0.012		ZBTB16	-1.9288	0.0011
SCF	1.4058	0.0123		CD200R1	-1.4752	0.0011
REN	1.4074	0.0137		TOP2B	-1.7634	0.0012
GCG	1.8922	0.0137		THY 1	-1.2913	0.0012
SERPINA9	1.5418	0.0151		PRKRA	-1.2761	0.0012
KAZALD1	1.2609	0.0154		ITGB1BP2	-1.8787	0.0013
SERPINA12	1.567	0.0155		CD48	-1.6022	0.0013
PODXL	1.2014	0.0163		CD244	-1.5176	0.0014
AMN	1.2517	0.017		HCLS1	-1.455	0.0014
IGF1R	1.2432	0.0171		MPO	-1.8431	0.0015
LTBP2	1.1874	0.0175		SIT1	-1.5501	0.0015
ANGPTL3	1.2673	0.0177		ICAM3	-1.464	0.0015
SCARA5	1.1342	0.0179		SOST	-1.3214	0.0015
B4GAT1	1.2795	0.0179		DDX58	-1.6381	0.0016
ROBO2	1.249	0.0181		TNF-R2	-1.5017	0.0016
PDGFC	1.223	0.0199		TRAF2	-1.4472	0.0016
CA12	1.247	0.0199		SMAD1	-1.3807	0.0016
DDC	1.5485	0.0203		LAIR-2	-1.8117	0.0017
EDIL3	1.12	0.0237		PIK3AP1	-1.7193	0.0018
XPNPEP2	1.285	0.0268		VSIG4	-1.5046	0.0018
PRTG	1.1026	0.0278		SIGLEC10	-1.4974	0.0019
NQO2	1.0895	0.0282		CD6	-1.758	0.002
AMBP	1.1635	0.0282		SKAP1	-1.8075	0.0021
ERBB2	1.1968	0.0283		FCRL5	-1.3113	0.0021
IL6	2.0047	0.0286		CD177	-1.768	0.0022
IL6	1.8649	0.0297		KLRD1	-1.8117	0.0023
MCP-1	1.4322	0.0301		ERBB2IP	-1.7337	0.0023
VEGFD	1.147	0.0314		MILR1	-1.3829	0.0023
GDF-2	1.3656	0.0326		MIF	-1.7486	0.0024
MUC-16	1.6356	0.0334		SNAP23	-1.5751	0.0024
KLK10	1.2102	0.0341		NUB1	-1.4966	0.0025
FAM3C	1.3109	0.0341		TIGAR	-1.3733	0.0026
uPA	1.1411	0.0346		STAMPB	-1.3721	0.0026
IL6	1.7278	0.0347		DSC2	-1.3652	0.0028
AGR2	1.4472	0.0376		LAIR1	-1.3173	0.0028
METRNL	1.2013	0.039		FKBP1B	-1.9994	0.0029

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
RTN4R	1.195	0.0391		RASSF2	-1.5477	0.003
IGF2R	1.1734	0.0395		FATC1	-1.5044	0.0031
NTRK2	1.118	0.0399		CBL	-1.7183	0.0033
ITGB6	1.152	0.0422		IgG Fc receptor II-b	-1.3893	0.0033
SCARF2	1.1639	0.0422		GLO1	-1.2571	0.0034
SCGB3A2	1.3677	0.0439		PVALB	-2.0291	0.0035
RGMB	1.1254	0.0449		SCAMP3	-1.7405	0.0035
EZR	1.1031	0.0454		SLAMF8	-1.492	0.0035
PROC	1.243	0.0456		STX16	-1.4673	0.0035
FURIN	1.2365	0.0464		TNF-R1	-1.3972	0.0035
PIgR	1.1476	0.049		DFFA	-1.31	0.0038
SMOC2	1.2842	0.0494		PPP1R2	-1.3339	0.0039
				ANG-1	-1.7898	0.004
				CCL5	-1.6357	0.0044
				MAP2K6	-1.8184	0.0046
				CRKL	-1.8003	0.0047
				CD38	-1.4181	0.0048
				CXCL5	-1.7254	0.0052
				PILRA	-1.2582	0.0052
				IRAK1	-1.2986	0.0053
				CA13	-1.8816	0.0054
				STX6	-1.4715	0.0055
				PRTN3	-1.7658	0.0056
				IL-5R-alpha	-1.6599	0.0058
				ESM-1	-1.4178	0.0058
				EGLN1	-1.3184	0.0062
				CLEC1B	-1.7033	0.0063
				TYMP	-1.7313	0.0066
				SNAP29	-1.6325	0.0067
				PDGF subunit A	-1.6021	0.0069
				TNFRSF11A	-1.3519	0.007
				gal-8	-1.3154	0.007
				GCNT1	-1.3034	0.0071
				STK4	-1.8393	0.0072
				TNC	-1.6915	0.0073
				THBS4	-1.7307	0.0075
				CLEC4D	-1.7084	0.0076
				SIGLEC6	-1.9024	0.0078
				WASF1	-1.5354	0.0078

