The Clinical Phenotype of Myelofibrosis Encompasses a Chronic Inflammatory State that is Favorably Altered by INCB018424, a Selective Inhibitor of JAK1/2

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1 Abstract

Background: Current evidence links some of the disease manifestations in myelofibrosis (MF) to abnormal cytokines, likely produced by clonally involved megakaryocytes and monocytes. Furthermore, the recent discovery of *JAK2/MPL* mutations in MF suggests the contribution of abnormal JAK-STAT signaling to both clonal myeloproliferation and cytokine-driven debility. In order to gain additional pathogenetic insight regarding cytokine-phenotype correlations in MF, we looked into the plasma cytokine profile of MF before and after treatment with INCB018424, a selective JAK1/2 inhibitor.

Methods: The current study includes subjects with MF enrolled in an ongoing phase 1-2 study of oral INCB018424 (doses ranging from 25 mg/day to 50 mg BID). Plasma samples were obtained prior to treatment and at intervals of 2 weeks, 1 month and 2 months following INCB018424 dosing. Samples were submitted to Rules Based Medicine Human MAP multiplexed immunoassay system to obtain plasma levels on a range of protein analytes.

Results:

(i) ***Plasma cytokine levels in MF patients (n=53) vs. normal healthy volunteers (n=15):*** Compared to normal controls, plasma levels of pro-inflammatory cytokines and markers of inflammation were significantly increased in MF patients (see table in slide 10; mean ± SD values). Furthermore, the observed inflammatory cytokine levels in MF were often higher than those seen in patients with rheumatoid arthritis or cancer-associated cachexia.

(ii) ***Correlation of plasma cytokine levels in MF with JAK2V617F mutational status, MF subtype and/or constitutional symptoms/cachexia:*** Comparison of *JAK2*V617F positive (n=40) and negative (n=13) MF cases suggested significantly (p<0.01) higher IL-1RA (mean ± SD = 5,575 ± 917 vs. 1291 ± 359 pg/ml) and CRP (17.4 ± 1.6 vs. 6.7 ± 1.9 µg/ml) levels for the former whereas the other cytokines were elevated to a similar extent. Plasma cytokine levels in PMF (n=30) vs. post-PV MF (n=15) vs. post-ET MF (n=8) were not significantly different. The presence of prior splenectomy did not appear to alter the specific MF cytokine profile (Table 1); the abnormal cytokine profile in MF is, therefore, not necessarily a consequence of marked splenomegaly. There was a direct correlation between the levels of pro-inflammatory cytokines and the presence or absence of constitutional symptoms/cachexia (Figure). Similarly, increased inflammatory cytokine levels in MF were accompanied with significantly decreased leptin levels, a surrogate for nutritional status (Table).

(iii) ***Post-INCB018424 treatment cytokine levels:*** Treatment with INCB018424 induced a rapid decrease in MF-associated inflammatory cytokine levels, in parallel with the observed clinical benefit of both reduced splenomegaly and improvement in constitutional symptoms/cachexia.

Conclusion: The plasma cytokine profile of MF is reminiscent of a chronic inflammatory state with levels of pro-inflammatory cytokines that are possibly higher than those seen in other inflammatory/malignant conditions. Furthermore, the current study suggests a fundamental link between these cytokines and MF-associated constitutional symptoms/cachexia. Cytokine modulation through JAK-STAT inhibition appears to be a mechanism of action for INCB018424 in MF.

