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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

IMPACT BIOMEDICINES, INC., **Plaintiff,** **v.** **TEVA PHARMACEUTICALS, INC.,** **Defendant.**	**Civil Action No. _____** **COMPLAINT FOR PATENT INFRINGEMENT** **(Filed Electronically)**

Plaintiff Impact Biomedicines, Inc. ("Impact"), by its undersigned attorneys, for its Complaint against Teva Pharmaceuticals, Inc. ("Teva") alleges as follows:

Nature of the Action

1. This is an action for patent infringement under the patent laws of the United States, 35 U.S.C. § 100, *et seq*., arising from Teva's submission of Abbreviated New Drug Application ("ANDA") No. 218907 ("Teva's ANDA") to the United States Food and Drug Administration ("FDA") seeking approval to manufacture, use, import, distribute, offer to sell, and/or sell generic versions of Impact's Inrebic® (fedratinib) drug product prior to the expiration of United States Patent Nos. 10,391,094 (the "'094 patent") and 11,400,092 (the "'092 patent") (together, "the patents-in-suit"), both owned by Impact.

The Parties

2. Impact is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 86 Morris Avenue, Summit, New Jersey 07901.

3. On information and belief, Defendant Teva is a corporation organized and existing under the laws of the State of Delaware, having its principal place of business at 400 Interpace Parkway, Parsippany, New Jersey 07054.

The Patents-in-Suit

4. On August 27, 2019, the United States Patent and Trademark Office ("USPTO") duly and lawfully issued the '094 patent, entitled, "Compositions and Methods for Treating Myelofibrosis." A copy of the '094 patent is attached hereto as Exhibit A.

5. On August 2, 2022, the USPTO duly and lawfully issued the '092 patent, entitled, "Methods of Treating Myeloproliferative Disorders." A copy of the '092 patent is attached hereto as Exhibit B.

The Inrebic® Drug Product

6. Impact holds an approved New Drug Application under Section 505(a) of the Federal Food Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 355(a) for fedratinib 100 mg capsules (NDA No. 212327), which is sold under the trade name Inrebic®.

7. The claims of the patents-in-suit cover, *inter alia*, pharmaceutical compositions comprising fedratinib and methods of using and administering fedratinib and pharmaceutical compositions comprising fedratinib.

8. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the patents-in-suit are listed in the FDA publication, "Approved Drug Products with Therapeutic Equivalence Evaluations" (the "Orange Book"), with respect to Inrebic®.

9. The FDA-approved prescribing information for Inrebic® instructs and encourages physicians, pharmacists, other healthcare workers, and patients to administer Inrebic® for the treatment of adults patients with intermediate-2 or high-risk primary or secondary (post-polycythemia vera or post-essential thrombocythemia) myelofibrosis (MF).

10. The FDA-approved prescribing information for Inrebic® instructs and encourages physicians, pharmacists, other healthcare workers, and patients to administer Inrebic® according to one or more of the methods claimed in the patents-in-suit.

Jurisdiction and Venue

11. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

12. On information and belief, Teva purposefully has conducted and continues to conduct business in this Judicial District.

13. On information and belief, Teva is in the business of, among other things, manufacturing, marketing, importing, offering for sale, and selling pharmaceutical products, including generic drug products, throughout the United States, including in this Judicial District.

14. On information and belief, Teva submitted ANDA No. 218907 seeking FDA approval to engage in the manufacturing, use, importation, distribution, offer to sell, and/or sale of the 100 mg fedratinib capsules that are the subject of Teva's ANDA ("Teva's Proposed Product"), throughout the United States, including in New Jersey and in this Judicial District, prior to the expiration of the patents-in-suit.

15. On information and belief, subject to the approval of ANDA No. 218907, Teva will make, use, import, sell, and/or offer for sale Teva's Proposed Product throughout the United States, including in New Jersey and in this Judicial District, prior to the expiration of the patents-

in-suit. On information and belief, this Judicial District is a likely destination for Teva's Proposed Product.

16. On information and belief, Teva is registered with the State of New Jersey's Division of Revenue and Enterprise Services as a business operating in New Jersey under Business ID No. 0450614134.

17. On information and belief, Teva has a regular and established, physical place of business in New Jersey at 400 Interpace Parkway, Parsippany, New Jersey, 07054. Teva's website states that its "US Headquarters" is located in Parsippany, New Jersey. *See* https://www.tevausa.com/contact-us (last visited October 23, 2023).

18. Teva has purposefully availed itself of the rights, benefits, and privileges of New Jersey, including by asserting counterclaims in this Court. *See, e.g.*, *GW Research Limited v. Teva Pharmaceuticals Inc., et al.*, No. 23-3914 (MEF)(AME) (D.N.J.) (D.I. 57).

19. Venue is proper in this Judicial District for Teva pursuant to 28 U.S.C. §§ 1391 and/or 1400(b).

Acts Giving Rise To This Suit

20. Pursuant to Section 505 of the FFDCA, Teva submitted its ANDA seeking approval to engage in the commercial manufacture, use, sale, offer for sale, or importation into the United States of Teva's Proposed Product before the patents-in-suit expire.

21. On information and belief, following FDA approval of Teva's ANDA, Teva will make, use, sell, or offer to sell Teva's Proposed Product throughout the United States, or import such generic products into the United States.

22. On information and belief, in connection with the submission of its ANDA as described above, Teva provided a written certification to the FDA pursuant to Section 505 of the

FFDCA, 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Teva's Paragraph IV Certification"), alleging that the claims of the patents-in-suit are invalid and/or will not be infringed by the activities described in Teva's ANDA.

23. No earlier than September 11, 2023, Teva sent to Impact a written notice of Teva's Paragraph IV Certification ("Teva's Notice Letter"). Teva's Notice Letter alleged that the claims of the '094 and '092 patents are invalid and/or will not be infringed by the activities described in Teva's ANDA. Teva's Notice Letter conveyed that Teva seeks approval to market Teva's Proposed Product before the patents-in-suit expire.

Count I: Infringement of the '094 Patent

24. Impact repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

25. Teva's submission of its ANDA, with the accompanying Paragraph IV Certification and notice to Impact of same, to engage in the commercial manufacture, use, sale, offer for sale, or importation into the United States of Teva's Proposed Product, prior to the expiration of the '094 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

26. There is a justiciable controversy between the parties hereto as to the infringement of the '094 patent.

27. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will infringe one or more claims of the '094 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States.

28. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will induce infringement of one or more claims of the '094 patent under 35 U.S.C. § 271(b) by

making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, upon FDA approval of Teva's ANDA, Teva will intentionally encourage acts of direct infringement with knowledge of the '094 patent and knowledge that its acts are encouraging infringement.

29. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will contributorily infringe one or more claims of the '094 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, Teva has had and continues to have knowledge that Teva's Proposed Product is especially adapted for a use that infringes one or more claims of the '094 patent and that there is no substantial non-infringing use for Teva's Proposed Product.

30. Impact will be substantially and irreparably damaged and harmed if Teva's infringement of the '094 patent is not enjoined.

31. Impact does not have an adequate remedy at law.

32. This case is an exceptional one, and Impact is entitled to an award of its reasonable attorneys' fees under 35 U.S.C. § 285.

Count II: Infringement of the '092 Patent

33. Impact repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

34. Teva's submission of its ANDA, with the accompanying Paragraph IV Certification and notice to Impact of same, to engage in the commercial manufacture, use, sale, offer for sale, or importation into the United States of Teva's Proposed Product, prior to the expiration of the '092 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

35. There is a justiciable controversy between the parties hereto as to the infringement of the ’092 patent.

36. Unless enjoined by this Court, upon FDA approval of Teva’s ANDA, Teva will infringe one or more claims of the ’092 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Teva’s Proposed Product in the United States.

37. Unless enjoined by this Court, upon FDA approval of Teva’s ANDA, Teva will induce infringement of one or more claims of the ’092 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Teva’s Proposed Product in the United States. On information and belief, upon FDA approval of Teva’s ANDA, Teva will intentionally encourage acts of direct infringement with knowledge of the ’092 patent and knowledge that its acts are encouraging infringement.

38. Unless enjoined by this Court, upon FDA approval of Teva’s ANDA, Teva will contributorily infringe one or more claims of the ’092 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Teva’s Proposed Product in the United States. On information and belief, Teva has had and continues to have knowledge that Teva’s Proposed Product is especially adapted for a use that infringes one or more claims of the ’092 patent and that there is no substantial non-infringing use for Teva’s Proposed Product.

39. Impact will be substantially and irreparably damaged and harmed if Teva’s infringement of the ’092 patent is not enjoined.

40. Impact does not have an adequate remedy at law.

41. This case is an exceptional one, and Impact is entitled to an award of its reasonable attorneys’ fees under 35 U.S.C. § 285.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff Impact respectfully requests the following relief:

(A) A Judgment that Teva has infringed the patents-in-suit by submitting ANDA No. 218907 with the accompanying Paragraph IV Certification and notice to Impact of same;

(B) A Judgment that Teva has infringed, and that Teva's making, using, selling, offering to sell, or importing Teva's Proposed Product will infringe one or more claims of the patents-in-suit;

(C) An Order that the effective date of FDA approval of ANDA No. 218907 be a date which is not earlier than the later of the expiration of the patents-in-suit, or any later expiration of exclusivity to which Impact is or becomes entitled;

(D) Preliminary and permanent injunctions enjoining Teva and its officers, agents, attorneys and employees, and those acting in privity and/or concert with them, from making, using, offering to sell, selling, or importing Teva's Proposed Product until after the expiration of the patents-in-suit, or any later expiration of exclusivity to which Impact is or becomes entitled;

(E) A permanent injunction, pursuant to 35 U.S.C. § 271(e)(4)(B), restraining and enjoining Teva, its officers, agents, attorneys, and employees, and those acting in privity and/or concert with them, from practicing any of the subject matter claimed in the patents-in-suit, or from actively inducing or contributing to the infringement of any claim of the patents-in-suit, until after the expiration of the patents-in-suit, or any later expiration of exclusivity to which Impact is or becomes entitled;

(F) A Judgment that the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of Teva's Proposed Product will directly infringe, induce infringement of, and/or contribute to infringement of the patents-in-suit;

(G) To the extent that Teva, its officers, agents, attorneys and/or employees, or those acting in privity and/or concert with them, has committed any acts with respect to the subject matter claimed in the patents-in-suit, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), a Judgment awarding Impact damages for such acts;

(H) If Teva, its officers, agents, attorneys, and/or employees, or those acting in privity and/or concert with them, engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of Teva's Proposed Product prior to the expiration of the patents-in-suit, a Judgment awarding damages to Impact resulting from such infringement, together with interest;

(I) A Judgment declaring that the patents-in-suit remain valid and enforceable;

(J) A Judgment that this is an exceptional case pursuant to 35 U.S.C. § 285 and awarding Impact its attorneys' fees incurred in this action;

(K) A Judgment awarding Impact its costs and expenses incurred in this action; and

(L) Such further and other relief as this Court may deem just and proper.

Dated: October 23, 2023

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CERTIFICATION PURSUANT TO L. CIV. R. 11.2 & 40.1

Pursuant to Local Civil Rules 11.2 and 40.1, I hereby certify that, to the best of my knowledge, the matter in controversy is not the subject of any other action pending in any court, or of any pending arbitration or administrative proceeding.

Dated: October 23, 2023

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EXHIBIT A

US010391094B2

(12) **United States Patent**
Jayan et al.

(10) **Patent No.: US 10,391,094 B2**
(45) **Date of Patent: Aug. 27, 2019**

(54) **COMPOSITIONS AND METHODS FOR TREATING MYELOFIBROSIS**

(71) Applicant: **Impact Biomedicines, Inc.**, San Diego, CA (US)

(72) Inventors: **Arvind Jayan**, La Jolla, CA (US); **Janice Cacace**, Miami, FL (US)

(73) Assignee: **Impact Biomedicines, Inc.**, Summit, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 204 days.

(21) Appl. No.: **13/888,096**

(22) Filed: **May 6, 2013**

(65) **Prior Publication Data**

US 2013/0243853 A1 Sep. 19, 2013

Related U.S. Application Data

(63) Continuation of application No. PCT/US2011/059643, filed on Nov. 7, 2011.

(60) Provisional application No. 61/410,924, filed on Nov. 7, 2010.

(51) **Int. Cl.**
A61K 31/506 (2006.01)
A61K 9/48 (2006.01)

(52) **U.S. Cl.**
CPC ***A61K 31/506*** (2013.01); ***A61K 9/48*** (2013.01); *A61K 9/4866* (2013.01)

(58) **Field of Classification Search**
CPC A61K 31/506; A61K 9/48; A61K 9/4866
USPC 424/452
See application file for complete search history.

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(57) **ABSTRACT**

Provided herein are compositions and methods for treating myelofibrosis in a subject. The methods comprise administering to the subject an effective amount of compound which is which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof.

26 Claims, 25 Drawing Sheets

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*Starting dose. Doses (mg/day) Cycle 1, 520-800; Cycles 2-6, 360-680 mg/day.
‡22-47% increase in 3 subjects with drug held for ~2-3 weeks immediately prior to measurement.

Doses at follow-up range from 360 to 680 mg/day. Last follow-up visit ranges from 8 to 24 weeks (median 24 weeks).
ULN = upper limit of normal.

Figure 3 TG101348 Effect on Thrombocytosis
(Baseline Platelet Count > 450 x 10⁹/L)
104031
104034
104038
106045
101047
104048
106049
ULN
Platelet Count (x10^9/L)
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Baseline
Follow-Up
Doses at follow-up range from 360 to 680 mg/day. Last follow-up visit ranges from 12 to 24 weeks (median 24 weeks).
ULN = upper limit of normal.

Figure 4 Effects of TG101348 on Constitutional Symptoms: Baseline to Last Visit

Last visit ranges from 4 to 24 weeks (median 20 weeks). Data reflect changes from symptoms present at baseline. 16 subjects reported new onset of ≥ 1 symptom during the study; of these, symptoms for 12 subjects were resolved by last follow-up visit.
Severity rated by subjects on scale of 1-10. For analysis, 0 = absent; 1-3 = mild; 4-7 = moderate; 8-10 = severe. Improved = downgrade to absent or to mild or moderate from more severe rating at baseline.

Figure 5 No Significant Changes in Cytokine Levels With TG101348

All values are medians.

Figure 6 TG101348 Effect on V617F Allele Burden in Subjects With Baseline > 20% (N = 22)*

*Subset of JAK2V617F positive subjects in the overall population (N = 48).
Doses at follow-up 360 to 680 mg/day. Last follow-up visit ranges from 20 to 72 weeks (median 24 weeks).

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Figure 7 Effect on BM Cellularity

Baseline: 60% cellularity

After 18 cycles: 5-10% cellularity

76-year-old male, V617F negative PMF; starting dose, 30 mg/day; dose at follow-up, 520 mg/day.

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Figure 8 Effect on BM Fibrosis

Baseline: 3+

After 18 cycles: 0

56-year-old male, V617F negative PMF; starting dose, 240 mg/day; dose at follow-up, 440 mg/day.

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Figure 9 A subject with JAK2V617F-positive PMF started at 680 mg/day (TG101348)

	Day -8	Screen -1	C1D8 *680mg*	C2D1 *680mg*	C2D15 *680mg*	C3D1 *360mg*	C6D1 *360mg*	C9D1 *360mg*
Hgb (g/dL)	9	9	9.4	7.9**	9.2	8	9.6	10.9
WBC (x 109/L)	13.5	14.9	18.6	7.1	6.2	7.3	5.4	5.2
Meta	2	0	n/a	0	0	0	0	0
Myelo	10	6		0	0	0	0	1
Blasts	0	1		0	1	0	0	0
nuRBC	18	10		0	0	0	1	0
Plt (x 109/L)	577	546	731	289	183	183	192	212
LDH U/L	943	928	1001	581	529	479	455	444

Figure 10A

Figure 10B

Figure 10C

Figure 10D

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Figure 10E

Figure 10F

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Figure 10G

Figure 11

Number of Subjects
35
30
25
20
15
10
5
0
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6
680
600
520
440
400
360

Figure 12A

Mean TE101349 Plasma Concentrations (ng/mL)
10000
1000
100
10
1
0
4
8
12
16
20
24
28
Time Post-Dose (hours)

Figure 12B

Mean TC101348 Plasma Concentrations (ng/mL)
10000
1000
100
10
1
0
4
8
12
16
20
24
28
Time Post-Dose (hours)
TC101348 (mg/kg)
9
60
120
240
360
720
960
800

Figure 13

Percentage of Subjects
80
70
60
50
40
30
20
10
0
Cycle 1 (n = 36)
Cycle 2 (n = 33)
Cycle 3 (n = 32)
Cycle 4 (n = 28)
Cycle 5 (n = 29)
Cycle 6 (n = 29)
≥ 50% Decrease
100% Decrease

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Figure 14A

Figure 14B

Figure 14C

Percentage of Subjects
100
90
80
70
60
50
40
30
20
10
0
Cycle 1 (n = 14)
Cycle 2 (n = 13)
Cycle 3 (n = 11)
Cycle 4 (n = 10)
Cycle 5 (n = 10)
Cycle 6 (n = 9)
Resolved From Mild
Resolved From Moderate or Severe

Figure 15

WBC Count (x10^9/L)
110
100
90
80
70
60
50
40
30
20
10
0
Baseline
Cycle 6
102001
102003
102004
101005
105007
104008
101014
104015
106016
105016
101020
103022
106024
105026
104030
106033
104034
101035
102037
104038
106042
106045
104048
106049
104052
104053
104055
103057

Figure 16A

Figure 16B

Figure 16C

Figure 16D

Absolute differences in IL-6 (-4719 pg/mL) and IL-2 (-1827 pg/mL) are omitted from panels A and D, respectively, for 1 subject (101-039) because they skewed presentation of data for other subjects.

Figure 17A

Figure 17B

Figure 17C

Figure 17D

Figure 18

30 mg
60 mg
120 mg
240 mg
360 mg
520 mg
680 mg
800 mg
680 mg
360 mg
Plasma Concentration (ng/mL)
4000
3000
2000
1000
0
0
4
8
12
16
20
24
28
Time (hr)
3x IC90
(3 uM)
IC90
(1 uM)
IC50
(0.3 uM)

COMPOSITIONS AND METHODS FOR TREATING MYELOFIBROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of International Application Number PCT/US2011/059643, filed Nov. 7, 2011, which claims the priority benefit of U.S. provisional application Ser. No. 61/410,924, filed Nov. 7, 2010, the disclosures of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

Provided herein are compositions and methods for treating myelofibrosis. The compositions and methods provided herein relate to treatment of myelofibrosis with compounds that inhibit JAK2 or a pharmaceutically acceptable salt thereof or a hydrate thereof.

BACKGROUND

Myelofibrosis ("MF") is a rare disease mainly affecting people of older age. 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"). Clinical features include progressive anemia, marked splenomegaly, constitutional symptoms (e.g. fatigue, night sweats, bone pain, pruritus, and cough) and weight loss (Tefferi A, N Engl J Med 342:1255-1265, 2000). 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). Mutations involving JAK2 (James C et al., Nature 434:1144-1148, 2005; Scott L M et al., N Engl J Med 356:459-468, 2007), MPL (Pikman Y et al., PLoS Med 3:e270, 2006), TET2 (Delhommeau F et al., N Engl J Med 360:2289-2301, 2009), ASXLI (Carbuccia N et al., Leukemia 23:2183-2186, 2009), IDH1/IDH2 (Green A et al., N Engl J Med 362:369-370, 2010; Tefferi A et al., Leukemia 24:1302-1309, 2010), CBL (Grand F H et al., Blood 113:6182-6192, 2009), IKZF1 (Jagcr R et al., Leukemia 24:1290-1298, 2010), LNK (Oh S T et al., Blood 116:988-992, 2010), or EZH2 (Ernst T et al., Nat Genet. 42:722-726) have been described in patients with MPN, including those with MF. Some mutations occur at high frequency in MF (e.g. JAK2 mutations in ~50% patients), and either directly (e.g. JAK2 or MPL mutations) or indirectly (e.g. LNK or CBL mutations) induce JAK-STAT hyperactivation.

The currently available treatments are not effective in reversing the process of MF, be it primary or secondary disease. The only potential for cure of the disease to date is bone marrow transplantation. However, most patients are not suitable bone marrow transplant candidates because of the older median age at diagnosis, in which transplant-related morbidity and mortality tends to be high. Thus management options of MF are currently inadequate to meet the needs of all patients. The main options for active intervention include cyto-reductive therapy, e.g. with hydroxyurea, treatment of anemia with androgens, erythropoietin and splenectomy. These options have not been shown to improve survival and are largely seen as palliative (Cervantes F., Myelofibrosis: Biology and treatment options, European Journal of Haematology, 2007, 79 (suppl. 68) 13-17). Therefore, there is a need to provide additional therapy options for MF patients.

SUMMARY OF THE INVENTION

Provided herein are capsules suitable for oral administration. In some embodiments, the capsule comprises an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate, wherein the admixture is contained in the capsule.

In some embodiments, the capsule contains about 10 mg to about 680 mg of the compound, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the capsule contains about 10 mg to about 500 mg of the compound. In some embodiments, the capsule contains about any of 10 mg, 40 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, or 600 mg of the compound. In some embodiments, the weight ratio of the compound to microcrystalline cellulose in the capsule is between about 1:1.5 to 1:15, wherein the weight for the compound in the weight ratio is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to sodium stearyl fumarate in the capsule is between about 5:1 to about 50:1, and wherein the weight for the compound in the weight ratio is the free base moiety weight of the compound. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. In some embodiments, the silicified microcrystalline cellulose is a combination of 98% microcrystalline cellulose and 2% colloidal silicon dioxide.

Also provided herein are unit dosage forms comprising an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino} pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the unit dosage forms are for treatment of myelofibrosis such as treatment of myelofibrosis according to a method described herein.

In some embodiments, the unit dosage form comprises an admixture of (i) about 10 mg to about 680 mg (or about 10 mg to about 500 mg) of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the specified weight is the free base moiety weight of the compound, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the unit dosage form is in the form of a capsule, and the admixture is contained in the capsule. In some embodiments, the compound in the admixture is about 10 mg to about 500 mg, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the admixture comprises (i) about 10 mg (or about any of 40 mg, 100 mg, 200 mg, 300 mg, 400 mg, or 500 mg) of the compound, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the weight ratio of the compound to microcrystalline cellulose in the capsule is between about 1:1.5 to 1:15, wherein the weight for the compound in the weight

ratio is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to sodium stearyl fumarate in the capsule is between about 5:1 to about 50:1, and wherein the weight for the compound in the weight ratio is the free base moiety weight of the compound. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. In some embodiments, the silicified microcrystalline cellulose is a combination of 98% microcrystalline cellulose and 2% colloidal silicon dioxide.

In some embodiments, sodium stearyl fumarate is about 1% w/w of capsule fill weight. In some embodiments, the weight ratio of the compound to microcrystalline cellulose such as silicified microcrystalline cellulose is about 40:60 to about 10:90 (e.g., about 40:60 or about 1:1.5, or about 10:90 or about 1:9).

In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the unit dosage form or capsule contains an admixture of about 12 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 122 mg of silicified microcrystalline cellulose, and about 1 mg of sodium stearyl fumarate. In some embodiments, the unit dosage form or capsule contains an admixture of about 47 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 448 mg of silicified microcrystalline cellulose, and about 5 mg of sodium stearyl fumarate. In some embodiments, the unit dosage form or capsule contains an admixture of about 117 mg of N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the unit dosage form or capsule contains an admixture of about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 357 mg of silicified microcrystalline cellulose, and about 6.00 mg of sodium stearyl fumarate. In some embodiments, the capsule is a hard gelatin capsule.

Also provided herein are methods of preparing a capsule drug product comprising a) blending a lubricant with a compound that is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof to generate granules and b) mixing the granules of a) with an excipient. In some embodiments, the lubricant is sodium stearyl fumarate. In some embodiments, the excipient is microcrystalline cellulose such as silicified microcrystalline cellulose. In some embodiments, sodium stearyl fumarate is about 1% w/w of capsule fill weight. In some embodiments, the weight ratio of the compound to silicified microcrystalline cellulose is about 1:1.5 to about 1:9. In some embodiments, the weight ratio of the compound to silicified microcrystalline cellulose is about 1:1.5. In some embodiments, the weight ratio of the compound to silicified microcrystalline cellulose is about 1:9. In some embodiments, the capsule is a hard gelatin capsule.

Also provided herein are methods of treating myelofibrosis in a subject, comprising orally administering a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and wherein the compound is in an admixture of (i) the compound, (ii) an excipient (e.g., a microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate). Any of the unit dosage forms or capsules described herein may be used. In some embodiments, there is provided a method of treating myelofibrosis in a subject comprising orally administering a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and wherein the compound is in a capsule containing an admixture of (i) the compound, (ii) a microcrystalline cellulose (e.g., silicified microcrystalline cellulose), and (iii) sodium stearyl fumarate.

Also provided herein are methods of treating myelofibrosis in a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject is negative 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.

Also provided herein are methods of treating myelofibrosis in a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy is a treatment with a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the previous therapy comprises administration of INCB018424 (ruxolitinib). In some embodiments, the subject is unresponsive to the previous therapy. In some embodiments, the previous therapy is a treatment with N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the previous therapy has been discontinued upon indication of elevated levels of amylase, lipase, aspartate aminotransferase ("AST"), alanine aminotransferase ("ALT"), and/or creatinine. In some embodiments, the previous therapy has been discontinued upon indication of a hematologic condition selected from the group consisting of anemia, thrombocytopenia, and neutropenia.

Also provided herein are methods of ameliorating bone marrow cellularity or bone marrow fibrosis associated with myelofibrosis in a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

Also provided herein are methods of improving pruritus associated with myelofibrosis in a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

Also provided herein are methods of monitoring treatment of myelofibrosis in a subject, comprising (a) administering to a subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof; (b) monitoring a non-hematologic parameter selected from the group consisting of amylase level, lipase level, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, and creatinine level in the subject; and (c) determining if the subject should continue or discontinue with the treatment. Also provided herein are methods of monitoring treatment of myelofibrosis to a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and discontinuing the treatment upon indication of elevated levels of one or more enzymes or molecules selected from the group consisting of amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine in the serum of the subject without prior dose reduction. In some embodiments, the one or more of the elevated levels are Grade 4 events.

Also provided herein are methods of monitoring a treatment of myelofibrosis to a subject, comprising (a) administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof; (b) monitoring a hematologic parameter selected from the group consisting of anemia, thrombocytopenia, and neutropenia in the serum of the subject; and (c) determining if the subject should continue or discontinue with the treatment. Also provided herein are methods of monitoring treatment of myelofibrosis to a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and discontinuing the treatment upon indication of one or more hematologic conditions selected from the group consisting of anemia, thrombocytopenia, and neutropenia without prior dose reduction. In some embodiments, the one or more hematologic conditions are grade 4 events.

