

USES OF REGULATORY T CELLS FOR TREATING MYELOFIBROSIS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/650,457, filed May 22, 2024, U.S. Provisional Patent Application No. 63/607,655, filed December 8, 2023, and U.S. Provisional Patent Application No. 63/546,612, filed October 31, 2023, the contents of each of which are incorporated by reference herein in their entirety for all purposes.

TECHNICAL FIELD

[0002] The disclosure is related to medical uses of T regulatory cells for treatment of myelofibrosis.

BACKGROUND

[0003] Myelofibrosis is a myeloproliferative neoplasm (MPN) characterized by bone marrow fibrosis, anemia, extramedullary hematopoiesis, and splenomegaly (Verstovsek et al., *J Hematol Oncol* 16, 82 (2023)). Janus kinase (JAK) inhibitors like ruxolitinib have been used for the treatment of myelofibrosis. However, many patients have a suboptimal response to ruxolitinib, and the disease remains incurable. Improved therapies targeted for patient populations with poor response to ruxolitinib are needed.

SUMMARY

[0004] Provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein the Treg cells are administered intravenously.

[0005] Provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$

CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein, after ruxolitinib monotherapy, the subject has at least one of the following: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination; and wherein the Treg cells are administered intravenously.

[0006] Provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein the subject has a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and/or (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*; and wherein the Treg cells are administered intravenously.

[0007] In some embodiments of the methods provided herein, the subject has a somatic mutation in the *JAK2* gene. In some embodiments of the methods provided herein, the subject has a W515L somatic mutation in the *MPL* gene. In some embodiments of the methods provided herein, the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

[0008] In some embodiments of the methods provided herein, the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time. In some embodiments of the methods provided herein, the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time.

[0009] In some embodiments of the methods provided herein, the subject is administered 10 mg or 15 mg of ruxolitinib twice daily.

[0010] In some embodiments of the methods provided herein, the symptom of myelofibrosis is splenomegaly, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

[0011] In some embodiments of the methods provided herein, the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days ± 3 days for up to 6

doses. In some embodiments of the methods provided herein, the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days for up to 6 doses.

[0012] In some embodiments of the methods provided herein, the Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days $\pm$ 3 days for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days $\pm$ 3 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days $\pm$ 3 days for 6 doses.

[0013] In some embodiments of the methods provided herein, the Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days for 6 doses.

[0014] Provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy.

[0015] Provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having at least one of the following after ruxolitinib monotherapy: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination.

[0016] Provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and (b) at

least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*.

[0017] In some embodiments of the pharmaceutical combinations provided herein, the subject has a V617F somatic mutation in the *JAK2* gene. In some embodiments of the pharmaceutical combinations provided herein, the subject has a W515L somatic mutation in the *MPL* gene. In some embodiments of the pharmaceutical combinations provided herein, the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

[0018] In some embodiments of the pharmaceutical combinations provided herein, the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time. In some embodiments of the pharmaceutical combinations provided herein, the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time.

[0019] In some embodiments of the pharmaceutical combinations provided herein, the symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1** depicts the change in transfusion requirement in subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy. “PRBC” = packed red blood cells.

[0021] **FIG. 2** depicts the change in spleen volume (best reduction in spleen volume at any time) in subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy.

[0022] **FIG. 3** depicts MPN-SAF TSS results from subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy. “PT” = patient. “C1D1” = cycle 1, day 1. “C1D8” = cycle 1, day 8. “C2D1” = cycle 2, day 1. “C3D1” = cycle 3, day 1. “C4D1” = cycle 4, day 1. “C5D1” = cycle 5, day 1. “C6D1” = cycle 6, day 1. “EOC6” = end of cycle 6.

[0023] **FIG. 4** depicts the change in total symptom score (TSS) (best reduction in TSS at any time) in subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy.

[0024] **FIG. 5** depicts results from a transwell migration assay measuring trafficking of CXCR4-enriched Treg cells towards SDF1 α .

[0025] **FIG. 6** depicts results of an IL-10 secretion assay.

[0026] **FIG. 7** depicts results of a cell suppression assay.

[0027] **FIG. 8A – FIG. 8B** depict results of studies measuring effect of Treg cells on expression of suppressive markers (**FIG. 8A**) and inflammatory markers (**FIG. 8B**). t-SNE algorithm maps Treg^{control} and Treg^{CXCR4} single-cell profiles.

[0028] **FIG. 9A – FIG. 9B** depict results of Treg cell localization 12 hours (**FIG. 9A**) and 24 hours (**FIG. 9B**) after infusion in a xenogeneic mouse model. “Per. Blood” = peripheral blood. Mean $\pm$ SD for a sample size of n = 4 mice per group at 12 hours. Mean $\pm$ SD for a sample size of n = 8 for BM and n = 5 for PB at 24 hours.

