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(54) **BIOMARKERS FOR GRAFT-VERSUS-HOST DISEASE**

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(58) **Field of Classification Search**

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(57) **ABSTRACT**

Biomarkers are provided that are predictive of a subject's responsiveness to a therapy comprising a JAK inhibitor. The biomarkers, compositions, and methods described herein are useful in selecting appropriate treatment modalities for a subject having, suspected of having, or at risk of developing Graft-Versus-Host Disease.

32 Claims, No Drawings

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BIOMARKERS FOR GRAFT-VERSUS-HOST DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 16/381,158, filed Apr. 11, 2019, issued as U.S. Pat. No. 11,372,003, which claims priority to U.S. Provisional Appl. No. 62/657,193, filed Apr. 13, 2018, and U.S. Provisional Appl. No. 62/773,308, filed Nov. 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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 concen-

tration, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, NFK-BIE, hOSCAR, LAT, PTPRJ, SIRT2, SIRPB1, AXIN1, EIF4G1, PTX3, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, IDUA, NCF2, SELP, ARHGEF12, CASP-3, CD27, MAP4K5, DAPP1, PRDX5, TLT-2, PARK7, IL2-RA, FOXO1, ST1A1, GRAP2, NBN, CD93, FCGR2A, DCTN1, IRF9, 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, TRIMS, 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, PL1N1, 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, TRIMS, 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, TRIMS, 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, CEACAM5, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, TRIMS, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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, CASA, 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 hav-

ing, 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

TABLE 1-continued

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
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
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

TABLE 2-continued

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
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, CASA, 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, TRIMS, 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, NINE, 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, TRIMS, 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, NINE, 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, immunochromatographic 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., U.S. Pat. Nos. 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 U.S. Pat. No. 7,598,257 (Example 67), filed Dec. 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 $—CH_3$).

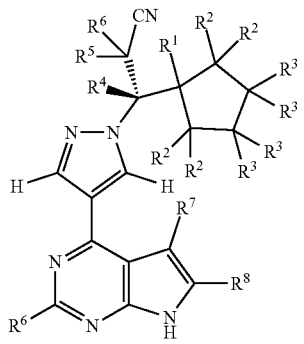
One or more constituent atoms of the compounds described herein can be replaced or substituted with isotopes of the atoms in natural or non-natural abundance. In some embodiments, the compound includes at least one deuterium atom. In some embodiments, the compound includes two or more deuterium atoms. 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 HID 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 U.S. Pat. No. 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

-continued

Compound	R^1	Each R^2	Each R^3	R^4	Each R^5
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 U.S. Pat. No. 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

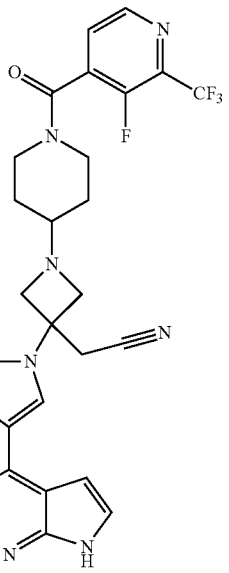
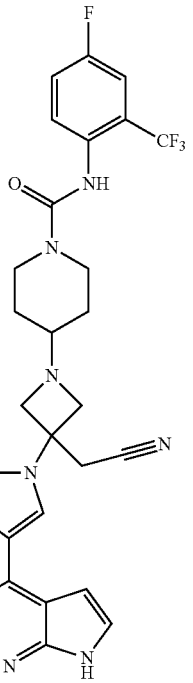
Comp. No.	Prep.	Name	Structure
1	US 2011/0224190 (Example 1)	{1-{1-[3-Fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile (itacitinib; ; also known as INCB039110)	
2	US 2011/0224190 (Example 154)	4-{3-(Cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-[4-fluoro-2-(trifluoromethyl)phenyl]piperidine-1-carboxamide	