In some embodiments of the methods of monitoring treatment provided herein, the methods further comprise administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof after the subject has been discontinued with the treatment for at least 2 weeks. In some embodiments, the subject has been discontinued with the treatment for at least 3 weeks. In some embodiments, the subject has been discontinued with the treatment for at least 4 weeks. In some embodiments, the treatment has been discontinued without prior dose reduction.

In some embodiments, the compound is administered to the human subject at a dose of about 240 mg per day to about 680 mg per day, and wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is administered at a dose of about 300 mg per day to about 500 mg per day (e.g., about 300 mg per day to about 400 mg per day, or about 400 mg per day to about 500 mg per day), and wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is administered at a dose of about any of 240 mg per day, 250 mg per day, 300 mg per day, 350 mg per day, 400 mg per day, 450 mg per day, 500 mg per day, 550 mg per day, 600 mg per day, 650 mg per day, or 680 mg per day, and wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is administered daily and/or orally. In some embodiments, the compound is administered over a period of at least 1 cycle, at least 2 cycles, at least 3 cycles, at least 4 cycles, at least 5 cycles, or at least 6 cycles (e.g., at least 7 cycles, at least 8 cycles, at least 9 cycles, at least 10 cycles, at least 11 cycles, at least 12 cycles, at least 15 cycles, at least 18 cycles, or at least 24 cycles) of a 28-day treatment cycle. In some embodiments, the compound is in a capsule and administered orally. In some embodiments, the compound is in a unit dosage form. Any of the capsules or unit dosage forms described herein may be administered. In some embodiments of the methods provided herein, the compound is in an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the weight ratio of the compound to microcrystalline cellulose in the admixture is between about 1:1.5 to 1:15, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to sodium stearyl fumarate in the admixture is between about 5:1 to about 50:1, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. In some embodiments, the subject is a human.

In some embodiments of the compositions and 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. In some embodiments of the compositions and methods provided herein, the subject has post essential thrombocythemia myelofibrosis. In some embodiments, the subject has high risk myelofibrosis. In some embodiments, the subject has intermediate risk myelofibrosis (such as intermediate risk level 2). In some embodiments of the compositions and methods provided herein, the subject is positive 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 of the compositions and methods provided herein, the subject is negative 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 of the compositions and methods provided herein, 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 of the compositions and methods provided herein, the subject is transfusion dependent. In some embodiments of the compositions and methods provided herein, the subject is not transfusion dependent.

In some embodiments of the methods provided herein, upon administration of the compound to a human subject, the Cmax of the compound is achieved within about 2 to about 4 hours post-dose. In some embodiments, upon administration of the compound to a human subject, the elimination half life of the compound is about 16 to about 34 hours. In some embodiments, the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 30 mg to about 800 mg per day. In some embodiments, the accumulation of the compound is about 1.25 to about 4.0 fold at steady state when the compound is dosed once daily. In some embodiments, the compound is in an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy) phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the weight ratio of the compound to microcrystalline cellulose in the admixture is between about 1:1.5 to 1:15, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to sodium stearyl fumarate in the admixture is between about 5:1 to about 50:1, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose.

Also provided herein are articles of manufacture or kits comprising (a) any one of the capsules provided herein, and (b) a package insert or a label indicating that the capsule is useful for treating myelofibrosis in a subject. Also provided herein are articles of manufacture or kits comprising (a) any one of the unit dosage forms provided herein, and (b) a package insert or a label indicating that the capsule is useful for treating myelofibrosis in a subject. In some embodiments, there is provided an article of manufacture or kit comprising (a) an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy) phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) microcrystalline cellulose, and (iii) sodium stearyl fumarate, and (b) a package insert or a label indicating that the admixture is useful for treating myelofibrosis in a subject.

Also provided herein are articles of manufacture or kits comprising (a) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl] amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and (b) a package insert or a label indicating that the compound can be used for treating myelofibrosis in a subject, wherein the subject is negative 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.

Also provided herein are articles of manufacture or kits comprising (a) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and (b) a package insert or a label indicating that the compound can be used for treating myelofibrosis in a subject, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy is a treatment with a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]ami- no}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

Also provided herein are articles of manufacture or kits comprising (a) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino} pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and (b) a package insert or a label indicating that the compound can be used for ameliorating bone marrow cellularity and/or bone marrow fibrosis.

Also provided herein are articles of manufacture or kits comprising (a) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and (b) a package insert or a label indicating that the compound can be used for improving pruritus associated with myelofibrosis.

Also provided herein are articles of manufacture or kits comprising a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and a package insert or a label indicating that the compound can be used for treating myelofibrosis in a subject, and that subject should discontinue the treatment upon indication of elevated levels of one or more enzymes or molecules selected from the group consisting of: amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine in the serum of the subject, and/or upon indication of one or more hematologic condition selected from the group consisting of anemia, thrombocytopenia, and neutropenia. In some embodiments, the package insert or the label further indicates that the compound can be discontinued without prior dose reduction. In some embodiments, the one or more of the elevated levels of the enzymes or molecules are Grade 4 events. In some embodiments, the one or more of the hematologic conditions are Grade 4 events.

In some embodiments, the package insert or the label is in a position which is visible to prospective purchasers. In some embodiments, the compound is in a unit dosage form or capsule form.

In some embodiments, the package insert or the label indicates that, upon administration of the admixture to a human subject, the Cmax of the compound is achieved within about 2 to about 4 hours post-dose. In some embodiments, the package insert or the label indicates that, upon administration of the compound to a human subject, the elimination half life of the compound is about 16 to about 34 hours. In some embodiments, the package insert or the label indicates that the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 30 mg to about 800 mg per day. In some embodiments, the package insert or the label indicates that the accumulation of the compound is about 1.25 to about 4.0 fold at steady state when the compound is dosed once daily. In some embodiments, the compound is in an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino] benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the weight ratio of the compound to microcrystalline cellulose

in the admixture is between about 1:1.5 to 1:15, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to sodium stearyl fumarate in the admixture is between about 5:1 to about 50:1, and wherein the weight for the compound is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose.

In some embodiments, there is provided use of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the compound is in an admixture of (i) the compound, (ii) an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate). In some embodiments, the compound is administered orally. In some embodiments, the use is according to a method described herein.

In some embodiments, there is provided use of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject is negative 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, there is provided use of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy comprises a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the use is according to a method described herein.

In some embodiments, there is provided a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the compound is in an admixture of (i) the compound, (ii) an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate). In some embodiments, the compound is administered orally. In some embodiments, the treatment is according to a method described herein.

In some embodiments, there is provided a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject is negative 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, there is provided a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy comprises a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the treatment is according to a method described herein.

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the compositions and methods provided herein. These and other aspects of the compositions and methods provided herein will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows decrease in palpable spleen size by cycle for patients treated with TG101348 680 mg/day (starting dose) (N=37). Doses for cycle 1 were 520-800 mg/day and doses for cycles 2-6 were 360-680 mg/day. For cycle 6≥50% subjects, there was 22-47% increase in 3 subjects with drug held for ~2-3 weeks immediately prior to measurement.

FIG. **2** shows WBC count in subjects treated with TG101348. The baseline WBC count was $>11\times10^9$/L. The doses at follow-up ranged from 360 to 680 mg/day. Last follow-up visit ranged from 8 to 24 weeks (median 24 weeks). "ULN" means upper limit of normal.

FIG. **3** shows platelet count in subjects treated with TG 101348. The baseline platelet count$>450\times10^9$/L. The doses at follow-up ranged from 360 to 680 mg/day. Last follow-up visit ranged from 12 to 24 weeks (median 24 weeks). "ULN" means upper limit of normal.

FIG. **4** shows the percentages of subjects with worsened, unchanged, improved or resolved constitutional symptoms (fatigue, early satiety, cough, night sweats, and pruritus) in subjects treated with TG101348. Last visit ranged from 4 to 24 weeks (median 20 weeks). The data here reflected changes from symptoms present at baseline. 18 subjects reported new onset of ≥1 symptom during the study; of these, symptoms for 12 subjects were resolved by last follow-up visit. Severity was rated by subjects on a scale of 1-10: 0=absent; 1-3=mild; 4-7=moderate; 8-10=severe. Improved=downgrade to absent or to mild or moderate from more severe rating at baseline.

FIG. **5** shows the cytokine levels (IL-6, IL-8, IL-2 and TNF-α) in subjects treated with TG101348. The values shown are median values.

FIG. **6** shows the change in V617F allele burden from baseline as a proportion of baseline in subjects with baseline>20% (N=22) treated with TG101348. The figure shows the subset of JAK2V617F positive subjects in the overall population (N=48). The doses at follow-up were 360 to 680 mg/day. Last follow-up visit ranged from 20 to 72 weeks (median 24 weeks).

FIG. **7** shows the bone marrow cellularity at baseline (60% cellularity) and after 18 cycles of TG101348 treatment (5-10% cellularity) in a 76-year-old male subject with

V617F negative PMF. The starting dose was 30 mg/day and the dose at follow-up was 520 mg/day.

FIG. **8** shows the bone marrow fibrosis at baseline (3+) and after 18 cycles of TG101348 treatment (0) in a 56-year-old male subject with V617F negative PMF. The starting dose was 240 mg/day and the dose at follow-up was 440 mg/day.

FIG. **9** shows various measurements of a subject with JAK2 V617F-positive PMF treated with TG101348 (starting dose at 680 mg/day).

FIGS. **10**A-**10**G show distribution of TG101348 doses at the end of each cycle for subjects who initiated dosing at 30 mg/day, 60 mg/day, 120 mg/day, 240 mg/day, 360 mg/day, 520 mg/day, and 800 mg/day, respectively, (n=25).

FIG. **11** shows distribution of TG101348 doses at the end of each cycle for subjects who initiated dosing at 680 mg/day (n=34).

FIG. **12**A shows plot of mean plasma TG101348 concentrations versus time on a semi-log scale (Cycle 1, Day 1). FIG. **12**B shows plot of mean plasma TG101348 concentrations versus time on a semi-log scale (Cycle 1, Day 28).

FIG. **13** shows splenomegaly response to TG101348 therapy. This figure shows decrease in palpable spleen size from baseline by cycle for subjects in the maximum tolerated dose cohort (n=37). The proportion of subjects with ≥50% and 100% decrease in palpable splenomegaly is shown. For subjects who completed 6 cycles of treatment, 90% had a ≥25% reduction in palpable spleen size, 66% had a ≥50% reduction, and in 31% the spleen became non palpable.

FIGS. **14**A-**14**C show effects of TG101348 on symptoms of myelofibrosis. (A): Proportion of subjects in maximum tolerated dose cohort with complete resolution of early satiety by cycle from a baseline symptom score of "mild" (score=11-3), "moderate" (score=4-7), or "severe" (score=8-10). Twenty-seven (79%) and 19 (56%) patients were evaluable for improvement in early satiety at the end of 1 and 6 cycles, respectively. After 2 cycles of treatment, 56% reported complete resolution of this symptom with durable benefit. (B): Proportion of subjects in maximum tolerated dose cohort with complete resolution of fatigue by cycle from a baseline symptom score of "mild" (score=1-3), or improvement in or complete resolution of fatigue from a baseline score of "moderate" (score=4-7) or "severe" (score=8-10). Twenty-four (71%) and 16 (47%) patients were evaluable for improvement in fatigue at the end of 1 and 6 cycles, respectively. After 6 cycles, 63% reported improvement and 25% had complete resolution of this symptom. (C): Proportion of subjects in maximum tolerated dose cohort with complete resolution of night sweats by cycle from a baseline symptom score of "mild" (score=1-3), "moderate" (score=4-7), or "severe" (score=8-10). Fourteen (40%) and 9 (26%) patients were evaluable for improvement in night sweats at the end of 1 and 6 cycles, respectively. After 1 cycle, 64% of subjects had complete resolution of this symptom; after 6 cycles, this proportion had increased to 89%.

FIG. **15** shows response of leukocytosis to TG101348 therapy. Changes in white blood cell (WBC) count after 6 cycles for subjects who entered the study with leukocytosis (WBC count>11×10^9/L). Following 6 cycles, 16 subjects across doses (57%) and 13 subjects in the MTD cohort (72%) achieved a normal WBC count, with durable benefit.

FIGS. **16**A-**16**D show effect of TG101348 therapy on JAK2V617F allele burden. Box plot representation of JAK2V617F allele burden data for all mutation-positive subjects (n=51; figures A and B) and for the subgroup with baseline allele burden>20% (n=23; FIGS. **16**C and **16**D). The y-axis represents the JAK2V617F allele burden from 1.0 (100%) to 0.0 (0%). The change in JAK2V617F allele burden per cycle of treatment (up to end of cycle 12; i.e. C13D1) as compared to pre-study baseline is shown for the 2 groups (FIGS. **16**A and **16**C); the change at the end of cycle 6 (i.e. C7D1) and cycle 12 is shown in FIGS. **16**B and **16**D. A significant decrease in JAK2V617F allele burden as compared to pre-study baseline was observed at the end of cycle 6 for the mutation-positive group (FIG. **16**B; p=0.04) and the subgroup with baseline allele burden>20% (FIG. **16**D; p=0.002); a similar significant decrease was seen at the end of cycle 12 for the former (FIG. **16**B; p=0.01) and latter (FIG. **16**D; $p=0.00^2$) groups. The Wilcoxon matched-pair signed-rank test was used to compare the median JAK2V617F allele burden for the comparisons.

FIG. **17** shows absolute changes in pro-inflammatory cytokine levels from baseline at cycle 6: IL-6 (A), TNF-α (B), IL-8 (C), and IL-2 (D). Absolute differences in IL-6 (−4719 pg/mL) and IL-2 (−1827 pg/mL) are omitted from FIGS. **17**A and **17**D, respectively, for 1 subject (101-039) because they skewed presentation of data for other subjects.

FIG. **18** shows a plot of mean plasma TG101348 concentrations versus time on a linear plot after once daily oral doses (Cycle 1; Day 28).

DETAILED DESCRIPTION

I. Definitions

As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. Beneficial or desired clinical results can include, but are not limited to, one or more of the following: decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals. In some embodiments, for the treatment of myelofibrosis, beneficial clinical results include one or more of reduction of splenomegaly, improvement in constitutional symptoms (such as early satiety, fatigue, night sweats, cough, and pruritus), reduction of leukocytosis, reduction of thrombocytosis, decrease of JAK2V617F allele burden, reduction of bone marrow fibrosis, and/or reduction of bone marrow cellularity.

As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as myelofibrosis) or symptoms of the disease, and can include "progression free survival". This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease.

As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results can include, for example, one or more results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results can include, include, for

example one or more clinical results such as decreasing one or more symptoms and pathological conditions resulting from or associated with the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of myelofibrosis, an effective amount of a drug may have the effect in reducing one or more of splenomegaly, improving constitutional symptoms (such as early satiety, fatigue, night sweats, cough, and pruritus), reducing leukocytosis, reducing thrombocytosis, decreasing JAK2V617F allele burden, reducing bone marrow fibrosis, and/or reducing bone marrow cellularity. An effective dosage can be administered in one or more administrations. An effective dosage of drug, compound, or pharmaceutical composition can be, for example, an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

As used herein, "ameliorating" bone marrow cellularity or bone marrow fibrosis refers to reducing the level of bone marrow cellularity or bone marrow fibrosis in a subject compared to the level of bone marrow cellularity or bone marrow fibrosis prior to commencing treatment with the compound provided herein. The reduction of bone marrow cellularity or bone marrow fibrosis can be at least by 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90%.

As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" can refer to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

As used herein, a "patient" or a "subject" refers to a mammal including a human, a dog, a horse, a cow or a cat, etc.

The term "pharmaceutically acceptable" refers to the fact that the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and can be administered to a subject.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise.

Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X."

It is understood that aspects and variations of the compositions and methods provided herein can include "consisting" and/or "consisting essentially of" aspects and variations.

II. Compounds and Pharmaceutical Compositions

Provided herein is a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}-pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. Also provided herein are pharmaceutical compositions comprising N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxyphenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and a pharmaceutically acceptable excipient or carrier. The compound and the pharmaceutical compositions described herein can be used for treating or delaying development of myelofibrosis in a subject. N-tert-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide has the following chemical structure:

The compound provided herein may be formulated into therapeutic compositions as natural or salt forms. Pharmaceutically acceptable non-toxic salts include the base addition salts (formed with free carboxyl or other anionic groups) which may be derived from inorganic bases such as, for example, sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, or ferric hydroxide, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, procaine, and the like. Such salts may also be formed as acid addition salts with any free cationic groups and will generally be formed with inorganic acids such as, for example, hydrochloric acid, sulfuric acid, or phosphoric acid, or organic acids such as acetic acid, citric acid, p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, tartaric acid, mandelic acid, and the like.

Salts of the compounds provided herein can include amine salts formed by the protonation of an amino group with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like. Salts of the compounds provided herein can also include amine salts formed by the protonation of an amino group with suitable organic acids, such as p-toluenesulfonic acid, acetic acid, methanesulfonic acid and the like. Additional excipients which are contemplated for use in the practice of the compositions and methods provided herein are those available to those of ordinary skills in the aft, for example, those found in the United States Pharmacopeia Vol. XXII and National Formulary Vol. XVII, U.S. Pharmacopeia Convention, Inc., Rockville, Md. (1989), the relevant contents of which are incorporated herein by reference.

In addition, the compounds provided herein can include polymorphs. The compound described herein may be in alternative forms. For example, the compound described herein may include a hydrate form. As used herein, "hydrate" refers to a compound provided herein which is associated with water in the molecular form, i.e., in which the H—OH bond is not split, and may be represented, for example, by the formula $R.H_2O$, where R is a compound provided herein. A given compound may form more than one hydrate including, for example, monohydrates ($R.H_2O$)

or polyhydrates ($R.nH_2O$ wherein n is an integer greater than 1) including, for example, dihydrates ($R.2H_2O$), trihydrates ($R.3H_2O$), and the like, or fractional hydrates, such as, for example, $R.n/2H_2O$, $R.n/3H_2O$, $R.n/4H_2O$ and the like wherein n is an integer.

The compounds described herein may also include acid salt hydrate forms. As used herein, "acid salt hydrate" refers to a complex that may be formed through association of a compound having one or more base moieties with at least one compound having one or more acid moieties or through association of a compound having one or more acid moieties with at least one compound having one or more base moieties, said complex being further associated with water molecules so as to form a hydrate, wherein said hydrate is as previously defined and R represents the complex herein described above.

In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate and has the following chemical structure:

The pharmaceutical compositions for the administration of the compound described herein, either alone or in combination with other therapeutic agents, may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy and methods described in Examples 4, 5 and 6. Such methods can include bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the pharmaceutical compositions are prepared by uniformly and intimately bringing the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. In the pharmaceutical composition the active object compound is included in an amount sufficient to produce the desired effect upon the process or condition of diseases. The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as hard or soft capsules. The suitable capsule shell may be hard gelatin or hydroxypropylmethyl cellulose ("HPMC").

Provided herein are formulations comprising (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) one or more excipients, and (iii) one or more lubricants. The formulations may be in capsule form and administered orally. The formulations may be in unit dosage form. In some embodiments, the excipient is lactose (such as Fast-Flo), mannitol (such as Parteck M200), microcrystalline cellulose ("MCC") (such as Avicel PH102), MCC (such as ProSolv 90 HD). In some embodiments, the lubricant is magnesium stearate, sodium stearyl fumarate (such as Pruv), or sodium laurel fumarate. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. In some embodiments, the capsule is hard gelatin capsule.

In some embodiments, there is provided a capsule suitable for oral administration comprising an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino] benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate), wherein the admixture is contained in the capsule. Methods known in the art and described herein may be used for making the capsules. See, e.g., Example 3. Microcrystalline cellulose may be used as a filler and/or diluent in the capsules provided herein. Sodium stearyl fumarate may be used as a lubricant in the capsules provided herein. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. For example, silicified microcrystalline cellulose may be composed of microcrystalline cellulose and colloidal silicon dioxide particles. In some embodiments, the silicified microcrystalline cellulose is a combination of 98% microcrystalline cellulose and 2% colloidal silicon dioxide.

In some embodiments, the capsule contains about 10 mg to about 680 mg of the compound, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the capsule contains about 10 mg to about 650 mg (or about 10 mg to about 550 mg or about 10 mg to about 500 mg), wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the capsule contains about 100 mg to about 600 mg (or about 200 mg to about 550 mg or about 300 mg to about 500 mg), wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the capsule contains about 10 mg, about 20 mg, about 40 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, or about 650 mg of the compound, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the capsule is a hard gelatin capsule. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

In some embodiments, the weight ratio of the compound to excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose) in the capsule is between about 1:1.5 to about 1:15 (e.g., between about 1:5 to about 1:10, between about 1:5 to about 1:12, or between about 1:10 to about 1:15), wherein the weight of the compound is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to lubricant (e.g., sodium stearyl fumarate) in the capsule is between about 5:1 to about 50:1 (e.g., between about 5:1 to about 10:1, between about 5:1 to about 25:1, between about 5:1 to about 40:1, between about 7:1 to about 34:1, or between about 8:1 to about 34:1), wherein the weight of the compound is the free base moiety weight of the compound.

In some embodiments, the capsule contains about 5% to about 50% (e.g., about 5% to about 10% or about 5% to about 35%) compound of the total fill weight of the capsule, wherein the weight of the compound is the free base moiety weight of the compound. In some embodiments, the capsule contains about 40% to about 95% (e.g., about 50% to about 90% or about 60% to about 90%) excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellu-

lose) of the total fill weight of the capsule. In some embodiments, the capsule contains about 0.2% to about 5% (e.g., about 0.2% to about 2% or about 0.5% to about 1.5%, or about 0.5%, about 1%, or about 1.5%) lubricant (e.g., sodium stearyl fumarate) of the total fill weight of the capsule.

Also provided herein are unit dosage forms comprising an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) an excipient (such as microcrystalline cellulose), and (iii) a lubricant (such as sodium stearyl fumarate). Any one of the capsules described herein may be used in a unit dosage form. In some embodiments, the unit dosage form is for treating myelofibrosis. In some embodiments, the treatment is according to a method described herein.

In some embodiments, the unit dosage form comprises an admixture of (i) about 10 mg to about 680 mg (or about 10 mg to about 500 mg) of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl] amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the specified weight is the free base moiety weight of the compound, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate. In some embodiments, the compound in the admixture is about 10 mg to about 500 mg, wherein the specified weight is the free base moiety weight of the compound.

In some embodiments, the unit dosage form is in the form of a capsule, and the admixture is contained in the capsule. In some embodiments, the unit dosage form comprises about 10 mg, about 20 mg, about 40 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, or about 650 mg of the compound, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy) phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the admixture comprises (i) about 10 mg (or about any of 40 mg, 100 mg, 200 mg, 300 mg, 400 mg, or 500 mg) of the compound, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate, wherein the specified weight is the free base moiety weight of the compound. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

In some embodiments, the weight ratio of the compound to excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose) in the unit dosage form is between about 1:1.5 to about 1:15 (e.g., between about 1:5 to about 1:10, between about 1:5 to about 1:12, or between about 1:10 to about 1:15), wherein the weight of the compound is the free base moiety weight of the compound. In some embodiments, the weight ratio of the compound to lubricant (e.g., sodium stearyl fumarate) in the unit dosage form is between about 5:1 to about 50:1 (e.g., between about 5:1 to about 10:1, between about 5:1 to about 25:1, between about 5:1 to about 40:1, between about 7:1 to about 34:1, or between about 8:1 to about 34:1), wherein the weight of the compound is the free base moiety weight of the compound. In some embodiments, the microcrystalline cellulose is silicified microcrystalline cellulose. In some embodiments, the silicified microcrystalline cellulose is a combination of 98% microcrystalline cellulose and 2% colloidal silicon dioxide.

In some embodiments, the lubricant (e.g., sodium stearyl fumarate) is about 0.1% to about 10%, about 0.5% to about 5%, about 0.5% to about 3%, about 0.5% to about 2%, about 0.75% to about 1.5% of the capsule fill weight. In some embodiments, the lubricant (e.g., sodium stearyl fumarate) is at least about any one of 0.1%, 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, or 5% of the capsule fill weight. In some embodiments, the lubricant (e.g., sodium stearyl fumarate) is about any one of 0.1%, 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, or 5% of the capsule fill weight.

In some embodiments, the weight ratio of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof to an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose) in a capsule or unit dosage form is about 40:60 to about 10:90. In some embodiments, the weight ratio of the compound to an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose) in a capsule of unit dosage form is about any one of 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, or 5:95. In some embodiments, the weight ratio of the compound to an excipient (e.g., microcrystalline such as silicified microcrystalline cellulose) is about 1:1.5 to about 1:9.5, such as about any of 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, 1:5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, or 1:9.5. In some embodiments, the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

In some embodiments, the capsule contains about 5% to about 50% (e.g., about 5% to about 10% or about 5% to about 35%) compound of the total weight of the admixture, wherein the weight of the compound is the free base moiety weight of the compound. In some embodiments, the capsule contains about 40% to about 95% (e.g., about 50% to about 90% or about 60% to about 90%) microcrystalline cellulose (such as silicified microcrystalline cellulose) of the total weight of the admixture. In some embodiments, the capsule contains about 0.2% to about 5% (e.g., about 0.2% to about 2% or about 0.5% to about 1.5%, or about 0.5%, about 1%, or about 1.5%) sodium stearyl fumarate of the total weight of the admixture.

In some embodiments, the capsule or unit dosage form contains an admixture of about 12 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 122 mg of silicified microcrystalline cellulose, and about 1 mg of sodium stearyl fumarate. In some embodiments, the capsule or unit dosage form contains an admixture of about 47 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 448 mg of silicified microcrystalline cellulose, and about 5 mg of sodium stearyl fumarate. In some embodiments, the capsule or unit dosage form contains an admixture of about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. In some embodiments, the capsule or unit dosage form contains an admixture of about 235 mg of N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)

phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 357 mg of silicified microcrystalline cellulose, and about 6.00 mg of sodium stearyl fumarate.