[0029] **FIG. 10A – FIG. 10B** depict results of Treg cell localization 12 hours and 24 hours after infusion in a xenogeneic mouse model in bone marrow (**d**) and peripheral blood (**FIG. 10B**).

[0030] **FIG. 11** depicts results of an analysis of suppression markers after Treg cell injection into mouse bone marrow.

[0031] **FIG. 12** depicts FlowSOM analysis grouping of human Treg^{control} and Treg^{CXCR4} samples into metaclusters. Each is identified by a unique color.

[0032] **FIG. 13** depicts a heatmap of FlowSOM metacluster 7 displaying Spearman’s r coefficients.

[0033] **FIG. 14A – FIG. 14B** depict results of Treg cell effects on TGF β -1 (**FIG. 14A**) and TGF β -2 (**FIG. 14B**) expression in bone marrow.

[0034] **FIG. 15** depicts the change in total symptom score (best reduction in TSS at any time) in subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy.

[0035] **FIG. 16** depicts the best reduction in spleen volume at any time in subjects with myelofibrosis and suboptimal response to ruxolitinib who have been treated with ruxolitinib and CK0804 add-on therapy.

[0036] **FIG. 17** depicts a heat map of inflammatory protein expression in a patient with SVR > 35% over time. In the x-axis labels, “C_D_” refers to the cycle number and the day number.

DETAILED DESCRIPTION

[0037] Provided herein are therapies for human subjects with myelofibrosis and a suboptimal response to ruxolitinib monotherapy. Ruxolitinib is a selective inhibitor of Janus kinase (JAK) 1 and 2. The therapies comprise administering CXCR4-enriched T regulatory (Treg) cells to a subject in combination with ruxolitinib. Also provided herein are pharmaceutical combinations comprising CXCR4-enriched Treg cells and ruxolitinib.

[0038] CXCR4 (C-X-C chemokine receptor type 4) is a rhodopsin-like G protein-coupled receptor. The canonical ligand of CXCR4 is CXCL12, also known as stromal cell-derived factor 1 (SDF-1). CXCR4/CXCL12 signaling is involved in cell migration, hematopoiesis and cell homing, and retention in the bone marrow. Exploiting CXCR4 expression on the Treg cell surface allows for Treg cell homing to the areas of dysregulation of the CXCR4/CXCL12 axis. In the case of myelofibrosis, these areas would be bone marrow and potentially the ongoing extramedullary hematopoiesis due to the clonal hematopoietic cells in spleen. Targeting of CXCR4/CXCL12 axis allows the repeated infusion of the Treg cells to capture any residual disease or escaped cells.

[0039] The methods and pharmaceutical combinations provided herein comprise use of a Treg cell population referred to as “CK0804”. CK0804 is a population of cryopreserved, allogeneic, cord blood-derived Treg cells that are CXCR4-enriched. CK0804 is cryopreserved at a dose of 100 million Treg cells per dose. CK0804 is produced from an umbilical cord blood unit that is not HLA-matched to the intended recipient.

[0040] CK0804 Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺ (T regulatory phenotype), wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺ (T-cytotoxic/suppressor phenotype). Treg cell phenotype is determined by flow cytometry analysis.

[0041] CK0804 Treg cells may be generated by the following method. CD25⁺ Treg cells are isolated and expanded from umbilical cord blood (UCB) units. UCB units are thawed and washed, and the resulting mononuclear cells are then subjected to CD25⁺ cell enrichment using magnetic-activated cell sorting (*e.g.*, Miltenyi Biotec, Germany) according to the manufacturer's instructions. Positively selected cells are stimulated with CD3/CD28 co-expressing Dynabeads (ClinExVivo CD3/CD28; Invitrogen Dynal

AS, Norway) at a ratio of one cell to three beads, resuspended at 1×10^6 cells/mL in expansion medium consisting of X-VIVO™ medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% penicillin-streptomycin and IL-2 and cultured in tissue culture flasks at 37°C in a 5% carbon dioxide in-air atmosphere continuously for 14 days for optimal expansion. In some embodiments, the amount of IL-2 is about 1000 IU/ml. On day 3 or 4, cultured cells are enriched for CXCR4⁺ cells using CD184 MicroBead Kit (Miltenyi Biotec, Germany) and then cultured for additional 11 or 12 days and harvested at day 14. At the end of the culture period, the Treg cells are cryopreserved. See, e.g., Lyu et al., *Cytotherapy*, 25(3):245-253 (2023).

