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Treatment Strategies and Survival after Ruxolitinib Discontinuation in Myelofibrosis Patients: the Italian RUX-MF Multicenter Study

Francesca Palandri[1], Massimo Breccia[2], Erika Morsia[3], Elena M. Elli[4], Giulia Benevolo[5], Mario Tiribelli[6], Eloise Beggiato[7], Mirko Farina[8], Giovanni Caocci[9], Novella Pugliese[10], Alessia Tieghi[11], Monica Crugnola[12], Gianni Binotto[13], Francesco Cavazzini[14], Alessandro Isidori[15], Alessandra Dedola[1,16], Emilia Scalzulli[2], Alessia Moioli[17], Fabrizio Cavalca[4], Santino Caserta[18], Andrea Duminuco[19], Bruno Martino[20], Roberto M. Lemoli[21,22], Daniela Cilloni[23], Monica Bocchia[24], Fabrizio Pane[10], Florian H. Heidel[25], Massimiliano Bonifacio[17], Giuseppe A. Palumbo[26], Filippo Branzanti[16], Alessandra Iurlo*[27], Elisabetta Abruzzese*[28]

*These authors contributed equally to this work

1) IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia “Seràgnoli”, Via Massarenti 9, 40138, Bologna, Italy
2) Hematology, Department of Translational and Precision Medicine, Az. Policlinico Umberto I-Sapienza University, Via Benevento 6, 00161, Rome, Italy
3) Hematology Unit, Department of Clinical and Molecular Sciences, DISCLIMO, Università Politecnica delle Marche, Via Tronto 10/A, 60020, Ancona, Italy
4) Fondazione IRCCS San Gerardo dei Tintori, Divisione di ematologia e unità trapianto di midollo, Via G.B. Pergolesi 33, 20900, Monza, Italy
5) University Hematology Division, Città della Salute e della Scienza Hospital, Corso Bramante 88, 10126, Torino, Italy
6) Division of Hematology and BMT, Department of Medical Area, University of Udine, P.le Santa Maria della Misericordia 15, 33100, Udine, Italy
7) Unit of Hematology, Department of Oncology, University of Torino, Via Verdi 8, 10124, Torino, Italy
8) Unit of Blood Diseases and Stem Cells Transplantation, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, P.le Spedali Civili 1, 25123, Brescia, Italy
9) Ematologia, Ospedale Businco, Università degli studi di Cagliari, Via Jenner, 09131, Cagliari, Italy
10) Department of Clinical Medicine and Surgery, Federico II University Medical School, Via S. Pansini 5, 80131, Naples, Italy
11) Department of Hematology, Azienda USL - IRCCS di Reggio Emilia, Viale Risorgimento 80, 42123, Reggio Emilia, Italy
12) Haematology and BMT Centre, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci 14, 43126, Parma, Italy
13) Unit of Hematology and Clinical Immunology, University of Padova, Via Giustiniani 2, 35128, Padova, Italy
14) Division of Hematology, University of Ferrara, Via Aldo Moro 8, 44124, Ferrara, Italy
15) Hematology and Stem Cell Transplant Center, AORMN Hospital, Via Lombroso 1, 61122, Pesaro, Italy
16) Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Università di Bologna, Via Massarenti 9, 40138, Bologna, Italy
17) Department of Engineering for Innovation Medicine, Section of Innovation Biomedicine, Hematology Area, University of Verona, P.le L.A. Scuro 10, 37134, Verona, Italy
18) Division of Hematology, Department of Human Pathology in Adulthood and Childhood "Gaetano Barresi", University of Messina, Via Consolare Valeria 98125 Messina, Italy
19) UO Ematologia, AOU Policlinico “G. Rodolico” – San Marco, Catania, Italy
20) Division of Hematology, Azienda Ospedaliera 'Bianchi Melacrino Morelli', Reggio Calabria, Italy
21) IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, 16132, Genoa, Italy
22) Clinic of Hematology, University of Genoa, Viale Benedetto XV, 6, 16132, Genoa, Italy
23) Department of Clinical and Biological Sciences, University of Turin, Largo Filippo Turati 62, 10128, Turin, Italy
24) Hematology Unit, Azienda Ospedaliera Universitaria Senese, University of Siena, Viale Bracci, 53100, Siena, Italy
25) Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School (MHH), Carl-Neuberg-Str.1, 30625, Hannover, Germany
26) Department of Scienze Mediche, Chirurgiche e Tecnologie Avanzate “G.F. Ingrassia”, University of Catania, Via S. Sofia 87, 95123, Catania, Italy
27) Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122, Milan, Italy
28) Division of Hematology, Ospedale S. Eugenio, Piazzale dell’Umanesimo 10, 00144, Roma, Italy

Corresponding Author
Filippo Branzanti
Alma Mater Studiorum Università di Bologna, Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale
Tel +39 051 214 3973
Fax +39 051 636 4037
e-mail: filippo.branzanti2@unibo.it

Keywords
Myelofibrosis; ruxolitinib; propensity score matching; second-line therapy