TABLE 3-continued

Examples of JAK inhibitors

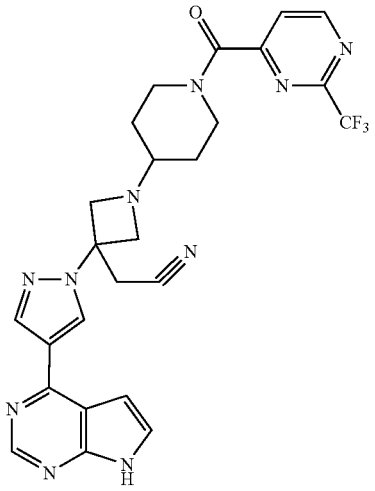
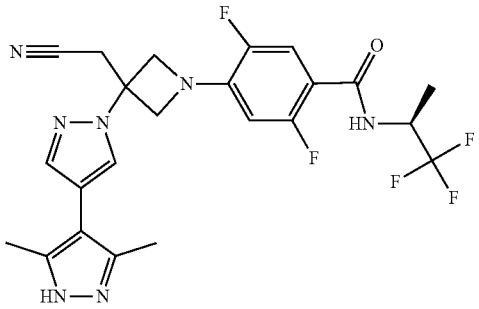
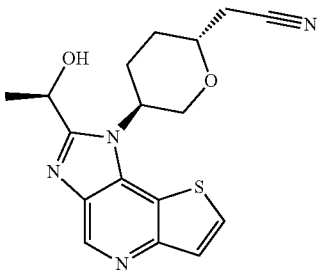
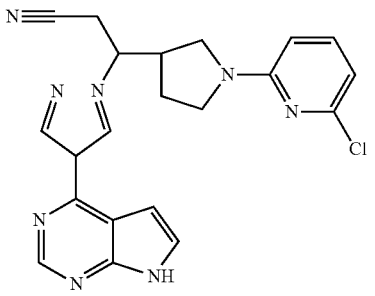
Comp. No.	Prep.	Name	Structure
3	US 2011/0224190 (Example 85)	[3-[4-(7H-pyrrolo[2,3 -d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-1-(1-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperidin-4-yl)azetidin-3-yl]acetonitrile	
4	US 2014/0343030 (Example 7)	4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]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	
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	

TABLE 3-continued

Examples of JAK inhibitors

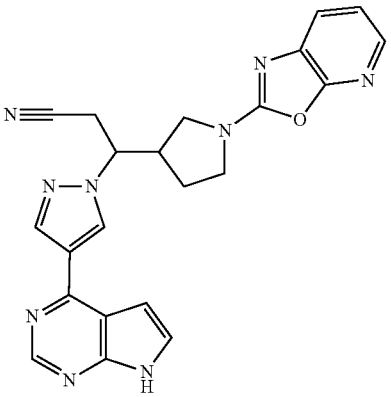
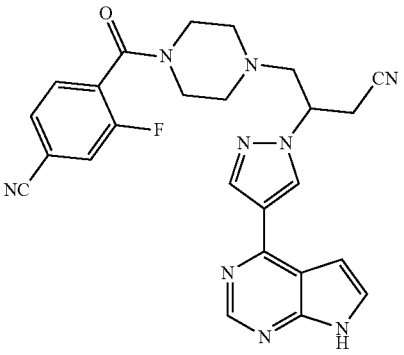
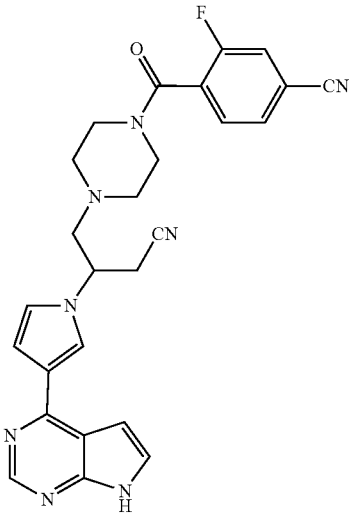
Comp. No.	Prep.	Name	Structure
7	US 2010/0298334 (Example 13c)	3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile	
8	US 2011/0059951 (Example 12)	4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile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

TABLE 3-continued

Examples of JAK inhibitors

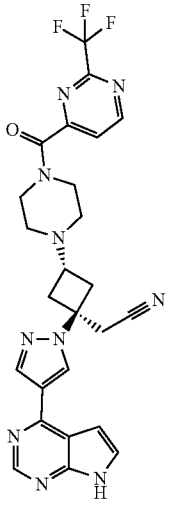
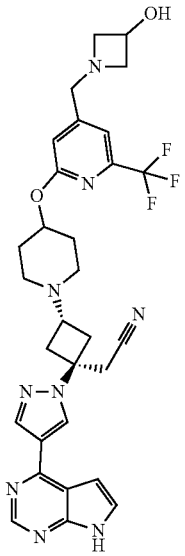
Comp. No.	Prep.	Name	Structure
10	US 2012/0149681 (Example 7b)	[trans-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-(4-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperazin-1-yl)cyclobutyl]acetonitrile	
11	US 2012/0149681 (Example 157)	{trans-3-(4-{[4-[(3-hydroxyazetidin-1-yl)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrro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