Also provided herein are oral solution formulations comprising a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the oral solution formulation further comprises methylcellulose. In some embodiments, the oral solution formulation further comprises methylcellulose and Tween 80. In some embodiments, the oral solution formulation comprises the compound at about 1 mg/ml to about 25 mg/ml, about 2 mg/ml to about 20 mg/ml, about 3 mg/ml to about 15 mg/ml, about 5 mg/ml to about 10 mg/ml. In some embodiments, the oral solution formulation comprises the compound at about any one of 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 6.25 mg/ml, 6.5 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, or 12.5 mg/ml, or 15 mg/ml. In some embodiments, the oral solution formulation comprises about 0.1% to about 5%, 0.2% to about 3%, about 0.25% to about 2%, about 0.25% to about 1%, or about 0.5% by weight of methylcellulose. In some embodiments, the oral solution formulation comprises about 0.01% to about 0.5%, 0.02% to about 0.3%, about 0.025% to about 0.2%, about 0.025% to about 0.1%, or about 0.05% by weight of Tween 80.

In some embodiments, the capsule does not comprise an absorption enhancer. In some embodiments, the capsule comprises an absorption enhancer (e.g., Vitamin E TPGS, Gelucire 44/14, Pluronic F127, or glyceryl monostearate).

A capsule or unit dosage form provided may comprise one or more of the following properties: (1) upon administration to a subject such as human subject, the Cmax of the compound is achieved within about 2 to about 4 hours post-dose; (2) upon administration to a human subject, the elimination half life of the compound is about 16 to about 34 hours; (3) the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 30 mg to about 800 mg per day; (4) the accumulation of the compound is about 1.25 to about 4.0 fold at steady state when the compound is dosed once daily.

Also provided are methods of preparing a capsule drug product comprising a) blending a lubricant with a compound that is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof to generate granules and b) mixing the granules of a) with an excipient. In some embodiments, the lubricant is sodium stearyl fumarate. In some embodiments, the excipient is microcrystalline cellulose such as silicified microcrystalline cellulose. Such method may be used to prepare a capsule or unit dosage form described herein. The weight (such as weight ratio or weight percentage) and components regarding the compound, excipient, and/or lubricant may be according to any described herein.

III. Methods of Treatment and Prevention of Myelofibrosis

Provided herein are methods for treating, delaying development, and/or preventing myelofibrosis in a subject comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino] benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof (e.g., N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate). 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. 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. In some embodiments, the compound is in an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) an excipient (such as microcrystalline cellulose), and (iii) a lubricant (such as sodium stearyl fumarate).

Myelofibrosis that may be treated by the compounds described herein includes primary myelofibrosis (MF) 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 compounds described herein also includes myelofibrosis of high risk, intermediate risk such as intermediate risk level 2. Methods for diagnosing various types of myelofibrosis are known in the art. See, e.g., Cervantes et al., Blood 2009. 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 a point mutation from valine 617 to phenylalanie in the Janus kinase 2 (JAK2 kinase) (JAK2V617F) if the subject is a human, or a point mutation corresponding to the valine 617 to phenylalanie 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 phenylalanie 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 treated with the methods described herein has previously received 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 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 14 days and discontinued the treatment for at least 30 days. In some embodiments, the previous therapy is a treatment with a compound 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. In some embodiments, the dose of the compound in the second treatment is the same or lower than the dose in the previous therapy.

The subject may be treated orally and/or daily. The subject (such as a human) may be treated by administering at a dose of about 240 mg per day to about 680 mg per day (or about 300 mg per day to about 500 mg per day), wherein the specified weight is the free base moiety weight of the compound. In some embodiment, the compound is administered at a dose of about any of 240 mg/day, 250 mg/day, 300 mg/day, 350 my/day, 400 mg/day, 450 mg/day, 500 mg/day, 550 mg/day, 600 mg/day, 650 mg/day, or 680 mg/day. The compound may be in a capsule and/or a unit dosage form described herein. In some embodiments, the compound administered is in an admixture with a microcrystalline cellulose and sodium stearyl fumarate, and the admixture is in a capsule. In some embodiments, the compound is administered orally.

Also provided herein are methods for ameliorating one or more symptoms associated with myelofibrosis. For example, the treatment using the compound described herein is effective in reducing spleen size, ameliorating constitutional symptoms (such as early satiety, fatigue, night sweats, cough, and pruritus), reducing leukocytosis, reducing thrombocytosis, decreasing JAK2V617F allele burden, reducing bone marrow fibrosis, improving pruritus, improving cachexia, and/or reducing 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 compound provided herein. 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 pruritus after treatment.

In some embodiments, the compound is administered to the subject daily for at least 1 cycle, at least 2 cycles, at least 3 cycles, at least 4 cycles, at least 5 cycles, or at least 6 cycles of a 28-day cycle. In some embodiments, the compound is administered to the subject daily 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 daily for at least one month, at least two month, at least three month, at least four month, at least five month, at least six month, at least eight month, or at least one year. In some embodiments, the compound is administered once a day.

In some embodiments, upon administration of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof to a subject such as human subject, the Cmax of the compound is achieved within about 1 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 12 to about 40 hours, about 16 to about 34 hours, or about 20 to about 30 hours. In some embodiments, the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 30 mg to about 800 mg per day. 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 daily.

In some embodiments, the method comprises instructing the subject to ingest the effective amount of the compound on an empty stomach. In some embodiments, the methods further comprise instructing the subject to avoid ingesting agents that are at least moderate inducers or inhibitors of CYP3A4. In some embodiments, the subject does not receive concomitant treatment with or use of drugs to herbal agents known to be at least moderate inhibitors or inducers of CYP3A4. Based on in vitro evaluations, N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide is metabolized by human CYP3A4. Agents that may increase N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide plasma concentrations (i.e., CYP3A4 inhibitors) or decrease N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide plasma concentrations (i.e., CYP3A4 inducers), including herbal agents and foods (e.g. grapefruit/grapefruit juice), should be avoided in subjects being treated as described herein. In addition, in vitro data have indicated that N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide inhibits CYP3A4 in a time-dependent fashion. Agents that are sensitive substrates for metabolism by CYP3A4 should be used with caution as coadministration with N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide may result in higher plasma concentrations of the coadministered agent. A list of clinically relevant substrates of CYP3A4 include alfentanil, Cyclosporine, Diergotamine, ethinyl estradiol, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, clarithromycin erythromycin, telithromycin, alprazolam, diazepam, midazolam, triazolam, indinavir, ritonavir, saquinavir, prokinetic, cisapride, astemizole, chlorpheniramine, amlodipine, diltiazem, felodipine, nifedipine, verapamil, atorvastatin, cerivastatin, lovastatin, simvastatin, aripiprazole, gleevec, haloperidol, sildenafil, tamoxifen, taxanes, trazodone, and Vincristine. A list of clinically relevant inducers of CYP3A4 include carbamazepine, phenobarbital, phenyloin, pioglitazone, rifabutin, rifampin, St. John's wort, and troglitazone. A list of clinically relevant inhibitors of CYP3A4 include indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, erythromycin, grapefruit juice, verapamil, diltiazem, cimetidine, amiodarone, fluvoxamine, mibefradil, and Troleandomycin. See reference Flockhart et al., http://medicine.iupui.edu/clinpharm/ddis/clinicaltable.aspx., 2009.

Also provided herein are methods of monitoring treatment of myelofibrosis to a subject, comprising (a) administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof; (b) monitoring a hematologic parameter and/or a non-hematologic parameter in the subject; and (c) determining if the subject should continue or discontinue with the treatment. In some embodiments, the hematologic parameter is selected from the group consisting of anemia, thrombocytopenia, and neutropenia. In some embodiments, the non-hematologic parameter is an enzyme or molecule in the

blood or serum wherein an elevated level of the enzyme or molecule is indicative of tissue or organ damage. In some embodiments, the serum enzyme or molecule can be, for example, amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, alkaline phosphatase, and calcium. Methods of monitoring these parameters are known in the art and are described herein. See Examples 1-3. In some embodiments, the method further comprises administering to the subject an effective amount of the compound described herein after the subject has been discontinued with the treatment for at least 2 week, at least 3 weeks, or at least 4 weeks. In some embodiments, the previous treatment has been discontinued without prior dose reduction.

Also provided herein are methods of monitoring treatment of myelofibrosis to a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy) phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and discontinuing the treatment upon indication of elevated levels of one or more enzymes or molecules selected from the group consisting of amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine and/or decreased level of calcium in the blood or serum of the subject without prior dose reduction. Also provided herein are methods of monitoring treatment of myelofibrosis to a subject, comprising administering to the subject an effective amount of a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy) phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, and discontinuing the treatment upon indication of one or more hematologic conditions selected from the group consisting of anemia, thrombocytopenia, and neutropenia without prior dose reduction. In some embodiments, the treatment is discontinued when one or more of the parameters (including hematologic and non-hematologic parameters) are grade 3 or 4 events.

Grade 3 or 4 adverse events for hematologic and non-hematologic parameters are known in the art and shown in the Table below. See, e.g. C Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, Published: May 28, 2009 (v4.03: Jun. 14, 2010).

RESPONSE (hematologic and non-hematologic)	Definition	Grade 3	Grade 4
Hyperlipasemia	A finding based on laboratory test results that indicate an increase in the level of lipase in a biological specimen.	>2.0-5.0 × ULN*	>5.0 × ULN
Serum amylase	A finding based on laboratory test results that indicate an increase in the levels of amylase in a serum specimen.	>2.0-5.0 × ULN	>5.0 × ULN
Alanine aminotransferase increased	A finding based on laboratory test results that indicate an increase in the level of alanine aminotransferase (ALT or SGPT) in the blood specimen.	>5.0-20.0 × ULN	>20.0 × ULN
Aspartate aminotransferase increased	A finding based on laboratory test results that indicate an increase in the level of aspartate aminotransferase (AST or SGOT) in a blood specimen.	>5.0-20.0 × ULN	>20.0 × ULN
Blood creatinine increased	A finding based on laboratory test results that indicate increased levels of creatinine in a biological specimen.	>3.0 baseline; >3.0	6.0 × ULN >6.0 × ULN
Blood alkaline phosphatase increased	A finding based on laboratory test results that indicate an increase in the level of alkaline phosphatase in a blood specimen.	>5.0-20.0 × ULN	>20.0 × ULN
Hypocalcemia	A disorder characterized by laboratory test results that indicate a low concentration of calcium (corrected for albumin) in the blood.	Corrected serum calcium of <7.0-6.0 mg/dL; <1.75-1.5 mmol/L; Ionized calcium <0.9-0.8 mmol/L; hospitalization indicated	Corrected serum calcium of <6.0 mg/dL; <1.5 mmol/L; Ionized calcium <0.8 mmol/L; life-threatening consequences
Anemia	A disorder characterized by a reduction in the amount of hemoglobin in 100 ml of blood. Signs and symptoms of anemia	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated	Life-threatening consequences; urgent intervention indicated

-continued

RESPONSE (hematologic and non-hematologic)	Definition	Grade 3	Grade 4
	may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.		
Thrombocytopenia	a platelet count below the normal range for the population ([+ or −] 2 standard deviations). In most laboratories, a normal platelet count is between 150,000 to 450,000/μL	25,000 to <50,000/μL	below 25,000/μL
Neutropenia	A finding based on laboratory test results that indicate a decrease in number of neutrophils in a blood specimen.	<1000-500/mm3; <1.0-0.5 × 10^9/L	<500/mm3; <0.5 × 10^9/L

*"ULN" refers to upper limit of normal.

IV. Articles of Manufactures and Kits

Also provided herein are articles of manufacture or kits containing a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the article of manufacture or the kit further includes instructions for using the compounds described herein in the methods provided herein. In some embodiments, the article of manufacture or the kit further comprises a label or a package insert providing the instructions. In some embodiments, the compound is in a capsule and/or a unit dosage form described herein.

In some embodiments, the article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic, and the container may hold the compound, for example in the formulation to be administered. The article of manufacture or the kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the compound. In some embodiments, the package insert or the label is in a position which is visible to prospective purchasers.

The label or package insert may further indicate that the compound is useful or intended for treating or preventing myelofibrosis in a subject. In some embodiments, the package insert or the label indicates that the compound can be used for ameliorating bone marrow cellularity and/or bone marrow fibrosis. In some embodiments, the package insert or the label indicates that the compound can be used for treating myelofibrosis in a subject, wherein the subject is negative for the valine 617 to phenylalanine mutation of human JAK2 (JAK2V617F) or negative for the mutation corresponding to the valine 617 to phenylalanine mutation of human JAK2. In some embodiments, the package insert or the label indicates that the compound can be used for treating myelofibrosis in a subject, and that subject should discontinue the treatment upon indication of elevated levels of one or more of amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and/or alkaline phosphatase and/or decreased level of calcium in the serum of the subject, and/or upon indication of one or more of anemia, thrombocytopenia, and/or neutropenia. In some embodiments, the package insert or the label further indicates that the compound can be discontinued without prior dose reduction.

In some embodiments, there is provided a kit or article of manufacture comprising (a) an admixture of (i) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, (ii) excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) lubricant (e.g., sodium stearyl fumarate), and (b) a package insert or a label indicating that the admixture is useful for treating myelofibrosis in a subject. In some embodiments, there is provided a kit or article of manufacture comprising (a) a compound which is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutical salt thereof or a hydrate thereof, and (b) a package insert or a label indicating that the compound can be used for treating myelofibrosis in a subject, wherein the subject has previously received another myelofibrosis therapy with a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

In some embodiments, the package insert or a label indicates that, upon administration of the compound to a human subject, the Cmax of the compound is achieved within about 1 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, the package insert or a label indicates that, upon administration of the compound to a human subject, the elimination half life of the compound is about 12 to about 40 hours, about 16 to about 34 hours, or about 20 to about 30 hours. In some embodiments, the mean AUC of the compound increases more than proportionally with increasing doses ranging from about 30 mg to

about 800 mg per day. 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 daily.

In some embodiments, the package insert or the label instructs the subject to ingest the effective amount of the compound on an empty stomach. In some embodiments, the package insert or the label instructs the subject to avoid ingesting agents that are at least moderate inducers or inhibitors of CYP3A4. In some embodiments, the inducer or inhibitor of CYP3A4 is any one of the inducers or inhibitors of CYP3A4 described herein.

Also provided are uses of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the use is according to a method described herein. In some embodiments, the compound is in an admixture of (i) the compound, (ii) an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate). In some embodiments, the compound is administered orally. In some embodiments, the use is according to a method described herein. In some embodiments, there is provided use of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject is negative 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, there is provided use of a compound in the manufacture of a medicament for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy comprises a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

Also provided is a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof. In some embodiments, the treatment is according to a method described herein. In some embodiments, the compound is in an admixture of (i) the compound, (ii) an excipient (e.g., microcrystalline cellulose such as silicified microcrystalline cellulose), and (iii) a lubricant (e.g., sodium stearyl fumarate). In some embodiments, the compound is administered orally. In some embodiments, the treatment is according to a method described herein. In some embodiments, there is provided a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject is negative 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, there is provided a compound for treating myelofibrosis in a subject, wherein the compound is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof, wherein the subject has previously received another myelofibrosis therapy. In some embodiments, the previous therapy comprises a JAK2 inhibitor which is not N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide or a pharmaceutically acceptable salt thereof or a hydrate thereof.

The following are examples of the methods and compositions provided herein. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLES

Example 1 Evaluation of TG101348 in Myelofibrosis

As used herein, "TG101348" refers to N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate. The subjects in this study were administered with capsule form of TG101348 as described in Example 5. TG101348 was evaluated in a Phase I study for the treatment of myelofibrosis. This study was ongoing at the time the data were collected.

Background:

TG101348 is a potent, orally bioavailable, JAK2-selective small molecule inhibitor, that was evaluated in a Phase I study for the treatment of myelofibrosis. The dose-limiting toxicity was asymptomatic grade 3 or 4 amylasemia/lipasemia that was reversible, and the maximum tolerated dose ("MTD") was 680 mg. The most frequent non-hematological toxicities were mild nausea, vomiting, and/or diarrhea that were easily controlled or resolved spontaneously. Grade 3/4 neutropenia and thrombocytopenia were observed in 14% and 25% of patients, respectively. TG101348 had activity in reducing spleen size, leukocyte count, and JAK2V617F ("VF") allele burden. This example describes the results with a focus on the data from the dose confirmation cohort who initiated treatment at a dose of 680 mg/day.

Results:

Fifty nine patients (median age=66 years; range 43-86) were treated—28 in the dose escalation phase, and 31 in the dose confirmation phase. Overall, 44 patients had PMF, 12 post-PV MF, and 3 post-ET MF; 86% were VF-positive. Median palpable spleen size was 18 cm and 22 patients were red blood cell ("RBC") transfusion-requiring at study enrollment. After a median follow-up of 12 weeks (range<1-76), 18 (31%) patients discontinued treatment due to toxicity (n=7; thrombocytopenia=3, neutropenia=1), comorbidities (n=5), withdrawal of consent (n=4), or non-compliance/lack of response (1 each). The remaining 41 patients were at the following dose levels when the data in this example were collected: 680 mg (n=14), 520-600 mg (n=16), 360-440 mg (n=10), and 240 mg (n=1). The cumulative drug exposure to the time when the data in this example were collected was

362 patient-months; exposure at or above MTD (≥680 mg) was 154 patient-months. Forty patients (68%) started treatment at ≥680 mg.

Toxicity:

TG101348 was well tolerated. Of the patients who started at ≥680 mg, Gr3/4 neutropenia was observed in 15/0% and Gr3/4 thrombocytopenia in 20/10%. Twenty four (60%) patients did not require RBC transfusions at baseline (median hemoglobin ("Hgb")=9.6 g/dL; range 7.4-13.1); of these, 42% and 8% of patients developed Grade 3 ("Gr3") and Grade 4 ("Gr4") anemia, respectively. The majority of patients who started at ≥680 mg developed mild nausea (1 Gr3), vomiting (1 Gr3), and/or diarrhea (3 Gr3) that were self-limited or easily controlled. Other non-hematological toxicities included Grade 1/2 ("Gr1/2") transaminitis (38%), Gr1/2 serum creatinine elevation (38%), and asymptomatic hyperlipasemia (33%).

Efficacy:

Thirty three patients who started at ≥680 mg completed at least 3 cycles of treatment; at 3 months, reduction in palpable spleen size (baseline median=18 cms; range 6-32) was at least 50% in 22 (67%) patients; the spleen became non-palpable in 9 (27%) patients. All 21 patients with leukocytosis at baseline (WBC range 11 to 203×10^9/L) who started at ≥680 mg experienced a marked reduction in their WBC count (range 4 to 90); 70% had a normal WBC count at their last follow-up visit. Overall, 48 of the 51 VF-positive patients completed at least 1 cycle and were evaluable for response in VF allele burden; at last available follow-up, the median decrease in granulocyte mutant allele burden was 48%; 21 (44%) patients had a ≥50% reduction, and in the group who started treatment at ≥680 mg, 48% have had a ≥50% reduction. Of those evaluable, there was clinically significant benefit or resolution of constitutional symptoms, including early satiety, fatigue, cough, pruritus, and night sweats.

Conclusions:

TG 101348 was well tolerated in patients with myelofibrosis. Spleen and lcukocyte responses were frequent, observed early, and produced substantial clinical benefit for patients. These responses were associated with significant decrease in VF allele burden and pointed to activity of TG101348 against the malignant clone in myelofibrosis.

Example 2 Evaluation of TG101348 in Myelofibrosis

The subjects in this study were administered with capsule form of TG101348. TG101348 was evaluated in a Phase I study for the treatment of myelofibrosis. This study is also described in Example 1. This example describes data available at the time of data collection.

This study was an open-label, multicenter, and dose-escalation study with expanded cohort dose confirmation at MTD. The primary objective of this study was to determine safety/tolerability, DLT, MTD, and pharmacokinetics of TG101348 in subjects with MF. The secondary objective of this study was to evaluate preliminary clinical and pharmacodynamic activity.

The key eligibility criteria for subjects included: Myelofibrosis (PMF or post-PV/ET MF); High-risk or intermediate-risk with symptomatic splenomegaly/unresponsive to available therapy; ECOG performance status ≤2; ANC $\geq1\times10^9$/L; Platelet count $\geq50\times10^9$/L; Serum creatinine ≤2 mg/dL; Total bilirubin ≤2 mg/dL; AST/ALT ≤3× upper limit of normal.

The subject disposition for this study is included in Table 1.

TABLE 1

Subject Disposition

	MTD*	Overall
Enrolled	40	59
Included in safety analysis	40	59
Included in drug activity analysis	37	55
Discontinued	11 (28%)	15 (25%)
Reasons for discontinuation		
Adverse event	5	6
Subject withdrew consent	4	6
Investigator discretion	2	3
Median (range) treatment duration	24 weeks (1-24 weeks)	24 weeks (0.3-84 weeks)**

*Includes all subjects who initiated treatment at 680 or 800 mg/day.

**Includes continued treatment in extension study.

The demographic and baseline characteristics for the subjects are included in Table 2.

TABLE 2

Demographic and Baseline Characteristics

	MTD (n = 40)	Overall (N = 59)
Age (median; years)	65 (43-85)	64 (43-85)
Male	22 (55%)	34 (58%)
JAK-2^{V617F} positive	35 (88%)	51 (86%)
PMF	31 (78%)	44 (75%)
Post-PV MF	6 (15%)	12 (20%)
Post-ET MF	3 (8%)	3 (5%)
High risk	20 (50%)	26 (44%)
Palpable splenomegaly	39 (98%)	58 (98%)
Transfusion dependent	16 (40%)	22 (37%)

This study was a dose-escalation study with expanded cohort dose confirmation at MTD. Below describes the data with a focus on the dose confirmation cohort who initiated treatment at a dose of 680 mg/day.

The decrease in palpable spleen size by cycle for subjects treated with TG101348 680 mg/day (starting dose) (N=37) is shown in FIG. **1**. The baseline spleen size was: median=18 cm; range=6-32 cm. 49% of subjects achieved clinical improvement based on reduction of palpable splenomegaly (IWG response) (56% of subjects by 12 weeks; 100% of subjects by 20 weeks). There was no relapse or disease progression at the time of data collection.

The effect of TG101348 on leukocytosis is shown in FIG. **2**. The baseline WBC count was $>11\times10^9$/L. 73% of subjects had normal WBC counts at their follow-up visit. The effect of TG101348 on thrombocytosis is shown in FIG. **3** (baseline platelet count$>450\times10^9$/L). TG101348 was able to reduce platelet counts. The effects of TG101348 on constitutional symptoms (baseline versus last visit) are shown in FIG. **4**. TG101348 was able to improve the MF-associated constitutional symptoms. TG101348 had no significant changes on cytokine levels (see FIG. **5**, all values shown are medians). FIG. **6** shows the effect of TG101348 on V617F allele burden in subjects with baseline>20% (N=22). FIG. **6** shows that TG101348 was able to decrease JAK2 V617F allele burden in 59% of the subjects with baseline>20%.

FIG. **7** shows the effects of TG101348 on bone marrow cellularity in a 76-year-old male with V617F negative PMF. The starting dose was 30 mg/day and the dose at follow-up

was 520 mg/day. FIG. **7** shows that TG101348 was able to reduce bone marrow cellularity in this subject from 60% bone marrow cellularity at baseline to 5-10% bone marrow cellularity after 18 cycles. FIG. **8** shows the effect of TG101348 on bone marrow fibrosis in a 56-year-old male with V617F negative PMF. The starting dose was 240 mg/day and the dose at follow-up was 440 mg/day. FIG. **8** shows that TG101348 was able to reduce bone marrow fibrosis in this subject from 3+ at baseline to 0 after 18 cycles.

The treatment-emergent grade 3 & 4 hematologic toxicities in MTD Subjects (N=40) is shown in Table 3. The treatment-emergent non-hematologic adverse events (reported for at least 5 subjects) in MTD Subjects (N=40) is shown in Table 4.

TABLE 3

Treatment-Emergent Grade 3 & 4 Hematologic Toxicities in MTD Subjects (N = 40)

Neutropenia (N = 40)		Thrombocytopenia (N = 40)		Anemia (N = 24)* New Transfusion
Grade 3	Grade 4	Grade 3	Grade 4	Requirement on Study‡
6 (15%)	0	8 (20%)	5 (13%)	16 (67%)

*Subjects who were not transfusion dependent at baseline.
‡Transfusion on at least 2 occasions for hemoglobin ("Hb") <10 g/dL.

TABLE 4

Treatment-Emergent Non-Hematologic Adverse Events in MTD Subjects (N = 40)

Event	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal disorders				
Diarrhea	21 (53%)	4 (10%)	5 (13%)	0
Nausea	20 (50%)	6 (15%)	2 (5%)	0
Vomiting	20 (50%)	7 (18%)	1 (3%)	0
Constipation	6 (15%)	1 (3%)	0	0
Abdominal pain	5 (13%)	0	0	0
Other				
Anorexia	7 (18%)	0	1 (3%)	0
Edema peripheral	7 (18%)	1 (3%)	0	0
Fatigue	2 (5%)	3 (8%)	1 (3%)	0
Contusion	5 (13%)	0	0	0
Headache	4 (10%)	1 (3%)	0	0
Proteinuria	2 (5%)	3 (8%)	0	0

The grade ≥2 treatment-emergent non-hematologic laboratory findings in MTD subjects (N=40) is shown in Table 5.

TABLE 5

Grade ≥2 Treatment-Emergent Non-Hematologic Laboratory Findings in MTD Subjects (N = 40)

Finding	Grade 2	Grade 3	Grade 4
Creatinine increased	11 (28%)	0	0
Hypocalcemia	8 (20%)	3 (8%)	0
AST increased	5 (13%)	1 (3%)	0
ALT increased	8 (20%)	2 (5%)	0
Hyperkalemia	3 (8%)	2 (5%)	1 (3%)
Hyperlipasemia	4 (10%)	3 (8%)	2 (5%)
Hyperamylasemia	0	1 (3%)	1 (3%)

Laboratory findings were transient and reversible, and resolved spontaneously or following dose interruption and/or reduction.

FIG. **9** shows various measurements in a subject with JAK2V617F-positive PMF that started at TG101348 680 mg/day. TG101348 was able to reduce the palpable spleen size from 9 cm to 0 cm and led to complete resolution of pruritus in this subject.

Conclusions:

TG101348 was generally well tolerated, with manageable, grade 1 gastrointestinal effects, especially at higher doses. The data indicated no long-term toxicities. The expected on-target myelosuppressive effect appeared to be mostly limited to erythropoiesis, which may be attenuated at lower, but still effective, doses. TG101348 had remarkable activity in MF-associated splenomegaly: ~two-thirds achieved ≥50% reduction in palpable splenomegaly; ~30% had complete response. TG101348 had significant anti-myeloproliferation activity with virtually all treated subjects experiencing complete resolution of leukocytosis and thrombocytosis. TG101348 had remarkable activity against MF-associated constitutional symptoms, pruritus and cachexia. TG101348 induced a significant decrease in JAK2V617F allele burden in a substantial proportion of treated subjects. TG101348 had minimal effect on serum levels of proinflammatory cytokines; this was consistent with the absence of immediate adverse cytokine-rebound phenomenon upon study drug discontinuation. Without wishing to be bound by any theory, the activity of TG101348 appeared to be a direct consequence of its JAK2 inhibitory activity and not an indirect effect from non-specific anti-cytokine activity. Furthermore, the preliminary observations showed reduction in BM cellularity and reticulinfibrosis with extended treatment.

