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(54) **Title:** METHODS FOR TREATING MYELOPROLIFERATIVE DISORDERS

(57) **Abstract:** In part, the disclosure relates to methods of treating myeloproliferative disorders by administering one or more Serum Amyloid Protein (SAP) proteins. In certain aspects, the method further comprises monitoring treatment efficacy by measuring change in mutant allele burden. In certain aspects, the disclosure relates to methods of treating myelofibrosis in patient sub-populations who carry myelofibrosis-associated mutations in some of their cells by administering an SAP protein.



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METHODS FOR TREATING MYELOPROLIFERATIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to and the benefit of U.S. provisional patent
5 application serial numbers 62/148,005 filed on April 15, 2015 and 62/218,869 filed on
September 15, 2015, the disclosures of which are incorporated herein by reference in their
entirety.

BACKGROUND OF THE INVENTION

Myeloproliferative disease (MPD), also referred to as myeloproliferative neoplasms
10 (MPN), refers to a group of disorders characterized by clonal abnormalities of the blood cells,
such as blood cells and precursors of the myeloid lineage. Such disorders may impact
myeloid, erythroid, and platelet cells. Myeloproliferative disorders can be challenging to
diagnose and treat.

In certain proliferative conditions, such as myelofibrosis (MF), replacement of healthy
15 organ tissue by fibrosis results in inadequate organ function, which contributes to the
symptoms of the disorder. Myelofibrosis (including primary myelofibrosis, post-
polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis) is a
clonal myeloproliferative neoplasm, characterized by progressive bone marrow fibrosis and
subsequent ineffective erythropoiesis, dysplastic megakaryocyte hyperplasia, and extramedullary
20 hematopoiesis. The typical clinical presentation includes marked splenomegaly, progressive
anemia, and constitutional associated with high morbidity and mortality. Moreover, most
patients are not suitable transplant candidates.

Until recently, there was no approved medical therapy for MF and most subjects were
managed with various combinations of growth factors, immunomodulatory agents, cytotoxic
25 chemotherapy, and steroids. None of these therapies produced significant responses in the
majority of subjects. For this reason, no medication has been approved for MF until recently.

Ruxolitinib is a Janus kinase inhibitor, recently approved in the US and EU for the
treatment of subjects with intermediate or high-risk myelofibrosis, including primary
myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF) and post-essential
30 thrombocythemia (post-ET MF) (JAKAFI® Full Prescribing Information 2011). Treatment with
ruxolitinib results in reduction in spleen volume and improvement in constitutional symptoms,
but does not appear to have an effect on bone marrow fibrosis or on allele burden.

There is a clear unmet medical need for new therapies that could improve bone marrow fibrosis in subjects with myelofibrosis with a resultant improvement in blood counts and other disease-related factors. While a number of drugs have been developed and evaluated in MF clinical trials, none of the drugs so far have displayed a selective anti-clonal effect, despite activity in alleviating other symptoms. Therefore, a need remains for developing additional therapeutic options for the treatment of myeloproliferative disorders such as myelofibrosis.

SUMMARY OF THE INVENTION

The disclosure provides various methods, such as various methods of treating a myeloproliferative disorder. The methods include various methods of administering serum amyloid P (SAP) protein or pentraxin-2, such as administering SAP protein to a subject in multiple doses according to a dosage regimen. Optionally, the methods include one or more steps in which one or more genes in a sample taken from the subject are evaluated to determine mutational status (e.g., whether some of the subject's cells comprise a mutation associated with the myeloproliferative disorder). In certain embodiments, subjects having a certain mutational status are specifically selected for treatment or the dosage regimen is adjusted based on mutational status. In other embodiments, mutational status is evaluated over the course of treatment to determine impact of treatment on allele burden. In some embodiments, the dosage regimen is adjusted based on the patient's responsiveness, such as impact on allele burden or impact on one or more other measures of symptom improvement.

In certain aspects, the disclosure provides a method of treating a myeloproliferative disorder, the method comprising administering to a subject in need thereof an effective amount of a serum amyloid P (SAP) protein, wherein some of the subject's cells comprise a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In other words, some of the subject's cells carry a mutation associated with the myeloproliferative disorder (e.g., the subject comprises cells carrying a mutation in one or more of the foregoing genes). In some embodiments, the subject comprises more than one mutation associated with the myeloproliferative disorder, such as a mutation in two or three (or more than three) of the foregoing genes. In certain embodiments, administering comprises administering the SAP protein in multiple doses according to a dosing schedule and/or dosage regimen, such as to achieve a therapeutic effect.

In certain aspects, the disclosure provides a method of treating a myeloproliferative disorder, comprising administering to a subject in need thereof an effective amount of a

serum amyloid P (SAP) protein, wherein some of the subject's cells comprise a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*, and wherein the SAP protein is administered according to a dosage regimen effective to reduce mutant allele burden of said gene in said subject. In other words, some of the subject's cells carry a mutation associated with the myeloproliferative disorder (e.g., the subject comprises cells carrying a mutation in one or more of the foregoing genes). In some embodiments, the subject comprises more than one mutation associated with the myeloproliferative disorder, such as a mutation in two or three (or more than three) of the foregoing genes. In certain embodiments, the dosage regimen is also effective to improve one or more other manifestations of the myeloproliferative disorder, such as effective to reduce bone marrow fibrosis by at least one grade.

In certain aspects, the disclosure provides a method for reducing mutant allele burden in a subject having a myeloproliferative disorder, the method comprising administering to a subject in need thereof an effective amount of a serum amyloid P (SAP) protein, wherein the subject comprises a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In other words, some of the subject's cells carry a mutation associated with the myeloproliferative disorder (e.g., the subject comprises cells carrying a mutation in one or more of the foregoing genes). In some embodiments, the subject comprises more than one mutation associated with the myeloproliferative disorder, such as a mutation in two or three (or more than three) of the foregoing genes. In certain embodiments, the dosage regimen is also effective to improve one or more other manifestations of the myeloproliferative disorder, such as effective to reduce bone marrow fibrosis by at least one grade. In certain embodiments, administering an effective amount comprises administering SAP protein according to a dosage regimen (e.g., multiple doses according to a dosing schedule).

In certain aspects, the disclosure provides a method for treating a myeloproliferative disorder with a serum amyloid P (SAP) protein, the method comprising: (i) measuring a first mutant allele burden of a mutation in one or more genes associated with the myeloproliferative disorder selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the

first mutant allele burden, wherein a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder. In some embodiments, mutant allele burden in more than one gene is measured (e.g., in two, three or more than three genes). In certain
5 embodiments, the measuring performed in step (ii) is performed following approximately 1 cycle of treatment and/or following about 1 month of treatment. In other embodiments, the measuring performed in step (ii) is performed following approximately 2 or 3 cycles of treatment and/or following about 2 or 3 months of treatment. However, step (ii) may be performed sooner or later in the course of treatment. Moreover, the disclosure contemplates
10 that allele burden may be evaluated over time of treatment to ascertain decrease in allele burden and durability of response.

In certain aspects, the disclosure provides a method for treating a myeloproliferative disorder, comprising: (i) determining whether the cells of a subject having a myeloproliferative disorder comprise a mutation associated with the myeloproliferative
15 disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*; and if the subject carries said mutant allele (ii) administering an effective amount of a serum amyloid P (SAP) protein to the subject. In other words, some of the subject's cells carry a mutation associated with the myeloproliferative disorder (e.g., the subject comprises cells carrying a mutation in one or more of the foregoing genes). In some
20 embodiments, the subject comprises more than one mutation associated with the myeloproliferative disorder, such as a mutation in two or three (or more than three) of the foregoing genes. In some embodiments, the mutation associated with the myeloproliferative disorder is not JAK2V617F. In certain embodiments, administering comprises administering the SAP protein in multiple doses according to a dosing schedule and/or dosage regimen,
25 such as to achieve a therapeutic effect. In certain embodiments, the dosage regimen is effective to decrease allele burden and/or to improve one or more other manifestations of the myeloproliferative disorder, such as effective to reduce bone marrow fibrosis by at least one grade.

In some embodiments of any of the foregoing or following, the mutation associated
30 with the myeloproliferative disorder is an activating mutation. In some embodiments, the subject comprises more than one mutation associated with a myeloproliferative disorder, such as a mutation in more than one of the foregoing genes (e.g., two, three, more than three). In some embodiments, the subject is a subject in need of treatment for a myeloproliferative disorder (e.g., a subject in need thereof).

In some embodiments of any of the foregoing or following, the subject comprises a mutation at codon 617 of JAK2 (e.g., the mutation associated with the myeloproliferative disorder is JAK2V617F). In some embodiments, the subject comprises a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject comprises a mutation at codon 515 of MPL. In some embodiments, the mutation results in a W515L, W515K, W515A, or W515R amino acid substitution in MPL. In some embodiments, the subject comprises a mutation in exon 10 of *MPL*. In some embodiments, the subject comprises a mutation a mutation in exon 9 of *CALR*. In some embodiments, the subject comprises a mutation in exon 12 of *ASXL1*. In some embodiments, the subject comprises a mutation in exon 12 of *ASXL1*. In some
10 embodiments, the subject comprises a mutation in exon 4 of *IDH1*. In some embodiments, the subject comprises a mutation at codon 132 of *IDH1*. In some embodiments, the subject comprises a mutation in exon 4 of *IDH2*. In some embodiments, the subject comprises a mutation at codon 140 of *IDH2*. In some embodiments, the subject comprises a mutation at codon 172 of *IDH2*. In some embodiments, the mutation is not JAK2V617F. In some
15 embodiments, if the mutation is JAK2V61F, the subject also comprises one or more additional mutations associated with the myeloproliferative disorder. In certain embodiments, the subject comprises more than one mutation associated with a myeloproliferative disorder, such as a mutation in more than one of the foregoing genes (e.g., two, three, more than three). In certain embodiments, the subject comprises or is evaluated
20 for mutations in JAK2, CALR and MPL and, optionally, one or more other genes. It should be understood that referring to “the subject comprises” also refers to the subject comprising cells that comprise the mutation, or the subject comprising cells that carry the mutation.

In some embodiments of any of the foregoing or following, the mutation associated with the myeloproliferative disorder is a deletion, insertion, point mutation, or translocation.
25 In some embodiments, the mutation results in the absence of expression of the one or more proteins encoded by the one or more genes or in the expression of a truncated protein. In some embodiments, the mutation is an activating mutation. In some embodiments, the activating mutation is JAK2V617F. In some embodiments, the activating mutation is a mutation at codon 515 of MPL.

30 In some embodiments of any of the foregoing or following, the mutation associated with the myeloproliferative disorder is present on one or both alleles of the one or more genes.

In some embodiment of any of the foregoing or following, SAP protein is administering in multiple doses, such as according to a dosing schedule and/or dosage regimen. Exemplary dosage regimens are provided herein.

5 In some embodiments of any of the foregoing or following, prior to and/or following administration of an SAP protein, one or more manifestations of the myeloproliferative disorder are measured in the subject, such as allele burden, bone marrow fibrosis and the like. Exemplary other manifestations/end points are provided herein. In some embodiments, administration of the SAP protein is effective to improve one or more of these manifestations/end points, optionally, without adverse myelosuppression.

10 In some embodiments of any of the foregoing or following, the decrease in mutant allele burden following treatment with SAP protein in one or more of the myeloproliferative disorder-associated genes is 10 to 90%. In some embodiments, the decrease in mutant allele burden is 25% to 50%. In some embodiments, the decrease in mutant allele burden is at least 50%, such as about 50-60%, 50-70%, 50-75%, or 50-80%. In some embodiments, a
15 complete molecular response is observed. In certain embodiments, the decrease in mutant allele burden is evaluated after one treatment cycle or after 30 days. In certain embodiments, the decrease in mutant allele burden is evaluated 60 days, 90 days or 120 days after initiation of treatment. In some embodiments, decrease in mutant allele burden is evaluated at multiple points. In some embodiments, in addition to mutant allele burden, one or more other
20 symptoms are evaluated before and/or during treatment.

In some embodiments of any of the foregoing or following, the biological sample is a blood sample. In some embodiments, the biological sample is bone marrow. In other words, in certain embodiments, when a sample is taken from a subject for, for example, the purpose of evaluating the presence of a mutation or for evaluating allele burden, the sample is a blood
25 sample or a bone marrow sample. In certain embodiments, a blood sample or bone marrow sample is taken from a subject to evaluate other manifestations of the myeloproliferative disorder, such as bone marrow fibrosis.

In some embodiments of any of the foregoing or following, the measuring step comprises amplifying nucleic acid comprising all or a portion of the one or more genes
30 associated with the myeloproliferative disorder. In other words, in certain embodiments, when a gene associated with myeloproliferative disorder is being assayed, for example, to determine whether a subject carries a mutation associated with the myeloproliferative disorder or to evaluate allele burden or changes in allele burden, the evaluation may comprise amplifying nucleic acid comprising all or a portion of the one or more genes associated with

the myeloproliferative disorder. In certain embodiments, multiple candidate genes are evaluated or measured as part of the assay or method (e.g., two, three or more than three).

In some embodiments of any of the foregoing or following, the measuring step comprises determining the proportion of mutant nucleic acid to wildtype nucleic acid of the one or more genes associated with the myeloproliferative disorder.

In certain embodiments of any of the foregoing or following, the SAP protein is an SAP protein comprising one or more protomers. In certain embodiments, the SAP protein is an SAP protein comprising five protomers. In certain embodiments, the SAP protein is provided as a composition comprising SAP protein, such as a pharmaceutical composition, and the SAP proteins and compositions may be used in any of the methods described herein.

In some embodiments of any of the foregoing or following, SAP protein is a glycosylated human SAP protein. By way of example, the SAP protein may comprise an SAP polypeptide, such as a glycosylated human SAP polypeptide, such as a glycosylated human SAP polypeptide having glycosylation that differs from SAP protein isolated from human serum (e.g., human SAP comprising an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety). In certain embodiments, the SAP protein is recombinant human SAP (e.g., rhSAP). In certain embodiments, the SAP protein comprises the recombinant human SAP also known in the art as PRM-151. Duffield and Luper, Drug News & Perspectives 2010, 23(5):305-315. Optionally, rhSAP may be prepared in CHO cells or in another suitable cell line. Any of the methods described herein comprise, in certain embodiments, administering the recombinant human SAP known as PRM-151.

In some embodiments of any of the foregoing or following, the SAP protein is a glycosylated human SAP protein comprising an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety. In some embodiments, all the sialylated branches of the oligosaccharide chain terminate with α 2,3-linked sialic acid moieties. In some embodiments, the oligosaccharide chain is substantially free of α 2,6-linked sialic acid moieties. By way of example, the SAP protein may comprise such a glycosylated human SAP protein. In some embodiments, the glycosylated human SAP comprises recombinant human SAP also referred to as recombinant human pentraxin-2 (hPTX-2), as described in Duffield and Luper, Drug News & Perspectives 2010, 23(5):305-315. In certain embodiments, the methods of the disclosure comprise administering a composition comprising glycosylated human SAP protein, wherein the human SAP protein comprises five SAP protomers. In certain embodiments, the

composition SAP protein comprising an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety. In certain embodiments, the SAP protein comprises five SAP protomers, wherein each protomer comprises an N-linked oligosaccharide chain, wherein at least one branch of the

5 oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety. In certain embodiments, the composition comprising the SAP protein or the SAP protein comprises 85% less α 2,6-linked sialic acid in comparison to serum-derived SAP. In some embodiments, all the sialylated branches of all of the oligosaccharide chains terminate with α 2,3-linked sialic acid moieties. In some embodiments, the oligosaccharide chains are

10 substantially free of α 2,6-linked sialic acid moieties.

In some embodiments of any of the foregoing or following, the SAP protein comprises an amino acid sequence at least 85% identical to SEQ ID NO: 1. In some embodiments, the SAP protein comprises an amino acid sequence at least 95% identical to SEQ ID NO: 1. In some embodiments, the SAP protein is a glycosylated SAP protein having

15 glycosylation that differs from human SAP purified from serum. In some embodiments, the SAP protein comprises five polypeptide chains each of which comprise an amino acid sequence at least 85%, at least 90%, 95%, 98%, or even 100% identical to SEQ ID NO: 1. In certain embodiments, the SAP protein comprises five SAP protomers and each protomer comprises an amino acid sequence that is at least 85% (or is at least 90, 95, 98, 99 or 100%)

20 identical to SEQ ID NO: 1.

In some embodiments of any of the foregoing or following, the SAP protein is a fusion protein comprising an SAP domain and one or more heterologous domains. In some embodiments, the one or more heterologous domains enhance one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of

25 protein complexes, and/or purification.

In some embodiments of any of the foregoing or following, the SAP protein comprises one or more modified amino acid residues. In some embodiments, the one or more modified amino acid residues comprise a PEGylated amino acid, a prenylated amino acid, an acetylated amino acid, a biotinylated amino acid, and/or an amino acid conjugated to an

30 organic derivatizing agent.

In some embodiments of any of the foregoing or following, the SAP protein is administered by a mode selected from: orally, topically, by injection, by intravenous injection, by subcutaneous injection, by inhalation, continuous release by depot or pump, or a combination thereof.

In some embodiments of any of the foregoing or following, the method further comprises administering to the patient an anti-cancer therapeutic (e.g., an additional anti-cancer therapeutic).

In some embodiments of any of the foregoing or following, the anti-cancer therapeutic is selected from: chemotherapy agents, antibody-based agents, kinase inhibitors (e.g., tyrosine kinase inhibitors, serine/threonine kinase inhibitors, etc.), immunomodulatory agents, biologic agents, and combinations thereof. A single additional agent or multiple additional agents or treatment modalities may be co-administered (at the same or differing time points and/or via the same or differing routes of administration and/or on the same or a differing dosing schedule). In certain embodiments, the combination of an SAP protein and the additional anti-cancer therapeutic is indicated for a condition, patient population or sub-population for which the additional anti-cancer therapeutic alone is not indicated. In certain embodiments, the SAP protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum.

In some embodiments of any of the foregoing or following, the chemotherapy agent is selected from but not limited to: actinomycin D, aldesleukin, alitretinoin, all-trans retinoic acid/ATRA, altretamine, amascrine, asparaginase, azacitidine, azathioprine, bacillus calmette-guerin/BCG, bendamustine hydrochloride, bexarotene, bicalutamide, bleomycin, bortezomib, busulfan, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, cisplatin/cisplatinum, cladribine, cyclophosphamide/cytosphosphane, cytarabine, dacarbazine, daunorubicin/daunomycin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil (5-FU), gemcitabine, goserelin, hydrocortisone, hydroxyurea, idarubicin, ifosfamide, interferon alfa, irinotecan CPT-11, lapatinib, lenalidomide, leuprolide, mechlorethamine/chlormethine/mustine/HN2, mercaptopurine, methotrexate, methylprednisolone, mitomycin, mitotane, mitoxantrone, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pegaspargase, pegfilgrastim, PEG interferon, pemetrexed, pentostatin, phenylalanine mustard, plicamycin/mithramycin, prednisone, prednisolone, procarbazine, raloxifene, romiplostim, sargramostim, streptozocin, tamoxifen, temozolomide, temsirolimus, teniposide, thalidomide, thioguanine, thiophosphoamide/thiotepa, thiotepa, topotecan hydrochloride, toremifene, tretinoin, valrubicin, vinblastine, vincristine, vindesine, vinorelbine, vorinostat, zoledronic acid, and combinations thereof. In certain embodiments, the method comprises administration of the SAP protein and an additional anti-cancer therapeutic, which additional anti-cancer therapeutic is a chemotherapeutic agent, such as a single chemotherapeutic agent or a

combination of two or more chemotherapeutic agents. In certain embodiments, the SAP protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

5 In some embodiments of any of the foregoing or following, the antibody-based agent is selected from but not limited to: alemtuzumab, bevacizumab, cetuximab, fresolimumab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, ofatumumab, panitumumab, rituximab, tositumomab, trastuzumab, trastuzumab DM1, and combinations thereof. In certain embodiments, the method comprises administration of the SAP protein and an
10 additional anti-cancer therapeutic, which additional anti-cancer therapeutic is an antibody-based agent. In certain embodiments, the SAP protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

15 In some embodiments of any of the foregoing or following, the kinase inhibitor (e.g., tyrosine kinase inhibitors, serine/threonine kinase inhibitors, etc.) is selected from but not limited to: axitinib, bafetinib, bosutinib, cediranib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, neratinib, nilotinib, pazopanib, ponatinib, quizartinib, regorafenib, sorafenib, sunitinib, vandetanib, vatalanib, vemurafinib, and combinations thereof. In certain
20 embodiments, the method comprises administration of the SAP protein and an additional anti-cancer therapeutic, which additional anti-cancer therapeutic is a kinase inhibitor. In certain embodiments, the SAP protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the
25 foregoing agents.

 In some embodiments of any of the foregoing or following, the immunomodulatory agent is selected from but not limited to: thalidomide, lenalidomide, pomalidomide, methotrexate, leflunomide, cyclophosphamide, cyclosporine A, minocycline, azathioprine, tacrolimus, methylprednisolone, mycophenolate mofetil, rapamycin, mizoribine,
30 deoxyspergualin, brequinar, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), lactoferrin, poly AU, polyI:polyC12U, poly-ICLC, imiquimod, resiquimod, unmethylated CpG dinucleotide (CpG-ODN), and ipilimumab. In certain embodiments, the method comprises administration of the SAP protein and an additional anti-cancer therapeutic, which additional anti-cancer therapeutic is an immunomodulatory agent. In certain embodiments, the SAP

protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

In some embodiments of any of the foregoing or following, the kinase inhibitor is a
5 Janus kinase inhibitor selected from but not limited to: AC-430, AZD1480, baricitinib, BMS-911453, CEP-33779, CYT387, GLPG-0634, INCB18424, lestaurtinib, LY2784544, NS-018, pacritinib, ruxolitinib, TG101348 (SAR302503), tofacitinib, VX-509, R-348, R723 and combinations thereof. In certain embodiments, the method comprises administration of the SAP protein and an additional anti-cancer therapeutic, which additional anti-cancer
10 therapeutic is a Janus kinase inhibitor. In certain embodiments, the SAP protein comprises a glycosylated SAP protein, such as an SAP protein having glycosylation that differs from human SAP purified from serum. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

In some embodiments of any of the foregoing or following, the biologic agent is
15 selected from but not limited to: IL-2, IL-3, erythropoietin, G-CSF, filgrastim, interferon alfa, bortezomib and combinations thereof. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

In some embodiments of any of the foregoing or following, the anti-cancer therapeutic is selected from but not limited to: AB0024, AZD1480, AT-9283, BMS-911543,
20 CYT387, everolimus, givinostat, imetelstat, lestaurtinib, LY2784544, NS-018, oral arsenic, pacritinib, panobinostat, peginterferon alfa-2a, pomalidomide, pracinostat, ruxolitinib, TAK-901, and TG101438 (SAR302503). In certain embodiments, the chemotherapeutic agent is selected from the group consisting of any of the foregoing agents.

In some embodiments, the SAP protein and the one or more additional active agents
25 (e.g., the additional anti-cancer therapeutic) are co-formulated. In some embodiments, the SAP protein and the one or more additional active agents are administered simultaneously. In some embodiments, the SAP protein and the one or more additional active agents are administered within a time of each other to produce overlapping therapeutic effects in the patient. When the SAP protein and the one or more additional active agents are administered
30 simultaneously or within a time of each other to produce overlapping therapeutic effects, the agents may be administered by the same or a different route of administration (e.g., oral versus infusion).

In some embodiments, the myeloproliferative disorder is myelofibrosis. In some embodiments, the myelofibrosis is primary myelofibrosis, post-polycythemia vera

myelofibrosis, or post-essential thrombocythemia myelofibrosis. In some embodiments, the myeloproliferative disorder is post-polycythemia vera or post-essential thrombocythemia. In certain embodiments, the methods of the present disclosure involve use of SAP as a monotherapy.

5 In certain embodiments, prior to initiation of treatment with SAP, the patient has clinically significant anemia and/or thrombocytopenia.

 In some embodiments, the myeloproliferative disorder is polycythemia vera, essential thrombocytosis, or chronic myelogenous leukemia.

 In certain embodiments, treatment comprises administering the SAP protein according
10 to a dosing schedule, such as any of the dosing schedules described herein. In certain
embodiments, administration and/or the therapeutically effective amount is understood in the
art to comprise administration according to a dose and dosing schedule effective to produce
therapeutic benefit as defined in a clinical study protocol, full prescribing information, the
Investigator's Brochure, or by improvement in measures generally understood by experts in
15 the field to be of benefit to patients with the respective disease. In certain embodiments, the
SAP protein, whether administered alone or as part of a combination therapy, can be
administered according to a dosing schedule providing administration less than once per
week. In certain embodiments, such less frequent dosing occurs following an initial loading
phase wherein, for example, during the first week of a treatment cycle, the SAP protein is
20 administered multiple times.

 In certain embodiments of any of the foregoing, treatment improves organ function
(e.g., therapeutic efficacy comprises improvement in organ function; SAP protein is
administered alone or in combination and improves organ function). In certain embodiments,
the organ is the bone marrow and improvement in organ function is evaluated by assessing
25 improvement in hemoglobin and/or platelets (e.g., improvement in one or both of these
metrics evinces improvement in organ function; in the case of platelets, improvement in
platelets refers to increasing platelets in subjects suffering from low platelet levels; in the
case of hemoglobin, improvement in hemoglobin refers to increasing hemoglobin in subjects
suffering from low hemoglobin levels). In certain embodiments, treatment restores normal
30 tissue, such as by decreasing fibrosis (e.g., therapeutic efficacy comprises restoration of
normal tissue). In certain embodiments, restoring normal tissue is evaluated by assessing
bone marrow fibrosis. In certain embodiments, treatment reduces mutant allele burden.

In certain aspects, the method comprises administering an SAP protein and an additional anti-cancer therapeutic according to a dosage regimen such that one or more side effects are reduced relative to treatment with the additional anti-cancer therapeutic alone.

5 In certain aspects, the disclosure provides a kit comprising: a) a composition or pharmaceutical composition comprising an SAP protein; b) one or more oligonucleotides capable of amplifying a region of one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*; and c) instructions for use. In some embodiments, the mutation detected is not JAK2V617F.

10 The disclosure contemplates all suitable combinations of any of the features of the invention, such as combinations of any of the aspects and embodiments described herein. For example, the disclosure contemplates that any of the foregoing aspects and embodiments may be combined with each other and/or with any of the embodiments disclosed herein. For example, SAP proteins described using any combination of functional and/or structural features may be used alone or in a combination therapy in any of the methods described
15 herein, to treat any of the conditions, patient populations, or sub-populations of patients described herein, such as patients described based on any one or more symptoms.

DETAILED DESCRIPTION OF THE INVENTION

Overview

20 The present disclosure provides methods and kits for evaluating allele burden in a subject having a myeloproliferative disorder, including methods and kits for reducing allele burden and/or using allele burden to evaluate treatment efficacy. The disclosure also provides methods for treating subjects having a myeloproliferative disorder, wherein the subject carries a mutation in one or more genes (e.g., the subject comprises cells comprising
25 one or more mutations associated with a myeloproliferative disorder).

A number of prognostically-relevant somatic mutations and cytogenetic abnormalities have been found to be associated with myeloproliferative disorders. Prognostically-relevant mutations have been identified in a diverse set of genes including *JAK2*, *MPL*, *CALR*, *ASXL1*, *SRSF2*, *EZH2*, *IDH1*, and *IDH2*. Presence of *JAK2*, *MPL*, *CALR*, *ASXL1*, and *SRSF2*
30 mutations were found to be independent predictors of shortened survival in primary myelofibrosis in a recent study (Tefferi et al. ASH 2014 Abstract 406) and triple-negativity for *JAK2*, *MPL* and *CALR* (TN), *JAK2* or *MPL* mutation, and mutations in *ASXL1*, *SRSF2*, *EZH2* or *IDH1/2* were identified as risk factors for inferior survival in another cohort

(Vanucchi et al. ASH 2015 Abstract 405). Thus, mutational status is a predictor of poor outcomes.

Cytogenetic abnormalities in primary myelofibrosis were recently stratified into four risk designations: very high risk (MK, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities), high
5 (complex without MK, two abnormalities not included in very high risk category, 5q-, +8, other autosomal trisomies except +9, and other sole abnormalities not included in other risk categories), intermediate (sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality) and low (normal or sole abnormalities of 13q- or +9) (Tefferi et al. ASH 2014 Abstract 631).

10 Allele burden is the ratio between mutant and wild-type nucleic acid in, for example, hematopoietic cells. So far, JAK inhibitor therapy in humans has had little effect on *JAK2V617F* allele burden or bone marrow fibrosis (Tefferi. Blood 119(12): 2721-2730). In addition to *JAK2V617F*, PMF and the other *BCR-ABL1*-negative myeloproliferative neoplasms are characterized by many other somatic mutations as described above, including
15 *MPL*, *TET2*, *ASXL1*, *CBL*, *IDH1*, *IDH2*, *IKZF1*, *LNK*, *EZH2*, *DNMT3A*, *CUX1*, and *SF3B1* mutations (Tefferi et al. Mayo Clin Proc. 2012, 87(1):25-33). Quantitative analyses of other prognostically-relevant somatic mutations and cytogenetic analyses will also be useful for monitoring and managing myeloproliferative disorders.

The present disclosure provides new genetics-based therapeutic regimens for treating
20 myeloproliferative disorders using an SAP protein.

Myelofibrosis is characterized by the presence of dense fibrotic tissue in the bone marrow. One goal of therapeutic intervention is to restore normal organ function by preventing or reducing excess fibrotic tissue. The regulation of events leading to fibrosis involves at least two major events. One is the proliferation and differentiation of fibrocytes.
25 Fibrocytes are a distinct population of fibroblast-like cells derived from peripheral blood monocytes that normally enter sites of tissue injury to promote angiogenesis and wound healing. Fibrocytes are important in the formation of tumors, particularly stromal tissue in tumors. Fibrocytes differentiate from CD14+ peripheral blood monocytes, and may differentiate from other PBMC cells. The presence of SAP, IL-12, Laminin-1, anti-FcγR
30 antibodies, crosslinked IgG and/or aggregated IgG may inhibit or at least partially delay this process.

The second major event is the formation and maintenance of fibrotic tissue. Fibrotic tissue may be formed and maintained by the differentiation of monocytes into fibrocytes, macrophages or myofibroblasts, the recruitment and proliferation of fibroblast cells, the

formation of new extracellular matrix, and the growth of new vascular tissue. In pathologic fibrosis, such as following chronic inflammation, injury, malignancy, or idiopathic fibrosis, it is this excess fibrotic tissue that can lead to tissue damage and destruction.

Recently, it has been suggested that serum amyloid P (SAP) or pentraxin-2 (PTX-2) can be used as a therapeutic agent to treat various disorders, including fibrosis-related disorders, hypersensitivity disorders, autoimmune disorders, mucositis, and inflammatory disorders such as those caused by microbial infection. See, for example, U.S. Patent Nos. 8,247,370 and 8,497,243 and U.S. Patent Application Nos. 12/720,845 and 12/720,847. SAP binding to Fc γ R provides an inhibitory signal for fibrocyte, fibrocyte precursor, myofibroblast precursor, and/or hematopoietic monocyte precursor differentiation. The use of SAP and SAP agonists as a therapeutic treatment for fibrosis is described in U.S. Patent Nos. 7,763,256, and 8,247,370, which are hereby incorporated by reference. In certain embodiments of any of the methods described herein, the method comprises administration of SAP protein (see the Examples). In certain embodiments, the SAP is recombinant human SAP, also referred to as recombinant human pentraxin-2, such as recombinant human SAP produced in CHO cells. In certain embodiments, the SAP protein comprises a human SAP protein, such as a human SAP protein having glycosylation that differs from that of SAP purified from human serum. In certain embodiments, the SAP protein comprises a human SAP protein wherein all the sialylated branches of the N-linked oligosaccharide chains terminate in α 2,3-linked sialic acid moieties and/or wherein the N-linked oligosaccharide chains are substantially free of α 2,6-linked sialic acid moieties. In certain embodiments, the methods of the disclosure comprise administering a composition comprising glycosylated human SAP protein, wherein the human SAP protein comprises five SAP protomers. In certain embodiments, at least one of the protomers or 1, 2, 3, 4 or 5 such protomers comprise an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety. In certain embodiments, the SAP protein comprises five SAP protomers, wherein each protomer comprises an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety or wherein all of the sialylated branches of the SAP protein terminate in an α 2, 3 linked sialic acid moiety. In certain embodiments, the composition comprising the SAP protein or the SAP protein comprises 85% less α 2,6-linked sialic acid in comparison to serum-derived SAP. In some embodiments, all the sialylated branches of the oligosaccharide chain(s) terminate with α 2,3-linked sialic acid moieties. In

some embodiments, the oligosaccharide chain(s) is substantially free of α 2,6-linked sialic acid moieties.

The present disclosure provides methods for treating myeloproliferative disorders. The method generally involves administering an effective amount of an anti-fibrotic agent
5 such as an SAP protein, as a single agent, or in combination with an additional agent.

In some embodiments, an effective amount of an SAP protein is an amount that, when administered alone, or in combination therapy, is effective to reduce mutant allele burden by at least about 10%, and more preferably at least about 15%, 20%, 25%, 30%, 35%, 40%, 45%, or even at least about 50%, 60%, 70%, 80%, 90% or more, compared with the mutant
10 allele burden in the individual prior to treatment with the SAP protein. In certain embodiments, the reduction in mutant allele burden is about 10%-90%, 10%-75%, 10%-50%, 20%-75%, 30%-75%, 20%-50%, 30%-60%, and the like. In certain embodiments, the SAP protein is administered in multiple dosing according to a dosing schedule and/or dosage regimen, and the reduction in allele burden is evaluated after multiple doses (e.g., after about
15 1, 2, 3, 4 or 5 months of treatment). In certain embodiments, the SAP protein is administered according to a dosing schedule and/or dosage regimen, and when administered alone or in a combination therapy, is effective to reduce fibrosis by at least about 10%, and more preferably at least about 15%, 20%, 25%, 30%, 35%, 40%, 45%, or even at least about 50%, or more, compared with the degree of fibrosis in the individual prior to treatment with SAP.
20 In certain embodiment fibrosis is bone marrow fibrosis, the effective treatment is a reduction in bone marrow fibrosis by at least one grade, optionally, at least 2 grades. In certain embodiments, the SAP protein is administered in multiple doses according to a dosing schedule and/or dosage regimen, and the reduction in fibrosis is evaluated after multiple doses (e.g., after about 1, 2, 3, 4 or 5 months of treatment).

25 Methods of the disclosure are useful in evaluating the interaction between selected genetic mutations and cytogenetic abnormalities prevalent in myeloproliferative disorders and an SAP protein. Methods of the disclosure are useful in evaluating the association of baseline mutational status or cytogenetic abnormalities and response to treatment with an SAP protein. In some embodiments, the SAP protein comprises a glycosylated SAP protein
30 (e.g., SAP comprising a glycosylated SAP protein, such as a glycosylated SAP protein having glycosylation that differs from that of SAP purified from human serum; recombinant human SAP, such as recombinant human pentraxin-2 or PRM-151).

Definitions

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics,
5 organic chemistry, and nucleic acid chemistry and hybridization are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references (e.g., Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring
10 Harbor, N.Y.), which are provided throughout this document.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "about" means plus or minus 10% of the numerical value of
15 the number with which it is being used. Therefore, about 50% means in the range of 45%-55%.

As used herein, the term "substantially" means being largely but not wholly what is specified. For example, the term "substantially similar" with regard to a nucleotide sequence indicates that the sequence is largely identical to another reported sequence for the same
20 protein or peptide; however, the nucleotide sequence may include any number of variations or mutations that do not affect the structure or function of the resulting protein.

"Administering," when used in conjunction with a therapeutic, means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient, whereby the therapeutic can impact the patient. Thus, as used herein, the term
25 "administering," when used in conjunction with an SAP protein can include, but is not limited to, providing an SAP protein to a subject systemically by, for example, intravenous injection (e.g., which may be intravenous infusion), subcutaneous delivery (e.g., subcutaneous injection or implantation of a subcutaneous delivery device), whereby the therapeutic reaches the target tissue.

30 "Administering" a composition may be accomplished by, for example, intravenous, subcutaneous, intramuscular, or intralesional injection, oral administration, topical administration, or by these methods in combination with other known techniques. Such combination techniques include heating, radiation, ultrasound and the use of delivery agents. When more than one different therapeutic agent is administered, the agents may be

administered by the same or different routes of administration and/or at the same or differing times. As is understood in the art, an agent can be administered according to a dosing schedule.

As used herein, the term “dosage regimen” encompasses both the dose or dosage (i.e., the amount of the SAP protein) and the dosing schedule (i.e., the frequency of administration or intervals between successive doses of the SAP protein).

“Providing,” when used in conjunction with a therapeutic, means to administer a therapeutic directly into or onto a target tissue, or to administer a therapeutic to a patient whereby the therapeutic can impact the patient.

The term “improves” is used to convey that the present disclosure changes either the characteristics and/or the physical attributes of the tissue to which it is being provided, applied or administered. The term “improves” may also be used in conjunction with a diseased state such that when a diseased state is “improved” the symptoms, manifestations, or physical characteristics associated with the diseased state are diminished, reduced or eliminated.

As used herein, “isolated” means altered or removed from the natural state through human intervention. For example, SAP naturally present in a living animal is not “isolated,” but a synthetic SAP protein or recombinant SAP protein, or an SAP protein partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated SAP protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a cell into which the SAP protein has been delivered.

The terms “mimetic,” “peptide mimetic” and “peptidomimetic” are used interchangeably herein, and generally refer to a peptide, partial peptide or non-peptide molecule that mimics the tertiary binding structure or activity of a selected native peptide or protein functional domain (e.g., binding motif or active site). These peptide mimetics include recombinantly or chemically modified peptides, as well as non-peptide agents such as small molecule drug mimetics, as further described below.

As used herein, the term “nucleic acid” refers to a polynucleotide such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotide.

“Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event occurs and instances where it does not.

The terms "peptides", "proteins" and "polypeptides" are used interchangeably herein.