TABLE 3-continued

Examples of JAK inhibitors

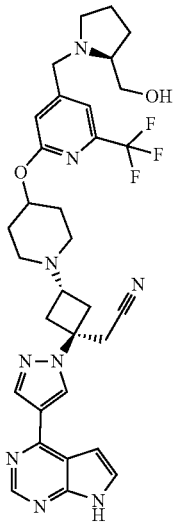
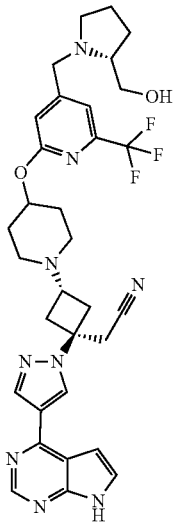
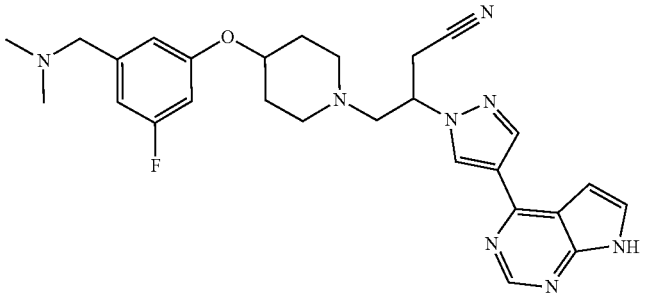
Comp. No.	Prep.	Name	Structure
12	US 2012/0149681 (Example 161)	{trans-3-(4-{[4-{[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile	
13	US 2012/0149681 (Example 162)	{trans-3-(4-{[4-{[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)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	

TABLE 3-continued

Examples of JAK inhibitors

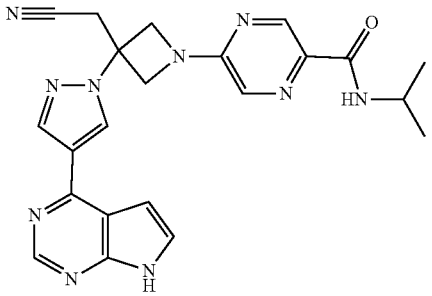
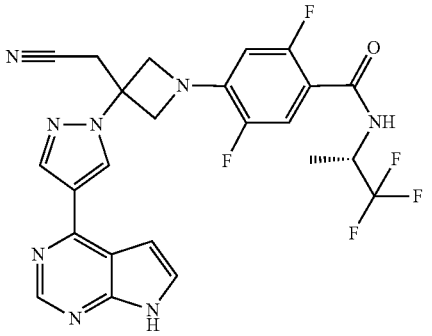
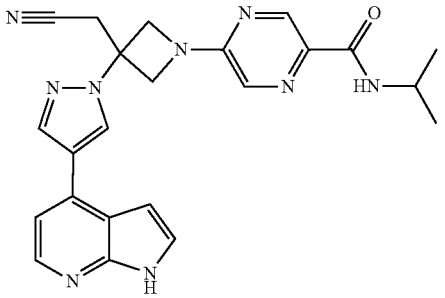
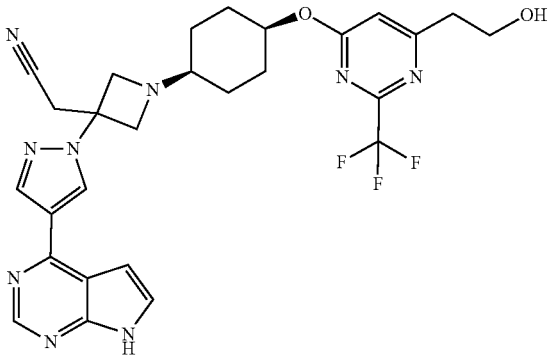
Comp. No.	Prep.	Name	Structure
15	US 2013/0018034 (Example 18)	5-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide	
16	US 2013/0018034 (Example 28)	4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide	
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	

TABLE 3-continued

Examples of JAK inhibitors

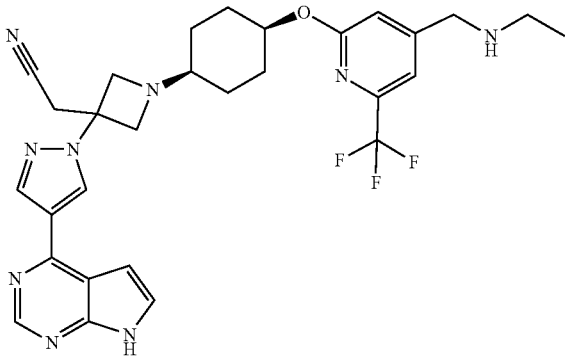
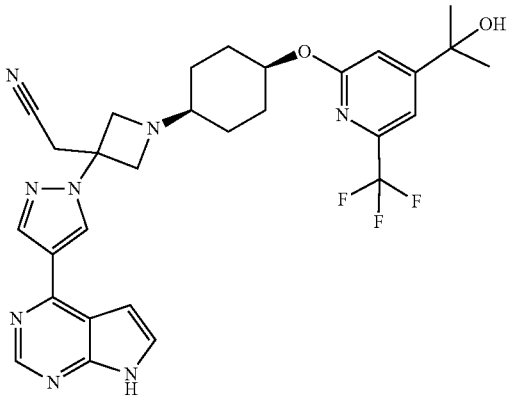
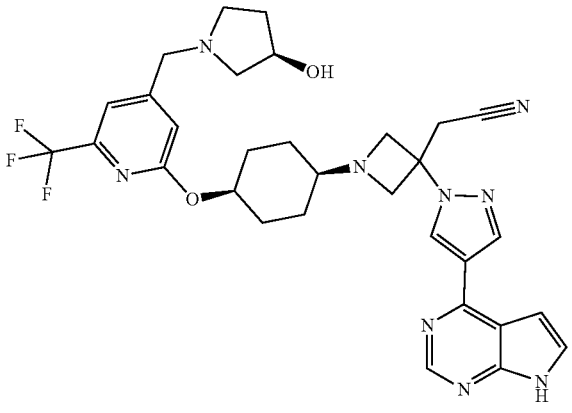
Comp. No.	Prep.	Name	Structure
19	US 2013/0045963 (Example 65)	{1-(cis-4-{[4-[(ethylamino)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
20	US 2013/0045963 (Example 69)	{1-(cis-4-{[4-(1-hydroxy-1-methylethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[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-yl}acetonitrile	

