SUPPLEMENTARY APPENDIX

A plasma microRNA signature as a biomarker for acquired aplastic anemia

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Supplemental Data:

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List of Supplementary Information

Supplemental Data

Table S1. Characteristics of patients and healthy controls

Characteristic	AA	MDS	HC
Discovery set (n = 35)			
No.	13	11	11
Median age (range)	30 (10-75)	64 (44-80)	34 (23-57)
Sex (M/F)	7/6	4/7	5/6
Disease status (%)	SAA 13 (100%)	WHO RCUD 5 (45.4%)	NA
		RCMD 1 (9.1%)	
		MDS-U 3 (27.3%)	
		RARS 2 (18.2%)	
		IPSS Low 1 (9.1%)	
		Int-1 8 (72.7%)	
		Int-2 2 (18.2%)	
Treatment (%)	rATG+CsA 8 (61.5%)	Alemtuzumab 4 (36.4%)	NA
	hATG+CsA 5 (38.5%)	TPO-RA 7 (63.6%)	
Transfusion dependency (%)	yes 13 (100%)	yes 8 (72.7%)	NA
		no 3 (27.3%)	
Validation set (n = 108)			
No.	41*	24	43
Median age (range)	25 (2-75)	54.5 (26-71)	38 (23-66)
Sex (M/F)	24/17	15/9	22/21
Disease status (%)	SAA 41 (100%)	WHO RCUD 7 (29.2%)	NA
		RCMD 7 (29.2%)	
		MDS-U 5 (20.8%)	
		RARS 2 (8.3%)	
		RAEB-1 3 (12.5%)	
		IPSS Low 2 (8.3%)	
		Int-1 18 (75.0%)	
		Int-2 4 (16.7%)	
Treatment (%)	rATG+CsA 18 (43.9%)	Alemtuzumab 24 (100%)	NA
	hATG+CsA 23 (56.1%)		
Transfusion dependency (%)	yes 41 (100%)	yes 18 (75%)	NA
		no 6 (25%)	

M, male; F, female; NA, not applicable; rATG, rabbit ATG; hATG, horse ATG; CsA, cyclosporine; IPSS, International Prognostic Scoring System; Int-1, Intermediate 1; Int-2, Intermediate 2; TPO-RA, thrombopoietin receptor agonist.

*In order to assess the effect of IST, 40 patients of 41 AA who had serial plasma samples collected both before and after 6 months of IST were analyzed.

Table S2. The Serum/Plasma Focus microRNA PCR Panel, 384 well (V4.M) for the discovery set

