Supplementary Information for

NPM1 Mutations define a Specific subgroup of MDS and MDS/MPN patients with improved Outcomes with Intensive Chemotherapy

Table of Contents

Content **Page**

Supplementary Methods

Supplementary Tables

Supplementary Figures

Flow cytometry analysis of bone marrow HSPC populations

BM mononuclear cells (MNC) were isolated using the standard gradient separation approach with Ficoll-Paque PLUS (GE Healthcare Lifesciences, Pittsburgh, PA). Quantitative flow cytometry analyses of live MNC or CD34^{+} cells was performed using previously described staining protocols (Will et al., 2012; Woll et al., 2014). Cells were acquired with BD Influx Cell Sorter (BD Biosciences), and population analysis was performed using FlowJo software.

See table for details on the antigen panels used for the identification of specific cell populations.

Table. Cell surface marker expression panel used for the identification and purification of human HSPC by flow cytometry

Population	**Gating strategy**
Live cells	Single cells/Sytox$^{\text{TM}}$ Green Nucleic Acid Stain (ThermoFisher Scientific, Waltham, MA) negative
Lineage negative cells (Lin^{-})	CD2^{-}, CD3^{-} CD4^{-}, CD7^{-}, CD10^{-}, CD11b^{-}, CD14^{-}, CD19^{-}, CD20^{-}, CD33^{-}, CD56^{-}, CD235a^{-}
HSC compartment	Live/Lin^{-}/CD34^{+}, CD38^{-}
LT-HSC	Live/Lin^{-}/CD34^{+}, CD38^{-}/CD90^{+}, CD45RA^{-}
MPP	Live/Lin^{-}/CD34^{+}, CD38^{-}/CD90^{-}, CD45RA^{-}
LMPP	Live/Lin^{-}/CD34^{+}, CD38^{-}/CD90^{-}, CD45RA^{+}
HPC compartment	Live/Lin^{-}/CD34^{+}, CD38^{+}
CMP	Live/Lin^{-}/CD34^{+}, CD38^{+}/CD123^{+}, CD45RA^{-}
GMP	Live/Lin^{-}/CD34^{+}, CD38^{+}/CD123^{+}, CD45RA^{+}
MEP	Live/Lin^{-}/CD34^{+}, CD38^{+}/CD123^{-}, CD45RA^{-}

Abbreviations:

HSC = Hematopoietic Stem Cell

LT-HSC = Long-term HSC

MPP = Multipotent Progenitors

LMPP = Lymphoid-primed MPP

HPC = Hematopoietic Progenitor Cell

CMP = Common Myeloid Progenitor

GMP = Granulocytic-Monocytic Progenitor

MEP = Megakaryocytic-Erythroid Progenitor

PCR-based next generation sequencing (NGS) 53-Gene panel

Exons (codons) tested

ABL1	4 (243-274), 5 (275-303), 6 (303-321), 6 (321-362), 7 (395-424)
AKT1	3 (16-49)
ALK	23 (1172-1175), 25 (1248-1275)
APC	16 (875-918), 16 (1113-1153), 16 (1257-1297), 16 (1288-1328), 16 (1318-1357), 16 (1349-1386), 16 (1377-1416), 16 (1416-1456), 16 (1456-1494), 16 (1493-1530), 16 (1530-1575)
ATM	8 (353-355), 9 (409-412), 12 (601-633), 17 (846-880), 26 (1308-1331), 34 (1678-1719), 35 (1741-1773), 36 (1792-1832), 39 (1940-1973), 50 (2441-2479), 54 (2665-2670), 55 (2694-2717), 56 (2725-2756), 59 (2889-2891), 61 (2946-2950), 63 (3007-3051)
BRAF	11 (439-471), 15 (581-606)
CDH1	3 (77-117), 8 (369-379), 9 (399-439)
CSF1R	7 (297-301), 22 (926-970)
CTNNB1	3 (12-50)
DNMT3A	23 (866-913)
EGFR	3 (108-142), 7 (288-297), 15 (598-627), 18 (708-728), 19 (729-761), 20 (762-775), 20 (775-817), 21 (857-875)
ERBB2	19 (754-769), 20 (772-818), 21 (839-883)
ERBB4	3 (98-140), 4 (153-186), 6 (208-244), 7 (248-287), 8 (295-306), 9 (333-350), 15 (579-619), 23 (907-936)
EZH2	16 (613-644)
FBXW7	5 (243-278), 8 (375-394), 9 (429-471), 10 (473-508), 11 (549-583)
FGFR1	4 (120-126), 7 (247-250)
FGFR2	7 (250-273), 7 (273-311), 7 (302-313), 9 (362-382), 12 (521-550)
FGFR3	7 (247-288), 9 (379-422), 14 (639-653), 15 (654-659), 18 (792-807)
FLT3	11 (437-456), 14 (569-605), 16 (648-683), 20 (807-843)
GNA11	4 (172-202), 6 (255-297), 7 (297-304), 7 (304-349)
GNAQ	4 (159-202), 5 (202-210), 5 (210-245), 5 (241-245), 6 (246-263), 6 (263-297), 6 (291-297), 7 (297-324), 7 (324-360), 7 (355-360)
GNAS	8 (200-220)
HNF1A	3 (205-238), 4 (271-314)
HRAS	2 (1-15), 3 (38-63)
IDH1	4 (90-132)
IDH2	4 (125-178)
JAK2	14 (615-622)
JAK3	13 (568-573), 16 (683-723)
KDR	6 (220-248), 7 (267-276), 11 (471-476), 19 (872-874), 21 (946-985), 26 (1135-1146), 27 (1171-1211), 30 (1308-1352), 30 (1352-1357)
KIT	2 (51-93), 9 (502-514), 10 (514-547), 10 (540-549), 11 (550-550), 11 (550-592), 13 (641-664), 14 (670-712), 15 (714-745), 17 (815-828), 18 (838-866)
KLHL6	1 (1-13), 1 (13-73), 1 (73-98)
KRAS	2 (1-22), 3 (38-63), 4 (103-147)
MET	2 (168-209), 2 (375-400), 14 (1008-1028), 16 (1110-1132), 19 (1247-1284)
MLH1	12 (383-426)
MPL	10 (514-522)
NOTCH1	26 (1562-1601), 27 (1673-1679)
NPM1	11 (283-295)
NRAS	2 (1-18), 3 (38-62)
PDGFRA	12 (552-592), 14 (659-668), 15 (673-717), 18 (823-854)
PIK3CA	2 (83-118), 5 (345-353), 8 (418-445), 10 (538-555), 14 (701-729), 21 (988-1027), 21 (1027-1069)
PTEN	1 (5-27), 3 (67-70), 6 (170-210), 7 (212-221), 7 (221-266), 8 (287-332), 8 (332-342)
PTPN11	3 (59-104), 13 (501-533)
RB1	4 (127-158), 6 (199-203), 11 (357-376), 18 (570-605), 20 (659-700), 21 (703-733), 22 (746-775)
RET	10 (610-627), 11 (628-667), 13 (766-798), 15 (880-910), 16 (918-934)
SMAD4	3 (119-142), 5 (167-208), 6 (243-263), 8 (310-319), 9 (329-373), 10 (385-424), 11 (443-480), 12 (496-535)
SMARCB1	2 (39-78), 4 (156-167), 5 (199-210), 9 (381-386)
SMO	3 (197-242), 5 (323-366), 6 (403-422)
STK11	6 (261-288), 8 (332-370)
TP53	2 (1-12), 4 (69-112), 5 (126-147), 5 (147-186), 5 (181-187), 6 (187-192), 6 (187-223), 6 (214-224), 7 (225-253), 8 (267-306), 10 (332-342)
VHL	1 (88-114), 2 (129-155), 3 (157-200)
XPO1	14 (501-522), 15 (523-539), 15 (539-575)