TABLE 3-continued

Examples of JAK inhibitors

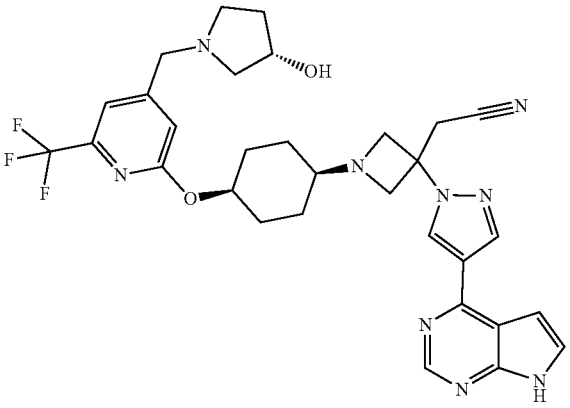
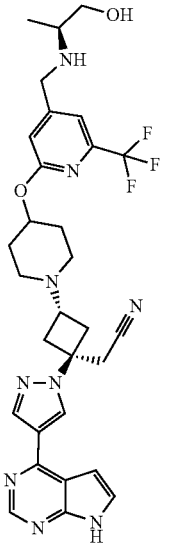
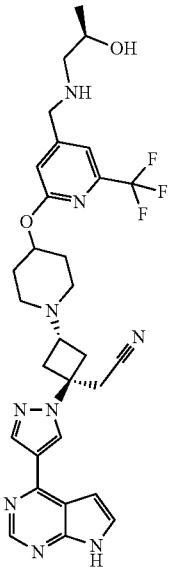
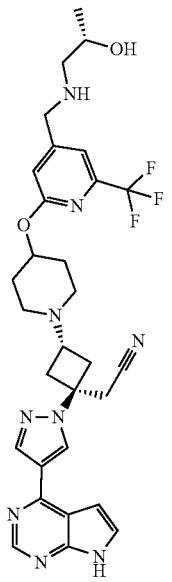
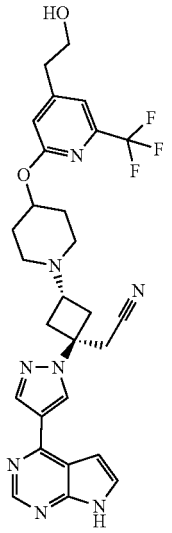
Comp. No.	Prep.	Name	Structure
22	US 2013/0045963 (Example 95)	{1-(cis-4-{[4-{[(3S)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile	
23	US 2014/0005166 (Example 1)	{trans-3-(4-{[4-({[(1S)-2-hydroxy-1-methylethyl]amino}methyl)-6-(trifluoromethyl)pyri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