Example 3 Evaluation of TG101348 in Myelofibrosis

The subjects in this study were administered with capsule form of TG101348.

Study Design:

The study constituted a Phase 1, dose-escalation trial (MF-TG101348-001). This study is also described in Examples 1 and 2. Study eligible patients were 218 years of age with high- or intermediate-risk primary myelofibrosis (PMF), post-PV MF, or post-ET MF (Tefferi A et al., Leukemia 22:14-22, 2008). Additional eligibility criteria and participating centers are listed in Table 6. All patients provided written informed consent. The primary endpoints were determination of safety and tolerability, dose-limiting toxicity ("DLT"), maximum tolerated dose ("MTD") and pharmacokinetic ("PK") behavior of TG101348. The secondary endpoint was assessment of therapeutic activity.

TABLE 6

Detailed enrollment criteria for MF-TG101348-001

Inclusion Criteria	Exclusion Criteria
1. Diagnosis of MF (PMF, post-PV MF, or post-ET MF) according to the revised WHO criteria.*	1. Any chemotherapy, immunomodulatory drug therapy, immunosuppressive therapy, corticosteroids >10 mg/day prednisone or equivalent, or growth factor treatment within 14 days (28 days in the case of darbepoetin) prior to initiation of TG101348.
2. High-risk MF (defined by Mayo PSS), or Mayo PSS intermediate-risk MF** accompanied by symptomatic splenomegaly and/or unresponsive to available therapy.	2. Major surgery or radiation therapy within 28 days prior to initiation of TG101348.
3. At least 18 years of age.	3. Concomitant treatment with agents known to inhibit or induce CYP3A4, unless approved by the sponsor.
4. Body weight ≥50 kg.	4. Known hypersensitivity to any ingredients in the study drug formulation.
5. ECOG performance status ≤2.	5. Active infection requiring antibiotics.
6. Within 4 days prior to initiation of TG101348: ANC ≥1 × 10^9/L Platelet count ≥50 × 10^9/L Serum creatinine ≤2.0 mg/dL Total bilirabin ≤2.0 mg/dL AST or ALT ≤3 times the ULN (unless clinically compatible with hepatic EMH)	6. Uncontrolled CHF (NYHA Classification 3 or 4), angina, MI, CVA, coronary/peripheral artery bypass graft surgery, TIA, or pulmonary embolism within 3 months prior to initiation of study drug.
7. Life expectancy ≥12 weeks.	7. Cardiac dysrhythmias requiring ongoing treatment, bundle branch block on ECG or QRS duration >120 ms, or prolongation of the QTc (Fridericia) interval to >450 ms for males or >470 ms for females.
8. Negative serum pregnancy test result for women of childbearing potential.	8. Pregnant or lactating females.
9. Absence of active malignancy other than MF, with the exception of adequately treated basal cell carcinoma and squamous cell carcinoma of the skin.	9. Women of childbearing potential, unless surgically sterile for at least 3 months (i.e., hysterectomy), postmenopausal for at least 12 months (FSH >30 U/mL), unless they agree to use effective, dual contraceptive methods (i.e., oral, injectable, or barrier method with male partner using a condom) while on study drug.
10. Provide written informed consent to participate.	10. Men who partner with a woman of childbearing potential, unless they agree to use effective, dual contraceptive methods (i.e., a condom, with female partner using oral, injectable, or barrier method) while on study drug.
11. Willing to comply with scheduled visits, treatment plans, laboratory assessments, and other study-related procedures.	11. Known HIV- or AIDS-related illness. 12. Clinically active hepatitis B or C. 13. Any severe, acute or chronic medical, neurological, or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration, may interfere with the informed consent process and/or with compliance with the requirements of the study, or may interfere with the interpretation of study results and, in the investigator's opinion, would make the patient inappropriate for entry into this study.

Abbreviations: AIDS = acquired immunodeficiency syndrome; ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; CHF = congestive heart failure; CVA = cerebrovascular accident; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EMH = extramedullary hematopoiesis; FSH = follicle stimulating hormone; HIV = human immunodeficiency virus; MF = myelofibrosis; MI = myocardial infarction; NYHA = New York Heart Association; PSS = prognostic scoring system; TIA = transient ischemic attack; WBC = white blood cell.

*Tefferi and Vardiman. Leukemia. 2008 January; 22(1): 14-22

**High-risk disease requires two and intermediate-risk disease requires one of the following prognostic factors: hemoglobin <10 g/dL, WBC count <4 or >30 × 10^9/L, platelet count <100 × 10^9/L, absolute monocyte count ≥1 × 10^9/L.

Patients were assigned to one of 8 dose cohorts, ranging from 30 to 800 mg per day, using standard 3+3 cohort design. TG101348 was administered orally once daily, with a treatment plan for continuous daily therapy for 24 weeks (6×28-day cycles). Intra-subject dose escalation was permitted after completion of at least 3 cycles of treatment at the starting dose. Once DLT was identified, a dose-confirmation cohort initiated treatment at the MTD. Treatment beyond 6 cycles was allowed on an extension study (MF-TG101348-002; NCT00724334) if deemed beneficial to the patient and if well tolerated.

Assessment of Toxicity and Response:

Safety assessments were performed weekly during cycle 1, every other week during cycles 2 and 3, and every 4 weeks thereafter. Toxicity was graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0.

Responses were measured every 4 weeks per International Working Group for MPN Research and Treatment (IWG-MRT) criteria (Tefferi A et al., Blood 108:1497-1503, 2006). Assessment of bone marrow histology was performed at baseline and every 24 weeks of therapy. Changes in JAK2V617F allele burden in the granulocyte fraction of peripheral blood were measured as previously described (Kittur J et al., Cancer 109:2279-2284, 2007); the assessments were at baseline and every 4 weeks during the first 6 cycles, and every 6th cycle of therapy in the extension study.

(Table 7). Forty-four subjects had PMF, 12 post-PV MF, and 3 post-ET MF; 86% were JAK2V617F-positive. The median duration of disease was 3.4 years (range 0.06 to 25.8). At study enrollment, the median palpable spleen size was 18 cm below the left costal margin (83% had a palpable spleen size >10 cm), median hemoglobin level was 9.2 g/dL (range 6.6 to 15.2) and 21 (36%) subjects were red cell transfusion-dependent by IWG-MRT criteria.

TABLE 7

Demographic and Baseline Subject Characteristics

	TG101348 Starting Dose (mg/day)								MTD	All
Characteristic	30 n = 4	60 n = 3	120 n = 3	240 n = 3	360 n = 3	520 n = 3	680 n = 34	800 n = 6	Cohort n = 40	Doses n = 59
Age - years	63.5	64.0	63.0	68.0	66.0	57.0	63.5	69.0	65.1 (10.47)†	64.5 (9.70)†
Range	55-76	56-66	53-71	55-79	61-71	50-66	43-83	50-85	43-85	43-85
Gender										
Male	2	3	1	2	2	2	18	4	22 (55.0%)	34 (57.6%)
Female	2	0	2	1	1	1	16	2	18 (45.0%)	25 (42.4%)
Race										
White	3	2	3	3	3	2	29	6	35 (87.5%)	51 (86.4%)
Black, African American	0	0	0	0	0	0	1	0	1 (2.5%)	1 (1.7%)
Asian	1	1	0	0	0	0	3	0	3 (7.5%)	5 (8.5%)
Other	0	0	0	0	0	1	1	0	1 (2.5%	2 (3.4%)
Diagnosis										
PMF	3	2	1	3	2	2	27	4	31 (77.5%)	44 (74.6%)
Post-PV MF	1	1	2	0	1	1	6	0	6 (15.0%)	12 (20.3%)
Post-ET MF	0	0	0	0	0	0	1	2	3 (7.5%)	3 (5.1%)
Risk Category (Mayo PSS)										
High	0	0	1	2	0	3	14	6	20 (50.0%)	26 (44.1%)
Not high*	4	3	2	1	3	0	20	0	20 (50.0%)	33 (55.9%)
$JAK2^{V617F}$ Positive	3	3	3	2	3	2	29	6	35 (87.5%)	51 (86.4%)
Transfusion Dependent	1	1	0	1	0	2	13	3	16 (40.0%)	21 (35.6%)
Spleen Size >10 cm	3	3	3	2	3	2	28	5	33 (82.5%)	49 (83.1%)

Abbreviations: ET, essential thrombocythemia; JAK, Janus kinase; MF, myelofibrosis; PMF, primary myelofibrosis; PV, polycythemia vera; PSS, prognostic scoring system.
*Equivalent to symptomatic/treatment refractory intermediate-risk disease.
†Mean (standard deviation)

Pharmacokinetics:

The concentration-time curves of TG101348 in plasma were evaluated by a non-compartmental analysis (with the use of WinNonlin® software, version 5.2).

Cytokine Assessment:

Samples for cytokine measurement were collected at baseline and every 4 weeks thereafter. Cytokine levels were measured using multiplexed sandwich ELISAs (Millipore, St. Charles, Mo.).

Results

Enrollment of Patients:

A total of 59 subjects were enrolled; 28 in the dose-escalation phase and 31 in the dose-confirmation phase

In the dose-escalation phase, the starting dose of TG101348 was 30 mg/day and subsequent dose levels were 60, 120, 240, 360, 520, 680 and 800 mg/day (Table 7). At 800 mg/day, 2 of 6 patients experienced DLT; consequently, the MTD was declared at 680 mg/day. In the dose-confirmation phase, all patients started treatment at the MTD. The "MTD cohort" (n=40; Table 7) included patients who received 680 mg/day as their starting dose (dose-escalation cohort, n=3; dose-confirmation cohort, n=31) and those whose drug dose was decreased from 800 mg/day (n-=6) to 680 mg/day after MTD was declared.

The median (range) exposure to TG101348 for the overall (n=59) and MTD (n=40) cohorts was 155 (2-172) and 147 (8-171) days, respectively. TG101348 doses at the end of each cycle per dose cohort are illustrated in FIGS. **10** and **11**. In the MTD cohort, 28 subjects (70%) required dose-reduction during the first 6 cycles; the primary reasons were: cytopenia(s) (20%), gastrointestinal adverse events (12.5%), amylase/lipase elevation (10%), ALT elevation (7.5%), investigator discretion (7.5%), or other adverse events (12.5%). The median cycle at dose-reduction for the MTD cohort was cycle 3 (range 1-7); the median (range) dose at the end of cycle 3 was 680 mg/day (360-680 mg/day); and 520 mg/day (360-680 mg/day) at the end of cycle 6.

Forty three (73%) subjects, including 28 (70%) from the MTD cohort, continued treatment on the extension study; at entry into the extension study, 31 (72%) subjects were receiving <680 mg/day of the drug (median 520 mg/day; range 120-680 mg/day). At data cutoff, the median (range) cumulative exposure to TG101348 for the 43 subjects was 380 days (170-767). The number of treatment cycles completed ranged from 7-29; 39 subjects (66%), including 27 (68%) from the MTD cohort completed 12 treatment cycles. At data cutoff, 28%, and 14% of subjects who entered the extension study had completed 18 and 24 treatment cycles, respectively. The median (range) treatment dose during the extension phase was 440 mg/day (120-680 mg/day).

Pharmacokinetics:

Peak plasma concentration of TG101348 was achieved 1-4 hours after dosing. TG101348 showed greater than dose-proportional increases in plasma PK parameters (C_{max} and AUC_{0-4}) (Table 8 and FIG. **12**). Mean steady-state C_{max} and AUC_{0-t} values increased approximately 54- and 88-fold, respectively, over a 27-fold increase in dose. The terminal phase half-life at steady state remained similar across all doses (16 to 34 hours), consistent with linear drug elimination. FIG. **18** shows a plot of mean plasma TG101348 concentrations versus time on a linear plot after once daily oral doses (Cycle 1; Day 28). The figure shows the IC50, IC90, and 3 times IC90 (3×IC90) values for TG101348 in relation to the plasma concentration of TG101348 over time. A dose of 520 mg/day exhibited a plasma concentration TG101348 that was above 3×IC90 over the course of at least 24 hours after the dose was administered. A dose of 360 mg/day exhibited a Cmax above 3×IC90 and a plasma concentration of TG101348 that was above IC90 over the course of at least 24 hours after the dose was administered.

Safety Profile:

The DLT in 2 of 6 patients treated at 800 mg/day was asymptomatic grade 3 or 4 hyperamylasemia (with or without hyperlipasemia) that was reversible. The most common non-hematologic adverse events at least possibly related to TG101348 included predominantly grade 1 nausea, diarrhea and vomiting; grade 3 events were reported overall/in the MTD cohort for 3%/5%, 10%/13%, and 3%/3% of subjects, respectively, and there were no Grade 4 events (Table 9). These adverse events were dose-dependent, with grade 3 occurrences observed almost exclusively with a TG101348 starting dose of ≥680 mg/day. The gastrointestinal symptoms were largely self-limited or controlled by symptomatic treatment and/or dose reduction. Other adverse events (Grades 3/4; overall/MTD cohort) included asymptomatic increases in serum lipase (10%/15%), AST (2%/3%), ALT (7%/8%), creatinine (0%/0%) and alkaline phosphatase (0%/0%) (Table 9).

TABLE 9

Treatment-Emergent Non-Hematologic Adverse Events Considered at Least Possibly Related to TG101348 and Reported for ≥10% of Subjects

	MTD Cohort (n = 40)		All Subjects (n = 59)	
Adverse Events	Severity Grade 1-2	Severity Grade 3-	Severity Grade 1-	Severity Grade 3-4
Gastrointestinal disorders				
Nausea	31	2 (5.0%)	39	2 (3.4%)
Diarrhea	25	5	32	6 (10.2%)
Vomiting	27	1 (2.5%)	32	2 (3.4%)
Abdominal pain	4 (10.0%)	0	6 (10.2%)	0
General disorders				
Anorexia	6 (15.0%)	0	8 (13.6%)	0
Edema peripheral	4 (10.0%)	0	6 (10.2%)	0
Abnormal laboratory values				
Hyperlipasemia	9 (22.5%)	6	10	6 (10.2%)
Alanine aminotransferase increased	9 (22.5%)	3 (7.5%)	11	4 (6.8%)
Aspartate aminotransferase increased	13	1 (2.5%)	15	1 (1.7%)
Blood creatinine increased	11	0	14	0

TABLE 8

Mean (SD) plasma pharmacokinetic parameters following multiple daily doses of TG101348 (Cycle 1, Day 28) in MF-TG101348-001

	Dose/Day							
Parameter	30 mg (n = 3)	60 mg (n = 3)	120 mg (n = 3)	240 mg (n = 3)	360 mg (n = 3)	520 mg (n = 3)	680 mg (n = 27)	800 mg (n = 5)
C_{max} (ng/mL)	81.85 (95.630)	257.33 (121.138)	556.67 (135.500)	1796.67 (648.254)	1717.33 (1558.705)	3886.67 (3560.707)	3064.07 (1129.671)	4380.00 (1764.809)
T_{max}* (hr)	2.00 (0.5, 4.0)	1.00 (1.0, 4.0)	2.00 (0.5, 4.0)	2.00 (2.0, 2.1)	2.00 (2.0, 4.0)	4.00 (4.0, 4.0)	4.00 (0.0, 8.3)	2.25 (2.0, 4.0)
$AUC_{(0-t)}$ (hr*ng/mL)	806.76 (806.973)	2426.53 (1048.264)	7645.69 (2810.740)	26193.40 (11767.460)	23879.05 (16898.162)	61749.22 (57240.295)	55111.68 (25702.038)	70840.97 (32668.886)
$T_{1/2}$ (hr)	20.94 (7.039)	15.68 (3.464)	24.42 (8.434)	20.77 (6.238)	21.39 (7.090)	20.94 (5.006)	33.71 (33.674)	23.99 (9.674)
λz (1/hr)	0.0354 (0.01016)	0.0456 (0.00918)	0.0305 (0.00932)	0.0352 (0.00903)	0.0353 (0.01309)	0.0343 (0.00723)	0.0301 (0.01421)	0.0331 (0.01321)

*T_{max} is presented as median (min, max)

SD indicates standard deviation; C_{max}, peak plasma concentration; T_{max}, the time to the maximal concentration; $AUC_{(0-t)}$, area under the concentration-time curve from time zero to the last measurable concentration; $T_{1/2}$, terminal half-life; and λz, the elimination rate constant.

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TABLE 9-continued

Treatment-Emergent Non-Hematologic Adverse Events Considered at Least Possibly Related to TG101348 and Reported for ≥10% of Subjects

	MTD Cohort (n = 40)		All Subjects (n = 59)	
Adverse Events	Severity Grade 1-2	Severity Grade 3-	Severity Grade 1-	Severity Grade 3-4
Blood alkaline phosphatase increased	9 (22.5%)	0	10	0
Hypocalcemia	6 (15.0%)	1 (2.5%)	7 (11.9%)	1 (1.7%)
Skin and subcutaneous tissue disorders				
Skin exfoliation	8 (20.0%)	0	8 (13.6%)	0
Dry skin	6 (15%)	0	6 (10.2%)	0

Grade 3/4 hematological adverse events considered related to TG101348 included anemia (35% of 37 subjects who were not transfusion dependent at baseline), thrombocytopenia (24%) and/or neutropenia (10%) (Table 10). The majority of treatment-emergent cytopenias were noted in the first three cycles of treatment. Of the 13 subjects who developed grade 3/4 anemia (all in the MTD cohort), 67% entered the study with grade 2 anemia. Emergence of transfusion requirement was significantly lower for subjects who initiated treatment at 240-520 mg/day (33%) as opposed to 680 mg/day (72%). Of the 14 subjects with grade 3/4 thrombocytopenia, 4 and 5 subjects entered the study with grade 1 and 2 thrombocytopenia, respectively.

TABLE 10

Treatment-Emergent Hematologic Adverse Events Considered at Least Possibly Related to TG101348 and Reported for ≥10% of Subjects

	MTD Cohort (n = 40)		All Subjects (n = 59)	
	Severity Grade 1-2	Severity Grade 3-4	Severity Grade 1-2	Severity Grade 3-4
Anemia*	2 (8.3%)	13 (54.2%)	3 (8.1%)	13 (35.1%)
Thrombocytopenia	8 (20.0%)	11 (27.5%)	10 (17.0%)	14 (23.7%)
Neutropenia	2 (5.0%)	4 (10.0%)	2 (3.4%)	6 (10.2%)

*Events reported only for subjects who were not transfusion dependent at study entry (MTD Cohort, n = 24; All Subjects, n = 37) are presented.

At data cutoff, no unique safety findings have emerged with continued dosing of TG101348 beyond 6 cycles of therapy.

Serious adverse events considered at least possibly related to TG101348 occurred in 8 subjects and included asymptomatic hyperlipasemia, thrombocytopenia/neutropenia, depression, tumor lysis syndrome, cerebrovascular accident, and dehydration (Table 11). One subject discontinued treatment due to Grade 4 thrombocytopenia; all other events were reversible and subjects were able to resume treatment at a lower dose after resolution of the adverse event.

TABLE 11

Serious Adverse Events Assessed by Investigators as at Least Possibly Related to Therapy (MF-TG101348-001 and MF-TG101348-002)

Subject #	Event	Starting Dose/Dose at Event (mg/day)	Onset From Start of Dosing (days)	CTCAE Severity Grade	Action Taken With Study Drug	Outcome
105-013	Thrombocytopenia	240/360	215	4	None	Recovered/resolved
	Thrombocytopenia	240/360	247	4	Permanently discontinued	Not recovered/not resolved
	Hyperlipasemia	240/0	356	4	None	Recovered/resolved
104-015	Depression	360/520	256	3*	Permanently discontinued	Not recovered/not resolved
106-024	Nausea	800/680	87	2	Stopped temporarily	Recovered/resolved
	Vomiting	800/680	87	2	Stopped temporarily	Recovered/resolved
	Diarrhea	800/680	87	3	Stopped temporarily	Recovered/resolved
	Dehydration	800/680	87	2	Stopped temporarily	Recovered/resolved
	Tumor lysis syndrome	800/440	366	3	Stopped temporarily	Recovered/resolved
	Dehydration	800/400	474	3	None	Recovered/resolved
106-033	Pleuritic pain	680/680	8	2	Stopped temporarily	Recovered/resolved
106-045	Dehydration	680/440	170	3	Stopped temporarily	Recovered/resolved
101-047	Neutropenia	680/680	52	2	Stopped temporarily	Recovered/resolved
105-056	Cerebrovascular accident	680/680	22	4	Stopped temporarily	Recovered/resolved
	Gallbladder pain	680/520	95	3	Stopped temporarily	Recovered/resolved with sequelae
105-059	Hyperlipasemia	680/680	8	3	Stopped temporarily	Recovered/resolved
	Hyperlipasemia	680/520	28	3	Stopped temporarily	Recovered/resolved
	Cardiac arrest	680/360	42	5	Permanently discontinued	Fatal

*Subject died (suicide) approximately 12 weeks after discontinuation of study drug.

One subject presented with severe pulmonary hypertension and right heart failure during cycle 4 (at 240 mg/day); the event was considered unrelated to TG101348 per the investigator.

Fifteen (25%) subjects discontinued treatment during the first 6 cycles of therapy (Table 12). Reasons for discontinuation included treatment-related adverse events (n=6); investigator decision/intercurrent illness (n=3) or withdrawal of consent (n=6). Eight of 43 subjects (19%) discontinued treatment during the extension study, including 3 because of adverse events following a total of 24 to 46 weeks on therapy (Table 12).

TABLE 12

Subjects discontinuing study due to death, toxicity, withdrawal of consent, or intercurrent illness

MF-TG101348-001 Reasons for Discontinuation (Table 12 A)

Subject	Starting Dose (mg/day)	Dose at Termination (mg/day)	Duration of Treatment (days)	Reason
102-002	30	30	2	Investigator discretion - previously undiagnosed cardiac condition with long QT_c interval
106-009	120	240	109	Patient withdrew consent
101-011	240	240	100	Patient withdrew consent
102-019	520	520	42	Adverse event - neutropenia (grade 3; probably related)
102-023	800	680	70	Investigator discretion - recurrent Waldenstrom' s macroglobulinemia
104-027	800	680	77	Adverse event - thrombocytopenia (grade 4; possibly related)
106-028	800	520	44	Adverse event - thrombocytopenia (grade 4; possibly related)
104-029	680	680	44	Adverse event - endocarditis (grade 3; not related), embolic stroke (grade 3; not related)
101-032	680	680	8	Investigator discretion - Acquired factor VIII inhibitor
101-040	680	520	24	Adverse events - diarrhea (grade 3; possibly related)
103-043	680	360	68	Patient withdrew consent
103-046	680	680	26	Patient withdrew consent
102-051	680	600	108	Patient withdrew consent
102-054	680	680	75	Patient withdrew consent
105-059	680	360	27	Adverse event - cardiac arrest (grade 5; possibly related)

MF-TG101348-002 Reasons for Discontinuation (Table 12B)

Subject	Starting Dose (mg/day)	Dose at Termination (mg/day)	Cumulative Duration of Treatment (days)	Reason
101-005	60	360	196	Investigator discretion - lack of response to treatment
106-010	120	520	185	Investigator discretion
105-013	240	360	321	Adverse event - thrombocytopenia (grade 4; probably related)
104-015	360	520	257	Adverse event - depression (grade 3; possibly related)
106-016	360	680	527	Investigator discretion - lack of response to treatment
104-017	520	200	309	Investigator discretion - disease progression
105-021	680	520	357	Patient withdrew consent
101-047	680	320	233	Adverse event - elevated creatinine (grade 2; possibly related)

Three subjects had disease progression (doses at study start and discontinuation are indicated): one each with progressive hepatosplenomegaly and ascites with superimposed endocarditis (cycle 2; 680 and 520 mg/day), accelerated myelofibrosis (cycle 13; 520 and 200 mg/day), and leukemic transformation (cycle 2; 520 and 520 mg/day).

Responses are shown below.

Splenomegaly:

The onset of spleen response was rapid, and generally seen within the first 2 cycles. By cycle 6, 36 subjects (61%) had experienced a minimum 25% decrease in palpable spleen size, including 65% in the MTD cohort (intent-to-treat analysis). By this time-point, a ≥50% decrease in palpable spleen size persistent for at least 8 weeks (i.e. Clinical Improvement ("CI") per IWG-MRT criteria) had been observed in 39% and 45% of subjects overall and in the MTD cohort, respectively. Spleen responses per treatment cycle for the MTD cohort are shown in FIG. **13**. Three of 4 subjects (75%) with JAK2V617F-negative MF who completed 6 cycles of treatment achieved CI. The lowest starting dose at which CT was observed was 240 mg/day. The median time (range) to CI across doses was 141 days (41 to

171), and 113 days (41-170) for the MTD cohort. By cycle 12, spleen responses (CI) were observed in 48% and 50% of subjects, for the overall and MTD cohorts, respectively. The mean (standard deviation) duration of spleen response per IWG-MRT criteria was 315 (±129) days and 288 (±76) days for the overall and MTD cohorts, respectively.

Constitutional Symptoms:

Thirty five subjects in the MTD cohort endorsed the presence and severity of early satiety, fatigue, night sweats, cough, and pruritus on an 11-point scale (0=absence of symptoms to 10=worst imaginable symptoms) at baseline and at the end of at least one cycle. Symptoms were categorized as "absent" (score=0), "mild" (score=1-3), "moderate" (score=4-7), or "severe" (score=8-10).

Early satiety was reported by 29 (85%) subjects at baseline. After 2 cycles of treatment (n=27), 56% reported complete resolution of this symptom (FIG. **14**A). Fatigue was reported at baseline by 26 (76%) subjects. After 6 cycles (n=16), 63% reported improvement and 25% complete resolution of this symptom (FIG. **14**B). Night sweats were reported at baseline by 14 (40%) subjects. After 1 cycle, 64% of subjects had complete resolution of this symptom; after 6 cycles, this proportion had increased to 89% (n=9) (FIG. **14**C). Cough was reported at baseline by 13 (37%) subjects. After 1 cycle (n=12), 75% reported improvement and 67% complete resolution of this symptom. Pruritus was reported by 8 (23%) subjects at baseline. After 1 cycle, 75% had improvement, with 50% reporting complete resolution. Responses in constitutional symptoms were durable in most instances.