Coverage by gene and codon(s) tested for indeterminate amplicons

CDKN2A	2 (51-70)
FGFR3	16 (692-723)
GNA11	4 (159-172), 5 (202-216), 7 (349-360)
NOTCH1	34 (2467-2526)
RB1	17 (550-565)
SMO	9 (533-551), 11 (639-646)
SRC	14 (530-537)
STK11	1 (36-77), 4 (193-199), 5 (200-211)

PCR-based next generation sequencing (NGS) 28-Gene panel

Gene	Exons (codons) tested
ABL1	1 (1-27), 2 (27-41), 2 (41-85), 3 (85-99), 3 (92-129), 3 (128-167), 3 (167-183), 4 (184-200), 4 (195-235), 4 (235-274), 5 (275-303), 6 (303-317), 6 (307-347), 6 (347-362), 7 (362-382), 7 (382-424), 8 (424-444), 8 (444-475), 9 (475-489), 9 (489-505), 10 (505-523), 11 (560-576), 11 (567-606), 11 (598-638), 11 (661-700), 11 (691-731), 11 (723-762), 11 (752-790), 11 (781-820), 11 (810-851), 11 (843-882), 11 (873-912), 11 (904-944), 11 (935-975), 11 (965-1003), 11 (995-1034), 11 (1070-1112), 11 (1112-1131)
ASXL1	2 (20-33), 2 (33-47), 3 (47-62), 3 (62-84), 4 (85-104), 4 (96-125), 5 (125-148), 5 (148-157), 6 (158-171), 6 (171-189), 7 (189-206), 7 (206-240), 8 (240-253), 8 (250-288), 8 (287-294), 9 (295-296), 9 (296-327), 10 (327-341), 10 (341-362), 11 (362-377), 11 (368-407), 11 (398-437), 11 (427-468), 11 (468-508), 11 (508-545), 11 (545-573), 12 (574-589), 12 (581-619), 12 (610-649), 12 (640-678), 12 (670-709), 12 (701-740), 12 (732-771), 12 (762-801), 12 (792-831), 12 (821-860), 12 (851-889), 12 (880-919), 12 (911-949), 12 (940-979), 12 (970-1009), 12 (999-1038), 12 (1031-1069), 12 (1061-1100), 12 (1091-1129), 12 (1123-1160), 12 (1153-1191), 12 (1182-1221), 12 (1212-1251), 12 (1241-1281), 12 (1271-1311), 12 (1301-1341), 12 (1336-1375), 12 (1369-1409), 12 (1408-1448), 12 (1448-1486), 12 (1486-1529), 12 (1529-1542)
BRAF	2 (47-73), 2 (73-80), 3 (81-119), 3 (119-157), 3 (147-168), 4 (169-187), 4 (187-203), 5 (203-234), 5 (234-237), 6 (238-273), 6 (273-287), 7 (287-311), 7 (311-327), 8 (327-351), 8 (351-380), 9 (381-386), 10 (393-425), 10 (425-438), 11 (439-465), 12 (478-493), 12 (493-506), 13 (506-518), 13 (518-555), 13 (555-565), 14 (565-581), 15 (581-619), 15 (619-620), 16 (621-654), 16 (654-664), 17 (665-697), 17 (697-709), 18 (710-729), 18 (729-767)
DNMT3A	2 (1-24), 3 (25-42), 3 (42-59), 4 (89-133), 4 (132-150), 5 (150-164), 7 (214-228), 7 (228-268), 7 (268-285), 8 (286-322), 8 (322-338), 9 (339-353), 9 (353-374), 10 (375-402), 10 (402-427), 11 (427-443), 11 (443-477), 12 (477-486), 12 (477-492), 13 (492-518), 14 (519-538), 14 (538-556), 15 (556-583), 15 (583-617), 16 (631-646), 17 (646-679), 17 (679-694), 18 (695-720), 18 (720-725), 19 (725-739), 19 (729-768), 19 (759-774), 20 (775-803), 22 (827-851), 22 (851-866), 23 (866-900), 23 (900-913)
EGFR	2 (30-42), 2 (42-80), 3 (81-96), 3 (89-128), 3 (128-142), 4 (142-158), 4 (158-187), 5 (187-210), 6 (210-234), 7 (250-273), 7 (273-297), 8 (297-317), 8 (317-336), 9 (336-353), 9 (343-378), 9 (377-378), 10 (378-385), 10 (385-403), 11 (403-433), 12 (433-444), 12 (438-478), 12 (478-500), 13 (521-544), 14 (544-559), 14 (559-574), 15 (575-591), 15 (591-627), 16 (627-640), 17 (640-659), 17 (659-687), 18 (688-702), 18 (702-728), 19 (729-743), 19 (743-761), 20 (762-784), 20 (784-823), 21 (824-849), 21 (849-875), 22 (876-901), 23 (901-916), 23 (916-950), 24 (950-982), 25 (983-998), 25 (998-1038), 26 (1039-1054), 27 (1055-1070), 27 (1070-1091), 28 (1091-1104), 28 (1096-1133), 28 (1171-1211)
EZH2	2 (1-18), 2 (18-39), 3 (40-69), 3 (69-82), 4 (83-114), 4 (114-121), 5 (122-139), 5 (139-162), 6 (162-196), 6 (196-209), 7 (209-227), 7 (227-243), 8 (243-283), 8 (283-303), 9 (303-321), 9 (321-333), 10 (334-364), 10 (364-404), 10 (395-414), 11 (414-429), 11 (429-466), 11 (460-470), 12 (471-487), 12 (487-502), 13 (502-516), 14 (516-529), 14 (529-558), 15 (558-569), 15 (569-610), 15 (606-617), 16 (618-634), 16 (634-649), 17 (650-677), 18 (677-704), 19 (704-728), 19 (719-732), 20 (732-752)
FLT3	2 (15-43), 2 (43-55), 3 (56-71), 3 (71-112), 3 (103-123), 4 (123-147), 4 (147-162), 5 (162-189), 5 (189-205), 6 (205-234), 6 (234-248), 7 (248-272), 7 (272-294), 8 (295-311), 8 (301-340), 8 (331-346), 9 (346-382), 9 (382-402), 10 (402-437), 11 (437-463), 11 (463-473), 12 (473-514), 12 (514-533), 13 (533-563), 13 (563-568), 14 (569-573), 14 (573-610), 14 (606-613), 15 (613-614), 15 (613-642), 15 (635-648), 16 (648-668), 16 (668-685), 17 (685-723), 17 (723-736), 18 (736-764), 19 (764-805), 19 (805-806), 20 (807-832), 20 (832-847), 21 (848-862), 21 (862-885), 22 (885-892), 22 (892-918), 23 (918-933), 23 (933-953), 24 (954-976), 24 (976-994)
GATA1	2 (1-16), 2 (13-51), 2 (51-74), 3 (74-89), 3 (81-120), 3 (111-151), 3 (142-182), 4 (200-227), 4 (227-248), 5 (249-265), 5 (265-290), 6 (291-306), 6 (297-336)
GATA2	2 (1-20), 3 (77-96), 3 (96-140), 3 (181-220), 3 (242-280), 3 (273-291), 4 (318-339), 5 (340-358), 5 (358-381), 6 (399-439), 6 (434-474), 6 (466-481)
HRAS	2 (18-37), 3 (38-63), 3 (63-97), 4 (97-129), 5 (151-190)
IDH1	3 (1-13), 4 (41-59), 4 (59-100), 4 (92-131), 4 (121-138), 5 (139-172), 5 (172-174), 6 (174-191), 6 (191-228), 6 (223-233), 7 (233-266), 7 (266-284), 8 (284-317), 8 (317-331), 9 (331-370), 9 (370-385), 10 (385-404), 10 (404-415)
IDH2	1 (1-39), 2 (39-69), 3 (106-125), 4 (125-167), 4 (167-178), 5 (179-181), 5 (181-220), 5 (212-226), 6 (227-262), 6 (262-272), 7 (272-281), 7 (278-317), 7 (308-323), 9 (361-370), 9 (370-393), 10 (393-417), 10 (408-424), 11 (424-453)
IKZF2	2 (1-12), 3 (12-23), 3 (23-47), 4 (47-60), 4 (60-99), 4 (91-129), 4 (121-136), 5 (136-178), 5 (178-192), 6 (192-223), 6 (223-238), 7 (238-270), 7 (270-286), 8 (286-312), 8 (312-351), 8 (351-393), 8 (393-432), 8 (424-463), 8 (454-494), 8 (484-524), 8 (514-527)
JAK2	3 (11-48), 3 (48-76), 4 (76-92), 4 (92-117), 5 (117-137), 5 (137-156), 6 (157-185), 6 (185-205), 7 (205-220), 7 (241-281), 7 (281-312), 8 (313-328), 8 (328-352), 9 (353-364), 9 (359-398), 9 (398-405), 10 (405-418), 10 (418-442), 11 (443-456), 11 (451-488), 11 (488-505), 12 (505-515), 12 (515-547), 13 (548-569), 13 (569-592), 14 (593-607), 14 (607-622), 15 (622-627), 15 (627-664), 16 (665-676), 16 (676-711), 17 (711-723), 17 (747-761), 18 (762-777), 18 (777-812), 19 (812-831), 19 (831-857), 20 (858-893), 20 (893-921), 21 (921-949), 21 (939-962), 22 (963-981), 22 (981-1020), 23 (1020-1039), 23 (1039-1059), 24 (1060-1079), 24 (1079-1097), 25 (1098-1111), 25 (1111-1133)