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
				WAS	-2.133	0.0079
				COMT	-1.4304	0.0082
				RETN	-1.8687	0.0084
				SH2D1A	-1.1574	0.0084
				RNASE3	-2.6612	0.0087
				PAR-1	-1.2074	0.0088
				CD69	-1.7621	0.0089
				SIGLEC1	-1.3842	0.0089
				FR-gamma	-1.2115	0.009
				ADAM 8	-1.3896	0.0091
				AZU1	-2.0976	0.0093
				AREG	-1.5881	0.0093
				SDC4	-1.4678	0.0094
				DCTN2	-1.5624	0.0096
				BID	-1.382	0.0097
				RELT	-1.3317	0.0099
				CLEC5A	-1.3618	0.0102
				APEX1	-1.5431	0.0103
				PSP-D	-1.2426	0.0106
				FGR	-1.4406	0.0108
				SELE	-1.5291	0.0112
				SELL	-1.4428	0.0112
				MESDC2	-1.7056	0.0114
				IQGAP2	-1.5317	0.012
				AREG	-1.5142	0.0121
				CRTAM	-1.5805	0.0124
				LILRB2	-1.2555	0.0126
				TANK	-1.3124	0.0127
				CPXM1	-1.4779	0.0131
				ARSB	-1.3432	0.0131
				SLAMF1	-1.2218	0.0133
				PEBP1	-1.307	0.0135
				STIP1	-1.2812	0.0144
				PDGF subunit B	-1.9124	0.0145
				SCARF1	-1.3509	0.0146
				DEFA1	-1.9173	0.0148
				EPHB4	-1.2339	0.015
				ARHGAP1	-1.6039	0.0155
				CLM-1	-1.3921	0.0156
				DAB2	-1.2548	0.0158

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
				LYN	-1.2337	0.0158
				CASP-8	-1.4795	0.016
				APBB1IP	-1.4021	0.0161
				ANXA11	-1.3456	0.0167
				ICAM1	-1.354	0.017
				PRKCQ	-1.3251	0.0171
				VCAM1	-1.2102	0.0173
				HDGF	-1.3392	0.0174
				CD2AP	-1.3188	0.0175
				TNFRSF6B	-1.3504	0.0177
				CLEC1A	-1.2841	0.0179
				TNFRSF14	-1.2658	0.0179
				TACC3	-1.7676	0.0181
				MMP-1	-1.4112	0.0186
				NRP1	-1.1237	0.0187
				ZBTB17	-1.2333	0.0189
				NADK	-1.3493	0.019
				PLXNA4	-1.405	0.0193
				MMP-9	-1.9306	0.0198
				NCR1	-1.3726	0.0202
				AMIGO2	-1.1962	0.0202
				FES	-1.4934	0.0204
				CD79B	-1.2372	0.0206
				TNXB	-1.156	0.0216
				TXNDC5	-1.4081	0.0217
				TRANCE	-1.4034	0.0222
				ARG1	-1.3036	0.0225
				PCDH17	-1.232	0.0228
				LRMP	-1.6365	0.0231
				C1QTNF1	-1.2979	0.0231
				CLM-6	-1.1356	0.0232
				CKAP4	-1.1904	0.0237
				APP	-1.5208	0.0244
				PGLYRP1	-1.6181	0.0255
				LILRA5	-1.342	0.0271
				CLEC10A	-1.274	0.028
				NMNAT1	-1.4212	0.0286
				IL-6RA	-1.1901	0.0287
				ATG4A	-1.3651	0.0289
				TIMP1	-1.2337	0.029