Body Weight:

At the end of 6 and 12 cycles, the median body weight was stable relative to baseline for the overall and MTD cohorts (Table 13).

normal platelet count; following 12 cycles, 7 of 8 subjects (88%) across doses and all 6 subjects in the MTD cohort had a normal platelet count.

JAK2V617F Allele Burden:

Fifty-one subjects (86%) were JAK2V617F-positive, with a median (range) allele burden of 20% (3%-100%); of these, 23 (45%) had a "significant" allele burden (defined as ≥20% at baseline) with a median (range) of 60% (23%-100%). For the overall mutation-positive subjects, there was a significant decrease in the JAK2V617F allele burden after 6 cycles (p=0.04) and 12 cycles of treatment (p=0.01) (FIGS. **16**A and **16**B). After 6 and 12 cycles of treatment, the median (range) allele burden was 17% (0%-100%) and 19% (0%-100%), respectively. Similarly, for the 23 subjects with baseline JAK2V617F allele burden of ≥20%, there was a significant and even more pronounced decrease in the JAK2V617F allele burden after 6 cycles ($p=0.00^2$) and 12 cycles of treatment (p=0.002) (FIGS. **16**C and **16**D). After 6 and 12 cycles of treatment, the median (range) allele burden was 31% (4%-100%) and 32% (7%-100%), respectively. After 6 cycles, 16 of 20 subjects (80%) with baseline allele burden>20% who reached this time-point exhibited a median 61% (range 6% to 96%) decrease, and 9 subjects (45%) had a ≥50% decrease in JAK2V617F allele burden. In contrast, 4 subjects (20%) exhibited an increase (18%, 21%, 30%, and 58%). Eighteen subjects (78%) of the group with allele burden ≥20% completed 12 cycles of treatment with a median 50% (range 29% to 82%) decrease, and 7 (39%) subjects had a ≥50% decrease in JAK2V617F. Three (17%) subjects exhibited an increase in allele burden (7%, 18%, and 22%), and 2 others with 100% allele burden at baseline exhibited no change.

TABLE 13

Change in weight during study treatment						
	Weight (kg)					
	Baseline		6 Cycles		12 Cycles	
	Overall (n = 57)	MTD Cohort (n = 38)	Overall (n = 43)	MTD Cohort (n = 28)	Overall (n = 36)	MTD Cohort (n = 26)
Median (range)	75.6 (48.2-105.2)	77.7 (48.2-96.1)	76.9 (51.4-105.8)	77.7 (51.4-97.6)	76.1 (49.8-106.8)	76.5 (49.8-99.5)
Change from baseline Median (range)	n/a	n/a	0.4 (−11.7-8.9)	0.6 (−9.2-8.9)	0.7 (−10.7-13.7)	0.35 (−10.7-13.7)

kg indicates kilograms; n, number, and MTD, maximum tolerated dose

Leukocytosis and Thrombocytosis:

Leukocytosis (WBC count>11×10^9/L) was present at baseline in 33 subjects (56%), 28 of whom completed 6 cycles of treatment; of these, 18 were in the MTD cohort. Following 6 cycles, 16 subjects across doses (57%) and 13 subjects in the MTD cohort (72%) achieved a normal WBC count (FIG. **15**); following 12 cycles, 14 of 25 (56%) across doses and 10 of 17 (59%) in the MTD cohort had normal WBC counts.

Thrombocytosis (platelet count>450×10^9/L) was noted at baseline for 10 (17%) subjects across doses and 7 (19%) in the MTD cohort (n=37), all of whom completed 6 cycles of therapy. At this time point, 90% and 100% of subjects across doses and in the MTD cohort, respectively, achieved a

Discussion:

A significant proportion of patients treated in this study experienced rapid, substantial, and durable control of symptomatic splenomegaly, leukocytosis, thrombocytosis, and constitutional symptoms. In addition, there was also evidence for a significant reduction in genomic disease burden that indicates potential for disease modifying activity. There were responses in MF patients who were JAK2V617F negative. It is unknown whether the subjects in this study have other mutations in the JAK-STAT signal transduction pathway such as MPL, LNK or as yet unknown alleles (Pardanani A D et al., Blood 108:3472-3476, 2006; Oh S T et al., Blood First Edition Paper, prepublished online Apr. 19, 2010; DOI 101182/blood-2010-02-270108 2010; Pardanani A et al., Leukemia In press: 2010).

The clinical study results show that TG101348 therapy can be discontinued without prior dose reduction or tapering. Subjects that were discontinued (whether or not recontinued at a later date) did not experience "cytokine rebound". This indicates that the treatment may be discontinued without prior dose reduction.

Cytokine rebound in the context of myelofibrosis is a phenomenon that has occurred in patients receiving therapy other than TG101348 therapy and were discontinued for any reason. In some cases, the discontinued patients experienced severe symptoms including acute spleen size enlargement and relapse of constitutional symptoms. In some cases, the discontinued patients experienced life-threatening hemodynamic disturbances (Wadleigh and Tefferi, Clinical Advances in Hematology & Oncology, 8:557-563, 2010).

Of note, among small molecule inhibitors of the JAK-STAT pathway in MF, TG101348 appeared to be unique in its ability to induce a significant and sustained decrease in JAK2V617F mutant allele burden. Without wishing to be bound by any theory, it appeared that the effect of JAK2 inhibition on disease burden was the basis for evidence of clinical efficacy in myelofibrosis with TG101348, as opposed to an indirect anti-cytokine effect that may play a major role in responses to JAK family antagonists that have off-target activity for JAK1 as well as for JAK2. In support of this, there were no consistent changes in levels of pro-inflammatory cytokines (interleukin ("IL")-6, IL-2, IL-8, and TNF-α) relative to baseline during the course of TG101348 treatment (FIG. **17**). In contrast, and consistent with the on-target activity of TG101348 for JAK2, increases in serum EPO and to a lesser extent TPO levels relative to baseline were observed after treatment initiation (data not shown).

The DLT (asymptomatic hypercramylasemia, sometimes with hyperlipasemia) for TG101348 was observed with other small molecule inhibitors including nilotinib (Kantarjian H M et al., Blood 110:3540-3546, 2007). Gastrointestinal adverse events were frequent in this study but accounted for treatment discontinuation in only one subject. These symptoms occurred as early as after the first administered dose, and demonstrated a clear dose-dependent relationship. The myelosuppressive effects of TG101348 were also dose-dependent.

While the MTD (680 mg/day) of TG101348 was the most efficacious dose, it was also associated with the highest incidence of adverse events. Therefore, a lower starting dose (e.g. 400-500 mg/day) may provide an optimal risk/benefit balance. Furthermore, because myelofibrosis is a heterogeneous disease, a dynamic dosing schedule may maximize the opportunity for identifying a patient-specific optimal dose.

These observations suggest that, in addition to MF, TG101348 may also have a potential role for the treatment of PV and ET.

Example 4 Synthesis of TG101348

Example 4.1 N-tert-Butyl-3-(2-chloro-5-methyl-pyrimidin-4-ylamino)-benzenesulfonamide (Intermediate)

Example 4.1(a)

A mixture of 2-chloro-5-methyl-pyrimidin-4-ylamine (1) (0.4 g, 2.8 mmol), 3-bromo-N-tert-butyl-benzenesulfonamide (2) (1.0 g, 3.4 mmol), $Pd_2(dba)_3$ (0.17 g, 0.19 mmol), Xantphos (0.2 g, 3.5 mmol) and cesium carbonate (2.0 g, 6.1 mmol) was suspended in dioxane (25 mL) and heated at reflux under the argon atmosphere for 3 h. The reaction mixture was cooled to room temperature and diluted with DCM (30 mL). The mixture was filtered and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc and hexanes added until solid precipitated. After filtration, the title compound (1.2 g, 98%) was obtained as a light brown solid. It was used in the next step without purification. MS (ES+): m/z 355 $(M+H)^+$.

Example 4.1(b)

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-continued

Intermediate

The Intermediate was synthesized from 2,4-dichloro-5-methylpyrimidine (SM1) and N-t-butyl-3-aminobenzenesulfonamide (SM2) in the following steps: (1) Mix MeOH (6.7 UOa) and SM1 (Combi Blocks) (UOa); (2) Add SM2 (1.15 UOa, 082 eq) and H2O (8.5 UOa); (3) Heat 45° C., 20 h, N_2, IPC CPL SM2<2%; (4) Cool 20° C.; (5) Centrifuge, N_2; (6) Wash H_2O (2.1 UOa)+MeOH (1.7 UOa); (7) Mix solid in H_2O (4.3 UOa)+MeOH (3.4 UOa); (8) Centrifuge, N_2; (9) Wash H_2O (2.1 UOa)+MeOH (1.7 UOa); and (10) Dry 45° C., vacuum, 15 h. Obtained Intermediate, mass 49.6 kg (UOb); Yield 79%; OP: 99.6%.

Example 4.2 N-tert-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide

Example 4.2(a)

A mixture of N-tert-Butyl-3-(2-chloro-5-methyl-pyrimidin-4-ylamino)-benzenesulfonamide (Intermediate) (0.10 g, 0.28 mmol) and 4-(2-pyrrolidin-1-yl-ethoxy)-phenylamine (3) (0.10 g, 0.49 mmol) in acetic acid (3 mL) was sealed in a microwave reaction tube and irradiated with microwave at 150° C. for 20 min. After cooling to room temperature, the cap was removed and the mixture concentrated. The residue was purified by HPLC and the corrected fractions combined and poured into saturated $NaHCO_3$ solution (30 mL). The combined aqueous layers were extracted with EtOAc (2×30 mL) and the combined organic layers washed with brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and the resulting solid dissolved in minimum amount of EtOAc and hexanes added until solid precipitated. After filtration, the title compound was obtained as a white solid (40 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d^6): δ 1.12 (s, 9H), 1.65-1.70 (m, 4H), 2.12 (s, 3H), 2.45-2.55 (m, 4H), 2.76 (t, J=5.8 Hz, 2H), 3.99 (t, J=6.0 Hz, 2H), 6.79 (d, J=9.0 Hz, 2H), 7.46-7.53 (m, 4H), 7.56 (s, 1H), 7.90 (s, 1H), 8.10-8.15 (m, 2H), 8.53 (s, 1H), 8.77 (s, 1H). MS (ES+): m/z 525 $(M+H)^+$.

Example 4.2(b)

N-tert-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate was prepared from 4-[2-(1-pyrrolidinyl)ethoxy]aniline dihydrochloride (SM3) and Intermediate following steps (A) and (B).

Step (A), preparation of free base of SM3 (3) from SM3, comprised steps (1)-(9): (1) Solubilize NaOH (0.42 UOb) in H2O (9 UOb); (2) Cool <20° C., N2; (3) Add TBME (6 UOb) then SM3 (Malladi Drugs) (1.06 UOb); (4) Mix>20 nm then stop; (5) Drain Aq Ph then extract by TBME (3 UOb); (6) Combine Or Ph; (7) Concentrate, vacuum, T<40° C., to an Oil; (8) Solubilize in IPA (2.5 UOb); and (9) Calculate dry extract 23%.

Step (B) comprised the steps (1)-(6): (1) Mix IPA (10.5 UOb) and Intermediate (UOb); (2) Add free base of SM3 (0.75 UOb, 1.33 eq/interm); (3) add HCl cone (0.413 UOb); (4) Heat 70° C., 20 h, N_2, IPC CPL Interm<2%; (5) Cool<20° C.; (2) Centrifuge, N_2; (3) Wash IPA (3 UOb); (4) Dry 50° C., vacuum, 26 h; (5) De-lump in Fitzmill; and (6) polybag (×2)/poly drum. Obtained TG101348 dihydrochloride monohydrate, mass 83.8 kg; Yield 98%; OP: 99.5%.

Example 5 Capsule Form of TG101348 and Process of Making TG101348

TG101348 drug products were provided as 10 mg, 40 mg, and 200 mg capsule strengths, where weights are specified for the amount of active (i.e., free base) moiety of TG101348. The quantitative composition of each strength of TG101348 drug product capsule is shown in Table 14.

TABLE 14

List of all components and unit formula for 10 mg, 40 mg, and 200 mg strengths of TG101348 drug product capsules

Component and Quality		Unit Formula		
Standard (and Grade, if Applicable)	Function	TG101348 10 mg Capsule	TG101348 40 mg Capsule	TG101348 200 mg Capsule
TG101348 (drug substance)*	Active ingredient	11.73 mg	46.90 mg	234.80 mg
Silicified Microcrystalline Cellulose (Prosolv SMCC 90HD ®)†	Filler/ Diluent	121.92 mg	448.10 mg	356.70 mg
Sodium Stearyl Fumarate (Pruv ®)	Lubricant	1.35 mg	5.00 mg	6.00 mg
Total Capsule Fill Weight	NA	135.00 mg	500.00 mg	597.50 mg
Hard Gelatin Capsule	Container	1 capsule (white opaque, size	1 capsule (white opaque, size	1 capsule (Swedish orange,

TABLE 14-continued

List of all components and unit formula for 10 mg, 40 mg, and 200 mg strengths of TG101348 drug product capsules

Component and Quality Standard (and Grade, if Applicable)	Function	Unit Formula TG101348 10 mg Capsule	TG101348 40 mg Capsule	TG101348 200 mg Capsule
		3, each capsule is 48 ± 3 mg)	00, each capsule is 118 ± 7 mg)	opaque, size 00, each capsule is 118 ± 7 mg)

*Adjusted to obtain full potency based on the purity of the TG101348 drug substance lot used.
†Adjusted to accommodate all component weights so as to ensure the total capsule fill weight is constant.
USP = United States Pharmacopoeia; NF = National Formulary; EP = European Pharmacopoeia; JP = Japanese Pharmacopoeia; NA = not applicable.

The components that were used in the manufacturing process for each capsule strength, on a per batch basis, are shown in Table 15.

TABLE 15

List of all components for manufacturing of the dosage forms

	Strength (Label Claim) TG101348 10 mg Capsule	TG101348 40 mg Capsule	TG101348 200 mg Capsule
Batch Size	1,620.000 g	6,000.000 g	5,975.000 g
Component and Quality Standard (and Grade, if Applicable)	Quantity per Batch (g)	Quantity per Batch (g)	Quantity per Batch (g)
INTRAGRANULAR COMPONENTS			
TG101348*	140.780	562.800	2,348.175
Silicified Microcrystalline Cellulose (Prosolv SMCC 90HD)†	214.160	856.800	3,567.075
Sodium Stearyl Fumarate (Pruv)	3.560	14.400	59.750
EXTRAGRANULAR COMPONENTS			
Silicified Microcrystalline Cellulose (Prosolv SMCC 90HD)	1248.860	4,520.400	Not included
Sodium Stearyl Fumarate (Pruv)	12.640	45.600	Not included
TOTAL OF INTRAGRANULAR COMPONENTS + EXTRAGRANULAR COMPONENTS			
Total Batch Weight	1, 620.000 g	6,000.000 g	5,975.000 g
CAPSULE SHELLS			
Capsule Shell Type	Hard gelatin	Hard gelatin	Hard gelatin
Capsule Size	Size 3	Size 00	Size 00
Capsule Color	White, opaque	White, opaque	Swedish orange, opaque
Total Batch Scale (Capsules)	12,000	12,000	10,000

*Adjusted to obtain full potency based on the purity of the TG101348 drug substance lot used.
†Adjusted to accommodate all component weights so as to ensure the total batch weight is constant.

The process for making TG101348 capsules is described below:

A. Dry granulation of intragranular components (implemented for all three drug product strengths): 1. TG101348 and intragranular sodium stearyl fumarate were blended within a V-blender for 5 minutes. 2. The blend was passed through a conical mill equipped with a round 18-mesh screen and round impeller. The blend was recharged into the V-blender. 3. Intragranular silicified microcrystalline cellulose was sifted through a 20-mesh screen and added to the blender. The mixture was blended for 15 minutes. 4. The blend was passed through a roller compactor. 5. The roller compacted ribbons were passed through a conical mill equipped with a round 16-mesh screen and round impeller. 6. The milled material was blended within the V-blender for 5 minutes. 7. In-process check (IPC) samples were withdrawn from the V-blender using a sample thief. Samples were subjected to potency analysis.

B. Addition of extragranular components (implemented for 10 mg and 40 mg capsules): 1. Where potency of granules (from Step 7 in A) was outside 98-102% (w/w) nominal, extragranular silicified microcrystalline cellulose was adjusted accordingly. 2. The V-blender was charged with TG101348 di-HCl monohydrate/silicified microcrystalline cellulose/sodium stearyl fumarate granules (from A). 3. The extragranular silicified microcrystalline cellulose was sifted through a 20-mesh screen and added to the V-blender. 4. The Extragranular sodium stearyl fumarate was added to the V-blender. 5. The intragranular and extragranular components were blended for 15 minutes. 6. IPC samples were withdrawn from the V-blender using a sample thief and analyzed for potency.

C. Capsule-filling (implemented for all three drug product strengths): 1. If potency (from Step 7 in A for the 200 mg capsules, or Step 6 in B for the 10 mg and 40 mg capsules)

was outside 98-102% (w/w) nominal, the capsule fill weight was adjusted accordingly. 2. The prepared material was encapsulated using automatic capsule filling machine. The prepared capsules were bottled and stores at 20-28° F. (68-82° C.) and ambient humidity.

Content uniformity and dissolution were examined. HPLC method validation was performed using a one-analyst, one-run-per-analyst design, and satisfied all required criteria for specificity, sensitivity, precision, accuracy, linearity, and sample stability. Specificity was evaluated and confirmed by comparing peak resolution between TG101348 and all of its related compounds, intermediates, and degradants (established from forced degradation studies). The limit of quantitation and limit of detection was established at 0.10 μg/mL and 0.03 μg/mL TG101348, respectively. Precision for content uniformity was evaluated via six injections of the mg and 200 mg strength capsules, prepared at the target assay concentration. RSD results were 3.7% and 5.8% for the 10 mg and 200 mg strength capsules, respectively. Precision for dissolution was evaluated via six injections at each dissolution timepoint of the 10 mg and 200 mg strength capsules. Relative standard deviation ("RSD") results for all strengths and corresponding time points were well within the acceptance criteria (±10%) specified in the validation protocol. Accuracy (defined by the recovery of the analyte spiked into a placebo solution for the 10 mg and 200 mg strength capsules) was evaluated at 70%, 100%, and 130% of the target assay standard concentration. Recovery values for all measurements were within the acceptance criteria (93%-105%) specified in the validation protocol. Linearity was demonstrated over the range of 50% to 120% of the target assay standard concentration, and exhibited an r2 of 1.00. Sample stability and method robustness were also demonstrated during method validation.

Example 6 Formulation Study for TG101348

The formulation study for N-tert-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide di-HCl monohydrate salt was conducted.

N-tert-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide di-HCl Monohydrate Salt Compatibility with Capsule Shells

It was unknown whether the caustic/acidic nature of N-tert-Rutyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide di-HCl monohydrate salt (TG101348 di-HCl monohydrate) would be incompatible with capsules due to the potential caustic/acidic nature of a di-HCl salt.

Hard gelatin and hydroxypropylmethyl cellulose (HPMC) capsule shells (size #00250) were filled with 250 mg of TG101348 di-HCl monohydrate. The filled capsules were placed on accelerated stability (40° C./75% relative humidity (RH) and 25° C./60% RH). The capsules were packaged into 30 mL (1 oz) amber high density polyethylene (HDPE) bottles. A summary of the formulations and accelerated stability protocol for the stability study is shown in Table 16.

TABLE 16

Formulations	(i) Size #00 Hard Gelatin (White Opaque) Capsule Filled with 250 mg [(1)] (ii) Size #00 Hard HPMC (White Opaque) Capsule Filled with 250 mg [(1)]			
Lot Numbers	191-031A: Gelatin capsule fills stored at 25° C./60% RH 191-031B: Gelatin capsule fills stored at 40° C./75% RH 191-031C: HPMC capsule fills stored at 25° C./60% RH 191-031D: HPMC capsule fills stored at 40° C./75% RH			
Storage	Timepoint (weeks)			
Condition	t = 0	t = 1 weeks	t = 2 weeks	t = 3 weeks
40° C./ 75% RH	1 capsule	1 capsule	1 capsule	1 capsule
25° C./ 60% RH		1 capsule	1 capsule	1 capsule
		Comments		
Storage	Nalgene 30 mL (1 oz) Amber Wide Mouth, HDPE Store capsules for each formulation at each condition in the same bottle			
Appearance/ Assay/ Impurities	1 capsules per pull			

(1) a free base assay content of 83.78%. 250 mg of TG101348 is equivalent to 209.45 mg of free base.

It was found that TG101348 di-HCL monohydrate was compatible with hard gelatine capsules. No appreciable changes in attributes (appearance, assay, impurities) were observed over the timepoints of the study (t=1, 2, and 3 weeks).

Drug Substance Blend Compatibility with Fillers and Lubricants

A matrix of formulation blends was designed to study the compatibility of TG101348 di-HCl monohydrate with four fillers and two lubricants (Table 17). Blends were prepared at a scale of 2.5 g each by prescreening all components through a 500 μm sieve, blending all components except the lubricant using a Turbula T2B blender for 10 min at 22 rpm, screening the blend through a 500 μm sieve, blending for 10 min, adding lubricant (weight adjusted) and blending for 5 min. The blends were manufactured and stored in 30 mL amber HDPE bottles under primary (60° C./ambient humidity) and backup conditions (40° C./75% RH, 25° C./60% RH, and 5° C.). A summary of the accelerated stability protocol is shown in Table 18. No appreciable changes in attributes (appearance, assay, impurities) were observed over the course of the study.

TABLE 17

	Formulation (% w/w)								
Ingredients	1	2	3	4	5	6	7	8	Control
TG101348 di-HCl monohydrate [(1)]	50.46%	50.46%	50.46%	50.46%	50.46%	50.46%	50.46%	50.46%	100%
Lactose (Fast-Flo)	48.54%	48.54%	—	—	—	—	—	—	—

TABLE 17-continued

Ingredients	Formulation (% w/w) 1	2	3	4	5	6	7	8	Control
Mannitol (Parteck M200)	—	—	48.54%	48.54%	—	—	—	—	—
MCC (Avicel PH102)	—	—	—	—	49.04%	49.04%	—	—	—
MCC (ProSolv 90 HD)	—	—	—	—	—	—	49.04%	49.04%	—
Magnesium Stearate	1.0%	—	1.0%	—	0.5%	—	0.5%	—	—
Sodium Stearyl Fumarate (Pruv)	—	1.0%	—	1.0%	—	0.5%	—	0.5%	—

(1) Based on TG101348 with a free-base assay content of 81.26% (equivalent to 41.00% w/w free base content in the formulation).

TABLE 18

Storage Condition	Timepoint (weeks) t = 0	t = 1 weeks	t = 2 weeks	t = 4 weeks	t = 12 weeks
60° C./ ambient humidity	X	X	X	X	X
40° C./ 75% RH		○	○	○	○
25° C./ 60% RH		○	○	○	○
5° C.		○	○	○	○

	Comments
Storage	Nalgene 30 mL (1 oz) Amber Wide Mouth, HDPE Store blend for each formulation at each condition in the separate bottles
Testing	Visual appearance Assay Impurities

○ = optional.

Powder-In-Capsule Development

Excipient Selection

Excipient compatibility testing of dry blends of TG101348 di-HCl monohydrate with four fillers (lactose, mannitol, microcrystalline cellulose (MCC) Avicel PH102, and MCC ProSolv 90 HD) and two lubricants (magnesium stearate and sodium stearyl fumarate (Pruv) (Table 17) indicated no incompatibilities. Prosolv SMCC 90HD (i.e., silicified microcrystalline cellulose) and Lactose Fast-Flo (i.e., spray dried lactose monohydrate) were selected as fillers for further testing based on properties amenable to direct dry blending processes. Magnesium stearate (vegetable grade) and Pruv were selected as lubricants for further testing. All excipients are global regulatory approved for use in solid oral dosage forms (USA, European Union, Japan).

Suitability for Dry Powder Process Development

True densities of TG101348 di-HCl monohydrate having particles of rounded/granular appearance, silicified microcrystalline cellulose (Prosolv SMCC 90HD), and sodium stearyl fumarate (Pruv) were measured using a helium pycnometer (Micromeritics Accupyc 1340). The true density of the drug substance and excipients (fillers/diluents) appeared well matched.

Capsule Formulations

A "matrix" of prototype capsule formulations for stability evaluation was designed, and is summarized in Table 19. Two dosage strengths were selected, 10 and 125 mg.

TABLE 19

Component	Prototype Formulation P1	P2	P3	P4	P5	P6	P7	P8
	% w/w composition							
Dry Blend								
TG101348 di-HCl monohydrate (1)	49.37	49.37	49.37	49.37	49.37	49.37	49.37	49.37
Prosolv SMCC 90HD	50.13	50.13	50.13	50.13	—	—	—	—
Lactose Fast-Flo	—	—	—	—	49.63	49.63	49.63	49.63
Magnesium Stearate	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0
Total	100%	100%	100%	100%	100%	100%	100%	100%
Capsule Fill								
Capsule Type	Gelatin Size #00	Gelatin Size #00	HPMC Size #00	HPMC Size #00	Gelatin Size #00	Gelatin Size #00	HPMC Size #00	HPMC Size #00

TABLE 19-continued

Component	Prototype Formulation							
	P1	P2	P3	P4	P5	P6	P7	P8
	% w/w composition							
Capsule Color	White Opaque	White Opaque	White Opaque	White Opaque	White Opaque	White Opaque	White Opaque	White Opaque
Capsule Fill	24.39 mg blend fill	304.86 mg blend fill	24.39 mg blend fill	304.86 mg blend fill	24.39 mg blend fill	304.86 mg blend fill	24.39 mg blend fill	304.86 mg blend fill
	Dose							
Dose (mg, i.e., free base)	10 mg	125 mg	10 mg	125 mg	10 mg	125 mg	10 mg	125 mg

(1) Based on a free-base assay content of 83.05% (equivalent to 41.00% w/w free base content in the formulation).

Accelerated Stability Testing Protocol

Table 20 summarizes the accelerated stability protocol applied for the capsule prototypes. No appreciable changes in attributes (appearance, assay, impurities, in vitro dissolution) were observed over the timepoints of the study (t=1, 2, 4, and 8 weeks at 40° C./75% RH and 25° C./60% RH). Based on these results, Prototypes P2 and P6 were selected for further evaluation.