TABLE 3-continued

Examples of JAK inhibitors

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 Mar. 9, 2011, US Patent Publ. No. 2013/0060026, filed Sep. 6, 2012, and US Patent Publ. No. 2014/0256941, filed Mar. 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 Oct. 31, 2013 and US Patent Publ. No. 2015/0344497, filed Apr. 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 Mar. 9, 2011, US Patent Publ. No. 2014/0343030, filed May 16, 2014, US Patent Publ. No. 2014/0121198, filed Oct. 31, 2013, US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed Aug. 31, 2010, US Patent Publ. No. 2012/0149681, filed Nov. 18, 2011, US Patent Publ. No. 2012/0149682, filed Nov. 18, 2011, US Patent Publ. 2013/0018034, filed Jun. 19, 2012, US Patent Publ. No. 2013/0045963, filed Aug. 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 Mar. 9, 2011, US Patent Publ. No. 2014/0343030, filed May 16, 2014, US Patent Publ. No. 2014/0121198, filed Oct. 31, 2013, US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed Aug. 31, 2010, US Patent Publ. No. 2012/0149681, filed Nov. 18, 2011, US Patent Publ. No. 2012/0149682, filed Nov. 18, 2011, US Patent Publ. 2013/0018034, filed Jun. 19, 2012, US Patent Publ. No. 2013/0045963, filed Aug. 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
			IL8	−4.0642	0.0216
LEP	3.0194	0.0441	IL6	−3.8938	0.0207
FKBP1B	2.9644	0.0357	SPINK1	−3.8821	0.012
MBL2	2.9639	0.0094	CXCL10	−3.8124	0.0065
SCF	2.9526	0.0026	SULT2A1	−3.5143	0.0149
GAL	2.936	0.0119	IL6	−3.4705	0.0258
SCF	2.9112	0.003	ENPP7	−3.3791	0.0388
ITGB1BP2	2.7985	0.0306	PLXNB1	−2.99	0.0105
PVALB	2.6979	0.0084	VAMP5	−2.9842	0.0315
THPO	2.6532	0.0056	CCL19	−2.9578	0.0041
CD40-L	2.645	0.041	CTSL1	−2.9056	0.0018
ANG-1	2.6308	0.0116	ACE2	−2.8742	0.0054
SCGB3A1	2.5332	0.0093	IL6	−2.8377	0.0121
CD69	2.4963	0.0119	CRTAM	−2.6741	0.0312
FAM3B	2.481	0.0235	ALDH1A1	−2.6667	0.0141
GH	2.4121	0.0155	SIGLEC10	−2.5993	0.0148
CCL5	2.3402	0.0064	KRT19	−2.5964	0.0312
MANF	2.3026	0.0174	SLAMF8	−2.5417	0.0189
SRC	2.1146	0.036	IL6	−2.5189	0.0141
CRISP2	2.0487	0.0148	CDCP1	−2.4173	0.0113
SAA4	2.0164	0.0348	N2DL-2	−2.3079	0.0075
CR2	2.0089	0.0197	GZMB	−2.307	0.0289
SERPINA5	2.0067	0.0044	TNFRSF6B	−2.3053	0.0037
PFKM	1.9756	8.93E−05	KYNU	−2.2368	0.0217
APOM	1.9231	0.0255	FOSB	−2.2367	0.0169
DCTN2	1.8684	0.0304	ALDH3A1	−2.1808	0.0104
HSD11B1	1.8131	0.0173	IGFBP-1	−2.1383	0.0472
PDGF subunit A	1.7697	0.0424	CLM-1	−2.1323	0.012
			NFATC3	−2.0978	0.0189
IGFBP3	1.7304	0.0287	HAVCR2	−2.0933	0.0196
HS3ST3B1	1.7175	0.0355	TNF-R2	−2.0374	0.0091
CDSN	1.7055	0.0227	DDAH1	−2.036	0.0289
APP	1.7003	0.0484	CD74	−1.9824	0.0051
TWEAK	1.6972	0.0338	CKAP4	−1.9573	0.0069
TN-R	1.653	0.0321	NINJ1	−1.9125	0.0043
AMBP	1.6302	0.0127	ENTPD2	−1.8643	0.0227
CNTN1	1.5831	0.0275	TNFRSF9	−1.8239	0.0276
GCP5	1.5583	0.0383	SIGLEC1	−1.8208	0.0229
CNDP1	1.555	0.0034	PREB	−1.8178	0.0327

TABLE 4-continued

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
NCAM1	1.5413	0.0011	AHCY	−1.7883	0.043
PROC	1.514	0.0289	IL12RB1	−1.7236	0.0186
F11	1.489	0.008	TNFRSF10A	−1.7188	0.0133
NCAN	1.4147	0.0259	SIRPB1	−1.7175	0.0467
NTRK3	1.3885	0.0255	DSC2	−1.6964	0.0261
TIMP4	1.3827	0.044	U-PAR	−1.687	0.0422
VEGFD	1.3649	0.0365	TNFRSF4	−1.677	0.0423
HSP27	1.3522	0.0446	TNFRSF10A	−1.6687	0.0145
GALNT10	1.3452	0.0207	IL-18R1	−1.668	0.0121
CCL11	1.3225	0.0111	IL-1ra	−1.6559	0.0334
LY75	1.2722	0.0376	CLEC7A	−1.6558	0.0316
DKKL1	1.2291	0.0499	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	−2.7388	0.001
GAL	2.0603	0.0052	subunit B		
THPO	1.8262	0.0094	FKBP1B	−2.5654	0.0036
FAM3B	1.7937	0.0011	ITGB1BP2	−2.1159	0.0047
CNDP1	1.5692	0.0134	CD69	−2.1102	0.0016
CCL11	1.401	0.0013	ANG-1	−2.0923	0.0062
SERPINA5	1.3313	0.0089	PVALB	−2.0632	0.0145
DKKL1	1.2705	0.0014	CD40-L	−1.9741	0.0256
NCAM1	1.2481	0.0284	CCL5	−1.9502	0.0065
SPON2	1.1003	0.0106	HS3ST3B1	−1.9303	0.0193
THBS2	1.0834	0.0051	MBL2	−1.9284	0.0215
GCG	2.3961	0.0168	CLEC7A	−1.8985	0.0006
GAL	2.0603	0.0052	APP	−1.8356	0.0053
THPO	1.8262	0.0094	PDGF	−1.7507	0.0068
FAM3B	1.7937	0.0011	subunit A		
CNDP1	1.5692	0.0134	DCTN2	−1.6367	0.0274