[0042] If necessary, the cells undergo CD8 depletion prior to harvest and final formulation to ensure that the CD4⁺CD8⁺ T-cytotoxic/suppressor cell content is less than 10%.

[0043] The ex-vivo expanded Treg cells are harvested, washed, depleted of residual CD3/CD28 beads, and formulated in infusable cryopreservation medium containing 5% or 10% dimethyl sulfoxide (DMSO). This product can then be cryopreserved in cryobags utilizing a controlled rate freezer and transferred to vapor phase LN2 (< minus 150°C).

[0044] In some embodiments, the CK0804 final product is the cryopreserved form of the liquid cell suspension consisting of the CK0804 Treg cells resuspended in CryoStor® cryoprotectant solution (5% DMSO), with a final volume of about 20mL.

[0045] CK0804 Treg cells show preferential homing to bone marrow and decrease inflammatory cytokines including TGFα, TNFβ, IL-13 and TGFβ when compared to unmanipulated control Treg cells.

[0046] In some embodiments, a subject treated by the methods and pharmaceutical combinations disclosed herein has primary myelofibrosis. In some embodiments, a subject treated by the methods and pharmaceutical combinations disclosed herein has myelofibrosis arising from polycythemia vera and essential thrombocythemia.

[0047] In some embodiments, the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the CK0804 Treg cells for the first time. In some embodiments, the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the CK0804 Treg cells for the first time. In some embodiments, the subject was on ruxolitinib monotherapy for at least 12 weeks and was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the CK0804 Treg cells for the first time.

[0048] In some embodiments, a subject has a suboptimal response to ruxolitinib monotherapy. In some embodiments, a subject unlikely to benefit from further ruxolitinib monotherapy in the opinion of a medical practitioner.

[0049] In some embodiments, a subject has evidence of evaluable residual burden of disease following ruxolitinib monotherapy. In some embodiments, evidence of evaluable residual burden of disease following ruxolitinib monotherapy comprises one or more of the following: wherein, after ruxolitinib monotherapy, the subject has at least one of the following: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination. The MPN-SAF TSS is a scoring system used to assess myeloproliferative neoplasm (including myelofibrosis) symptom burden (see, e.g., Emanuel et al., *J Clin Oncol* 30:4098-4103 (2012)).

[0050] In some embodiments, a subject has one or more mutations associated with poor prognosis in myelofibrosis. In some embodiments, a subject has one or more mutations associated with inferior survival in myelofibrosis. In some embodiments, a subject has one or more mutations associated with resistance to ruxolitinib monotherapy in myelofibrosis.

[0051] In some embodiments, a subject has a somatic mutation in: (a) at least one of the following human genes: *JAK2* (Janus Kinase 2), *MPL* (Thrombopoietin receptor), and *CALR* (calreticulin); and/or (b) at least one of the following human genes: *ASXL1* (additional sex comb-like 1), *EZH2* (Enhancer of Zeste Homolog 2), *SF3B1* (splicing factor 3b subunit 1), *KRAS*, *TET2* (tet methylcytosine dioxygenase 2), *CREBBP* (cAMP-response element binding protein (CREB) binding protein), *SMZ3*, *SUZ12* (SUZ12 polycomb repressive complex 2 subunit), and *CBL* (Cbl proto-oncogene). In some embodiments, the subject has a V617F somatic mutation in the human *JAK2* gene. In some embodiments, the subject has a W515L somatic mutation in the human *MPL* gene. In some embodiments, the subject has a *CALR1* mutation in the human *CALR* gene (characterized by 52 base pair (bp) deletions). In some embodiments, the subject has a *CALR2* mutation in the human *CALR* gene (characterized by 5 bp insertions). In some embodiments, the subject has a Q157 mutation in the human *U2AF1* gene.

[0052] Somatic mutations in the human *JAK2*, *MPL*, and *CALR* genes may be classified as “driver” mutations for myelofibrosis. In some embodiments, the subject has a somatic mutation in the human *JAK2* gene, the human *MPL* gene, or the human *CALR* gene. In some embodiments, the subject has a somatic mutation in the human *JAK2* gene and a somatic mutation in the human *MPL* gene. In some embodiments, the subject has a somatic mutation in the human *JAK2* gene and a somatic mutation in the human *CALR* gene. In some embodiments, the subject has a somatic mutation in the human *MPL* gene and a somatic mutation in the human *CALR* gene.

[0053] In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *ASXL1* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *EZH2* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *SF3B1* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *KRAS* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *TET2* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *CREBBP* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *SMZ3* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *SUZ12* gene. In some embodiments, the subject has a somatic mutation in at least one of the following human genes: *JAK2*, *MPL*, and *CALR*; and a somatic mutation in the human *CBL* gene.