TABLE 20

Storage Condition	Timepoint (weeks)				
	t = 0	2 weeks	4 weeks	8 weeks	Contingency
40° C./75% RH	16 capsules	5 capsules	5 capsules	5 capsules	7 capsules
25° C./60% RH		5 capsules	5 capsules	5 capsules	7 capsules
	Comments				
Storage	Store each pull separately into individual bottles Nalgene 30 mL (1 oz) Amber Wide Mouth, HDPE				
Appearance/ Assay/Impurities	2 capsules per pull				
In vitro Dissolution	3 capsules per pull				
Notes	16 capsules for t = 0 to include support for in vitro dissolution methodology development				

In Vitro Dissolution Performance Considerations

On in vitro dissolution testing, prototype formulations filled into gelatin capsule shells (P1, P2, P5, P6) demonstrated ≥85% drug release within 15 minutes. Prototypes formulations filled into HPMC capsule shells (e.g., P3, P4, P7, P8) typically demonstrated <60% drug release after 60 minutes. Prototypes in HPMC capsules were therefore not progressed beyond t=0 testing.

Absorption Enhancer Capsule Development

TG101348 is on the borderline between "low" and "high" permeability based on caco-2 permeability data. In addition, bioavailability in multiple species was typically 20-25%. Therefore, it was not known whether an "absorption enhancer" would be required in the formulation to achieve adequate bioavailability.

Excipient Selection

Based on excipient compatibility described above, silicified microcrystalline cellulose (Prosolv SMCC 90IID) was used as the primary filler/carrier excipient for the absorption enhancement formulation. Four absorption enhancement excipient candidates were selected for further testing (Table 21).

TABLE 21

Absorption Enhancement Excipient	Excipient Manufacturer	Absorption Enhancement Mechanism
Vitamin E D-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS)	Eastman Chemical Company	Lipid excipient (surfactant), P-glycoprotein (PGP) inhibitor
Gelucire 44/14 (PEG-32 glyceryl laurate)	Gattefossé S.A.	Lipid excipient (amphiphile)
Pluronic F127	BASF Chemical Company	Polymeric amphiphilic surfactant/ micellar solubilization/ P-glycoprotein (PGP) inhibitor
Glyceryl monostearate 40-55	Gattefossé S.A.	Lipid excipient (emulsifier) P-glycoprotein (PGP) inhibitor

Formulations and Manufacturing Processes

Table 22 summarizes the absorption enhancement formulations tested. Melt granulation, as opposed to a direct blend manufacturing process, was selected to produce the formulation.

TABLE 22

Component	Absorption Enhancer Formulations A	B	C	D
	% w/w composition			
Granules				
TG101348 di-HCl monohydrate [(1)]	50.17%	50.17%	50.17%	50.17%
Vitamin E TPGS	25.00%	—	—	—
Gelucire 44/14	—	25.00%	—	—
Pluronic F127	—	—	3.33%	—
Glyceryl monostearate	—	—	—	3.33%
ProSolv SMCC 90HD	24.33%	24.33%	46.00%	46.00%
Magnesium Stearate	0.50%	0.50%	0.50%	0.50%
Capsule Fill				
Capsule Type	Gelatin Size #00	Gelatin Size #00	Gelatin Size #00	Gelatin Size #00
Capsule Color	White Opaque	White Opaque	White Opaque	White Opaque
Capsule Fill	300 mg of granules	300 mg of granules	300 mg of granules	300 mg of granules
Dose				
Dose (mg, i.e. free base)	125 mg	125 mg	125 mg	125 mg

[(1)] Based on TG101348 with a free-base assay content of 83.05% (equivalent to 41.67% w/w free base content in the formulation).

Crossover Beagle Dog PK Study

A crossover beagle dog PK study was performed testing five formulations: an oral solution as described below, two capsule formulations without absorption enhancer and two capsule formulations with absorption enhancer.

Five beagle dogs were dosed with each formulation at a TG101348 dose of 125 mg, or approximately 11 mg/kg based on mean body weights, with a 'washout' of one week in between doses. The formulations administered are summarized in Table 23.

TABLE 23

Phase	Dose	Formulation	Dose Strength	Dose Amount	Matrix Collected
1	Oral solution	Aqueous, 0.5% MC, 6.25 mg/mL	125 mg	20 mL	Plasma
2	Prototype Capsule P2	Capsule fill containing dry blend of: 49.37% TG101348 Lot K-18 50.13% Prosolv SMCC 90HD 0.5% Magnesium Stearate	125 mg	One capsule	Plasma
3	Prototype Capsule P6	Capsule fill containing dry blend of: 49.37% TG101348 Lot K-18 49.63% Lactose Fast-Flo 1.0% Magnesium Stearate	125 mg	One capsule	Plasma
4	Prototype Capsule A	Capsule fill containing granules composed of: 50.17% TG101348 Lot K-18 3.33% Glyceryl Monostearate	125 mg	One capsule	Plasma

TABLE 23-continued

Phase	Dose	Formulation	Dose Strength	Dose Amount	Matrix Collected
		46.00% Prosolv SMCC 90HD 0.5% Magnesium Stearate			
5	Prototype Capsule D	Capsule fill containing granules composed of: 50.17% TG101348 Lot K-18 25.00% Vitamin E TPGS 24.33% Prosolv SMCC 90HD 0.5% Magnesium Stearate	125 mg	One capsule	Plasma

All four capsule formulations displayed immediate release characteristics with a demonstration of bioequivalence to the reference solution dose. Therefore, despite the boarderline permeability in human caco-2 cells and the 20-25% bioavailability in various animal species, capsule formulations without an absorption enhancer formulation demonstrated immediate release characteristics.

Process Development

Drug Substance Particle Morphology

Different particle morphology, from rounded, granular particles (mean particle size≈25 μm), to that of small needles (mean particle size=7-10 μm) was found between different lots of the drug substance. The needle form was found to be highly static, which could negatively affect drug product manufacturing and also negatively affect drug product content uniformity.

Dry Granulation Process

The initial formulation, developed with the drug substance having rounded, granular particles with a mean particle size of 25 μm was 50:50 ratio by weight of

TG101348 drug substance and filler, with 0.5% w/w lubricant. Prior to roller compaction, a blend of drug, filler, and lubricant was prepared. As described herein, the drug substance was passed through a co-mil to de-agglomerate prior to blending with formulation excipients. The drug substance having small needles demonstrated a high tendency to agglomerate on storage. After deagglomerating the drug substance having small needles, significant reagglomeration or 'clumping' would occur almost instantaneously. This reagglomeration was significantly reduced through blending the drug with lubricant prior to milling.

The initial formulation of TG101348 di-HCL monohydrate comprised about a 50:50 ratio by weight of TG101348 drug substance and filler, with 0.5% w/w lubricant. This formulation exhibited a poor flow and significant sticking to the metal rolls within the roller compactor.

The amount of magnesium stearate lubricant could be increased in the formulation, however increasing the concentration within the formulation could adversely affect drug release kinetics. The lubricant sodium laurel fumarate was also shown to be compatible with TG101348 di-HCl monodyrate and is less hygroscopic than magnesium stearate and was added (at a weight ratio of 2.0% w/w) instead of magnesium stearate which minimized sticking of the formulation to the metal rolls of the roller compactor. However, powder flow remained poor.

The ratio of TG101348 drug substance to filler was reduced from about 50:50 to about 40:60. The lubricant (Pmruv) content was also reduced to 1% w/w which provided acceptable flow and minimal sticking within the roller compactor.

Development of Multi-Dose Formulations

On capsule hand-filling trials, with aggressive tamping, into size #00 gelatin capsule shells, approximately 600 mg of granules appeared to be the maximum achievable fill. With a drug substance loading of 40% w/w in the formulation, and with the TG101348 di-HCL monohydrate batch comprising 83.78% free base content, it therefore appeared that an upper capsule strength of 200 mg strength was feasible.

The dry granulation process developed as described herein to produce a particles having intragranular TG101348 di-HCL monohydrate and sodium laurel fumarate allowed the preparation of a range of capsule dosages using dry blending processes.

The mean granule size was approximately 300 μm and the silicified microcrystalline cellulose mean particle size was approximately 100 μm. Therefore, 40 mg and 10 mg capsule strength formulations were produced through dilution of the granules with extragranular silicified microcrystalline cellulose. The general, particles sizes within a The size of the granules and the extragranular silicified microcrystalline cellulose are similar enough to allow homogeneous mixing.

40 mg strength capsules were produced using a formulation comprising comparable fill volume to the 200 mg formulation, within the same capsule shell (size #00 hard gelatin capsule). For the 10 mg strength capsules, a common blend with the 40 mg capsule strength formulation was used by filing a smaller capsule size.

Oral Solution Formulation

An oral solution formulation was developed that contained the drug substance, 0.5% methylcellulose (MC) and 0.05% Tween 80. A pH-stability study was conducted at 60° C. on formulations passed through a 0.22 μm polyethersulfone (PES) filter. No appreciable changes in attributes (appearance, assay, impurities) were observed over the course of the study (14 days). A second oral solution formulation was developed that contained the drug substance and 0.5% MC. The second oral solution formulation was used in the dog PK study.

Although the foregoing examples have been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

What is claimed is:

1. A capsule comprising a formulation comprising (i) a compound that is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate,

wherein:

the weight ratio of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:9, and

sodium stearyl fumarate is about 1% by weight of the formulation.

2. The capsule unit of claim **1**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

3. The capsule of claim **1**, wherein the to formulation comprises about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl) amino]benzenesulfonamide dihydrochloride monohydrate.

4. The capsule of claim **1**, wherein the formulation comprises about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, about 357 mg of silicified microcrystalline cellulose, and about 6 mg of sodium stearyl fumarate.

5. The capsule of claim **1**, wherein the capsule is a hard gelatin capsule.

6. A method of treating myelofibrosis in a subject comprising administering a capsule comprising a formulation comprising (i) a compound that is N-tert-butyl-3-[(5-methyl-2-{[4(2-pyrrolidin-1-ylethoxy)phenyl] amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate,

wherein:

the weight ratio of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:9, and

sodium stearyl fumarate is about 1% by weight of the formulation.

7. A method of preparing a capsule comprising a formulation, wherein the method comprises blending a microcrystalline cellulose and sodium stearyl fumarate with a compound that is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate,

wherein:

the weight ratio of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:9, and

sodium stearyl fumarate is about 1% by weight of the formulation.

8. An article of manufacture comprising:

(a) a capsule comprising a formulation comprising (i) a compound that is N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate, (ii) a microcrystalline cellulose, and (iii) sodium stearyl fumarate,

wherein:

the weight ratio of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:9, and

sodium stearyl fumarate is about 1% by weight of the formulation; and

(b) a package insert or a label indicating that the formulation is useful for treating myelofibrosis in a subject.

9. The capsule or claim **3**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

10. The article of manufacture of claim **8**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

11. The method of claim **7**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

12. The capsule of claim **1**, wherein the weight ratio or N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:2.

13. The capsule of claim **12**, wherein the formulation comprises about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

14. The capsule of claim **12**, wherein the formulation comprises about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

15. The capsule of claim **12**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

16. The capsule of claim **15**, wherein the formulation comprises about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

17. The capsule of claim **15**, wherein the formulation comprises about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

18. The capsule of claim **4**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

19. The article of manufacture of claim **8**, wherein the formulation comprises about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

20. The article of manufacture of claim **19**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

21. The article of manufacture of claim **8**, wherein the weight ratio of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide to microcrystalline cellulose in the formulation is about 1:1.5 to about 1:2.

22. The article of manufacture of claim **21**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

23. The article or manufacture of claim **22**, wherein the formulation comprises about 117 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino)}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

24. The article of manufacture of claim **8**, wherein the formulation comprises about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

25. The article of manufacture of claim **22**, wherein the formulation comprises about 235 mg of N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide dihydrochloride monohydrate.

26. The article of manufacture of claim **24**, wherein the microcrystalline cellulose is silicified microcrystalline cellulose.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 10,391,094 B2
APPLICATION NO. : 13/888096
DATED : August 27, 2019
INVENTOR(S) : Arvind Jayan et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 2, Lines 35-37: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino} pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 3, Lines 33-35: Please replace "N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 8, Lines 1-3: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]ami- no}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 8, Lines 6-8: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino} pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 10, Line 30: Please replace "6≥50%" with --6 ≥50%--.

Column 10, Line 39: Please replace "count>450×10^9/L" with --count > 450 × 10^9/L--.

Column 10, Line 60: Please replace "baseline>20%" with --baseline > 20%--.

Column 11, Line 35: Please replace "(score=11-3)" with --(score=1-3)--.

Column 11, Line 61: Please replace "count>11×10^9/L" with --count >11 × 10^9/L--.

Column 12, Line 1: Please replace "burden>20%" with --burden >20%--.

Signed and Sealed this
Fifth Day of May, 2020

Andrei Iancu

Andrei Iancu
Director of the United States Patent and Trademark Office

Column 12, Line 11: Please replace "burden>20%" with --burden >20%--.

Column 12, Line 14: Please replace "p=0.00^2" with --p=0.002--.

Column 13, Line 67 to Column 14, Line 1: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino} - pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 14, Lines 4-6: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxyphenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 14, Line 52: Please replace "aft" with --art--.

Column 16, Lines 43-45: Please replace "N-tert-butyl-3-[(5-methyl-2-([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 17, Lines 8-10: Please replace "N-tert-butyl-3-[(5-methyl-2-[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 18, Line 67 to Column 19, Line 1: Please replace "N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 20, Lines 10-12: Please replace "N-tert-butyl-3-[(5-methyl-2-{([4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 22, Line 47: Please replace "phenyloin" with --phenytoin--.

Column 28, Line 59: Please replace "range<1-76" with --range <1-76--.

Column 29, Line 39: Please replace "TG 101348" with --TG101348--.

Column 29, Line 40: Please replace "lcukocyte" with --leukocyte--.

Column 30, Line 55: Please replace "count>450×10^9/L" with --count > 450 × 10^9/L--.

Column 30, Line 62: Please replace "baseline>20%" with --baseline > 20%--.

Column 30, Line 64: Please replace "baseline>20%" with --baseline > 20%--.

Column 32, Line 56: Please replace "218" with --≥18--.

Column 36, Line 66: Please replace "(n-=6)" with --(*n*=6)--.

Column 43, Line 54: Please replace "count>11×10^9/L" with --count >11 × 10^9/L--.

Column 43, Line 63: Please replace "count>450×10^9/L" with --count >450 × 10^9/L--.

Column 44, Line 18: Please replace "(p=0.00^2)" with --(p=0.002)--.

Column 44, Line 24: Please replace "burden>20%" with --burden >20%--.

Column 44, Line 29: Please replace "≥20%" with -->20%--.

Column 45, Line 43: Please replace "hypercramylasemia" with --hyperamylasemia--.

Column 48, Lines 21-22: Please replace "Mix>20nm" with --Mix >20mn--.

Column 51, Line 17: Please replace "the mg" with --the 10 mg--.

Column 51, Lines 49-50: Please replace "N-tert-Rutyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-Butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Column 55, Line 48: Please replace "≥85%" with --> 85%--.

Column 58, Line 59: Please replace "=" with --≈--.

Column 59, Line 29: Please replace "Pmruv" with --Pruv--.

In the Claims

Claim 2, Column 60, Line 25: Please delete the term "unit".

Claim 3, Column 60, Line 27: Please delete the term "to".

Claim 6, Column 60, Lines 41-43: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

Claim 9, Column 61, Line 17: Please replace "or" with --of--.

Claim 12, Column 61, Line 24: Please replace "or" with --of--.

Claim 23, Column 62, Line 25: Please replace "or" with --of--.

Claim 23, Column 62, Lines 26-28: Please replace "N-tert-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino)}pyrimidin-4-yl)amino]benzenesulfonamide" with --*N-tert*-butyl-3-[(5-methyl-2-{[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino}pyrimidin-4-yl)amino]benzenesulfonamide--.

EXHIBIT B

US011400092B2

(12) **United States Patent**
Berry et al.

(10) **Patent No.: US 11,400,092 B2**
(45) **Date of Patent: Aug. 2, 2022**

(54) **METHODS OF TREATING MYELOPROLIFERATIVE DISORDERS**

(71) Applicant: **Impact Biomedicines, Inc.**, Summit, NJ (US)

(72) Inventors: **Tymara Berry**, Morristown, NJ (US); **John Hood**, Del Mar, CA (US); **Catriona Jamieson**, La Jolla, CA (US); **Curtis L. Scribner**, Oakland, CA (US)

(73) Assignee: **Impact Biomedicines, Inc.**, Summit, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **17/560,373**

(22) Filed: **Dec. 23, 2021**

(65) **Prior Publication Data**

US 2022/0133724 A1 May 5, 2022

Related U.S. Application Data

(63) Continuation of application No. 17/279,765, filed as application No. PCT/US2019/052608 on Sep. 24, 2019.

(60) Provisional application No. 62/783,076, filed on Dec. 20, 2018, provisional application No. 62/736,369, filed on Sep. 25, 2018.

(51) **Int. Cl.**

A61K 31/51	(2006.01)
A61P 35/02	(2006.01)
A61K 31/675	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/51*** (2013.01); ***A61K 31/675*** (2013.01); ***A61P 35/02*** (2018.01)

(58) **Field of Classification Search**

CPC A61K 2300/00; A61K 31/506; A61K 31/519; A61K 31/635; A61P 35/00

See application file for complete search history.

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(57) **ABSTRACT**

The present disclosure provides methods of mitigating thiamine deficiency.

25 Claims, No Drawings

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METHODS OF TREATING MYELOPROLIFERATIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation application of U.S. patent application Ser. No. 17/279,765, filed Mar. 25, 2021, which is a 371 national phase application of PCT/US19/52608, filed Sep. 24, 2019, which claims priority to U.S. Provisional Patent Application Nos. 62/736,369, filed on Sep. 25, 2018, and 62/783,076, filed on Dec. 20, 2018, each of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention provides methods of treating, stabilizing or lessening the severity or progression of a myeloproliferative disorder.

BACKGROUND OF THE INVENTION

The search for new therapeutic agents has been greatly aided in recent years by a better understanding of the structure of enzymes and other biomolecules associated with diseases. One important class of enzymes that has been the subject of extensive study is protein kinases.

Protein kinases constitute a large family of structurally related enzymes that are responsible for the control of a variety of signal transduction processes within the cell. Protein kinases are thought to have evolved from a common ancestral gene due to the conservation of their structure and catalytic function. Almost all kinases contain a similar 250-300 amino acid catalytic domain. The kinases may be categorized into families by the substrates they phosphorylate (e.g., protein-tyrosine, protein-serine/threonine, lipids, etc.).

In general, protein kinases mediate intracellular signaling by effecting a phosphoryl transfer from a nucleoside triphosphate to a protein acceptor that is involved in a signaling pathway. These phosphorylation events act as molecular on/off switches that can modulate or regulate the target protein biological function. These phosphorylation events are ultimately triggered in response to a variety of extracellular and other stimuli. Examples of such stimuli include environmental and chemical stress signals (e.g., osmotic shock, heat shock, ultraviolet radiation, bacterial endotoxin, and H_2O_2), cytokines (e.g., interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α)), and growth factors (e.g., granulocyte macrophage-colony-stimulating factor (GM-CSF), and fibroblast growth factor (FGF)). An extracellular stimulus may affect one or more cellular responses related to cell growth, migration, differentiation, secretion of hormones, activation of transcription factors, muscle contraction, glucose metabolism, control of protein synthesis, and regulation of the cell cycle.

Many diseases are associated with abnormal cellular responses triggered by protein kinase-mediated events as described above. These diseases include, but are not limited to, autoimmune diseases, inflammatory diseases, bone diseases, metabolic diseases, neurological and neurodegenerative diseases, cancer, cardiovascular diseases, allergies and asthma, Alzheimer's disease, and hormone-related diseases. Accordingly, there remains a need to find protein kinase inhibitors useful as therapeutic agents.

SUMMARY OF THE INVENTION

The present disclosure provides methods of treating, stabilizing or lessening the severity or progression of one or more myeloproliferative disorders. In certain embodiments, provided methods comprise mitigating one or more adverse events associated with treatment of a myeloproliferative disorder. In some such embodiments, the one or more adverse event is a thiamine deficiency.

In some aspects, the present disclosure provides methods of treating, stabilizing or lessening the severity or progression of one or more myeloproliferative disorders comprising administering to a patient a pharmaceutically acceptable composition comprising a compound of formula I:

I

or a pharmaceutically acceptable salt or hydrate thereof. The compound of formula I is also referred to herein as "Compound I". In some embodiments, Compound I is in the form of a dihydrochloride salt. Compound I, or a pharmaceutically acceptable salt thereof, may also exist in a hydrate form. In some such embodiments, Compound I is in the form of a dihydrochloride monohydrate. Accordingly, in some embodiments, provided methods comprise administering to a patient in need thereof Compound II:

II

In some embodiments, the present disclosure provides a method of treating a myeloproliferative disorder, comprising

(i) administering to a patient in need thereof Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II); and

(ii) monitoring the patient's thiamine levels;

wherein the patient's thiamine levels are adjusted if the level of thiamine is below a reference standard.

In some embodiments, the patient's thiamine levels are adjusted if the thiamine levels are about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more below a reference standard. In some embodiments, a reference standard is the patient's baseline level of thiamine prior to administration of Compound I. In some embodiments, a reference standard is a thiamine level that is from about 74 nM/L to about 222 nM/L of whole blood.

In some embodiments, the patient is at risk for developing Wernicke's encephalopathy.

In some embodiments, the patient's thiamine levels are assessed by analyzing one or more biomarkers for thiamine deficiency. In some embodiments, a biomarker for thiamine deficiency is a serum thiamine level.

In some embodiments, the patient's thiamine levels are adjusted by administering thiamine or a thiamine equivalent to the patient.

In some embodiments, the present disclosure provides a method for treating a patient comprising:

(i) administering to the patient Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II);

(ii) analyzing the thiamine level in the patient; and

(iii) administering to the patient thiamine or a thiamine equivalent if the patient's thiamine level is less than about 74 nM/L to about 222 nM/L of whole blood but greater than about 30 nM/L of whole blood.

In some embodiments, the patient is administered 100 mg thiamine per day. In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 100 mg of thiamine per day. In some such embodiments, the thiamine or thiamine equivalent is administered orally.

In some embodiments, the present disclosure provides a method for treating a patient comprising:

(i) administering to the patient Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II);

(ii) analyzing the thiamine level in the patient; and

(iii) administering to the patient thiamine or a thiamine equivalent if the patient's thiamine level is less than or equal to about 30 nM/L of whole blood.

In some embodiments, the thiamine or thiamine equivalent is administered intravenously. In some embodiments, thiamine is administered to the patient at an amount of about 250 mg. In some embodiments, thiamine is administered to the patient at an amount of about 250 mg per day (QD). In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 250 mg of thiamine per day.

In some embodiments, thiamine is administered to the patient at an amount of about 500 mg. In some embodiments, thiamine is administered to the patient at an amount of about 500 mg per day (QD). In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 500 mg of thiamine QD. In some embodiments, thiamine is administered to the patient at an amount of about 500 mg three times daily (TID). In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 500 mg of thiamine TID.

In some embodiments, thiamine is administered to the patient at an amount of about 500 mg TID for 2 or 3 days, followed by administration of thiamine at an amount of about 250 mg to about 500 mg daily (QD) for 3-5 days, followed by administration of thiamine at an amount of about 100 mg QD for 90 days.

In some embodiments, provided methods further comprise increasing the patient's magnesium level.

In some embodiments, the patient has a myeloproliferative disorder. In some such embodiments, the myeloproliferative disorder is myelofibrosis. In some embodiments, the myelofibrosis is primary myelofibrosis. In some embodiments, the primary myelofibrosis is Dynamic International Prognostic Scoring System (DIPSS) intermediate or high-risk primary myelofibrosis. In some embodiments, the myelofibrosis is secondary myelofibrosis. In some embodiments, the myelofibrosis is post-essential thrombocythemia myelofibrosis. In some embodiments, the myelofibrosis is post-polycythemia vera myelofibrosis. In some embodiments, the myeloproliferative disorder is polycythemia vera. In some embodiments, the myeloproliferative disorder is essential thrombocythemia. In some embodiments, the myeloproliferative disorder is acute myeloid leukemia (AML).

In some embodiments, the present disclosure provides a method for treating a patient comprising:

(i) administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), and

(ii) conducting a cognitive assessment.

In some embodiments, the cognitive assessment occurs during the 2nd 28-day cycle. In some embodiments, the cognitive assessment occurs during the 3rd 28-day cycle. In some embodiments, the cognitive assessment occurs during at least every 3rd 28-day cycle. In some embodiments, the cognitive assessment comprises a mini-mental state examination. In some embodiments, the method further comprises analyzing thiamine level in the patient.

Definitions

The term "about" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/−10% or less, preferably +/−5% or less, more preferably +/−1% or less, and still more preferably +/−0.1% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. As an example, when the term "about" is used in combination with a certain number of days, it includes said specific number of days plus or minus 1 day, e.g., "about 6 days" includes any number of days between 5 and 7. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

The term "biomarker" is used herein to refer to a to an entity, event, or characteristic whose presence, level, degree, type, and/or form, correlates with a particular biological event or state of interest, so that it is considered to be a "marker" of that event or state. To give but a few examples, in some embodiments, a biomarker may be or comprise a marker for a particular disease state, or for likelihood that a particular disease, disorder or condition may develop, occur, or reoccur. In some embodiments, a biomarker may be or comprise a marker for a particular disease or therapeutic outcome, or likelihood thereof. Thus, in some embodiments, a biomarker is predictive, in some embodiments, a biomarker is prognostic, in some embodiments, a biomarker is diagnostic, of the relevant biological event or state of interest. A biomarker may be or comprise an entity of any chemical class, and may be or comprise a combination of entities. For example, in some embodiments, a biomarker may be or comprise a nucleic acid, a polypeptide, a lipid, a carbohydrate, a small molecule, an inorganic agent (e.g., a metal or ion), or a combination thereof. In some embodiments, a biomarker is a cell surface marker. In some embodiments, a biomarker is intracellular. In some embodiments, a biomarker is detected outside of cells (e.g., is secreted or is otherwise generated or present outside of cells, e.g., in a body fluid such as blood, urine, tears, saliva, cerebrospinal fluid, etc. In some embodiments, a biomarker may be or comprise a genetic or epigenetic signature. In some embodiments, a biomarker may be or comprise a gene expression signature.

As used herein, the term "combination therapy" refers to situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents, including one or more compounds as described herein). The two or more regimens may be administered simultaneously or sequentially (e.g., all doses of a first regimen are administered prior to administration of any doses of a second regimen). In other embodiments, such compounds are administered in overlapping dosing regimens. "Administration" of a combination therapy may involve administration of one or more compounds to a subject receiving the other compound(s) in the combination. For clarity, combination therapy does not require that individual compounds be administered together in a single composition (or even necessarily at the same time or by the same route of administration), although in some embodiments, two or more compounds may be administered together in a combination composition, or even in a combination compound (e.g., as part of a single chemical complex or covalent entity).