TABLE 5-continued

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
CCL11	1.401	0.0013	SLAMF8	−1.5899	0.0244
SERPINA5	1.3313	0.0089	VAMP5	−1.5851	0.0053
DKKL1	1.2705	0.0014	SIGLEC10	−1.4859	0.0226
NCAM1	1.2481	0.0284	CLM-1	−1.4729	0.0319
SPON2	1.1003	0.0106	DSC2	−1.4423	0.0017
THBS2	1.0834	0.0051	HAVCR2	−1.3876	0.0126
			SIRPB1	−1.3861	0.0072
			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.

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	HSP27	−1.1778	0.1094
CNTN1	1.2158	0.1831	SAA4	−1.1593	0.5157
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			

TABLE 6-continued

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
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 REG3a, TNFR1, and ST2 levels at baseline. Correlation refers to potential biomarkers showing similar change or distribution as REG3a, TNFR1, and ST2. Table 7 identifies proteins that significantly ($p<0.1$) correlate with REG3a, TNFR1, and ST2 at baseline. Table 8 identifies proteins that do not significantly ($p>0.1$) correlate with REG3a, 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	HSP27	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
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

TABLE 8-continued

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
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
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	HSP27	−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 REG3a, 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

TABLE 9-continued

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
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
HSP27	1.3522	0.0446	DDAH1	−2.036	0.0289
GALNT10	1.3452	0.0207	NINJ1	−1.9125	0.0043
			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
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 log 2 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
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
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

TABLE 11-continued

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
			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
			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
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

TABLE 12-continued

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
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

TABLE 13-continued

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
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
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			
GAL	1.6888	0.0008	CNTNAP2	−1.3091	0.0001
KIM1	1.757	0.0009	ZBTB16	−1.9166	0.0002
CPM	1.1479	0.0011	CD163	−1.7206	0.0002
VWC2	1.3331	0.0011	TXLNA	−1.5277	0.0002
PPY	1.9703	0.0012	MEPE	−1.4941	0.0002
PAM	1.1653	0.0014	BACH1	−1.4794	0.0002
PVR	1.1964	0.0015	MAX	−1.449	0.0002
SERPINA5	1.2683	0.0015	NFKBIE	−1.2885	0.0002
ST3GAL1	1.3415	0.0016	hOSCAR	−1.2603	0.0002
CST5	1.3521	0.002	LAT	−2.049	0.0003
CES2	1.4212	0.0022	PTPRJ	−1.7421	0.0003
CNDP1	1.3856	0.0024	SIRT2	−1.6014	0.0003
CX3CL1	1.5454	0.0024	SIRPB1	−1.3991	0.0003
HO-1	1.3293	0.0028	AXIN1	−1.8046	0.0004
PRELP	1.1833	0.0029	EIF4G1	−1.5969	0.0004
ADM	1.2035	0.003	PTX3	−1.5567	0.0004
VSIG2	1.2163	0.0031	TRIM5	−1.4743	0.0004
FABP2	3.5893	0.0031	IDUA	−1.3887	0.0004
CEACAM5	1.6422	0.0039	NCF2	−2.4951	0.0005
SLITRK2	1.2986	0.004	SELP	−1.9244	0.0005
MCP-1	1.6302	0.0044	ARHGEF12	−1.8347	0.0005
NTRK3	1.2526	0.0045	CASP-3	−1.787	0.0005
CLUL1	1.3108	0.0046	CD27	−1.6873	0.0005
CXCL16	1.2028	0.0053	MAP4K5	−1.658	0.0005
SCF	1.4714	0.0056	DAPP1	−1.3805	0.0005
TMPRSS5	1.3307	0.0057	PRDX5	−2.0131	0.0006
REG4	2.1118	0.0059	TLT-2	−1.8372	0.0006
hK11	1.2975	0.0061	PARK7	−1.4104	0.0006
SCF	1.3943	0.0061	IL2-RA	−2.2452	0.0007
SCGB3A1	1.5691	0.0061	FOXO1	−1.3557	0.0007
DKKL1	1.1495	0.0071	ST1A1	−1.9523	0.0008
NEP	1.8376	0.0077	GRAP2	−1.6468	0.0008
CPA2	1.7185	0.0088	NBN	−1.5879	0.0008
Ep-CAM	1.403	0.0089	CD93	−1.3029	0.0008
THBS2	1.0963	0.0091	FCGR2A	−1.5278	0.0009
GPNMB	1.2117	0.0092	DCTN1	−1.4726	0.0009
ITGB5	1.229	0.0104	IRF9	−1.4384	0.0009
GT	1.8699	0.0104	HAVCR2	−1.312	0.0009
APLP1	1.4933	0.0117	CD84	−1.7208	0.001
TACSTD2	1.1931	0.0119	STX8	−1.4796	0.001
NINJ1	1.2723	0.012	LY9	−1.4447	0.001
SCF	1.4058	0.0123	ZBTB16	−1.9288	0.0011
REN	1.4074	0.0137	CD200R1	−1.4752	0.0011
GCG	1.8922	0.0137	TOP2B	−1.7634	0.0012
SERPINA9	1.5418	0.0151	THY 1	−1.2913	0.0012
KAZALD1	1.2609	0.0154	PRKRA	−1.2761	0.0012
SERPINA12	1.567	0.0155	ITGB1BP2	−1.8787	0.0013
PODXL	1.2014	0.0163	CD48	−1.6022	0.0013
AMN	1.2517	0.017	CD244	−1.5176	0.0014
IGF1R	1.2432	0.0171	HCLS1	−1.455	0.0014
LTBP2	1.1874	0.0175	MPO	−1.8431	0.0015
ANGPTL3	1.2673	0.0177	SIT1	−1.5501	0.0015
SCARA5	1.1342	0.0179	ICAM3	−1.464	0.0015
B4GAT1	1.2795	0.0179	SOST	−1.3214	0.0015
ROBO2	1.249	0.0181	DDX58	−1.6381	0.0016
PDGFC	1.223	0.0199	TNF-R2	−1.5017	0.0016
CA12	1.247	0.0199	TRAF2	−1.4472	0.0016
DDC	1.5485	0.0203	SMAD1	−1.3807	0.0016
EDIL3	1.12	0.0237	LAIR-2	−1.8117	0.0017
XPNPEP2	1.285	0.0268	PIK3AP1	−1.7193	0.0018
PRTG	1.1026	0.0278	VSIG4	−1.5046	0.0018
NQO2	1.0895	0.0282	SIGLEC10	−1.4974	0.0019