[0054] Mutational analysis to determine the presence of mutations associated with myelofibrosis may be performed on DNA from a subject’s peripheral blood granulocytes.

[0055] In some embodiments, a subject is transfusion dependent. In some embodiments, a subject is PRBC (packed red blood cells) transfusion dependent. In

some embodiments, PRBC dependence is defined as $\geq$ one PRBC per month for at least 4 consecutive months. In some embodiments, a subject has advanced bone marrow fibrosis.

[0056] CK0804 Treg cells are administered to the subject intravenously. In some embodiments, CK0804 Treg cells are thawed immediately before administration. In some embodiments, CK0804 Treg cells are infused by gravity, intravenously and within 60 minutes of thawing.

[0057] In some embodiments, the CK0804 Treg cells are administered to the subject at a dose of about 1×10^8 Treg cells every 28 days $\pm$ 1 day, 28 days $\pm$ 2 days, or 28 days $\pm$ 3 days for up to 6 doses. In some embodiments, the CK0804 Treg cells are administered to the subject at a dose of about 1×10^8 Treg cells every 28 days $\pm$ 1 day, 28 days $\pm$ 2 days, or 28 days $\pm$ 3 days for 6 doses.

[0058] In some embodiments, the CK0804 Treg cells are administered to the subject at a dose of about 1×10^8 Treg cells every 28 days for up to 6 doses. In some embodiments, the CK0804 Treg cells are administered to the subject at a dose of about 1×10^8 Treg cells every 28 days for 6 doses.

[0059] In some embodiments, the CK0804 Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days $\pm$ 1 day, 7 days $\pm$ 2 days, or 7 days $\pm$ 3 days, for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days $\pm$ 1 day, 21 days $\pm$ 2 days, or 21 days $\pm$ 3 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days $\pm$ 1 day, 28 days $\pm$ 2 days, or 28 days $\pm$ 3 days for 6 doses.

[0060] In some embodiments, the CK0804 Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days for 6 doses.

[0061] In some embodiments, a subject's ruxolitinib dose is unchanged during combination therapy with CK0804 Treg cells compared to the subject's ruxolitinib dose during ruxolitinib monotherapy. In some embodiments, a subject is administered 10 mg of oral ruxolitinib twice daily. In some embodiments, a subject is administered 15 mg of oral ruxolitinib twice daily.

[0062] In some embodiments, provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells,

wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$; wherein the Treg cells are administered intravenously.

[0063] In some embodiments, provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$; wherein, after ruxolitinib monotherapy, the subject has at least one of the following: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination; and wherein the Treg cells are administered intravenously.

[0064] In some embodiments, provided herein is a method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$; wherein the subject has a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and/or (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*; and wherein the Treg cells are administered intravenously.

[0065] In some embodiments, provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy.

[0066] In some embodiments, provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+$

CD8⁺, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having at least one of the following after ruxolitinib monotherapy: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination.

[0067] In some embodiments, provided herein is a pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and/or (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*.

[0068] In some embodiments, a symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

[0069] Unless otherwise noted, the terms used herein have definitions as ordinarily used in the art. Some terms are defined below, and additional definitions can be found within the rest of the detailed description.

[0070] The term “a” or “an” refers to one or more of that entity, *i.e.*, can refer to plural referents. As such, the terms “a,” “an,” “one or more,” and “at least one” are used interchangeably herein. In addition, reference to “an element” by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there is one and only one of the elements.

[0071] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device or the method being employed to determine the value, or the variation that exists among the samples being measured. Unless otherwise stated or otherwise evident from the context, the term “about” means within 10% above or below the reported numerical value (except where such number would exceed 100% of a possible value or go below 0%). When used in conjunction with a range or series of values, the term “about” applies to the endpoints of the range

or each of the values enumerated in the series, unless otherwise indicated. As used in this application, the terms “about” and “approximately” are used as equivalents.

[0072] As used herein, the terms “treat,” “treating” or “treatment of” (and grammatical variations thereof) mean that the severity of the subject's condition is reduced, at least partially improved or stabilized and/or that some alleviation, mitigation, decrease or stabilization in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder.

[0073] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. The use of the alternative (*e.g.*, “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms “include” and “comprise” are used synonymously.

[0074] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

NUMBERED EMBODIMENTS

[0075] Notwithstanding the appended claims, the disclosure sets forth the following numbered embodiments:

[0076] Embodiment 1. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the

CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein the Treg cells are administered intravenously.

[0077] Embodiment 2. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein, after ruxolitinib monotherapy, the subject has at least one of the following: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination; and wherein the Treg cells are administered intravenously.