The term "thiamine equivalent" refers to an agent that delivers or is capable of delivering a bioequivalent amount of thiamine. Such thiamine equivalents include prodrugs of thiamine as well as derivatives of thiamine such as thiamine monophosphate, thiamine pyrophosphate (also known as thiamine diphosphate), and thiamine triphosphate. In some embodiments, a thiamine equivalent is a dietary form of thiamine such as that found in vegetables or other food sources.

The terms "treat" or "treating," as used herein, refers to partially or completely alleviating, inhibiting, delaying onset of, preventing, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder or condition. As used herein, the terms "treatment," "treat," and "treating" refer to partially or completely alleviating, inhibiting, delaying onset of, preventing, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder or condition, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In some embodiments, the term "treating" includes preventing or halting the progression of a disease or disorder. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence. Thus, in some embodiments, the term "treating" includes preventing relapse or recurrence of a disease or disorder.

The expression "unit dosage form" as used herein refers to a physically discrete unit of inventive formulation appropriate for the subject to be treated. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular subject or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active agent employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active agent employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Myelofibrosis

Myeloproliferative neoplasm (MPN)-associated myelofibrosis (MF) is a serious and life-threatening disease that can present as a de novo or primary myelofibrosis (PMF) or evolve from previous polycythemia vera (post PV-MF) or essential thrombocythemia (post ET-MF) (Swerdlow S H, Campo E, Harris N L, Jafie E S, Pileri S A, Stein H, et al. World Health Organization classification of tumors of haematopoietic and lymphoid tissues. Lyon: IARC Press 2008). The disease is characterized by clonal myeloproliferation, ineffective erythropoiesis, bone marrow stromal changes, hepatosplenic extramedullary hematopoiesis, and aberrant cytokine expression (Tefferi A, Pardanani A. JAK inhibitors in myeloproliferative neoplasms: rationale, current data and perspective. Blood Rev. 2011 September; 25(5):229-37). Patients typically present with splenomegaly, constitutional symptoms, moderate to severe anemia, thrombocytopenia, and leukocytosis.

Primary myelofibrosis is a member of a group of Philadelphia chromosome (Ph1)-negative MPN which also includes PV and ET (Tefferi A. The recent advances in classic BCR-ABL-negative myeloproliferative disorders. Clin Adv Hematol Oncol. 2007a; 5:113-5). Almost all patients with PV and about one-half of patients with ET and PMF have a JAK2 mutation, typically JAK2V617F. Other mutations in patients with PMF include CALR and MPL. About 20% of patients with PMF have no detectable mutation in JAK2, CALR, or MPL and are termed triple negative (Levine R L, Wadleigh M, Cools J, Ebert B L, Wernig G, Huntly B J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005; 7:387-97; Werning G, Mercher T, Okabe R, Levine L, Lee B H, Gilliland G L. Expression of JAK2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood. 2006; 107:4274-81). Mutations in JAK2, CALR, and MPL result in activation of the JAK/STAT signaling pathway resulting in cell proliferation and inhibiting cell death. The result is clonal expansion (Ilhe J N, Gilliland D G. JAK2: normal function and role in hematopoietic disorders. Curr Opin Genet Dev. 2007; 17:8-14). Thus, a JAK2 inhibitor that can down regulate the JAK/STAT pathway is expected to be helpful in reducing cell proliferation.

Polycythemia vera (PV) and essential thrombocythemia (ET) are characterized by increased levels of red blood cells (RBC) and platelets. However, about 10% of affected patients develop bone marrow fibrosis morphologically indistinguishable from PMF. These conditions are termed post-PV-MF and post-ET-MF (Campbell P J, Green A R. Management of polycythemia vera and essential thrombocythemia. Hematology Am Soc Hematol Educ Program. 2005; 201-8), and are clinically named MPN associated myelofibrosis. Patients with MPN-associated myelofibrosis have similar survival prognoses to that of the PMF and about a 10% cumulative risk of transformation to acute myeloid leukemia (AML).

There are several prognostic scoring systems predicting survival of patients with PMF. The International Prognostic Scoring System (IPSS) is used to predict survival at diagnosis and the Dynamic International Prognostic Scoring System (DIPSS) at any time in the disease course (Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the

International Working Group for Myelofibrosis Research and Treatment. Blood. 2009; March 26; 113(13):2895-901; Passamonti F, Cervantes F, Vannucchi A M, Morra E, Rumi E, Pereira A, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood. 2010 Mar. 4; 115(9):1703-8). Variables included in the IPSS are age >65 years, constitutional symptoms, hemoglobin level <10 g/dL, and white blood cell (WBC) counts. Additional recent prognostic scoring systems include the Dynamic International Prognostic Scoring System Plus (DIPSS Plus) and scoring systems incorporating data from mutation analyses. There is a strong association between overall survival for MF patients and the DIPSS risk category for patients with low, intermediate risk 1, intermediate risk 2, or high risk with median survival of 15.4, 6.5, 2.9, and 1.3 years, respectively (Tefferi A. Primary myelofibrosis: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol. 2016 December; 91(12):1262-1271).

Approximately 70% of individuals with MF are in the intermediate-2 or high-risk categories (Gangat N, Caramazza D, Vaidya R, George G, Begna K, Schwager S, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol. 2011 Feb. 1; 29(4):392-7), representing the greatest unmet medical need. Symptomatic enlargement of the spleen and liver, the necessity for RBC transfusions, cachexia, and the other MF-associated symptoms result in greatly compromised quality of life in these patients (Mesa R A, Camoriano J K, Geyer S M, Wu W, Kaufmann S H, Rivera C E, et al. A phase II trial of tipifarnib in myelofibrosis: primary, post-polycythemia vera and post-essential thrombocythemia. Leukemia. 2007 September; 21(9):1964-70).

Compound I

The synthesis of the Compound I is disclosed in Example 90 of U.S. Pat. No. 7,528,143, issued May 5, 2009, which is hereby incorporated by reference in its entirety. Compound I, also known as fedratinib, is a potent and selective inhibitor of JAK2 kinase activity that in cellular assays inhibits JAK2 signaling, cellular proliferation driven by mutant JAK2 or mutant MPL, and induces apoptosis in cells expressing constitutively active JAK2. Compound I also inhibits erythroid colony formation of hematopoietic progenitors isolated from myeloproliferative neoplasm (MPN) patients.

Myelofibrosis (MF) is a clonal disease resulting from mutations in CD34+ hematopoietic stem cells that promote abnormal proliferation and myeloid differentiation (Mead A J, 2017). In addition to JAK2V617F, several other mutations, in JAK2 and other genes, are found in MF patients and have been associated with prognosis, AML progression, and response to the JAK inhibitor ruxolitinib (Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017 Feb. 9; 129(6):667-679, Tefferi A, Guglielmelli P, Nicolosi M, Mannelli F, et al. GIPSS: genetically inspired prognostic scoring system for primary myelofibrosis. Leukemia. 2018 Mar. 23; Spiegel J Y, McNamara C, Kennedy J A, Panzarella T, et al. Impact of genomic alterations on outcomes in myelofibrosis patients undergoing JAK1/2 inhibitor therapy. Blood. 2017 Sep. 8; 1(20):1729-1738; Newberry K J, Patel K, Masarova L, Luthra R, et al. Clonal evolution and outcomes in myelofibrosis after ruxolitinib discontinuation. Blood. 2017 Aug. 31; 130(9):1125-1131; Patel K P, Newberry K J, Luthra R, Jabbour E, et al. Correlation of mutation profile and response in patients with myelofibrosis treated with ruxolitinib. Blood. 2015 Aug. 6; 126(6):790-7; Levine R L, Wadleigh M, Cools J, Ebert B L, Wernig G, Huntly B J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005; 7:387-97; Werning G, Mercher T, Okabe R, Levine L, Lee B H, Gilliland G L. Expression of JAK2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood. 2006; 107:4274-81; Mercher T, Wernig G, Moore S A, Levine R L, Gu T L, Frohling S, Cullen D, Polakiewicz R D, Bernard O A, Boggon T J, Lee B H, Gilliland D G. JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model. Blood. 2006 Oct. 15; 108(8):2770-9; Scott L M, Tong W, Levine R L, Scott M A, Beer P A, Stratton M R, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007 Feb. 1; 356(5):459-68; Pardanani A, Tefferi A, Jamieson C, Gabrail N Y, et al. A phase 2 randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. Blood Cancer J. 2015 Aug. 7; 5:e335).

Phosphorylated STAT3 (pSTAT3) is a downstream effector of activated JAK2. Tracking pSTAT3 level in circulating cells has proven a useful pharmacodynamics (PD) biomarker of fedratinib-JAK2 engagement in MF patients (Pardanani A, Tefferi A, Jamieson C, Gabrail N Y, et al. A phase 2 randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. Blood Cancer J. 2015 Aug. 7; 5:e335). Preclinical data indicates that fedratinib is also able to inhibit pSTAT5; and basal levels of pSTAT5 in AML cells have been reported to predict response to fedratinib in AML xenograft models (Chen W C, Yuan J S, Xing Y, Mitchell A, Mbong N, et al. An Integrated Analysis of Heterogeneous Drug Responses in Acute Myeloid Leukemia That Enables the Discovery of Predictive Biomarkers. Cancer Res. 2016 Mar. 1; 76(5): 1214-24). Abnormal cytokine expression and bone marrow fibrosis are hallmarks of MF (Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017 Feb. 9; 129(6): 667-679; Mondet J, Hussein K, Mossuz P. Circulating Cytokine Levels as Markers of Inflammation in Philadelphia Negative Myeloproliferative Neoplasms: Diagnostic and Prognostic Interest. Mediators Inflamm. 2015; 2015: 670580). High levels of pro-inflammatory and fibrogenic cytokines have been reported to contribute to bone marrow (BM) stromal changes, ineffective erythropoiesis/extramedullary hematopoiesis and constitutional symptoms in MF (Mondet J, Hussein K, Mossuz P. Circulating Cytokine Levels as Markers of Inflammation in Philadelphia Negative Myeloproliferative Neoplasms: Diagnostic and Prognostic Interest. Mediators Inflamm. 2015; 2015:670580; Tefferi A, Pardanani A. JAK inhibitors in myeloproliferative neoplasms: rationale, current data and perspective. Blood Rev. 2011 September; 25(5):229-37). Fedratinib was found to modulate circulating cytokines in MF patients not previously treated with JAK inhibitors (Pardanani A, Tefferi A, Jamieson C, Gabrail N Y, et al. A phase 2 randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. Blood Cancer J. 2015 Aug. 7; 5:e335). Cytokine modulation correlated with sustained viral response (SVR) and improvement in constitutional symptoms in these patients (Pardanani A, Tefferi A, Jamieson C, Gabrail N Y, et al. A phase 2

randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. Blood Cancer J. 2015 Aug. 7; 5:e335).

Recent studies are starting to unveil immune regulatory roles for JAK2V617F, as well as for JAK inhibitors like ruxolitinib. For instance, JAK2V617F was found to contribute to immune evasion of MPN myeloid cells by upregulation of program death-ligand 1 (PD-L1) (Prestipino A, Emhardt A J, Aumann K, O'Sullivan D, et. al. Oncogenic JAK2V617F causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018 Feb. 21; 10(429)), and ruxolitinib has been reported to modulate PD-L1 expression in these cells (Prestipino A, Emhardt A J, Aumann K, O'Sullivan D, et. al. Oncogenic JAK2V617F causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018 Feb. 21; 10(429)). However, preclinical and clinical data indicate that ruxolitinib is also a potent immunosuppressive drug, which can suppress graft-versus-host disease (GVHD), decrease frequency and impair activation of T- and NK-cells of MF patients (Betts B C, Bastian D, Iamsawat S, Nguyen H, et al. Targeting JAK2 reduces GVHD and xenograft rejection through regulation of T cell differentiation. Proc Natl Acad Sci USA. 2018 Feb. 13; 115(7):1582-1587. Epub 2018; Schonberg K, Rudolph J, Vonnahme M, Parampalli et al. Cancer JAK Inhibition Impairs NK Cell Function in Myeloproliferative Neoplasms. Res. 2015 Jun. 1; 75(11):2187-99; Parampalli Yajnanarayana S, Stithig T, Cornez I, Alchalby H, et al. JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. Br J Haematol. 2015 June; 169(6):824-33). Preclinical data suggest that fedratinib is able to modulate PD-L1 expression in lymphoma tumor cells (Hao Y, Chapuy B, Monti S, Sun H H, Rodig S J, Shipp M A. Selective JAK2 inhibition specifically decreases Hodgkin lymphoma and mediastinal large B-cell lymphoma growth in vitro and in vivo. Clin Cancer Res. 2014 May 15; 20(10):2674-83), however, it is neither a potent suppressor of GVHD, nor able to impair human T cell development in xenograft mouse models (Betts B C, Veerapathran A, Pidala J, Yang H, et al. Targeting Aurora kinase A and JAK2 prevents GVHD while maintaining Treg and antitumor CTL function. Sci Transl Med. 2017 Jan. 11; 9(372)). The selective activity of fedratinib on JAK2 raises the possibility that fedratinib might have immune-modulatory effects without impairing T- or NK-cell function in MF patients.

In some embodiments, the present disclosure provides a method for preventing and/or mitigating thiamine deficiency in a patient receiving Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II). In some embodiments, the present disclosure provides a method for treating a patient comprising administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), and monitoring the thiamine levels in the patient.

In some embodiments, the present disclosure provides a method of treating a myeloproliferative disorder, comprising

(i) administering to a patient in need thereof a JAK1 and/or JAK2 inhibitor; and

(ii) monitoring the patient's thiamine levels.

In some such embodiments, the method further comprises adjusting the patient's thiamine levels if the level of thiamine is below a reference standard (e.g., baseline level). In some embodiments, the JAK1 and/or JAK2 inhibitor is Compound I, or a pharmaceutically acceptable salt thereof, (e.g., Compound II).

Accordingly, in some embodiments, the present disclosure provides a method of treating a myeloproliferative disorder, comprising

(i) administering to a patient in need thereof Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II); and

(ii) monitoring the patient's thiamine levels;

wherein the patient's thiamine levels are adjusted if the level of thiamine is below a reference standard.

In some embodiments, the patient's thiamine levels are assessed by analyzing one or more biomarkers for thiamine deficiency. In some embodiments, a biomarker for thiamine deficiency is a serum thiamine level.

In some embodiments, the level of the biomarker is compared to a reference standard. In some embodiments, the reference standard is a baseline level of the biomarker (e.g., the level of the biomarker prior to administration of Compound I or Compound II). In some embodiments, the reference standard is a level of the biomarker that is considered to be within a range typically observed in a healthy population (e.g., a population that is not afflicted with the target disease or disorder). In some embodiments, the reference standard is a level of the biomarker that is considered to be within a range typically observed in a population that has been diagnosed with the target disease or disorder.

In some embodiments, the level of the biomarker following administration of Compound I or Compound II is lower than the reference standard (e.g., lower than the baseline level of the biomarker).

In some embodiments, the patient's thiamine levels are adjusted if the thiamine levels are about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more below a reference standard. In some embodiments, a reference standard is the patient's baseline level of thiamine prior to administration of Compound I or Compound II. In some embodiments, a reference standard is a thiamine level that is from about 74 nM/L to about 222 nM/L of whole blood.

In some embodiments, the patient is at risk for developing Wernicke's encephalopathy.

In some embodiments, the patient's thiamine levels are adjusted by administering thiamine or a thiamine equivalent to the patient.

In some embodiments, the present disclosure provides a method for treating a patient comprising:

(i) administering to the patient Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II),

(ii) analyzing the thiamine level in the patient, and

(iii) administering to the patient thiamine or a thiamine equivalent if the patient's thiamine level is less than or equal to about 30 nM/L of whole blood.

In some embodiments, the present disclosure provides a method of mitigating thiamine deficiency, the method comprising administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), to a patient at risk for developing thiamine deficiency, wherein the patient is simultaneously exposed to thiamine or a thiamine equivalent.

In some such embodiments, the patient is administered about 100 mg thiamine per day. In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 100 mg of thiamine per day. In some embodiments, the thiamine or thiamine equivalent is administered orally.

In some embodiments, the patient is administered a thiamine equivalent that delivers about 100 mg thiamine per day.

In some embodiments, the present disclosure provides a combination therapy comprising (i) Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), and (ii) thiamine or a thiamine equivalent.

In some embodiments, the present disclosure provides a method of treating myelofibrosis comprising administering to a patient in need thereof a combination therapy comprising Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), and thiamine or a thiamine equivalent. In some such embodiments, thiamine is administered in an amount of about 100 mg per day (QD). In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 100 mg of thiamine per day. In some embodiments, the thiamine or thiamine equivalent is administered orally.

In some such embodiments, thiamine is administered in an amount of about 500 mg thiamine TID. In some such embodiments, the patient is administered about 250 or about 500 mg thiamine QD. In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 250 mg or about 500 mg thiamine per day (QD). In some embodiments, the about 250 mg or about 500 mg thiamine is administered orally. In some embodiments, the thiamine equivalent is administered orally.

In some embodiments, a thiamine equivalent is thiamine pyrophosphate.

In some embodiments, provided methods further comprise increasing the patient's magnesium level. In some embodiments, the patient's magnesium level is increased by administering a magnesium supplement.

In some embodiments, the patient has a myeloproliferative disorder.

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

In some embodiments, the myeloproliferative disorder is polycythemia vera. In some embodiments, the myeloproliferative disorder is essential thrombocythemia. In some embodiments, the myeloproliferative disorder is acute myeloid leukemia.

In some embodiments, Compound I is administered in the form of a dihydrochloride monohydrate (e.g., Compound II).

In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered to the patient in a unit dosage form. In some embodiments, the unit dosage form of Compound I or Compound II is based on the free base weight of the compound. For example, a 100 mg dose of the free base form of Compound I equates to about 117.30 mg of Compound I in its dihydrochloride monohydrate form (i.e., Compound II). In some embodiments, the unit dosage form of Compound I or Compound II is about 50 mg, about 100 mg, about 150 mg, or about 200 mg, based on the free base weight of the compound. In some embodiments, the unit dosage form of Compound I or Compound II is 100 mg, based on the free base weight of the compound.

In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered in an oral dosage form. In some such embodiments, the oral dosage form is a capsule. In some embodiments, the oral dosage form is a tablet.

In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily (QD). In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered at a total daily dose of about 200 mg, about 300 mg or about 400 mg. In some embodiments, Compound I or Compound II is administered to the patient at a total daily dose of about 400 mg. In some embodiments, Compound I or Compound II is administered to the patient at a total daily dose of about 300 mg. In some embodiments, Compound I or Compound II is administered to the patient at a total daily dose of about 200 mg. In some embodiments, the total daily dose of Compound I or Compound II is modified due to an adverse event. In some embodiments, the total daily dose of Compound I or Compound II is reduced. In some embodiments, the total daily dose of Compound I or Compound II is reduced from about 400 mg to about 300 mg. In some embodiments, the total daily dose of Compound I or Compound II is reduced to about 200 mg.

In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily for a 28 day cycle. In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily for two 28-day cycles. In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily for three, four, five, or more 28-day cycles. In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily for six, seven, eight, nine, ten, eleven, twelve or more 28-day cycles. In some embodiments, Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), is administered once daily until symptoms of disease are no longer measureable. In some embodiments, Compound I or Compound II is administered for the duration of a patient's life. In some embodiments, Compound I or Compound II is administered once daily for one or more 28-day cycles, followed by a dose holiday. A "dose holiday" as used herein refers to a period of time wherein Compound I or Compound II is not administered to the patient. In some embodiments, a dose holiday is one day, one week, or one 28-day cycle. In some embodiments, Compound I or Compound II is administered once daily for one or more 28-day cycles, followed by a dose holiday, and then resumption of administration of Compound I or Compound II once daily at the same dose level prior to the dose holiday. In some embodiments, Compound I or Compound II is administered once daily for one or more 28-day cycles, followed by a dose holiday, and then resumption of administration of Compound I or Compound II once daily at a dose level that is 100 mg less than the dose of Compound I or Compound II prior to the dose holiday. In some embodiments, the total daily dose of Compound I or Compound II is titrated upward by 100 mg following a prior dose reduction.

In some embodiments, provided methods comprise administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), once daily for two or more 28-day cycles. In some such embodiments, the patient's thiamine levels are assessed at the beginning of the 2nd 28-day cycle.

In some embodiments, provided methods comprise administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), once daily to the patient for two or more 28-day cycles, wherein the patient's thiamine levels are assessed at the beginning of every 28-day cycle. In some embodiments, provided meth-

ods comprise administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), once daily to the patient for two or more 28-day cycles, wherein the patient's thiamine levels are assessed at the end of each 28-day cycle. In some embodiments, provided methods comprise administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), once daily to the patient for two or more 28-day cycles, wherein the patient's thiamine levels are assessed at the beginning of the 2nd 28-day cycle and at the beginning of the 3rd 28-day cycle. In some such embodiments, the patient's thiamine levels are assessed every 3rd 28-day cycle thereafter. For example, if a patient is treated for twelve 28-day cycles, thiamine levels are assessed on day 1 (±3 days) of each of the 2nd cycle, 3rd cycle, 6th cycle, 9th cycle and 12th cycle. In some embodiments, provided methods comprise administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), once daily to the patient for six or more 28-day cycles, wherein the patient's thiamine levels are assessed at the beginning of each of the 1st cycle, 2nd cycle, 3rd cycle, 4th cycle, 5th cycle and 6th cycle.

In some embodiments, provided methods comprise administering thiamine or a thiamine equivalent to the patient when the patient's thiamine levels are below normal (e.g., 74-222 nM/L of whole blood) but greater than or equal to 30 nM/L of whole blood. In some such embodiments, the thiamine or thiamine equivalent is administered orally. In some embodiments, thiamine is administered to the patient at a dose of about 100 mg per day. In some embodiments, the patient is administered a thiamine equivalent sufficient to deliver about 100 mg of thiamine per day.

In some embodiments, provided methods comprise administering thiamine or a thiamine equivalent when the patient's thiamine levels are 30 nM/L of whole blood. In some such embodiments, the thiamine is administered to the patient according to the following schedule:

(i) about 500 mg three times daily (TID) for 2 or 3 days,
(ii) about 250 mg to about 500 mg daily (QD) for 3 to 5 days, and
(iii) about 100 mg daily (QD) for at least 90 days.

In some embodiments, a patient whose thiamine levels are 30 nM/L of whole blood is administered a thiamine equivalent sufficient to deliver an amount of thiamine according to the following schedule:

(i) about 500 mg three times daily (TID) for 2 or 3 days,
(ii) about 250 mg to about 500 mg daily (QD) for 3 to 5 days, and
(iii) about 100 mg daily (QD) for at least 90 days.

In some embodiments, thiamine or thiamine equivalent is administered intravenously.

In some embodiments, provided methods further comprise monitoring the patient's magnesium levels. In some such embodiments, provided methods comprise increasing the patient's magnesium level.

In some embodiments, the patient has a myeloproliferative disease or condition. In some embodiments, the myeloproliferative disease or condition is selected from primary myelofibrosis, secondary myelofibrosis, polycythemia vera, essential thrombocythemia, post polycythemia vera, and post essential thrombocythemia. In some embodiments, the myeloproliferative disorder is acute myeloid leukemia (AML). In some embodiments, the primary myelofibrosis is Dynamic International Prognostic Scoring System (DIPSS) intermediate or high-risk primary myelofibrosis. In some embodiments, said method comprises administering to a patient in need thereof a composition according to the present invention.

In some embodiments, the present disclosure provides a method for treating a patient comprising:

(i) administering Compound I, or a pharmaceutically acceptable salt or hydrate thereof, (e.g., Compound II), and
(ii) conducting a cognitive assessment.

In some embodiments, the cognitive assessment occurs during the 2nd 28-day cycle. In some embodiments, the cognitive assessment occurs during the 3rd 28-day cycle. In some embodiments, the cognitive assessment occurs during at least every 3rd 28-day cycle. In some embodiments, the cognitive assessment comprises a mini-mental state examination. In some embodiments, the method further comprises analyzing thiamine level in the patient.

EXEMPLIFICATION

Protocol Summary. A multicenter, single-arm, open-label efficacy and safety study of fedratinib in subjects previously treated with ruxolitinib and with DIPSS (Dynamic International Prognostic Scoring System) intermediate or high-risk primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF), or post-essential thrombocythemia myelofibrosis (post-ET MF).

Objectives. The primary objective of the study is to evaluate the percentage of subjects with at least a 35% spleen volume reduction with fedratinib. The secondary objectives are:

To evaluate the safety of fedratinib
To evaluate the reduction of spleen size by palpation
To evaluate myelofibrosis (MF)-associated symptoms as measured by the Myelofibrosis Symptom Assessment Form (MFSAF)
To evaluate duration of spleen response by MRI (magnetic resonance imaging)/CT (computed tomography) and by palpation
To evaluate the duration of symptoms response
To assess the effectiveness of the risk mitigation strategy for gastrointestinal (GI) events
To assess the risk mitigation strategy for Wernicke's encephalopathy (WE)

The exploratory objectives are:

To evaluate Overall Survival (OS)
To assess the effect of study treatment on selected treatment-related symptoms from the subject's perspective (diarrhea, nausea, vomiting, dizziness, headache), assessed by the Patient Reported Outcome Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)
To explore prognostic markers (e.g., gene mutations, cytogenics) at baseline and in relation to efficacy parameters
To explore biomarkers related to mechanisms of action of fedratinib (eg, circulating cytokines)

Study Population. The study will enroll approximately 110 subjects with intermediate- or high-risk primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF), or post-essential thrombocythemia myelofibrosis (post-ET MF).

All subjects discontinued from protocol-prescribed therapy for any reason will be followed for survival, subsequent therapies, and progression of myelofibrosis to acute myeloid leukemia (AML) every 3 months until death or up to 12 months after End of Treatment (EOT), lost to follow-up, withdrawal of consent for further data collection, or study closure, whichever comes first.

Study Design. The study will consist of the following 3 phases:

A 28-day Screening Period

Fedratinib Treatment Period including a 30-Day Follow-up After Last Dose Visit

A 12 month Survival Follow-up Period

Length of Study. The expected duration of study is approximately 4 years, which includes approximately 18 months to fully enroll, and 24 months for treatment and follow-up.

The End of Trial is defined as either the date of the last visit of the last subject to complete the Survival Follow-up, or the date of receipt of the last data point from the last subject that is required for primary, secondary and/or exploratory analysis, as prespecified in the protocol, whichever is the later date.