To The Editor,

Myelofibrosis (MF) is a *BCR::ABL* negative chronic myeloproliferative neoplasm (MPN) characterized by progressive bone marrow fibrosis, leading to impaired hematopoiesis, cytopenias, splenomegaly, and debilitating symptoms such as fatigue, night sweats, and weight loss. These features significantly impact quality of life and contribute to a reduced life expectancy.[1,2] The introduction of ruxolitinib, a *JAK1/JAK2* inhibitor, marked a major advancement in the treatment of MF, offering symptom relief, reduction in spleen size, and improved overall survival (OS).[3,4] However, ruxolitinib is not curative and becomes less effective over time for most patients. Many eventually require discontinuation due to resistance and/or intolerance, at which point OS is significantly reduced, highlighting the need for effective post-ruxolitinib treatment strategies.[5,6]

Second-line therapies for MF patients who discontinue ruxolitinib have undergone significant development in recent years. Novel JAK inhibitors (JAKi), like fedratinib, momelotinib, and pacritinib, have provided alternative options tailored to specific clinical challenges, including progressive splenomegaly, anemia, or thrombocytopenia.[7–9] Fedratinib has shown significant efficacy in reducing spleen size and alleviating symptoms, particularly after ruxolitinib failure.[7,10] Momelotinib, uniquely targeting *JAK1*, *JAK2*, and *ACVR1*, is effective in managing anemia with considerable hemoglobin improvements in many patients.[8,11] Pacritinib offers a valuable option for individuals with severe thrombocytopenia, a condition where therapeutic choices are limited.[9] Multiple agents with novel mechanisms of action are in ongoing clinical investigation.[12] Finally, allogeneic transplantation remains the only potentially curative strategy for eligible patients.[13]

Despite, but also thanks to, these advances, the management of patients who discontinue ruxolitinib remains challenging. In this evolving scenario, understanding the prognostic impact of differential second-line therapies is relevant for improving outcomes.

To address this issue, we report the findings from a retrospective cohort of 397 MF patients who discontinued ruxolitinib in chronic phase, with a particular focus on the impact of subsequent therapies on survival.

The study cohort was extracted from the "RUX-MF" (NCT06516406) study, which now includes 1055 MF patients treated with ruxolitinib in a real-life setting, as previously described.[14] Baseline characteristics, clinical and laboratory parameters at ruxolitinib initiation and discontinuation, and details of subsequent treatments were recorded. Only the patients who were in chronic phase and who received a second-line therapy within one year from ruxolitinib discontinuation were included. This specified time frame was intended to minimize variability in disease progression and treatment patterns, thereby facilitating more accurate assessment of therapeutic outcomes and prognostic factors within a consistent clinical context.

Second-line therapies were grouped into three groups: conventional therapy (CT), novel therapy (NT), and allogeneic stem cell transplantation (ASCT). Specifically, CT included all therapies that were commercially available and/or standard clinical practice at the time of ruxolitinib discontinuation, namely: hydroxyurea, steroids, danazol, busulfan, ruxolitinib-rechallenge, splenectomy, interferon and red blood cell/platelets transfusions. In order not to include patients in whom ruxolitinib discontinuation was only temporary, the minimum interval between withdrawal and restart of ruxolitinib was 3 months. NT encompassed investigational agents (n.23) and newer JAKi, including fedratinib (n.34), momelotinib (n.12), and pacritinib (n.2). ASCT was performed in accordance with the clinical practices of the referring center.

Statistical analyses included Kaplan-Meier survival curve, with delayed-entry considering different time periods between ruxolitinib discontinuation and second-line therapy start (log-rank tests).

A propensity score (PS) matching analysis was performed to balance patients who received conventional therapy or novel therapy. A 1:1 matching was achieved by creating matched sets of one patient treated with CT and one randomly sampled patient treated with NT (1:1 matching) who shared a similar PS. The PS was estimated using a logistic regression of exposure to NT on baseline covariates at the end of ruxolitinib treatment. Matching was performed using the nearest-neighbor method without replacement and with a caliper of width equal to 0.1 of the pooled standard deviation of the PS logit.

Tests were 2-sided, and P-values <0.05 were considered significant. Analyses were performed using STATA/SE software version 18.0 (StataCorp).

As shown in **Supplementary Figure 1**, the study cohort includes 1055 patients, of whom 272 (25.8%) received a second-line therapy within one year from ruxolitinib failure and represents the focus of this analysis.

Overall, 119 patients (43.8%) received a CT (mainly, HU: 35.3%; ruxolitinib rechallenge: 20.2%). A NT was administered to 71 patients (26.1%), while 82 (30.1%) patients underwent ASCT with no prior use of novel agents. In the ASCT cohort, 12 (14.6%) patients underwent to transplant while in good response. The mean time from ruxolitinib discontinuation and the start of a second-line therapy was 1.59 months (range: 0–11.2), and it was comparable in CT and NT patients (p=0.15).

Compared to ASCT patients, CT and NT patients showed larger spleen size, lower hemoglobin levels and older age at ruxolitinib stop. In addition, CT patients had significantly lower platelets count than NT and ASCT patients. Finally, the use of CT was more frequent in patients who discontinued ruxolitinib in the period 2014-2019, compared to patients treated from 2020 onwards (**Supplementary Table 1** and **Table 1**).