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	hsa-miR-652-3p	hsa-miR-502-3p	UniSp3 IPC	hsa-miR-339-3p	hsa-miR-221-3p	hsa-miR-409-3p	hsa-let-7f-5p	hsa-miR-154-5p	hsa-miR-27b-3p	hsa-miR-155-5p	hsa-miR-374b-5p	hsa-miR-140-3p	hsa-miR-93-5p	hsa-miR-92b-3p	hsa-miR-200a-3p	hsa-miR-505-3p	UniSp2	hsa-miR-23b-3p	hsa-miR-484	hsa-miR-141-3p	hsa-miR-181a-5p	hsa-miR-361-5p	hsa-miR-106a-5p	hsa-miR-27a-3p
B	hsa-miR-652-3p	hsa-miR-502-3p	UniSp3 IPC	hsa-miR-339-3p	hsa-miR-221-3p	hsa-miR-409-3p	hsa-let-7f-5p	hsa-miR-154-5p	hsa-miR-27b-3p	hsa-miR-155-5p	hsa-miR-374b-5p	hsa-miR-140-3p	hsa-miR-93-5p	hsa-miR-92b-3p	hsa-miR-200a-3p	hsa-miR-505-3p	UniSp2	hsa-miR-23b-3p	hsa-miR-484	hsa-miR-141-3p	hsa-miR-181a-5p	hsa-miR-361-5p	hsa-miR-106a-5p	hsa-miR-27a-3p
C	hsa-miR-145-5p	hsa-miR-1	UniSp3 IPC	hsa-miR-382-5p	hsa-miR-486-5p	hsa-miR-32-5p	hsa-miR-26a-5p	hsa-miR-133b	hsa-miR-143-3p	hsa-let-7d-5p	hsa-miR-30a-5p	hsa-miR-133a-3p	hsa-miR-222-3p	hsa-miR-20b-5p	hsa-miR-342-3p	hsa-miR-106b-5p	UniSp4	hsa-miR-328-3p	hsa-miR-324-5p	hsa-miR-532-3p	hsa-miR-185-5p	hsa-miR-15a-5p	hsa-let-7e-5p	hsa-miR-532-5p
D	hsa-miR-145-5p	hsa-miR-1	UniSp3 IPC	hsa-miR-382-5p	hsa-miR-486-5p	hsa-miR-32-5p	hsa-miR-26a-5p	hsa-miR-133b	hsa-miR-143-3p	hsa-let-7d-5p	hsa-miR-30a-5p	hsa-miR-133a-3p	hsa-miR-222-3p	hsa-miR-20b-5p	hsa-miR-342-3p	hsa-miR-106b-5p	UniSp4	hsa-miR-328-3p	hsa-miR-324-5p	hsa-miR-532-3p	hsa-miR-185-5p	hsa-miR-15a-5p	hsa-let-7e-5p	hsa-miR-532-5p
E	hsa-miR-139-5p	hsa-miR-194-5p	UniSp3 IPC	hsa-miR-660-5p	hsa-miR-451a	hsa-miR-574-3p	hsa-let-7a-5p	hsa-miR-320c	hsa-miR-128-3p	hsa-miR-130a-3p	hsa-miR-125a-5p	hsa-miR-28-5p	hsa-miR-485-3p	hsa-miR-497-5p	hsa-let-7b-3p	hsa-miR-425-3p	UniSp5	hsa-miR-132-3p	hsa-miR-25-3p	hsa-let-7c-5p	hsa-miR-375	hsa-miR-18a-5p	hsa-miR-33a-5p	hsa-miR-29b-3p