Screening Period. All enrolled subjects will undergo screening procedures during the screening period which must be completed within 28 days prior to the start of study treatment. This will serve to determine subject eligibility based on all inclusion and exclusion criteria defined in the protocol. For subjects that are receiving ruxolitinib during the screening period or that have potentially reversible laboratory abnormalities (or other criteria that excludes the subject from enrollment) detected during screening, the screening period may be extended to 35 days (additional 7 days).

Treatment Period. Upon confirmation of eligibility, subjects will be enrolled and receive treatment with fedratinib at a dose of 400 mg once daily orally continuously. Fedratinib is administered as the dihydrochloride monohydrate form (i.e., Compound II). Cycles are defined for administrative purposes as 4-week (28-day) periods. Unless otherwise noted, visit windows are ±3 days except for MRI/CT scan procedures which have a visit window of ±7 days. For the first 3 cycles, site visits will be performed on Day 1 and Day 15 and thereafter on Day 1 for the subsequent cycles. At Cycle 1 Day 8 the site will contact the subject by telephone to assess occurrence and discuss management of nausea, vomiting and diarrhea. Subjects may continue treatment with fedratinib until unacceptable toxicity, lack of therapeutic effect or withdraws consent. All subjects will be monitored for adverse events during the study. All subjects discontinued from protocol prescribed therapy for any reason will be followed for a period of 30 days following the last dose of fedratinib.

Fedratinib is self-administered orally once daily continuously on an outpatient basis, preferably together with food at dinner in the evening, the same time each day. In case a dose is missed, the next dose should be taken the following day at the same time of day as previously taken before the dose was missed.

For the first 3 cycles, site visits will be performed on Day 1 and Day 15 and thereafter on Day 1 for the subsequent cycles. At Cycle 1 Day 8 the site will contact the subject by telephone to assess occurrence and discuss management of nausea, vomiting and diarrhea.

Subjects may continue treatment with fedratinib until unacceptable toxicity, lack of therapeutic effect, progression of disease, or until consent is withdrawn.

All subjects will be monitored for adverse events during the study.

All subjects discontinued from protocol-prescribed therapy for any reason will be followed for at least a period of 30 days following the last dose of fedratinib.

The most common adverse events associated with fedratinib are hematological and gastrointestinal. Hematological adverse events associated with JAK inhibitors are dose dependent, mechanism-based and their managed through dose reductions, dose interruptions and transfusions.

The fedratinib dose in this study is 400 mg/day. If a subject experience a drug toxicity as specified in the Dose Modification Schedule table (Table 1), the dosing should be interrupted and the dose may need to be modified.

If a subject does not tolerate fedratinib therapy after 2 dose level reductions from the starting dose, he/she must be withdrawn from the study treatment. If the toxicity does not resolve in the time period as specified in the Dose Modification Schedule table (Table 1), subjects must be withdrawn from the study treatment. Reescalation of doses is possible in certain cases as defined in the Dose Modification Schedule table (Table 1). The daily dose of fedratinib cannot exceed 400 mg/day.

Subjects may continue treatment with fedratinib until unacceptable toxicity, lack of therapeutic effect or until a subject is not compliant with treatment or withdraws consent.

Dose Modification Schedule

A flexible dose modification regimen may be employed to minimize drug toxicity for individual subjects, with possible daily doses are of 200 mg, 300 mg, or 400 mg.

The most common adverse events associated with fedratinib are hematological and gastrointestinal events. Hematological adverse events associated with JAK inhibitors are dose dependent, mechanism-based and are managed through dose reductions, dose interruptions and transfusions.

If a subject experiences a drug toxicity as specified in Table 1, infra, the dosing must be interrupted; in some cases (i.e. when it is not a liver function test (LFT) abnormality) the dose can be titrated by a 100 mg/day decrement during the study, depending upon the Investigator's judgment, down to a minimum dose of 200 mg/day. For subjects with severe impairment of renal function and co-administration of strong or moderate CYP3A4 inhibitors the fedratinib dose is adjusted.

If a subject does not tolerate fedratinib therapy after 2 dose level reductions from the starting dose, he/she must be withdrawn from the study treatment. If the toxicity does not resolve in the time period as specified in Table 1, subjects must be withdrawn from the study treatment. Reescalation of doses is possible in certain cases. The daily dose of fedratinib cannot exceed 400 mg/day (based on the free base weight).

TABLE 1

Dose Modification Schedule

Adverse Event	Fedratinib Management	Recovery	Fedratinib Dose After Recovery
Hematological			
Grade 4 or Grade 3 thrombocytopenia with major bleeding	Hold fedratinib up to 28 days	Grade ≤3 thrombocytopenia without bleeding	Dose decrement by 1 dose level: 100 mg/daily decrease
Grade 4 neutropenia	Hold fedratinib up to 28 days	Grade ≤2 neutropenia	Dose decrement by 1 dose level: 100 mg/daily decrease

TABLE 1-continued

Dose Modification Schedule			
Adverse Event	Fedratinib Management	Recovery	Fedratinib Dose After Recovery
Grade 4 hematological toxicity with dose reduction in subsequent cycle	—	Toxicity resolves for at least 1 cycle	Subsequent upward dose titration possible of 1 dose level (100 mg daily) per cycle as per the Investigator's discretion
Recurrence of a grade 4 hematological toxicity	—	—	Subsequent upward dose titration not permitted Fedratinib discontinuation as per the Investigator's discretion
Non-hematological			
Drug-related non-hematological Grade 4 or unmanageable Grade 3 toxicity with dose reduction in subsequent cycle	—	—	Subsequent upward dose titration not permitted Fedratinib discontinuation as per the Investigator's discretion
Hepatic (LFT abnormalities)			
Grade ≥3 AST or ALT or total bilirubin	Hold fedratinib Weekly monitoring of LFTs, until resolution, After fedratinib resumed, LFT monitoring every 2 weeks for the 3 subsequent cycles at a minimum	Grade ≤1	Fedratinib Hold ≤14 days: Dose decrement by 1 dose level: 100 mg daily decrease Subsequent upward dose titration not permitted Fedratinib Hold >14 days (AE did not return to Grade ≤1): fedratinib permanently discontinued Grade 4 in the absence of demonstrable cause: permanently discontinue fedratinib
Recurrence of LFT abnormality (i.e. ≥Grade 3 toxicity) after dose reduction	Discontinue fedratinib permanently	—	—
Gastrointestinal			
Grade 2 nausea, vomiting, diarrhea, or constipation that does not respond to adequate therapeutic or supportive measures within 48 hours	Hold fedratinib up to 14 days	Toxicity resolves to Grade ≤1	Consider resuming the dose at the same level after resolution of adverse event
Grade ≥3 or recurrence of Grade 2 nausea vomiting, diarrhea, or constipation that does not respond to adequate therapeutic or supportive measures within 48 hours	Hold fedratinib up to 14 days	Toxicity resolves to Grade ≤1	Consider reducing one dose level after resolution of adverse event
Other Adverse Events Not Described Above			
Grade ≥3 or recurrence of Grade 2 that does not respond to adequate therapeutic or supportive measures within 48 hours	Hold fedratinib up to 14 days	Toxicity resolves to Grade ≤1	Consider reducing one dose level after resolution of adverse event

TABLE 1-continued

Dose Modification Schedule			
Adverse Event	Fedratinib Management	Recovery	Fedratinib Dose After Recovery
Grade ≥3 non-hematological toxicity, non-gastrointestinal toxicity or Grade ≥2 peripheral neuropathies	Hold fedratinib up to 14 days	Toxicity resolved to Grade ≤1	Dose decrement by 1 dose level: 100 mg daily decrease

Dose Adjustment for Co-Administration with Strong and Moderate CYP3A4 Inhibitors

Concomitant administration of fedratinib with strong or moderate CYP3A4 inhibitors can increase fedratinib exposure. Increased fedratinib exposure may increase the risk of exposure-related AEs and need to be considered carefully.

For subjects with a co-administration of a strong CYP3A4 inhibitor a dose reduction of the starting dose of fedratinib from 400 mg to 200 mg is recommended. In case a strong CYP3A4 inhibitor is required to be introduced during fedratinib treatment consider dose reduction by 2 decrement dose level (e.g. 300 mg to 100 mg). Strong CYP3A4 inhibitors include, but are not limited to, boceprevir, cobicistat, conivaptan, danoprevir and ritonavir, elvitegravir and ritonavir, grapefruit juice, indinavir and ritonavir, itraconazole, ketoconazole, lopinavir and ritonavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, troleandomycin, and voriconazole.

For subjects with a co-administration of a moderate CYP3A4 inhibitor a dose reduction from the starting dose of fedratinib from 400 mg to 300 mg is recommended. In case a moderate CYP3A4 inhibitor is required to be introduced during fedratinib treatment consider dose reduction by 1 decrement dose level (e.g. 300 mg to 200 mg). Moderate CYP3A4 inhibitors include, but are not limited to, aprepitant, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, and verapamil.

If the fedratinib dose needs to be reduced below 100 mg daily based on any fedratinib related AEs due to a potentially increased plasma concentration of fedratinib, consider a lower average daily dose by administering, for example, 100 mg fedratinib every other day that is equivalent to an average daily dose of 50 mg.

If AEs are still not resolved after reducing fedratinib dose, consider interrupting dosing of either fedratinib or strong CYP3A4 inhibitors based on overall benefit/risk for a patient.

In cases where co-administration with the CYP3A4 inhibitor is discontinued, the fedratinib dose should be re-escalated accordingly.

Dose adjustment for renal impairment. No dose adjustment is recommended in subjects with mild and moderate renal impairment. In subjects that develop severe renal impairment during the study the fedratinib dose should be adjusted by one dose decrement level (e.g. from 400 mg to 300 mg once a day [QD]). Subject on a planned dose of 200 mg QD are allowed to reduce to 100 mg.

Management of Potential Wernicke's Encephalopathy (WE)

A potential case of WE is a medical emergency. Screening for WE and management of potential cases of WE during treatment with fedratinib will be done according the following steps:

Clinical and Cognitive Assessment. Interval history: including a review of the subject's history for confusion, memory problems, vision problems (e.g. double vision) as well as poor nutrition, signs and symptoms of malabsorption, and alcohol use

- Physical examination: including assessment for abnormal eye movements, cerebellar abnormalities and body weight (weight loss compared to previous examination or patient history) during screening and Day 1 of every treatment cycle, at the End of Treatment (EOT), and the 30-Day Follow-up visit
- Mini-Mental State Examination (MMSE): to objectively assess for signs/symptoms of encephalopathy during screening, on Day 1 of Cycles 2 and 3 and every third cycle thereafter, at EOT, and more frequently as clinically indicated

Management of Potential WE. In case of signs or symptoms that may indicate WE:

- Hold fedratinib until WE is ruled out
- Obtain sample for thiamine level
- Empirically start thiamine supplementation
- Report the event as an AESI to the Sponsor
- Obtain a neurological consult
- Perform a brain MRI
- If WE is confirmed discontinue fedratinib permanently

Thiamine Monitoring and Correction. Thiamine levels (for whole blood) will be monitored and thiamine supplementation will be administered to all subjects with thiamine levels below the normal range.

- Thiamine levels are assessed at screening and need to be corrected and retested before starting fedratinib treatment
- While on treatment with fedratinib, thiamine levels are assessed at start of Cycle 2, 3 and every third cycle thereafter or as clinically indicated:
  - If a subject is on thiamine supplementation, thiamine levels should be assessed in a fasting state for thiamine supplementation and thiamine given after the blood draw
  - If a thiamine level result is below normal, the site will contact the subject as soon as possible to start thiamine supplementation
  - For thiamine levels below the normal range but ≥30 nM/L without signs or symptoms of WE:
    - Supplementation with 100 mg oral thiamine must be started
  - If the results were obtained by a local laboratory, report the event as an Adverse Event of Special Interest (AESI) to the Sponsor

For thiamine level <30 nM/L with or without signs or symptoms of WE:

- Immediate treatment with thiamine (preferably intravenous (IV)) at therapeutic dosages (e.g., 500 mg infused over 30 minutes 3 times daily for 2 to 3 days) or alternatively intramuscular (IM) in equivalent doses according to local standard of care
- Report the event as an Adverse Event of Special Interest (AESI) to the Sponsor
- This will be followed by 250 mg to 500 mg IV thiamine infused once a day for 3 to 5 days or alternatively IM in equivalent doses according to local standard of care
- And then continue at an oral daily dose of 100 mg thiamine for at least 90 days
- Fedratinib must be held until thiamine levels are restored to normal range.

Thiamine supplementation should be administered as a thiamine only formulation.

- If thiamine levels are low, ensure that magnesium levels are normal or corrected if low
- An Adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring and rapid communication by the Investigator to the sponsor.

The following are considered to be Adverse Events of Special Interest (AESI):

- Wernicke's encephalopathy (WE) or suspected cases of WE associated with thiamine levels below normal range.
- Thiamine levels below normal range with or without signs or symptoms of WE
- New malignancy after start of study treatment
- Progression of myelofibrosis to acute myeloid leukemia (AML)
- Cardiac failure or cardiomegaly
- Grade 3 and 4 hyperlipasemia or Grade 3 and 4 hyperamylasemia according to CTCAE criteria, v 5.0 or events of pancreatitis
- Grade 3 or 4 alanine transaminase (ALT), aspartate transaminase (AST), or total bilirubin elevation or events of hepatotoxicity

Management of Nausea and Vomiting. Management of nausea and vomiting during treatment with fedratinib will be done according the following steps:

- Subjects will be provided management instructions (including when to contact the study site) before the start of treatment
- In order to mitigate for nausea and vomiting events, it is recommended to take fedratinib with food during an evening meal.
- It is highly recommended to use anti-nausea/vomiting treatment prophylactically according to local practice for the first 8 weeks of treatment (e.g., ondansetron). If dimenhydrinate or other muscarinic receptor antagonists are used for nausea and vomiting, administer these agents in the evening to minimize drowsiness and other potential neurological AEs
- Hold/reduce the dose of fedratinib according to Table 1
- Hospitalization may be indicated for Grade 3 or higher nausea or vomiting or events that persist
- For medications that are administered for prophylactic use of nausea and vomiting, if no clinically significant nausea and vomiting occurs during the first 8 weeks of fedratinib treatment, consider weaning the subject off these medications

Management of Diarrhea. Management of diarrhea during treatment with fedratinib will be done according the following steps:

- Subjects should have loperamide available at home and should be provided with diarrhea management instructions (including when to contact the study site) before the start of treatment
- Loperamide should not be given as prevention in case the subject does not experience diarrhea
- Treat with loperamide as per local practice at the onset of diarrhea. Consider starting loperamide at a 4 mg loading dose and then 2 mg after each diarrheal bowel movement without exceeding 16 mg/24 hours
- Dietary modifications including adequate hydration, avoidance of lactose containing foods and alcohol, small meals with rice, bananas, bread, etc.
- Hold/reduce the dose of fedratinib according to Table 1
- Hospitalization may be indicated for Grade 3 or higher persisting diarrhea.
- Management of nausea, vomiting and diarrhea will be assessed during the subject's visit on Day 1 of every following 28-day cycle, at Day 15 of the first three cycles and by a mandatory telephone contact at Day 8 of the first cycle.

Overview of Key Efficacy Assessments

Unless otherwise specified, analysis of spleen volume response will be performed on the efficacy evaluable population, myelofibrosis symptom response analyses will be performed on the MFSAF population and spleen size response analyses will be performed on the safety population.

Spleen Volume Response Rate (RR) by MRI/CT. Response rate of reduction in spleen volume is defined as proportion of subjects who have a ≥35% reduction in spleen volume at the end of Cycle 6 as compared to baseline. The response rate and 95% confidence interval will be provided. In addition, a descriptive summary of spleen volumes measurements and percentage change from baseline will be provided. Subjects with a missing MRI/CT spleen volume at the end of Cycle 6 including those who meet the criteria for progression of splenomegaly before the end of Cycle 6 will be considered non-responders.

A sensitivity analysis will be conducted for response rate of subjects who have a ≥25% reduction in spleen volume at the end of Cycle 6 as compared to baseline.

Spleen Response Rate by Palpation (RRP). Spleen response rate by palpation is the proportion of subjects with a spleen response according to the IWG-MRT 2013 at the end of Cycle 6 as compared to baseline. This will be calculated for subjects that have an enlarged spleen (≥5 cm below LCM) at baseline. Subjects with a missing spleen size assessment at the end of Cycle 6 including those who meet the criteria for progression of splenomegaly before the end of Cycle 6 will be considered not to be responders. The response rate and 95% confidence interval will be provided.

Symptom Response Rate (SRR). Symptom response rate (SRR) is defined as the proportion of subjects with ≥50% reduction from baseline to the end of Cycle 6 in total symptom score (TSS) measured by MFSAF version 4.0. The SRR and 95% confidence interval will be provided. The TSS will be defined as the sum of each of the 7 symptom scores (Gwaltney C, Paty J, Kwitkowski V E, Mesa R A, Dueck A C, Papadopoulos E J, et al. Development of a harmonized patient-reported outcome questionnaire to assess myelofibrosis symptoms in clinical trials. Leuk Res. 2017 August; 59:26-31). To allow indirect comparison with previous MF studies, a modified TSS (Mesa R A, Gotlib J, Gupta V,

Catalano J V, Deininger M W, Shields A L, et al. Effect of ruxolitinib therapy on myelofibrosis-related symptoms and other patient-reported outcomes in COMFORT-I: a randomized, double-blind, placebo-controlled trial. J Clin Oncol. 2013 Apr. 1; 31(10):1285-92) will also be derived from the 6 symptoms considered (night sweats, pruritus, abdominal discomfort, early satiety, pain under ribs on left side, bone or muscle pain) and analysis of SRR will be also performed.

At each timepoint, the TSS (based on 7 symptoms) and the modified TSS will be calculated. Descriptive summary statistics (size, mean, standard deviation, median, range) will be provided for baseline scores, postbaseline scores and change from baseline for TSS, modified TSS and symptom scores.

Subjects without a baseline TSS>0 will be considered non-evaluable (due to no place for symptom reduction) for the SRR analysis. Subjects with a missing TSS at the end of Cycle 6 or who had disease progression before the end of the Cycle 6 will be considered non-responders.

Duration of Spleen Volume Response by MRI/CT (DR). Duration of spleen volume response (DR) by MRI/CT is defined as time from the first documented spleen response (ie, ≥35% reduction in spleen volume) to the first documented spleen volume reduction <35%. In the absence an event (ie subsequent spleen volume reduction <35% before the analysis is performed), the DR will be censored at the date of the last valid assessment performed before the analysis performed date.

Duration of spleen volume response by MRI/CT scan will be analyzed using Kaplan-Meier method. K-M estimates of the 25th, 50th, and 75th percentiles and the 95% confidence interval of median will be provided. K-M curves will be plotted.

Duration of Spleen Response by Palpation (DRP). Duration of spleen response by palpation (DRP) is defined as time from the first documented palpable spleen response, according to the IWG-MRT 2013 to the time of the first documented loss of response according to the IWG-MRT 2013. Duration of spleen response by palpation according to the IWG-MRT 2013 criteria will be calculated for subjects that have an enlarged spleen at baseline (≥5 cm below LCM), and that have a spleen response by palpation. In the absence of an event (ie no loss of spleen response by palpation) before the analysis is performed, the DRP will be censored at the date of the last valid assessment performed before the analysis performed date.

Duration of Symptoms Response (DSR). Duration of symptoms response is defined as time from the first documented response in TSS (ie, reduction in TSS≥50%) measured by MFSAF version 4.0 to the first documented TSS reduction <50%. In the absence of TSS reduction <50% before the analysis performed, the DSR will be censored at the date of the last valid assessment performed before the analysis performed date. DRS will be analyzed using K-M method. The K-M estimates of the 25th, 50th, and 75th percentiles and the 95% confidence intervals of median will be provided, and K-M curves will be plotted.

TABLE 2

Study Endpoints

Endpoint	Name	Description	Timeframe
Primary	Spleen volume response rate	Proportion of subjects who have a ≥35% reduction in spleen volume at the end of Cycle 6	From Screening to the end of Cycle 6
Secondary	Safety profile of fedratinib	Incidence and severity of all Grade adverse events (AEs) per NCI CTC Incidence and severity of Grade 3-4 AEs as per the NCI CTC, including laboratory parameters	From ICF signature up until 30 days post last dose For fedratinib related AEs, anytime until the last study visit
	Spleen response rate by palpation	Proportion of subjects who have a ≥50% reduction in spleen size by palpation	From C1D1 to the end of cycle 6
	Symptom response rate	Proportion of subjects with a ≥50% reduction in total symptom scores measured by MFSAF	From C1D1 to the end of cycle 6
	Durability of spleen response	Duration of ≥35% reduction in spleen volume	From screening to the End of Treatment visit
	Durability of spleen response by palpation	Duration of ≥50% reduction in spleen size by palpation for subjects with a palpable spleen at least 5 cm below the left costal margin (LCM) at C1D1	From C1D1 until the 30-day follow-up after last dose visit
	Durability of symptoms response	Duration of ≥50% reduction in total symptom scores measured by MFSAF	From C1D1 until the 30-day follow-up after last dose visit
	Assessment of risk mitigation strategy for gastrointestinal adverse events and potential Wernicke's encephalopathy (WE)	Incidence of patients with a CTCAE grade ≥3 of nausea, diarrhea, or vomiting, or occurrence of WE (confirmed by brain MRI or autopsy). Assessment of thiamine levels at screening, on Day 1 of the first 3 cycles and every third cycle thereafter, and at the End of Treatment visit	From ICF signature to the 30-day follow-up after last dose visit

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TABLE 2-continued

Study Endpoints

Endpoint	Name	Description	Timeframe
	HRQoL measured by EORTC QOL-C30 domains	Mean changes in the HRQoL function and symptom domain scores over the study compared with C1D1	From C1D1 to Day 1 of each cycle, at the End of Treatment visit up to the 30-day follow-up after last dose visit
	PRO measured by EQ-5D-5L	Mean changes in the health utility scores over the study compared with enrollment measured by EQ-5D-5L	From C1D1 to Day 1 of each cycle up to the 30-day follow-up after last dose visit
Exploratory	Overall Survival	Time from C1D1 to death due to any reason	From enrollment to 12 months after the End of Treatment visit
	Treatment-related symptoms from the subject's perspective	Assessment of the five selected treatment-related symptoms from the subject's perspective (diarrhea, nausea, vomiting, dizziness, headache) using the PRO-CTCAE	From C1D1 to Day 1 of each cycle, at the End of Treatment visit up to the 30-Day Follow-up after last dose visit
	Prognostic markers	Genetic alterations, including cytogenetics and gene mutations in peripheral blood	From C1D1 to End of Treatment visit
	Mechanism of Action	Circulating proteins, including cytokines, immune and hematopoietic markers	From C1D1 to End of Treatment visit

AEs = adverse events; C1D1 = Cycle 1 Day 1; CTC = Common Terminology Criteria for Adverse Events; ICF = informed consent form; MFSAF = Myelofibrosis Symptom Assessment Form; MRI = magnetic resonance imaging; NCI = National Cancer Institute; PRO-CTCAE = Patient Reported Outcome Version of Common Terminology Criteria of Adverse Events; SVR = spleen volume reduction.

Survival Follow-up Phase. All subjects discontinued from protocol-prescribed therapy for any reason will be followed for survival, subsequent therapies, new malignancy and progression of myelofibrosis to acute myeloid leukemia (AML) every 3 months until death or up to 12 months after end of treatment (EOT), lost to follow-up, withdrawal of consent for further data collection, or study closure.

The invention claimed is:

1. A method for treating a patient comprising:

administering to the patient Compound I

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or a pharmaceutically acceptable salt and/or hydrate thereof;

(ii) monitoring thiamine level in the patient; and

(iii) administering to the patient thiamine or a thiamine equivalent if the patient's thiamine level is below a reference standard, wherein the reference standard is from about 74 to about 222 nM/L of whole blood wherein the patient has a myeloproliferative disorder.

2. The method of claim **1**, wherein the patient's thiamine level is monitored by analyzing one or more biomarkers for thiamine deficiency.

3. The method of claim **1**, wherein thiamine is administered to the patient at an amount of about 100 mg per day.

4. The method of claim **1**, wherein thiamine is administered to the patient at an amount of about 250 mg to about 500 mg.

5. The method of claim **4**, wherein thiamine is administered to the patient at an amount of about 250 mg.

6. The method of claim **4**, wherein thiamine is administered to the patient at an amount of about 500 mg.

7. The method of claim **1**, wherein thiamine is administered intravenously.

8. The method of claim **1**, wherein thiamine is administered according to the following schedule:

about 500 mg TID for 2 or 3 days;

about 250 mg to about 500 mg daily (QD) for 3-5 days; and

about 100 mg QD for 90 days.

9. The method of claim **1**, wherein the patient is administered a thiamine equivalent sufficient to deliver about 100 mg of thiamine mg per day.

10. The method of claim **1**, wherein thiamine or a thiamine equivalent is administered orally.

11. The method of claim **1**, wherein the patient is administered a thiamine equivalent sufficient to deliver about 250 mg to about 500 mg of thiamine.

12. The method of claim **11**, wherein the thiamine equivalent is sufficient to deliver about 250 mg of thiamine.

13. The method of claim **11**, wherein the thiamine equivalent is sufficient to deliver about 500 mg of thiamine.

14. The method of claim **11**, wherein the thiamine equivalent is sufficient to deliver an amount of thiamine according to the following schedule:

about 500 mg TID for 2 or 3 days;

about 250 mg to about 500 mg daily (QD) for 3-5 days; and

about 100 mg QD for 90 days.

15. The method of claim **1**, further comprising increasing the patient's magnesium level.

16. The method of claim **1**, wherein the myeloproliferative disorder is myelofibrosis.

17. The method of claim **16**, wherein the myelofibrosis is primary myelofibrosis.

18. The method of claim **17**, wherein the primary myelofibrosis is selected from intermediate risk primary myelofibrosis and high risk primary myelofibrosis.

19. The method of claim **16**, wherein the myelofibrosis is secondary myelofibrosis.

20. The method of claim **16**, wherein the myelofibrosis is post essential thrombocythemia myelofibrosis.

21. The method of claim **16**, wherein the myelofibrosis is post polycythemia vera myelofibrosis.

22. The method of claim **1**, wherein the myeloproliferative disorder is acute myeloid leukemia (AML).

23. The method of claim **1**, wherein the myeloproliferative disorder is polycythemia vera.

24. The method of claim **1**, wherein the myeloproliferative disorder is essential thrombocythemia.

25. The method of claim **1**, wherein Compound I is in the form of a dihydrochloride monohydrate salt.

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