5 The term "purified protein" refers to a preparation of a protein or proteins that are preferably isolated from, or otherwise substantially free of, other proteins normally associated with the protein(s) in a cell or cell lysate. The term "substantially free of other cellular proteins" or "substantially free of other contaminating proteins" is defined as encompassing individual preparations of each of the proteins comprising less than 20% (by dry weight) contaminating
10 protein, and preferably comprises less than 5% contaminating protein. Functional forms of each of the proteins can be prepared as purified preparations by using a cloned gene as is well known in the art. By "purified", it is meant that the indicated molecule is present in the substantial absence of other biological macromolecules, such as other proteins (particularly other proteins which may substantially mask, diminish, confuse or alter the characteristics of
15 the component proteins either as purified preparations or in their function in the subject reconstituted mixture). The term "purified" as used herein preferably means at least 80% by dry weight, more preferably in the range of 85% by weight, more preferably 95-99% by weight, and most preferably at least 99.8% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having
20 a molecular weight of less than 5000, can be present). The term "pure" as used herein preferably has the same numerical limits as "purified" immediately above.

By “pharmaceutically acceptable,” “physiologically tolerable,” and grammatical variations thereof, as they refer to compositions, carriers, diluents, and reagents or other ingredients of the formulation, can be used interchangeably and indicate that the materials are
25 capable of administration without the production of undesirable physiological effects such as nausea, dizziness, rash, gastric upset or other deleterious effects to the recipient thereof.

“Pharmaceutically acceptable salts” include both acid and base addition salts.

“Pharmaceutically acceptable acid addition salt” refers to those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or
30 otherwise undesirable and formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, carbonic acid, phosphoric acid, and the like. Organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic, and sulfonic classes of organic acids, such as formic acid, acetic acid, propionic acid, glycolic acid, gluconic acid, lactic acid, pyruvic acid, oxalic acid, malic

acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, aspartic acid, ascorbic acid, glutamic acid, anthranilic acid, benzoic acid, cinnamic acid, mandelic acid, embonic acid, phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

5 As used herein, the term “pharmaceutically acceptable salts, esters, amides, and prodrugs” refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for
10 their intended use, as well as the zwitterionic forms, where possible, of the compounds of the disclosure.

 As used herein, the term “therapeutic” means an agent utilized to treat, combat, ameliorate, prevent or improve an unwanted condition or disease of a patient. In part, embodiments of the present disclosure are directed to the treatment of myeloproliferative
15 diseases, or the aberrant proliferation of cells.

 An “effective amount” of a composition is a predetermined amount calculated to achieve the desired result. An effective amount is an amount that is consistent with a dosage regimen that, over a period of time, yields a desired therapeutic effect. For an effective amount to be therapeutically effective, multiple doses over time may be required. In certain
20 embodiments herein, when an effective amount is specific a therapeutically effective amount may be referred to as well. A desired result may be the maintenance, amelioration or resolution of symptoms, manifestations, or any of the effects described herein, or any of the effects commonly recognized in the art as a useful effect. The activity contemplated by the present methods includes both medical therapeutic and/or prophylactic treatment, as
25 appropriate. The specific dose of a compound administered according to this disclosure to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the compound administered, the route of administration, and the condition being treated. An effective amount of compound of this disclosure is typically an amount such that when it is administered in a physiologically
30 tolerable excipient composition, it is sufficient. “Therapeutically effective amounts” may be administered according to a dosing schedule. It is understood that when administering a drug according to a dosing schedule, it may take some period of time before improvement in symptoms or manifestations is observed. Nevertheless, administration in one or more doses that, alone or in combination, results in or is intended to result in improvement in symptoms

or manifestations and/or decrease in allele burden are exemplary of administering an effective amount.

"N-linked" oligosaccharides are those oligosaccharides that are linked to a peptide backbone through asparagine, by way of an asparagine-N-acetylglucosamine linkage. N-linked oligosaccharides are also called "N-glycans." Naturally occurring N-linked oligosaccharides have a common pentasaccharide core of Man[(α 1,6-)-(Man(α 1,3))-Man(β 1,4)-GlcNAc(β 1,4)-GlcNAc(β 1,N)]. They differ in the presence of, and in the number of branches (also called antennae) of peripheral sugars such as N-acetylglucosamine, galactose, N-acetylgalactosamine, fucose, and sialic acid. Optionally, this structure may also contain a core fucose molecule and/or a xylose molecule.

The term "sialic acid" refers to any member of a family of nine-carbon carboxylated sugars. The most common member of the sialic acid family is N-acetyl-neuraminic acid (often abbreviated as Neu5Ac, NeuAc, or NANA). A second member of the family is N-glycolyl-neuraminic acid (Neu5Gc or NeuGc), in which the N-acetyl group of NeuAc is hydroxylated. A third sialic acid family member is 2-keto-3-deoxy-nonulosonic acid (KDN) (Nadano et al. (1986) *J. Biol. Chem.* 261: 11550-11557; Kanamori et al., *J. Biol. Chem.* 265: 21811-21819 (1990)). Also included are 9-substituted sialic acids such as a 9-O-C₁C₆-acyl-Neu5Ac like 9-O-lactyl-Neu5Ac or 9-O-acetyl-Neu5Ac, 9-deoxy-9-fluoro-Neu5Ac and 9-azido-9-deoxy-Neu5Ac. For a review of the sialic acid family, see, e.g., Varki, *Glycobiology* 2: 25-40 (1992); *Sialic Acids: Chemistry, Metabolism and Function*, R. Schauer, Ed. (Springer-Verlag, New York (1992)).

A "genetically engineered" or "recombinant" cell is a cell having one or more modifications to the genetic material of the cell. Such modifications include, but are not limited to, insertions of genetic material, deletions of genetic material and insertion of genetic material that is extrachromosomal whether such material is stably maintained or not.

As used herein, the term "modified sugar," refers to a naturally- or non-naturally-occurring carbohydrate that is enzymatically added onto an amino acid or a glycosyl residue of a peptide in a process of the disclosure. The modified sugar is selected from a number of enzyme substrates including, but not limited to, sugar nucleotides (mono-, di-, and tri-phosphates), activated sugars (e.g., glycosyl halides, glycosyl mesylates) and sugars that are neither activated nor nucleotides. A "modified sugar" may be covalently functionalized with a "modifying group." Useful modifying groups include, but are not limited to, water-soluble and -insoluble polymers, therapeutic moieties, diagnostic moieties, and biomolecules. The locus of functionalization with the modifying group is selected such that it does not prevent

the "modified sugar" from being added enzymatically to a peptide or glycosyl residue of the peptide.

The term "zygosity status" as used herein refers to a sample, a cell population, or an organism as appearing heterozygous, homozygous, or hemizygous as determined by testing methods known in the art and described herein. The term "zygosity status of a nucleic acid" means determining whether the source of nucleic acid appears heterozygous, homozygous, or hemizygous. The "zygosity status" may refer to differences in a single nucleotide in a sequence. In some methods, the zygosity status of a sample with respect to a single mutation may be categorized as homozygous wild-type, heterozygous (i.e., one wild-type allele and one mutant allele), homozygous mutant, or hemizygous (i.e., a single copy of either the wild-type or mutant allele). Because direct sequencing of plasma or cell samples as routinely performed in clinical laboratories does not reliably distinguish between hemizygosity and homozygosity, in some embodiments, these classes are grouped. For example, samples in which no or a minimal amount of wild-type nucleic acid is detected are termed "hemizygous/homozygous mutant." In some embodiments, a "minimal amount" may be between about 1-2%. In other embodiments, a minimal amount may be between about 1-3%. In still other embodiments, a "minimal amount" may be less than 1%.

Treatment Methods

In part, the disclosure provides new genetics-based therapeutic regimens for treating a myeloproliferative disorder (MPD) using an SAP protein. In one aspect, the disclosure provides methods of treating an MPD in a subject carrying one or more somatic mutations or cytogenetic abnormalities associated with an MPD. In some embodiments, the method comprises administering a therapeutically effective amount of a serum amyloid P (SAP) protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) associated with the MPD in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. The SAP proteins of the disclosure are used, alone or in combination with an additional agent, to treat an MPD. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the MPD is not JAK2V617F. The

disclosure contemplates any of the methods described herein having any combination of features described herein.

In one aspect, the disclosure provides methods of treating an MPD in a subject carrying one or more somatic mutations and/or cytogenetic abnormalities associated with an MPD by administering an SAP protein according to a dosing schedule and/or dosage regimen effective to reduce mutant allele burden and/or cytogenetic abnormalities. In some embodiments, the method comprises administering a therapeutically effective amount of a serum amyloid P (SAP) protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*, and wherein the SAP protein is administered according to a dosage regimen effective to reduce mutant allele burden of said gene in said subject. The SAP proteins of the disclosure are used, alone or in combination with an additional agent, to treat the MPD. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the MPD is not JAK2V617F.

In one aspect, the disclosure provides methods of reducing mutant allele burden and/or cytogenetic abnormalities associated with an MPD by administering an SAP protein. In some embodiments, the disclosure provides a method for reducing mutant allele burden in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. The SAP proteins of the disclosure are used, alone or in combination with an additional agent, to treat the MPD. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the MPD is not JAK2V617F.

In one aspect, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on quantitative and qualitative analyses of the mutational status and/or cytogenetic information of a subject receiving the SAP protein. The quantitative analysis of the mutational status may comprise measuring the mutant allele burden and may comprise a comparison of the mutational status prior to starting SAP protein

therapy and at different time points during the course of therapy. Similarly, cytogenetic analyses are carried out prior to starting therapy and at different time points during the course of therapy. In some embodiments, the method comprises: (i) measuring a first mutant allele burden of a mutation in one or more genes associated with the MPD selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the MPD and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. The SAP proteins of the disclosure are used, alone or in combination with an additional agent, to treat the MPD. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the myeloproliferative disorder is not JAK2V617F.

In one aspect, the disclosure provides methods of determining responsiveness to SAP protein or agonist therapy based on the presence or absence of one or more somatic mutations and/or cytogenetic abnormalities associated with an MPD. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of a serum amyloid P (SAP) protein to the subject. The SAP proteins of the disclosure are used, alone or in combination with an additional agent, to treat the MPD. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the MPD is not JAK2V617F. In some embodiments of any of the above aspects of the disclosure, the subject comprises a mutation in one or more genes

selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and *SF3B1* and/or the methods of the disclosure further comprise carrying out mutational analyses of one or more genes selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and *SF3B1*.

Any of the above aspects of the disclosure can be further combined with cytogenetic analyses to assay for one or more of MPD-associated cytogenetic abnormalities such as, but not limited to, monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. Tefferi et al. ASH 2014 Abstract 631. In some embodiments, cytogenetic analysis is carried out according to the International System for Human Cytogenetic Nomenclature (*Cytogenetic and genome research*. 2013. Prepublished on 2013/07/03 as DOI 10.1159/000353118). In some embodiments, assignment to “normal” karyotype requires a minimum of 10 metaphases analyzed. In some embodiments, a complex karyotype is defined as the presence of 3 or more distinct structural or numeric abnormalities. In some embodiments, a monosomal karyotype is defined as 2 or more distinct autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality (JCO. 2008; 26:4791; Tefferi et al. ASH 2014 Abstract 631).

Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic cells leading to excess production of various blood cells in the bone marrow. Since the hematopoietic stem cell gives rise to myeloid, erythroid, and platelet cells, qualitative and quantitative changes can be seen in any or all of these cell lines. Myeloproliferative disorders can be challenging to diagnose and treat. The term “myeloproliferative disorder (MPD)” or “myeloproliferative disease” is meant to include non-lymphoid dysplastic or neoplastic conditions arising from a hematopoietic stem cell or its progeny. “MPD patient” includes a patient who has been diagnosed with an MPD. “Myeloproliferative disease” is meant to encompass the specific, classified types of myeloproliferative diseases including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) or primary myelofibrosis (PMF). Also included in the definition are hypereosinophilic syndrome (HES), chronic neutrophilic leukemia (CNL), myelofibrosis with myeloid metaplasia (MMM), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, chronic basophilic leukemia, chronic eosinophilic leukemia, and systemic mastocytosis (SM). “Myeloproliferative disorder” is also meant to encompass any unclassified myeloproliferative diseases (UMPD or MPD-NC).

In certain aspects, the disclosure encompasses the use of an SAP protein, as a single agent or in combination with another agent, for a genetics-based treatment of myelofibrosis. Myelofibrosis ("MF") is a BCR-ABL1-negative myeloproliferative neoplasm ("MPN") that presents de novo (primary) or may be preceded by polycythemia vera ("PV") or essential thrombocythemia ("ET"). Primary myelofibrosis (PMF) (also referred to in the literature as idiopathic myeloid metaplasia, and Agnogenic myeloid metaplasia) is a clonal disorder of multipotent hematopoietic progenitor cells of monocytic lineage (reviewed in Abdel-Wahab, O. et al. (2009) *Annu. Rev. Med.* 60:233-45; Varicchio, L. et al. (2009) *Expert Rev. Hematol.* 2(3):315-334; Agrawal, M. et al. (2011) *Cancer* 117(4):662-76). Myelofibrosis originates from acquired mutations that target the hematopoietic stem cell and induce dysregulation of kinase signaling, clonal myeloproliferation, and abnormal cytokine expression (Tefferi. *Blood* 2011, 117(13): 3494-3504). The disease is characterized by anemia, splenomegaly and extramedullary hematopoiesis, and is marked by progressive marrow fibrosis and atypical megakaryocytic hyperplasia. CD34+ stem/progenitor cells abnormally traffic in the peripheral blood and multi organ extramedullary erythropoiesis is a hallmark of the disease, especially in the spleen and liver. The bone marrow structure is altered due to progressive fibrosis, neoangiogenesis, and increased bone deposits. Median survival ranges from less than 2 years to over 15 years based on currently identified prognostic factors (Cervantes F et al., *Blood* 113:2895-2901, 2009; Hussein K et al. *Blood* 115:496-499, 2010; Patnaik M M et al., *Eur J Haematol* 84:105-108, 2010).

It is known in the literature that inhibitors of JAK2 are useful in the treatment and/or prevention of MPDs. See, e.g., Tefferi, A. and Gilliland, D. G. *Mayo Clin. Proc.* 80(7): 947-958 (2005); Fernandez-Luna, J. L. et al. *Haematologica* 83(2): 97-98 (1998); Harrison, C. N. *Br. J. Haematol.* 130(2): 153-165 (2005); *Leukemia* (2005) 19, 1843-1844; and Tefferi, A. and Barbui, T. *Mayo Clin. Proc.* 80(9): 1220-1232 (2005). However, the management options of MF are currently inadequate to meet the needs of all patients. Therefore, there is a need to provide additional therapy options for MF patients, such as those provided herein.

In some embodiments of the methods provided herein, the subject has primary myelofibrosis. In some embodiments of the compositions and methods provided herein, the subject has post polycythemia vera myelofibrosis (post-PV MF). In some embodiments, the subject has post essential thrombocythemia myelofibrosis (post-ET MF). In some embodiments, the subject has high risk myelofibrosis. In some embodiments, the subject has intermediate risk myelofibrosis (such as intermediate risk level 1 or intermediate risk level 2). In some embodiments, the subject has low risk myelofibrosis. In some embodiments, the

subject has PV or ET without fibrosis. In some embodiments, the subject is positive (e.g., the mutation is present) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or positive for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject is negative (e.g., the mutation is absent) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In some embodiments, prior to initiation of treatment with an SAP protein of the disclosure, the subject has bone marrow fibrosis and the fibrosis is measurable according to the grading system of the European Consensus on Grading of Bone Marrow Fibrosis. In some embodiments, prior to initiation of treatment with an SAP protein of the disclosure, the subject has bone marrow fibrosis of greater than or equal to Grade 2. In other embodiments, prior to initiation of treatment with an SAP protein of the disclosure, the subject has bone marrow fibrosis of Grade 3. In other embodiments, prior to initiation of treatment with an SAP protein of the disclosure, the subject has bone marrow fibrosis of Grade 1.

In certain embodiments, the fibrotic condition of the bone marrow is an intrinsic feature of a chronic myeloproliferative neoplasm of the bone marrow, such as primary myelofibrosis. In other embodiments, the bone marrow fibrosis is associated with a malignant+- condition or a condition caused by a clonal proliferative disease or a hematologic disorder such as but not limited to hairy cell leukemia, lymphoma (e.g., Hodgkin or non-Hodgkin lymphoma), multiple myeloma or chronic myelogenous leukemia (CML). In yet other embodiments, the bone marrow fibrosis is associated with a solid tumor metastasis to the bone marrow.

In certain embodiments, the SAP proteins of the disclosure (e.g., an SAP protein comprising a glycosylated SAP protein; recombinant human SAP protein; etc.) are used to

treat myelofibrosis by decreasing fibrosis to restore organ function. Administering an SAP protein of the disclosure as a single agent or as part of a combination therapy, resulted in a decrease in organ fibrosis (e.g. bone marrow fibrosis), leading to improvements and/or restoration of organ function, improvement in hemoglobin, improvement in blood counts such as platelets or white blood cells, or improvement in symptoms. Improvement in organ function can be evaluated for example, by assessing improvement in platelet levels and/or hemoglobin in the subject over the course of treatment, such as over 12, 20, 24, or greater than 24 weeks of treatment (e.g., greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

As described herein, in certain embodiments, an SAP protein is used as a monotherapy in a subject who has a mutation in one or more MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) or one or more MPD-associated cytogenetic abnormalities. Optionally, the subject is characterized based on other features (e.g., level of manifestations, such as initial level of bone marrow fibrosis or other symptoms). In some embodiments, the subject is positive (e.g., the mutation is present) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (*JAK2*) or positive for the mutation corresponding to the valine 617 to phenylalanine mutation of human *JAK2*. In some embodiments, the subject is negative (e.g., the mutation is absent) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (*JAK2*) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human *JAK2*. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In some embodiments, the mutation associated with the MPD is not JAK2V617F. In some embodiments, the subject has one or more mutations in one or more genes selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and *SF3B1*. In some embodiments, the subject has one or more cytogenetic abnormalities selected from monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities,

complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities.

As described herein, in certain embodiments, addition of SAP to a therapeutic regimen or replacement of a therapeutic regimen with SAP therapy is used in a subject who is
 5 unresponsive, resistant or otherwise refractory to a treatment (in the absence of the SAP) or for whom efficacy of the treatment is or has waned. In certain embodiments, the addition of or substitution with SAP is used to expand the patient population for which treatment with another therapeutic agent is suitable (e.g., SAP expands the therapeutic window or patient
 10 population for another drug). In some embodiments, the patients are intolerant of a treatment or ineligible for it (e.g. ruxolitinib therapy in the absence of the SAP). By way of example, certain cancers are known to be unresponsive to chemotherapy. Without being bound by theory, fibrosis may hinder effective access of the drugs to the tumor. In some embodiments, the subject is positive (e.g., the mutation is present) for the valine 617 to phenylalanine
 15 mutation of human Janus Kinase 2 (JAK2) or positive for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject is negative (e.g., the mutation is absent) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject has a mutation in
 20 exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the
 25 subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In some embodiments, the mutation associated with the MPD is not JAK2V617F. In some embodiments, the subject has
 30 one or more mutations in one or more genes selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and *SF3B1*. In some embodiments, the subject has one or more cytogenetic abnormalities selected from monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex

chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities.

In certain embodiments, an SAP protein is used as a monotherapy and/or is used to treat naïve patients. In certain embodiments, an SAP protein is used in patients whose
5 disease has a certain fibrotic score, such as bone marrow fibrosis of Grade 2 or Grade 3, as assessed by the European Consensus on Grading of Bone Marrow Fibrosis. In some embodiments, the subject is positive (e.g., the mutation is present) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or positive for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some
10 embodiments, the subject is negative (e.g., the mutation is absent) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the
15 subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some
20 embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In some embodiments, the mutation associated with the MPD is not JAK2V617F. In some embodiments, the subject has one or more mutations in one or more genes selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and
25 *SF3B1*. In some embodiments, the subject has one or more cytogenetic abnormalities selected from monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities.

30 In certain embodiments, an SAP protein is used as a monotherapy and/or is used to treat patients who are anemic or thrombocytopenic. In some embodiments, the subject is positive (e.g., the mutation is present) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or positive for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject is negative (e.g.,

the mutation is absent) for the valine 617 to phenylalanine mutation of human Janus Kinase 2 (JAK2) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In some embodiments, the mutation associated with the MPD is not JAK2V617F. In some embodiments, the subject has one or more mutations in one or more genes selected from *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, and *SF3B1*. In some embodiments, the subject has one or more cytogenetic abnormalities selected from monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities.

In certain aspects, the myeloproliferative disease is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

In any of the above aspects, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) may be used in combination with any of the additional therapeutic agents described herein. In some embodiments, the additional therapeutic agent is an anti-cancer agent.

Exemplary mutations associated with myeloproliferative disorders

JAK2

Janus kinase 2 (JAK2) is a non-receptor tyrosine kinase and acts as an intermediary between membrane-bound cytokine receptors, and down-stream members of the signal transduction pathway such as STAT (Signal Transducers and Activators of Transcription

protein) molecules which then act as transcription factors in the nucleus. It has long been hypothesized that perturbation of protein tyrosine kinase (PTK) signaling by mutations and other genetic alterations is associated with MPDs. Mutant PTKs such as, for example, Janus kinase 2 (JAK2) gene mutations, can lead to constitutive activity in patients with MPDs or to other defects. As such, in certain embodiments, the subject being treated has a mutation in JAK2, such as a mutation described herein and/or the subject is evaluated for allele burden prior to and/or during treatment.

The JAK2 V617F substitution relieves the auto-inhibition of its kinase activity, leading to a constitutively active kinase and augments downstream JAK2-STAT signaling pathways (see e.g., Saharinen et al. Mol Cell Biol. 2000, 20:3387-3395; Saharinen et al., Mol Biol Cell 2003, 14(4):1448-1459). The mutation has been detected from blood samples, bone marrow and buccal samples and contributes to the pathogenesis of MPD (see, e.g., Baxter et al. Lancet 2005, 365:1054-1060; James et al. Nature 2005, 438: 1144-1148; Zhao et al. J. Biol. Chem. 2005, 280(24):22788-22792 ; Levine et al. Cancer Cell 2005, 7:387-397; Kralovics et al. New Eng. J. Med. 2005, 352(17):1779-1790), and homozygous and heterozygous cell populations have been reported in MPD patients (Baxter et al., Lancet 2005, 365:1054-1060). Other *JAK2* mutations in humans including translocations, point mutations, deletions, and insertions have been reported. See e.g., Scott et al., N Engl J Med. 2007, 356:459-468; Li et al. Blood 2008, 111:3863-3866.

Over 10 different sequence variations, mostly occurring between codons 536 and 544, and involving a deletion of three to six nucleotides have been found in exon 12 of *JAK2*. Some duplications and some 2-bp replacements have also been found (Laughlin et al. J Mol Diagn. 2010, 12(3): 278-282).

Other exemplary mutations in JAK2 include: exon 12 missense mutations such as T514M, N533Y, L545V, F547L; exon 13 missense mutations such as F556V, R564L, R564Q, V567L, V567A, G571S, G571R, L579F, H587N, S591L, exon 14 missense mutation H606Q, exon 14 deletion S593-N622; exon 15 missense mutations L624P, I645V (see, e.g., U.S. Patent Application Publication No. 2010/0112571); K539L, V617I, C618R, L624P, whole exon 14-deletion (Lee et al. BMC Struct Biol. 2009, 9:58).

JAK2 genomic nucleic acid is located in human chromosome 9. An exemplary sequence of all or portions of human *JAK2* mRNA includes but is not limited to GenBank Accession number NM_004972 (SEQ ID NO: 5). These sequences are incorporated herein by reference.

For the *JAK2* nucleic acid sequence, a “mutation” means a *JAK2* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_004972 (SEQ ID NO: 5). A mutation in *JAK2* nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be silent
5 with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_004963 (SEQ ID NO: 6) as a reference amino acid sequence.

In some embodiments, the *JAK2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *JAK2* include
10 exon 12 mutations or exon 14 mutations. In some embodiments the *JAK2* mutation is *JAK2*V617F. In some embodiments, the *JAK2* mutation is T514M, N533Y, L545V, F547L, F556V, R564L, R564Q, V567L, V567A, G571S, G571R, L579F, H587N, S591L, H606Q, L624P, I645V, K539L, V617L, C618R, L624P, exon 14 deletion S593-N622, or whole exon 14-deletion.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *JAK2* (e.g., some of the hematopoietic cells of the subject carry a *JAK2* mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject
20 carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *JAK2*, according to a dosage regimen effective to reduce mutant *JAK2* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *JAK2* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an
25 SAP protein therapy for an MPD based on *JAK2* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *JAK2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii)
30 identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative

embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein or agonist therapy based on the presence or absence of one or more somatic mutations in *JAK2*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *JAK2*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using a *JAK2* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *JAK2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *JAK2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *JAK2* include exon 12 mutations or exon 14 mutations. In some embodiments the *JAK2* mutation is JAK2V617F. In some embodiments, the *JAK2* mutation is T514M, N533Y, L545V, F547L, F556V, R564L, R564Q, V567L, V567A, G571S, G571R, L579F, H587N, S591L, H606Q, L624P, I645V, K539L, V617I, C618R, L624P, exon 14 deletion S593-N622, or whole exon 14-deletion. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In some embodiments, the mutation associated with the myeloproliferative disorder is not JAK2V617F. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-

associated genes such as but not limited to, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more

5 cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD

10 is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *JAK2*

15 mutations. The sample may be any suitable biological sample including, for example, whole blood (e.g., *JAK2* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *JAK2* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *JAK2* nucleic acid mutation may be inferred by assessing the *JAK2* protein from the individual. For example, identification of a mutant *JAK2* protein is indicative of a mutation in the *JAK2* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more

20 additional MPD-associated genes (e.g., *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *JAK2* nucleic acid mutation. In some embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to a *JAK2* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

30 *MPL*

Myeloproliferative leukemia protein (MPL) is the receptor for thrombopoietin that regulates the production of platelets by bone marrow. Recently, acquired mutations in the transmembrane-juxtamembrane region of MPL (MPLW515 mutations) have been reported in approximately 5% of *JAK2*V617F-negative PMF and about 1% of all cases of ET (Pardanani

et al. Blood. 2006, 108(10): 3472-3476 and Pikman et al. PLoS Med. 2006, 3:e270). Like JAK2V617F, the MPL mutations confer constitutive activation of the JAK-STAT pathway.

Other exemplary *MPL* mutations include mutations in the *MPL* nucleic acid such as deletion/insertion mutations in exons 10 and 11 described in U.S. Patent Application

5 Publication No. 2013/0053262.

MPL genomic nucleic acid is located in human chromosome 1. An exemplary sequence of all or portions of human *MPL* mRNA includes but is not limited to GenBank Accession number NM_005373 (SEQ ID NO: 7). These sequences are incorporated herein by reference.

10 For the *MPL* nucleic acid sequence, a “mutation” means a *MPL* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_005373 (SEQ ID NO: 7). A mutation in *MPL* nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined
15 as compared to NP_005364 (SEQ ID NO: 8) as a reference amino acid sequence.

In some embodiments, the *MPL* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *MPL* include insertion/deletion mutations in exon 10 of *MPL*. In some embodiments, mutations in *MPL* include insertion/deletion mutations in exon 11 of *MPL*. In some embodiments, the mutation
20 in *MPL* includes a mutation in codon 515. In some embodiments, mutations in *MPL* include MPLW515L, MPLW515K, MPLW515A or MPLW515R.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *MPL* (e.g., some of the hematopoietic cells of the subject carry a *MPL*
25 mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *MPL*, according to a dosage regimen effective to reduce mutant *MPL* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant
30 allele burden in *MPL* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *MPL* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *MPL*, wherein said first mutant allele burden is measured before administration of the SAP protein;

(ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *MPL*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *MPL*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *MPL* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *MPL*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *MPL* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *MPL* include insertion/deletion mutations in exon 10 of *MPL*. In some embodiments, mutations in *MPL* include insertion/deletion mutations in exon 11 of *MPL*. In some embodiments, the mutation in *MPL* includes a mutation in codon 515. In some embodiments, mutations in *MPL* include MPLW515L, MPLW515K, MPLW515A or MPLW515R. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative

disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In

5 certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods

10 further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the

15 above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *MPL*

20 mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *MPL* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *MPL* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *MPL* nucleic acid

25 mutation may be inferred by assessing the *MPL* protein from the individual. For example, identification of a mutant *MPL* protein is indicative of a mutation in the *MPL* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more

30 additional myeloproliferative disorder-associated genes (e.g., *JAK2*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *MPL* nucleic acid mutation. In some embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *MPL* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

CALR

Calreticulin (CALR) is a highly conserved, multifunctional endoplasmic reticulum (ER) protein and plays an integral role in calcium homeostasis and protein folding inside the ER. Outside the ER, CALR regulates various integrin-mediated cell adhesion, gene nuclear
5 transport, programmed cell removal, and immunogenic cell death. *CALR* encoding CALR protein is located on chromosome 19p13.2, contains 9 exons, and spans a 4.2-kb region. Somatic insertions or deletions in exon 9 of *CALR* were found in as high as 70% to 84% of samples of myeloproliferative neoplasms with nonmutated *JAK2* (Klampfl et al. N Engl J Med. 2013, 369(25):2379-90 and Nangalia et al. N Engl J Med. 2013, 369(25):2391-2405).
10 The *CALR* mutations cause a frameshift resulting in a novel C-terminus containing a number of positively charged amino acids whereas the wildtype C-terminus is mostly negatively charged.

In essential thrombocythemia and primary myelofibrosis, *CALR* mutations and *JAK2* and *MPL* mutations were mutually exclusive (Klampfl et al. N Engl J Med. 2013, 369(25):2379-90). *CALR* mutations are a useful diagnostic marker for *JAK2/MPL*-negative
15 ET or PMF patients due to their relative high frequency. Moreover, the phenotypic manifestations are different from those of *JAK2* mutations.

CALR genomic nucleic acid is located in human chromosome 19. An exemplary sequence of all or portions of human *CALR* mRNA includes but is not limited to GenBank
20 Accession number NM_004343 (SEQ ID NO: 9). These sequences are incorporated herein by reference.

For the *CALR* nucleic acid sequence, a "mutation" means a *CALR* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_004343 (SEQ ID NO: 9). A mutation in *CALR* nucleic acid
25 may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_004334 (SEQ ID NO: 10) as a reference amino acid sequence.

In some embodiments, the *CALR* mutation is a missense mutation, a deletion
30 mutation, an insertion mutation or a translocation. In some embodiments, mutations in *CALR* include insertion/deletion mutations in exon 9 of *CALR*.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *CALR* (e.g., some of the hematopoietic cells of the subject carry a

CALR mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *CALR*, according to a dosage regimen effective to reduce mutant *CALR* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *CALR* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *CALR* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *CALR*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *CALR*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *CALR*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using a *CALR* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *CALR*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an

alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *CALR* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *CALR* include

5 insertion/deletion mutations in exon 9 of *CALR*. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an

10 SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods

15 further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other

20 translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

25 Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *CALR* mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *CALR* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *CALR*

30 nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *CALR* nucleic acid mutation may be inferred by assessing the *CALR* protein from the individual. For example, identification of a mutant *CALR* protein is indicative of a mutation in the *CALR* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer

extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more additional MPD-associated genes (e.g., *JAK2*, *MPL*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *CALR* nucleic acid mutation. In some
5 embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to a *CALR* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

ASXL1

Additional sex combs like transcriptional regulator 1 (*ASXL1*) is needed for normal
10 hematopoiesis and is thought to be involved in activation of transcription factors and transcriptional repression. *ASXL1* mutations are thought to contribute to epigenetic dysregulation of effects in myeloproliferative neoplasms. *ASXL1* mutations involve exon 12 and truncate the pleckstrin homology domain of *ASXL1*. In a recent study, *ASXL1* mutational frequencies were 13% in PMF, 23% in post-PV/ET MF, and 18% in blast-phase
15 MPN (The same study demonstrated co-occurrence of mutant *ASXL1* with *TET2*, *JAK2*, *EZH2*, *IDH* and *MPL* mutations (Abdel-Wahab et al. ASH Annu Meet Abstr. 2010, 116:3070).

ASXL1 genomic nucleic acid is located in human chromosome 20. An exemplary sequence of all or portions of human *ASXL1* mRNA includes but is not limited to GenBank
20 Accession number NM_001164603 (SEQ ID NO: 11). These sequences are incorporated herein by reference.

For the *ASXL1* nucleic acid sequence, a “mutation” means a *ASXL1* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_001164603 (SEQ ID NO: 11). A mutation in *ASXL1*
25 nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_001158075 (SEQ ID NO: 12) as a reference amino acid sequence.

In some embodiments, the *ASXL1* mutation is a missense mutation, a deletion
30 mutation, an insertion mutation or a translocation. In some embodiments, mutations in *ASXL1* include insertion/deletion mutations in exon 12 of *ASXL1*.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *ASXL1* (e.g., some of the hematopoietic cells of the subject carry an

ASXL1 mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *ASXL1*, according to a dosage regimen effective to reduce mutant *ASXL1* allele

5 burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *ASXL1* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *ASXL1*/mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *ASXL1*, wherein

10 said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to

15 the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or

20 frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *ASXL1*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *ASXL1*; and if the subject carries said

25 mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *ASXL1* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *ASXL1*, wherein said first mutant allele burden is measured before administration

30 of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an

alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *ASXL1* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *ASXL1* include

5 insertion/deletion mutations in exon 12 of *ASXL1*. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an

10 SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *CALR*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods

15 further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other

20 translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

25 Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *ASXL1* mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *ASXL1* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *ASXL1*

30 nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *ASXL1* nucleic acid mutation may be inferred by assessing the *ASXL1* protein from the individual. For example, identification of a mutant *ASXL1* protein is indicative of a mutation in the *ASXL1* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer

extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more additional MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *ASXL1* nucleic acid mutation. In some
5 embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *ASXL1* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

EZH2

Enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) is part of a
10 methyltransferase (polycomb-repressive complex 2 associated with H3 Lys-27 trimethylation). In one study, mutational frequencies of *EZH2* were 13% in CMML, 13% in atypical CML, 13% in MF (PMF or post-PV/ET MF), 10% in MDS/MPN-U, 6% in MDS, 3% in PV and 3% in hypereosinophilic syndrome/chronic eosinophilic leukemia (Ernst et al. Nat Genet. 2010, 42:722–726). *EZH2* variants in this study included missense, frameshift or
15 stop mutations expected to result in premature chain termination or truncation of critical domains. It is thought that the MPN-associated *EZH2* mutations have a tumor suppressor activity.

EZH2 genomic nucleic acid is located in human chromosome 7. An exemplary sequence of all or portions of human *EZH2* mRNA includes but is not limited to GenBank
20 Accession number NM_001203247 (SEQ ID NO: 13). These sequences are incorporated herein by reference.

For the *EZH2* nucleic acid sequence, a “mutation” means a *EZH2* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_001203247 (SEQ ID NO: 13). A mutation in *EZH2* nucleic
25 acid may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_001190176 (SEQ ID NO: 14) as a reference amino acid sequence.

In some embodiments, the *EZH2* mutation is a missense mutation, a deletion
30 mutation, an insertion mutation or a translocation.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *EZH2* (e.g., some of the hematopoietic cells of the subject carry an *EZH2* mutation). In some embodiments, the disclosure provides a method of treating an

MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *EZH2*, according to a dosage regimen effective to reduce mutant *EZH2* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *EZH2* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *EZH2* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *EZH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein (e.g., an SAP protein) is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *EZH2*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *EZH2*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *EZH2* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *EZH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an alternative embodiment, if there is no change in the second mutant allele burden relative to

the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *EZH2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *CALR*, *ASXL1*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *EZH2* mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *EZH2* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *EZH2* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *EZH2* nucleic acid mutation may be inferred by assessing the *EZH2* protein from the individual. For example, identification of a mutant *EZH2* protein is indicative of a mutation in the *EZH2* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more

additional MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *SRSF2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *EZH2* nucleic acid mutation. In some embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *EZH2* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

SRSF2

Serine/arginine-rich splicing factor 2 (*SRSF2*) is a component of the RNA splicing machinery. *SRSF2* mutations alter pre-mRNA splicing, are relatively common in primary myelofibrosis, and appear to be predictive of poor outcome. In one study, 187 PMF patients were studied and it was found that 17% harbored *SRSF2* mutations, including missense mutations such as P95H, P95L, P95R, and P95S, a 24-bp deletion (delP95-R102) and an insertion mutation 274-275insACC (G93D;P95R). *SRSF2* mutations clustered with *IDH* mutations (Lasho et al. Blood 2012, 120(20):4168-71).

SRSF2 genomic nucleic acid is located in human chromosome 17. An exemplary sequence of all or portions of human *SRSF2* mRNA includes but is not limited to GenBank Accession number NM_001195427 (SEQ ID NO: 15). These sequences are incorporated herein by reference.

For the *SRSF2* nucleic acid sequence, a “mutation” means a *SRSF2* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_001195427 (SEQ ID NO: 15). A mutation in *SRSF2* nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_001182356 (SEQ ID NO: 16) as a reference amino acid sequence.

In some embodiments, the *SRSF2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *SRSF2* include P95H, P95L, P95R, P95S, delP95-R102 or G93D;P95R insertion.

In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *SRSF2* (e.g., some of the hematopoietic cells of the subject carry an *SRSF2* mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *SRSF2*, according to a dosage regimen effective to reduce mutant *SRSF2* allele

burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *SRSF2* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *SRSF2* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *SRSF2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *SRSF2*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *SRSF2*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *SRSF2* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *SRSF2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *SRSF2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *SRSF2* include P95H, P95L,

P95R, P95S, delP95-R102 or G93D;P95R insertion. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *IDH1*, or *IDH2*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *SRSF2* mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *SRSF2* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *SRSF2* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *SRSF2* nucleic acid mutation may be inferred by assessing the *SRSF2* protein from the individual. For example, identification of a mutant *SRSF2* protein is indicative of a mutation in the *SRSF2* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more additional MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *IDH1*, or *IDH2*) either simultaneously or prior to screening for the *SRSF2* nucleic acid mutation. In some

embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *SRSF2* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

IDH1 and *IDH2*

5 Isocitrate dehydrogenase (IDH) mutations involve exon 4 and affect three arginine residues, R132 and R172 in *IDH1* and R140 in *IDH2*. *IDH1* mutations result in loss of isocitrate to 2-ketoglutarate conversion activity and a gain of 2-ketoglutarate to 2-hydroxyglutarate conversion activity. One study identified *IDH* mutational frequencies of ~2% in PV, 1% in ET, 4% in PMF and 22% in blast-phase MPN (Tefferi et al. Leukemia. 10 2010, 24:1302–1309). *IDH*-mutated patients were more likely to be nullizygous for *JAK2* 46/1 haplotype and less likely to display complex karyotype.