At ruxolitinib stop, 59.2% of patients exhibited resistance (lack of spleen response: 31.5% loss of spleen response: 16.9%, lack of symptom response: 16.5% loss of symptom response: 22.1%), while 37.9% exhibited intolerance (grade 3-4 anaemia: 18.8%, thrombocytopenia: 16.2%, non-haematological toxicity: 10.3%). Notably, 20.2% of patients had both criteria of resistance and intolerance at the time of permanent withdrawal. According to clinical-laboratory characteristics, the distribution of reasons for discontinuation varied between groups. Resistance was reported in 56.3% of CT patients, 77.46% of NT patients, and 47.6% of ASCT patients (global p-value=0.001). By Delphi panel consensus study[15], 45.4% of patients experienced a "true" ruxolitinib failure (CT, 49.0%; NT, 55.7%; ASCT, 31.6%, p=0.004). Intolerance was more frequently observed in the CT group (53.8%) compared to the NT (26.8%) and ASCT (24.45%) groups (global p-value=0.001).

OS at 3 years was 22.8%, 49.1% and 62.5%, while median OS was 1.5, 2.9 and 7.3 years in CT, NT and ASCT patients, respectively (**Supplementary Figure 2A**) (p<0.001). These outcomes were confirmed after adjustment for age, hemoglobin level, platelet count and spleen length, the four parameters that were significantly different across the groups (p<0.001) (**Supplementary Figure 2B**). Within the NT group, patients treated with new JAKi and those receiving non-JAKi investigational agents showed comparable OS (3-year OS, JAKi: 50.6%; non-JAKi: 45.4%, p=0.23).

In multivariable Cox analysis including older age, male sex, ruxolitinib dose, hematology parameters, severity of splenomegaly and symptoms and use of CT, only age >65 years (HR: 2.40, 95%CI: 1.58-3.65, p<0.001), a cytopenic phenotype, including hemoglobin <10g/dL (HR: 1.35, 95%CI: 1.03-1.90, p=0.035) and platelet count <100x10^9/L (HR: 1.65, 95%CI: 1.16-2.20, p=0.028), and use of conventional therapies (HR: 1.62, 95%CI: 1.11-2.36, p=0.013) remained associated with a worse prognosis (**Supplemental Table 2**).

To ensure comparability between patients treated with CT and NT, we also performed a PS matching analysis. Patient characteristics before and after PS matching are shown in **Table 1**. Median OS was 2.91 years (95% CI: 2.29–4.93) in patients treated with NT compared with 1.78 years (95% CI: 1.01–2.42) in patients treated with CT. The 3-year OS was 29.7% (95% CI: 18.2%–42.2%) for patients receiving CT and 49.0% (95% CI: 31.6%–64.4%) for NT patients (p=0.04) (**Figure 1**).

This study shows that post-ruxolitinib therapeutic strategies have a significant impact on survival outcomes in patients with myelofibrosis. Most patients rapidly transitioned to second-line therapies. This indicates how the failure of ruxolitinib therapy represents a significant turning point, with an urgent need for specific therapeutic interventions. While conventional therapies, such as hydroxyurea and supportive care, were commonly employed, they provided limited survival benefits and failed to control disease progression. In contrast, novel therapies and allogeneic stem cell transplantation demonstrated marked improvements in survival, with ASCT offering the most favorable outcomes in eligible patients. These results underscore the critical importance of tailoring treatment approaches to individual patient needs and the clinical phenotype at the time of ruxolitinib discontinuation.

ASCT remains the only potentially curative option for MF and demonstrated the most favorable outcomes in the present cohort. However, its optimal timing requires further and collective clinical research, and its feasibility is constrained by older age and comorbidities.[16-17]

Despite the inherent limitations of this study, which are attributable to its observational nature and the variability in data quality, the database's long-standing history, that started a decade ago and is characterized by continuous updating and meticulous data verification, along with its substantial sample size, serves to considerably minimize potential biases, such as the 'center effect'. While differences in outcomes may result from differences in clinical expertise, patient management

protocols, and resources available at different centers, we observed homogeneous management of second-line therapies between more and less experienced hematology centers. This finding was certainly due to the high cooperation level between centers, with patients being referred as needed, and the large volume of the study, which contributed to more reliable and robust results. Additionally, the use of the propensity score matching to compare patients receiving CT and NT allows for the creation of well-balanced cohorts, reducing potential confounding factors and strengthening the validity of our findings. This approach helps to limit biases intrinsic to retrospective studies and enhances the reliability of our results in assessing post-ruxolitinib treatment strategies.

In conclusion, our study highlights the critical importance of selecting effective therapies following ruxolitinib discontinuation in MF. The availability of second-generation JAK2 inhibitors and the development of emerging therapies provide hope for further improving outcomes in this high-risk population. Early referral to specialized centers with access to advanced therapies and clinical trials is essential to optimize care after ruxolitinib discontinuation.

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Authorship Contributions

Fr.P.: Conceptualization; Investigation; Funding acquisition; Writing - original draft; Writing - review & editing; Data curation; Resources; and Visualization. **F.B.**: Conceptualization, Writing - original draft; Writing - review & editing; Data curation; Formal analysis; and Visualization. **E.M., A.De., G.Be., M.T., G.A.P., M.Br., E.M.E., M.Bo.**: Conceptualization; Investigation; Visualization; Writing - review & editing; Resources. **E.B., M.F., B.M., G.C., N.P., A.T., M.C., G.Bi., F.C., E.A., A.I., E.S., A.M., V.G., S.C., A.I., A.Du., R.M.L., D.C., M.Bo., Fa.P.:** Investigation; Writing - review & editing; and Resources. **F.H.H**: Visualization; Writing - review & editing