F	hsa-miR-139-5p	hsa-miR-194-5p	UniSp3 IPC	hsa-miR-660-5p	hsa-miR-451a	hsa-miR-574-3p	hsa-let-7a-5p	hsa-miR-320c	hsa-miR-128-3p	hsa-miR-130a-3p	hsa-miR-125a-5p	hsa-miR-28-5p	hsa-miR-485-3p	hsa-miR-497-5p	hsa-let-7b-3p	hsa-miR-425-3p	UniSp5	hsa-miR-132-3p	hsa-miR-25-3p	hsa-let-7c-5p	hsa-miR-375	hsa-miR-18a-5p	hsa-miR-33a-5p	hsa-miR-29b-3p
G	hsa-miR-16-5p	hsa-miR-136-5p	hsa-let-7b-5p	hsa-miR-1260a	hsa-miR-152-3p	UniSp3 IPC	hsa-miR-30c-5p	hsa-miR-15b-3p	hsa-miR-197-3p	hsa-miR-142-5p	hsa-miR-99b-5p	hsa-miR-100-5p	hsa-miR-30e-5p	hsa-miR-326	hsa-miR-146a-5p	hsa-miR-362-3p	cel-miR-39-3p	hsa-miR-421	hsa-miR-424-5p	hsa-miR-223-5p	UniSp6	hsa-miR-146b-5p	hsa-miR-107	hsa-miR-205-5p
H	hsa-miR-16-5p	hsa-miR-136-5p	hsa-let-7b-5p	hsa-miR-1260a	hsa-miR-152-3p	UniSp3 IPC	hsa-miR-30c-5p	hsa-miR-15b-3p	hsa-miR-197-3p	hsa-miR-142-5p	hsa-miR-99b-5p	hsa-miR-100-5p	hsa-miR-30e-5p	hsa-miR-326	hsa-miR-146a-5p	hsa-miR-362-3p	cel-miR-39-3p	hsa-miR-421	hsa-miR-424-5p	hsa-miR-223-5p	UniSp6	hsa-miR-146b-5p	hsa-miR-107	hsa-miR-205-5p
I	hsa-miR-148b-3p	hsa-miR-339-5p	hsa-miR-20a-5p	Blank (H2O)	hsa-miR-17-5p	UniSp3 IPC	hsa-miR-30d-5p	hsa-miR-378a-3p	hsa-miR-186-5p	hsa-miR-425-5p	hsa-let-7i-5p	hsa-miR-454-3p	hsa-miR-26b-5p	hsa-miR-874-3p	hsa-miR-34a-5p	hsa-miR-193a-5p	hsa-miR-320b	hsa-miR-885-5p	hsa-miR-590-5p	hsa-miR-127-3p	hsa-miR-191-5p	hsa-miR-208a-3p	hsa-miR-99a-5p	hsa-miR-16-2-3p
J	hsa-miR-148b-3p	hsa-miR-339-5p	hsa-miR-20a-5p	Blank (H2O)	hsa-miR-17-5p	UniSp3 IPC	hsa-miR-30d-5p	hsa-miR-378a-3p	hsa-miR-186-5p	hsa-miR-425-5p	hsa-let-7i-5p	hsa-miR-454-3p	hsa-miR-26b-5p	hsa-miR-874-3p	hsa-miR-34a-5p	hsa-miR-193a-5p	hsa-miR-320b	hsa-miR-885-5p	hsa-miR-590-5p	hsa-miR-127-3p	hsa-miR-191-5p	hsa-miR-208a-3p	hsa-miR-99a-5p	hsa-miR-16-2-3p
K	hsa-miR-301a-3p	hsa-miR-140-5p	hsa-miR-151a-5p	hsa-miR-130b-3p	hsa-miR-122-5p	UniSp3 IPC	hsa-miR-423-5p	hsa-miR-629-5p	hsa-miR-101-3p	hsa-miR-200c-3p	hsa-miR-365a-3p	hsa-miR-501-3p	hsa-miR-23a-3p	hsa-miR-423-3p	hsa-miR-215-5p	hsa-miR-376a-3p	hsa-miR-320a	hsa-miR-22-5p	hsa-miR-338-3p	hsa-miR-2110	hsa-miR-223-3p	hsa-miR-376c-3p	hsa-miR-103a-3p	hsa-miR-93-3p
