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

<u>Gene</u>	<u>Exons (codons) tested</u>
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)

Gene	Exons (coordinates)
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)

Table S1. Identified mutations by NGS or Sanger sequencing

UPN	WHO subtype	Gene	c.DNA change	Amino acid change
3	MDS-EB	DNMT3A	c.2645G>A	p.R882H
		U2AF1	c.101C>T	p.S34F
8	MDS-MLD	U2AF1	c.101C>T	p.S34F
11	MDS-SLD	TP53	c.644G>T	p.S215I
12	CMML	NRAS	c.35G>A	p.G12D
15	CMML	ASXL1	c.3053_3055delinsG	p.T1018fs
		SRSF2	c.284C>G	p.P95R
17	MDS-MLD	DNMT3A	c.2695C>T	p.R899C
20	MDS-EB	GNAQ	c.515A>G	p.Y172C
21	MDS del(5q)	SF3B1	c.2098A>G	p.K700E
22	MDS-SLD	APC	c.3920T>A	p.I1307K
23	MDS-EB	SF3B1	c.1852A>G	p.D618G
25	MDS-MLD	U2AF1	c.470A>C	p.Q157P
27	MDS-MLD	RUNX1	c.146_147insC	p.G50fs
		TET2	c.3811del	p.C1271fs
		U2AF1	c.470A>C	p.Q157P
		ZRSR2	c.18542A del	
29	CMML	NRAS	c.181C>G	p.Q61E
		NRAS	c.34G>C	p.G12R
		TP53	c.869G>A	p.R290H
		SRSF2	c.284C>A	p.P95H
30	MDS-RS	SF3B1	c.1866G>C	p.E622D
32	MDS-MLD	IDH2	c.419G>A	p.R140Q
35	CMML	RUNX1	c.318G>A	p.W106*
		TET2	c.3840del	p.C1281fs
		TET2	c.5026del	p.H1676fs
36	MDS-EB	TET2	c.1621del	p.L541*
		TET2	c.5125del	p.C1709fs
37	MDS-MLD	SF3B1	c.2098A>G	p.K700E
38	MDS-RS	SF3B1	c.2098A>G	p.K700E
43	MDS-EB	U2AF1	c.101C>A	p.S34Y
44	MDS-EB	DNMT3A	c.2645G>A	p.R882H
48	MDS-MLD	TP53	c.28G>A	p.V10I
52	MDS-EB	RUNX1	c.784C>T	p.Q262*
53	MDS/MPD	ASXL1	c.1934_1935insG	p.G646fs
		TET2	c.2662C>T	p.Q888*
		SRSF2	c.284C>T	p.P95L
54	CMML	ASXL1	c.1878_1879del	p.A627fs
		TET2	c.4546C>T	p.R1516*
		TET2	c.5219T>A	L1740*
57	MDS-EB	IDH2	c.419G>A	p.R140Q
58	CMML	TET2	c.3646C>T	p.R1216*