KIT	1 (1-23), 2 (23-41), 2 (41-80), 2 (80-113), 3 (113-128), 3 (119-157), 3 (152-189), 3 (189-207), 4 (207-219), 4 (219-252), 5 (253-292), 5 (253-254), 5 (292-309), 6 (309-321), 6 (318-353), 6 (353-372), 7 (372-386), 7 (386-411), 8 (411-428), 8 (428-449), 9 (449-460), 9 (454-493), 9 (493-514), 10 (514-532), 10 (523-549), 11 (550-565), 11 (565-592), 12 (592-606), 12 (597-627), 13 (627-643), 13 (643-664), 14 (664-681), 14 (681-714), 15 (714-727), 15 (727-745), 16 (745-765), 16 (765-787), 17 (788-806), 17 (806-828), 18 (829-842), 18 (834-866), 19 (866-873), 19 (873-899), 20 (899-914), 20 (914-934), 21 (935-948), 21 (948-977)
KRAS	2 (1-26), 2 (26-37), 3 (38-43), 3 (43-87), 3 (81-97), 4 (97-135), 4 (135-150), 5 (151-180), 5 (180-189)
MDM2	1 (1-5), 2 (5-22), 2 (22-33), 3 (34-58), 4 (59-62), 4 (62-103), 5 (103-109), 5 (109-120), 7 (143-156), 7 (156-175), 8 (175-199), 8 (199-228), 9 (229-251), 9 (251-280), 10 (281-298), 10 (298-306), 11 (307-323), 11 (313-352), 11 (342-379), 11 (370-408), 11 (403-444), 11 (444-483), 11 (483-498)
MLL	2 (145-168), 3 (168-182), 3 (178-215), 3 (206-244), 3 (236-274), 3 (265-303), 3 (293-332), 3 (325-362), 3 (353-392), 3 (390-427), 3 (418-456), 3 (476-515), 3 (506-544), 3 (534-573), 3 (564-603), 3 (594-632), 3 (624-662), 3 (653-691), 3 (682-722), 3 (714-753), 3 (743-780), 3 (771-809), 3 (799-837), 3 (831-870), 3 (870-910), 3 (910-950), 3 (949-988), 3 (988-1029), 3 (1029-1052), 4 (1053-1064), 4 (1063-1103), 4 (1103-1112), 5 (1112-1129), 5 (1121-1161), 5 (1161-1190), 6 (1190-1212), 7 (1218-1257), 7 (1257-1296), 7 (1296-1338), 8 (1338-1362), 9 (1363-1381), 9 (1381-1406), 10 (1407-1422), 10 (1422-1444), 11 (1445-1458), 11 (1458-1493), 12 (1494-1507), 13 (1526-1566), 14 (1566-1578), 14 (1578-1604), 15 (1604-1617), 15 (1610-1647), 15 (1647-1665), 16 (1666-1691), 16 (1691-1723), 17 (1724-1737), 17 (1737-1760), 18 (1761-1774), 18 (1774-1785), 19 (1785-1805), 19 (1796-1836), 19 (1836-1850), 20 (1850-1866), 20 (1866-1885), 21 (1886-1907), 21 (1907-1931), 22 (1932-1943), 22 (1940-1979), 23 (1985-1999), 23 (1999-2024), 24 (2032-2050), 25 (2050-2063), 25 (2063-2104), 26 (2104-2114), 26 (2111-2151), 26 (2151-2166), 27 (2166-2186), 27 (2176-2214), 27 (2204-2244), 27 (2236-2274), 27 (2264-2303), 27 (2295-2334), 27 (2324-2362), 27 (2354-2392), 27 (2382-2422), 27 (2413-2451), 27 (2443-2480), 27 (2471-2511), 27 (2502-2542), 27 (2533-2570), 27 (2561-2600), 27 (2591-2629), 27 (2619-2660), 27 (2651-2690), 27 (2710-2747), 27 (2798-2836), 27 (2827-2864), 27 (2856-2894), 27 (2885-2924), 27 (2914-2953), 27 (2946-2983), 27 (2974-3012), 27 (3003-3041), 27 (3032-3072), 27 (3063-3102), 27 (3092-3131), 27 (3123-3161), 27 (3152-3190), 27 (3183-3222), 27 (3222-3261), 27 (3261-3300), 27 (3299-3338), 27 (3338-3376), 27 (3376-3414), 27 (3414-3454), 27 (3453-3492), 27 (3492-3530), 27 (3530-3570), 27 (3570-3582), 28 (3582-3609), 29 (3609-3631), 30 (3631-3645), 30 (3645-3688), 31 (3688-3713), 32 (3713-3729), 32 (3720-3758), 32 (3755-3771), 33 (3771-3775), 33 (3775-3807), 34 (3807-3810), 34 (3810-3835), 35 (3835-3852), 35 (3852-3878), 36 (3879-3891), 36 (3882-3921), 36 (3918-3958), 36 (3958-3970)
MPL	1 (1-17), 1 (7-27), 2 (27-61), 2 (61-71), 3 (71-88), 3 (81-122), 4 (131-147), 4 (146-189), 4 (189-230), 5 (231-246), 5 (246-285), 6 (285-303), 6 (303-327), 7 (327-346), 7 (336-375), 7 (370-389), 8 (389-396), 8 (396-436), 9 (455-490), 11 (522-551), 12 (552-566), 12 (559-600), 12 (600-636)
MYD88	1 (10-50), 1 (41-82), 1 (82-123), 2 (123-143), 2 (143-168), 3 (168-184), 3 (174-213), 3 (211-228), 4 (228-229), 4 (229-259), 5 (259-275), 5 (275-310)
NOTCH1	1 (1-21), 2 (21-47), 3 (47-88), 3 (88-128), 3 (120-135), 4 (197-237), 5 (248-266), 5 (266-289), 6 (318-357), 6 (350-367), 8 (419-443), 8 (434-475), 8 (466-481), 9 (481-504), 9 (504-519), 10 (519-538), 10 (538-557), 11 (632-635), 12 (635-650), 12 (650-672), 13 (716-736), 14 (736-770), 14 (770-785), 16 (823-847), 17 (863-885), 18 (914-940), 18 (940-981), 21 (1126-1166), 21 (1157-1170), 22 (1171-1196), 23 (1253-1294), 24 (1304-1338), 24 (1335-1338), 25 (1373-1413), 25 (1449-1491), 25 (1481-1523), 25 (1513-1529), 26 (1529-1549), 26 (1549-1591), 29 (1803-1824), 30 (1825-1843), 30 (1835-1874), 30 (1865-1880), 31 (1895-1935), 31 (1965-1978), 32 (2012-2028), 33 (2028-2052), 33 (2043-2060), 34 (2085-2131), 34 (2131-2170), 34 (2170-2210), 34 (2210-2251), 34 (2251-2293), 34 (2293-2335), 34 (2326-2364), 34 (2356-2398), 34 (2388-2430), 34 (2420-2460), 34 (2451-2489), 34 (2481-2521), 34 (2540-2556)
NPM1	1 (1-20), 2 (20-31), 2 (31-46), 3 (47-61), 3 (61-86), 4 (87-100), 4 (100-118), 5 (118-131), 5 (123-153), 6 (154-164), 6 (164-175), 7 (175-194), 8 (195-223), 9 (224-243), 9 (243-257), 10 (258-282), 11 (283-295)
NRAS	2 (1-21), 2 (21-37), 3 (77-97), 4 (97-134), 4 (134-150), 5 (151-169), 5 (169-190)
PTPN11	2 (5-7), 2 (7-46), 3 (46-60), 3 (54-92), 3 (92-111), 4 (111-134), 4 (134-175), 5 (176-196), 5 (196-214), 6 (215-240), 6 (240-252), 7 (253-268), 7 (268-285), 8 (285-299), 9 (312-317), 9 (317-355), 9 (355-364), 10 (365-377), 10 (377-408), 11 (409-423), 11 (423-460), 12 (460-483), 13 (483-496), 13 (496-533), 14 (534-564), 14 (564-571), 15 (571-594)
RUNX1	3 (20-31), 3 (31-33), 4 (62-101), 4 (97-117), 5 (118-142), 5 (142-170), 6 (170-187), 6 (187-205), 7 (205-249), 8 (269-310), 8 (310-323), 9 (323-354), 9 (354-397), 9 (396-435), 9 (460-481)
TET2	3 (1-22), 3 (13-52), 3 (44-82), 3 (73-112), 3 (103-140), 3 (132-171), 3 (162-200), 3 (191-229), 3 (220-259), 3 (250-288), 3 (279-318), 3 (308-347), 3 (337-376), 3 (369-408), 3 (399-439), 3 (429-468), 3 (459-498), 3 (488-527), 3 (518-557), 3 (549-586), 3 (577-616), 3 (636-674), 3 (665-704), 3 (694-732), 3 (723-762), 3 (752-790), 3 (783-823), 3 (823-860), 3 (859-896), 3 (896-935), 3 (935-975), 3 (975-1014), 3 (1013-1051), 3 (1051-1090), 3 (1090-1127), 3 (1127-1137), 4 (1137-1145), 4 (1145-1167), 5 (1167-1182), 5 (1182-1198), 6 (1199-1216), 6 (1207-1249), 6 (1249-1268), 7 (1268-1283), 7 (1283-1318), 8 (1319-1321), 8 (1321-1348), 9 (1349-1369), 9 (1369-1394), 10 (1395-1408), 10 (1399-1438), 10 (1432-1474), 10 (1474-1513), 11 (1513-1533), 11 (1523-1563), 11 (1555-1593), 11 (1585-1622),