IDH1 genomic nucleic acid is located in human chromosome 2. An exemplary sequence of all or portions of human *IDH1* mRNA includes but is not limited to GenBank Accession number NM_005896 (SEQ ID NO: 17). These sequences are incorporated herein 15 by reference.

 For the *IDH1* nucleic acid sequence, a “mutation” means a *IDH1* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence GenBank accession number NM_005896 (SEQ ID NO: 17). A mutation in *IDH1* nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be 20 silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_005887 (SEQ ID NO: 18) as a reference amino acid sequence.

 In some embodiments, the *IDH1* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *IDH1* 25 include insertion/deletion mutations in exon 4 of *IDH1*. In some embodiments, mutations in *IDH1* include missense mutations at R132.

 In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *IDH1* (e.g., some of the hematopoietic cells of the subject carry an 30 *IDH1* mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of the subject) in *IDH1*, according to a dosage regimen effective to reduce mutant *IDH1* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing

mutant allele burden in *IDH1* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *IDH1* mutational status. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *IDH1*, wherein
5 said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to
10 the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or
15 frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein or agonist therapy based on the presence or absence of one or more somatic mutations in *IDH1*. In some embodiments, the method comprises (i) determining whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *IDH1*; and if the subject carries
20 said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *IDH1* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *IDH1*, wherein said first mutant allele burden is measured before administration
25 of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an
30 alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *IDH1* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *IDH1* include insertion/deletion mutations in exon 4 of *IDH1*. In some embodiments, mutations in *IDH1*

include missense mutations at R132. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, or *IDH2*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, inv(3), i(17q), -7/7q-, 11q or 12p abnormalities, complex non-monosomal, 5q-, +8, other autosomal trisomies except +9, sole abnormalities of 20q-, 1q duplication or any other translocation, and -Y or other sex chromosome abnormality, normal or sole abnormalities of 13q- or +9, or other sole abnormalities. In some embodiments of any of the above aspects of the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *IDH1* mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *IDH1* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *IDH1* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *IDH1* nucleic acid mutation may be inferred by assessing the *IDH1* protein from the individual. For example, identification of a mutant *IDH1* protein is indicative of a mutation in the *IDH1* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more additional MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, or *IDH2*) either simultaneously or prior to screening for the *IDH1* nucleic acid mutation. In some

embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *IDH1* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

IDH2 genomic nucleic acid is located in human chromosome 15. An exemplary
5 sequence of all or portions of human *IDH1* mRNA includes but is not limited to GenBank Accession number NM_001289910 (SEQ ID NO: 19). These sequences are incorporated herein by reference.

For the *IDH2* nucleic acid sequence, a “mutation” means a *IDH2* nucleic acid sequence that includes at least one nucleic acid variation as compared to reference sequence
10 GenBank accession number NM_001289910 (SEQ ID NO: 19). A mutation in *IDH2* nucleic acid may result in a change in the encoded polypeptide sequence or the mutation may be silent with respect to the encoded polypeptide sequence. A change in an amino acid sequence may be determined as compared to NP_001276839 (SEQ ID NO: 20) as a reference amino acid sequence.

15 In some embodiments, the *IDH2* mutation is a missense mutation, a deletion mutation, an insertion mutation or a translocation. In some embodiments, mutations in *IDH2* include insertion/deletion mutations in exon 4 of *IDH2*. In some embodiments, mutations in *IDH2* include missense mutations at R172 or R140.

In some embodiments, the disclosure provides a method of treating an MPD
20 comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation in *IDH2* (e.g., some of the hematopoietic cells of the subject carry an *IDH2* mutation). In some embodiments, the disclosure provides a method of treating an MPD comprising administering a therapeutically effective amount of an SAP protein to a subject carrying a mutation (e.g., the mutation may be found in some hematopoietic cells of
25 the subject) in *IDH2*, according to a dosage regimen effective to reduce mutant *IDH2* allele burden in said subject. In some embodiments, the disclosure provides a method for reducing mutant allele burden in *IDH2* in a subject suffering from a myeloproliferative disorder. In some embodiments, the disclosure provides methods of monitoring the effectiveness of an SAP protein therapy for an MPD based on *IDH2* mutational status. In one embodiment, the
30 method comprises: (i) measuring a first mutant allele burden of a mutation in *IDH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) identifying a difference between the second mutant allele burden and the first mutant allele

burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder and the dosage regimen may be maintained or modified to decrease the dosage and/or frequency of administration. In an alternative
5 embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden, the dosage regimen may be modified to increase the dosage and/or frequency of administration. In one aspect, the disclosure provides methods of determining responsiveness to SAP protein therapy based on the presence or absence of one or more somatic mutations in *IDH2*. In some embodiments, the method comprises (i) determining
10 whether the cells of a subject having a myeloproliferative disorder carry a mutation associated with the myeloproliferative disorder in *IDH2*; and if the subject carries said mutant allele (ii) administering a therapeutically effective amount of an SAP protein to the subject. In one aspect, the disclosure provides a method of using an *IDH2* mutation as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure. In one
15 embodiment, the method comprises: (i) measuring a first mutant allele burden of a mutation in *IDH2*, wherein said first mutant allele burden is measured before administration of the SAP protein; (ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and (iii) measuring the difference between the second mutant allele burden and the
20 first mutant allele burden. In a further embodiment, a decrease in the second mutant allele burden relative to the first mutant allele burden indicates a positive prognosis. In an alternative embodiment, if there is no change in the second mutant allele burden relative to the first mutant allele burden indicates a neutral or negative prognosis. In some embodiments, the *IDH2* mutation is a missense mutation, a deletion mutation, an insertion
25 mutation or a translocation. In some embodiments, mutations in *IDH2* include insertion/deletion mutations in exon 4 of *IDH2*. In some embodiments, mutations in *IDH2* include missense mutations at R172 or R140. The SAP proteins of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) are used, alone or in combination with an additional agent, to treat a myeloproliferative disorder. In some
30 embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used as a monotherapy. In some embodiments, an SAP protein of the disclosure (such as a recombinant human SAP protein, such as a glycosylated SAP protein) is used in combination with an anti-cancer agent. In certain embodiments, any of the foregoing methods further comprise assaying for one or more

mutations in one or more MPD-associated genes such as but not limited to, *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, or *IDH1*. In some embodiments, any of the foregoing methods further comprise assaying for one or more mutations in *TET2*, *CBL*, *IKZF1*, *LNK*, *DNMT3A*, *CUX1*, *U2AF1*, or *SF3B1*. In some embodiments, any of the foregoing methods further
5 comprise assaying for one or more cytogenetic abnormalities such as monosomal karyotype, *inv(3)*, *i(17q)*, *-7/7q-*, *11q* or *12p* abnormalities, complex non-monosomal, *5q-*, *+8*, other autosomal trisomies except *+9*, sole abnormalities of *20q-*, *1q* duplication or any other translocation, and *-Y* or other sex chromosome abnormality, normal or sole abnormalities of *13q-* or *+9*, or other sole abnormalities. In some embodiments of any of the above aspects of
10 the disclosure, the MPD is polycythemia vera, essential thrombocythemia, myelofibrosis, or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

Methods of the disclosure involve evaluating a sample containing nucleic acids from an individual having or suspected of having an MPD for the presence or absence of *IDH2*
15 mutations. The sample may be any suitable biological sample including, for example, whole blood (i.e., *IDH2* nucleic acid being extracted from the cellular fraction), plasma, serum, bone marrow, and tissue samples (e.g., biopsy and paraffin-embedded tissue). The *IDH2* nucleic acid may be any convenient nucleic acid type including, for example, genomic DNA, RNA (e.g., mRNA), or cDNA prepared from subject RNA. Alternatively, the *IDH2* nucleic
20 acid mutation may be inferred by assessing the *IDH2* protein from the individual. For example, identification of a mutant *IDH2* protein is indicative of a mutation in the *IDH2* gene. Suitable detection methodologies include oligonucleotide probe hybridization, primer extension reaction, nucleic acid sequencing, and protein sequencing. In some embodiments, the individual is screened for the presence of other pathological mutations in one or more
25 additional MPD-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, or *IDH1*) either simultaneously or prior to screening for the *IDH2* nucleic acid mutation. In some embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, or 8) mutations in addition to an *IDH2* mutation are used as a prognostic marker for measuring response to treatment with an SAP protein of the disclosure.

30 In certain methods of any of the foregoing, the disclosure provides methods of treating a subject determined to comprise a mutation associated with an MPD, such as a mutation in one or more of the foregoing genes (e.g., some of the subject's cells carry the mutation). In certain embodiments of any of the foregoing, the subject is heterozygous or

homozygous. In certain embodiments, the subject carries more than one mutation associated with an MPD (e.g., 2, 3 or more than 3).

Detection Methods

The methods of the disclosure can also be used to detect mutations in a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) or to detect other genetic alterations such as cytogenetic abnormalities. In certain embodiments, the methods include detecting, in a sample of cells (e.g., bodily fluid cells such as blood cells, bone marrow cells, etc.) from the subject, the presence or absence of a genetic mutation in a myeloproliferative disorder-associated gene. For example, such genetic mutations can be detected by ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from one or more genes; 2) an addition of one or more nucleotides to one or more genes; 3) a substitution of one or more nucleotides of one or more genes; 4) a chromosomal rearrangement (e.g., translocation) of one or more genes; 5) an alteration in the level of a messenger RNA transcript of one or more genes; 6) aberrant modification of one or more genes, such as of the methylation pattern of the genomic DNA; 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of one or more genes; 8) a non-wild type level of a one or more proteins; 9) allelic loss of one or more genes; and 10) inappropriate post-translational modification of one or more proteins. As described herein, there are a large number of assays known in the art which can be used for detecting mutations in one or more genes. In some embodiments, the mutational status of gene is measured by collecting peripheral blood samples, extracting DNA from the samples, and analyzing by PCR. In some embodiments, genomic DNA is extracted from peripheral blood leukocytes. In some embodiments, genomic DNA is extracted from peripheral blood granulocytes. In some embodiments, the mutations in one or more genes are detected by PCR. In some embodiments, the mutations in one or more genes are detected by whole exome sequencing. In some embodiments, the mutations in one or more genes are detected by Sanger sequencing. In some embodiments, the mutations in one or more genes are detected by whole genome sequencing.

In certain embodiments, detection of the genetic mutation involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202 and 5,854,033), such as real-time PCR, COLD-PCR, anchor PCR, recursive PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077; Prodromou and Pearl (1992) Protein Eng. 5:827; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. USA 91:360), the latter of which can be particularly useful

for detecting point mutations in a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) (see Abravaya et al. (1995) Nucleic Acids Res. 23:675). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, 5 contacting the nucleic acid sample with one or more primers which specifically hybridize to myeloproliferative disorder-associated gene under conditions such that hybridization and amplification of the gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a 10 preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al., (1990) Proc. Natl. Acad. Sci. USA 87:1874), transcriptional amplification system (Kwoh et al., (1989) Proc. Natl. Acad. Sci. USA 86:1173), Q-Beta Replicase (Lizardi 15 et al. (1988) Bio-Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in one or more myeloproliferative disorder-associated genes (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) from a 20 sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, optionally amplified, digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and 25 control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in one or more of the myeloproliferative disorder-associated genes (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) 30 described herein can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) Human Mutation 7: 244; Kozal et al. (1996) Nature Medicine 2:753). For example, genetic mutations in a nucleic acid can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M. T. et al. supra.

Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the
5 characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art
10 can be used to directly sequence a myeloproliferative disorder-associated gene and detect mutations by comparing the sequence of the sample gene with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) Proc. Natl. Acad. Sci. USA 74:560) or Sanger ((1977) Proc. Natl. Acad. Sci. USA 74:5463). It is also contemplated that any of a variety of
15 automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) Biotechniques 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen et al. (1996) Adv. Chromatogr. 36:127-162; and Griffin et al. (1993) Appl. Biochem. Biotechnol. 38:147).

Other methods for detecting mutations in a myeloproliferative disorder-associated
20 gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) Science 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type sequence with potentially mutant RNA or
25 DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to base pair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA
30 duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton et al. (1988) Proc. Natl. Acad. Sci. USA 85:4397;

Saleeba et al. (1992) *Methods Enzymol.* 217:286. In one embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657). According to an exemplary embodiment, a probe based on a myeloproliferative disorder-associated gene sequence, e.g., a wild-type sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in myeloproliferative disorder-associated genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73). Single-stranded DNA fragments of sample and control nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet.* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 by of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing

gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) Biophys. Chem. 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification or selective primer
5 extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) Nature 324:163; Saiki et al. (1989) Proc. Natl. Acad. Sci. USA 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the
10 oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant disclosure. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of
15 the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) Nucl. Acids Res. 17:2437) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) Tibtech 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) Mol. Cell Probes
20 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification. In some embodiments, quantitative real-time allele-
25 specific PCR, for example, in which allelic discrimination is enhanced by the synergistic effect of a mismatch in the -1 position, and a locked nucleic acid (LNA) at the -2 position, is used to detect and/or quantify the presence or absence of a mutation (Nussenzveig et al. (2007) Exp Hematol. 35(1):32-8).

In certain exemplary embodiments, the level of mRNA corresponding to the
30 myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) can be determined either by *in situ* and/or by *in vitro* formats in a biological sample using methods known in the art. In some embodiments, the level of an MPD-associated miRNA can be determined either by *in situ* and/or by *in vitro* formats in a biological sample using methods known in the art. In some embodiments, an MPD-

associated mRNA or miRNA is present in an exosome. Many expression detection methods use isolated RNA. For *in vitro* methods, any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA from blood cells (see, e.g., Ausubel et al, ed., Current Protocols in Molecular Biology, John Wiley & Sons, New York 1987 1999). Additionally, large numbers of cells and/or samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (1989, U.S. Pat. No. 4,843,155).

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. In certain exemplary embodiments, a diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding a myeloproliferative disorder-associated gene. Other suitable probes for use in the diagnostic assays of the disclosure are described herein. Hybridization of an mRNA with the probe indicates that the mutation in question is being expressed.

In one format, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in a gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in detecting the level of mRNA encoded by a myeloproliferative disorder-associated gene.

An alternative method for determining the level of mRNA corresponding to a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) in a sample involves the process of nucleic acid amplification, e.g., by rtPCR (the experimental embodiment set forth in U.S. Pat. Nos. 4,683,195 and 4,683,202), quantitative real-time allele-specific PCR (Borowczyk et al. (2015) *Thromb Res.* 135(2):272-80), COLD-PCR (Li et al. (2008) *Nat. Med.* 14:579), ligase chain reaction (Barany, 1991, *Proc. Natl. Acad. Sci. USA*, 88:189), self sustained sequence replication (Guatelli et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:1874), transcriptional amplification system (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173), Q-Beta Replicase (Lizardi et al. (1988)

Bio/Technology 6:1197), rolling circle replication (U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, mRNA does not need to be isolated from the sample (e.g., a bodily fluid (e.g., blood cells)) prior to detection. In such methods, a cell or tissue sample is prepared/processed using known histological methods. The sample is then immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to an mRNA of the disclosure.

Determinations may be based on the normalized expression level of a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*). Expression levels are normalized by correcting the absolute expression level of a marker by comparing its expression to the expression of a gene that is not a marker, e.g., a housekeeping gene that is constitutively expressed. Suitable genes for normalization include housekeeping genes such as the actin gene, or epithelial cell-specific genes. This normalization allows the comparison of the expression level in a patient sample from one source to a patient sample from another source.

According to the disclosure, the presence or absence of myeloproliferative disorder-associated mutations can also be determined by analyzing the proteins (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) encoded by the mutated genes. Detection of mutations at the protein level can be detected by any method well known in the field. In one embodiment, detection of mutation in the myeloproliferative disorder-associated protein is carried out by isolating the protein and subjecting it to amino acid sequence determination. This may require fragmenting the protein by proteolytic or chemical means prior to sequencing. Methods of determining an amino acid sequence are well known in the art.

Detection of mutated proteins can be accomplished using, for example, antibodies, aptamers, ligands; substrates, other proteins or protein fragments, other protein-binding

agents, or mass spectrometry analysis of fragments. Preferably, protein detection agents are specific for the mutated proteins of the present disclosure and can therefore discriminate between a mutated protein and the wild-type protein or another variant form. This can generally be accomplished by, for example, selecting or designing detection agents that bind to the region of a protein that differs between the variant and wild-type protein.

One preferred agent for detecting a mutated protein is an antibody capable of selectively binding to a variant form of the protein. Antibodies capable of distinguishing between wild-type and mutated proteins may be created by any suitable method known in the art. The antibodies may be monoclonal or polyclonal antibodies, single chain or double chain, chimeric or humanized antibodies or portions of immunoglobulin molecules containing the portions known in the state of the art to correspond to the antigen binding fragments.

Methods for manufacturing polyclonal antibodies are well known in the art. Typically, antibodies are created by administering (e.g., via subcutaneous injection) the mutated protein immunogenic fragment containing the mutation to white New Zealand rabbits. The antigen (e.g. mutant JAK2, MPL, CLR, ASXL1, EZH2, SRSF2, IDH1 or IDH2) is typically injected at multiple sites and the injections are repeated multiple times (e.g., approximately bi-weekly) to induce an immune response. Desirably, the rabbits are simultaneously administered an adjuvant to enhance immunity. The polyclonal antibodies are then purified from a serum sample, for example, by affinity chromatography using the same antigen to capture the antibodies. The antibodies can be made specific to the mutation by removing antibodies cross-reacting with native protein.

In vitro methods for detection of the mutated proteins (e.g. mutant JAK2, MPL, CLR, ASXL1, EZH2, SRSF2, IDH1 or IDH2) also include, for example, enzyme linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), Western blots, immunoprecipitations, immunofluorescence, and protein arrays chips (e.g., arrays of antibodies or aptamers). For further information regarding immunoassays and related protein detection methods, see Current Protocols in Immunology, John Wiley & Sons, N.Y., and Hage, "Immunoassays", Anal Chem: 1999 Jun. 15; 71(12):294R-304R. Additional analytic methods of detecting amino acid variants include, but are not limited to, altered electrophoretic mobility (e.g., 2-dimensional electrophoresis), altered tryptic peptide digest, altered JAK2 kinase activity in cell-based or cell-free assay, alteration in ligand or antibody-binding pattern, altered isoelectric point, and direct amino acid sequencing.

The disclosure also encompasses kits for detecting the presence of one or more mutations in one or more myeloproliferative disorder-associated genes (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting marker genomic DNA, polypeptide, protein mRNA, and the like in a biological sample; and means for determining the presence or absence of the mutation in the sample. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the mutations in the marker peptide or nucleic acid.

Biological Sample Collection and Preparation

The methods and compositions of this disclosure may be used to detect mutations in a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) and/or myeloproliferative disorder-associated protein (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) using a biological sample obtained from an individual. Methods of obtaining test samples are well known to those of skill in the art and include, but are not limited to, aspirations, tissue sections, drawing of blood or other fluids, surgical or needle biopsies, and the like. The test sample may be obtained from an individual or patient diagnosed as having a myeloproliferative disorder or suspected being afflicted with a myeloproliferative disorder or undergoing therapy for a myeloproliferative disorder. The test sample may be a cell-containing liquid or a tissue. Samples may include, but are not limited to, amniotic fluid, biopsies, blood, blood cells, bone marrow, fine needle biopsy samples, peritoneal fluid, amniotic fluid, plasma, pleural fluid, saliva, semen, serum, tissue or tissue homogenates, frozen or paraffin sections of tissue. Samples may also be processed, such as sectioning of tissues, fractionation, purification, or cellular organelle separation. If necessary, the sample may be collected or concentrated by centrifugation and the like. The cells of the sample may be subjected to lysis, such as by treatments with enzymes, heat, surfactants, ultrasonication, or a combination thereof. The lysis treatment is performed in order to obtain a sufficient amount of nucleic acid derived from the individual's cells to detect using for example, polymerase chain reaction. Alternatively, mutations in the myeloproliferative disorder-associated gene may be detected using an acellular bodily fluid according to the methods described in U.S. patent application Ser. No. 11/408,241 (Publication No. US 2007-0248961), hereby incorporated by reference.

Methods of plasma and serum preparation are well known in the art. Either "fresh" blood plasma or serum, or frozen (stored) and subsequently thawed plasma or serum may be used. Frozen (stored) plasma or serum should optimally be maintained at storage conditions

of -20 to -70 degrees centigrade until thawed and used. "Fresh" plasma or serum should be refrigerated or maintained on ice until used, with nucleic acid (e.g., RNA, DNA or total nucleic acid) extraction being performed as soon as possible. Exemplary methods are described below. Blood can be drawn by standard methods into a collection tube, typically
5 siliconized glass, either without anticoagulant for preparation of serum, or with EDTA, sodium citrate, heparin, or similar anticoagulants for preparation of plasma. If preparing plasma or serum for storage, although not an absolute requirement, it is preferable that plasma or serum is first fractionated from whole blood prior to being frozen. This reduces the burden of extraneous intracellular RNA released from lysis of frozen and thawed cells
10 which might reduce the sensitivity of the amplification assay or interfere with the amplification assay through release of inhibitors to PCR such as porphyrins and hematin. "Fresh" plasma or serum may be fractionated from whole blood by centrifugation, using gentle centrifugation at 300-800 times gravity for five to ten minutes, or fractionated by other standard methods. High centrifugation rates capable of fractionating out apoptotic bodies
15 should be avoided. Since heparin may interfere with RT-PCR, use of heparinized blood may require pretreatment with heparanase, followed by removal of calcium prior to reverse transcription. Imai, H., et al., J. Virol. Methods 36:181-184, (1992). Thus, EDTA is a suitable anticoagulant for blood specimens in which PCR amplification is planned.

Nucleic Acid Extraction and Amplification

20 The nucleic acid to be amplified may be from a biological sample such as an organism, cell culture, tissue sample, and the like. The biological sample can be from a subject which includes any animal, preferably a mammal. A preferred subject is a human, which may be a patient presenting to a medical provider for diagnosis or treatment of a disease. The biological sample may be obtained from a stage of life such as a fetus, young
25 adult, adult, and the like. Particularly preferred subjects are humans being tested for a mutation in a myeloproliferative disorder-associated gene (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*).

Various methods of extraction are suitable for isolating the DNA or RNA. Suitable methods include phenol and chloroform extraction. See Maniatis et al., Molecular Cloning,
30 A Laboratory Manual, 2d, Cold Spring Harbor Laboratory Press, page 16.54 (1989). Numerous commercial kits also yield suitable DNA and RNA including, but not limited to, QIAamp™ mini blood kit, Agencourt Genfind™, Roche Cobas® Roche MagNA Pure® or phenol:chloroform extraction using Eppendorf Phase Lock Gels®, and the NucliSens extraction kit (Biomerieux, Marcy l'Etoile, France). In other methods, mRNA may be

extracted from patient blood/bone marrow samples using MagNA Pure LC mRNA HS kit and Mag NA Pure LC Instrument (Roche Diagnostics Corporation, Roche Applied Science, Indianapolis, Ind.).

Numerous methods are known in the art for isolating total nucleic acid, DNA and
 5 RNA from blood, serum, plasma and bone marrow or other hematopoietic tissues. In fact, numerous published protocols, as well as commercial kits and systems are available. By way of example but not by way of limitation, examples of such kits, systems and published protocols are described below. Commercially available kits include Qiagen products such as the QiaAmp DNA Blood MiniKit (Cat.# 51104, Qiagen, Valencia, Calif.), the QiaAmp RNA
 10 Blood MiniKit (Cat.# 52304, Qiagen, Valencia, Calif.); Promega products such as the Wizard Genomic DNA Kit (Cat.# A1620, Promega Corp. Madison, Wis.), Wizard SV Genomic DNA Kit (Cat.# A2360, Promega Corp. Madison, Wis.), the SV Total RNA Kit (Cat.# X3100, Promega Corp. Madison, Wis.), PolyAtract System (Cat.# Z5420, Promega Corp. Madison, Wis.), or the PurYield RNA System (Cat.# 23740, Promega Corp. Madison, Wis.).

15 Extraction of RNA from Plasma or Serum

Plasma RNA is highly sensitive and may replace DNA-based testing because of the relative abundance of the RNA and the ease in detecting deletions such as, for example, deletion of *JAK2* exon 14. Circulating extracellular deoxyribonucleic acid (DNA), including tumor-derived or associated extracellular DNA, is also present in plasma and serum. See
 20 Stroun, M., et al., *Oncology* 46:318-322, (1989). Since this DNA will additionally be extracted to varying degrees during the RNA extraction methods described above, it may be desirable or necessary (depending upon clinical objectives) to further purify the RNA extract and remove trace DNA prior to proceeding to further RNA analysis. This may be accomplished using DNase, for example by the method as described by Rashtchian, A., *PCR*
 25 *Methods Applic.* 4:S83-S91, (1994), as follows.

Glass beads, Silica particles or Diatom Extraction: RNA may be extracted from plasma or serum using silica particles, glass beads, or diatoms, as in the method or adaptations of Boom, R., et al., *J. Clin. Micro.* 28:495-503, (1990); Cheung, R. C., et al., *J. Clin Micro.* 32:2593-2597, (1994).

30 Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction: As an alternative method, RNA may be extracted from plasma or serum using the Acid Guanidinium Thiocyanate-Phenol-chloroform extraction method described by Chomczynski, P. and Sacchi, N., *Analytical Biochemistry* 162:156-159, (1987), as follows.

Alternative Nucleic Acid Extraction Methods: Alternative methods may be used to extract RNA from body fluids including but not limited to centrifugation through a cesium chloride gradient, including the method as described by Chirgwin, J. M., et al., *Biochemistry* 18:5294-5299, (1979), and co-precipitation of extracellular RNA from plasma or serum with gelatin, such as by adaptations of the method of Fournie, G. J., et al., *Analytical Biochemistry* 158:250-256, (1986), to RNA extraction.

Cytogenetic Analyses

Metaphase cytogenetic analysis may be carried out by standard karyotype methods, fluorescence *in situ* hybridization (FISH), spectral karyotyping or multiplex-FISH (M-FISH), multicolor FISH (mFISH), and comparative genomic hybridization), and/or *in situ* hybridization. It will be understood that any of the commercially available probes for conducting FISH analyses (e.g., Abbott Molecular VYSIS FISH Technology) may be employed in the methods of the disclosure. In one embodiment, the method includes: contacting a sample, e.g., a chromosomal sample or a fractionated, enriched or otherwise pre-treated sample) obtained from the subject with a probe (e.g., a probes specific for the desired sequence) under conditions suitable for hybridization, and determining the presence or absence of one or more of the abnormalities in the gene (e.g., genomic DNA in chromosomal regions associated with cytogenetic abnormalities). In some embodiments, cytogenetic analysis is performed on metaphases obtained from unstimulated bone marrow aspirate cultures using standard techniques known in the art (Tam et al. *Blood* 113(18): 4171-4178). The method can, optionally, include enriching a sample for the gene or gene product. In some embodiments, cytogenetic analysis is carried out according to the International System for Human Cytogenetic Nomenclature. (Cytogenetic and genome research. 2013. Prepublished on 2013/07/03 as DOI 10.1159/000353118).

In certain embodiments of any of the foregoing or following, the method of treatment may be effective to improve one or more manifestations of the MPD, such as bone marrow fibrosis or other manifestations described herein. In certain embodiments, the method of treatment is effective to both decrease allele burden and to improve one or more additional manifestations. In certain embodiments, improvement over time is evaluated by determining change in one or more manifestations, such as bone marrow fibrosis.

Methods of Administration

In one aspect, the disclosure provides methods for treating a myeloproliferative disorder in a patient by administering a therapeutically effective amount of an SAP protein of

the disclosure to a patient in need thereof. In one aspect, the disclosure provides methods for treating a myeloproliferative disorder in a patient carrying a mutation associated with the myeloproliferative disorder (e.g., a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) by administering a therapeutically effective amount of an SAP protein of the disclosure. The dosage and frequency of treatment can be determined by one skilled in the art and will vary depending on the symptoms, age and body weight of the patient, and the nature and severity of the disorder to be treated or prevented. The present disclosure has identified dosing regimens that are effective in treating myelofibrosis.

In one aspect, the disclosure provides methods for treating a myeloproliferative disorder in a patient carrying a mutation associated with the myeloproliferative disorder (e.g. a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) by administering a therapeutically effective amount of an SAP protein of the disclosure to a patient in need thereof according to a dosage regimen effective to reduce mutant allele burden. In some embodiments, the patient, prior to administration of the SAP protein, was not receiving any therapy other than transfusions. In some embodiments, the patient is anemic or thrombocytopenic.

As used herein, the term “dosage regimen” encompasses both the dose or dosage (i.e., the amount of the SAP protein) and the dosing schedule (i.e., the frequency of administration or intervals between successive doses of the SAP protein).

Administration of an SAP protein of the disclosure, singly or in combination with another agent such as an additional anti-cancer agent, according to either a weekly dosing schedule or a less frequent dosing schedule (e.g., less than weekly, such as every 4 weeks), resulted in significant improvements in myeloproliferative disorder symptoms. Moreover, the methods of the disclosure are also based on the finding that an SAP protein of the disclosure was well tolerated both alone and in combination with another anti-cancer therapeutic, with no evidence of clinically significant myelosuppression induced by the SAP treatment, e.g., treatment-related myelosuppression.

In certain aspects, the disclosure provides methods for treating a myeloproliferative disorder in a patient by administering to a patient in need thereof an SAP protein of the disclosure in an amount effective to reduce mutant allele burden. In certain aspects, the disclosure provides methods for treating a myeloproliferative disorder in a patient by administering to a patient in need thereof an SAP protein of the disclosure in an amount effective to improve the functioning of an affected organ. Improvement in function may be evaluated by, for example, evaluating a decrease in organ fibrosis, an improvement in platelet

levels, and/or an increase in hemoglobin. In some embodiments, the fibrotic organ is the bone marrow. In some embodiments, the myeloproliferative disorder is myelofibrosis.

In some embodiments, an SAP protein is administered to a patient once or twice per day, once or twice per week, once or twice per month, or once per month, or just prior to or at the onset of symptoms. In some embodiments, the SAP protein is administered to a patient more frequently at the onset of the treatment regimen (e.g., every other day for the first week and once every four weeks thereafter). In some embodiments, an SAP protein is administered to a patient with PV or ET who has not yet developed fibrosis, to prevent development of fibrosis. In some embodiments, an SAP protein is administered to a patient who has been determined to carry a mutation or cytogenetic abnormality associated with the myeloproliferative disorder (e.g., a mutation in one or more of *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) prior to the onset of symptoms.

Dosages may be readily determined by techniques known to those of skill in the art or as taught herein. Toxicity and therapeutic efficacy of an SAP protein may be determined by standard pharmaceutical procedures in experimental animals, for example, determining the LD₅₀ and the ED₅₀. The ED₅₀ (Effective Dose 50) is the amount of drug required to produce a specified effect in 50% of an animal population. The LD₅₀ (Lethal Dose 50) is the dose of drug which kills 50% of a sample population.

In certain aspects, an SAP protein is administered as a single agent for treating a myeloproliferative disorder in a subject. In certain aspects, administering a combination of an SAP protein (e.g., a variant SAP protein of the disclosure) and an additional anti-cancer therapeutic (e.g., a chemotherapeutic agent or a kinase inhibitor) optionally provides synergistic effects for treating a myeloproliferative disorder, e.g., myelofibrosis in a subject. In some embodiments, the SAP protein and the additional anti-cancer therapeutic (e.g., a chemotherapeutic agent or a kinase inhibitor) act on different aspects of the disease. Such an approach, combination or co-administration of the two types of agents, can be useful for treating individuals suffering from myeloproliferative disorders who do not respond to or are resistant to currently-available therapies. The combination therapy provided herein is also useful for improving the efficacy and/or reducing the side effects of currently-available therapies for individuals who do respond to such therapies.

In certain embodiments, the disclosure provides methods of treating myelofibrosis in a patient carrying a mutation in one or more myelofibrosis-associated genes (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) and/or one or more MPD-associated cytogenetic abnormalities, comprising administering an amount of an SAP protein, to a

subject in need thereof according to a dosing regimen (e.g., a dose and dosing schedule) and/or dosing schedule effective to ameliorate one or more symptoms of myelofibrosis and reduce mutant allele burden, wherein the subject in need thereof is not receiving therapy for myelofibrosis other than transfusions. In some embodiments, the subject is anemic or thrombocytopenic. In some embodiments, the methods of the disclosure do not induce treatment-related myelosuppression [e.g., the SAP protein does not induce clinically significant myelosuppression and/or does not increase (and may even decrease) myelosuppression present at baseline]. In other words, in certain embodiments, methods of the present disclosure do not induce or result in worsening of myelosuppression in comparison to, for example, that observed prior to initiation of treatment. Myelosuppression may be assessed according to the Common Terminology for Coding of Adverse Events (CTCAE) on a scale of Grade 0-Grade 5 (See National Cancer Institute Common Terminology Criteria for Adverse Events v4.0, NCI, NIH, DHHS. May 29, 2009 NIH publication # 09-7473). In some embodiments, one or more measures of myelosuppression, such as anemia, do not deteriorate (e.g., from a Grade 3 to Grade 4 adverse event; from a Grade 2 to Grade 3 or 4 adverse event; from a Grade 1 to a Grade 2, 3, or 4 adverse event; from a Grade 0 to a Grade 1, 2, 3, or 4 adverse event) as a result of treatment.

In one aspect, the disclosure provides a method for treating a myeloproliferative disorder in an individual carrying a myeloproliferative disorder-associated mutation (e.g., a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2* in some of his cells and/or one or more myeloproliferative disorder-associated cytogenetic abnormalities, comprising: a) obtaining a sample from said individual, wherein said sample comprises the nucleic acid of interest, b) evaluating a sample from the individual for the presence or absence of one or more mutations and/or cytogenetic abnormalities in the nucleic acid of interest, and c) administering an SAP protein of the disclosure to the individual carrying a myeloproliferative disorder-associated mutation and/or cytogenetic abnormality (e.g., a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*).

In one aspect, the disclosure provides a method for treating a myeloproliferative disorder in an individual carrying a myeloproliferative disorder-associated mutation (e.g., a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) in some his cells and/or one or more myeloproliferative disorder-associated cytogenetic abnormalities, comprising: a) obtaining a sample from said individual, wherein said sample comprises *JAK2* nucleic acid, b) evaluating a sample from the individual for the presence or absence of one or

more mutations in *JAK2* nucleic acid, and c) administering an SAP protein of the disclosure according to a dosage regimen effective to reduce *JAK2* mutant allele burden.

In one aspect, the disclosure provides a method for reducing mutant allele burden in a patient having a myeloproliferative disorder, comprising administering an SAP protein of the disclosure.

In one aspect, the disclosure provides a method of determining the efficacy of treatment in an individual diagnosed with a myeloproliferative disease, the method comprising: (a) determining the presence of one or more mutations in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2* nucleic acid sample; b) administering an SAP protein of the disclosure and (c) identifying the treatment as having been effective when the mutant allele burden of one or more mutations present in the nucleic acid sample is decreased. In some embodiments, eradication of a pre-existing abnormality (e.g., the allele burden of a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) is considered to be a complete response while a >50% reduction in allele burden is considered to be a partial response. In some embodiments, partial response only applies to patients with at least 20% mutant allele burden at baseline (Tefferi et al. Blood 2013, 122:1395-1398).

In one aspect, the disclosure provides a method of determining the efficacy of treatment in an individual diagnosed with a myeloproliferative disease, the method comprising: (a) determining the presence of one or more cytogenetic abnormalities; b) administering an SAP protein of the disclosure and (c) identifying the treatment as having been effective when the one or more cytogenetic abnormalities are decreased. In some embodiments, eradication of a pre-existing abnormality (e.g., a cytogenetic abnormality) is considered to be a complete response while a >50% reduction in abnormal metaphases is considered to be a partial response. In some embodiments, partial response only applies to patients with at least 10 abnormal metaphases at baseline (Tefferi et al. Blood 2013, 122:1395-1398).

In another aspect, the disclosure provides a method for selecting therapy for an individual with a hematopoietic disorder comprising evaluating a sample containing nucleic acids from the individual for the presence or absence of one or more mutations in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2* nucleic acid and selecting the therapy based on the presence of the one or more mutations.

One or more of the following determinations may be used to select a treatment plan: determining the presence or absence of a specific myeloproliferative disorder-associated mutation (e.g. mutations in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*),

determining the zygosity status of the sample, and determining the ratio of mutant to wild-type nucleic acid or mRNA in the sample (e.g. mutant to wild-type ratios for *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*). For example, patients found to carry a specific *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2* mutation by the methods of the disclosure may be recommended for treatment with an SAP protein of the disclosure, or detection of the mutation may be used to measure the effectiveness of treatment with an SAP protein of the disclosure. Similarly, methods of the disclosure may be used to treat patients who are asymptomatic for MPD, for example patients who are in the very early stages of an MPD. Mutations may also be detected in MPD patients who are undergoing treatment; if the ratio of mutant to wild-type nucleic acid or the zygosity status of the sample changes during treatment, a different diagnosis may be made.

One or more of the following determinations may be used to treat a patient with an SAP protein of the disclosure: determining the presence or absence of a specific myeloproliferative disorder-associated mutation (e.g. mutations in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*), determining the zygosity status of the sample, and determining the ratio of mutant to wild-type nucleic acid in a sample (e.g. mutant to wild-type ratios for *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*). A physician or treatment specialist may administer, forego or alter a treatment or treatment regime based on one or more of the determinations. Alternatively, a physician or treatment specialist may decide to maintain the treatment regime as is based on one or more of the determinations. Further, the number of cells carrying the mutation may change during the course of an MPD and monitoring the ratio, the zygosity status, and/or the presence or absence of a mutation may be an indication of disease status or treatment efficacy. For example, treatment may reduce the number of mutant cells, or the disease may become worse with time, and the number of diseased cells may increase. Additionally, one or more of the determinations may aid in patient prognosis and quality of life decisions. For example, decisions about whether to continue or for how long to continue a painful, debilitating treatment such as chemotherapy may be made.