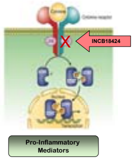
2 Cytokine Association with MF

- Myelofibrosis (MF) is associated with profound constitutional symptoms that are typically associated with chronic inflammation
  - Fatigue, night sweats, fever, and unintended weight loss
- Pro-inflammatory cytokines mediate chronic inflammation
  - Elevated cytokines are known to be responsible for systemic inflammation, hypercatabolic state, muscle and adipose tissue wasting and fever in advanced cancer patients with cachexia, chronic infections and inflammatory diseases*
  - Elevated plasma cytokines are associated with shortened survival in cancer patients†
- Many pro-inflammatory cytokines use JAK-STAT pathway for signaling
  - Mutations in JAK2 and elevated cytokines result in hyperactivation of JAK signaling in myelofibrosis and hence targeting JAK-STAT pathway is an attractive approach for the treatment of myelofibrosis

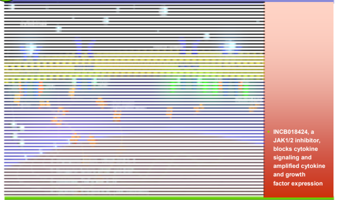
Source:* Ravasco et al. (2007). †*Source:*** Mantovani et al. (2001)

3 Objectives

- To characterize the plasma levels of cytokines and growth factors in MF using an unbiased proteomic analysis
- To evaluate the relationship between plasma cytokine levels and disease status in MF
- To study the effect of INCB018424, a potent selective inhibitor of JAK1 and JAK2, on JAK/STAT signaling in MF patients
- To study the effect of INCB018424 treatment on plasma cytokines and growth factor levels in MF patients
- To assess the effect of INCB018424 on constitutional symptoms in MF



4 INCB018424 Mechanism of Action



5 Methods

- **Patient Samples:** Plasma samples were obtained from 53 patients enrolled in the phase I/II study INCB018424-251. Patient plasma samples were collected at different time points including pre-treatment and at intervals of 2 weeks, 1 month, 2 months, 3 months, 6 months, and 9 months following the initiation of INCB018424 dosing.

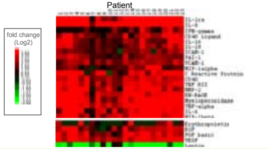
  Plasma samples were also obtained from 15 normal healthy volunteers using the same sample collection protocol.
- **Cytokine Analysis:** The HumanMAP multiplexed immunoassay system (Rules Based Medicine, Austin, TX) was used to evaluate all plasma samples. The protein analytes included in this assay are listed below. All data are presented as the average and all comparisons were run using a two-tailed Student t-test, with p<0.05 considered significant.
- **Heat Map Analysis:** MF patient data were analyzed for differences at baseline compared with normal healthy volunteers and for changes on day 28 after initiation of 25 mg BID INCB018424 treatment compared to baseline using clustering software. The antigens were clustered along the X-axis and the patients are clustered down the Y-axis.
  - **Green** denotes decreased levels
  - **Red** denotes increased levels
  - A scale is provided for each graph with numbers being shown in log 2 (e.g., 3.5 = 11.3-fold change).

6 Protein Analytes in RBM HumanMAP® Panel

1. Adiponectin
2. Alpha-1 antitrypsin
3. Alpha-2 macroglobulin
4. Alpha-fetoprotein
5. Apolipoprotein A-1
6. Apolipoprotein C-III
7. Apolipoprotein H
8. Aspartate transaminase
9. Beta-2 microglobulin
10. Brain-derived neurotrophic factor
11. Calcitonin
12. Cancer Antigen 125
13. Cancer Antigen 19-9
14. Carcinoembryonic antigen
15. CD40
16. CD40 ligand
17. Creatine kinase-MB
18. Complement C3
19. C-reactive protein
20. ENA-78
21. Endothelin-1
22. ENRAGE
23. Eotaxin
24. Epidermal growth factor
25. Erythropoietin
26. Factor VII
27. Fatty acid binding protein
28. Ferritin
29. FGF-basic
30. Fibrinogen
31. G-CSF
32. Glutathione S-transferase
33. GM-CSF
34. Growth hormone
35. Haptoglobin
36. ICAM-1
37. Immunoglobulin A
38. Immunoglobulin E
39. Immunoglobulin M
40. Insulin
41. Insulin-like growth factor-1
42. Interferon-gamma
43. Interleukin-1 alpha
44. Interleukin-1 beta
45. Interleukin-1 Receptor Antagonist
46. Interleukin-10
47. Interleukin-12 p40
48. Interleukin-12 p70
49. Interleukin-13
50. Interleukin-15
51. Interleukin-16
52. Interleukin-2
53. Interleukin-3
54. Interleukin-4
55. Interleukin-5
56. Interleukin-6
57. Interleukin-7
58. Interleukin-8
59. Leptin
60. Lipoprotein (a)
61. Lymphotactin
62. MCP-1
63. MDC
64. MIP-1 alpha
65. MIP-1 beta
66. MMP-2
67. MMP-3
68. MMP-9
69. Myeloperoxidase
70. Myoglobin
71. PAI-1
72. Pregnancy-associated plasma protein A
73. Prostatic Acid Phosphatase
74. PSA, Free
75. RANTES
76. Serum Amyloid P
77. Sex Hormone Binding Globulin
78. Stem Cell Factor
79. Thrombopoietin
80. Thyroid Binding Globulin
81. Thyroid Stimulating Hormone
82. TIMP-1
83. Tissue Factor
84. Tumor Necrosis Factor RII
85. Tumor Necrosis Factor-alpha
86. Tumor Necrosis Factor-beta
87. VCAM-1
88. VEGF
89. von Willebrand Factor