	11 (1612-1651), 11 (1643-1683), 11 (1674-1712), 11 (1703-1740), 11 (1736-1775), 11 (1774-1813), 11 (1813-1850), 11 (1850-1891), 11 (1890-1929), 11 (1929-1969), 11 (1969-2003)
TP53	4 (41-80), 4 (72-112), 4 (107-125), 5 (126-162), 5 (162-187), 6 (187-214), 6 (210-224), 7 (234-261), 8 (261-276), 8 (276-307), 9 (307-324), 9 (324-331), 10 (332-349), 10 (349-367)
WT1	1 (25-63), 1 (163-204), 1 (197-216), 2 (216-240), 2 (240-257), 4 (291-304), 4 (304-317), 5 (317-334), 6 (334-352), 6 (352-366), 7 (367-400), 7 (400-417), 8 (417-433), 8 (433-447), 9 (477-478), 10 (478-505), 10 (505-518)

PCR-based next generation sequencing (NGS) 81-Gene panel

Gene	**Exons (codons) tested**
ANKRD26	1 (1-6)
ASXL1	11-12 (362-1442), 12 (1450-1542)
ASXL2	11-12 (381-1436)
BCOR	2-4 (1-511), 4-12 (515-1547), 13-15 (1550-1644), 15 (1663-1722)
BCORL1	1-6 (1-1261), 6 (1292-1323), 6-12 (1326-1700), 12 (1706-1712)
BRAF	11 (439-478), 15 (581-620)
BRINP3	2-8 (1-767)
CALR	9 (352-418)
CBL	7-9 (336-477)
CBLB	7-10 (282-469)
CBLC	7-9 (336-454), 10 (465-475)
CEBPA	1 (1-90), 1 (249-358), 1 (128-175), 1 (178-201)
CREBBP	1-8 (1-608), 9-30 (615-1724), 31 (2238-2443), 31 (2049-2235), 31 (1725-1943), 31 (1950-2042)
CRLF2	6 (217-256)
CSF3R	14 (575-622), 17 (681-800), 17 (822-864)
CUX1	2-6 (11-172), 6-9 (174-241), 10-14 (248-408)
DDX41	1-11 (1-410), 12 (416-418), 12-17 (420-623)
DNMT3A	8-22 (286-862), 23 (866-913)
EED	1-2 (1-69), 2-8 (71-287), 9-12 (289-442)
ELANE	1-2 (1-48), 2 (69-75), 4-5 (123-268)
ETNK1	3 (228-275)
ETV6	1-8 (1-453)
EZH2	2-5 (1-158), 5-6 (160-205), 7 (209-217), 8-19 (243-732), 20 (752)
FBXW7	9-12 (413-708)
FLT3	11-20 (437-847)
GATA1	2-3 (1-84)
GATA2	2 (2-5), 2-5 (22-377), 5-6 (379-481)
GFI1	2 (2-39)
GNAS	8 (200-202), 11 (315-324)
HNRNPK	3-17 (1-465)
HRAS	2-3 (1-59), 3-4 (87-135), 4 (137-150)
IDH1	4 (132-133)
IDH2	4 (125-178)
IKZF1	2-8 (1-443), 8 (445-518)
IL2RG	1-2 (1-45), 2-8 (51-340), 8 (352-370)
IL7R	5-7 (180-292)
JAK1	3-22 (3-1023), 22-24 (1026-1123)
JAK2	10 (405-442), 12-14 (505-622), 16 (665-711), 18 (762-812)