L	hsa-miR-301a-3p	hsa-miR-140-5p	hsa-miR-151a-5p	hsa-miR-130b-3p	hsa-miR-122-5p	UniSp3 IPC	hsa-miR-423-5p	hsa-miR-629-5p	hsa-miR-101-3p	hsa-miR-200c-3p	hsa-miR-365a-3p	hsa-miR-501-3p	hsa-miR-23a-3p	hsa-miR-423-3p	hsa-miR-215-5p	hsa-miR-376a-3p	hsa-miR-320a	hsa-miR-22-5p	hsa-miR-338-3p	hsa-miR-2110	hsa-miR-223-3p	hsa-miR-376c-3p	hsa-miR-103a-3p	hsa-miR-93-3p
M	hsa-miR-331-3p	hsa-miR-144-5p	hsa-miR-142-3p	hsa-miR-210-3p	hsa-let-7d-3p	hsa-miR-199a-5p	hsa-miR-126-5p	hsa-miR-766-3p	hsa-miR-19a-3p	hsa-miR-584-5p	hsa-miR-144-3p	hsa-miR-92a-3p	hsa-miR-126-3p	hsa-miR-363-3p	hsa-miR-148a-3p	hsa-miR-374a-5p	hsa-miR-10b-5p	hsa-miR-483-5p	hsa-miR-195-5p	hsa-miR-7-1-3p	hsa-miR-125b-5p	hsa-miR-877-5p	hsa-miR-192-5p	hsa-miR-151a-3p
N	hsa-miR-331-3p	hsa-miR-144-5p	hsa-miR-142-3p	hsa-miR-210-3p	hsa-let-7d-3p	hsa-miR-199a-5p	hsa-miR-126-5p	hsa-miR-766-3p	hsa-miR-19a-3p	hsa-miR-584-5p	hsa-miR-144-3p	hsa-miR-92a-3p	hsa-miR-126-3p	hsa-miR-363-3p	hsa-miR-148a-3p	hsa-miR-374a-5p	hsa-miR-10b-5p	hsa-miR-483-5p	hsa-miR-195-5p	hsa-miR-7-1-3p	hsa-miR-125b-5p	hsa-miR-877-5p	hsa-miR-192-5p	hsa-miR-151a-3p
O	hsa-miR-18b-5p	hsa-miR-28-3p	hsa-miR-335-5p	hsa-miR-324-3p	hsa-miR-320d	hsa-miR-136-3p	hsa-let-7g-5p	hsa-miR-15b-5p	hsa-miR-22-3p	hsa-miR-106b-3p	hsa-miR-199a-3p	hsa-miR-29c-3p	hsa-miR-19b-3p	hsa-miR-335-3p	hsa-miR-29a-3p	hsa-miR-21-5p	hsa-miR-150-5p	hsa-miR-30e-3p	hsa-miR-30b-5p	hsa-miR-543	hsa-miR-24-3p	hsa-miR-7-5p	Blank (H2O)	hsa-miR-495-3p
P	hsa-miR-18b-5p	hsa-miR-28-3p	hsa-miR-335-5p	hsa-miR-324-3p	hsa-miR-320d	hsa-miR-136-3p	hsa-let-7g-5p	hsa-miR-15b-5p	hsa-miR-22-3p	hsa-miR-106b-3p	hsa-miR-199a-3p	hsa-miR-29c-3p	hsa-miR-19b-3p	hsa-miR-335-3p	hsa-miR-29a-3p	hsa-miR-21-5p	hsa-miR-150-5p	hsa-miR-30e-3p	hsa-miR-30b-5p	hsa-miR-543	hsa-miR-24-3p	hsa-miR-7-5p	Blank (H2O)	hsa-miR-495-3p