		TET2	c.4621C>T	p.Q1541*
62	MDS-EB	TP53	c.711G>A	p.M237I
63	MDS-EB	FLT3	c.2503G>T	p.D835Y
		TET2	c.4964del	p.P1655fs
64	MDS-EB	IDH2	c.419G>A	p.R140Q
66	CMML	IDH2	c.419G>A	p.R140Q
		RUNX1	c.1070dupC	p.T358fs
68	CMML	TET2	c.3812dupG	p.C1271fs
70	MDS-EB	TET2	c.2746C>T	p.Q916*
		TET2	c.1337T>G	p.L446*
71	MDS-RS	TET2	c.3501-2A>C	
72	MDS-SLD	ASXL1	c.3637_3640del	p.L1213fs
73	MDS-EB	KRAS	c.436G>C	p.A146P
74	MDS-U	RUNX1	c.485G>A	p.R162K
		SRSF2	c.284C>A	p.P95H
75	MDS-EB	SF3B1	c.2098A>G	p.K700E
76	MDS-MLD	TET2	c.1763C>G	p.S588*
77	MDS-MLD	ASXL1	c.1773C>A	p.Y591*
78	CMML	RUNX1	c.496C>G	p.R166G
		TET2	c.2746C>T	p.Q916*
		TET2	c.553C>T	p.Q185*
79	CMML	KIT	c.2447A>T	p.D816V
80	MDS-U	TP53	c.814G>A	p.V272M
		TP53	c.528C>A	p.C176*
81	CMML	TET2	c.3415del	p.I1139fs
		TET2	c.4940del	p.G1647fs
82	MDS/MPD	JAK2	c.1849G>T	p.V617F
		SF3B1	c.2098A>G	p.K700E
83	MDS-MLD	TET2	c.4854C>A	p.Y1618*
86	MDS-MLD	MPL	c.1775G>A	p.R592Q
		MPL	c.775G>A	p.E259K
		TET2	c.3646C>T	p.R1216*
		TET2	c.1612C>T	p.Q538*
		TET2	c.3092del	p.H1031fs
88	MDS-U	RUNX1	c.484A>G	p.R162G
		TP53	c.1009C>T	p.R337C
		TP53	c.613T>C	p.Y205H
89	MDS-EB	RUNX1	c.477_499dupTGACCTCAGGTTTGTCGGTCGAA	p.S167fs
92	MDS-EB	TET2	c.5219T>A	p.L1740*
93	MDS-RS	IDH2	c.419G>A	p.R140Q
		SF3B1	c.2098A>G	p.K700E
94	MDS-SLD	BRAF	c.1406G>C	p.G469A
96	MDS-EB	NPM1	c.863_864insCCTG	p.W288fs
97	MDS-SLD	DNMT3A	c.1903C>T	p.R635W

		NPM1	c.860_863dupTCTG	p.W288fs
98	CMML	*KRAS*	c.436G>A	p.A146T
99	MDS-EB	*RUNX1*	c.485G>A	p.R162K
102	MDS/MPD	*JAK2*	c.1849G>T	p.V617F
103	MDS-U	*ASXL1*	c.1900_1922del	p.E635fs
105	MDS-U	*DNMT3A*	c.2053_2063delinsTG	p.G685_R688delinsC
107	MDS-EB	*ASXL1*	c.1934dupG	p.G646fs*
		TET2	c.3699G>A	p.W1233*
108	MDS-MLD	*TP53*	c.398T>G	p.M133R
109	MDS-RS	*SF3B1*	c.2098A>G	p. K700E
110	MDS-MLD	*ASXL1*	c.1888_1910del	p.E635fs
		EZH2	c.952del	p.T318fs
		TET2	c.1842del	p.G614fs
		TET2	c.4767T>G	p.Y1589*
112	MDS-RS	*JAK2*	c.1849G>T	p.V617F
113	MDS-MLD	*TET2*	c.4546C>T	p.R1516*
114	MDS-MLD	*ZRSR2*	c.26036Gdel	
117	MDS-RS	*EZH2*	c.371A>T	p.D124V
		TET2	c.1676_1677del	p.Y559fs
		SF3B1	c.1996A>G	p.K666E
		SRSF2	c.284C>A	p. P95H
		ZRSR2	c.26036Gdel	

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 S2. Clinical characteristics of the 38 patients with no detectable mutations

Variable	N(%)/Median [Range]
Age (years)	73 [44-83]
Male	23 (61)
Hgb (g/dL)	10.2 [7.3-14.8]
WBC (x10^9/L)	3.1 [0.7-20.3]
ANC (x10^9/L)	1.3 [0.2-11.5]
Platelets (x10^9/L)	78 [7-404]
Bone marrow blasts (%)	3 [0-9]
WHO diagnosis	
MDS-SLD	9 (24)
MDS-RS	1 (3)
MDS-MLD	18 (47)
MDS-EB	5 (13)
MDS/MPN-U	1 (3)
CMML	4 (11)
Therapy-related MDS	7 (18)
≥2 cytopenias	21 (55)
Transfusion dependence	19 (50)
BM blasts percentage	
Blasts≥5%	9 (24)
Karyotype	
Normal	16 (42)
Del(20q)	2 (5)
Del(11q)	1 (3)
-Y	2 (5)
Trisomy 8	4 (11)
Del(5q)	1 (3)
Del(7q)/-7	2 (5)
Other	9 (24)
Unavailable	1 (3)
IPSS risk group	
Low	5 (13)
Intermediate-1	33 (88)
MDACC LR-MDS score	
Low	4 (11)
Intermediate	20 (53)
High	14 (36)
Prior therapy	
Growth factors	6 (16)
Others	2 (5)
Time from diagnosis (weeks)	7 [1-205]