The zygosity status and the ratio of wild-type to mutant nucleic acid in a sample may be determined by methods known in the art including sequence-specific, quantitative detection methods. Other methods may involve determining the area under the curves of the sequencing peaks from standard sequencing electropherograms, such as those created using ABI Sequencing Systems, (Applied Biosystems, Foster City Calif.). For example, the presence of only a single peak such as a "G" on an electropherogram in a position

representative of a particular nucleotide is an indication that the nucleic acids in the sample contain only one nucleotide at that position, the “G.” The sample may then be categorized as homozygous because only one allele is detected. The presence of two peaks, for example, a “G” peak and a “T” peak in the same position on the electropherogram indicates that the sample contains two species of nucleic acids; one species carries the “G” at the nucleotide position in question, the other carries the “T” at the nucleotide position in question. The sample may then be categorized as heterozygous because more than one allele is detected.

The sizes of the two peaks may be determined (e.g., by determining the area under each curve), and a ratio of the two different nucleic acid species may be calculated. A ratio of wild-type to mutant nucleic acid (e.g. mutant to wild-type ratios for *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) may be used to monitor disease progression, determine treatment, or to make a diagnosis. For example, the number of cancerous cells carrying a specific *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2* mutation may change during the course of an MPD. If a base line ratio is established early in the disease, a later determined higher ratio of mutant nucleic acid relative to wild-type nucleic acid may be an indication that the disease is becoming worse or a treatment is ineffective; the number of cells carrying the mutation may be increasing in the patient. A lower ratio of mutant relative to wild-type nucleic acid may be an indication that a treatment is working or that the disease is not progressing; the number of cells carrying the mutation may be decreasing in the patient.

In some embodiments, the subject carries mutations in one or more myeloproliferative disorder-associated genes such as but not limited to *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. In some embodiments, the subject has a mutation at codon 617 of *JAK2*. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*.

In certain embodiments of any of the above aspects of the disclosure, the myeloproliferative disease is polycythemia vera, essential thrombocythemia, myelofibrosis,

or an unclassified myeloproliferative disease. In some embodiments, the myelofibrosis is primary myelofibrosis, post-PV myelofibrosis, or post-ET myelofibrosis.

In some embodiments, evaluating or determining the presence or absence of one or more mutations in a nucleic acid sample of interest includes performing allele-specific PCR.

5 In other embodiments of any of the above aspects of the disclosure, evaluating or determining the presence or absence of one or more mutations in a nucleic acid sample of interest includes amplifying the nucleic acid of interest and performing direct sequencing analysis of the amplified nucleic acid. Other suitable detection methodologies include primer extension reaction, and protein sequencing. In certain embodiments of the above aspects, evaluating a
10 sample or determining the presence or absence of one or more mutations in a myeloproliferative disorder-associated polypeptide includes using an antibody that specifically binds to the mutated JAK2 polypeptide. In some embodiments of any of the above aspects of the disclosure, the nucleic acid and/or polypeptide sample is from a suitable biological sample including, for example, whole blood (i.e., JAK2 nucleic acid being
15 extracted from the cellular fraction), plasma, serum, and tissue samples (e.g., biopsy and paraffin-embedded tissue).

In one aspect, the disclosure provides methods for treating myeloproliferative disorders (e.g., myelofibrosis) by administering an SAP protein in combination with one or more additional agents such as another anti-cancer therapeutic. As used herein, “in
20 combination with” or “conjoint administration” refers to any form of administration such that the one or more additional agents is still effective in the body (e.g., the two agents, the three agents, the four agents, etc. are simultaneously effective in the patient, which may include synergistic effects of the two compounds). Effectiveness may not correlate to measurable concentration of the agent in blood, serum, or plasma. For example, the different therapeutic
25 agents can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially, and on different schedules. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents. The SAP protein can be administered concurrently with, prior to, or subsequent to, one or more other additional agents.

30 In general, each therapeutic agent will be administered at a dose and/or on a time schedule determined for that particular agent. The particular combination to employ in a regimen will take into account compatibility of the SAP protein with the agent and/or the desired therapeutic effect to be achieved.

Anti-cancer therapeutics of the disclosure may include, but are not limited to chemotherapy agents, antibody-based agents, kinase inhibitors (e.g., tyrosine kinase inhibitors, serine/threonine kinase inhibitors, etc.), immunomodulatory agents and biologic agents or combinations thereof. Chemotherapy agents include, but are not limited to

5 actinomycin D, aldesleukin, alitretinoin, all-trans retinoic acid/ATRA, altretamine, amascrine, asparaginase, azacitidine, azathioprine, bacillus calmette-guerin/BCG, bendamustine hydrochloride, bexarotene, bicalutamide, bleomycin, bortezomib, busulfan, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, cisplatin/cisplatinum, cladribine, cyclophosphamide/cytophosphane, cytarabine, dacarbazine,

10 daunorubicin/daunomycin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil (5-FU), gemcitabine, goserelin, hydrocortisone, hydroxyurea, idarubicin, ifosfamide, interferon alfa, irinotecan CPT-11, lapatinib, lenalidomide, leuprolide, mechlorethamine/chlormethine/mustine/HN2, mercaptopurine, methotrexate, methylprednisolone, mitomycin, mitotane, mitoxantrone,

15 octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pazopanib, pegaspargase, pegfilgrastim, PEG interferon, pemetrexed, pentostatin, phenylalanine mustard, plicamycin/mithramycin, prednisone, prednisolone, procarbazine, raloxifene, romiplostim, sargramostim, streptozocin, tamoxifen, temozolomide, temsirolimus, teniposide, thalidomide, thioguanine, thiophosphoamide/thiotepa, thiotepa, topotecan hydrochloride, toremifene,

20 tretinoin, valrubicin, vinblastine, vincristine, vindesine, vinorelbine, vorinostat, zoledronic acid, or combinations thereof. Antibody-based agents include, but are not limited to alemtuzumab, bevacizumab, cetuximab, fresolimumab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ofatumumab, panitumumab, rituximab, tositumomab, trastuzumab, trastuzumab DMI, and combinations thereof. Immunomodulatory compounds include, but

25 are not limited to small organic molecules that inhibit TNF α , LPS induced monocyte IL1 β , IL12, and IL6 production. In some embodiments, immunomodulatory compounds include but are not limited to methotrexate, leflunomide, cyclophosphamide, cyclosporine A, minocycline, azathioprine, an antibiotic (e.g., tacrolimus), methylprednisolone, a corticosteroid, a steroid, mycophenolate mofetil, rapamycin, mizoribine, deoxyspergualin,

30 brequinar, a T cell receptor modulator, or a cytokine receptor modulator, and a Toll-like receptor (TLR) agonist. In some embodiments, immunomodulatory compounds include 5,6-dimethylxanthene-4-acetic acid (DMXAA), thalidomide, lenalidomide, pomalidomide, lactoferrin, polyadenosine-polyuridylic acid (poly AU), rintatolimod (polyI:polyC12U; Hemispherx Biopharma), polyinosinic-polycytidylic acid stabilized with poly-L-lysine and

carboxymethylcellulose (Poly-ICLC, Hiltonol®), imiquimod (3M) and resiquimod (R848; 3M), unmethylated CpG dinucleotide (CpG-ODN), and ipilimumab. Biologic agents include monoclonal antibodies (MABs), CSFs, interferons and interleukins. In some embodiments, the biologic agent is IL-2, IL-3, erythropoietin, G-CSF, filgrastim, interferon alfa, 5 alemtuzumab, bevacizumab, cetuximab, gemtuzumab, ozogamicin, ibritumomab tiuxetan, ofatumumab, panitumumab, rituximab, tositumomab or trastuzumab.

Kinase inhibitors (e.g., tyrosine kinase inhibitors, serine/threonine kinase inhibitors, etc.) include, but are not limited to axitinib, bafetinib, bosutinib, cediranib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, neratinib, nilotinib, ponatinib, quizartinib, 10 regorafenib, sorafenib, sunitinib, vandetanib, vatalanib, vemurafenib, and combinations thereof.

In some embodiments, the anti-cancer therapeutic is a JAK kinase inhibitor such as, but not limited to AC-430, AZD1480, baricitinib, BMS-911453, CEP-33779, CYT387, GLPG-0634, lestaurtinib, LY2784544, NS-018, pacritinib, R-348, R723, ruxolitinib, 15 TG101348 (SAR302503), tofacitinib, and VX-509.

In certain embodiments, the anti-cancer therapeutic includes but is not limited to anti-metabolites (e.g., 5-fluoro-uracil, cytarabine, methotrexate, fludarabine and others), antimicrotubule agents (e.g., vinca alkaloids such as vincristine, vinblastine; taxanes such as paclitaxel and docetaxel), alkylating agents (e.g., cyclophosphamide, melphalan, carmustine, 20 nitrosoureas such as bischloroethylnitrosourea and hydroxyurea), platinum agents (e.g. cisplatin, carboplatin, oxaliplatin, satraplatin and CI-973), anthracyclines (e.g., doxorubicin and daunorubicin), antitumor antibiotics (e.g., mitomycin, idarubicin, adriamycin and daunomycin), topoisomerase inhibitors (e.g., etoposide and camptothecins), anti-angiogenesis agents (e.g., sunitinib, sorafenib and bevacizumab) or any other cytotoxic agents, (e.g. 25 estramustine phosphate, prednimustine), hormones or hormone agonists, antagonists, partial agonists or partial antagonists, kinase inhibitors (such as imatinib), and radiation treatment.

Any treatment method of the disclosure may be repeated as needed or required. For example, the treatment may be done on a periodic basis. The frequency of administering treatment may be determined by one of skill in the art. For example, treatment may be 30 administered once a week for a period of weeks, once every four weeks, or multiple times a week for a period of time (e.g., 3 times over the first week of treatment). In some embodiments, an initial loading dose period is followed by a maintenance dose. In some embodiments, the loading dose is periodically repeated. In some embodiments, the initial loading dose period includes administering the treatment multiple times a week (e.g., 3 times

over the first week of treatment). In some embodiments, the loading dose may be repeated every other week, every month, every two months, every 3 months, or every 6 months, or as needed, with or without continued periodic dosing between loading doses. Generally, the amelioration of the cancer-associated fibrosis persists for some period of time, preferably at least months, but maintenance of the anti-fibrotic effect and/or prevention of recurrence of fibrosis may require continued periodic dosing of an SAP protein over an unlimited period of time. Over time, the patient may experience a relapse of symptoms, at which point the treatments may be repeated.

In certain aspects, methods are provided herein for treating, delaying development, and/or preventing myelofibrosis in a subject comprising administering to the subject an effective amount of an SAP protein, or a pharmaceutically acceptable salt thereof, alone or in combination with an anti-cancer therapeutic. In some embodiments, the subject has myelofibrosis. In some embodiments, the subject is at risk of developing myelofibrosis. In some embodiments, the subject is a human subject. In some embodiments, the subject has been determined carry a mutation associated with the myeloproliferative disorder (e.g., a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*). Any one of the formulations described herein such as capsule or unit dosage forms described herein may be used to treat a subject with myelofibrosis.

Myelofibrosis that may be treated by the methods described herein includes primary myelofibrosis (PMF) and secondary myelofibrosis (e.g., myelofibrosis arising from antecedent polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF)). Myelofibrosis that may be treated by the methods described herein also includes myelofibrosis of high risk, intermediate risk such as intermediate risk level 1 or intermediate risk level 2, and low risk. Methods for diagnosing various types of myelofibrosis are known in the art. See, e.g., Cervantes et al., *Blood* 2009, 113(13):2895-901. In some embodiments, a dynamic prognostic model that accounts for modifications to the risk profile after diagnosis may prove useful. See, e.g., Passamonti et al., *Blood* 2010, 115:1703-1708. In some embodiments, the subject has palpable splenomegaly. In some embodiments, the subject with myelofibrosis has spleen of at least 5 cm below costal margin as measured by palpation. In some embodiments, the subject has anemia and/or thrombocytopenia and/or leukopenia. In some embodiments, the subject does not have anemia or thrombocytopenia or leukopenia. In some embodiments, the subject is transfusion dependent. In some embodiments, the subject is not transfusion dependent. In some embodiments, the subject has a pathologically confirmed diagnosis of PMF as per the WHO diagnostic criteria or post ET/PV MF,

including the presence of at least Grade 2 marrow fibrosis with intermediate -1, intermediate -2, or high risk disease according to the IWG-MRT Dynamic International Prognostic Scoring System. In some embodiments, the subject has a pathologically confirmed diagnosis of PMF as per the WHO diagnostic criteria or post ET/PV MF, with Grade 0 or 1 bone marrow fibrosis and low risk, intermediate -1, intermediate -2, high risk, or low risk disease according to the IWG-MRT Dynamic International Prognostic Scoring System. In some embodiments, the subject has "prefibrotic" myelofibrosis. In some embodiments, the subject has PV or ET and receives an SAP protein to prevent development of myelofibrosis. In some embodiments, the subject has a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*.

In some embodiments, the subject has a point mutation from valine 617 to phenylalanine in the Janus kinase 2 (JAK2 kinase) (JAK2V617F) if the subject is a human, or a point mutation corresponding to the valine 617 to phenylalanine in the Janus kinase 2 (JAK2 kinase) if the subject is not a human. In some embodiments, the subject is negative for the valine 617 to phenylalanine mutation of JAK2 if the subject is a human, or negative for a mutation corresponding to the valine 617 to phenylalanine in the Janus kinase 2 (JAK2 kinase) if the subject is not a human. Whether a subject is positive or negative for JAK2V617F can be determined by a polymerase chain reaction ("PCR") analysis using genomic DNA from bone marrow cells or blood cells (e.g., whole blood leukocytes). The PCR analysis can be an allele-specific PCR (e.g., allele-specific quantitative PCR) or PCR sequencing. See Kittur J et al., Cancer 2007, 109(11):2279-84 and McLornan D et al., Ulster Med J. 2006, 75(2): 112-9, each of which is expressly incorporated herein by reference. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*.

In some embodiments, the subject treated with the methods described herein has previously received or is currently receiving another myelofibrosis therapy or treatment. In

some embodiments, the subject is a non-responder to the other myelofibrosis therapy or has a relapse after receiving the other myelofibrosis therapy. The previous therapy may be a JAK2 inhibitor (e.g. INCB018424 (also known as ruxolitinib, available from Incyte), CEP-701 (lestaurtinib, available from Cephalon), or XL019 (available from Exelixis)) (See Verstovsek S., Hematology Am Soc Hematol Educ Program. 2009:636-42) or a non-JAK2 inhibitor (such as hydroxyurea). In some embodiments, the previous therapy may be JAK kinase inhibitor such as, but not limited to AC-430, AZD1480, baricitinib, BMS-911453, CEP-33779, CYT387, GLPG-0634, INCB18424, lestaurtinib, LY2784544, NS-018, pacritinib, ruxolitinib, TG101348 (SAR302503), tofacitinib, VX-509, R-348, or R723. In some

embodiments, the subject has received ruxolitinib treatment for primary myelofibrosis, post-polycythemia vera myelofibrosis (Post-PV MF), post-essential thrombocythemia myelofibrosis (Post-ET MF), polycythemia vera, or essential thrombocythemia for at least three months. In some embodiments, the subject has received ruxolitinib treatment for primary myelofibrosis, post-polycythemia vera myelofibrosis (Post-PV MF), post-essential thrombocythemia myelofibrosis (Post-ET MF), polycythemia vera, or essential thrombocythemia for less than three months. In some embodiments, the subject has received ruxolitinib treatment for primary myelofibrosis, post-polycythemia vera myelofibrosis (Post-PV MF), post-essential thrombocythemia myelofibrosis (Post-ET MF), polycythemia vera, or essential thrombocythemia for at least three months. In some embodiments, at least one or more symptoms have ceased to improve on continued ruxolitinib therapy. In some

embodiments, the subject is no longer responsive to ruxolitinib. In some embodiments, the subject has previously received another myelofibrosis therapy for at least 6 months, at least 5 months, at least 4 months, at least 3 months, at least 2 months, at least 1 month, at least 3 weeks, or at least 2 weeks. In some embodiments, the subject is no longer responsive to the other myelofibrosis therapy. In some embodiments, the previous therapy is an anti-cancer therapeutic described herein and the previous therapy has been discontinued upon indication of one or more elevated levels of amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and/or creatinine in the serum from the subject, and/or upon indication of a hematologic condition selected from the group consisting of anemia, thrombocytopenia, and neutropenia, or for any other reason based on a decision by the treating physician or the patient's request. In some embodiments, the dose of the compound in the second treatment is the same or lower than the dose in the previous therapy. In some embodiments, the subject has not received any therapy other than transfusions. In some embodiments, the subject has not received any prior therapy.

In some embodiments, the SAP protein is administered in combination with a JAK kinase inhibitor such as, but not limited to AC-430, AZD1480, baricitinib, BMS-911453, CEP-33779, CYT387, GLPG-0634, INCB18424, lestaurtinib, LY2784544, NS-018, pacritinib, ruxolitinib, TG101348 (SAR302503), tofacitinib, VX-509, R-348, or R723 (See
5 Kontzias et al. *Curr Opin Pharmacol.* 2012, 12(4):464-470). In some embodiments, the SAP protein is administered in combination with an agent known to reduce the symptoms of myelofibrosis, such as, but not limited to AB0024, AZD1480, AT-9283, BMS-911543, CYT387, everolimus, givinostat, imetelstat, lestaurtinib, LY2784544, oral arsenic, NS-018, pacritinib, panobinostat, peginterferon alfa-2a, pomalidomide, pracinostat, ruxolitinib, TAK-
10 901, and TG101438 (SAR302503) (Mesa, *Leuk Lymphoma* 2013, 54(2):242-251; Gupta et al. 2012, 2(3):170-186; Kucine and Levine 2011, 2(4):203-211).

The subject (such as a human) may be treated by administering the SAP protein at a dose of about 0.1 mg/kg to about 40 mg/kg. In some embodiments, the SAP protein is administered at a dose of 0.3 mg/kg. In some embodiments, the SAP protein is administered
15 at a dose of 3 mg/kg. In some embodiments, the SAP protein is administered at a dose of 10 mg/kg. In some embodiments, the compound is administered at a dose of about any of 0.1mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, 15 mg/kg, 18 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, or 40 mg/kg. In some
20 embodiments, the SAP protein is administered at a dose of about 0.1-0.3, 0.3-0.5, 0.5-0.8, 0.8-1, 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, or 35-40 mg/kg. The compound may be in a capsule and/or a unit dosage form described herein. In some embodiments, the compound is administered intravenously (IV). In some embodiments, the compound is administered by injection (e.g. subcutaneous (SubQ), intramuscular (IM), intraperitoneal
25 (IP)), by inhalation or insufflation (either through the mouth or the nose) or the administration is oral, buccal, sublingual, transdermal, nasal, parenteral or rectal. In some embodiments, the SAP protein is administered by intravenous infusion. In certain embodiments, for each dose, infusion is over a period of approximately one hour. However, longer or shorter infusion periods may be used (e.g., 30 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, 1 hour ten minutes, 1 hour fifteen minutes, 90 minutes, and
30 the like). When the method comprises administering an additional anti-cancer therapeutic, that therapeutic may be administered by the same route of administration or by a different route of administration. In certain embodiments, an additional anti-cancer therapeutic is administered orally.

In some embodiments, the SAP protein is administered at a dose of 0.3 mg/kg by IV infusion. In some embodiments, the SAP protein is administered at a dose of 0.3 mg/kg subcutaneously. In some embodiments, the SAP protein is administered at a dose of less than 0.3 mg/kg subcutaneously. In some embodiments, the SAP protein is administered at a dose of 3 mg/kg by IV infusion. In some embodiments, the SAP protein is administered at a dose of 10 mg/kg by IV infusion. In some embodiments, the SAP protein is administered once every four weeks. In some embodiments, an initial loading dose period is followed by a maintenance dose (e.g., three times a week for the first week and once every four weeks thereafter).

Also provided herein are methods for ameliorating one or more manifestations or symptoms associated with myelofibrosis. For example, the treatment using the methods described herein is effective in reducing mutant allele burden in one or more myelofibrosis-associated genes (e.g., *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*). In some embodiments, the treatment using the methods described herein is effective in reducing spleen size, ameliorating constitutional symptoms (such as early satiety, fatigue, night sweats, cough, and pruritus), reducing the MPN-SAF Total Symptom Score, improving quality of life as measured by the EORTC QLQ-C30, reducing leukocytosis, reducing thrombocytosis, improving anemia, improving thrombocytopenia, improving leukopenia, reducing transfusion dependence, decreasing JAK2V617F allele burden, decreasing MPL515W allele burden, decreasing CALR mutant allele burden, decreasing ASXL1 mutant allele burden, decreasing EZH2 mutant allele burden, decreasing SRSF2 mutant allele burden, decreasing IDH1 mutant allele burden, decreasing IDH2 mutant allele burden, decrease in peripheral blood blasts, decrease in bone marrow blasts, reducing bone marrow fibrosis, inducing a change in metabolic activity as measured by FDG or FLT PET-CT scan indicative of reduction in fibrosis in the bone marrow, spleen, and/or liver, improving pruritus, improving cachexia, and/or reducing or increasing bone marrow cellularity. The reduction, decrease, amelioration, or improvement can be at least by 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, bone marrow fibrosis is reduced in the subject after treatment. In some embodiments, bone marrow fibrosis becomes Grade 0 after treatment. In some embodiments, bone marrow fibrosis becomes Grade 1 after treatment. In some embodiments, the bone marrow fibrosis is reduced by at least one Grade, e.g. from Grade 3 to Grade 2 or Grade 1, or from Grade 2 to Grade 1 or Grade 0. In some embodiments, the bone marrow fibrosis is reduced by a measurable percent from baseline as measured by quantitative image analysis or

other quantitative means. In some embodiment, the spleen becomes non-palpable in the subject after treatment. In some embodiments, the subject has complete resolution of leukocytosis and/or thrombocytosis after treatment. In some embodiments, the subject has complete resolution of anemia, thrombocytopenia, and/or leukopenia after treatment. In some embodiments, the subject becomes transfusion independent (e.g., red blood cell transfusions or platelet transfusions) after treatment. In some embodiments, the subject has a 50% reduction in transfusions. In some embodiments, the subject has a 40%-60%, 30%-70%, 40%-50%, 50%, 60% reduction in transfusions. In some embodiments, the subject has complete resolution of pruritus after treatment. In some embodiments, efficacy of the treatment will be assessed by evaluation of the overall response rate (ORR) categorized according to the International Working Group (IWG) criteria modified to include stable disease with improvement in bone marrow fibrosis by at least one grade as a response. In some embodiments, efficacy of the treatment will be assessed by evaluation of improvement in bone marrow fibrosis score by at least one grade according to the European Consensus on Grading of Bone Marrow Fibrosis. In some embodiments, efficacy of treatment will be assessed by evaluating the molecular effect on a pre-existing abnormality such as a genetic mutation. Eradication of a pre-existing abnormality (e.g., the allele burden of a mutation in *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) is considered to be a complete response while a >50% reduction in allele burden is considered to be a partial response. Partial response only applies to patients with at least 20% mutant allele burden at baseline (Tefferi et al. Blood 2013, 122:1395-1398). In some embodiments, efficacy of the treatment will be assessed by evaluating changes in levels of circulating plasma cytokine levels including but not limited to CRP, IL-1Ra, MIP-1 β , TNF α , IL-6 and VEGF. In some embodiments, efficacy of the treatment will be assessed by evaluating changes in levels of PBMC mRNA and miRNA expression levels. In some embodiments, efficacy of the treatment will be assessed by lack of progression of PV or ET to myelofibrosis. In some embodiments, efficacy of the treatment will be assessed by lack of increase in bone marrow fibrosis by at least one grade.

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in reducing mutant allele burden in one or more myeloproliferative disorder-associated genes (e.g. *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*) by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% compared to the allele burden prior to commencing treatment with the methods

provided herein (e.g., compared to baseline). In some embodiments, the treatment is effective in reducing mutant allele burden by about 20-70%, about 20-60%, about 25-60%, about 25-55%, or about 25%-50%. In some embodiments, the mutant allele burden is decreased by 25% to 50%. In some embodiments, the mutant allele burden is decreased by at least 50%. In some embodiments, the treatment is effective in achieving a complete molecular response. In some embodiments, allele burden may be measured by PCR performed on nucleic acid samples extracted from blood. It would be understood by one of skill in the art that other known methods to measure allele burden may also be employed. In certain embodiments, the disclosure provides methods for decreasing allele burden in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein according to a dosing schedule effective to decrease allele burden by at least 25%, at least 30%, at least 35%, at least 40%, or at least 50%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, allele burden is decreased by about 25-55%, by about 25-50%, or by about 25-40%. In some embodiments, the subject has a JAK2V617F mutation. In some embodiments, the subject has a mutation in exon 12 or exon 14 of *JAK2*. In some embodiments, the subject has a mutation at codon 515 of *MPL*. In some embodiments, the subject has a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*. In some embodiments, the subject has a mutation in exon 10 of *MPL*. In some embodiments, the subject has a mutation in exon 9 of *CALR*. In some embodiments, the subject has a mutation in exon 12 of *ASXL1*. In some embodiments, the subject has a mutation in exon 4 of *IDH1*. In some embodiments, the subject has a mutation at codon 132 of *IDH1*. In some embodiments, the subject has a mutation in exon 4 of *IDH2*. In some embodiments, the subject has a mutation at codon 140 of *IDH2*. In some embodiments, the subject has a mutation at codon 172 of *IDH2*. In certain embodiments, the reduction in allele burden is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in reducing spleen volume by at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% compared to the level prior to commencing treatment with the methods provided herein (e.g., compared to baseline). In some embodiments, the treatment is effective in reducing spleen volume by at

least 10%. In some embodiments, the treatment is effective in reducing spleen volume by at least 25%. In some embodiments, the treatment is effective in reducing spleen volume by at least 35%. In some embodiments, the treatment is effective in reducing spleen volume by at least 50%. In some embodiments, the treatment is effective in reducing spleen volume by about 20-70%, about 20-60%, about 25-60%, about 25-55%, or about 25%-50%. In some
5 embodiments, spleen volume may be measured by manual palpation. It would be understood by one of skill in the art that other known methods to measure spleen volume may also be employed, such as measurement by magnetic resonance imaging. In certain embodiments, the disclosure provides methods for decreasing spleen volume in a patient in need thereof,
10 wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein according to a dosing schedule effective to decrease spleen volume by at least 10%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, or at least 55%. In certain embodiments, the SAP protein, comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer
15 therapeutic is a JAK kinase inhibitor. In certain embodiments, spleen volume is decreased by about 10-25%, by about 25-55%, by about 25-50%, or by about 25-40%. In certain embodiments, the reduction in spleen volume by at least 10%, at least 25%, or at least 50% is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

20 In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in reducing the Myeloproliferative Neoplasms Symptom Assessment Form (MPN-SAF) Total Symptom Score by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% compared to the score
25 prior to commencing treatment with the methods provided herein. See Emanuel et al., 2012, Journal of Clinical Oncology, volume 30, number 33, pages 4098-4013, for a description and discussion of the myeloproliferative neoplasm symptom assessment form total symptom score. In some embodiments, the treatment is effective in reducing the MPN-SAF Total Symptom Score by at least 25%. In some embodiments, the treatment is effective in reducing
30 the MPN-SAF Total Symptom Score by at least 50%. In some embodiments, the symptoms were assessed using the MPN-SAF patient reported outcome tool (Emanuel et al. 2012, Journal of Clinical Oncology 30(33): 4098- 4103). In certain embodiments, the disclosure provides methods for reducing the MPN-SAF Total Symptom Score in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an

amount of an SAP protein, according to a dosing schedule effective to reduce the MPN-SAF Total Symptom Score by at least about 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, or at least 60%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, the MPN-SAF Total Symptom Score is reduced by about 25-60%, by about 25-55%, or by about 25-50%. In certain embodiments, the reduction in the MPN-SAF Total Symptom Score is reduced by at least 25% or at least 50% for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in improving quality of life based on the EORTC QLQ-C30 score, by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% compared to the score prior to commencing treatment with the methods provided herein. See EORTC QLQ-C30 (version 3) 1995, EORTC Quality of Life Group, for a description and discussion of the EORTC QLQ-C30 questionnaire and scoring system. In some embodiments, the treatment is effective in improving the EORTC QLQ-C30 score by at least 25%. In some embodiments, the treatment is effective in improving the EORTC QLQ-C30 score by at least 50%. In some embodiments, the score was assessed using the questions and scoring system outlined in EORTC QLQ-C30 (version 3) 1995, EORTC Quality of Life Group. In certain embodiments, the disclosure provides methods for improving the EORTC QLQ-C30 score in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to improve the EORTC QLQ-C30 score by at least about 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, or at least 60%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, the EORTC QLQ-C30 score is improved by about 25-60%, by about 25-55%, or by about 25-50%. In certain embodiments, the improvement in the EORTC QLQ-C30 score is at least 25% or at least 50% for ≥ 12 consecutive weeks following treatment.

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in increasing hemoglobin

levels by at least about 500 mg/L, 1 g/L, 2 g/L, 3 g/L, 5 g/L, 10 g/L, or 20 g/L compared to the level prior to commencing treatment with the methods provided herein (e.g., compared to baseline). In some embodiments, the treatment is effective in increasing hemoglobin levels by 500-1000 mg/L, 1-2 g/L, 2-3 g/L, or 3-5 g/L compared to the level prior to commencing treatment with the methods provided herein (e.g., compared to baseline). In some
5 embodiments, the treatment is effective in increasing hemoglobin levels by 1 g/L. In some embodiments, the treatment is effective in increasing the hemoglobin levels to at least 80 g/L, at least 90 g/L, at least 100 g/L, at least 110 g/L, at least 120 g/L, at least 130 g/L, or at least 140 g/L. In some embodiments, the treatment is effective in increasing hemoglobin levels to
10 at least 100 g/L. In some embodiments, the hemoglobin levels are measured as part of a routine Complete Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure hemoglobin levels may also be employed. In certain embodiments, the disclosure provides methods for increasing the hemoglobin levels in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising
15 administering an amount of an an SAP protein, according to a dosing schedule effective to increase the hemoglobin levels by at least about 500 mg/L, 1 g/L, 2 g/L, 3 g/L, or 5 g/L. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, the hemoglobin levels are
20 increased by about 500-1000 mg/L, 1-2 g/L, 2-3 g/L, or 3-5 g/L. In certain embodiments, the hemoglobin levels are increased to at least about 80 g/L, 90 g/L, 100 g/L, 110 g/L, 120 g/L, 130 g/L, or 140 g/L. In some embodiments, the increase in hemoglobin levels is seen for $\geq$ 12 consecutive weeks following treatment. In some embodiments, the hemoglobin levels are increased by about $\geq$ 10 g/L for $\geq$ 12 consecutive weeks following treatment (e.g., greater
25 than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks) without transfusions. In some embodiments, the hemoglobin levels are increased by about $\geq$ 20 g/L is seen for $\geq$ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks) without transfusions. In some embodiments, the increase in
30 hemoglobin levels to at least about 100 g/L is seen for $\geq$ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in reducing red blood cell

(RBC) transfusions by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, or at least 60% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in reducing RBC transfusions by at least 25%. In some embodiments, the treatment is effective in reducing RBC transfusions by at least 50%. In some embodiments, the treatment is effective in achieving RBC transfusion independence. In certain embodiments, the disclosure provides methods for reducing RBC transfusions in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to reduce RBC transfusions by at least about 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, or at least 60%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, RBC transfusions are reduced by about 25-60%, by about 25-55%, or by about 25-50%. In certain embodiments, the patient becomes transfusion independent following treatment. In certain embodiments, the patient becomes transfusion independent for ≥ 12 consecutive weeks following treatment. In certain embodiments, the patient has a $\geq 50\%$ reduction in transfusions for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in ameliorating thrombocytopenia when present. In some embodiments, the treatment increases platelets by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment increases platelets by at least 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, at least 60%-70%, at least 70%-80%, at least 80%-90%, or at least 90%-100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in increasing platelets by at least 100%. In some embodiments, the treatment increases platelets to at least $25 \times 10^9/L$, $30 \times 10^9/L$, $40 \times 10^9/L$, $50 \times 10^9/L$, $60 \times 10^9/L$, $70 \times 10^9/L$, $80 \times 10^9/L$, $90 \times 10^9/L$, or $100 \times 10^9/L$. In some embodiments, the treatment increases platelets to at least $25-50 \times 10^9/L$, $50-75 \times 10^9/L$, $75-100 \times 10^9/L$, or $100-150 \times 10^9/L$. In some embodiments, the treatment increases platelets to $50 \times 10^9/L$. In some embodiments, the treatment increases platelets to $100 \times 10^9/L$. In some embodiments, platelets are measured as part of a routine Complete

Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure platelets may also be employed. In certain embodiments, the disclosure provides methods for increasing platelets in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to increase platelets by at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum, and the additional anticancer therapeutic is a JAK kinase inhibitor. In certain embodiments, platelets are increased by about 50%-60%, 60%-70%, 70%-80%, 80%-90%, or 90%-100%. In certain embodiments, the patient has a platelet count $> 25 \times 10^9/L$ for ≥ 12 consecutive weeks following treatment. In certain embodiments, the patient has a platelet count $> 50 \times 10^9/L$ for ≥ 12 consecutive weeks following treatment. In certain embodiments, the patient has a platelet count $> 100 \times 10^9/L$ for ≥ 12 consecutive weeks following treatment. In certain embodiments, the patient has a doubling of baseline platelet count for ≥ 12 consecutive weeks without transfusions. In certain embodiments, the patient has a $\geq 50\%$ reduction in transfusions for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in improving either or both of hemoglobin levels or platelet levels in subjects with both hemoglobin < 100 g/L and platelets $< 50 \times 10^9/L$, with no worsening of hemoglobin or platelets from baseline. In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in improving hemoglobin levels in subjects with hemoglobin < 100 g/L, with no worsening of platelets to $< 50 \times 10^9/L$. In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in improving platelet levels in subjects with platelets to $< 50 \times 10^9/L$, with no worsening of hemoglobin to < 100 g/L or new transfusion dependence.

In certain embodiments, the treatment using the methods described herein (e.g., single agent or combination therapy using SAP) increases progression-free survival and/or overall survival, such as versus the standard of care. In certain embodiments, progression-free survival and/or overall survival is measured versus no treatment, or versus a standard of care therapy.

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective at decreasing platelet transfusions by at least 25%, 30%, 40%, 50%, 60%, 75%, or 100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment decreases platelet transfusions by at least 50%. In certain embodiments, the disclosure provides methods for decreasing platelet transfusions in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease platelet transfusions by at least 30%, at least 40%, at least 50%, at least 60%, or at least 70%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, platelet transfusions are decreased by about 25%-40%, 25%-50%, 50%-70%, or 70%-100%. In certain embodiments, the patient becomes transfusion independent for ≥ 12 consecutive weeks following treatment. In certain embodiments, the patient has a $\geq 50\%$ reduction in transfusions for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in ameliorating thrombocytosis when present. In some embodiments, the treatment decreases platelets by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 50% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment decreases platelets by 25%. In some embodiment, the treatment decreases platelets to the normal levels. In some embodiments, platelets are measured as part of a routine Complete Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure platelets may also be employed. In certain embodiments, the disclosure provides methods for decreasing platelets in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease platelets by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 50%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, platelets are decreased by about 10%-15%, at least 15%-25%, or at least 25%-35%. In certain embodiments, the reduction in platelet count

is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in ameliorating neutropenia when present. In some embodiments, the treatment increases the absolute neutrophil count (ANC) by at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment increases ANC by at least 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, at least 60%-70%, at least 70%-80%, at least 80%-90%, or at least 90%-100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in increasing ANC by at least 50%. In some embodiments, the treatment increases ANC to at least 1000 cells/ μL , at least 1250 cells/ μL , at least 1500 cells/ μL , at least 1750 cells/ μL , or at least 2000 cells/ μL . In some embodiments, the treatment increases ANC to at least 1250-1500 cells/ μL , at least 1500-1750 cells/ μL , or at least 1750-2000 cells/ μL . In some embodiments, the treatment increases ANC to at least 1500 cells/ μL . In some embodiments, ANC is measured as part of a routine Complete Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure ANC may also be employed. In certain embodiments, the disclosure provides methods for increasing ANC in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to increase ANC by at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. In certain embodiments, SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, ANC is increased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, at least 60%-70%, at least 70%-80%, at least 80%-90%, or at least 90%-100%. In some embodiments, the treatment increases ANC to at least 1500 cells/ μL for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in ameliorating leukopenia when present. In some embodiments, the treatment increases the white blood cells (WBC) by at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%,

at least 90%, or at least 100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment increases WBC by at least 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, at least 60%-70%, at least 70%-80%, at least 80%-90%, or at least 90%-100% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in increasing WBC by at least 50%. In some embodiments, the treatment increases WBC to at least $4 \times 10^9/\text{L}$, $5 \times 10^9/\text{L}$, $7.5 \times 10^9/\text{L}$, or $10 \times 10^9/\text{L}$. In some embodiments, the treatment increases WBC to $10 \times 10^9/\text{L}$. In some embodiments, the treatment increases WBC to the normal range. In some embodiments, WBC is measured as part of a routine Complete Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure WBC may also be employed. In certain embodiments, the disclosure provides methods for increasing WBC in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to increase WBC by at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, WBC is increased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, at least 60%-70%, at least 70%-80%, at least 80%-90%, or at least 90%-100%. In some embodiments, the increase in WBC is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in ameliorating leukocytosis when present. In some embodiments, the treatment decreases ANC by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, or at least 70% compared to the level prior to commencing treatment with the methods provided herein, without decreasing ANC below $1500/\mu\text{L}$. In some embodiments, the treatment decreases ANC by 25%. In some embodiments, the treatment decreases ANC by 50%. In some embodiments, the treatment decreases ANC to normal levels. In some embodiments, the treatment decreases white blood cells (WBC) by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, or at least 70% compared to the level prior to commencing treatment with the methods provided herein, without decreasing WBC below the lower limit of normal. In some

embodiments, the treatment decreases WBC by 25%. In some embodiments, the treatment decreases WBC by 50%. In some embodiments, the treatment decreases WBC to $< 35 \times 10^9/L$, $< 30 \times 10^9/L$, $< 25 \times 10^9/L$, $< 20 \times 10^9/L$, or $< 15 \times 10^9/L$. In some embodiments, the treatment decreases WBC to $< 25 \times 10^9/L$. In some embodiments, the treatment decreases WBC to the normal range. In some embodiments, ANC and WBC are measured as part of a routine Complete Blood Count (CBC). It would be understood by one of skill in the art that other known methods to measure ANC or WBC may also be employed. In certain embodiments, the disclosure provides methods for decreasing ANC or WBC in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease ANC or WBC by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 60%, or at least 70% without decreasing WBC below the lower limit of normal. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, ANC or WBC is decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70% without decreasing WBC below the lower limit of normal. In some embodiments, the treatment decreases WBC to $< 25 \times 10^9/L$ for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in decreasing peripheral blood blasts by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, or at least 70% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in decreasing peripheral blood blasts by at least 50%. In some embodiments, the treatment is effective in decreasing peripheral blood blasts from $\geq 1\%$ to $< 1\%$. It would be understood by one of skill in the art that any of the methods known in the art to measure peripheral blood blasts may be employed. In certain embodiments, the disclosure provides methods for decreasing peripheral blood blasts in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease peripheral blood blasts by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, or at least 70%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human

SAP purified from serum. In certain embodiments, peripheral blood blasts are decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, peripheral blood blasts are decreased from $\geq 1\%$ to $< 1\%$. In certain embodiments, peripheral blood blasts are decreased from $\geq 1\%$ to $< 1\%$ for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in decreasing bone marrow fibrosis from Grade 3 to Grade 2. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis from Grade 3 to Grade 1. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis from Grade 3 to Grade 0. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis from Grade 2 to Grade 1. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis from Grade 2 to Grade 0. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis by at least by 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. It would be understood by one of skill in the art that any of the methods known in the art to evaluate bone marrow fibrosis may be employed. In certain embodiments, the disclosure provides methods for decreasing bone marrow fibrosis in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease bone marrow fibrosis by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow fibrosis is decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, the decrease in bone marrow fibrosis by greater than 1 grade is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in decreasing bone marrow fibrosis as measured by quantitative image analysis. In some embodiments, the treatment is effective in decreasing bone marrow fibrosis by at least by 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. It would be understood by one of skill in the art that any of quantitative image analysis

methods known in the art to evaluate bone marrow fibrosis may be employed. In some embodiments, computer assisted image analysis (CIA) is performed on whole slide scans from serial bone marrow specimens from a patient for objective quantification of overall fibrosis level and osteosclerosis of all post-treatment samples compared to baseline samples.