Declaration of interests

Francesca Palandri participated in the speakers bureau and advisory board of Novartis, BMS, AOP, Sierra Oncology, Incyte, Telios, Abbvie, Constellation-Morphosys, Sobi and GSK. **Giulia Benevolo** reports honoraria from Novartis, Janssen, Amgen, Takeda, and BMS. **Massimo Breccia** reports honoraria from Novartis, BMS, Pfizer, Incyte. **Massimiliano Bonifacio** reports honoraria from Novartis, BMS, Pfizer, and Incyte. **Monica Crugnola** reports honoraria from Novartis and Amgen. **Gianni Binotto** reports honoraria from Novartis, Incyte, BMS-Celgene, and Pfizer. **Roberto M. Lemoli** reports honoraria from Jazz, Pfizer, AbbVie, BMS, Sanofi, and StemLine. **Fabrizio Pane** reports

honoraria from Incyte, Novartis, Jazz, BMS-Celgene, Amgen, and Gilead. **Giuseppe A. Palumbo** reports consultancy and honoraria from Abbvie, AOP, AstraZeneca, BMS, Incyte, GSK, Morphosys, and Novartis. **Mario Tiribelli** reports honoraria from and has served on speakers' bureaus for Novartis, BMS, Pfizer, and Incyte.

Data Availability Statement

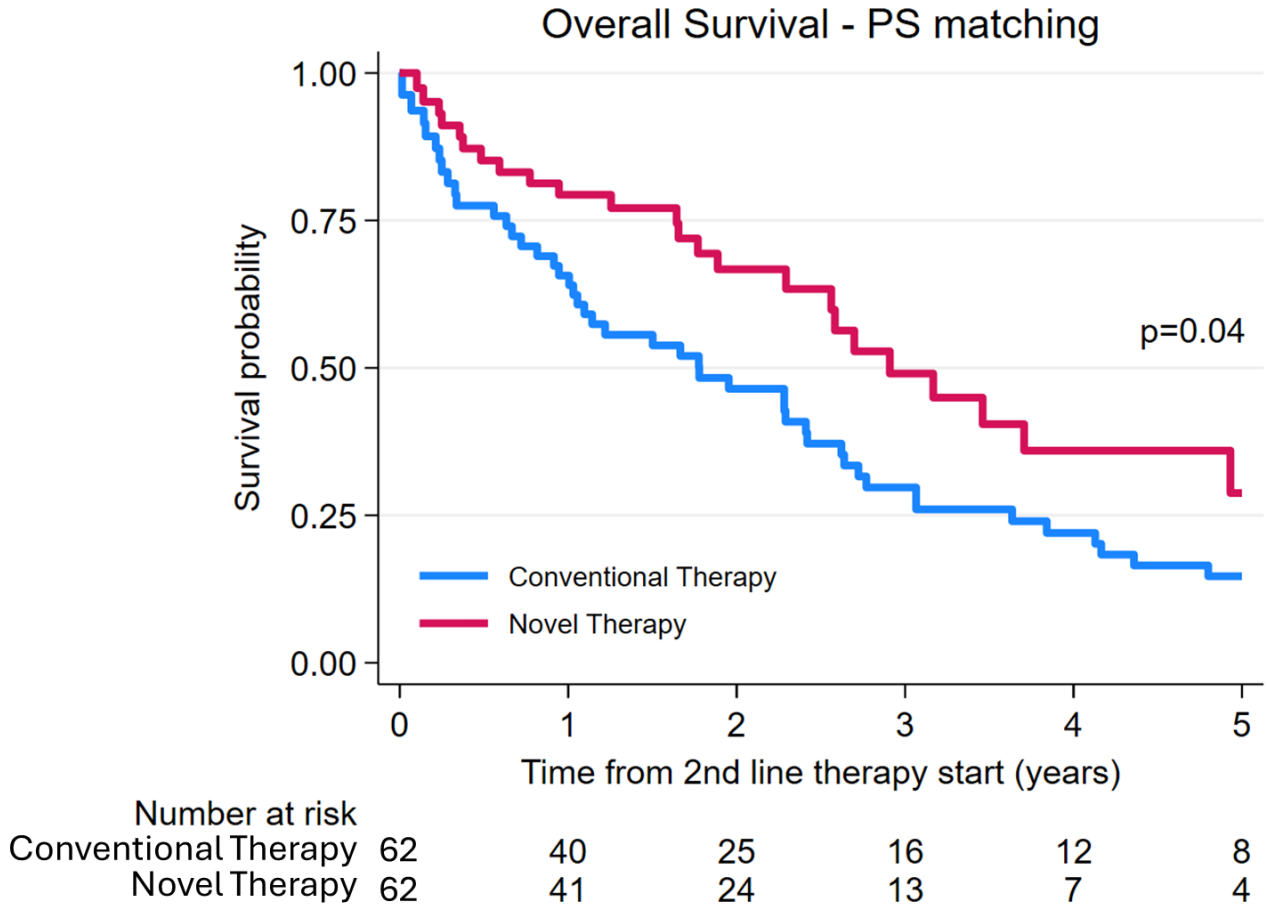
The data that support the findings of this study are available from the corresponding author upon reasonable request to the corresponding author (filippo.branzanti2@unibo.it), at the following DOI: 10.5281/zenodo.13902218

Table 1: Characteristics at ruxolitinib discontinuation comparison between Conventional Therapy and Novel Therapy patients, before and after propensity score matching