Table S3. Erythropoietin levels and red blood cell transfusion requirements of anemic patients without prior erythropoietin-stimulating agent exposure (n=38)

		Erythropoietin level		
		<100 U/L	100-500 U/L	>500 U/L
Red blood cell transfusion requirements	<2 units/month	6 (16%)	11 (29%)	1 (3%)
	≥2 units/month	7 (18%)	6 (16%)	7 (18%)

Putative erythroid responses to G-CSF plus erythropoietin-stimulating agent (ESA) are 74% in the high response group (green), 23% in the intermediate response group (yellow), and 7% in the low response group (red). This predicted response with G-CSF plus ESA is based on published data from Hellström-Lindberg E, Negrin R, Stein R et al. Erythroid response to treatment with G-CSF plus erythropoietinfor the anaemia of patients with myelodysplastic syndromes:proposal for a predictive model. *Br J Haematol*. 1997;99:344-351.

Table S4. Univariate analysis for response based on detected mutations. Only mutations detected in >5% of evaluated patients who were evaluable for response were included in this analysis.

Gene	Overall response N (%)		OR [95% CI]	p value
	Wildtype	Mutated		
ASXL1	34/50 (68)	6/8 (75)	1.412 [0.256-7.783]	0.692
DNMT3A	60/96 (63)	2/5 (40)	0.400 [0.064-2.509]	0.328
IDH2	58/96 (60)	4/5 (80)	2.621 [0.282-24.352]	0.397
RUNX1	34/49 (69)	6/9 (67)	0.882 [0.194-4.007]	0.871
SF3B1	26/41 (63)	5/9 (56)	0.721 [0.167-3.106]	0.661
SRSF2	27/45 (60)	4/5 (80)	2.667 [0.275-25.838]	0.397
TET2	26/39 (67)	14/19 (74)	1.400 [0.414-4.737]	0.589
TP53	57/94 (61)	5/7 (71)	1.623 [0.299-8.806]	0.575
U2AF1	28/45 (62)	3/5 (60)	0.911 [0.138-6.016]	0.923

Table S5. Univariate analysis for survival for detected mutations. Only mutations detected in >5% of evaluated patients where included in this analysis.

Gene	Deaths N (%)		Median OS (months)		HR [95% CI]	p value
	Wildtype	**Mutated**	**Wildtype**	**Mutated**		
ASXL1	12/51 (24)	2/8 (25)	25.3	24.1	0.902 [0.201-4.045]	0.893
DNMT3A	24/97 (25)	3/5 (60)	NR	13	5.719 [1.621-20.181]	0.007
IDH2	26/97 (27)	1/5 (20)	NR	NR	0.549 [0.074-4.057]	0.557
RUNX1	11/50 (22)	3/9 (33)	25.3	24.6	1.173 [0.327-4.212]	0.807
SF3B1	10/42 (24)	0/10 (0)	NR	NR	0.033 [0.00-18.828]	0.291
SRSF2	8/46 (17)	2/6 (33)	NR	NR	2.183 [0.457-10.436]	0.328
TET2	10/39 (26)	4/20 (20)	24.7	25.3	0.571 [0.178-1.831]	0.346
TP53	23/95 (24)	4/7 (57)	NR	13.7	3.682 [1.228-11.035]	0.020
U2AF1	8/47 (17)	2/5 (40)	NR	NR	2.211 [0.468-10.442]	0.316
ZRSR2	9/49 (18)	1/3 (33)	NR	6.8	45.475 [2.843-727.310]	0.007

Table S6. Univariate analysis for leukemia-free survival based on detected mutations.
Only mutations detected in >5% of evaluated patients where included in this analysis.