- 5 The areas occupied with bone trabeculae (% of total core biopsy) and reticulin fibers (% of hematopoietic areas excluding the fat) are calculated. In certain embodiments, the disclosure provides methods for decreasing bone marrow fibrosis in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease bone marrow fibrosis by at least
- 10 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow fibrosis is decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, a decrease in bone marrow fibrosis of greater than 10% is seen for ≥ 12 consecutive weeks
- 15 following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

- In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in improving bone marrow morphology indicative of healing and restoration of hematopoiesis. In some embodiments,
- 20 the treatment is effective in improving megakaryocytic topography by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. In some embodiments, the treatment is effective in normalizing the myeloid to erythroid (M:E) ratio by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. In
- 25 some embodiments, the treatment is effective in reducing collagen and osteosclerosis by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. It would be understood by one of skill in the art that any of the methods known in the art to evaluate bone marrow morphology may be employed. In certain embodiments, the disclosure provides methods for improving bone
- 30 marrow morphology (e.g., one or more of: improving megakaryocytic topography, normalizing M:E ratio, reducing collagen and osteosclerosis) in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to improve bone marrow morphology by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90%. In certain embodiments, the

SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow morphology is improved by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, an improvement in bone marrow morphology of greater than 10% is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in decreasing fibrosis in bone marrow, spleen, and liver. In some embodiments, the fibrosis is measured by Positron Emission Tomography/Computed Tomography (PET/CT). In some embodiments, the fibrosis is measured by 3'-¹⁸Fluoro-3'-deoxy-L-thymidine (¹⁸F-FLT) PET (Andreoli et al. ASH 2014 Abstract 3195). In some embodiments, the fibrosis is measured by 1866 ¹⁸F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography ¹⁸FDG-PET/CT (Derlin et al. ASH 2014 Abstract 1866). In some embodiments, the treatment is effective in decreasing bone marrow, spleen, or liver fibrosis by at least by 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90% compared to the level prior to commencing treatment with the methods provided herein. In certain embodiments, the disclosure provides methods for decreasing bone marrow, spleen, or liver fibrosis in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein, according to a dosing schedule effective to decrease bone marrow, spleen, or liver fibrosis by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow, spleen, or liver fibrosis is decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, the decrease in bone marrow, spleen, or liver fibrosis by greater than 10% is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) is effective in decreasing bone marrow blasts from $\geq 5\%$ to $<5\%$. It would be understood by one of skill in the art that any of the methods known in the art to measure bone marrow blasts be employed. In certain embodiments, the disclosure provides methods for decreasing bone marrow blasts in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein according to a dosing schedule effective to

decrease bone marrow blasts by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, or at least 70%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow blasts are
5 decreased by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at least 60%-70%. In certain embodiments, the decrease in bone marrow blasts is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment is effective in improving bone marrow
10 cellularity. The improvement can be at least by 20, 30, 40, 50, 60, or 70% compared to the level prior to commencing treatment with the methods provided herein. In certain embodiments, the disclosure provides methods for improving bone marrow cellularity in a patient in need thereof, wherein the patient in need thereof has myelofibrosis, comprising administering an amount of an SAP protein according to a dosing schedule effective to
15 improve bone marrow cellularity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, or at least 70%. In certain embodiments, the SAP protein comprises an SAP protein with glycosylation that differs from that of human SAP purified from serum. In certain embodiments, bone marrow cellularity is improved by about 20%-30%, at least 30%-40%, at least 40%-50%, at least 50%-60%, or at
20 least 60%-70%. In certain embodiments, the improvement in bone marrow cellularity is seen for ≥ 12 consecutive weeks following treatment (e.g., greater than 24 weeks, greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, the treatment is effective in decreasing leukoerythroblastosis. In some embodiments, the treatment is effective in eliminating leukoerythroblastosis. In
25 some embodiments, the treatment is effective in decreasing or eliminating leukoerythroblastosis for ≥ 12 consecutive weeks following treatment. In certain embodiments, the treatment using the methods described herein (e.g. single agent or combination therapy using an SAP protein) results in at least one of the effects described herein (e.g. reduction in mutant allele burden, reduction in spleen volume, reduction in MPN-
30 SAF Total Symptom Score, improving quality of life as measured by the EORTC QLQ-C30, increase in hemoglobin, reduction in RBC transfusions, improvement in thrombocytopenia, decrease in platelet transfusions, improvement in thrombocytosis, improvement in neutropenia, improvement in leukocytosis, decrease in peripheral blood blasts, decrease in bone marrow fibrosis, decrease in bone marrow blasts, decrease in peripheral blood blasts, or

improvement in bone marrow cellularity). In some embodiments, the treatment using the methods described herein results in at least two of the effects described herein. In some embodiments, the treatment using the methods described above results in at least three, four, five, six, seven, eight, nine, or ten of the effects described herein. In certain embodiments of any of the foregoing, evaluation of whether a particular degree of improvement of a symptom or therapeutic effect has been achieved is evaluated at one or more points over time, such as following at least 12, 18, 20, or at least 24 weeks of treatment, or following greater than 24 weeks of treatment (e.g., greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

In some embodiments, treatment using one or more of the methods described herein (e.g. single agent or combination therapy using an SAP protein of the disclosure) results in at least one of the effects described herein (e.g. reduction in spleen volume, reduction in MPN-SAF Total Symptom Score, improving quality of life as measured by the EORTC QLQ-C30, increase in hemoglobin, reduction in RBC transfusions, achievement of transfusion independence, improvement in thrombocytopenia, decrease in platelet transfusions, improvement in thrombocytosis, improvement in neutropenia, improvement in leukocytosis, decrease in peripheral blood blasts, decrease in bone marrow fibrosis, decrease in bone marrow blasts or improvement in bone marrow cellularity), without causing or inducing clinically significant myelosuppression. In some embodiments, treatment using one or more of the methods described herein results in at least two of the effects described herein, without causing or inducing clinically significant myelosuppression. In some embodiments, treatment using one or more of the methods described above results in at least three, four, five, six, seven, eight, nine, or ten of the effects described herein, without causing or inducing clinically significant myelosuppression. In some embodiments, treatment using one or more of the methods described herein results in no myelosuppression. In certain embodiments, any of the foregoing methods comprise administering SAP comprising an SAP protein having glycosylation that differs from that of SAP purified from human serum, such as recombinant human SAP (e.g., recombinant human pentraxin-2 produced in CHO cells). In certain embodiments, any of the foregoing methods comprise administering the SAP protein according to a dosing schedule, wherein any of the foregoing therapeutic effects are achieved following administration according to the dosing schedule. In certain embodiments, one or more of the foregoing therapeutic effects are achieved following administration according to a dosing schedule (e.g., administering comprises administering according to a dosing schedule). Improvement in any of the foregoing parameters (e.g., reduction in symptoms) is

evaluated at one or more time points during treatment, for example, following at least 12, at least 18, at least 20, at least 24, or greater than 24 weeks of treatment (e.g., greater than 30 weeks, greater than 36 weeks, greater than 42 weeks, greater than 48 weeks).

For any of the foregoing examples of improvement in a patient, such as an
5 improvement in one or more symptoms, in certain embodiments, the disclosure provides that the treatment comprises administering an SAP protein at a dose and on a dosing schedule effective to have the therapeutic effect. In certain embodiments, such as certain embodiments of any of the foregoing, SAP is administered without an additional anti-cancer therapeutic.

In some embodiments, the SAP protein is administered at a dosing schedule
10 comprising administration of the SAP protein every 4 weeks for at least 1 cycle, at least 2 cycles, at least 3 cycles, at least 4 cycles, at least 5 cycles, at least 6 cycles, at least 7 cycles or at least 8 cycles of a 28-day or 4-week cycle. In some embodiments, the SAP protein is administered to the subject once every 4 weeks for at least 6 cycles of a 28-day cycle, at least 8 cycles of a 28-day cycle, at least 10 cycles of a 28-day cycle, at least 12 cycles of a 28-day
15 cycle, at least 15 cycles of a 28-day cycle, at least 18 cycles of a 28-day cycle, or at least 24 cycles of a 28-day cycle. In some embodiments, the compound is administered to the subject once every 4 weeks for at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least eight months, at least one year, or at least two years, and possibly administered chronically over the life of the patient. In a
20 further embodiment, the SAP protein is administered every other day in the first week of treatment. In some embodiments, the SAP protein is administered several days (e.g. days 1, 3 and 5) every 4 weeks for at least 6 cycles of a 28-day cycle, at least 8 cycles of a 28-day cycle, at least 10 cycles of a 28-day cycle, at least 12 cycles of a 28-day cycle, at least 15 cycles of a 28-day cycle, at least 18 cycles of a 28-day cycle, or at least 24 cycles of a 28-day
25 cycle. In some embodiments, the compound is administered to the subject for several days (e.g., days 1, 3, 5) every 4 weeks for at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least eight months, at least one year, or at least two years, and possibly administered chronically over the life of the patient. In some embodiments, the SAP protein is administered to the subject at a dosing
30 schedule comprising administration of the SAP protein at least once a week for at least 1 cycle, at least 2 cycles, at least 3 cycles, at least 4 cycles, at least 5 cycles, at least 6 cycles, at least 7 cycles or at least 8 cycles of a 28-day cycle. In some embodiments, the SAP protein is administered to the subject at least once a week for at least 6 cycles of a 28-day cycle, at least 8 cycles of a 28-day cycle, at least 10 cycles of a 28-day cycle, at least 12 cycles of a 28-day

cycle, at least 15 cycles of a 28-day cycle, at least 18 cycles of a 28-day cycle, or at least 24 cycles of a 28-day cycle. In some embodiments, the compound is administered to the subject once a week for at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least eight months, at least one year, or at least two years. In further embodiments, the compound is administered every other day in the first week of treatment. In certain embodiments, the dosing schedule results in at least one of the effects (e.g., improvement in one or more symptoms or parameters) described herein (e.g. reduction in spleen volume, reduction in MPN-SAF Total Symptom Score, increase in hemoglobin, reduction in RBC transfusions, achievement of transfusion independence, improvement in thrombocytopenia, decrease in platelet transfusions, improvement in thrombocytosis, improvement in neutropenia, improvement in leukocytosis, decrease in peripheral blood blasts, decrease in bone marrow fibrosis, decrease in bone marrow blasts or improvement in bone marrow cellularity). In some embodiments, the dosing schedule results in at least two of the effects described herein. In some embodiments, the dosing schedule results in at least three, four, five, six, seven, eight, nine, or ten of the effects described herein. In certain embodiments, the SAP agonist comprises recombinant human SAP.

In certain embodiments, the disclosure provides methods for administering an amount of an SAP protein, according to a dosing schedule comprising administering an SAP protein using a dosage regimen comprising administering 10 mg/kg of an SAP protein, such as an SAP protein with glycosylation that differs from that of human SAP purified from serum, on days 1, 3, 5 of Cycle 1 and Day 1 each subsequent 28 day cycle.

In certain embodiments, the disclosure provides methods for administering an amount of an SAP protein, according to a dosing schedule comprising administering an SAP protein using a dosage regimen comprising administering 3 mg/kg of an SAP protein on Days 1, 3, and 5 of Cycle 1 and Day 1 of each subsequent 28 day cycle.

In certain embodiments, the disclosure provides methods for administering an amount of an SAP protein, according to a dosing schedule comprising administering an SAP protein using a dosage regimen comprising administering 0.3 mg/kg of an SAP protein on Days 1, 3, and 5 of Cycle 1 and Day 1 of each subsequent 28 day cycle.

In some embodiments, the SAP protein is administered multiple times during the first week (e.g., days 1, 3 and 5), followed by administration every week, every two weeks, every three weeks, or every 4 weeks. In some embodiments, the SAP protein is administered multiple times a week every other week, every three weeks, every month, every other month,

every three months, every six months, or as needed. In some embodiments, the SAP protein is administered by IV infusion. In some embodiments, the SAP protein is administered subcutaneously. In some embodiments, the SAP protein is administered at a dose of 10 mg/kg. In some embodiments, the SAP protein is administered at a dose of 3 mg/kg. In some embodiments, the SAP protein is administered at a dose of 0.3 mg/kg. In some embodiments, the SAP protein is administered at any of the dosages described herein. In some embodiments, the dosage regimens described herein are adjusted as needed to achieve one of the treatment outcomes described herein.

In some embodiments, the methods disclosed herein comprise administering one or more additional doses of the SAP protein after achieving an initial response. In some embodiments, a subsequent response is achieved following the administration of one or more additional doses of the SAP protein after achieving an initial response in a subject. A subsequent response may be an additional response (e.g. any of the responses described herein not initially observed), the maintenance of the initial response, or an improvement upon the initial response. In some embodiments, the administration of one or more additional doses substantially maintains the initial response. In some embodiments, the administration of one or more additional doses provides further improvement relative to the initial response. In some embodiments, the administration of one or more additional doses provides one or more additional responses that were not initially observed. In certain embodiments, the SAP protein comprises recombinant human SAP.

In some embodiments, upon administration of an SAP protein or a pharmaceutically acceptable salt thereof to a subject such as human subject, the C_{max} (maximum drug concentration) of the compound is achieved within about 0.5 to about 5 hours, about 1.5 to about 4.5 hours, about 2 to about 4 hours, or about 2.5 to about 3.5 hours post-dose. In some embodiments, upon administration of the compound to a human subject, the elimination half life of the compound is about 11 to 110 hours, 20-72 hours, 12 to about 40 hours, about 16 to about 34 hours, or about 20 to about 40 hours. In some embodiments, the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 0.1 mg to about 40 mg per kg. In some embodiments, the accumulation of the compound is about 1.1 to about 5 fold, about 1.25 to about 4.0 fold, about 1.5 to about 3.5 fold, about 2 to about 3 fold at steady state when the compound is dosed once weekly. In some embodiments, the compound does not accumulate when dosed weekly.

SAP proteins and SAP agonists

One aspect of the disclosure provides SAP proteins useful in the treatment of myeloproliferative disorders. One aspect of the disclosure provides SAP agonists useful in the treatment of myeloproliferative disorders. SAP agonists encompass all compounds and compositions that increase or otherwise mimic endogenous SAP signaling, including compounds that increase SAP activity. In certain embodiments of the any of the foregoing methods, an SAP agonist can be used wherever an SAP protein is being used, alone or in combination with an SAP protein of the disclosure. Exemplary SAP agonists are described below. Throughout the disclosure, "SAP proteins" are referred to. Unless otherwise specified, such reference contemplates the use of any of the SAP proteins disclosed herein, including use of recombinant SAP, such as SAP protein comprising human SAP, which SAP protein has a glycosylation that differs from that of SAP isolated from human serum. The disclosure contemplates use of any of the SAP proteins and SAP agonists disclosed herein in any of the methods described herein, including use alone or as a combination therapy.

SAP

SAP or pentraxin-2 is a naturally occurring serum protein in mammals composed of five identical subunits, or protomers, which are non-covalently associated in a disk-like complex. SAP belongs to the pentraxin superfamily of proteins, which are characterized by this cyclic pentameric structure. The classical short pentraxins include SAP as well as C-reactive protein (Osmand, A.P., *et al.*, Proc. Nat. Acad. Sci., 74: 739-743, 1997). The long pentraxins include pentraxin-3. SAP is normally synthesized in the liver and has a physiological half-life of twenty-four hours. Human SAP (hSAP) circulates at approximately 20-40 µg/ml in plasma as a homopentamer. The sequence of the human SAP subunit is disclosed in SEQ ID NO: 1, which corresponds to amino acids 20-223 of Genbank Accession NO. NP_001630 (signal sequence not depicted).

Previous research demonstrates that SAP has an important role in both the initiation and resolution phases of the immune response. hSAP functions in innate resistance to microbes and in the scavenging and phagocytosis of cellular debris and appears to play a role in regulation of wound healing and fibrosis. These functions may involve (i) binding to ligands associated with microbes and cellular debris, as specified above, and various extracellular matrix proteins in a Ca^{2+} -dependent manner, (ii) binding to Clq for complement activation by promoting opsonization by C3b and iC3b, (iii) binding to Fcγ receptors to initiate direct opsonization and subsequent phagocytosis or endocytosis, and (iv) subsequent regulation of monocyte function and differentiation. Accordingly, hSAP molecules localize

to sites of injury and repair and may target and/or concentrate in these locations through binding these molecules.

The 3D structure of hSAP has been determined by X-ray crystallography and several crystal structures complexed with different ligands have also been reported. The pentameric structure of hSAP has 5-fold rotational symmetry and is fairly rigid with a pore. The diameter of the hSAP pentamer is approximately 100Å, and the central pore is 20 Å in diameter and 35 Å deep. Each protomer is constructed of antiparallel β-strands arranged in two sheets, with a hydrophobic core with a jellyroll topology. The hSAP pentamer has 2 faces, an A-face, which possesses five α helices, one on each protomer, and a B face with 5 sets of double calcium-binding sites. The B-face is thought to provide a calcium-dependent ligand binding face, and several calcium-dependent ligands that bind the B-face have been identified, including phosphorylethanolamine, DNA, heparan sulfate, dermatan sulfate and dextran sulfate, laminin and collagen IV. The A-face of hSAP also appears to bind molecules such as Clq and may mediate phagocytosis through binding to Fcγ receptors. Each protomer may be glycosylated at Asn32, a single site.

N- and C-termini are solvent accessible and are located on the inner edge of each protomer molecule. The N- terminus is located on the outer edge of each protomer and on the perimeter of the ring formed by the 5 protomers. The C-terminus is located more toward the inner perimeter and pore of the pentamer ring but is directed outward toward the A face. N- and C-termini within one protomer are about 25 Å apart. The termini do not appear to be involved in subunit interactions and they are away from the glycan chain attached at Asn32. The subunits of hSAP are held together non-covalently with approximately 15% of the surface of each subunit involved in these interactions. These extensive interactions account for the considerable stability of the hSAP pentamer.

The SAP encompassed by embodiments described herein includes SAP from any source such as, for example, human SAP or isomers or analogs from other vertebrate or mammalian sources. SAP further encompasses SAP molecules having modifications from the native PTX-2 amino acid sequence introduced by, for example, site-directed mutagenesis. Such modification may alter specific amino acids and/or other features of the molecule, while retaining the general pentameric pentraxin nature of the molecule. The “SAP protein” may be used to encompass both SAP pentamers and SAP protomers. “SAP pentamer” or “pentameric SAP” refers to a protein complex at least including five SAP protomers, and “SAP protomer” refers to one individual protein unit of the SAP pentamer. In certain embodiments of any of the aspects and embodiments of the disclosure, the disclosure

comprises administration of an SAP protein comprising one or more protomers comprising the amino acid sequence set forth in SEQ ID NO: 1. In certain embodiments, the SAP protein is a protein comprising five protomers. In certain embodiments, the SAP protein comprises recombinant human SAP. An exemplary recombinant human SAP comprises PRM-151. In certain embodiments, the SAP agonist comprises recombinant SAP. Methods of making proteins generally, and human pentraxin-2 specifically, recombinantly are known in the art. Suitable cells for recombinant expression, such as insect or mammalian cells may be selected. SEQ ID NO: 1 corresponds to the amino acid sequence of a human SAP protomer.

Modification of a glycan structure on a human SAP protein can increase the biological activity of the SAP protein relative to a corresponding sample of wild-type SAP isolated from human serum. Isolated SAP from human serum contains only $\alpha 2,6$ -linked sialic acid residues. In contrast, recombinant human SAP (rhSAP) produced in CHO cells contains only $\alpha 2,3$ -linked sialic acid residues. In *in vitro* cell-based bioassays, $\alpha 2,3$ -linked sialic acid SAP proteins demonstrate consistently higher activity than wild-type SAP (i.e., $\alpha 2,6$ -linked sialic acid) isolated from human serum. The variant SAP proteins of the disclosure would be more effective as therapeutic agents due to their increased biological potency. For example, more potent SAP variants may require lower dosing and/or less frequent dosing relative to wild-type SAP isolated from human serum. The disclosure provides both variant human SAP proteins and methods for making the same. In particular, the present disclosure includes methods and compositions for *in vitro* and *in vivo* addition, deletion, or modification of sugar residues to produce a human SAP protein having a desired glycosylation pattern.

Variant SAP proteins

In part, the disclosure provides variant Serum Amyloid P (SAP) polypeptides for use in treatment of myeloproliferative disorders. In particular, SAP variants of the disclosure include glycosylated human SAP proteins that comprise one or more N-linked or O-linked oligosaccharide chains each independently having one, two, three, four, or five branches terminating with an $\alpha 2,3$ -linked sialic acid moiety. In some embodiments, all the sialylated branches of the N-linked or O-linked oligosaccharide chains terminate in $\alpha 2,3$ -linked moieties. In some embodiments, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%, or even at least 95% of the sialylated branches of the N-linked or O-linked oligosaccharide chains terminate in $\alpha 2,3$ -linked moieties. Other SAP variants of the disclosure include glycosylated human SAP proteins that contain an N-linked or O-linked

oligosaccharide chains having at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%, or even at least 95% fewer α 2,6-linked sialic acid moieties than a wild-type SAP protein derived from human serum. In some embodiments, the N-linked or O-linked oligosaccharide chains are substantially free of α 2,6-linked sialic acid moieties.

5 Glycovariant SAP proteins of the disclosure may comprise an N-linked oligosaccharide or O-linked chain having one or more branches (e.g., having a bi-antennary, tri-antennary, tetra-antennary, penta-antennary, etc. structure). In certain embodiments, SAP proteins of the disclosure comprise an N-linked or O-linked oligosaccharide chain wherein one, two, three, four, or five branches of the oligosaccharide chain are substantially free of galactose and N-acetylglucosamine. Certain SAP proteins of the disclosure have N-linked or O-linked
10 oligosaccharide chains that are substantially free of galactose and N-acetylglucosamine. In some embodiments, SAP proteins of the disclosure comprise an N-linked or O-linked oligosaccharide chain wherein one, two, three, four, or five branches of the oligosaccharide chain contain one or more mannose residues. In certain embodiments, the SAP protein of the
15 disclosure comprises an N-linked oligosaccharide having a pentasaccharide core of Man[(α 1,6-)-(Man(α 1,3)]-Man(β 1,4)-GlcNAc(β 1,4)-GlcNAc(β 1,N)-Asn. This pentasaccharide core also may comprise one or more fucose or xylose residues. In certain embodiments, SAP proteins of the disclosure comprise an N-linked oligosaccharide chain wherein one, two, three, four, or five branches of the oligosaccharide chain have the structure
20 NeuNAc2 α 3Gal β 4GlcNAc β 2Man α 6. SAP proteins of the disclosure also may comprise an N-linked oligosaccharide chain wherein all branches have the structure NeuNAc2 α 3Gal β 4GlcNAc β 2Man α 6.

Variant SAP proteins of the disclosure may comprise one or more “modified” sugar residues. Modified sugars are substituted at any position that allows for the attachment of the
25 modifying moiety or group, yet which still allows the sugar to function as a substrate for the enzyme used to couple the modified sugar to the peptide. A modifying group can be attached to a sugar moiety by enzymatic means, chemical means or a combination thereof, thereby producing a modified sugar, e.g., modified galactose, fucose, or sialic acid. Modifying groups suitable for use in the present disclosure as well as methods for conjugating these
30 modifying groups to sugar residues are described in the following section.

In some embodiments, the SAP proteins of the disclosure may comprise amino acid sequences at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 1, as determined using the FASTDB computer program based on

the algorithm of Brutlag et al. (Comp. App. Biosci., 6:237-245 (1990)). In a specific embodiment, parameters employed to calculate percent identity and similarity of an amino acid alignment comprise: Matrix=PAM 150, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5 and Gap Size Penalty=0.05.

Polypeptides sharing at least 95% identity with SEQ ID NO: 1 may include polypeptides having conservative substitutions in these areas of divergence. The term "SAP protein" encompasses functional fragments and fusion proteins comprising any of the preceding. Generally, an SAP protein will be soluble in aqueous solutions at biologically relevant temperatures, pH levels and osmolarity. The SAP protomers that non-covalently associate together to form a pentameric SAP complex may have identical amino acid sequences and/or post-translational modifications or, alternatively, individual SAP protomers within a single complex may have different sequences and/or modifications. The term SAP protein includes polypeptides comprising any naturally occurring SAP protein as well as any variant thereof (including mutants, fragments, and fusions). An SAP protein of the disclosure may be a recombinant polypeptide. In preferred embodiments, the SAP protein of the disclosure is a human SAP protein.

In some embodiments, pharmaceutical compositions are provided comprising a variant SAP protein of the disclosure, or a functional fragment thereof. In some aspects, the amino acid sequence of an SAP variant may differ from SEQ ID NO: 1 by one or more conservative or non-conservative substitutions. In other aspects, the amino acid sequence of an SAP variant may differ from SEQ ID NO: 1 by one or more conservative substitutions. As used herein, "conservative substitutions" are residues that are physically or functionally similar to the corresponding reference residues, i.e., a conservative substitution and its reference residue have similar size, shape, electrical charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Preferred conservative substitutions are those fulfilling the criteria defined for an accepted point mutation in Dayhoff et al., Atlas of Protein Sequence and Structure 5:345-352 (1978 & Supp.). Examples of conservative substitutions are substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. Additional guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie et al., Science 247:1306-1310 (1990).

Variant SAP proteins and fragments thereof that retain biological function are useful in the pharmaceutical compositions and methods described herein. In some embodiments, a variant SAP protein or fragment thereof binds to one or more Fcγ receptors. In some embodiments, the Fcγ receptor is FcγRI, FcγRIIA, and/or FcγRIIIB. In some embodiments, a
5 variant SAP protein or fragment thereof inhibits one or more of fibrocyte, fibrocyte precursor, myofibroblast precursor, and/or hematopoietic monocyte precursor differentiation. In some embodiments, a variant SAP protein or fragment thereof inhibits the differentiation of monocytes into fibrocytes. Measuring the expression of Macrophage Derived Chemokine (MDC) is an effective method of determining fibrocyte differentiation. SAP variants may be
10 generated by modifying the structure of an SAP protein for such purposes as enhancing therapeutic efficacy or stability (e.g., *ex vivo* shelf life and resistance to proteolytic degradation *in vivo*).

In certain aspects, the variant SAP proteins of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the SAP
15 protein. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation (e.g., O-linked oligosaccharides, N-linked oligosaccharides, etc.), phosphorylation, lipidation, and acylation. As a result, the modified SAP protein may contain non-amino acid elements, such as polyethylene glycols, lipids, poly- or mono-saccharides, and phosphates.

20 Methods of producing variant hSAP proteins with altered N-glycosylation are described in U.S. Patent Application No. 12/794,132, which is hereby incorporated by reference. In some embodiments, one or more protomers of variant SAP proteins comprise an amino acid at position 32 of SEQ ID NO: 1 that is not asparagine, resulting in altered glycosylation patterns. In some embodiments, one or more of the SAP promoters are
25 substantially free of N-linked or O-linked glycans.

In certain aspects, one or more modifications to the SAP protein described herein may enhance the stability of the SAP protein. For example, such modifications may enhance the *in vivo* half-life of the SAP protein or reduce proteolytic degradation of the SAP protein.

In certain aspects, variant SAP proteins of the disclosure include fusion proteins
30 having at least a portion of the human SAP protein and one or more fusion domains or heterologous portions. Well known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, and immunoglobulin heavy chain constant region (Fc), maltose binding protein

(MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel-, or cobalt-conjugated resins are used. As another example, a fusion domain may be selected so as to facilitate detection of the SAP proteins. Examples of such detection domains include the various fluorescent protein (e.g., GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus hemagglutinin (HA) and c-myc tags. In some cases, the fusion domains have a protease cleavage site that allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant protein therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In some cases, the SAP protein may be fused to a heterologous domain that stabilizes the SAP protein *in vivo*. By "stabilizing" is meant anything that increases serum half-life, regardless of whether this is because of decreased destruction, decreased clearance by the liver and/or kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin and serum albumin are known to confer increased stability.

It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, an SAP protein may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to an SAP protein. The SAP protein and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences (e.g., linker sequences) may be included C- or N-terminal to either domain or between the domains.

SAP proteins of the disclosure may comprise one or more "modified" sugar residues. A modifying group can be attached to a sugar moiety by enzymatic means, chemical means or a combination thereof, thereby producing a modified sugar, e.g., modified galactose, fucose, or sialic acid. When a modified sialic acid is used, either a sialyltransferase or a trans-sialidase can be used in these methods. The sugars may be substituted at any position that allows for the attachment of the modifying moiety, yet which still allows the sugar to function as a substrate for the enzyme used to couple the modified sugar to the peptide.

In general, the sugar moiety and the modifying group are linked together through the use of reactive groups, which are typically transformed by the linking process into a new organic functional group or unreactive species. The sugar reactive functional group(s) may

be located at any position on the sugar moiety. Reactive groups and classes of reactions useful in practicing the present disclosure are generally those that are well known in the art of bioconjugate chemistry. Currently favored classes of reactions available with reactive sugar moieties are those which proceed under relatively mild conditions. These include, but are not limited to nucleophilic substitutions (e.g., reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (e.g., enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (e.g., Michael reaction, Diels-Alder addition). These and other useful reactions are discussed in, for example, Smith and March, *Advanced Organic Chemistry*, 5th Ed., John Wiley & Sons, New York, 2001; Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, 1996; and Feeney et al., *Modification of Proteins*; *Advances in Chemistry Series*, Vol. 198, American Chemical Society, Washington, D.C., 1982.

Useful reactive functional groups pendent from a sugar nucleus or modifying group include, but are not limited to: (a) carboxyl groups and various derivatives thereof (e.g., N-hydroxysuccinimide esters, N-hydroxybenzotriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters); (b) hydroxyl groups, which can be converted to, e.g., esters, ethers, aldehydes, etc.; (c) haloalkyl groups, wherein the halide can be later displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the functional group of the halogen atom; (d) dienophile groups, which are capable of participating in Diels-Alder reactions such as, for example, maleimido groups (e) aldehyde or ketone groups, such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkyllithium addition; (f) sulfonyl halide groups for subsequent reaction with amines, for example, to form sulfonamides; (g) thiol groups, which can be, for example, converted to disulfides or reacted with alkyl and acyl halides; (h) amine or sulfhydryl groups, which can be, for example, acylated, alkylated or oxidized; (i) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, metathesis, Heck reaction, etc.; (j) epoxides, which can react with, for example, amines and hydroxyl compounds.

The reactive functional groups can be chosen such that they do not participate in, or interfere with, the reactions necessary to assemble the reactive sugar nucleus or modifying group. Alternatively, a reactive functional group can be protected from participating in the reaction by the presence of a protecting group. Those of skill in the art understand how to

protect a particular functional group such that it does not interfere with a chosen set of reaction conditions. For examples of useful protecting groups, see, for example, Greene et al., *Protective Groups in Organic Synthesis*, John Wiley & Sons, New York, 1991.

In some embodiments, the modified sugar is an activated sugar. Activated modified
5 sugars useful in the present disclosure are typically glycosides which have been synthetically altered to include an activated leaving group. As used herein, the term "activated leaving group" refers to those moieties which are easily displaced in enzyme-regulated nucleophilic substitution reactions. Many activated sugars are known in the art. See, for example, Vocadlo et al., In *Carbohydrate Chemistry and Biology*, Vol. 2, Ernst et al. Ed., Wiley-VCH
10 Verlag: Weinheim, Germany, 2000; Kodama et al., *Tetrahedron Lett.* 34: 6419 (1993); Loughheed, et al., *J. Biol. Chem.* 274: 37717 (1999)). Examples of such leaving groups include fluoro, chloro, bromo, tosylate, mesylate, triflate and the like. Preferred activated leaving groups for use in the present disclosure are those that do not significantly sterically encumber the enzymatic transfer of the glycoside to the acceptor. Accordingly, preferred
15 embodiments of activated glycoside derivatives include glycosyl fluorides and glycosyl mesylates, with glycosyl fluorides being particularly preferred. Among the glycosyl fluorides, α -galactosyl fluoride, α -mannosyl fluoride, α -glucosyl fluoride, α -fucosyl fluoride, α -xylosyl fluoride, α -sialyl fluoride, α -N-acetylglucosaminyl fluoride, α -N-acetylgalactosaminyl fluoride, β -galactosyl fluoride, β -mannosyl fluoride, β -glucosyl
20 fluoride, β -fucosyl fluoride, β -xylosyl fluoride, β -sialyl fluoride, β -N-acetylglucosaminyl fluoride and β -N-acetylgalactosaminyl fluoride are most preferred.

In certain aspects, a modified sugar residue is conjugated to one or more water-soluble polymers. Many water-soluble polymers are known to those of skill in the art and are useful in practicing the present disclosure. The term water-soluble polymer encompasses
25 species such as saccharides (e.g., dextran, amylose, hyaluronic acid, poly(sialic acid), heparans, heparins, etc.); poly(amino acids); nucleic acids; synthetic polymers (e.g., poly(acrylic acid), poly(ethers), e.g., poly(ethylene glycol)); peptides, proteins, and the like. The present disclosure may be practiced with any water-soluble polymer with the sole limitation that the polymer must include a point at which the remainder of the conjugate can
30 be attached.

Methods and chemistry for activation of water-soluble polymers and saccharides as well as methods for conjugating saccharides and polymers to various species are described in the literature. Commonly used methods for activation of polymers include activation of functional groups with cyanogen bromide, periodate, glutaraldehyde, biepoxydes,

epichlorohydrin, divinylsulfone, carbodiimide, sulfonyl halides, trichlorotriazine, etc. (see, R. F. Taylor, (1991), Protein Immobilisation, Fundamentals and Applications, Marcel Dekker, N.Y.; S. S. Wong, (1992), Chemistry of Protein Conjugation and Crosslinking, CRC Press, Boca Raton; G. T. Hermanson et al., (1993), Immobilized Affinity Ligand Techniques, Academic Press, N.Y.; Dunn, R. L., et al., Eds. Polymeric Drugs and Drug Delivery Systems, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991).

In certain aspects, a modified sugar residue is conjugated to one or more water-insoluble polymers. In some embodiments, conjugation to a water-insoluble polymer can be used to deliver a therapeutic peptide in a controlled manner. Polymeric drug delivery systems are known in the art. See, for example, Dunn et al., Eds. Polymeric drugs and Drug Delivery Systems, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991. Those of skill in the art will appreciate that substantially any known drug delivery system is applicable to the conjugates of the present disclosure.

Representative water-insoluble polymers include, but are not limited to, polyphosphazines, poly(vinyl alcohols), polyamides, polycarbonates, polyalkylenes, polyacrylamides, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butyl methacrylate), poly(isobutyl methacrylate), poly(hexyl methacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl acetate), polyvinyl chloride, polystyrene, polyvinyl pyrrolidone, pluronics, and polyvinylphenol, and copolymers thereof.

These and the other polymers discussed herein can be readily obtained from commercial sources such as Sigma Chemical Co. (St. Louis, Mo.), Polysciences (Warrenton, Pa.), Aldrich (Milwaukee, Wis.), Fluka (Ronkonkoma, N.Y.), and BioRad (Richmond, Calif.), or else synthesized from monomers obtained from these suppliers using standard techniques. Representative biodegradable polymers useful in the conjugates of the disclosure include, but are not limited to, polylactides, polyglycolides and copolymers thereof poly(ethylene terephthalate), poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), poly(lactide-co-glycolide), polyanhydrides, polyorthoesters, blends and copolymers thereof. Of particular use are compositions that form gels, such as those including collagen, and pluronics.

In a preferred embodiment, one or more modified sugar residues are conjugated to one or more PEG molecules.