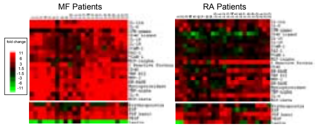
7 Plasma Cytokine and Growth Factor Levels in MF Patients and Healthy Volunteers

- MF is characterized by extremely high levels of a broad range of pro-inflammatory cytokines and growth factors



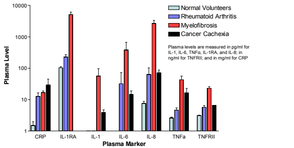
8 Cytokine and Growth Factor Levels in Patients with MF and Active Rheumatoid Arthritis

- The elevated levels of pro-inflammatory cytokines and growth factors in MF exceeds that observed in RA in terms of both breadth and degree of elevation



9 Cytokine Elevation in MF Patients

- Inflammatory cytokine elevation in MF patients is more pronounced than in other disease states associated with chronic inflammation


Normal Volunteers
Rheumatoid Arthritis
Myelofibrosis
Cancer Cachexia
Plasma levels are measured in pg/ml for IL-1, IL-6, TNFα, IL-1RA, and IL-8; in ng/ml for TNFRII; and in mg/ml for CRP
Plasma Level
10000
1000
100
10
1
CRP
IL-1RA
IL-1
IL-6
IL-8
TNFa
TNFRII
Plasma Marker

10 Cytokine Levels in Subgroups of MF

- Markedly elevated levels of pro-inflammatory cytokines characterize all subgroups of MF

	Normal volunteers (N = 15)	Post-PV MF (N = 15)	Post-ET MF (N = 8)	PMF (N = 30)
IL-1β (pg/ml)	0.6	104	4.6	19
IL-1RA* (pg/ml)	103	6,721	2,350	4,005
IL-6 (pg/ml)	0	49	66	44
IL-8† (pg/ml)	7.6	4,019	1,652	1,597
TNFα (pg/ml)	2.6	63	32	39
TNFRII (ng/ml)	3.1	30	22	23

Note: ***All*** cytokine levels in ***all*** MF subgroups are elevated significantly relative to normal volunteers.
*IL-1RA is higher in post-PV MF vs post-ET MF.
†IL-8 is higher in post-PV MF vs PMF.

11 Cytokine Levels in V617F+/– MF

- Marked elevation in levels of inflammatory cytokines is observed in V617F-positive and -negative MF patients

	Normal volunteers (N = 15)	V617F negative (N = 13)	V617F positive (N = 40)
IL-1β (pg/ml)	0.6	16	49
IL-1RA* (pg/ml)	103	1,291	5,575
IL-6 (pg/ml)	0	101	34
IL-8 (pg/ml)	7.6	1,770	2,460
TNFα (pg/ml)	2.6	44	45
TNFRII (ng/ml)	3.1	22.6	25.8

Note: ***All*** cytokine levels in MF are significantly elevated relative to normal volunteers.
*IL-1RA is further elevated in V617F positive patients compared to V617 negative patients.