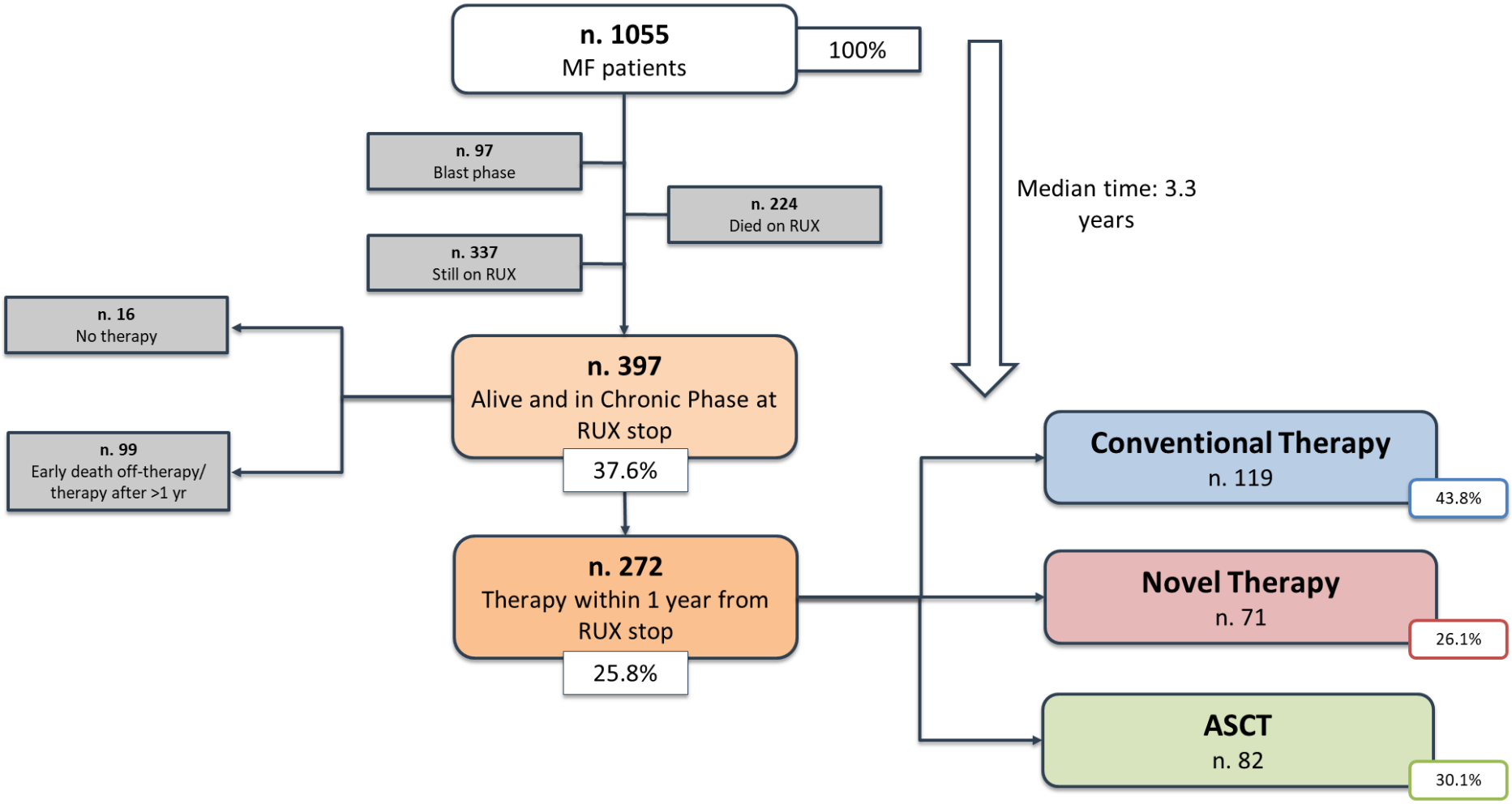
Characteristics at ruxolitinib discontinuation	Before 1:1 Propensity Score-Matching			After 1:1 Propensity Score-Matching		
	Conventional Therapy (n. 119)	**Novel Therapy (n. 71)**	**p-value**	**Conventional Therapy (n. 62)**	**Novel Therapy (n. 62)**	**p-value**
Age, median (range), years	73.8 (40.1 – 89.6)	70.3 (46.7 – 85.1)	**0.03**	69.8 (40.1 – 83.7)	71.5 (49.4 – 85.1)	0.52
Age > 65 years, n. (%)	95 (79.8%)	52 (73.2%)	0.29	44 (71.0%)	47 (75.8%)	0.54
Male Sex, n. (%)	68 (57.1%)	45 (63.4%)	0.40	37 (61.2%)	38 (61.3%)	0.85
High Molecular Risk mutation anytime, n. (%)	17/32 (53.1%)	14/24 (58.3%)	0.70	10/17 (58.8%)	11/19 (57.9%)	0.96
Dose at discontinuation						
5-10 BID	81 (68.1%)	43 (60.6%)	0.20	42 (67.7%)	39 (62.9%)	0.56
15-20 BID	38 (31.9%)	28 (39.4%)		20 (32.3%)	23 (37.1%)	
Grade of fibrosis (*on 56 evaluable*)						
0-1	2/17 (11.8%)	3/22 (13.6%)	0.86	1/12 (8.3%)	3/19 (15.8%)	0.55
2-3	15/17 (88.2%)	19/22 (86.4%)		11/12 (91.7%)	16/19 (84.2%)	
Platelet count, median (range), x 10^9/L	92 (3 – 891)	139.5 (7 – 698)	**0.04**	112 (4 – 891)	138.5 (7 – 656)	0.35
Platelet count < 100 x 10^9/L, n. (%)	62 (52.1%)	27 (38.0%)	0.08	31 (50.0%)	25 (40.3%)	0.28
Leukocytes, median (range), x 10^9/L	8.9 (1.5 – 118.0)	8.8 (1.0 – 108)	0.50	7.3 (1.5 – 115.8)	8.8 (1.0 – 108)	0.60
Leukocytes > 25 x 10^9/L, n. (%)	30 (25.9%)	10 (14.1%)	0.07	13 (21.0%)	9 (14.5%)	0.25
Hemoglobin, median (range), g/dL	9.0 (5.7 – 15)	9.4 (1.6 – 14.2)	0.13	8.8 (5.7 – 15)	9.5 (6.4 – 14.2)	0.19
Hemoglobin < 10 g/dL, n. (%)	81 (68.1%)	42 (59.2%)	0.28	45 (72.6%)	39 (62.9%)	0.22
Blasts, mean (range), %	1.4 (0 – 12)	1.6 (0 – 10)	0.23	1.5 (0 – 12)	1.6 (0 – 10)	0.22
Blasts ≥ 1%, n. (%)	54 (45.4%)	39 (55.0%)	0.26	31 (50.0%)	36 (58.0%)	0.23
Spleen length below costal margin, median (range), cm	11 (0 – 40)	12 (0 – 30)	0.57	11 (0 – 38)	13.5 (0 – 30)	0.38
Spleen length below costal margin ≥ 10 cm, n. (%)	60 (50.4%)	37 (52.1%)	0.85	34 (54.8%)	37 (59.7%)	0.57
Total Symptoms Score, median (range)	18 (0 – 100)	17 (0 – 100)	0.61	18 (0 – 67)	14 (0 – 100)	0.75
Total Symptoms Score ≥ 20, n. (%), (on 244 evaluable)	50/101 (49.5%)	31/66 (47.0%)	0.75	26/54 (48.2%)	27/59 (45.8%)	0.80
Time on RUX, median (range), years	1.7 (0.2 – 6.6)	1.9 (0.3 – 8.2)	0.23	1.6 (0.2 – 6.3)	1.9 (0.3 – 8.2)	0.20
Time from RUX discontinuation to 2nd line therapy start, median (range), months	2.0 (0 – 11.6)	4.9 (0 – 9.5)	0.15	3.3 (0 – 11.6)	5.1 (0 – 9.5)	0.27
Treated in a Referral Center*, n. (%)	94 (79.0%)	56 (78.9%)	0.87	51 (82.3%)	49 (79.0%)	0.65
Ruxolitinib discontinuation between 2014 and 2019, n. (%)	104 (87.4%)	54 (76.1%)	**0.04**	52 (83.9%)	52 (83.9%)	0.99