In certain aspects, the modified sugar is conjugated to a biomolecule. Biomolecule of the disclosure may include, but are not limited to, functional proteins, enzymes, antigens, antibodies, peptides, nucleic acids (e.g., single nucleotides or nucleosides, oligonucleotides, polynucleotides and single- and higher-stranded nucleic acids), lectins, receptors or a combination thereof.

Some preferred biomolecules are essentially non-fluorescent, or emit such a minimal amount of fluorescence that they are inappropriate for use as a fluorescent marker in an assay. Other biomolecules may be fluorescent.

In some embodiments, the biomolecule is a targeting moiety. A "targeting moiety" and "targeting agent", as used herein, refer to species that will selectively localize in a particular tissue or region of the body. In some embodiments, a biomolecule is selected to direct the SAP protein of the disclosure to a specific intracellular compartment, thereby enhancing the delivery of the peptide to that intracellular compartment relative to the amount of underivatized peptide that is delivered to the tissue. The localization is mediated by specific recognition of molecular determinants, molecular size of the targeting agent or conjugate, ionic interactions, hydrophobic interactions and the like. Other mechanisms of targeting an agent to a particular tissue or region are known to those of skill in the art.

In some embodiments, the modified sugar includes a therapeutic moiety. Those of skill in the art will appreciate that there is overlap between the category of therapeutic moieties and biomolecules, i.e., many biomolecules have therapeutic properties or potential.

Classes of useful therapeutic moieties include, for example, non-steroidal anti-inflammatory drugs; steroidal anti-inflammatory drugs; adjuvants; antihistaminic drugs; antitussive drugs; antipruritic drugs; anticholinergic drugs; anti-emetic and antinauseant drugs; anorexic drugs; central stimulant drugs; antiarrhythmic drugs; β -adrenergic blocker drugs; cardiogenic drugs; antihypertensive drugs; diuretic drugs; vasodilator drugs; vasoconstrictor drugs; antiulcer drugs; anesthetic drugs; antidepressant drugs; tranquilizer and sedative drugs; antipsychotic drugs; and antimicrobial drugs.

Other drug moieties useful in practicing the present disclosure include antineoplastic drugs, cytotoxic agents, anti-estrogens, and antimetabolites. Also included within this class are radioisotope-based agents for both diagnosis (e.g., imaging) and therapy, and conjugated toxins.

The therapeutic moiety can also be a hormone, a muscle relaxant, an antispasmodic, bone activating agent, endocrine modulating agent, modulator of diabetes, androgen, antidiuretics, or calxitonin drug.

Other useful modifying groups include immunomodulating drugs,
5 immunosuppressants, etc. Groups with anti-inflammatory activity, such as sulindac, etodolac, ketoprofen and ketorolac, are also of use. Other drugs of use in conjunction with the present disclosure will be apparent to those of skill in the art.

The altered N-glycosylation SAP proteins produced by the methods of the disclosure can be homogeneous (i.e., the sample of SAP protein is uniform in specific N-glycan
10 structure) or substantially homogeneous. By "substantially homogeneous" is meant that at least about 25% (e.g., at least about 27%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, or at least about 99%) of the SAP proteins
15 contain the same specific N-glycan structure.

In some embodiments, variant SAP proteins of the disclosure have an IC_{50} for inhibiting the differentiation of monocytes into fibrocytes *in vitro* that is less than 1/2, less than 1/3, less than 1/4, less than 1/10, or less than 1/100 that of a corresponding sample of wild-type SAP isolated from human serum. In some embodiments, variant SAP proteins of
20 the disclosure have an IC_{50} for inhibiting the differentiation of monocytes into fibrocytes *in vitro* that is less than one-half that of a corresponding sample of wild-type SAP isolated from human serum. There are many well characterized methods for determining the responsiveness of Peripheral Blood Mononuclear Cells (PBMCs) or monocyte cells to SAP for fibrocyte differentiation. In some embodiments, the SAP protein of the disclosure inhibits
25 production of IL-8. In some embodiments, the inhibitory effect of an SAP protein of the disclosure to block phorbol myristate acetate (PMA)-induced production of IL-8 is measured. These methods may be used to determine the relative potency of any of the SAP variant polypeptides of the disclosure in comparison to a sample of human serum-derived SAP, any other SAP variant polypeptide, or other fibrocyte suppressant or activating agent. PBMCs or
30 monocytes suitable for use in these methods may be obtained from various tissue culture lines. Alternatively, suitable cells for fibrocyte differentiation assays may be obtained from any biological sample that contains PBMC or monocyte cells. The biological sample may be obtained from serum, plasma, healthy tissue, or fibrotic tissue. In general, fibrocyte differentiation assays are conducted by incubating PBMC or monocyte cells in media with

various concentrations of an SAP protein to determine the degree of fibrocyte differentiation. The concentration of SAP can range from 0.0001 µg/mL to 1 mg/ml, and in some embodiments is 0.001 µg/mL, 1.0 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL, 45 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, or 500 µg/mL. In some assays, the media can be supplemented with between 1-100 ng/ml hMCSF; the preferred concentration of hMCSF being 25 ng/mL. The indication that PBMC and monocytes have differentiated into fibrocytes can be determined by one skilled in the art. In general, fibrocytes are morphologically defined as adherent cells with an elongated spindle-shape and the presence of an oval nucleus. In some assays, cells are fixed and stained with Hema 3 before enumerating fibrocytes by direct counting, e.g., using an inverted microscope. The amount of fibrocyte differentiation is interpreted by one skilled in the art as an indication of a cell's responsiveness to SAP. A greater suppression of fibrocyte differentiation indicates a greater degree of SAP responsiveness. An alternative method of measuring fibrocyte differentiation involves determining the expression of fibrocyte-specific cell surface markers or secreted factors, e.g., cytokines (such as IL-1ra, ENA-78/CXCL-5, PAI-1), fibronectin, collagen-1). Methods of detecting and/or quantifying cell surface markers or secreted factors are well known in the art, including but not limited to various ELISA- and FACS-based techniques using immunoreactive antibodies against one or more fibrocyte-specific markers. Measuring the expression of Macrophage Derived Chemokine (MDC) is an effective method of determining fibrocyte differentiation.

Methods for detecting and/or characterizing N-glycosylation (e.g., altered N-glycosylation) of an SAP protein include DNA sequencer-assisted (DSA), fluorophore-assisted carbohydrate electrophoresis (FACE) or surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). For example, an analysis can utilize DSA-FACE in which, for example, glycoproteins are denatured followed by immobilization on, e.g., a membrane. The glycoproteins can then be reduced with a suitable reducing agent such as dithiothreitol (DTT) or β-mercaptoethanol. The sulfhydryl groups of the proteins can be carboxylated using an acid such as iodoacetic acid. Next, the N-glycans can be released from the protein using an enzyme such as N-glycosidase F. N-glycans, optionally, can be reconstituted and derivatized by reductive amination. The derivatized N-glycans can then be concentrated. Instrumentation suitable for N-glycan analysis includes, for example, the ABI PRISM® 377 DNA sequencer (Applied Biosystems). Data analysis can be performed using, for example, GENESCAN® 3.1 software (Applied Biosystems). Optionally, isolated mannoproteins can be further treated with one or more enzymes to confirm their N-glycan

status. Exemplary enzymes include, for example, α -mannosidase or α -1,2 mannosidase. Additional methods of N-glycan analysis include, for example, mass spectrometry (e.g., MALDI-TOF-MS), high-pressure liquid chromatography (HPLC) on normal phase, reversed phase and ion exchange chromatography (e.g., with pulsed amperometric detection when
5 glycans are not labeled and with UV absorbance or fluorescence if glycans are appropriately labeled). See also Callewaert et al. (2001) *Glycobiology* 11(4):275-281 and Freire et al. (2006) *Bioconjug. Chem.* 17(2):559-564, the disclosures of each of which are incorporated herein by reference in their entirety.

Anti-Fc γ R Antibodies as SAP Agonists

10 In one aspect of the disclosure, one or more compounds are provided that mimic SAP signaling. In some embodiments, the SAP signaling agonists are anti-Fc γ R antibodies, wherein the antibodies are selected from a class of anti-Fc γ RI, anti-Fc γ RIIA, and anti-Fc γ RIII antibodies that are able to bind to either Fc γ RI, Fc γ RIIA, or Fc γ RIII, respectively. Anti-Fc γ R antibodies are antibodies that bind to receptors for the Fc portion of IgG
15 antibodies (Fc γ R). The anti-Fc γ R antibodies bind through their variable region, and not through their constant (Fc) region. Anti-Fc γ R antibodies may include any isotype of antibody. The anti-Fc γ R antibodies may be further cross-linked or aggregated with or without additional antibodies or other means. This process initiates intracellular signaling events consistent with Fc γ R activation. In some embodiments, the SAP signaling agonist
20 may be a cross-linked Fc γ R.

Aggregated Fc Domains and Fc-Containing Antibodies

In some embodiments, the SAP signaling agonists are cross-linked or aggregated IgG. Cross-linked or aggregated IgG may include any IgG able to bind the target Fc γ R through its Fc region, provided that at least two such IgG antibodies are physically connected to one
25 another.

Cross-linked or aggregated IgG may include whole antibodies or a portion thereof, preferably the portion functional in suppression of fibrotic disorders. For example, they may include any antibody portion able to cross-link Fc γ R. This may include aggregated or cross-linked antibodies or fragments thereof, such as aggregated or cross-linked whole antibodies,
30 Fab fragments, F(ab')₂ fragments, Fab' fragments, and possibly even Fc fragments.

Aggregation or cross-linking of antibodies may be accomplished by any known method, such as heat or chemical aggregation. Any level of aggregation or cross-linking may be sufficient, although increased aggregation may result in increased fibrotic disorder suppression. Antibodies may be polyclonal or monoclonal, such as antibodies produced from

hybridoma cells. Compositions and methods may employ mixtures of antibodies, such as mixtures of multiple monoclonal antibodies, which may be cross-linked or aggregated to like or different antibodies.

SAP Peptidomimetic

5 In certain embodiments, the SAP agonists include peptidomimetics. As used herein, the term "peptidomimetic" includes chemically modified peptides and peptide-like molecules that contain non-naturally occurring amino acids, peptoids, and the like. Methods for identifying a peptidomimetic are well known in the art and include the screening of databases that contain libraries of potential peptidomimetics. For example, the Cambridge Structural
10 Database contains a collection of greater than 300,000 compounds that have known crystal structures (Allen et al., Acta Crystallogr. Section B, 35:2331 (1979)). Where no crystal structure of a target molecule is available, a structure can be generated using, for example, the program CONCORD (Rusinko et al., J. Chem. Inf. Comput. Sci. 29:251 (1989)). Another database, the Available Chemicals Directory (Molecular Design Limited, Informations
15 Systems; San Leandro Calif.), contains about 100,000 compounds that are commercially available and also can be searched to identify potential peptidomimetics of SAP proteins.

Increase SAP Activity

In some embodiments, an SAP agonist increases SAP activity. SAP activity can be increased by increasing the concentration of SAP by, for example, increasing SAP
20 transcription, increasing translation, increasing SAP secretion, increasing SAP RNA stability, increasing SAP protein stability, or decreasing SAP protein degradation. SAP activity can also be increased by increasing specifically the "free concentration" of SAP, or rather the unbound form by, for example, decreasing SAP endogenous binding partners.

FcγR Crosslinkers

25 In some embodiments, fibronectin-based scaffold domain proteins may be used as SAP agonists to crosslink FcγRs. Fibronectin-based scaffold domain proteins may comprise a fibronectin type III domain (Fn3), in particular a fibronectin type III tenth domain (¹⁰Fn3).

In order to crosslink FcγRs, multimers of FcγR binding Fn3 domains may be generated as described in U.S. Pat. No. 7,115,396.

30 Fibronectin type III (Fn3) domains comprise, in order from N-terminus to C-terminus, a beta or beta-like strand, A; a loop, AB; a beta or beta-like strand, B; a loop, BC; a beta or beta-like strand C; a loop CD; a beta or beta-like strand D; a loop DE; a beta or beta-like strand, E; a loop, EF; a beta or beta-like strand F; a loop FG; and a beta or beta-like strand G. The BC, DE, and FG loops are both structurally and functionally analogous to the

complementarity-determining regions (CDRs) from immunoglobulins Fn3 domains can be designed to bind almost any compound by altering the sequence of one or more of the BC, DE, and FG loops. Methods for generating specific binders have been described in U.S. Pat. No. 7,115,396, disclosing high affinity TNF α binders, and U.S. Publication No.

- 5 2007/0148126, disclosing high affinity VEGFR2 binders. An example of fibronectin-based scaffold proteins are Adnectins™ (Adnexus, a Bristol-Myers Squibb R&D Company).

In some embodiments, the SAP agonist is an aptamer. In order to crosslink Fc γ R_s, multimers of Fc γ R binding aptamers may be generated.

- Aptamers are oligonucleotides, which can be synthetic or natural, that bind to a particular target molecule, such as a protein or metabolite. Typically, the binding is through interactions other than classic Watson-Crick base pairing. Aptamers represent a promising class of therapeutic agents currently in pre-clinical and clinical development. Like biologics, e.g., peptides or monoclonal antibodies, aptamers are capable of binding specifically to molecular targets and, through binding, inhibiting target function. A typical aptamer is 10-15 kDa in size (i.e., 30-45 nucleotides), binds its target with sub-nanomolar affinity, and discriminates among closely related targets (e.g., will typically not bind other proteins from the same gene family) (Griffin, et al. (1993), *Gene* 137(1): 25-31; Jenison, et al. (1998), *Antisense Nucleic Acid Drug Dev.* 8(4): 265-79; Bell, et al. (1999), *In vitro Cell. Dev. Biol. Anim* 35(9): 533-42; Watson, et al. (2000), *Antisense Nucleic Acid Drug Dev.* 10(2): 63-75; Daniels, et al. (2002), *Anal. Biochem.* 305(2): 214-26; Chen, et al. (2003), *Proc. Natl. Acad. Sci. U.S.A.* 100(16): 9226-31; Khati, et al. (2003), *J. Virol.* 77(23): 12692-8; Vaish, et al. (2003), *Biochemistry* 42(29): 8842-51).

- Aptamers have a number of attractive characteristics for use as therapeutics. In addition to high target affinity and specificity, aptamers have shown little or no toxicity or immunogenicity in standard assays (Wlotzka, et al. (2002), *Proc. Natl. Acad. Sci. U.S.A.* 99(13): 8898-902). Indeed, several therapeutic aptamers have been optimized and advanced through varying stages of pre-clinical development, including pharmacokinetic analysis, characterization of biological efficacy in cellular and animal disease models, and preliminary safety pharmacology assessment (Reyderman and Stavchansky (1998), *Pharmaceutical Research* 15(6): 904-10; Tucker et al., (1999), *J. Chromatography B.* 732: 203-212; Watson, et al. (2000), *Antisense Nucleic Acid Drug Dev.* 10(2): 63-75).

A suitable method for generating an aptamer to a target of interest is with the process entitled "Systematic Evolution of Ligands by EXponential Enrichment" ("SELEX™"). The SELEX™ process is a method for the *in vitro* evolution of nucleic acid molecules with highly

specific binding to target molecules and is described in, e.g., U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, now abandoned, U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands", and U.S. Pat. No. 5,270,163 (see also WO 91/19813) entitled "Nucleic Acid Ligands". Each SELEX™-identified nucleic acid ligand is a specific ligand of a given target
5 compound or molecule. The SELEX™ process is based on the insight that nucleic acids can form a variety of two- and three-dimensional structures and have sufficient chemical versatility available within their monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric. Molecules of any size or composition can serve as targets. The SELEX™ method applied to the application of
10 high affinity binding involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX™ method includes steps of contacting the mixture with the target
15 under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly
20 specific high affinity nucleic acid ligands to the target molecule. SELEX™ is a method for making a nucleic acid ligand for any desired target, as described, e.g., in U.S. Pat. Nos. 5,475,096 and 5,270,163, and PCT/US91/04078, each of which is specifically incorporated herein by reference.

In some embodiments, SAP agonists are Nanobodies®. Nanobodies® are antibody-
25 derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. The Nanobody® technology was originally developed following the discovery that camelidae (camels and llamas) possess fully functional antibodies that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3). Importantly, the cloned
30 and isolated VHH domain is a stable polypeptide harboring the full antigen-binding capacity of the original heavy-chain antibody. These VHH domains with their unique structural and functional properties form the basis of a new generation of therapeutic antibodies.

Pharmaceutical Preparations and Formulations

In certain embodiments, the methods described herein involve administration of at least one SAP protein of the disclosure to a subject as a therapeutic agent. The therapeutic agents of the disclosure may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. For example, therapeutic agents and their physiologically acceptable salts and solvates may be formulated for administration by, for example, intravenous infusion (IV), injection (e.g. SubQ, IM, IP), inhalation or insufflation (either through the mouth or the nose) or oral, buccal, sublingual, transdermal, nasal, parenteral or rectal administration. In certain embodiments, therapeutic agents may be administered locally, at the site where the target cells are present, i.e., in a specific tissue, organ, or fluid (e.g., blood, cerebrospinal fluid, tumor mass, etc.). In other words, the disclosure contemplates that for any of the methods described herein, the method comprises administration of SAP or a composition comprising SAP (e.g., a pharmaceutical composition). In certain preferred embodiments, the composition is a composition comprising SAP, wherein the activity and/or SAP sialylation across the composition differs from that in human serum.

The present disclosure further provides use of any SAP protein of the disclosure in the manufacture of a medicament for the treatment or prevention of a disorder or a condition, as described herein, in a patient, for example, the use of an SAP protein in the manufacture of medicament for the treatment of a disorder or condition described herein. In some aspects, an SAP protein of the disclosure may be used to make a pharmaceutical preparation for the use in treating or preventing a disease or condition described herein.

Therapeutic agents can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For parenteral administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. In some embodiments, the therapeutic agents can be administered to cells by a variety of methods known to those familiar in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For administration by inhalation (e.g., pulmonary delivery), therapeutic agents may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

In the methods of the disclosure, the pharmaceutical compounds can also be administered by intranasal or intrabronchial routes including insufflation, powders, and aerosol formulations (for examples of steroid inhalants, see Rohatagi (1995) *J. Clin. Pharmacol.* 35:1187-1193; Tjwa (1995) *Ann. Allergy Asthma Immunol.* 75:107-111). For example, aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations such as in a nebulizer or an atomizer. Typically, such administration is in an aqueous pharmacologically acceptable buffer.

Pharmaceutical compositions suitable for respiratory delivery (e.g., intranasal, inhalation, etc.) of SAP proteins may be prepared in either solid or liquid form.

SAP proteins of the disclosure may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. In certain embodiments, the SAP proteins are formulated for intravenous delivery. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

SAP proteins of the disclosure may be formulated for subcutaneous delivery, e.g., by injection. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with or without an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

In addition, SAP proteins of the disclosure may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Controlled release formula also includes patches.

In certain embodiments, SAP proteins of the disclosure are incorporated into a topical formulation containing a topical carrier that is generally suited to topical drug administration and comprising any such material known in the art. The topical carrier may be selected so as to provide the composition in the desired form, e.g., as an ointment, lotion, cream, microemulsion, gel, oil, solution, or the like, and may be comprised of a material of either naturally occurring or synthetic origin. It is preferable that the selected carrier not adversely affect the active agent or other components of the topical formulation. Examples of suitable topical carriers for use herein include water, alcohols and other nontoxic organic solvents, glycerin, mineral oil, silicone, petroleum jelly, lanolin, fatty acids, vegetable oils, parabens, waxes, and the like.

Pharmaceutical compositions (including cosmetic preparations) may comprise from about 0.00001 to 100% such as from 0.001 to 10% or from 0.1% to 5% by weight of one or more of the SAP proteins described herein. In certain topical formulations, the active agent is present in an amount in the range of approximately 0.25 wt. % to 75 wt. % of the formulation, preferably in the range of approximately 0.25 wt. % to 30 wt. % of the formulation, more preferably in the range of approximately 0.5 wt. % to 15 wt. % of the formulation, and most preferably in the range of approximately 1.0 wt. % to 10 wt. % of the formulation.

Therapeutic agents described herein may be stored in oxygen-free environment according to methods in the art.

Exemplary compositions comprise an SAP protein with one or more pharmaceutically acceptable carriers and, optionally, other therapeutic ingredients. The carrier(s) must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the composition and not eliciting an unacceptable deleterious effect in the subject. Such carriers are described herein or are otherwise well known to those skilled in the art of pharmacology. In some embodiments, the pharmaceutical compositions are pyrogen-free and are suitable for administration to a human patient. In some embodiments, the pharmaceutical compositions are irritant-free and are suitable for administration to a human patient. In some embodiments, the pharmaceutical compositions are allergen-free and are suitable for administration to a human patient. The compositions may be prepared by any of the methods well known in the art of pharmacy.

In some embodiments, an SAP protein is administered in a time release formulation, for example in a composition which includes a slow release polymer. An SAP protein can be prepared with carriers that will protect against rapid release. Examples include a controlled release vehicle, such as a polymer, microencapsulated delivery system, or bioadhesive gel. Alternatively, prolonged delivery of an SAP protein may be achieved by including in the composition agents that delay absorption, for example, aluminum monostearate hydrogels and gelatin.

In certain embodiments, the methods of the disclosure comprise administration via any of the foregoing routes of administration, such as intravenous or subcutaneous. In certain embodiments, administration is subcutaneous, particularly when each dose being administered is in a small volume. In certain embodiments, administration is intravenous.

The following examples serve to more fully describe the manner of using the above-described disclosure, as well as to set forth the best modes contemplated for carrying out

various aspects of the disclosure. It is understood that these examples in no way serve to limit the true scope of this disclosure, but rather are presented for illustrative purposes.

EXEMPLIFICATION

5 Example 1. Treatment of myelofibrosis with recombinant human SAP (rhSAP)

Patients diagnosed as having myelofibrosis, including PMF, post-PV MF, or post ET-MF are tested for their baseline mutational status in one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*. To measure the baseline mutational status, peripheral blood samples are collected from the patients and DNA is extracted from the
10 samples and analyzed by real-time quantitative allele-specific PCR to measure the mutational status (i.e., identify the presence or absence of one or more mutations) of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. Patients receive human, α 2,3-sialic acid-containing SAP recombinantly expressed in CHO cells (rhSAP expressed in
15 CHO cells; SAP comprising at least one α 2,3 linkage and differing in glycosylation from SAP derived from human serum; an exemplary SAP protein of the disclosure). Efficacy is assessed by evaluation of the bone marrow response rate, defined as a reduction of one grade in the WHO myelofibrosis grade as described in the EU Consensus Criteria. Reponse rate to SAP treatment will be correlated to the mutational status of the patient. This method can be used to identify subpopulations of myelofibrosis patients (e.g., patients with one or more
20 specific combinations of mutations in genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*) who are particularly appropriate for treatment with rhSAP. Further, change in mutational status of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2* is measured at regular intervals to evaluate any difference in responsiveness or change in allele burden. The dosage regimen is maintained if a reduction
25 in allele burden in one or more genes is observed. Subjects responding to therapy will continue receiving it as long as there is a benefit.

Example 2. Treatment of myelofibrosis with recombinant human SAP (rhSAP)

Patients diagnosed as having myelofibrosis, including PMF, post-PV MF, or post ET-MF are tested for their baseline mutational status in one or more genes such as *JAK2*, *MPL*,
30 *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*. To measure the baseline mutational status, peripheral blood samples are collected from the patients and DNA is extracted from the samples and analyzed by real-time quantitative allele-specific PCR to measure the mutational status (i.e., identify the presence or absence of one or more mutations) of one or more genes

such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. Patients receive human, α 2,3-sialic acid-containing SAP recombinantly expressed in CHO cells (rhSAP expressed in CHO cells; SAP comprising at least one α 2,3 linkage and differing in glycosylation from SAP derived from human serum). Efficacy is assessed by evaluation of the bone marrow response rate, defined as a reduction of one grade in the WHO myelofibrosis grade as described in the EU Consensus Criteria. Change in mutational status of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2* is measured at regular intervals to evaluate any difference in responsiveness or change in allele burden. If no change in the allele burden in any of the tested genes is observed, the dosage regimen is modified to increase the dosage of rhSAP and/or increase the frequency of rhSAP administration.

Example 3. Reduction of mutant allele burden in myelofibrosis with recombinant human SAP (rhSAP)

Patients diagnosed as having myelofibrosis, including PMF, post-PV MF, or post ET-MF are tested for their baseline mutational status in one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*. To measure the baseline mutational status, peripheral blood samples are collected from the patients and DNA is extracted from the samples and analyzed by real-time quantitative allele-specific PCR to measure the mutational status (i.e., identify the presence or absence of one or mutations) of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. Patients who have a mutation in one or more genes receive human, α 2,3-sialic acid -containing SAP recombinantly expressed in CHO cells (rhSAP expressed in CHO cells; SAP comprising at least one α 2,3 linkage and differing in glycosylation from SAP derived from human serum). Dosage is adjusted to be effective to reduce mutant allele burden in one or more genes. Subjects responding to therapy continue receiving it as long as there is a benefit.

Example 4. Reduction of mutant allele burden as an indicator of treatment efficacy with recombinant human SAP (rhSAP)

Patients diagnosed as having myelofibrosis, including PMF, post-PV MF, or post ET-MF are tested for their baseline mutational status in one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*. To measure the baseline mutational status, peripheral blood samples are collected from the patients and DNA is extracted from the samples and analyzed by real-time quantitative allele-specific PCR to measure the allele burden of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or

IDH2. Patients receive human, α 2,3-sialic acid -containing SAP recombinantly expressed in CHO cells (rhSAP expressed in CHO cells; SAP comprising at least one α 2,3 linkage and differing in glycosylation from SAP derived from human serum). A second mutant allele burden of the same mutation is measured after administration of the SAP protein. A decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder. Mutant allele burden may be measured at one or more time points following initiation of treatment, such as after about one month, two months, three months, four months or five months of treatment. Mutant allele levels may be subsequently monitored to evaluate durability of response.

Example 5. Treatment of myelofibrosis patients with mutations in one or more of *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2* with recombinant human SAP (rhSAP)

Patients diagnosed as having myelofibrosis, including PMF, post-PV MF, or post ET-MF are tested for their mutational status in one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*. To measure the baseline mutational status, peripheral blood samples are collected from the patients and DNA is extracted from the samples and analyzed by real-time quantitative allele-specific PCR to measure the mutational status (i.e., identify the presence or absence of one or mutations) of one or more genes such as *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*. Patients who carry a mutation in one or more of the genes receive human, α 2,3-sialic acid -containing SAP recombinantly expressed in CHO cells (rhSAP expressed in CHO cells; SAP comprising at least one α 2,3 linkage and differing in glycosylation from SAP derived from human serum). Efficacy is assessed by evaluation of the bone marrow response rate, defined as a reduction of one grade in the WHO myelofibrosis grade as described in the EU Consensus Criteria. Subjects responding to therapy continue receiving it as long as there is a benefit. Optionally, additional criteria are measured, such as hemoglobin, platelet count, symptoms, mutant allele status and the like. Any one or more of these criteria may be measured at one or more time points following initiation of treatment, such as after about one month, two months, three months, four months or five months of treatment. Any one or more of these criteria may be subsequently monitored to evaluate durability of response.

Example 6. Treatment of myelofibrosis patients with PRM-151 alone: Stage 2

This study evaluates an every four week dosing schedule, following a loading period. Recombinant human SAP, in this case the recombinant human SAP known as PRM-151, is administered to intermediate-2 or high risk patients with PMF, post-PV PMF, or post-ET PMF to evaluate safety and efficacy of three different doses of PRM-151 in reducing bone marrow fibrosis by ≥ 1 grade. Patients who are anemic or thrombocytopenic and are not receiving therapy for MF other than transfusions are eligible for this study. Patients are not candidates for ruxolitinib based on either a platelet count $< 50 \times 10^9/L$ or Hgb < 100 g/L, have received ≥ 2 units PRBC in the 12 weeks prior to study entry, and be intolerant of or had inadequate response to ruxolitinib.

84 patients with intermediate-2 or high risk MF who meet the eligibility requirements are randomized to one of 3 groups receiving treatment with single agent PRM-151. Group 1: patients who have received no MF-directed drug treatment for MF in at least four weeks receive (i) an initial loading dose of PRM-151 at 0.3 mg/kg by intravenous infusion on days 1, 3, and 5 of cycle 1 (a 28-day cycle), and (ii) thereafter were administered a dose of PRM-151 at 0.3 mg/kg by intravenous infusion on day 1 of each subsequent 28-day cycle for nine cycles. Group 2: patients who have received no MF-directed drug treatment for MF in at least four weeks receive (i) an initial loading dose of PRM-151 at 3 mg/kg by intravenous infusion on days 1, 3, and 5 of cycle 1 (a 28-day cycle), and (ii) thereafter were administered a dose of PRM-151 at 3 mg/kg by intravenous infusion on day 1 of each subsequent 28-day cycle for nine cycles. Group 3: patients who have received no MF-directed drug treatment for MF in at least four weeks receive (i) an initial loading dose of PRM-151 at 10 mg/kg by intravenous infusion on days 1, 3, and 5 of cycle 1 (a 28-day cycle), and (ii) thereafter were administered a dose of PRM-151 at 10 mg/kg by intravenous infusion on day 1 of each subsequent 28-day cycle for nine cycles. In certain embodiments, the randomization is stratified according to type of subject (subjects with Hgb < 100 g/L and having received ≥ 2 units PRBC in the 12 weeks prior to study entry OR subjects with platelet count $< 50 \times 10^9/L$) and ensures that the final study population will include at least 50% of subjects from the second stratum (platelet count $< 50 \times 10^9/L$). All subjects may switch to an open label extension and receive PRM-151 10 mg/kg every 4 weeks completing 9 cycles of the originally assigned treatment. After study completion and data analysis, all subjects remaining on PRM-151 switch to the dose that has been selected for future development based on study results. Enrolled subjects are considered evaluable for response if they are on study drug for at least twelve weeks.

Patients in each cohort are monitored for improvements in bone marrow fibrosis (BMF) (by quantitative image analysis, for example) and disease related anemia, thrombocytopenia, peripheral blood blasts, constitutional symptoms, and spleen size. The IWG-MRT response (complete response, partial response, clinical improvement), and the effect on PRM-151 on the rate of stable and progressive disease is assessed. Further, patients are monitored for changes in other disease related hematologic abnormalities, changes in prognostic factors associated with increased mortality as measured by the DIPSS (Dynamic International Prognostic Scoring System), changes in bone marrow morphology, changes in bone marrow metabolism as measured by PET imaging. In addition, the interaction between genetic mutations and cytogenetic abnormalities and response to PRM-151 is evaluated and biomarkers of PRM-151 activity in bone marrow samples are evaluated. Correlation between baseline SAP levels and patient outcomes are assessed. The relationship between bone marrow fibrosis reduction and hematologic improvements is assessed. Patients are also monitored for overall response rate according to the International Working Group consensus criteria for treatment response in myelofibrosis with myeloid metaplasia (Tefferi A, Cervantes F, Mesa R, et al. Revised response criteria for myelofibrosis: International Working Group -Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. *Blood*. 2013; 122:1395-1398). Patients are also monitored for incidence of adverse events, changes in bone marrow fibrosis by WHO criteria as described in the European Consensus of Grading Bone Marrow Fibrosis (Thiele J, Kvasnicka HM, Facchetti F, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica* 2005; 90:1128-1132.), changes in the modified Myeloproliferative Neoplasia Symptom Assessment Form (MPN-SAF) Score (Emanuel et al. 2012, *Journal of Clinical Oncology* 30(33): 4098- 4103), and changes in quality of life as measured by the EORTC QLQ-C30 score (EORTC QLQ-C30 (version 3) 1995, EORTC Quality of Life Group). Progression-free and overall survival is measured.

Optionally, the patients in each cohort are monitored for one or more of the effects described herein, for example, the effect of the treatment on a reduction in bone marrow fibrosis score by at least one grade according to WHO criteria is evaluated as determined by a central adjudication panel of expert hematopathologists, blinded to subject, treatment, and time of biopsy. Optionally, the effect of the treatment on hematologic improvements such as RBC transfusion independence, platelet transfusion dependence, or a 10-20 g/L increase in hemoglobin levels is further monitored.

Mutational status and allele burden is optionally evaluated prior to initiation of treatment. Allele burden is optionally evaluated following initiation of treatment, and may be evaluated multiple times over the course of treatment (e.g., following 1, 2, 3, 4, 5 or 6 cycles). At time points throughout the study, blood samples are collected and DNA is
5 isolated from peripheral whole blood for the purpose of associating baseline mutational status with select primary and secondary endpoints. Samples are analyzed for mutational status of *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and/or *IDH2*. Samples are also analyzed to assess changes in allele burden of *JAK2V617F* at, for example, week 36 (Cycle 1 Day 1 to Cycle 9 Day 29). Samples are also analyzed to assess changes in the allele burden of
10 *MPLW515*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and/or *IDH2* at, for example, week 36.

Immunohistochemical analysis of additional bone marrow biopsy samples for disease and mechanism related proteins and cellular markers is optionally performed.