Increased Expression from Baseline to Day 28				Decreased Expression from Baseline to Day 28		
				COCH	-1.22	0.0294
				DKN1A	-1.4302	0.0303
				CD1C	-1.5651	0.0305
				DECR1	-1.4327	0.0316
				DAG1	-1.2406	0.0317
				IGFBP-2	-1.2058	0.0321
				RET	-1.4592	0.0329
				GSAP	-1.4153	0.0338
				PILRB	-1.3019	0.0338
				CLEC6A	-1.3248	0.0343
				PECAM-1	-1.2009	0.0347
				PXN	-1.329	0.0359
				ADGRG1	-1.1823	0.0378
				DPP7	-1.1582	0.038
				TDRKH	-1.2785	0.0385
				Siglec-9	-1.1514	0.0387
				CD40-L	-1.5868	0.0388
				VEGFC	-1.1727	0.04
				LYVE1	-1.227	0.0403
				FADD	-1.546	0.041
				FCRL1	-1.3733	0.0416
				EGF	-1.7729	0.0419
				HGF	-1.5542	0.0426
				GZMH	-1.494	0.0428
				CLEC4G	-1.1865	0.045
				LY75	-1.1401	0.0452
				PRDX3	-1.199	0.0465
				COL4A1	-1.2699	0.0466
				CEACAM8	-1.6177	0.0471
				SEMA7A	-1.1335	0.0475
				NUDT5	-1.5449	0.0476
				FCRL6	-1.3556	0.0476
				PAPPA	-1.3491	0.0485
				FASLG	-1.3614	0.0486
				GRN	-1.2448	0.0486
				MATN3	-1.3384	0.049

Example 7: Protein Expression Levels for Selected Biomarkers in Complete Responder and Progressive Disease/Death Populations

Targeted proteomic analysis of MCP-3 (CCL7), Reg3A, TNFRSF6B, SCF, CXCL10, IL-8, ST2, CALCA, TNF-R1, IL-6, CCL19, IL-2Ra, and PON3 was conducted using the OLINK proximity extension assay platform. Table 14 provides expression information for each of the proteins within the Complete Responder (CR) and Progressive Disease/Death (PD/Death) groups. For each protein, Table 14 includes the median and mean protein expression levels (pg/ml) within each group, standard error, range, and statistical differences between the CR and PD/Death groups. Statistical differences between the groups were identified using an unpaired T test.

Table 14: Protein Expression in Complete Responder and Progressive Disease/Death Populations

Analyte	CR (N=10) (pg/ml)				PD/Death (N=7) (pg/ml)				p value (unpaired t test)
	median	mean	SEM	range	median	mean	SEM	range	
MCP-3 (CCL7)	2.343	2.613	0.4318	0.7949 - 4.862	16.66	21.35	5.705	3.907 - 42.27	0.0013
Reg3A	4150	9252	3597	728.8 - 28209	49259	47951	8907	19613 - 81454	0.0006
TNFRSF6B	159	184.7	26.38	67.97 - 308.7	413.6	411.9	72.41	203 - 681.7	0.0043
SCF	627.6	639	90.92	185.2 - 1171	318.4	283.7	38.16	151.2 - 398.6	0.0071
CXCL10	200.6	307.2	81.94	96.17 - 879.4	941.8	920.4	231.6	147.6 - 1991	0.0121
IL-8	5.332	9.346	2.458	2.629 - 23	42.87	54.49	17.53	15.03 - 155.7	0.0079
ST2	47037	70902	19650	24630 - 205075	142056	163519	38569	55016 - 318173	0.0339
CALCA	1456	1826	392.6	858.5 - 5026	3130	5996	2494	1375 - 19759	0.0669
TNF-R1	9199	8822	1056	3713 - 15630	12659	13129	1265	10015 - 20208	0.0195
IL-6	1.068	0.9521	0.1724	0.1551 - 1.791	3.753	7.156	4.24	0.9551 - 32.2	0.0969
CCL19	439.1	484.9	80.79	156.5 - 973.4	1036	1751	663.3	203.6 - 5450	0.0377
IL-2Ra	355	469.8	94.9	63.64 - 1178	612.6	577.4	96.43	213.8 - 856.9	0.4771
PON3	386584	438566	61426	254507 - 885782	93702	148321	37162	50041 - 284920	0.0025

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.