[0078] Embodiment 3. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and $\leq 10\%$ CD4⁺CD8⁺; wherein the subject has a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and/or (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*; and wherein the Treg cells are administered intravenously.

[0079] Embodiment 4. The method of embodiment 3, wherein the subject has a somatic mutation in the *JAK2* gene.

[0080] Embodiment 5. The method of embodiment 3 or 4, wherein the subject has a W515L somatic mutation in the *MPL* gene.

[0081] Embodiment 6. The method of any one of embodiments 3-5, wherein the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

[0082] Embodiment 7. The method of any one of embodiment 1-6, wherein the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time.

[0083] Embodiment 8. The method of any one of embodiment 1-7, wherein the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time.

[0084] Embodiment 9. The method of any one of embodiments 1-8, wherein the subject is administered 10 mg or 15 mg of ruxolitinib twice daily.

[0085] Embodiment 10. The method of any one of embodiments 1-9, wherein the symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

[0086] Embodiment 11. The method of any one of embodiments 1-10, wherein the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days $\pm$ 3 days for up to 6 doses.

[0087] Embodiment 12. The method of any one of embodiments 1-10, wherein the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days for up to 6 doses.

[0088] Embodiment 13. The method of any one of embodiments 1-10, wherein the Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days $\pm$ 3 days for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days $\pm$ 3 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days $\pm$ 3 days for 6 doses.

[0089] Embodiment 14. The method of any one of embodiments 1-10, wherein the Treg cells are administered: (a) at a dose of about 1×10^8 Treg cells once every 7 days for 4 doses; (b) at a dose of about 1×10^8 Treg cells once every 21 days for 6 doses; and (c) at a dose of about 1×10^8 Treg cells once every 28 days for 6 doses.

[0090] Embodiment 15. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy.

[0091] Embodiment 16. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$, for use in treating

myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having at least one of the following after ruxolitinib monotherapy: (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia; (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination.

[0092] Embodiment 17. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of: $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and $\leq 10\%$ $CD4^+CD8^+$, for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having a somatic mutation in: (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*.

[0093] Embodiment 18. The pharmaceutical combination of embodiment 17, wherein the subject has a V617F somatic mutation in the *JAK2* gene.

[0094] Embodiment 19. The pharmaceutical combination of embodiment 17 or 18, wherein the subject has a W515L somatic mutation in the *MPL* gene.

[0095] Embodiment 20. The pharmaceutical combination of any one of embodiments 17-19, wherein the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

[0096] Embodiment 21. The pharmaceutical combination of any one of embodiment 15-20, wherein the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time.

[0097] Embodiment 22. The pharmaceutical combination of any one of embodiment 15-21, wherein the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time.

[0098] Embodiment 23. The pharmaceutical combination of any one of embodiments 15-22, wherein the symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

[0099] The disclosure will be further clarified by the following examples, which are intended to be purely exemplary of the disclosure and in no way limiting.

EXAMPLES**Example 1. Phase Ib, open-label study of add on therapy with CK0804 in participants with myelofibrosis and suboptimal response to ruxolitinib**

[0100] Ruxolitinib leads to improvements in symptom burden and spleen volume in patients with myelofibrosis (MF), but suboptimal response represents a major unmet clinical need.

[0101] This phase Ib study evaluates the safety and activity of CK0804 (non-HLA matched, cryopreserved, multi-dose) Treg therapy in patients with suboptimal response to ruxolitinib. The study design consists of a safety run-in phase of 9 patients followed by expansion of additional 15 patients. Participants continue ruxolitinib and receive infusion of CK0804 at fixed dose of 100 million Treg cells every 28 days up to 6 cycles. Participants will continue to be followed for 6 months after the last infusion. Patients with MF on ruxolitinib for ≥ 12 weeks and stable dose for ≥ 8 weeks, who have palpable splenomegaly, active symptoms or development of grade 2 of cytopenia or thrombocytopenia are eligible. Primary objective is safety, secondary objective includes overall response per IWG-MRT criteria at 24 weeks. Exploratory objectives evaluate pharmacodynamics, pharmacokinetics, markers of immunogenicity and inflammation.

[0102] Five patients were enrolled (median age 68 years [range 60-78], 60% males; **Table 1**. Median number of previous MF therapies was 1.5 (range 1-4), median duration of previous ruxolitinib therapy was 38 months (range, 10-132). At enrollment, all patients were symptomatic with median MPN-SAF TSS of 35 (range, 24-40), 3 patients had worsening splenomegaly and 3 patients were transfusion dependent. All patients had advanced marrow fibrosis, 40% had JAK2 V617F and 80% had additional co-mutations (**Table 1**).