JAK3	2-23 (1-1069)
KDM6A	1-29 (1-1402)
KIT	8-9 (411-514), 11 (550-592), 17 (788-828)
KMT2A	2 (145-168), 3-4 (176-1075), 4 (1081-1112), 5 (1117-1184), 6 (1190-1212), 7 (1224-1325), 8-13 (1338-1560), 14-15 (1566-1665), 27 (2186-2195), 27 (2201-2355), 27 (2373-3215), 27 (3223-3324), 27 (3339-3575)
KRAS	2-4 (1-150)
MAP2K1	2 (27-90), 3 (98-146)
MPL	10 (490-522), 12 (552-636)
NF1	2-5 (21-189), 6 (201-218), 8-13 (244-467), 13-24 (478-1066), 25-26 (1082-1146), 26-31 (1160-1378), 31-35 (1380-1550), 35-38 (1564-1868), 39 (1870-1884), 39-47 (1886-2322), 47-52 (2325-2555), 52-58 (2568-2840)
NOTCH1	26-28 (1529-1795), 34 (2069-2273), 34 (2290-2556), 34 (2069-2273), 34 (2290-2556)
NPM1	11 (283-295)
NRAS	2-4 (1-150)
PAX5	1-10 (14-392)
PHF6	2-10 (1-366)
PIGA	2 (1-6), 2-6 (16-485)
PML	3 (201-255)
PRPF40B	2-19 (2-609), 19-20 (611-658), 20-26 (661-893)
PTEN	7-8 (212-285), 8 (290-342)
PTPN11	3-4 (46-125), 7 (253-285), 12 (460-462), 12-13 (465-533)
RAD21	2-14 (1-632)
RARA	6-7 (211-338)
RUNX1	2-9 (1-437), 9 (456-474)
SETBP1	4 (838-885)
SF1	1-13 (1-640)
SF3A1	1-7 (1-322), 7-9 (328-424), 9-16 (427-794)
SF3B1	13-16 (574-790)
SH2B3	2 (1-118), 2 (132-164), 2-8 (211-576)
SMC1A	1-19 (1-983), 20-25 (992-1234)
SMC3	1-6 (1-110), 6-16 (113-504), 16-17 (507-580), 17-29 (591-1217)
SRSF2	1 (1-38), 1 (45-121)
STAG1	2 (1-5), 3-20 (10-703), 21-22 (718-738), 22-27 (740-953), 27-34 (955-1259)
STAG2	2-15 (1-512), 16-33 (521-1232)
STAT3	17 (521-534), 17 (489-503), 17 (506-508), 18-22 (534-715)
STAT5A	3-7 (1-214), 8-9 (264-286), 9-20 (303-795)
STAT5B	16 (636-693)
SUZ12	1-2 (20-107), 4-5 (129-169), 7-16 (198-740)
TERT	1 (1-24), 2 (80-172), 2-4 (258-630), 4-5 (633-677), 5-6 (683-749), 6-8 (753-800), 8-16 (805-1133)
TET2	3 (1-77), 3 (91-826), 3 (829-853), 3-11 (867-2003)
TP53	2 (1-25), 4-11 (80-394)
U2AF1	2 (15-44), 6 (117-161)
U2AF2	1-5 (1-161), 6-12 (163-473)
WT1	1 (122-216), 1 (2-44), 1 (56-58), 2-10 (216-518)
ZRSR2	1-4 (1-90), 5 (108-131), 6-9 (134-263), 9-11 (267-483)

Table S1. Characteristics of Individual patients with *NPM1mut* MDS and MDS/MPN

UPN	Age	WHO 2016 Subtype	BM Blast%	Karyotype	IPSS-R	*NPM1*$_{mut}$ VAF	Other mutations	Initial Therapy	Response	Transplant	OS (months)
1	33	MDS-EB	10	46,XX[20]	H	0.41	-	Decitabine	HI	Yes	106
2	62	MDS-EB	12	46,XX,del(9)(q13q22)[9]; 46,XX[11]	H	0.405	*PTPN11 S502L*	Azacitidine+Lenalidomide	Died	No	2
3	80	CMML	6	46,XY[20]	Int	0.143	-	Azacitidine+Vorinostat	mCR+HI	No	5
4	85	MDS-EB	15	46,XY[20]	Int	0.1703	*IDH2 R140Q*	Azacitidine+Rigosertib	CR	No	22
5	47	MDS-EB	8	46,XX,del(9)(q13q22)[20]	H	0.4184	*NRAS G13R, PTPN11 G503R, PTPN11 A72T, WT1 L378fs, FLT3 ITD*	Azacitidine+Rigosertib	mCR	Yes	40
6	56	MDS-EB	6	47,XY,+21[1]/46,XY[19]	H	0.4411	*FLT3 D835*	Decitabine	HI	No	7
7	65	MDS-EB	10	46,XY[20]	H	0.2384	-	Azacitidine	mCR	No	7
8	86	MDS-EB	16	46,XY[20]	Int	0.35	-	Azacitidine	CR	No	58
9	77	MDSdel5q	4	46,XY,del(5q)[7],46,XY[13]	Int	0.09	-	Lenalidomide	HI	No	19
10	44	MDS-EB	10	46,XX[20]	Int	0.36	-	IAx1 and BIDFAx4	CR	No	14
11	35	MDS-EB	17	46,XY[20]	VH	0.48	*NRAS G12D*	IA+Vorinostat	CR	Yes	91
12	19	MDS-EB	8	46,XX[20]	Int	0.43	*NRAS G12D*	ADE	CR	Yes	9
13	58	MDS-EB	17	46,XX[20]	H	0.42	*IDH1 R132H*	Azacitidine	PR	Yes	83
14	60	MDS-EB	2	46,XY[20]	Int	0.48	*NRAS G13D, FLT3-ITD*	Decitabine	PD	No	10
15	55	MDS-EB	15	46,XY[20]	VH	0.17	-	FIA	CR	Yes	60
16	66	T-MDS	0	48,XY,+8,+8,dup(13)(q12q34)[2] 48,XY,add(2)(q37),+8,+8[4]/48,XY,+8,+8,add(15)(q26)[2] 48,XY,+8,+8[cp2] 46,XY[10]	VH	0.4475	*DNMT3A R882H, TET2 R1951W*	Guadecitabine	CR	No	34
17	75	MDS-EB	7	46,XY[20]	H	0.3366	-	Azacitidine	NE	No	0
18	43	MDS-EB	15	46,XX[20]	H	0.4363	*DNMT3A S352fs, NRAS G12D*	FIA	CR	No	24
19	51	T-MDS	2	46,XX[20]	Int	0.4939	*DNTM3A R635W*	Decitabine	PD	Yes	16
20	62	MDS-EB	6	46,XY[20]	H	0.401	*WT1 C428fs*	Azacitidine	mCR+HI	No	2

21	39	CMML	7	46,XY[20]	Int	0.4192	_DNMT3A F752L, NRAS G12D, WT1 Q422*_	Azacitidine + Nivolumab	mCR	Yes	30
22	68	MDS-EB	19	46,XX[20]	VH	0.1404	_KRAS G12S, TET2 Q649*, TET2 C470*, TET2 E887*, RUNX1 F336fs*625_	BIDFA	mCR	Yes	30
23	48	MDS/MPN	13	46,XX[20]	Int	0.33	_NRAS G12D, TET2 C1378Y_	CLIA	CR	Yes	24
24	63	CMML	15	47,XX,+21[16]/46,XX[4]	H	0.382	_NRAS G12D_	Azacitidine	PR	No	26
25	66	CMML	16	46,XY[20]	Int	0.2776	_TP53 E358V, IDH2 R140Q ,DNMT3A R882H_	Decitabine	CR	Yes	12
26	73	MDS/MPN	15	46,XX[20]	VH	0.355	_SF3B1 G740E, DNMT3A R771*, DNMT3A R320*_	Azacitidine+Ruxolitinib	mCR	No	6
27	71	MDS/MPN	4	46,XX,del(20)(q11.2q13.3)[20]	H	0.382	_RUNX1 R166*, ASXL1 G646fs_	BuFlu	CR	Yes	22
28	78	CMML	4	46,XY[20]	Int	0.526	_NRAS G12D, PTPN11 N308D_	Decitabine	PD	No	14
29	79	MDS-EB	11	46,XY[20]	VH	0.4481	-	Guadecitabine	CR	No	7
30	59	MDS-EB	18	46,XX[20]	H	0.443	_NRAS G12D, WT1 R380fs_	CLIA	CR	No	18
31	63	CMML	10	46,XX,inv(9)(p12q13)[20]	Int	0.3295	_TET2 Y1255*_	CLIA	CR	Yes	10