INCORPORATION BY REFERENCE

15 All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will be apparent to those
20 skilled in the art upon review of this specification and the below-listed claims. The full scope of the disclosure should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

SEQUENCE LISTING

SEQ ID NO: 1 human serum amyloid protein P

HTDLSGKVFVFPRESVTDHVNLTIPLEKPLQNFTLCFRAYS DLSRAYS LFSYNTQGRD
 NELLVYKERVGEYSLYIGRHKVTSKVIKFPAPVHICVSWESSSGIAEFWINGTPLVK
 KGLRQGYFVEAQPKIVLGQEQDSYGGKFDRSQSFVGEIGDLYMWDSVLPENILSAY
 QGTPLPANILDWQALNYEIRGYVIKPLVWV

SEQ ID NO: 2 *Gallus gallus* serum amyloid protein P

QEDLYRKVFVFPREDPSDAYVLLQVQLERPLLNTVCLRSYTDLTRPHSLFSYATKAQ
 DNEILLFKPKPGEYRFYVGGKYVTFRVPENRGWEHVCASWESGSGIAEFWLNGRP
 WPRKGLQKGYEVGNEAVVMLGQEQDAYGGGFDVYNSFTGEMADVHLWDAGLSP
 DKMRSAYLALRLPPAPLAWGRLRYEAKGDVVVKPRLREALGA

SEQ ID NO: 3 *Bos taurus* serum amyloid protein P

QTDLRGKVFVFPRESSTDHVTLTITKLEKPLKNLTCLRAYSDLSRGYSLFSYNIHSD
 NELLVFKNGIGEYSLYIGKTKVTVRATEKFPSPVHICTSWESSTGIAEFWINGKPLVKR
 GLKQGYAVGAHPKIVLGQEQDSYGGGFDKNQSFMGEIGDLYMWDSVLSPEEILLVY
 QGSSSISPTILDWQALKYEIKGYVIVKPMVWG

SEQ ID NO: 4 *Cricetulus migratorius* serum amyloid protein P

QTDLTGKVFVFPRESESDYVKLIPRLEKPLENFTLCFRITYTDLSRPHSLFSYNTKNKD
 NELLIYKERMGEYGLYIENVGAIVRGVEEFASPVHFCTSWESSSGIADFVWNGIPWV
 KKGLKKGYTVKTQPSIILGQEQDNYGGGFDKKSQSFVGEMGDLNMWDSVLTPEEIKS
 VYEGSWLEPNILDWRALNYEMSGYAVIRPRVWH

(SEQ ID NO: 5) NM_004972 Homo sapiens Janus kinase 2 (JAK2), mRNA

1 ctgcagggaag gagagaggaa gaggagcaga agggggcagc agcggacgcc gctaaccggcc
 61 tccctcggcg ctgacaggct gggccggcgc ccggctcget tgggtgttcg cgtcgccaact
 121 tcggcttctc ggcgggtcgg gccctcggc ccgggcttgc ggcgcgcgtc ggggctgagg
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 481 gaaaaagact ctgcatggga atggcctgcc ttacgatgac agaaatggag ggaacatcca
 541 cctcttctat atatcagaat ggtgatattt ctggaaatgc caattctatg aagcaaatag
 601 atccagttct tcagggtgat ctttaccatt cccttgggaa atctgaggca gattatctga
 661 cctttccatc tggggagtat gttgcagaag aaatctgtat tgctgcttct aaagcttgtg
 721 gtatcacacc tgtgtatcat aatatgtttg ctttaattgag tgaaacagaa aggatctggt

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781 atccacccaa ccatgtcttc catatagatg agtcaaccag gcataatgta ctctacagaa
841 taagattttta ctttcctcgt tggatttgca gtggcagcaa cagagcctat oggcatggaa
901 tatctcgagg tgctgaagct cctcttcttg atgactttgt catgtcttac ctctttgctc
961 agtggcggca tgattttgtg cacggatgga taaaagtacc tgtgactcat gaaacacagg
1021 aagaatgtct tgggatggca gtgttagata tgatgagaat agccaaagaa aacgatcaaa
1081 ccccaactggc catctataac tctatcagct acaagacatt cttaccaaaa tgtattcgag
1141 caaagatcca agactatcat attttgacaa ggaagcgaat aaggtacaga tttcgcagat
1201 ttattcagca attcagccaa tgcaaagcca ctgccagaaa cttgaaactt aagtatctta
1261 taaatctgga aactctgcag tctgccttct acacagagaa atttgaagta aaagaacctg
1321 gaagtgggtcc ttcagggtgag gagatttttg caaccattat aataactgga aacgggtgaa
1381 ttcagtggttc aagagggaaa cataaagaaa gtgagacact gacagaacag gatttacagt
1441 tatattgcga ttttcctaatt attattgatg tcaglattaa gcaagcaaac caagagggtt
1501 caaatgaaaag ccgagttgtg actatccata agcaagatgg taaaaatctg gaaattgaac
1561 ttagctcatt aagggaagct ttgtctttcg tgtcattaat tgatggatat tatagattaa
1621 ctgcagatgc acatcattac ctctgtaaag aagtagcacc tccagccgtg cttgaaaaaa
1681 tacaagcaaa ctgtcatggc ccaatttcga tggattttgc cattagttaa ctgaagaaag
1741 caggtaatca gactggactg tatgtacttc gatgcagtcc taaggacttt aataaatatt
1801 ttttgacttt tgctgtcgag cgagaaaatg tcattgaata taaacactgt ttgattacaa
1861 aaaatgagaa tgaagagtac aacctcagtg ggacaaagaa gaacttcagc agtcttaag
1921 atcttttgaa ttgttaccag atggaaactg ttcgctcaga caatataatt ttccagttta
1981 ctaaatgctg tccccaaaag ccaaaagata aatcaaacct tctagtcttc agaacgaatg
2041 gtgtttctga tgtaccaacc tcaccaacat tacagaggcc tactcatatg aaccaaatgg
2101 tgtttcacaa aatcagaaat gaagatttga tatttaatga aagccttggc caaggcactt
2161 ttacaaagat ttttaagggc gtacgaagag aagtaggaga ctacggtcaa ctgcatgaaa
2221 cagaagttct tttaaaagtt ctggataaag cacacagaaa ctattcagag tctttctttg
2281 aagcagcaag tatgatgagc aagctttctc acaagcattt ggttttaaat tatggagtat
2341 gtgtctgtgg agacgagaat attctgggtc aggagtttgt aaaatttggg tccactagata
2401 catatctgaa aaagaataaa aattgtataa atatatattg gaaacttgaa gttgctaasc
2461 agttggcatg ggccatgcat tttctagaag aaaacaccct tattcatggg aatgtatgtg
2521 ccaaaaatat tctgcttatt agagaagaag acaggaagac aggaaatcct cctttcatca
2581 aacttagtga tcttgccatt agtattacag ttttgccaaa ggacattctt caggagagaa
2641 taccatgggt accacctgaa tgcattgaaa atcctaataa tttaaatttg gcaacagaca
2701 aatggagttt tggtagcaat ttgtgggaaa tctgcagtgg aggagataaa cctctaagtg
2761 ctctggattc tcaaagaaaag ctacaatttt atgaagatag gcatcagctt cctgcaccaa
2821 agtgggcaga attagcaaac cttataaata attgtattgg tttatgaacca gatttcaggc
2881 cttctttcag agccatcata cgagatctta acagtttgtt tactccagat tatgaactat
2941 taacagaaaa tgacatgtta ccaaatatga ggataggtgc cctggggttt tctggtgcct
3001 ttgaagaccg ggaacctaca cagtttgaa agagacattt gaaattctt cagcagagaa
3061 gcaagggtaa ttttgggagt gtggagatgt gccggtatga cctctacag gacaacactg
3121 gggagggtgt cgctgtaaaa aagcttcagc atagtactga agagcaccta agagactttg
3181 aaagggaaat tgaaatcctg aaatccctac agcatgacaa cattgtaaag tacaaggag
3241 tgtgctacag tgcgtgtcgg cgtaatctaa aatttaattt ggaatattta ccatatggaa
3301 gtttacgaga ctatcttcaa aaacataaag aacggataga tcacataaaa cttctgcagt
3361 acacatctca gatatgcaag ggtatggagt atcttggtag aaaaaggtag atccacaggg
3421 atctggcaac gagaaatata ttggtggaga acgagaacag agttaaaatt ggagattttg
3481 ggtttaaccaa agtcttgcca caagacaaag aatactataa agtaaaaagaa cctggtgaaa
3541 gtcccatatt ctggtatgct ccagaatcac tgacagagag caagttttct gtggcctcag
3601 atgtttggag ctttggagtg gttctgtatg aacttttcac atacattgag aagagtaaaa
3661 gtccaccagc ggaatttatg cgtatgattg gcaatgacaa acaaggacag atgatcgtgt
3721 tccattttgat agaacttttg aagaataatg gaagattacc aagaccagat ggatgccag
3781 atgagatcta tatgatcatg acagaatgct ggaacaataa tgtaaatcaa cgccctcct
3841 ttagggatct agctcttcga gtggatcaaa taagggataa catggctgga tgaaagaaat
3901 gaccttcatt ctgagaccaa agtagattta cagaacaaag ttttatattt cacattgctg
3961 tggactatta ttacatata cttattata taaatcatga tgctagccag caaagatgtg
4021 aaaatatctg ctcaaaactt tcaaagttaa gtaagtttt cttcatgagg ccaccagtaa
4081 aagacattaa tgagaattcc ttagcaagga ttttgtaaga agtttcttaa acattgtcag
4141 ttaacatcac tcttgtctgg caaaagaaaa aaaatagact ttttcaactc agctttttga
4201 gacctgaaaa aattattatg taaattttgc aatgttaaag atgcacagaa tatgtatgta
4261 tagtttttac cacagtggat gtataatacc ttggcatctt gtgtgatgtt ttacacacat
4321 gagggctggg gttcaataat aatttttcca tagttaattt ataatttaatt
4381 acttcactat acaaacaaat taagatgttc agataattga ataagtaacct ttgtgtcctt

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4441 gttcattttat atcgctggcc agcattataa gcagggtgtat acttttagct tgtagtccca
4501 tgtactgttaa atatttttca cataaaggga acaaatgtct agttttatct gtataggaaa
4561 tttccctgac cctaaataat acattttgaa atgaaacaag cttacaaaga tataatctat
4621 tttattatgg tttcccttgt atctatttgt ggtgaatgtg ttttttaaat ggaactatct
4681 ccaaattttt ctaagactac tatgaacagt tttcttttaa aattttgaga ttaagaatgc
4741 caggaatatt gtcattcctt gagctgctga ctgccaataa cattcttcga tctctgggat
4801 ttatgctcat gaactaaatt taagcttaag ccataaaata gatttagattg ttttttaaaa
4861 atggatagct cattaagaag tgcagcaggt taagaatttt ttccataaga ctgtatatct
4921 gaggggtttc agaattttgc attgcagtea tagaagagat ttatttcctt tttagagggg
4981 aaatgaggta aataagtaaa aaagtatgct tgtaattttt attcaagaat gccagtagaa
5041 aattcataac gtgtatcttt aagaaaaatg agcatacatc ttaaactctt tcaattaagt
5101 ataaggggtt gttcgttgtt gtcatttgtt atagtgtctac tccactttag acaccatagc
5161 taaaataaaa tatgggtggg tttgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg
5221 tgttattttat acaaaactta aaatacttgc tgttttgatt aaaaagaaaa tagttttctta
5281 cttt

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(SEQ ID NO: 6) NP_004963 tyrosine-protein kinase JAK2 [Homo sapiens]

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1 mgmacitnte megtstssiy qngdisgnan smkqidpvlq vylyhslgks eadyltfpsg
61 eyvaeieicia askacgitpv yhnmfalmse teriwyppnh vfhidestrh nvlyrirfyf
121 prwycsgsnr ayrhgisrga eapllddfvm sylfaqwrhd fvhgwikvpv thetgeecldg
181 mavldmmria kendqtplai ynsisyktfl pkcirakiqd yhiltrkrir yrfrffiqqf
241 sqckatarnl klkylinlet lqsafytekf evkepgsgps geeifatiii tngnggiqwsr
301 gkhkesetlt egdlqlycdf pniidvsikq anqegsnesr vvtihkqdgk nleielsslr
361 ealsfvslid gyyrltadah hylckeavapp avleniqsnc hgpismdfai sklkkagnqt
421 glyvlrcspk dfnkyfltf a verenvieyk hclitknene eynlsgtkkn fsslkdlinc
481 yqmetvrdsn iifqftkccp pkpkdksnll vfrtngvsdv ptsptlqrp hmnmqmvfhki
541 nredlifnes lqggtftkif kgvrrevgdy gqihetevll kvldkahrny sesffeaasm
601 msklshkhlv lnygvvcvgd enilvqefvk fgsltdylkk nkncinilwk levakqlawa
661 mhfleentli hgnvcaknil lireedrktg nppfiklsdp gisitvlpkd ilgeripwvp
721 pecienpknl nlatdkwsfg ttlweicsgg dkplsaldsq rklqfyedrh qlpapkwael
781 anlinncmdy epdfrrsfra iirdlnslft pdyelltend mlpnmrigal gfsgafedrd
841 ptqfeerhik flqqlgkgnf gsvemcrydp lqdnrtgeva vkkqlhstee hlrdfereie
901 ilkslqhdni vkykgvcysa grniklime ylpyslrdy lqkhkeridh ikllqytsqi
961 ckgmeylgtk ryihrdlatr nilvenenrv kigdfltkv lpqdkeyykv kepgespiw
1021 yapesltesk fsvasdvwsf gvlyyelfty iekskspdae fmrnmigndkq ggmivfhlle
1081 llnkngrlpr pdgcpdeiy mtecwnnnv nqrpsfrdla lrvdqirdnm ag

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(SEQ ID NO: 7) NM_005373 Homo sapiens MPL proto-oncogene, thrombopoietin receptor (MPL), mRNA

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1 cctgaaggga ggatgggcta aggcaggcac acagtggcgg agaagatgcc ctctctgggc
61 ctcttcatgg tcacctcctg cctcctcctg gccctcaca acctggccca agtcagcagc
121 caagatgtct ccttgctggc atcagactca gagccctga agtgtttctc ccgaacattt
181 gaggacctca cttgcttctg ggatgaggaa gaggcagcgc ccagtgggac ataccagctg
241 ctgtatgcct acccgcgagg gaagcccgct gcttgcccc tgagttccca gagcatgccc
301 cacttttgaa cccgatacgt gtgcaagtct ccagaccagg aggaagtgcg tctcttcttt
361 ccgctgcacc tctgggtgaa gaatgtgttc ctaaaccaga ctcggaatca gcgagtcctc
421 tttgtggaca gtgtaggcct gccggctccc ccagtatca tcaaggccat ggggtgggagc
481 cagccagggg aacttcagat cagctgggag gagccagctc cagaaatcag tgatttctctg
541 aggtacgaac tccgctatgg cccagagat cccaagaact ccactggtcc caccgtcata
601 cagctgattg ccacagaaac ctgctgccct gctctgcaga ggccctcactc agcctctgct
661 ctggaccagt ctccatgtgc tcagcccaca atgccctggc aagatggacc aaagcagacc
721 tccccagta gagaagcttc agctctgaca gcagagggtg gaagctgcct catctcagga
781 tcccagcctg gcaactccta ctggctgcag ctgctgcagc aacctgatgc gatctccctc
841 ggtggctcct ggggatcctg gtccctccct gtgactgtgg acctgctgg agatgcagtg
901 gcacttggac tgcaatgctt taccttggac ctgaagaatg ttacctgtca atggcagcaa
961 caggaccatg ctagctccca aggtctcttc taccacagca gggcacggtg ctgccccaga
1021 gacaggtacc ccactctggg gaactgcgaa gaggaagaga aaacaaatcc aggactacag
1081 accccacagt tctctcgtct ccacttcaag tcacgaaatg acagcattat tcacatcctt
1141 gtggagggtga ccacagcccc gggtaactgt cacagctacc tgggctcccc tttctggatc

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1201 caccaggctg tgcgcctccc ccccccaaac ttgcaactgga gggagatctc cagtgggcat
1261 ctggaattgg agtggcagca cccatcgctc tgggcagccc aagagacctg ttatcaactc
1321 cgatacacag gagaaggcca tcaggactgg aagggtgctg agccgcctct cggggcccgga
1381 ggagggaccc tggagctgcg cccgcgatct cgtaccgtt tacagctgcg cggcaggctc
1441 aacggcccca cctaccaagg tccctggagc tctgtgctgg acccaactag ggtggagacc
1501 gccaccgaga ccgcctggat ctcttgggtg accgctctgc atctagtgtt gggcctcagc
1561 gccgtcctgg gcctgctgct gctgaggtgg cagtctcctg cacactacag gagactgagg
1621 catgccctgt ggcctcact tccagacctg caccgggtcc taggccagta ccttagggac
1681 actgcagccc tgagcccgcc caaggccaca gtctcagata cctgtgaaga agtggaaacc
1741 agcctccttg aaatcctccc caagtcctca gagaggactc ctttgccctt gtgttctctc
1801 caggcccgaga tggactaccg aagattgcag ccttcttgcc tggggaccat gccctgtctt
1861 gtgtgcccac ccatggctga gtcagggtcc tgcgtacca cccacattgc caaccattcc
1921 tacctaccac taagctattg gcagcagcct tgaggacagg ctctcactc ccagttccct
1981 ggacagagct aaactctcga gacttctctg tgaacttccc taccctaccc ccacaacaca
2041 agcacccccag acctcacctc catccccctc tgtctgccc cacaattagg cttcattgca
2101 ctgatctttac tctactgctg ctgacataaa accaggacct tttctccaca ggcaggctca
2161 tttcactaag ctctcctttt actttctctc tctctttga tgtcaaacgc cttgaaaaaca
2221 agcctccact tccccacact tcccatttac tcttgagact acttcaatta gttcccttac
2281 tacacttttg tagtgaaact gccaggcaa agtgacctc aaatcttcta attccaagat
2341 ccaataggat ctggttaatc atcagttcct ttgatctgc tgtaagattt gtcaaggctg
2401 actactcact tctcctttta attcttctct acctgggtcc tgcctctttg agtatattag
2461 taggtttttt ttatttggtt gagacagggt ctactctgt caccaggct gcagtgaat
2521 ggcgcgatct cagctcactg caacctccac ctccgggttc aagcgattct tgtgctcgg
2581 cctccctagt agctgggatt acagcgccac accaccacac acagctaat tttttttttt
2641 tttttttttt ttttttttag acggagcctt gctctgttgc cagactggag tgcagtggca
2701 cgatctcggc tcaactgcaac ctctgcctcc cgggttcaag ccattctgct tcagcctccc
2761 aagtagctgg gactacaggc gtctgcaacc atgcctaatt ttttctatt tttaggagag
2821 accggttttc accacgttgg ccaggatggt ctcgatatcc tgatctctg atccgcctgc
2881 ctctgcctcc caaagtgtg ggattacagg tgtgaccac tgcgcacagc ccagctaat
2941 tttcatattt ttagtagaga cagggttttg ccatgttgc caggctgttc ttgaactcct
3001 aacctcgggt gatccacca cttggcctc ccaaagtgtt aggtatcag gcattagcca
3061 ctgcgcccgg ctgagtgtac tagtagttaa gagaataaac tagatctga atcagagctg
3121 gattcaattc ctgtccttca catttactag ctgtgcaacc ttgggcacat aacttaatgt
3181 ctttgagcct tagttttttc atctgtaaaa cagggataat aacagacccc catagagttg
3241 tgacgaggat tgagataatc taagtaaagc acagtcctta ggacatagta aatgattcat
3301 atatccgaac tactgttata attattcctt cttactctcc tcttctagca tttcttccaa
3361 ttattacagt cttcaagat tccatttctt aacagctctc aatcccctt attctctgcc
3421 tttactatat gttgaccatt ccaaagttct tatctctagc tcagacacac actacagcac
3481 tgtgatgctt tatgcaacta actgtttaca tatctgtccc ctgctactag attgtgagct
3541 ccttgaggga aaggaacatg atttatttgt ccttttcccc cagcacctag agtagtgctt
3601 ggtgcatgat agtaggcctt caataaattt tttctaaatg aatga

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(SEQ ID NO: 8) NP_005364 thrombopoietin receptor precursor [Homo sapiens]

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1 mpswalfmvt sclllapqnl aqvssqdvsl lasdseplkc fsrtfedltc fwdeeeaaaps
61 gtyqllyayp rekpracpls sqsmphfgtr yvcqfpdqe vrlffplhlw vknvflnqtr
121 tqrvlfdsv glpappsiiik amggsqpgel qisweepape isdflryelr ygprdpknst
181 gptviqliat etccpalqrp hsasaldqsp cagptmpwqd gpkqtspsre asaltaeggs
241 clisglqp gn sywlqlrsep dgislgsgsw swslpvtvdl pgdavalglq cftldlknvt
301 cqwqqqdh as sqgffyhsra rccprdrypi wenceeeekt npglqtpqfs rchfksrnds
361 iihilvevtt apgtvhsylg spfwihqavr lptpnlh wre issghlelew qhpsswaaqe
421 tcyqlrytge ghqdwkvlep plgarpgtle lrprsrlyrlq lrarlngpty qgpwsswsdp
481 trvetateta wislvtahl vlglsavigl llirwqfpah yrirlhalwp slpdlhrvlg
541 qylrdaals ppkatvsdte eevepsllel lpkssertpl plcssaqamd yrirlqpsclg
601 tmlsvcpmm aesgscctth ianhsylpls ywqqp

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(SEQ ID NO: 9) NM_004343 Homo sapiens calreticulin (CALR), mRNA

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1 gccgcgtccg tccgtactgc agagccgctg ccggagggtc gttttaaagg gccgcgcgt
61 tgcgccccc tcggcccgcc atgctgctat ccgtgccgct gctgctcgcc ctctcggcc
121 tggccgctgc cgagcctgcc gtctacttca aggagcagtt tctggacgga gacgggtgga
181 cttcccgcgt gatcgaaatc aaacacaagt cagattttgg caaattcgtt ctcagttccg

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241 gcaagtctcta cgggtgacgag gagaagata aaggtttgca gacaagccag gatgcacgct
301 tttatgctct gtcggccagt ttcgagcctt tcagcaacaa aggccagacg ctgggtggtgc
361 agttcacggt gaaacatgag cagaacatcg actgtggggg cggctatgtg aagctgtttc
421 ctaatagttt ggaccagaca gacatgcacg gagactcaga atacaacatc atgttttggtc
481 ccgacatctg tggccctggc accaagaagg ttcattgcat cttcaactac aagggcaaga
541 acgtgctgat caacaaggac atccgttgca aggatgatga gtttacacac ctgtacacac
601 tgattgtgcg gccagacaac acctatgagg tgaagattga caacagccag gtggagtccg
661 gctccttgga agacgattgg gacttcctgc caccacagaa gataaaggat cctgatgctt
721 caaaaccgga agactgggat gagcgggcca agatcgatga tcccacagac tccaagcctg
781 aggactggga caagcccgag catatccctg acctgatgc taagaagccc gaggactggg
841 atgaagagat ggacggagag tgggaacccc cagtgattca gaacctgag tacaaggggtg
901 agtgggaagc ccggcagatc gacaacccag attacaaggg cacttggtac caccagaaa
961 ttgacaaccc cgagtattct cccgatccca gtatctatgc ctatgataac tttggcgtgc
1021 tgggcttgga cctctggcag gtcaagtctg gcacctctt tgacaacttc ctcatcacca
1081 acgatgaggg atacgctgag gaggttggca acgagacgtg gggcgtaaca aaggcagcag
1141 agaaacaaat gaaggacaaa caggacgagg agcagaggct taaggaggag gaagaagaca
1201 agaaacgcaa agaggaggag gaggcagagg acaaggagga tgatgaggac aaagatgagg
1261 atgaggagga tgaggaggac aaggagggaag atgaggagga agatgtcccc ggccaggcca
1321 aggacgagct gtagagaggg ctgctccag ggctggactg aggcctgagc gctcctgccg
1381 cagagctggc ccgcccacaa atgtctctg tgagactcga gaactttcat tttttccag
1441 gctgggtcgg atttgggggt gattttgggt ttgttccct cctccactct ccccccccc
1501 cccccccccc tttttttttt ttttttttaa actggtattt tatctttgat tctccttcag
1561 cctccacccc tggttctcat ctttcttgat caacatcttt tcttgctctt gtccccctt
1621 ctcatctctt agctccctc caacctgggg gccagtgggt tggagaagcc acaggcctga
1681 gatttcatct gctctccttc ctggagccca gaggagggca gcagaagggg gtgggtgtctc
1741 caacccccca gcactgagga agaacggggc tcttctcatt tcacccctcc ctttctcccc
1801 tgcccccagg actggggcac ttctgggtgg gccagtgggt ccagattgg ctccactga
1861 gaatgtaaga actacaaaca aaatttctat taaattaaat tttgtgtctc caaaaaaaaa
1921 aaaaaaaaaa

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(SEQ ID NO: 10) NP_004334calreticulin precursor [Homo sapiens]

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1 mllsvplllg llglavaepa vyfkeqfldg dgwtswies khksdfgkfv lssgkfygde
61 ekdkglqtsq darfyalsas fepfsnkqgt lvvqftvkhe qnidcgggyv klfpnsldqt
121 dmhgdseynt mfgpdicgpg tkkvhvfifny kgknvlinkd irckddefth lytlivrpdn
181 tyevkidnsq vesgsleddw dflppkkikd pdaskpedwd erakiddptd skpedwdkpe
241 hipdpdakpp edwdeemdge weppviqnpe ykgewkprqi dnpdykgtwi hpeidnpeys
301 pdpsiyaydn fgvlglldlw vksgtifdnf litndeayae efgnetwgtv kaaekqmkdk
361 qdeeqrlkee eedkkrkeee eaedkedded kdedeedeed keedeedevp qqakdel

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(SEQ ID NO: 11) NM_001164603 Homo sapiens additional sex combs like transcriptional regulator 1(ASXL1), transcript variant 2, mRNA

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1 cacacccacg gcagacacgc acgcacccgg gcgcgaagg gaaagccgag tctcgccctc
61 ccgccccgccc gtcggctctg tctcagtcct tcagcagagc gggaaagcgg aggccggagc
121 cgtgacctct gaccccggtg tlatgaggag ccgcccatt ccttagcgat cgcggggcag
181 ccgcgcgtgc cgcgctgggc gactgacgca gcgcgggcgc gtggagccgc cgcgcgccct
241 cccccacgac cgctctcgcg ccagccggtc cccgctgccc ccccccctct cccggccgag
301 accccagagc tcgcgcgcgc ccgctggccc gcgcgccccc caccgctgac cccgccccag
361 cccgcgcgca ccgccccagc ccgcccagcc cggaggctcc gcgtggagct gcgcgcgcgc
421 ccggggagaa ggatgaagga caaacagaag aagaagaagg agcgcacgtg ggccgaggcc
481 gcgcgccttg tattagaaaa ctactcggat gctccaatga caccacaaac gattctgcag
541 gtcataagag cagaaggact aaaggaaatg agaagtggga cttccctctc cgcattgctc
601 aatgctatgc tacattccaa ttcaagagga ggagaggggt tgttttataa actgcctggc
661 cgaatcagcc ttttcacgct caaggtgtga gccactgcac caggccccct catcttaatt
721 ttaatatatc tttgaataaa caccattgta tgaacctgct gtaagcttgg gagtggctctg
781 ttagtctaca gcttgtgtct gagatgtgct aattgaatat ttgctcagta cctcatctta
841 actgcctttg gctttatgtt gcttatcctt catagtatct tgttcatttg ccttttacat
901 ccatagggcat cacttctctg atattcgttg tgctctttta atggattaat ggtttgcttg
961 gttgggttct ctagttagac tgtaaacctc ttgagagcag agtctgtatt ttattaatta
1021 cccacagtac taggtacata gttgccttca ataaatatat atttaatgaa aaaaaaaaaa
1081 aaaa

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(SEQ ID NO: 12) NP_001158075 putative Polycomb group protein ASXL1 isoform 2 [Homo sapiens]

1 mkdkqkkkrtwaearlv lenysdapmt pkqilqvlea egkemrsgt splaclnaml
61 hnsnrggegl fyklpgrisl ftlkv

(SEQ ID NO: 13) NM_001203247 Homo sapiens enhancer of zeste homolog 2 (Drosophila) (EZH2), transcript variant 3, mRNA

1 ggcgggcgctt gattgggctg gggggggccaa ataaaagcga tggcgattgg gctgcccgcgt
61 ttggcgctcg gtccgggtcg gtccgacacc cgggtggact cagaaggcag tggagccccg
121 gcgcgggcgg cgccggcgcg cgggggcgac gcgcgggaac aacgcgagtc ggcgcgcggg
181 acgaagaata atcatggggc agactgggaa gaaatctgag aaggggaccag tttgttggcg
241 gaagcgtgta aaatcagagt acatgcgact gagacagctc aagaggttca gacgagctga
301 tgaagtaaaag agtatgttta gttccaatcg tcagaaaatt ttggaaaagaa cggaaaatctt
361 aaaccaagaa tggaaacagc gaaggatata gcctgtgcac atcctgactt ctgtgagctc
421 attgcgcggg actagggagt gttcgggtgac cagtgcactt gattttccaa cacaagtcac
481 cccattaaag actctgaatg cagtgtcttc agtaccata atgtattctt ggtctccctt
541 acagcagaat tttatgggtg aagatgaaac tgttttacct aacattctt atattggaga
601 tgaagtttta gatcaggatg gtactttcat tgaagaacta ataaaaaatt atgatgggaa
661 agtacacggg gatagagaat gtgggtttat aaatgatgaa atttttgttg agttgggtgaa
721 tgcccttggg caatataatg atgatgacga tgatgatgat ggagacgac ctgaagaaag
781 agaagaaaag cagaaagatc tggaggatca ccgagatgat aaagaaagcc gccacacctg
841 gaaatcttct tctgataaaa tttttgaagc catttctcca atgtttccag ataagggcac
901 agcagaagaa ctaaaggaaa aatataaaga actcacgaa cagcagctcc caggcgact
961 tctctctgaa tgtaccccca acatagatgg accaaatgct aaatctgttc agagagagca
1021 aagctttcac tcttttcata cgcttttctg taggcgatgt tttaaatag actgcttctt
1081 acatcttttt catgcaacac ccaacactta taagcggaag aacacagaaa cagctctaga
1141 caacaaacct tgtggaccac agtgttacca gcatttggag ggagcaaagg agtttgcctg
1201 tgcctctcacc gctgagcgga taaagacccc accaaaacgt ccaggaggcc gcagaagagg
1261 acggttctcc aataacagta gcaggccacg cccccccacc attaatgtgc tggaaatcaa
1321 ggatacagac agtgataggg aagcaggggc tgaacgggg ggagagaaca atgataaaga
1381 agaagaagag aagaaagatg aaacttcgag ctctctgaa gcaaatcttc ggtgtcaaac
1441 accaataaag atgaagccaa atattgaacc tcttgagaat gtggagtgga gtgtgtctga
1501 agcctcaatg tttagagtcc tcattggcac ttactatgac aatttctgtg ccattgtctg
1561 gtttaattggg accaaaacat gtagacaggt gtatgagttt agagtcaaag aatctagcat
1621 catagctcca gctcccgctg aggatgtgga tactctcca aggaaaaaga agaggaaaaca
1681 ccggttgttg gctgcacact gcagaaagat acagctgaaa aaggacggct cctctaacca
1741 tgtttacaac tatcaaccct gtgatcatcc acggcagcct tgtgacagtt cgtgccctt
1801 tgtgatagca caaaattttt gtgaaaagtt ttgtcaatgt agttcaggt gtcaaaaccg
1861 ctttccggga tgcgctgca aagcacagtg caacaccaag cagtgcggct gctacctggc
1921 tgtccgagag tgtgacctg acctctgtct tacttgtgga gccgctgacc attgggacag
1981 taaaaatgtg tcctgcaaga actgcagtat tcagcggggc tccaaaaagc atctattgct
2041 ggcaccatct gacgtggcag gctgggggat ttttatcaaa gatcctgtgc agaaaaatga
2101 attcatctca gaatactgtg gagagattat ttctcaagat gaagctgaca gaagagggaa
2161 agtgtatgat aaatacatgt gcagctttct gttcaacttg aacaatgatt ttgtgggtga
2221 tgcaaacccg aagggttaaca aaattcgttt tgcaaatcat tcggtaaatc caaactgcta
2281 tgcaaaaagtt atgatggtta acggtgatca caggataggt atttttgcca agagagccat
2341 ccagactggc gaagagctgt tttttgatta cagatacagc caggctgatg cctgaagta
2401 tgtcggcatc gaaagagaaa tggaaatccc ttgacatctg ctacctctc cccctctctc
2461 tgaaacagct gccttagctt caggaaacct gagtactgtg ggcaatttag aaaaagaaca
2521 tgcagtttga aattctgaat ttgcaaagta ctgtaagaat aattttatagt aatgagttta
2581 aaaatcaact ttttattgcc ttctcaccag ctgcaaagtg tttgtacca gtgaattttt
2641 gcaataatgc agtatggtac atttttcaac tttgaataaa gaatacttga acttgtcctt
2701 gttgaatc

(SEQ ID NO: 14) NP_001190176 histone-lysine N-methyltransferase EZH2 isoform c [Homo sapiens]

1 mgqtgksek gpvcwrkrvk seymrlrlqk rfradevks mfssnrqkil erteilnqew

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61 kqrriqpvhi ltsvsslrgrt recsvtsdld fptqviplkt lnavasvpim yswsplqqnf
121 mvedetvlhn ipymgdevld qdgtfieeli knydgkvhgd recgfindei fvelvnlqg
181 ynddddddgd ddpeereekq kdledhrddk esrpprkfps dkifeaissm fpdkgtaeel
241 kekykelteq qlpgalppcc tpnidgpnak svqreqslhs fhtlfcrrcf kydcflhpfh
301 atpntykrkn tetaldnkpc gpqcyqhleg akefaaalta eriktpkrp grrrrgrlpn
361 nssrpstpti nvleskdtts dreagtetgg enndkeeeek kdetsssseas nsrcttpikm
421 kpnieppenv ewsgaeasmf rvligttydn fcaiarligt ktrqvyeifr vkessiiapa
481 paedvdtppr kkrkrhrlwa ahcrkiqlkk dgssnhvyny qpcdhprqpc dsscpvciaq
541 nfcekfcqcs secqnrfgpc rckagcntkq cpcylavrec dpdlcltcca adhwdsknvs
601 cknsciqrgs kkhlllpsd vagwgifikd pvqknefise ycgeiisgde adrrgkvydk
661 ymcsflfnln ndfvvdatrk gnkirfanhs vnpncyakvm mvngdhrigi fakraigtge
721 elffdyrysq adalkyvgie remeip

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(SEQ ID NO: 15) NM_001195427 Homo sapiens serine/arginine-rich splicing factor 2 (SRSF2), transcript variant 2, mRNA

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1 agaaggtttc atttccgggt ggcgcggggc ccattttgtg aggagcgata taaacggggc
61 cagaggcccg ctgcccgccc agttgttact caggtgcgct agcctgcgga gcccgctccgt
121 gctgtttctgc ggcaaggcct ttcccagtggt cccacgcggg aaggcaactg cctgagaggc
181 gcggcgctgc accgcccaga gctgaggaag ccggcgccag ttccgggggc tccggggccgc
241 cactcagagc tatgagctac ggcgcggccc ctcccgatgt ggagggtatg accctccca
301 aggtggacaa cctgacgtac cgcacgtcgc ccgacacgct gaggcgctgc ttcgagaagt
361 acggggcgct cggcgacgtg tacatccgcg gggaccgcta caccaaggag tccgcgggt
421 tcgccttcgt tcgctttcac gacaagcgcg acgctgagga cgctatggat gccatggacg
481 gggccgtgct ggacggcgcg gagctgcggg tgcaaatggc gcgctacggc cggcccccg
541 actcacacca cagccgcggg ggaccgccac ccgcgaggta cggggcgcggt ggctacggac
601 gccggagccg cagccctagg cggcgctgcc gcagccgata ccggagtcgg agccgttcca
661 ggtctcgcag ccgatctcgc tacagccgct cgaagtctcg gtcccgcat cgttctcgat
721 ctcggtcgac ctccaagtcg agatccgcac gaaggtccaa gtccaagtcg tcgtcggtct
781 ccagatctcg ttccgggtcc aggtcccggt ctcggtccag gagtctctcc ccagtgtcca
841 agagggaatc caaatccagg tcgcgatcga agagtccccc caagtctcct gaagaggaag
901 gagcgggtgt ctcttaagaa aatgatgtat cggcaagcag tgtaaacgga ggacttgggg
961 aaaaaggacc acatagtcca tcgaagaaga gtccctggaa caagcaactg gctattgaaa
1021 aggttatttt gtaacatttg tctaactttt tacttgttta agctttgcct cagttggcaa
1081 acttcatttt atgtgccatt ttgtgtctgt tattcaaat tcttgtaatt tagtgaggtg
1141 aacgacttca gatttcttta ttggtatttg atatttgagg taaaatttca tttgttata
1201 tagtgctgac tttttttgtt tgaaattaaa cagattggta acctaatgtg tggcctcctg
1261 acttttaagg aaaacgtgtg cagccattac acacagccta aagctgtcaa gagattgact
1321 cggcattgcc ttcatctcct aaaattaaaa acctacaaa gttgggtgtaa atttgtatat
1381 gttattttacc ttcatgtcta aatggtaatc tgaaccaaa tttgtataaa gacttttcag
1441 gtgaaaagac ttgatttttt gaaaggattg tttatcaaac acaattctaa tctcttctct
1501 tatgtatttt tgtgcactag gcgcagttgt gtagcagttg agtaatgctg gttagctgtt
1561 aaggtggcgt gttgcagtg cagtgctttg gctgttctcc gtttctcctc gttgtctcct
1621 gtgtaaagat gccttgctgt gcagaaacaa atggctgtcc agtttattaa aatgcctgac
1681 aactgcactt ccagtcaccc gggccttgca tataaataac ggagcataca gtgagcacat
1741 ctactctgat ataaatacac ctttttttcc ctcttcccc taaaaatggt aaatctgatc
1801 atatctacat gtatgaactt aacatggaaa atgttaagga agcaaatggt tgtaactttg
1861 taagtactta taacatggtg tatctttttg cttatgaata ttctgtatta taaccattgt
1921 ttctgtagtt taattaaaaa attttcttg tgttagcttt tctcagaaaa aaaaaaaaaa
1981 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

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(SEQ ID NO: 16) NP_001182356 serine/arginine-rich splicing factor 2 [Homo sapiens]

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1 msygrpppdv egmtslkvdn ltyrtspdtl rrvfkygrv gdvyipdry tkesrgfafv
61 rfhdkrdaed amdandgavl dgrelrvqma rygrppdshh srrgpppry ggggyrrsr
121 sprrrrrsrs rrrrrrrsrs rrrrrrrsrs srtrrrrst skrrsarrsk sksssvrrsr
181 rrrrrrrsrs spppvskres krrrrsksp kspeeravgs s

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(SEQ ID NO: 17) NM_005896 Homo sapiens isocitrate dehydrogenase 1 (NADP+), soluble (IDH1), transcript variant 1, mRNA

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1 gggctgagga ggcggggcct gggaggggac aaagccggga agaggaaaag ctccgaccta

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61 cccgtgtggtc ccgggtttct gcagagtcta cttcagaagc ggaggcactg ggagtcgggt
121 ttgggattgc caggctgtgg ttgtgagtc gagcttgtga ggggctgtgg cggcccaact
181 cttgccagc ataatcatccc ggcaggcgat aaactacatt cagttgagtc tgcaagactg
241 ggaggaaactg ggggtgataag aaatctattc actgtcaagg tttattgaag tcaaaatgtc
301 caaaaaaatc agtggcggtt ctgtggtaga gatgcaagg gatgaaatga cacgaatcat
361 ttgggaattg attaaagaga aactcatttt tccctacgtg gaattggatc tacatagcta
421 tgatttaggc atagagaatc gtgatgccac caacgaccaa gtcaccaagg atgctgcaga
481 agctataaag aagcataatg ttggcgtaaa atgtgccact atcactcctg atgagaagag
541 gggttgaggag ttcaagttga aacaaatgtg gaaatcacca aatggacca tacgaaatat
601 tctgggtggc acggtcttca gagaagccat tatctgcaaa aatatcccc ggcttgtgag
661 tggatgggta aaacctatca tcataggctg tcatgcttat ggggatcaat acagagcaac
721 tgattttgtt gtccctgggc ctggaaaagt agagataacc tacacaccaa gtgacggaa
781 ccaaaagggtg acataacotg tacataactt tgaagaagg ggtggtgtg ccatggggat
841 gtataatcaa gataagtcaa ttgaagattt tgcacacagt tccctccaaa tggctctgtc
901 taagggttgg cctttgtatc tgagcaccaa aaacactatt ctgaagaaat atgatggcg
961 ttttaaaagc atctttcagg agatatatga caagcagta aagtcacagt ttgaagctca
1021 aaagatctgg tatgagcata ggctcatcga cgacatgggt gcccaatcga tgaatcaga
1081 gggagggttc atctgggctt gtaaaaaacta tgatggtgac gtgcagtcgg actctgtggc
1141 ccaagggtat ggctctctcg gcatgatgac cagcgtgctg gtttgcacg atggcaagac
1201 agtagaagca gaggctgccc acgggactgt aaccctcac taccgcatgt accagaaagg
1261 acaggagacg tccaccaatc ccattgcttc catttttggc tggaccagag ggttagccca
1321 cagagcaaaag cttgataaca ataaagagct tgccttcttt gcaaagtctt tggagaagt
1381 ctctattgag acaattgagg ctggcttcat gaccaaggac ttggctgctt gcattaaagg
1441 tttaccaaat gtgcaacggt ctgactactt gaatacattt gagttcatgg ataaacttgg
1501 agaaaacttg aagatcaaac tagctcaggc caaactttaa gttcatacct gagctaagaa
1561 ggataattgt cttttggtta ctaggctctac aggtttacat tttctgtgt tactactcaag
1621 gataaaggca aaatcaattt tgtaatttgt ttgaagcca gagtttatct tttctataag
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1801 gtatttactg tcaactttttc tcatgttcta attataaatg accaaaatca agattgtca
1861 aaagggtaaa tgatagccac agtattgctc cctaaaatat gcataaagta gaaattcact
1921 gccttccctt cctgtccatg accttgggca cagggaagt ctggtgcat agatatccg
1981 ttttgtgagg tagagctgtg cattaaactt gcacatgact ggaacgaagt atgagtcaa
2041 ctcaaatgtg ttgaagatac tgcagtcatt tttgtaaaga ccttgctgaa tgtttccaat
2101 agactaaata ctgttttagc cgcaggagag tttggaatcc ggaataaata ctacctggag
2161 gtttgtcctc tccatttttc tctttctcct cctggcctgg cctgaatatt atactactct
2221 aaatagcata tttcatccaa gtgcaataat gtaagctgaa tcttttttgg acttctgtc
2281 gctgttttta tttcttttat ataaatgtga tttctcagaa attgatatta aacactatct
2341 tatcttctcc tgaactgttg attttaatta aaattaagt ctaattacca ttaaaaaaaa
2401 aa

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(SEQ ID NO: 18) NP_005887 isocitrate dehydrogenase [NADP] cytoplasmic [Homo sapiens]