[0103] Two patients received all 6 doses of CK0804, 2 patients were ongoing, and one patient withdrew consent. The infusions were tolerated with no adverse reactions in 5 patients. One patient had an infusion reaction to her second dose of CK0804 likely due to the excipient dimethylsulfoxate used for cryopreservation of the cells. No hematologic adverse events were observed. Ruxolitinib dose remained unchanged in all patients throughout the study.

[0104] Among the three patients who were transfusion dependent, two were evaluable for response, and their monthly transfusion requirements by the end of 6th cycle improved from baseline value of 4 units and 1.2 units to 2.8 and 0.8 units, respectively (FIG. 1). Spleen volume at 4 months assessment (3 patients) remained stable. One in four evaluable patients treated with therapy had a spleen volume reduction of 35% compared to baseline by week 12 (FIG. 2). Spleen volume was measured by magnetic resonance imaging (MRI). MPN-SAF TSS showed improvement in all patients on therapy to date, and there will be additional follow-up (FIG. 3). Two in four evaluable patients treated with therapy had a TSS reduction of 50% compared to baseline by week 12 (FIG. 4). Correlative studies including longitudinal analysis of inflammatory cytokines and immune reconstitution were performed.

[0105] The analysis of this study evaluating CK0804 (CXCR4-enriched Treg cell therapy) as addition to ruxolitinib showed safety with no myelosuppressive adverse events and promising clinical activity.

Table 1. Patient characteristics

Patient's characteristics	N (%); median (range)
Median age, years	68 (60-78)
Males	3 (60)
Primary MF	2 (40)
Body mass index	26.3 (23.9-33.2)
Previous ET, time to MF [years]	1 [23 years]
Previous PV, time to MF [years]	2 [5; 7 years]
Time to enrollment from MF, months	40 (14-135)
Previous MF therapies	1.5 (1-4)
Ruxolitinib dose: 10 mg twice daily	4 (80)
15 mg twice daily	1 (20)
Time on ruxolitinib prior to enrollment, months	38 (10-132)
White blood cells x 10 ⁹ /L	5.1 (4.2-10.6)
Hemoglobin g/dL	8.8 (7.8-11.5)
Hemoglobin < 10 g/dL	4 (80)
PRBC dependent	3 (60)
Platelets x10 ⁹ /L	177 (148-311)
Peripheral blasts ≥1%	3 (60)
Lactate dehydrogenase	620 (440-1288)
Palpable splenomegaly > 5 cm BCM	4 (80)
Spleen volume, cubic cm	810 (782-1446)
Liver volume, cubic cm	1991 (1516-2348)
Peripheral blasts ≥1%	3 (60)
Lactate dehydrogenase	620 (440-1288)

MPN-SAF TSS score	35 (24-40)
Reticulin fibrosis - grade 2	3 (60)
- grade 3	2 (40)
Collagen +	4 (80)
Osteosclerosis +	2 (40)
Bone marrow cellularity, %	60 (10-95)
Karyotype diploid	3 (60)
del(20)(q11.2q13)	1 (20)
del(20)(q11.2q13); add(21)(q22)	1 (20)
Driver mutation: JAK2 V617F	2 (40)
MPL W515L	1 (20)
CALR1 / CALR2	1 (20) / 1 (20)
Additional co-mutations	4 (80)
ASXL1	3 (60)
EZH2	2 (40)
SF3B1/KRAS/TET2/CREBBP/SMZ3/SUZ12	1 each (20)

Example 2. *In vitro* and *in vivo* studies of CXCR4-enriched T regulatory cells

[0106] CXCR4-enriched Treg cells demonstrated faster trafficking towards SDF1 α (ligand of CXCR4) as early as 15 minutes in a transwell migration assay (**FIG. 5**). CXCR4-enriched Treg cells show comparable IL-10 secretion to Treg control cells (**FIG. 6**). CXCR4-enriched Treg cells show superior cell suppression compared to Treg control cells in a CELLTRACE™ Violet assay (ThermoFisher) (**FIG. 7**). CXCR4-enriched Treg cells maintained their regulatory identity as shown by upregulated expression of suppressive markers (**FIG. 8A**) and downregulated expression of inflammatory markers (**FIG. 8B**).

[0107] Using a xenogeneic mouse model, 12 and 24 hours after Treg cell infusion, CXCR4-enriched Treg cells showed marked preference for bone marrow over Treg control (**FIG. 9A – FIG. 9B**). A summary of these results is shown in **FIG. 10A – FIG. 10B**.

[0108] viSNE was applied to assess the profile of human Treg^{control} and Treg^{CXCR4} post-injection into mouse bone marrow. CD39 and CD73 - conversion of ATP to immunosuppressive adenosine. Images are shown in **FIG. 11**. Bone marrow recipients showed higher expression of suppression markers.