MDS-EB: MDS with excess blasts. CMML: chronic myelomonocytic leukemia. MDS/MPN-U: MDS/MPN unclassifiable. T-MDS: Therapy related MDS.. CR: complete response. mCR: marrow complete response. PR: partial response. HI: Hematological improvement. NR: No response. PD: progressive disease. NE: Not evaluable. ADE: Cytarabine, daunarubicine, etoposoide. IA: Idarubicin and cytarabine. CLIA: Cladribine, idarubicin and cytarabine. FIA: Fludarabine, idarubicin, cytarabine. BuFlu: Busulphan, fludarabine.

Table S2. Characteristics of Patients with *NPM1*$_{mut}$ compared to *NPM1*$_{wt}$ MDS and MDS/MPN

Variable	*NPM1*$_{mut}$ MDS (n=31) n (%) /mean[SD]	*NPM1*$_{wt}$ MDS (n=558) n (%) /mean[SD]	p value
Median age (years)	62 [19-86]	69 [22-93]	<0.001
Male	14 (45)	371 (67)	0.020
Hgb (g/dL)	8.7 [8.2-9.2]	9.9 [9.7-10.1]	0.001
WBC (x10^9/L)	7.9 [4.5-11.3]	7.9 [6.5-9.3]	0.996
Platelets (x10^9/L)	85 [63-107]	117 [109-126]	0.076
ANC (x10^9/L)	2.9 [0.5-5.2]	3.9 [0.738]	0.716
Median bone marrow blasts %	10 [0-19]	5 [0-19]	<0.001
WHO Diagnosis			<0.001
MDS-SLD	1* (4)	38 (7)	
MDS-RS	0 (0)	57 (10)	
MDS-MLD	1* (4)	127 (23)	
MDS-EB	19 ()	196 (35)	
MDS-U	0 (0)	25 (5)	
MDS/MPN-U	3 ()	6 (1)	
CMML	6 ()	109 (20)	
MDS del(5q)	1 ()	0 (0)	
IPSS-R			<0.001
Very Low	0 (0)	50 (9)	
Low	0 (0)	151 (27)	
Intermediate	13 (42)	125 (22)	
High	12 (39)	105 (19)	
Very high	6 (19)	127 (23)	

* Also classifiable as therapy-related MDS.

MDS-EB: MDS with excess blasts. CMML: chronic myelomonocytic leukemia. MDS/MPN-U: MDS/MPN unclassifiable. MDS-MLD: MDS with multilineage dysplasia. MDS-SLD: MDS with single lineage dysplasia.

Table S3. Univariate analysis for survival based on mutations. Only genes with mutations present in at least 3 patients were included in the analysis to prevent bias associated with small patient numbers. Survival impact calculated by Cox regression univariate analysis.

Gene	Frequency	Overall Survival		Time to transformation	
		HR [95% CI]	p value	HR [95% CI]	p value
DNMT3A	6/22 (19)	0.36 [0.08-1.66]	0.191	0.81 [0.20-3.27]	0.766
FLT3	3/31 (10)	1.71 [0.38-7.71]	0.485	0.41 [0.00-159.25]]	0.448
NRAS	10/31 (32)	0.39 [0.11-1.41]	0.153	0.79 [0.24-2.63]	0.703
PTPN11	3/22 (10)	1.19 [0.26-5.51]	0.822	0.83 [0.10-6.67]	0.862
TET2	4/19 (13)	0.25 [0.03-2.04]	0.195	0.91 [0.18-4.53]	0.909
WT1	4/22 (13)	0.03 [0.00-8.09]	0.217	0.99 [0.20-4.79]	0.988
NPM1 VAF <20%	5/31 (16)	0.66 [0.21-2.08]	0.481	1.05 [0.23-4.79]	0.953
NPM1 subclonal	3/16 (10)	0.462 [0.11-1.95]	0.293	0.97 [0.11-8.43]	0.979

Figure S1. Changes in bone marrow blast percentages between pre-treatment bone marrow aspirations. X axis represents time in days from initial bone marrow (time point 0) to second bone marrow aspiration prior to starting therapy. Dots represent pre-treatment blast percentage for each given patient.

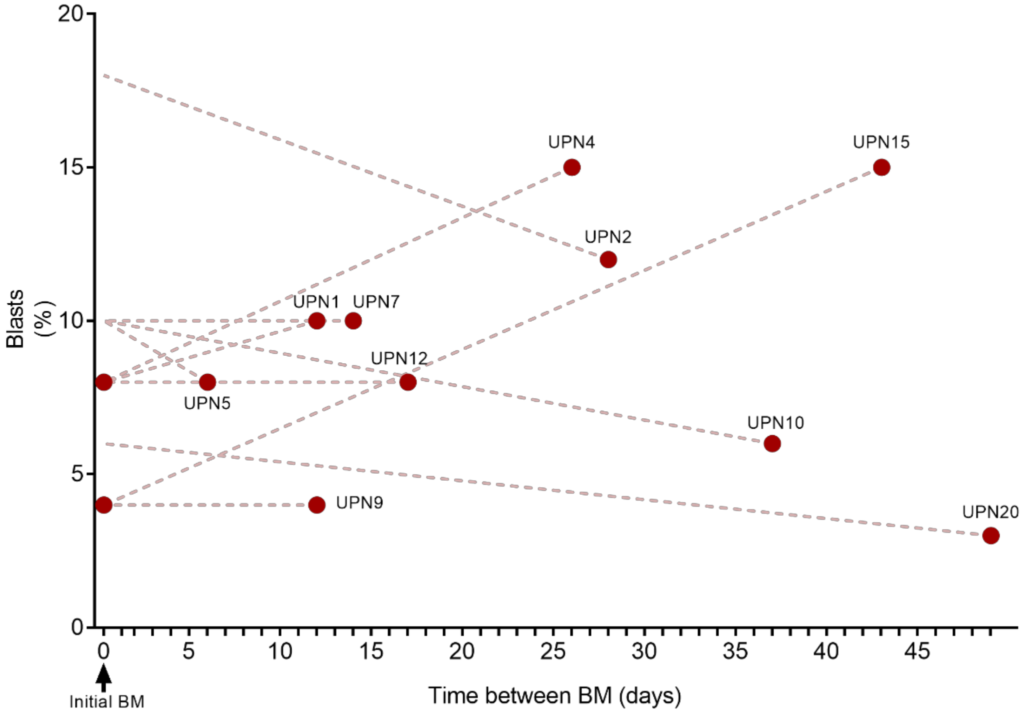


Figure S2. Kaplan-Meier estimate curve for overall survival of patients with *NPM1* mutant MDS.