TABLE 13-continued

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
AMBP	1.1635	0.0282	CD6	−1.758	0.002
ERBB2	1.1968	0.0283	SKAP1	−1.8075	0.0021
IL6	2.0047	0.0286	FCRL5	−1.3113	0.0021
IL6	1.8649	0.0297	CD177	−1.768	0.0022
MCP-1	1.4322	0.0301	KLRD1	−1.8117	0.0023
VEGFD	1.147	0.0314	ERBB2IP	−1.7337	0.0023
GDF-2	1.3656	0.0326	MILR1	−1.3829	0.0023
MUC-16	1.6356	0.0334	MIF	−1.7486	0.0024
KLK10	1.2102	0.0341	SNAP23	−1.5751	0.0024
FAM3C	1.3109	0.0341	NUB1	−1.4966	0.0025
uPA	1.1411	0.0346	TIGAR	−1.3733	0.0026
IL6	1.7278	0.0347	STAMPB	−1.3721	0.0026
AGR2	1.4472	0.0376	DSC2	−1.3652	0.0028
METRNL	1.2013	0.039	LAIR1	−1.3173	0.0028
RTN4R	1.195	0.0391	FKBP1B	−1.9994	0.0029
IGF2R	1.1734	0.0395	RASSF2	−1.5477	0.003
NTRK2	1.118	0.0399	FATC1	−1.5044	0.0031
ITGB6	1.152	0.0422	CBL	−1.7183	0.0033
SCARF2	1.1639	0.0422	IgG Fc	−1.3893	0.0033
SCGB3A2	1.3677	0.0439	receptor II-b		
RGMB	1.1254	0.0449	GLO1	−1.2571	0.0034
EZR	1.1031	0.0454	PVALB	−2.0291	0.0035
PROC	1.243	0.0456	SCAMP3	−1.7405	0.0035
FURIN	1.2365	0.0464	SLAMF8	−1.492	0.0035
PIgR	1.1476	0.049	STX16	−1.4673	0.0035
SMOC2	1.2842	0.0494	TNF-R1	−1.3972	0.0035
			DFFA	−1.31	0.0038
			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
			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

TABLE 13-continued

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
			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
			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
			COCH	−1.22	0.0294
			DKN1A	−1.4302	0.0303
			CDIC	−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

TABLE 13-continued

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
			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
			NUDTS	−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									
	CR (N = 10) (pg/ml)				PD/Death (N = 7) (pg/ml)				p value (unpaired
Analyte	median	mean	SEM	range	median	mean	SEM	range	t test)
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

TABLE 14-continued

Protein Expression in Complete Responder and Progressive Disease/Death Populations

	CR (N = 10) (pg/ml)				PD/Death (N = 7) (pg/ml)				p value (unpaired
Analyte	median	mean	SEM	range	median	mean	SEM	range	t test)
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.