Gene	Deaths N (%)		Median LFS (months)		HR [95% CI]	p value
	Wildtype	Mutated	Wildtype	Mutated		
ASXL1	15/51 (29)	2/8 (25)	23.3	24.2	0.602 [0.137-2.651]	0.502
DNMT3A	28/97 (29)	3/5 (60)	NR	13	4.484 [1.300-15.470]	0.018
IDH2	29/97 (30)	2/5 (40)	NR	22.6	1.130 [0.269-4.743]	0.867
RUNX1	14/50 (28)	3/9 (33)	24.2	22.6	0.948 [0.272-3.308]	0.933
SF3B1	13/42 (31)	0/10 (0)	NR	NR	0.032 [0.000-8.262]	0.225
SRSF2	11/46 (24)	2/6 (33)	NR	NR	1.529 [0.336-6.960]	0.583
TET2	13/39 (33)	4/20 (20)	22.7	NR	0.415 [0.134-1.284]	0.127
TP53	26/95 (24)	5/7 (71)	NR	13.7	3.974 [1.471-10.734]	0.006
U2AF1	11/47 (23)	2/5 (40)	NR	NR	1.481 [0.327-6.712]	0.611
ZRSR2	12/49 (24)	1/3 (33)	NR	6.8	22.802 [2.066-251.606]	0.011

Table S7. Univariate and Multivariate analysis for leukemia-free survival.

Variable	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age ≤60 years >60 years	1.576	0.554-4.487	0.394			
WHO Diagnosis MDS MDS/MPN	1.168	0.526-2.591	0.703			
Cytopenias 0-1 ≥2	1.659	0.816-3.373	0.162			
BM Blasts <5% ≥5	1.311	0.644-2.668	0.455			
Cytogenetic risk Good Others	0.717	0.341-1.508	0.381			
Transfusion Dependent Independent	1.756	0.873-3.534	0.114			
IPSS Risk Low Intermediate-1	1.353	0.475-3.856	0.572			
MDA LR-MDS High Others	1.086	0.547-2.156	0.813			
Response CR Non-CR	0.448	[0.208-0.969]	0.041	0.244	0.053-1.131	0.071
DNMT3A Wildtype Mutated	4.484	1.300-15.470	0.018	17.626	1.235-251.603	0.034
TP53 Wildtype Mutated	3.974	1.471-10.734	0.006	29.348	4.384-196.471	<0.001
ZRSR2 Wildtype Mutated	22.802	2.066-251.606	0.011	54.493	3.431-865.433	0.005

Figure S1


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                        ┌─────────────────────────┐
                        │ Assessed for eligibility │
                        │      (n = 119)          │
                        └─────────────────────────┘
                                    │
                                    │        ┌──────────────────────────────────┐
                                    │        │ Excluded                         │
                                    ├────────│                                  │
                                    │        │ Incorrect diagnosis (n = 1)      │
                                    │        │ Higher-risk disease (n = 3)      │
                                    │        │ Withdrew consent (n=1)           │
                                    │        │ Denied financial approval (n=1)  │
                                    │        └──────────────────────────────────┘
                        ┌───────────────────────────────┐
                        │ Adaptive Randomization (n = 113)│
                        └───────────────────────────────┘
                          /                          \
         ┌──────────────────────────┐    ┌──────────────────────────┐
         │ Allocated to Azacytidine │    │ Allocated to Decitabine  │
         │      (n = 40)            │    │      (n = 73)            │
         └──────────────────────────┘    └──────────────────────────┘
         ┌──────────────────────────┐    ┌──────────────────────────┐
         │ Lost to follow up (n = 0)│    │ Lost to follow up (n = 0)│
         │                          │    │                          │
         │ Discontinued treatment   │    │ Discontinued treatment   │
         │       (n =0)             │    │       (n = 0)            │
         └──────────────────────────┘    └──────────────────────────┘
         ┌──────────────────────────┐    ┌──────────────────────────┐
         │ Analyzed for efficacy    │    │ Analyzed for efficacy    │
         │       (n = 40)           │    │       (n =73)           │
         │ Analyzed for safety      │    │ Analyzed for safety      │
         │       (n = 40)           │    │       (n = 73)          │
         └──────────────────────────┘    └──────────────────────────┘
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Enrollment

Allocation

Follow up

Analysis