*Table 1: *Referral Center, defined as a center that has contributed to the study with a patient case load of over 30 patients.*

Figure 1: Overall Survival comparison between patients treated with Conventional Therapies and patients treated with Novel Therapies after propensity score matching



Supplementary Figure 1: Patients' disposition



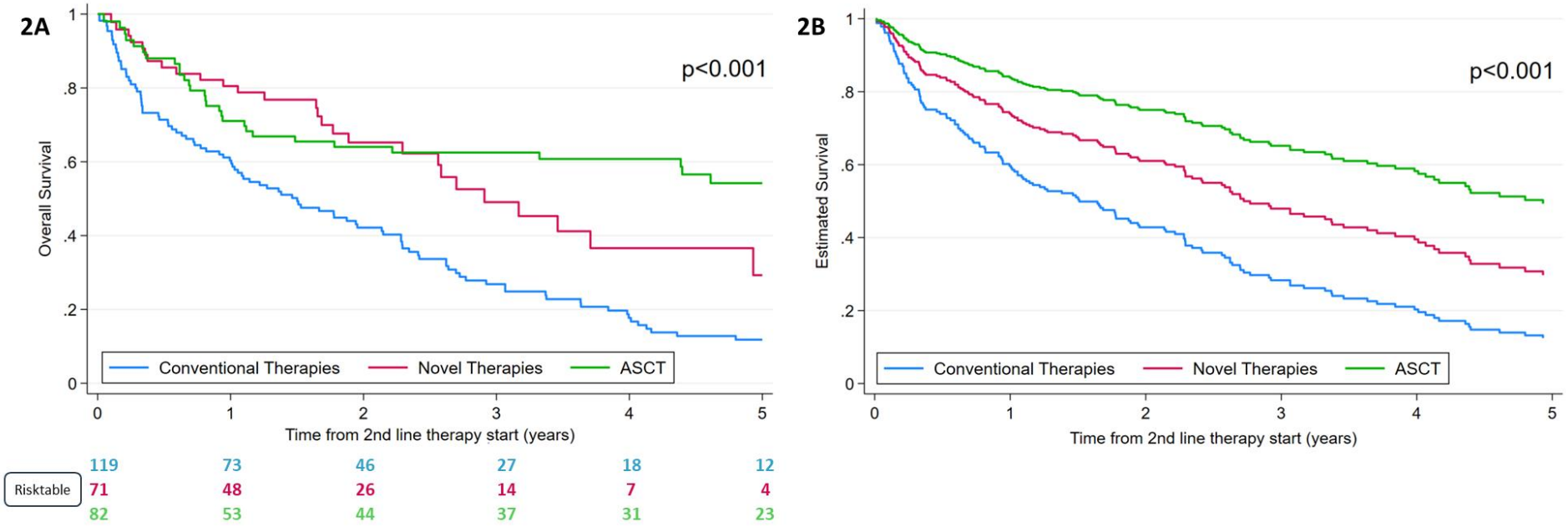
Supplementary Figure 1: MF, Myelofibrosis; RUX, Ruxolitinib; yr, year; ASCT, Allogenic Stem Cells Transplant