[0109] Bone marrow metacluster analysis revealed a higher Treg percentage in Treg^{CXCR4} (**FIG. 12**). Human Treg^{CXCR4} in mouse bone marrow recipients showed stronger correlation with CD39 and lower correlation with CD95, HLADR and CCR4 in metacluster 7 (**FIG. 13**). A positive correlation with CD39 suggests enhanced

function in bone marrow. A negative correlation with CD95, CCR4 and HLADR suggests the Treg cells are less susceptible to FasLigand mediated apoptosis.

[0110] CXCR4-enriched Treg cells decreased TGF β expression in bone marrow *in vivo* (FIG. 14A – FIG. 14B).

Example 3. Additional results from phase Ib, open-label study of add on therapy with CK0804 in participants with myelofibrosis and suboptimal response to ruxolitinib

[0111] The study design and objectives are provided in Example 1. Patient characteristics are shown in Table 2.

Table 2. Patient characteristics

Patient's characteristics, N=9	N (%); median (range)
Median age, years	68 (55-84)
Males	4 (44)
Primary MF	4 (44)
Body mass index	28.8 (23.9-33.7)
White blood cells x 10 ⁹ /L	10.6 (3.1-70.4)
Hemoglobin g/dL	8.8 (7.8-12.0)
Hemoglobin < 10 g/dL	6 (67)
PRBC dependent	3 (33)
Platelets x10 ⁹ /L	177 (109-311)
Peripheral blasts $\geq$ 1%	5 (56)
Lactate dehydrogenase	659 (440-1288)
Palpable splenomegaly > 5 cm BCM	6 (67)
Spleen volume, cubic cm	1356.5 (176-2562)
Liver volume, cubic cm	2120 (1516-2472)
Lactate dehydrogenase	620 (440-1288)
MPN-SAF TSS score	25 (21-40)

Previous ET, time to MF [years]	4 [7-35 years]
Previous PV, time to MF [years]	1 [6 years]
Time to enrollment from MF diagnosis, months	41 (12-135)
Previous no. of MF therapies	2 (1-6)
Ruxolitinib dose: 10 mg twice daily	4 (44)
15 mg twice daily	4 (44)
20 mg twice daily	1 (12)
Time on ruxolitinib prior to enrollment, months	35 (10-132)

[0112] Eight patients have completed therapy, where 6 patients got all 6 doses. One patient has ongoing therapy and has completed 3 doses. One patient experienced infusion reaction to second dose of CK0804 likely due to the excipient dimethylsulfoxide and withdrew consent. One patient died of unrelated cause prior to the infusion number six. There were no other nonhematologic or hematologic adverse events. There was no observed increase in donor specific antibodies or evidence of allo-sensitization.

[0113] All patients showed improvement in symptom burden. 4 out of 8 evaluable patients had > 50% reduction in TSS (see **FIG. 15**). Evaluable patients had at least 12 week follow up. Calculated value = (observed-pretreatment)/ pretreatment x 100.

[0114] 3 out of 5 evaluable patients had reduction in spleen volume (see **FIG. 16**). 1 in 5 evaluable patients had >35% SVR (spleen volume reduction). Evaluable patients had baseline spleen volume > 300 cc, and at least 12 week follow up.

[0115] There was a trend towards decreased inflammatory burden for the patient with SVR > 35% (see **FIG. 17**).

[0116] Two PRBC transfusion-dependent patients who were evaluable for response had decreased monthly need for transfusions by the end of the sixth cycle: 4 units/mo decreased to 2.8 units/mo; and 1.2 units/mo decreased to 0.8 units /mo.

[0117] Multiple doses of CK0804 can be administered safely in an outpatient setting. No HLA matching or lymphodepletion is needed before CK0804 allogeneic Treg cell product administration. Safety run-in of 9 patients shows early efficacy signal. Planned expansion cohort of 6 patients examines a more intensive regimen of CK0804:

- Induction: 4 weekly doses
- Consolidation: 5 monthly doses
- Efficacy endpoint: 24 weeks

CLAIMS

1. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and

$\leq 10\%$ $CD4^+CD8^+$;

wherein the Treg cells are administered intravenously.

2. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and

$\leq 10\%$ $CD4^+CD8^+$;

wherein, after ruxolitinib monotherapy, the subject has at least one of the following:

(a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia;

(b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and

(c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination; and

wherein the Treg cells are administered intravenously.

3. A method for treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject in need thereof, the method comprising administering to the subject ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

$\geq 60\%$ CD3⁺CD4⁺CD25⁺, wherein at least 80% of the CD3⁺CD4⁺CD25⁺ Treg cells are CXCR4⁺; and

$\leq 10\%$ CD4⁺CD8⁺;

wherein the subject has a somatic mutation in:

(a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and/or

(b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*; and

wherein the Treg cells are administered intravenously.