What is claimed is:

1. A method of treating a human subject having Graft-Versus-Host Disease (GvHD), comprising administering to the human subject a therapy comprising a JAK inhibitor, wherein the human subject has been previously determined to have a baseline concentration of at least one protein selected from the group consisting of MCP-3, CALCA, and REG3A in a biological sample obtained from the human subject that is lower than a control, wherein the control is the concentration of the at least one protein in a sample or samples obtained from one or more subjects having GvHD that have not responded to treatment with the JAK inhibitor.

2. The method of claim **1**, wherein the human subject has been previously determined to have a baseline concentration of each of the proteins MCP-3, CALCA, and REG3A in the biological sample obtained from the human subject that is lower than a control, wherein the control is the concentration of the proteins MCP-3, CALCA, and REG3A, respectively, in a sample or samples obtained from one or more subjects having GvHD that have not responded to treatment with the JAK inhibitor.

3. A method of treating a human subject having Graft-Versus-Host Disease (GvHD), comprising:

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, CALCA, and REG3A; and

administering a therapy comprising a JAK inhibitor to the human subject,

wherein the control is the concentration of the at least one protein in a sample or samples obtained from one or more subjects having GvHD that have not responded to treatment with the JAK inhibitor.

4. The method of claim **3**, comprising:

measuring in the biological sample the reduced concentration, as compared to a control, of each of the proteins MCP-3, CALCA, and REG3A, wherein the control is the concentration of each of the proteins MCP-3, CALCA, and REG3A, respectively, in a sample or samples obtained from one or more subjects having GvHD that have not responded to treatment with the JAK inhibitor; and

administering the therapy comprising the JAK inhibitor to the human subject.

5. The method of claim **1**, wherein a second therapeutic agent is administered to the human subject in combination with the JAK inhibitor.

6. The method of claim **5**, wherein the second therapeutic agent is a corticosteroid, methotrexate, cyclosporine, mycophenolate mofetil, tacrolimus, sirolimus, everolimus, antithymocyte globulin, alemtuzumab, cyclophosphamide, ibrutinib, imatinib, infliximab, etanercept, tocilizumab, basiliximab, daclizumab, rituximab, denileukin diftitox, pentostatin, thalidomide, halofuginone, hydroxychloroquine, or mesenchymal stem cells.

7. The method of claim **5**, wherein the second therapeutic agent is a corticosteroid.

8. The method of claim **7**, wherein the corticosteroid is methylprednisolone or prednisone.

9. The method of claim **1**, wherein the biological sample is blood, serum, plasma, urine, spinal fluid, saliva, lacrimal fluid, or sweat.

10. The method of claim **1**, wherein the biological sample is blood, serum, or plasma.

11. The method of claim **1**, wherein the concentration of the at least one protein is measured by an immunological method.

12. The method of claim **11**, wherein the immunological method is selected from the group consisting of enzyme-linked immunosorbent assay, enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immunochromatographic assay, and western blotting.

13. The method of claim **1**, wherein the concentration of the at least one protein is measured by mass spectrometry.

14. The method of claim **1**, wherein the JAK inhibitor is itacitinib.

15. The method of claim **1**, wherein 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.

16. The method of claim **1**, wherein the GvHD is acute GvHD.

17. The method of claim **1**, wherein the GvHD is chronic GvHD.

18. The method of claim **3**, wherein a second therapeutic agent is administered to the human subject in combination with the JAK inhibitor.

19. The method of claim 18, wherein the second therapeutic agent is a corticosteroid, methotrexate, cyclosporine, mycophenolate mofetil, tacrolimus, sirolimus, everolimus, antithymocyte globulin, alemtuzumab, cyclophosphamide, ibrutinib, imatinib, infliximab, etanercept, tocilizumab, basiliximab, daclizumab, rituximab, denileukin diftitox, pentostatin, thalidomide, halofuginone, hydroxychloroquine, or mesenchymal stem cells.

20. The method of claim 18, wherein the second therapeutic agent is a corticosteroid.

21. The method of claim 20, wherein the corticosteroid is methylprednisolone or prednisone.

22. The method of claim 3, wherein the biological sample is blood, serum, plasma, urine, spinal fluid, saliva, lacrimal fluid, or sweat.

23. The method of claim 3, wherein the biological sample is blood, serum, or plasma.

24. The method of claim 3, wherein the concentration of the at least one protein is measured by an immunological method.

25. The method of claim 24, wherein the immunological method is selected from the group consisting of enzyme-linked immunosorbent assay, enzyme immunoassay, radioimmunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, latex turbidimetric immunoassay, latex photometric immunoassay, immunochromatographic assay, and western blotting.

26. The method of claim 3, wherein the concentration of the at least one protein is measured by mass spectrometry.

27. The method of claim 3, wherein the JAK inhibitor is itacitinib.

28. The method of claim 3, wherein 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.

29. The method of claim 3, wherein the GvHD is acute GvHD.

30. The method of claim 3, wherein the GvHD is chronic GvHD.

31. The method of claim 2, wherein the JAK inhibitor is itacitinib.

32. The method of claim 4, wherein the JAK inhibitor is itacitinib.

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