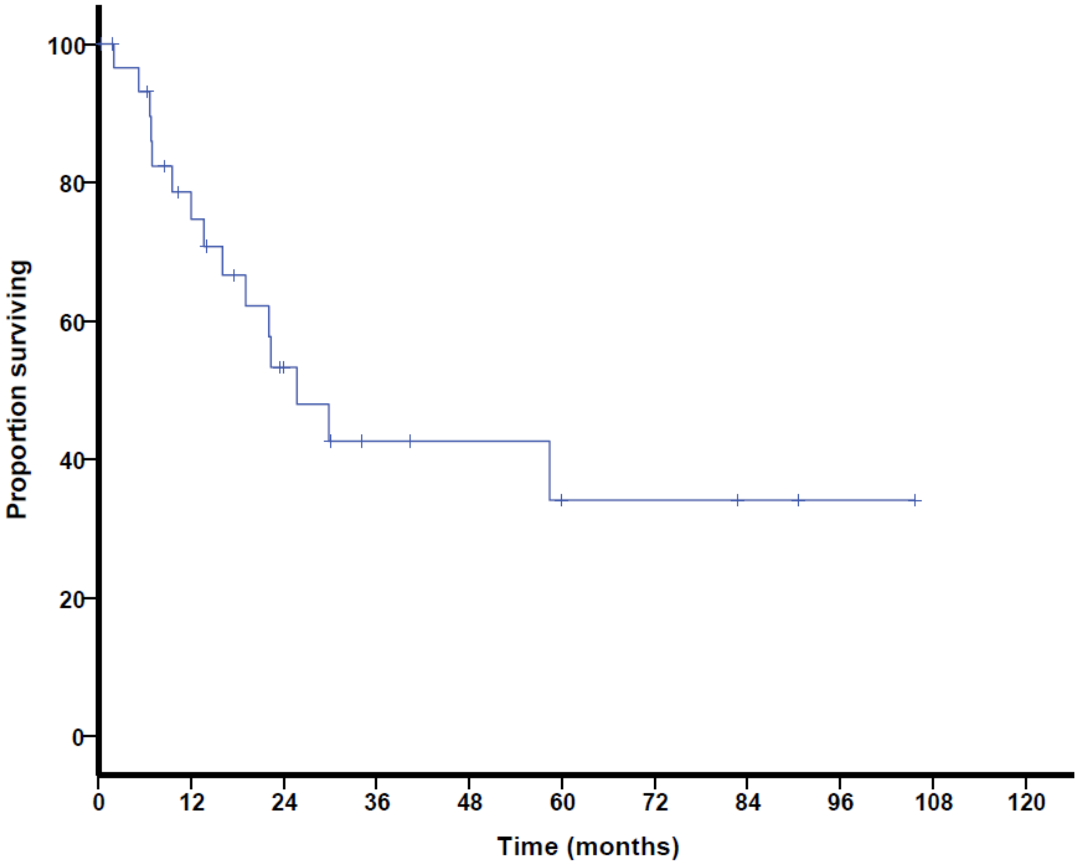


Figure S3. Kaplan-Meier estimate curves for progression-free survival in patients with *NPM1* mutant MDS

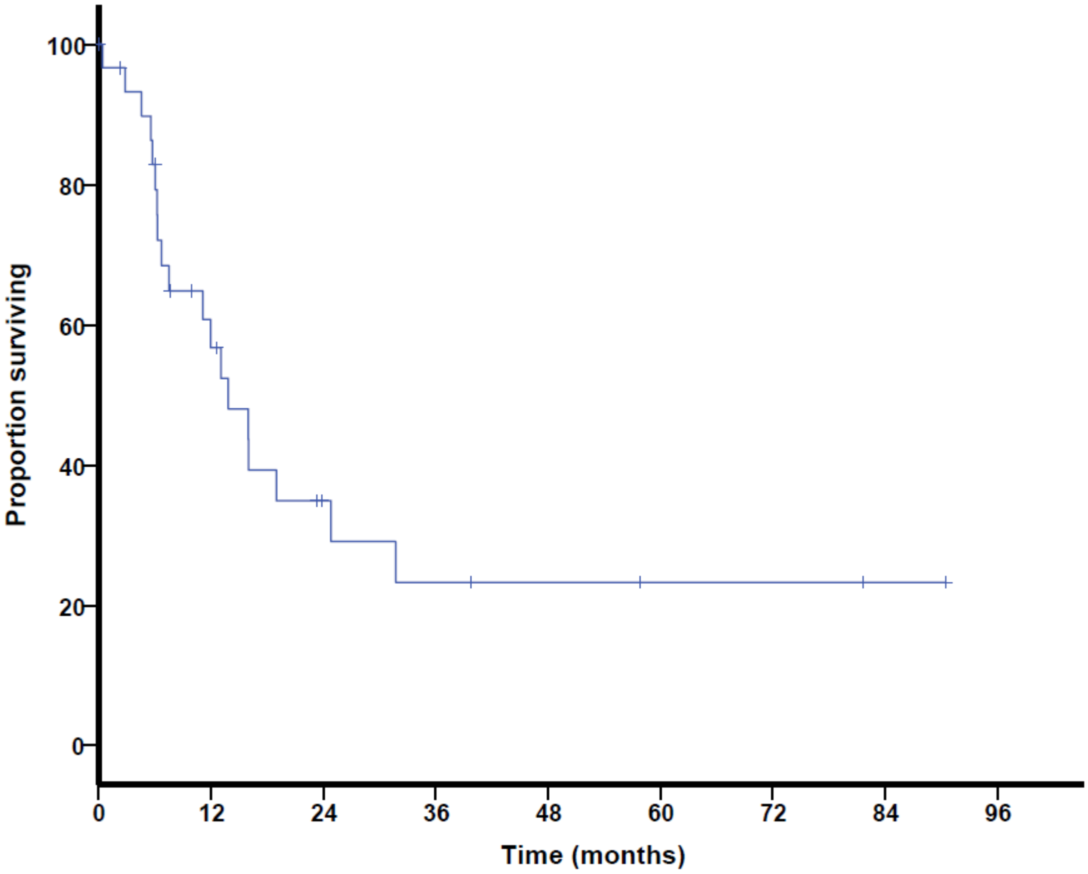


Figure S4. Morphologic and immunophenotypic features of *NPM1* mutated MDS and MDS/MPN. A) Bone marrow image shows a hypercellular bone marrow with dysmegakaryopoiesis as observed by the presence of megakaryocytes with hypolobated and dispersed nuclei (arrow). B) Bone marrow aspirate images show marked dyserythropoiesis with intercytoplasmatic bridging (arrow), nuclear picnosis and abnormal hemoglobination (star), as well as dysgranulopoiesis characterized by hypogranulation (asterisk) and abnormal segmentation and chromatin compaction (cross). C) Bone marrow progenitor cell distribution by 6 color multiparametric flow cytometry evaluating CD123 and CD45RA expression profile among the CD34+/CD38+ compartment of a representative patient. Capture shows a GMP pattern of differentiation characterized by increased frequency of GMP population and a sharp decrease in the number of MEPs. CMP = Common myeloid progenitor. MEP = Megakaryocytic-erythroid progenitor. GMP = Granulo-monocytic progenitor.