4. The method of claim 3, wherein the subject has a somatic mutation in the *JAK2* gene.

5. The method of claim 3 or 4, wherein the subject has a W515L somatic mutation in the *MPL* gene.

6. The method of any one of claims 3-5, wherein the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

7. The method of any one of claim 1-6, wherein the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time.

8. The method of any one of claim 1-7, wherein the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time.

9. The method of any one of claims 1-8, wherein the subject is administered 10 mg or 15 mg of ruxolitinib twice daily.

10. The method of any one of claims 1-9, wherein the symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.

11. The method of any one of claims 1-10, wherein the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days $\pm$ 3 days for up to 6 doses.
12. The method of any one of claims 1-10, wherein the Treg cells are administered at a dose of about 1×10^8 Treg cells every 28 days for up to 6 doses.
13. The method of any one of claims 1-10, wherein the Treg cells are administered:
(a) at a dose of about 1×10^8 Treg cells once every 7 days $\pm$ 3 days for 4 doses;
(b) at a dose of about 1×10^8 Treg cells once every 21 days $\pm$ 3 days for 6 doses;
and
(c) at a dose of about 1×10^8 Treg cells once every 28 days $\pm$ 3 days for 6 doses.
14. The method of any one of claims 1-10, wherein the Treg cells are administered:
(a) at a dose of about 1×10^8 Treg cells once every 7 days for 4 doses;
(b) at a dose of about 1×10^8 Treg cells once every 21 days for 6 doses; and
(c) at a dose of about 1×10^8 Treg cells once every 28 days for 6 doses.
15. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:
 $\geq 60\% \text{ CD3}^+ \text{CD4}^+ \text{CD25}^+$, wherein at least 80% of the $\text{CD3}^+ \text{CD4}^+ \text{CD25}^+$ Treg cells are CXCR4^+ ; and
 $\leq 10\% \text{ CD4}^+ \text{CD8}^+$,
for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject with suboptimal response to ruxolitinib monotherapy.
16. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:
 $\geq 60\% \text{ CD3}^+ \text{CD4}^+ \text{CD25}^+$, wherein at least 80% of the $\text{CD3}^+ \text{CD4}^+ \text{CD25}^+$ Treg cells are CXCR4^+ ; and
 $\leq 10\% \text{ CD4}^+ \text{CD8}^+$,
for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having at least one of the following after ruxolitinib monotherapy:

- (a) presence of $\geq$ grade 2 anemia or thrombocytopenia or neutropenia;
- (b) presence of disease-related symptoms, as determined by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) score of ≥ 10 points; and
- (c) documented splenomegaly of at least 5 cm below the costal margin as measured by physical examination.

17. A pharmaceutical combination comprising ruxolitinib and cryopreserved, allogeneic, cord blood-derived T regulatory (Treg) cells, wherein the Treg cells have the phenotype of:

- $\geq 60\%$ $CD3^+CD4^+CD25^+$, wherein at least 80% of the $CD3^+CD4^+CD25^+$ Treg cells are $CXCR4^+$; and
- $\leq 10\%$ $CD4^+CD8^+$,

for use in treating myelofibrosis or ameliorating a symptom of myelofibrosis in a subject having a somatic mutation in:

- (a) at least one of the following genes: *JAK2*, *MPL*, and *CALR*; and
- (b) at least one of the following genes: *ASXL1*, *EZH2*, *SF3B1*, *KRAS*, *TET2*, *CREBBP*, *SMZ3*, *SUZ12*, and *CBL*.

18. The pharmaceutical combination of claim 17, wherein the subject has a V617F somatic mutation in the *JAK2* gene.

19. The pharmaceutical combination of claim 17 or 18, wherein the subject has a W515L somatic mutation in the *MPL* gene.

20. The pharmaceutical combination of any one of claims 17-19, wherein the subject has a *CALR1* or a *CALR2* somatic mutation in the *CALR* gene.

21. The pharmaceutical combination of any one of claim 15-20, wherein the subject was on ruxolitinib monotherapy for at least 12 weeks prior to administration of the Treg cells for the first time.

22. The pharmaceutical combination of any one of claim 15-21, wherein the subject was on a stable dose of ruxolitinib monotherapy for at least 8 weeks prior to administration of the Treg cells for the first time

23. The pharmaceutical combination of any one of claims 15-22, wherein the symptom of myelofibrosis is splenomegaly, transfusion requirement, fatigue, fever, anemia, weight loss, bone pain, night sweats, itching, abdominal discomfort, early satiety, or problems with concentration.