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1 mskkisggs vcmqgdemtr iivelielkl ifpyveldlh sydlgienrd atndqvtkda
61 aeaikkhvng vkcatitpde krveefklkq mwkspngrtir nilggtvfre aiickniprl
121 vsqgwvkpiii grhaygdqyr atdfvvpqpg kveitytpsd gtqkvtylvh nfeeggvgam
181 gmyndqksie dfahssfqma lskgwplyls tkntilkkyd grfkdfiqei ydkqyksqfe
241 aqkiwyehrl iddmvaqamk seggfiwack nydgdvqsds vaqgygslgm mtsvlvcpdg
301 ktveaeaaahg tvtrrhymyq kgqetstnpi asifawtrgl ahrakldnnk elaffanale
361 evsietieag fmktdlaaci kglpnvqrsd ylnthfemdk lgenlkikla qakl

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(SEQ ID NO: 19) NM_001289910 Homo sapiens isocitrate dehydrogenase 2 (NADP+), mitochondrial(IDH2), transcript variant 2, mRNA

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1 attttgcaac gccataggct tccagcgact gctggtgatg tttctgatgc cgacaaaagg
61 atcaagggtg cgaagcccggt ggtggagatg gatggtgatg agatgaccog tattatctgg
121 cagttcatca aggagaagct catcctgccc cactgtgaca tccagctaaa gtattttgac
181 ctccgggctcc caaacccgtga ccagactgat gaccagggtca ccattgactc tgcactggcc
241 acccagaagt acagtgtggc tgtcaagtgat gccaccatca cccctgatga ggcccggtgtg
301 gaagagttca agctgaagaa gatgtggaaa agtcccaatg gaactatcog gaacatcctg
361 ggggggactg tcttcgggga gccatcatc tgcaaaaaca tcccacgcct agtccctggc
421 tggaccaagc ccatcaccat tggcaggcac gcccatggcg accagtacaa ggccacagac

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481 tttgtggcag accggggccgg cactttcaaa atggtcttca ccccaaaaga tggcagtggt
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601 accgacgagt ccatctcagg ttttgcgcac agctgcttcc agtatgcat ccagaagaaa
661 tggccgctgt acatgagcac caagaacacc atactgaaag cctacgatgg gcgtttcaag
721 gacatcttcc aggagatctt tgacaagcac tataagaccg acttcgacaa gaataagatc
781 tgggtatgag accggctcat tgatgacatg gtggctcagg tcctcaagtc ttccgggtggc
841 tttgtgtggg cctgcaagaa ctatgacgga gatgtgcagt cagacatcct ggcccagggc
901 tttggctccc ttggcctgat gacgtccgtc ctggtctgcc ctgatgggaa gacgattgag
961 gctgagggcg ctcatgggac cgtcaccgcg cactatcggg agcaccagaa gggccggccc
1021 accagcacca accccatcgc cagcatcttt gectggacac gtggcctgga gcaccggggg
1081 aagctggatg ggaaccaaga cctcatcagg tttgccaga tgctggagaa ggtgtgcgtg
1141 gagacggtgg agagtggagc catgaccaag gacctggcgg gctgcattca cggcctcagc
1201 aatgtgaagc tgaacgagca ctctctgaac accacggact tcctcgacac catcaagagc
1261 aacctggaca gagccctggg caggcagtag ggggagggcg caccatggc tgcagtggag
1321 gggccagggc tgagccggcg ggtcctcctg agcgcggcag agggtagacc tcacagcccc
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1441 tgtgtttccc ctcatggtga cgtgaggcag gagcagtgcg ttttacctca gccagtcagt
1501 atgttttgca tactgtaatt tatattgccc ttggaaacaca tgggtgccata tttagctact
1561 aaaaagctct tcacaaaa

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(SEQ ID NO: 20) NP_001276839 isocitrate dehydrogenase [NADP], mitochondrial isoform
2 [Homo sapiens]

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1 mdgdemtrii wqfikeklil phvdiqlkyf dlglpnrdqt ddqvttidsal atqkysvavk
61 catitpdear veefklkkmw kspngtirni lggtvfrepj ickniprlvp gwtkpitigr
121 hahgdqykat dfvadragtf kmvftpkdgs gvkewevynf paggvqmgmy ntdeisigfa
181 hscfqyiaiq kwplymstkn tilkaydgrf kdifgeifdk hyktdfdknk iwyehrlidd
241 mvaqvlkssg gfvwacknyd gdvqsdilaq gfgslglmts vlvcpdgkti eaeaahgtvt
301 rhyrehqkgr ptstnpiasi fawtrglehr gkldgnqdli rfaqmlekvc vetvesgamt
361 kdlagcihgl snvklnehfl nttdfldtik snldralgrq

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We claim:

1. A method for treating a myeloproliferative disorder, comprising:
(i) determining whether the cells of a subject having a myeloproliferative disorder
comprise a mutation associated with the myeloproliferative disorder in one or more
5 genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*; and
if the subject comprises said mutant allele
(ii) administering an effective amount of a serum amyloid P (SAP) protein to the
subject.
2. The method of claim 1, wherein the myeloproliferative disorder comprises
10 myelofibrosis.
3. The method of claim 2, wherein the myelofibrosis comprises primary myelofibrosis,
post-polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis.
4. The method of claim 1, wherein the myeloproliferative disorder comprises
polycythemia vera, essential thrombocytosis, or chronic myelogenous leukemia (CML).
- 15 5. The method of any one of claims 1 to 4, wherein the method comprises determining
whether the subject comprises a mutation at codon 617 of *JAK2*.
6. The method of claim 5, wherein the mutation results in a V617F amino acid
substitution in *JAK2*.
7. The method of any one of claims 1 to 4, wherein the method comprises determining
20 whether the subject comprises a mutation in exon 12 or exon 14 of *JAK2*.
8. The method of any one of claims 1 to 7, wherein the method comprises determining
whether the subject comprises a mutation at codon 515 of *MPL*.
9. The method of claim 8, wherein the mutation results in a W515L, W515K, W515A,
or W515R amino acid substitution in *MPL*.
- 25 10. The method of any one of claims 1 to 7, wherein the method comprises determining
whether the subject comprises a mutation in exon 10 of *MPL*.

11. The method of any one of claims 1 to 10, wherein the method comprises determining whether the subject comprises a mutation in exon 9 of *CALR*.
12. The method of any one of claims 1 to 11, wherein the method comprises determining whether the subject comprises a mutation in exon 12 of *ASXL1*.
- 5 13. The method of any one of claims 1 to 12, wherein the method comprises determining whether the subject comprises a mutation in exon 4 of *IDH1*.
14. The method of any one of claims 1 to 12, wherein the method comprises determining whether the subject comprises a mutation at codon 132 of *IDH1*.
15. The method of any one of claims 1 to 14, wherein the method comprises determining
10 whether the subject comprises a mutation in exon 4 of *IDH2*.
16. The method of any one of claims 1 to 14, wherein the method comprises determining whether the subject comprises a mutation at codon 140 of *IDH2*.
17. The method of any one of claims 1 to 16, wherein the method comprises determining whether the subject comprises a mutation at codon 172 of *IDH2*.
- 15 18. The method of any one of claims 1 to 17, wherein the mutation associated with the myeloproliferative disorder in one or more genes is a deletion, insertion, point mutation, or translocation.
19. The method of claim 18, wherein said mutation results in the absence of expression of the one or more proteins encoded by the one or more genes or in the expression of a truncated
20 protein.
20. The method of any one of claims 1 to 19, wherein the mutation associated with the myeloproliferative disorder in one or more genes is present on one or both alleles of the one or more genes.
21. A method for treating a myeloproliferative disorder with a serum amyloid P (SAP)
25 protein, the method comprising:
- (i) measuring a first mutant allele burden of a mutation in one or more genes associated with the myeloproliferative disorder selected from: *JAK2*, *MPL*, *CALR*,

ASXL1, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*, wherein said first mutant allele burden is measured before administration of the SAP protein;

(ii) measuring a second mutant allele burden of the same mutation measured in (i), wherein said second mutant allele burden is measured after administration of the SAP protein; and

(iii) identifying a difference between the second mutant allele burden and the first mutant allele burden,

wherein a decrease in the second mutant allele burden relative to the first mutant allele burden indicates that the administration of the SAP protein is effective in treating the myeloproliferative disorder.

22. The method of claim 21, wherein the decrease in the second mutant allele burden relative to the first mutant allele burden is 10% to 90% or the difference between the second mutant allele burden and the first mutant allele burden is a reduction by 10% to 90%.

23. The method of claim 21, wherein the decrease in the second mutant allele burden relative to the first mutant allele burden is 25%-50% or the difference between the second mutant allele burden and the first mutant allele burden is a reduction by 25% to 50%.

24. The method of claim 21, wherein the decrease in the second mutant allele burden relative to the first mutant allele burden is at least 50% or the difference between the second mutant allele burden and the first mutant allele burden is a reduction by at least 50%.

25. The method of claim 21, wherein the difference is a complete molecular response.

26. The method of any one of claims 21 to 25, wherein the method comprises obtaining a first biological sample and measuring the first mutant allele burden in the first biological sample and obtaining a second biological sample and measuring the second mutant allele burden in the second biological sample.

27. The method of claim 26, wherein the first and second biological samples comprise blood samples and/or bone marrow samples.

28. The method of any one of claims 21 to 27, wherein the measuring step comprises amplifying nucleic acid comprising all or a portion of the one or more genes associated with the myeloproliferative disorder.
29. The method of any one of claims 21 to 28, wherein the measuring step comprises
5 determining the proportion of mutant nucleic acid to wildtype nucleic acid of the one or more genes associated with the myeloproliferative disorder.
30. The method of any one of claims 21 to 29, further comprising increasing, decreasing, or maintaining the dosage regimen of the SAP protein based on the effectiveness of the treatment.
- 10 31. The method of any one of claims 21 to 30, wherein at least one of the mutations in one or more genes is a mutation at codon 617 of JAK2.
32. The method of claim 31, wherein the mutation results in a V617F amino acid substitution in JAK2.
33. The method of any one of claims 21 to 30, wherein at least one of the mutations in
15 one or more genes is in exon 12 or exon 14 of *JAK2*.
34. The method of any one of claims 21 to 33, wherein at least one of the mutations in one or more genes is at codon 515 of *MPL*.
35. The method of claim 34, wherein the mutation results in a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*.
- 20 36. The method of any one of claims 21 to 35, wherein at least one of the mutations in one or more genes is in exon 10 of *MPL*.
37. The method of any one of claims 21 to 36, wherein at least one of the mutations one or more genes is in exon 9 of *CALR*.
38. The method of any one of claims 21 to 37, wherein at least one of the mutations in
25 one or more genes is in exon 12 of *ASXL1*.
39. The method of any one of claims 21 to 38, wherein at least one of the mutations in one or more genes is in exon 4 of *IDH1*.

40. The method of any one of claims 21 to 38, wherein at least one of the mutations in one or more genes is at codon 132 of *IDH1*.
41. The method of any one of claims 21 to 40, wherein at least one of the mutations in one or more genes is in exon 4 of *IDH2*.
- 5 42. The method of any one of claims 21 to 40, wherein at least one of the mutations in one or more genes is at codon 140 of *IDH2*.
43. The method of any one of claims 21 to 42, wherein at least one of the mutations in one or more genes is at codon 172 of *IDH2*.
44. The method of any one of claims 21 to 43, wherein the mutation in one or more genes
10 is a deletion, insertion, point mutation, or translocation.
45. The method of claim 44, wherein said mutation results in the absence of expression of the one or more proteins encoded by the one or more genes or in the expression of a truncated protein.
46. The method of any one of claims 21 to 45, wherein the mutation in one or more genes
15 is present on one or both alleles of the one or more genes.
47. A method of treating a myeloproliferative disorder, the method comprising administering to a subject in need thereof an effective amount of a serum amyloid P (SAP) protein, wherein some of the subject's cells comprise a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*,
20 *EZH2*, *SRSF2*, *IDH1*, or *IDH2*.
48. A method of treating a myeloproliferative disorder, comprising administering to a subject in need thereof an effective amount of a serum amyloid P (SAP) protein, wherein some of the subject's cells comprise a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*,
25 *IDH1*, or *IDH2*, and wherein the SAP protein is administered according to a dosage regimen effective to reduce mutant allele burden of said gene in said subject.
49. The method of claim 47 or 48, wherein the myeloproliferative disorder comprises myelofibrosis.

50. The method of claim 49, wherein the myelofibrosis comprises primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis.
51. The method of claim 47 or 48, wherein the myeloproliferative disorder comprises polycythemia vera, essential thrombocytosis, or chronic myelogenous leukemia (CML).
- 5 52. The method of any one of claims 47 to 51, wherein the subject comprises a mutation at codon 617 of *JAK2*.
53. The method of claim 52, wherein the mutation results in a V617F amino acid substitution in *JAK2*.
54. The method of any one of claims 47 to 51, wherein the subject comprises a mutation
10 in exon 12 or exon 14 of *JAK2*.
55. The method of any one of claims 47 to 54, wherein the subject comprises a mutation at codon 515 of *MPL*.
56. The method of claim 55, wherein the mutation results in a W515L, W515K, W515A, or W515R amino acid substitution in *MPL*.
- 15 57. The method of any one of claims 47 to 54, wherein the subject comprises a mutation in exon 10 of *MPL*.
58. The method of any one of claims 47 to 57, wherein the subject comprises a mutation in exon 9 of *CALR*.
59. The method of any one of claims 47 to 58, wherein the subject comprises a mutation
20 in exon 12 of *ASXL1*.
60. The method of any one of claims 47 to 59, wherein the subject comprises a mutation in exon 4 of *IDH1*.
61. The method of any one of claims 47 to 59, wherein the subject comprises a mutation at codon 132 of *IDH1*.
- 25 62. The method of any one of claims 47 to 61, wherein the subject comprises a mutation in exon 4 of *IDH2*.

63. The method of any one of claims 47 to 61, wherein the subject comprises a mutation at codon 140 of IDH2.
64. The method of any one of claims 47 to 63, wherein the subject comprises a mutation at codon 172 of IDH2.
- 5 65. The method of any one of claims 47 to 64, wherein the mutation associated with the myeloproliferative disorder in one or more genes is a deletion, insertion, point mutation, or translocation.
66. The method of claim 65, wherein said mutation results in the absence of expression of the one or more proteins encoded by the one or more genes or in the expression of a truncated
10 protein.
67. The method of any one of claims 47 to 66, wherein the mutation associated with the myeloproliferative disorder in one or more genes is present on one or both alleles of the one or more genes.
68. A method for reducing mutant allele burden in a subject having a myeloproliferative
15 disorder, the method comprising administering to a subject in need thereof an effective amount of a serum amyloid P (SAP) protein, wherein the subject comprises a mutation associated with the myeloproliferative disorder in one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, or *IDH2*.
69. The method of claim 68, wherein the myeloproliferative disorder comprises
20 myelofibrosis.
70. The method of claim 69, wherein the myelofibrosis comprises primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis
71. The method of any one of claims 1 to 70, wherein the SAP protein comprises an SAP polypeptide comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1.
- 25 72. The method of any one of claims 1 to 71, wherein the SAP protein comprises a glycosylated recombinant human SAP polypeptide comprising an N-linked oligosaccharide chain, wherein at least one branch of the oligosaccharide chain terminates with a α 2,3-linked sialic acid moiety.

73. The method of claim 72, wherein all sialylated branches of the oligosaccharide chain terminate with α 2,3-linked sialic acid moieties.
74. The method of claim 72, wherein the oligosaccharide chain is substantially free of α 2,6-linked sialic acid moieties.
- 5 75. The method of any one of claims 71 to 74, wherein the polypeptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 1.
76. The method of any one of claims 71 to 74, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 1.
77. The method of any one of claims 71 to 76, wherein the polypeptide is a fusion protein
10 comprising an SAP domain and one or more heterologous domains.
78. The method of claim 77, wherein the one or more heterologous domains enhance one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification.
79. The method of any one of claims 71 to 77, wherein the polypeptide comprises one or
15 more modified amino acid residues.
80. The method of claim 79, wherein the one or more modified amino acid residues comprise a PEGylated amino acid, a prenylated amino acid, an acetylated amino acid, a biotinylated amino acid, and/or an amino acid conjugated to an organic derivatizing agent.
81. The method of any one of claims 1 to 80, wherein the SAP protein is administered by
20 a mode selected from: by injection, by intravenous injection or infusion, by subcutaneous injection, by continuous release by depot or pump, or a combination thereof.
82. The method of any one of claims 1 to 81, wherein the method further comprises administering to the patient an additional anti-cancer therapeutic.
83. The method of claim 82, wherein the additional anti-cancer therapeutic is selected
25 from: chemotherapy agents, antibody-based agents, kinase inhibitors (e.g. ruxolitinib), immunomodulatory agents, biologic agents, or combinations thereof.

84. The method of claim 83, wherein the chemotherapy agent is selected from: actinomycin D, aldesleukin, alitretinoin, all-trans retinoic acid/ATRA, altretamine, amascrine, asparaginase, azacitidine, azathioprine, bacillus calmette-guerin/BCG, bendamustine hydrochloride, bexarotene, bicalutamide, bleomycin, bortezomib, busulfan,
- 5 capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, cisplatin/cisplatinum, cladribine, cyclophosphamide/cytophosphane, cytarabine, dacarbazine, daunorubicin/daunomycin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil (5-FU), gemcitabine, goserelin, hydrocortisone, hydroxyurea, idarubicin, ifosfamide, interferon alfa, irinotecan CPT-11,
- 10 lapatinib, lenalidomide, leuprolide, mechlorethamine/chlormethine/mustine/HN2, mercaptopurine, methotrexate, methylprednisolone, mitomycin, mitotane, mitoxantrone, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pegaspargase, pegfilgrastim, PEG interferon, pemetrexed, pentostatin, phenylalanine mustard, plicamycin/mithramycin, prednisone, prednisolone, procarbazine, raloxifene, romiplostim, sargramostim, streptozocin,
- 15 tamoxifen, temozolomide, temsirolimus, teniposide, thalidomide, thioguanine, thiophosphoamide/thiotepa, thiotepa, topotecan hydrochloride, toremifene, tretinoin, valrubicin, vinblastine, vincristine, vindesine, vinorelbine, vorinostat, zoledronic acid, or combinations thereof.
- 20 85. The method of claim 83, wherein the antibody-based agent is selected from: alemtuzumab, bevacizumab, cetuximab, fresolimumab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, ofatumumab, panitumumab, rituximab, tositumomab, trastuzumab, trastuzumab DMI, or combinations thereof.
- 25 86. The method of claim 83, wherein the kinase inhibitor is selected from: axitinib, bafetinib, bosutinib, cediranib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, neratinib, nilotinib, pazopanib, ponatinib, quizartinib, regorafenib, sorafenib, sunitinib, vandetanib, vatalanib, vemurafinib, or combinations thereof.
87. The method of claim 83, wherein the immunomodulatory agent is selected from:
- 30 thalidomide, lenalidomide, pomalidomide, methotrexate, leflunomide, cyclophosphamide, cyclosporine A, minocycline, azathioprine, tacrolimus, methylprednisolone, mycophenolate mofetil, rapamycin, mizoribine, deoxyspergualin, brequinar, 5,6-dimethylxanthenone-4-

acetic acid (DMXAA), lactoferrin, poly AU, polyI:polyC12U, poly-ICLC, imiquimod, resiquimod, unmethylated CpG dinucleotide (CpG-ODN), or ipilimumab.

88. The method of claim 83, wherein the kinase inhibitor is a Janus kinase inhibitor selected from: AC-430, AZD1480, baricitinib, BMS-911453, CEP-33779, CYT387, GLPG-
5 0634, INCB18424, lestaurtinib, LY2784544, NS-018, pacritinib, ruxolitinib, TG101348 (SAR302503), tofacitinib, VX-509, R-348, R723 or combinations thereof.

89. The method of claim 83, wherein the biologic agent is selected from: IL-2, IL-3, erythropoietin, G-CSF, filgrastim, interferon alfa, bortezomib or combinations thereof.

90. The method of claim 82, wherein the additional anti-cancer therapeutic is selected
10 from: AB0024, AZD1480, AT-9283, BMS-911543, CYT387, everolimus, givinostat, imetelstat, lestaurtinib, LY2784544, NS-018, oral arsenic, pacritinib, panobinostat, peginterferon alfa-2a, pomalidomide, pracinostat, ruxolitinib, TAK-901, or TG101438 (SAR302503).

91. The method of any one of claims 1 to 90, wherein the SAP protein is administered
15 according to a dosage regimen effective to reduce spleen volume by at least 25% relative to baseline.

92. The method of claim 91, wherein the SAP protein, alone or in combination with an additional anti-cancer therapeutic, is administered according to a dosage regimen effective to reduce spleen volume by at least 50% relative to baseline.

20 93. The method of any one of claims 1 to 92, wherein the SAP protein is administered according to a dosage regimen effective to reduce MPN-SAF Total Symptom Score by at least 25% relative to baseline.

94. The method of claim 93, wherein the SAP protein is administered according to a dosage regimen effective to reduce MPN-SAF Total Symptom Score by at least 50% relative
25 to baseline.

95. The method of any one of claims 1 to 94, wherein the SAP protein is administered according to a dosage regimen effective to increase hemoglobin levels by at least 1 g/L relative to baseline.

96. The method of any one of claims 1 to 95, wherein the SAP protein is administered according to a dosage regimen effective to increase hemoglobin levels to at least 100 g/L wherein baseline hemoglobin was less than 100 g/L at baseline.
97. The method of any one of claims 1 to 96, wherein the SAP protein is administered according to a dosage regimen effective to reduce red blood cell (RBC) transfusions by at least 25% relative to baseline.
98. The method of any one of claims 1 to 97, wherein the SAP protein is administered according to a dosage regimen effective to reduce red blood cell (RBC) transfusions by at least 50% relative to baseline.
99. The method of any one of claims 1 to 98, wherein the SAP protein is administered according to a dosage regimen effective to achieve RBC transfusion independence.
100. The method of any one of claims 1 to 99, wherein the SAP protein is administered according to a dosage regimen effective to reduce platelet transfusions by at least 25%.
101. The method of any one of claims 1 to 100, wherein the SAP protein, alone or in combination with an additional anti-cancer therapeutic, is administered according to a dosage regimen effective to reduce platelet transfusions by at least 50%.
102. The method of any one of claims 1 to 101, wherein the SAP protein, alone or in combination with an additional anti-cancer therapeutic, is administered according to a dosage regimen effective to cause a decrease in bone marrow fibrosis by 1 grade.
103. The method of any one of claims 1 to 101, wherein the method comprises administering an SAP protein and an additional anti-cancer therapeutic according to a dosage regimen such that one or more side effects are reduced relative to treatment with the additional anti-cancer therapeutic alone.
104. A kit comprising: a) an SAP protein; b) one or more oligonucleotides capable of amplifying a region of one or more genes selected from: *JAK2*, *MPL*, *CALR*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1*, and *IDH2*; and c) instructions for use.

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SEQUENCE LISTING

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<120> METHODS FOR TREATING MYELOPROLIFERATIVE DISORDERS

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Lys Ser Glu Ala Asp Tyr Leu Thr Phe Pro Ser Gly Glu Tyr Val Ala
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Glu Glu Ile Cys Ile Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
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Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
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Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asn Val
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Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser Gly Ser
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Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu
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Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp
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Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
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Glu Cys Leu Gly Met Ala Val Leu Asp Met Met Arg Ile Ala Lys Glu
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Asn Asp Gln Thr Pro Leu Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr
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Phe Leu Pro Lys Cys Ile Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
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Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
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260 265 270

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Pro Asn Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser
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Asn Glu Ser Arg Val Val Thr Ile His Lys Gln Asp Gly Lys Asn Leu
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Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu
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Tyr Gln Met Glu Thr Val Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr

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490

495

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Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
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 <213> Homo sapiens

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 35 40 45

Phe Val Leu Ser Ser Gly Lys Phe Tyr Gly Asp Glu Glu Lys Asp Lys
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Gly Leu Gln Thr Ser Gln Asp Ala Arg Phe Tyr Ala Leu Ser Ala Ser
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Phe Glu Pro Phe Ser Asn Lys Gly Gln Thr Leu Val Val Gln Phe Thr
 85 90 95

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Asn	Ile	Met	Phe	Gly	Pro	Asp	Ile	Cys	Gly	Pro	Gly	Thr	Lys	Lys	Val
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Ile	Arg	Cys	Lys	Asp	Asp	Glu	Phe	Thr	His	Leu	Tyr	Thr	Leu	Ile	Val
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			180					185					190		
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		195					200					205			
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Ile	Asp	Asp	Pro	Thr	Asp	Ser	Lys	Pro	Glu	Asp	Trp	Asp	Lys	Pro	Glu
225					230					235					240
His	Ile	Pro	Asp	Pro	Asp	Ala	Lys	Lys	Pro	Glu	Asp	Trp	Asp	Glu	Glu
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Ala Glu Lys Gln Met Lys Asp Lys Gln Asp Glu Glu Gln Arg Leu Lys
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Glu Glu Glu Glu Asp Lys Lys Arg Lys Glu Glu Glu Glu Ala Glu Asp
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Gln Ile Leu Gln Val Ile Glu Ala Glu Gly Leu Lys Glu Met Arg Ser
 35 40 45

Gly Thr Ser Pro Leu Ala Cys Leu Asn Ala Met Leu His Ser Asn Ser
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Arg Gly Gly Glu Gly Leu Phe Tyr Lys Leu Pro Gly Arg Ile Ser Leu
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Phe Thr Leu Lys Val
 85

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 <212> DNA
 <213> Homo sapiens

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<400> 14

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35 40 45

Ile Leu Glu Arg Thr Glu Ile Leu Asn Gln Glu Trp Lys Gln Arg Arg
50 55 60

Ile Gln Pro Val His Ile Leu Thr Ser Val Ser Ser Leu Arg Gly Thr
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Arg Glu Cys Ser Val Thr Ser Asp Leu Asp Phe Pro Thr Gln Val Ile
85 90 95

Pro Leu Lys Thr Leu Asn Ala Val Ala Ser Val Pro Ile Met Tyr Ser
100 105 110

Trp Ser Pro Leu Gln Gln Asn Phe Met Val Glu Asp Glu Thr Val Leu
115 120 125

His Asn Ile Pro Tyr Met Gly Asp Glu Val Leu Asp Gln Asp Gly Thr
130 135 140

Phe Ile Glu Glu Leu Ile Lys Asn Tyr Asp Gly Lys Val His Gly Asp
145 150 155 160

Arg Glu Cys Gly Phe Ile Asn Asp Glu Ile Phe Val Glu Leu Val Asn
165 170 175

Ala Leu Gly Gln Tyr Asn Asp Asp Asp Asp Asp Asp Gly Asp Asp
180 185 190

Pro Glu Glu Arg Glu Glu Lys Gln Lys Asp Leu Glu Asp His Arg Asp

195

200

205

Asp Lys Glu Ser Arg Pro Pro Arg Lys Phe Pro Ser Asp Lys Ile Phe
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 Lys Glu Lys Tyr Lys Glu Leu Thr Glu Gln Gln Leu Pro Gly Ala Leu
 245 250 255
 Pro Pro Glu Cys Thr Pro Asn Ile Asp Gly Pro Asn Ala Lys Ser Val
 260 265 270
 Gln Arg Glu Gln Ser Leu His Ser Phe His Thr Leu Phe Cys Arg Arg
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 Cys Phe Lys Tyr Asp Cys Phe Leu His Pro Phe His Ala Thr Pro Asn
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 Thr Tyr Lys Arg Lys Asn Thr Glu Thr Ala Leu Asp Asn Lys Pro Cys
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 Gly Pro Gln Cys Tyr Gln His Leu Glu Gly Ala Lys Glu Phe Ala Ala
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 Ala Leu Thr Ala Glu Arg Ile Lys Thr Pro Pro Lys Arg Pro Gly Gly
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 Arg Arg Arg Gly Arg Leu Pro Asn Asn Ser Ser Arg Pro Ser Thr Pro
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 Thr Ile Asn Val Leu Glu Ser Lys Asp Thr Asp Ser Asp Arg Glu Ala
 370 375 380
 Gly Thr Glu Thr Gly Gly Glu Asn Asn Asp Lys Glu Glu Glu Glu Lys
 385 390 395 400
 Lys Asp Glu Thr Ser Ser Ser Ser Glu Ala Asn Ser Arg Cys Gln Thr
 405 410 415
 Pro Ile Lys Met Lys Pro Asn Ile Glu Pro Pro Glu Asn Val Glu Trp
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 Ser Gly Ala Glu Ala Ser Met Phe Arg Val Leu Ile Gly Thr Tyr Tyr
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Gln Val Tyr Glu Phe Arg Val Lys Glu Ser Ser Ile Ile Ala Pro Ala
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Pro Ala Glu Asp Val Asp Thr Pro Pro Arg Lys Lys Lys Arg Lys His
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Arg Leu Trp Ala Ala His Cys Arg Lys Ile Gln Leu Lys Lys Asp Gly
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Ser Ser Asn His Val Tyr Asn Tyr Gln Pro Cys Asp His Pro Arg Gln
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Pro Cys Asp Ser Ser Cys Pro Cys Val Ile Ala Gln Asn Phe Cys Glu
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Lys Phe Cys Gln Cys Ser Ser Glu Cys Gln Asn Arg Phe Pro Gly Cys
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Arg Cys Lys Ala Gln Cys Asn Thr Lys Gln Cys Pro Cys Tyr Leu Ala
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Val Arg Glu Cys Asp Pro Asp Leu Cys Leu Thr Cys Gly Ala Ala Asp
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His Trp Asp Ser Lys Asn Val Ser Cys Lys Asn Cys Ser Ile Gln Arg
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Gly Ser Lys Lys His Leu Leu Leu Ala Pro Ser Asp Val Ala Gly Trp
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Gly Ile Phe Ile Lys Asp Pro Val Gln Lys Asn Glu Phe Ile Ser Glu
 625 630 635 640

Tyr Cys Gly Glu Ile Ile Ser Gln Asp Glu Ala Asp Arg Arg Gly Lys
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Val Tyr Asp Lys Tyr Met Cys Ser Phe Leu Phe Asn Leu Asn Asn Asp
 660 665 670

Phe Val Val Asp Ala Thr Arg Lys Gly Asn Lys Ile Arg Phe Ala Asn
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His Ser Val Asn Pro Asn Cys Tyr Ala Lys Val Met Met Val Asn Gly
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Asp His Arg Ile Gly Ile Phe Ala Lys Arg Ala Ile Gln Thr Gly Glu
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Glu Leu Phe Phe Asp Tyr Arg Tyr Ser Gln Ala Asp Ala Leu Lys Tyr
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Val Gly Ile Glu Arg Glu Met Glu Ile Pro
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Val Phe Glu Lys Tyr Gly Arg Val Gly Asp Val Tyr Ile Pro Arg Asp
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Arg Tyr Thr Lys Glu Ser Arg Gly Phe Ala Phe Val Arg Phe His Asp
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Lys Arg Asp Ala Glu Asp Ala Met Asp Ala Met Asp Gly Ala Val Leu
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Asp Gly Arg Glu Leu Arg Val Gln Met Ala Arg Tyr Gly Arg Pro Pro
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Asp Ser His His Ser Arg Arg Gly Pro Pro Pro Arg Arg Tyr Gly Gly
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Gly Gly Tyr Gly Arg Arg Ser Arg Ser Pro Arg Arg Arg Arg Arg Ser
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 <213> Homo sapiens

<400> 18

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Glu	Met	Thr	Arg	Ile	Ile	Trp	Glu	Leu	Ile	Lys	Glu	Lys	Leu	Ile	Phe
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Pro Tyr Val₃₅ Glu Leu Asp Leu His₄₀ Ser Tyr Asp Leu Gly₄₅ Ile Glu Asn
 Arg Asp₅₀ Ala Thr Asn Asp₅₅ Gln Val Thr Lys Asp₆₀ Ala Ala Glu Ala Ile
 Lys₆₅ Lys His Asn Val₇₀ Gly Val Lys Cys Ala Thr₇₅ Ile Thr Pro Asp₈₀ Glu
 Lys Arg Val Glu₈₅ Glu Phe Lys Leu Lys₉₀ Gln Met Trp Lys Ser Pro₉₅ Asn
 Gly Thr Ile Arg₁₀₀ Asn Ile Leu Gly₁₀₅ Gly Thr Val Phe Arg₁₁₀ Glu Ala Ile
 Ile Cys Lys₁₁₅ Asn Ile Pro Arg Leu₁₂₀ Val Ser Gly Trp Val₁₂₅ Lys Pro Ile
 Ile Ile₁₃₀ Gly Arg His Ala Tyr₁₃₅ Gly Asp Gln Tyr Arg₁₄₀ Ala Thr Asp Phe
 Val₁₄₅ Val Pro Gly Pro Gly₁₅₀ Lys Val Glu Ile Thr₁₅₅ Tyr Thr Pro Ser Asp₁₆₀
 Gly Thr Gln Lys Val₁₆₅ Thr Tyr Leu Val His₁₇₀ Asn Phe Glu Glu Gly₁₇₅ Gly
 Gly Val Ala Met₁₈₀ Gly Met Tyr Asn Gln₁₈₅ Asp Lys Ser Ile Glu Asp Phe₁₉₀
 Ala His Ser₁₉₅ Ser Phe Gln Met Ala₂₀₀ Leu Ser Lys Gly Trp₂₀₅ Pro Leu Tyr
 Leu Ser₂₁₀ Thr Lys Asn Thr Ile₂₁₅ Leu Lys Lys Tyr Asp₂₂₀ Gly Arg Phe Lys
 Asp₂₂₅ Ile Phe Gln Glu Ile Tyr Asp Lys Gln Tyr₂₃₅ Lys Ser Gln Phe Glu₂₄₀
 Ala Gln Lys Ile Trp₂₄₅ Tyr Glu His Arg Leu₂₅₀ Ile Asp Asp Met Val₂₅₅ Ala
 Gln Ala Met Lys₂₆₀ Ser Glu Gly Gly Phe₂₆₅ Ile Trp Ala Cys Lys₂₇₀ Asn Tyr

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Gly Met Met Thr Ser Val Leu Val Cys Pro Asp Gly Lys Thr Val Glu
290 295 300

Ala Glu Ala Ala His Gly Thr Val Thr Arg His Tyr Arg Met Tyr Gln
305 310 315 320

Lys Gly Gln Glu Thr Ser Thr Asn Pro Ile Ala Ser Ile Phe Ala Trp
325 330 335

Thr Arg Gly Leu Ala His Arg Ala Lys Leu Asp Asn Asn Lys Glu Leu
340 345 350

Ala Phe Phe Ala Asn Ala Leu Glu Glu Val Ser Ile Glu Thr Ile Glu
355 360 365

Ala Gly Phe Met Thr Lys Asp Leu Ala Ala Cys Ile Lys Gly Leu Pro
370 375 380

Asn Val Gln Arg Ser Asp Tyr Leu Asn Thr Phe Glu Phe Met Asp Lys
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Leu Gly Glu Asn Leu Lys Ile Lys Leu Ala Gln Ala Lys Leu
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<212> DNA
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Lys Leu Ile Leu Pro His Val Asp Ile Gln Leu Lys Tyr Phe Asp Leu
20          25          30

```

```
Gly Leu Pro Asn Arg Asp Gln Thr Asp Asp Gln Val Thr Ile Asp Ser
35          40          45

```

```
Ala Leu Ala Thr Gln Lys Tyr Ser Val Ala Val Lys Cys Ala Thr Ile
50          55          60

```

```
Thr Pro Asp Glu Ala Arg Val Glu Glu Phe Lys Leu Lys Lys Met Trp
65          70          75          80

```

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Lys Ser Pro Asn Gly Thr Ile Arg Asn Ile Leu Gly Gly Thr Val Phe
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Arg Glu Pro Ile Ile Cys Lys Asn Ile Pro Arg Leu Val Pro Gly Trp
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Thr Lys Pro Ile Thr Ile Gly Arg His Ala His Gly Asp Gln Tyr Lys
115 120 125

Ala Thr Asp Phe Val Ala Asp Arg Ala Gly Thr Phe Lys Met Val Phe
130 135 140

Thr Pro Lys Asp Gly Ser Gly Val Lys Glu Trp Glu Val Tyr Asn Phe
145 150 155 160

Pro Ala Gly Gly Val Gly Met Gly Met Tyr Asn Thr Asp Glu Ser Ile
165 170 175

Ser Gly Phe Ala His Ser Cys Phe Gln Tyr Ala Ile Gln Lys Lys Trp
180 185 190

Pro Leu Tyr Met Ser Thr Lys Asn Thr Ile Leu Lys Ala Tyr Asp Gly
195 200 205

Arg Phe Lys Asp Ile Phe Gln Glu Ile Phe Asp Lys His Tyr Lys Thr
210 215 220

Asp Phe Asp Lys Asn Lys Ile Trp Tyr Glu His Arg Leu Ile Asp Asp
225 230 235 240

Met Val Ala Gln Val Leu Lys Ser Ser Gly Gly Phe Val Trp Ala Cys
245 250 255

Lys Asn Tyr Asp Gly Asp Val Gln Ser Asp Ile Leu Ala Gln Gly Phe
260 265 270

Gly Ser Leu Gly Leu Met Thr Ser Val Leu Val Cys Pro Asp Gly Lys
275 280 285

Thr Ile Glu Ala Glu Ala Ala His Gly Thr Val Thr Arg His Tyr Arg
290 295 300

Glu His Gln Lys Gly Arg Pro Thr Ser Thr Asn Pro Ile Ala Ser Ile
305 310 315 320

Phe Ala Trp Thr Arg Gly Leu Glu His Arg Gly Lys Leu Asp Gly Asn
325 330 335

Gln Asp Leu Ile Arg Phe Ala Gln Met Leu Glu Lys Val Cys Val Glu

340

345

350

Thr Val Glu Ser Gly Ala Met Thr Lys Asp Leu Ala Gly Cys Ile His
355 360 365

Gly Leu Ser Asn Val Lys Leu Asn Glu His Phe Leu Asn Thr Thr Asp
370 375 380

Phe Leu Asp Thr Ile Lys Ser Asn Leu Asp Arg Ala Leu Gly Arg Gln
385 390 395 400