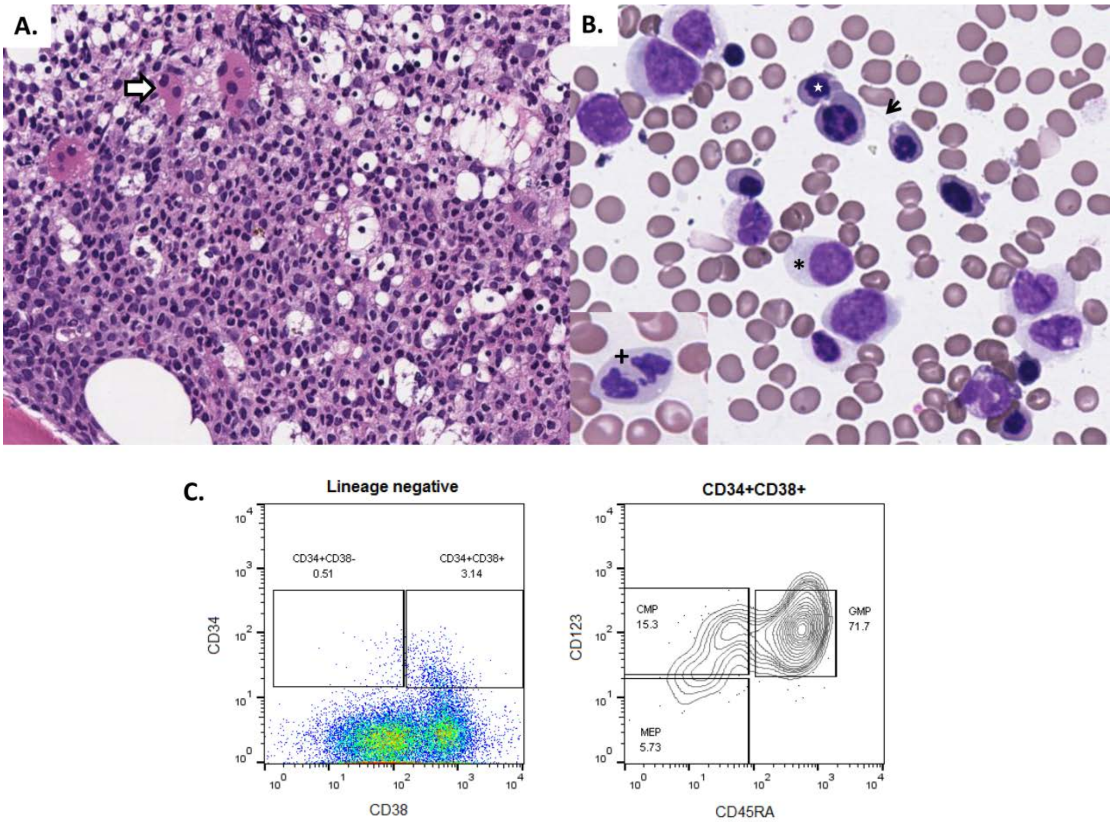
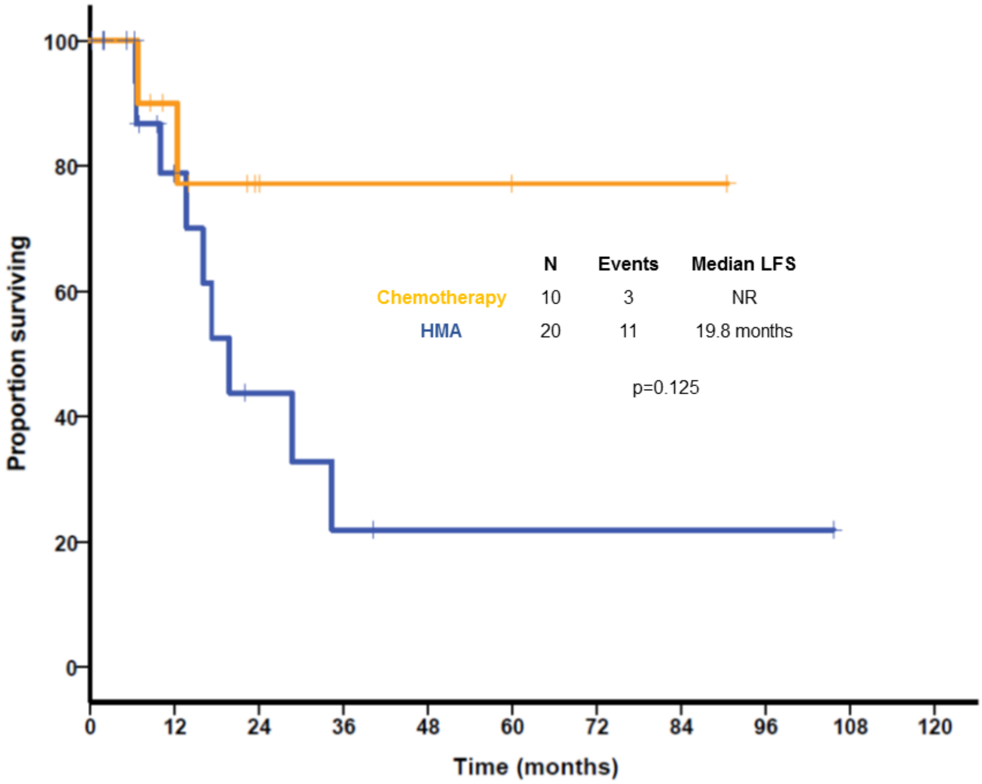


Figure S5. Kaplan-Meier estimates for time to transformation based on therapy in patients with *NPM1* mutated MDS or MDS/MPN.



	N	Events	Median LFS
Chemotherapy	10	3	NR
HMA	20	11	19.8 months

Figure S6. Kaplan-Meier estimates for overall-free survival based on IPSS-R in patients with *NPM1* mutated MDS or MDS/MPN.

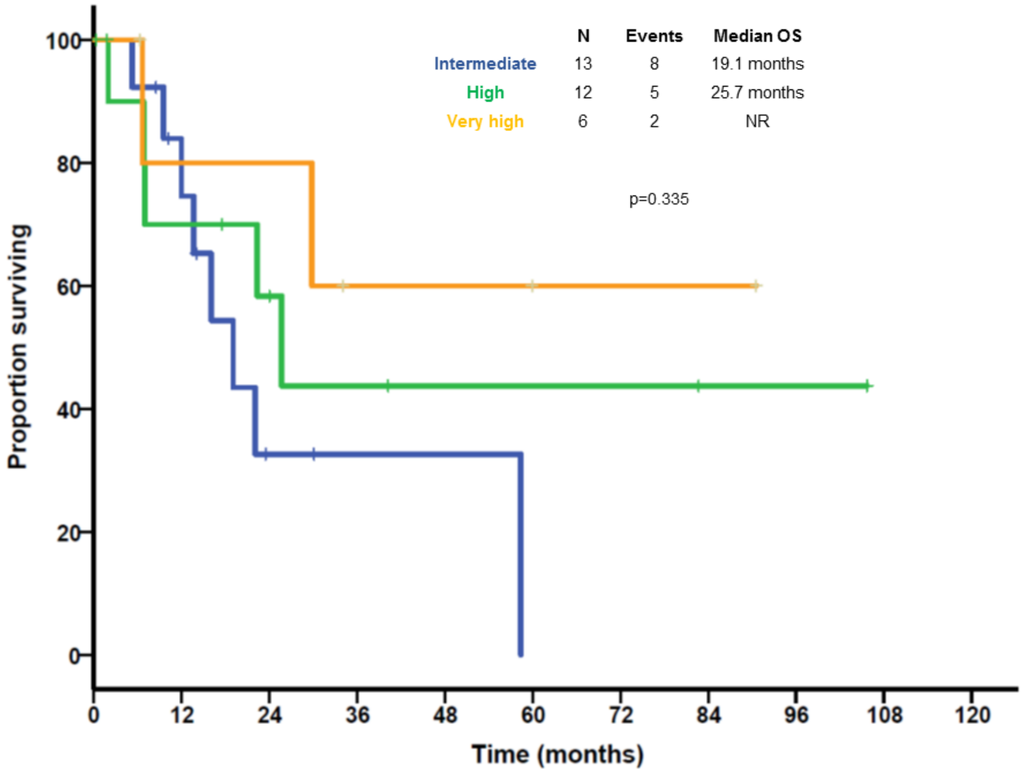


Figure S7. Kaplan-Meier estimates for time to transformation based on IPSS-R in patients with *NPM1* mutated MDS or MDS/MPN.