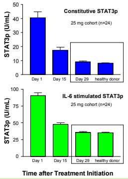
12 Cytokine Levels and Splenectomy

- High levels of cytokines are present in MF patients regardless of splenectomy

	Normal volunteer (N = 15)	Spleen present (N = 47)	Post-splenectomy (N = 6)
IL-1β (pg/ml)	0.6	41	44
IL-1RA (pg/ml)	103	4111	7759
IL-6* (pg/ml)	0	54	9.7
IL-8 (pg/ml)	7.6	2376	1618
TNFα (pg/ml)	2.6	45	38
TNFRII (ng/ml)	3.1	25	27

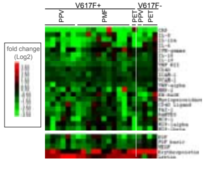
Note: ***All*** cytokine levels are significantly elevated in MF patients relative to normal volunteers
*IL-6 is higher in patients with spleen vs. post-splenectomy

13 Effects of INCB018424 in MF Patients



- MF patients present with:
  - Constitutive JAK activation in their peripheral blood cells, identified by spontaneously elevated levels of unstimulated, phosphorylated STAT3 ***and***
  - Enhanced STAT3 phosphorylation in response to IL-6 stimulation
- Treatment with INCB18424 returns the constitutive hyperactivation of JAK-STAT pathway to normal and normalizes the response to IL-6

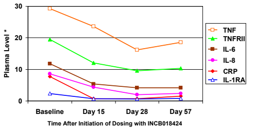
14 Effects of INCB18424 in MF Patients



- INCB018424 suppresses pro-inflammatory cytokines and angiogenic/fibrogenic growth factors in MF patients
- Treatment with INCB018424 25 mg BID for 28 days decreases the plasma levels of multiple cytokines and growth factors
- This effect is observed regardless of V617F status or MF subgroup

15 Effects of INCB018424 in MF Patients

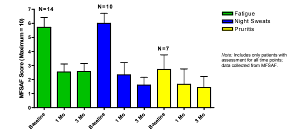
- Treatment of MF patients with INCB018424 results in rapid decreases of pro-inflammatory cytokine levels



*Plasma levels are given in pg/ml for TNF and IL-6; in ng/ml for TNFRII, IL-8, and IL-1RA; and in mg/ml for CRP.

16 Effects of INCB018424 in MF Patients

- INCB018424 treatment is followed by prompt and sustained improvement in MF symptoms associated with elevated levels of pro-inflammatory cytokines



17 Conclusions

- ***MF is a chronic inflammatory state***, in which pro-inflammatory cytokine levels are more profoundly elevated than in classic inflammatory diseases, like RA, or in other advanced malignancies
- ***Elevated levels of pro-inflammatory cytokines are ubiquitous in MF***, regardless of a patient's JAK mutational status, the presence of splenomegaly, or disease etiology
- Surgical splenectomy does not appear to reduce levels of pro-inflammatory cytokines – consistent with its lack of effect on constitutional symptoms
- ***Treatment with INCB018424 rapidly decreases pro-inflammatory cytokine levels in all MF patients***, regardless of their JAK mutational status or disease etiology
- ***INCB018424 directly inhibits the signaling of key cytokines***, such as IL-6, that are implicated in hypermetabolic state, fever, and weight loss, and treatment results in rapid resolution of cytokine-associated constitutional symptoms

18 References

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DISCLOSURES:
Drs. Tefferi, Kantarjian, Pardanani, Mesa, and Verstovsek: *Have all acted in advisory and/or consulting capacities to Incyte Corp. and have received research funding from the Company.*
Drs. Newton, Scherle, and Burn: *Are all current employees of Incyte Corp. and have equity in the Company.*

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