Supplementary Table 1: Patients characteristics at ruxolitinib discontinuation comparison between Conventional Therapy, Novel Therapy and Allogenic Stem Cell Transplant (ASCT) patients

Characteristics at ruxolitinib discontinuation	Conventional Therapy (n. 119)	Novel Therapy (n. 71)	ASCT (n. 82)	p-value
Age, median (range), years	73.8 (40.1 – 89.6)	70.3 (46.7 – 85.1)	57.6 (24.3 – 70.8)	**<0.001**
Age > 65 years, n. (%)	95 (79.8%)	52 (73.2%)	20 (24.4%)	**<0.001**
Male Sex, n. (%)	68 (57.1%)	45 (63.4%)	52 (63.4%)	0.59
High molecular risk mutation anytime, n. (%) (*on 85 evaluable*)	17/32 (53.1%)	14/24 (58.3%)	21/29 (72.4%)	0.29
Dose at discontinuation				
5-10 BID	81 (68.1%)	43 (60.6%)	35 (42.7%)	**0.001**
15-20 BID	38 (31.9%)	28 (39.4%)	47 (57.8%)	
Grade of fibrosis (*on 56 evaluable*)				
0-1	2/17 (11.8%)	3/22 (13.6%)	1/17 (5.9%)	0.73
2-3	15/17 (88.2%)	19/22 (86.4%)	16/17 (94.1%)	
Platelet count, median (range), x 10^9/L	92 (3 – 891)	139.5 (7 – 698)	146.5 (6 – 834)	**0.02**
Platelet count < 100 x 10^9/L, n. (%)	62 (52.1%)	27 (38.0%)	28 (34.1%)	**0.03**
Leukocytes, median (range), x 10^9/L	8.9 (1.5 – 118.0)	8.8 (1.0 – 108)	10.3 (2 – 100.8)	0.71
Leukocytes > 25 x 10^9/L, n. (%)	30 (25.9%)	10 (14.1%)	18 (22.0%)	0.19
Hemoglobin, median (range), g/dL	9.0 (5.7 – 15)	9.4 (1.6 – 14.2)	9.9 (6 – 14.3)	**0.02**
Hemoglobin < 10 g/dL, n. (%)	81 (68.1%)	42 (59.2%)	43 (52.4%)	0.07
Blasts, mean (range), %	1.4 (0 – 12)	1.6 (0 – 10)	3.5 (0 – 80)	0.41
Blasts ≥ 1%, n. (%)	54 (45.4%)	39 (55.0%)	36 (43.9%)	0.39
Spleen length below costal margin, median (range), cm	11 (0 – 40)	12 (0 – 30)	7 (0 – 28)	**0.001**
Spleen length below costal margin ≥ 10 cm, n. (%)	60 (50.4%)	37 (52.1%)	27 (32.9%)	**0.02**
Total Symptoms Score, median (range)	18 (0 – 100)	17 (0 – 100)	10 (0 – 70)	0.1
Total Symptoms Score ≥ 20, n. (%), (on 244 evaluable)	50/101 (49.5%)	31/66 (47.0%)	24/77 (31.3%)	0.07
Time from RUX discontinuation to 2nd line therapy start, median (range), months	2.0 (0 – 11.6)	4.9 (0 – 9.5)	2.3 (0 – 11.2)	0.34
Treated in a Referral Center*, n. (%)	94 (79.0%)	56 (78.9%)	70 (85.4%)	0.47
Ruxolitinib discontinuation between 2014 between 2019, n. (%)	104 (87.4%)	54 (76.1%)	70 (85.4%)	0.11

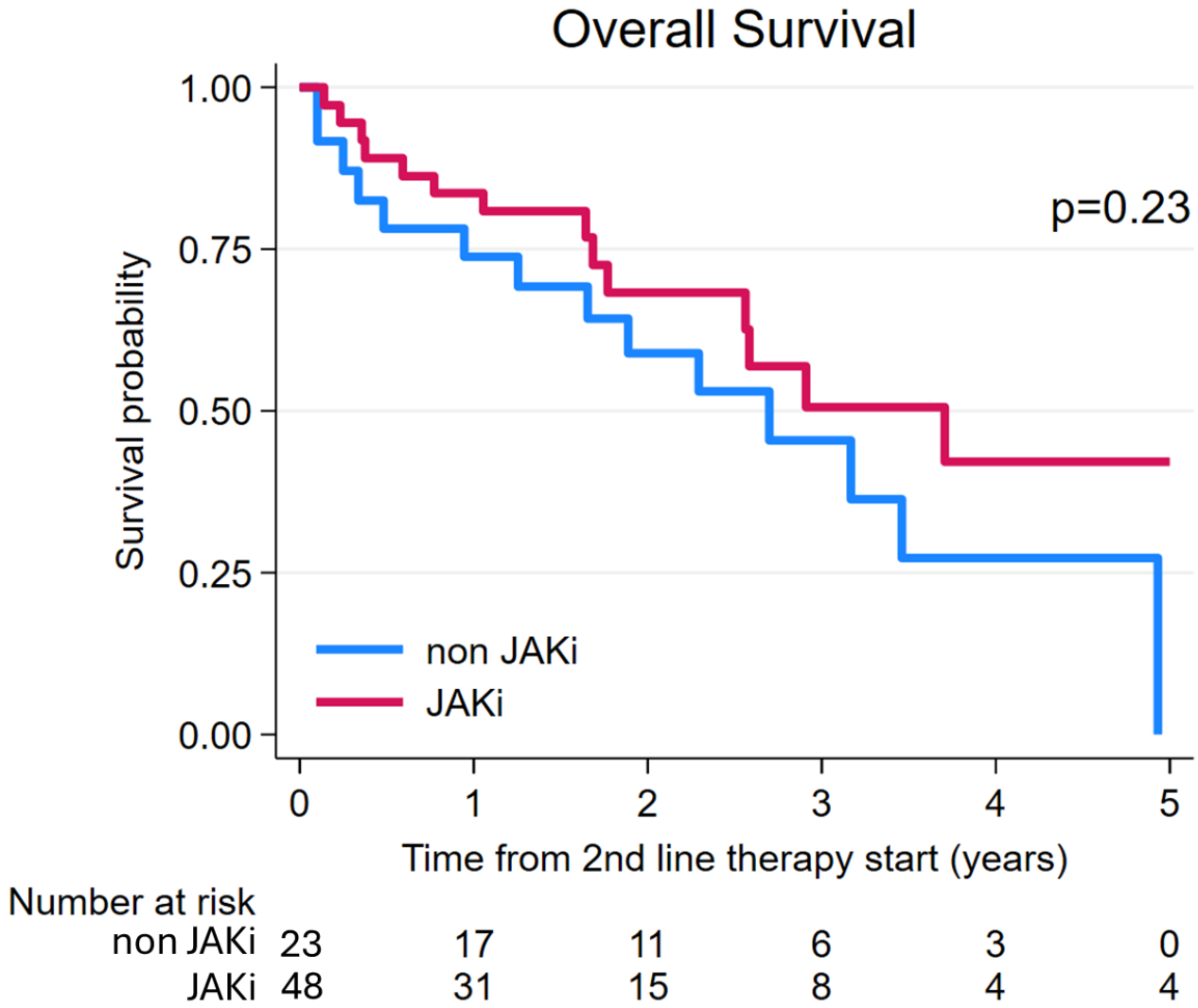
*Supplementary Table 1: *Referral Center, defined as a center that has contributed to the study with a patient case load of over 30 patients*

Supplementary Figure 2: Treatment Strategies Impact on Overall Survival



Supplementary Figure 2: (A) Conventional Therapy vs Novel Therapy vs ASCT after 2nd line therapy start; (B) Estimated Survival Plots of Conventional Therapy vs Novel Therapy vs ASCT after 2nd line therapy start, adjusted for age >65 years, hemoglobin <10 g/dL, spleen length ≥10 cm BCM and platelet count <100 x109/L at time of ruxolitinib discontinuation. Conventional therapy included hydroxyurea, steroids, danazol, ruxolitinib rechallenge, and red blood cells transfusions; Novel therapy included investigational agents and newer JAK inhibitors such as fedratinib, momelotinib, and pacritinib. ASCT: allogenic stem cells transplant.

Supplementary Figure 3: Kaplan-Meier survival curves comparing patients receiving JAK inhibitors (JAKi) vs. non-JAK inhibitors investigational (non-JAKi) agents



Supplementary Table 2: Univariate and Multivariate Cox Analysis of Risk Factors for Worse Overall Survival After Discontinuation of Ruxolitinib

Variables at discontinuation	Univariate (HR, 95% CI, p-value)	Multivariate (HR, 95% CI, p-value)
Age >65 years	**2.87, 1.98-4.17, <0.001**	**2.40, 1.58-3.65, <0.001**
Male sex	1.09, 0.79-1.51, 0.59	
Dose RUX <15mg BID	1.06, 0.77-1.46, 0.73	
Grade of fibrosis ≥2	7.28, 0.98-53.70, 0.06	
Platelet Count <100x10^9/L	**1.72, 1.25-2.36, 0.001**	**1.48, 1.04-2.11, 0.028**
Leukocytes >25 x 10^9/L	**1.52, 1.05-2.18, 0.03**	1.59, 0.98-2.60, 0.06
Hemoglobin <10 g/dL	**1.45, 1.04-2.04, 0.03**	**1.35, 1.03-1.90, 0.035**
Blasts ≥1%	**1.65, 1.18-2.31, 0.004**	1.18, 0.71-1.96, 0.52
Spleen length BCM ≥ 10 cm	**1.59, 1.16-2.20, 0.004**	1.23, 0.86-1.75, 0.26
Total Symptoms Score ≥ 20	1.38, 0.98-1.93, 0.06	
Use of Conventional Therapy	**2.50, 1.81-3.46, <0.001**	**1.62, 1.11-2.36, 0.013**

Supplementary Table 2: HR, Hazard Ratio; CI, Confidence of Interval; BID, bis in die; BCM, below costal margin