Table S3. The custom microRNA PCR Panel for the validation set

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p	hsa-miR-103a-3p	hsa-miR-106a-5p
B	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p	hsa-miR-23a-3p	hsa-miR-148b-3p
C	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p	hsa-miR-451a	hsa-miR-191-5p
D	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1	hsa-miR-320a	hsa-miR-1
E	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p	hsa-let-7f-5p	hsa-let-7a-5p
F	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p	hsa-let-7g-5p	hsa-miR-143-3p
G	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a	hsa-miR-29b-3p	hsa-miR-1260a
H	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p	hsa-miR-30e-5p	hsa-miR-146b-5p
I	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p	hsa-miR-20a-5p	hsa-miR-26b-5p
J	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p	hsa-miR-501-3p	hsa-miR-21-5p
K	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p	hsa-miR-150-5p	hsa-miR-22-3p
L	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p	hsa-miR-29c-3p	hsa-miR-424-5p
M	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p	hsa-miR-181a-5p	hsa-miR-144-5p
N	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP	Cel-miR-39-3p CP	UniSp2 CP
O	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP	UniSp4 CP	UniSp5 CP
P	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC	UniSp6 CP	UniSp3 IPC

Table S4. Differentially expressed miRNAs in the discovery set

AA vs HC		MDS vs HC		AA vs MDS	
miRNAs	*P*	miRNAs	*P*	miRNAs	*P*
let-7f-5p	.04	miR-339-3p	.02	miR-339-3p	.03
miR-1	.02	miR-409-3p	.009	miR-409-3p	.005
miR-143-3p	.05	**let-7f-5p**	.001	miR-154-5p	.03
miR-29b-3p	.005	miR-200a-3p	.04	miR-484	.003
miR-1260a	.05	miR-484	.006	miR-141-3p	.02
miR-30e-5p	<.001	**miR-181a-5p**	.001	**miR-181a-5p**	<.001
miR-146b-5p	.002	miR-382-5p	.01	miR-382-5p	<.001
miR-20a-5p	.01	miR-32-5p	<.001	miR-32-5p	.001
miR-26b-5p	.02	let-7d-5p	.01	**miR-143-3p**	.01
miR-501-3p	.02	miR-342-3p	.03	let-7d-5p	.006
miR-21-5p	.02	miR-139-5p	.003	miR-342-3p	.02
miR-150-5p	.05	miR-660-5p	.004	miR-139-5p	.05
		miR-574-3p	.02	**miR-29b-3p**	.01
		let-7a-5p	.05	**miR-30e-5p**	.05
		miR-28-5p	.03	**miR-424-5p**	.01
		miR-1260a	.003	**miR-146b-5p**	.04
		miR-424-5p	<.001	miR-425-5p	<.001
		miR-30d-5p	.01	miR-34a-5p	.02
		miR-378a-3p	.007	miR-191-5p	.04
		miR-425-5p	.04	miR-151a-5p	.05
		miR-34a-5p	.01	miR-101-3p	.01
		miR-127-3p	.03	miR-22-5p	.006
		miR-423-5p	.02	miR-376c-3p	.03
		miR-629-5p	.004	miR-331-3p	.03
		miR-101-3p	.03	miR-766-3p	.02
		miR-501-3p	.02	miR-19a-3p	.02
		miR-23a-3p	.03	miR-148a-3p	.05
		miR-376a-3p	.02	miR-195-5p	.002
		miR-22-5p	.006	**let-7g-5p**	<.001
		miR-376c-3p	.005	**miR-22-3p**	<.001
		miR-331-3p	.05	miR-199a-3p	.03
		miR-126-5p	.05	miR-29a-3p	.006
		miR-766-3p	.02	**miR-29c-3p**	<.001
		miR-584-5p	.01	**miR-150-5p**	.004
		miR-483-5p	.04	miR-543	.04
		miR-195-5p	.01	miR-495-3p	.04
		miR-28-3p	.05		
		let-7g-5p	<.001		
		miR-22-3p	<.001		
		miR-199a-3p	.002		
		miR-335-3p	.007		
		miR-29a-3p	.03		
		miR-29c-3p	<.001		
		miR-21-5p	.003		
		miR-30b-5p	.02		
		miR-543	.02		
		miR-495-3p	.008		

Probability values of significantly ($P < .05$) and differentially expressed miRNAs based on 1-way ANOVA followed by pair-wise group comparisons in the discovery set.
MiRNAs in bold text were tested using qPCR in the validation set.

Table S5. Association between miRNAs and clinical and laboratory parameters

	ANC		Plt		ARC	
miRNA	**Pearson correlation coefficients**	***P***	**Pearson correlation coefficients**	***P***	**Pearson correlation coefficients**	***P***
miR-1	0.296	**.038**	0.010	.476	0.243	.073
miR-146b-5p	-0.130	.222	-0.174	.151	-0.340	**.020**
miR-150-5p	-0.095	.287	-0.325	**.025**	-0.227	.088

Positive coefficients indicate that an increase in the cycle number is associated with an increase in ANC, Plt, or ARC.miRNA: microRNA, ANC: Absolute neutrophil count, Plt: Platelet count, ARC: Absolute reticulocyte count

Table S6. Change in miR-150-5p and clinical status after IST in AA patients

UPN	Age	Sex	% Change in miR-150-5p after IST	IST	Response at 6 months
1	19	M	-23.4	h-ATG	PR
2	3	M	-74.7	r-ATG	NR
3	9	M	-26.1	h-ATG	PR
4	53	M	-74.2	h-ATG	CR
5	43	F	NA	r-ATG	PR
6	17	M	-79.7	r-ATG	NR
7	8	M	-72.3	h-ATG	PR
8	11	F	-86.1	r-ATG	NR
9	22	M	-93.6	r-ATG	NR
10	25	F	88.6	h-ATG	PR
11	42	M	-66.3	h-ATG	CR
12	28	M	109.3	h-ATG	NR
13	20	M	109.1	h-ATG	NR
14	39	M	-78.9	r-ATG	PR
15	52	F	-74.0	h-ATG	PR
16	4	F	13.2	h-ATG	NR
17	7	F	133.1	r-ATG	NR
18	22	M	-60.5	h-ATG	PR
19	59	M	4.6	h-ATG	NR
20	33	M	-81.0	r-ATG	PR
21	39	F	-74.1	r-ATG	PR
22	61	F	-53.0	h-ATG	PR
23	11	M	-37.6	r-ATG	NR
24	57	M	29.3	h-ATG	NR
25	17	F	-75.8	r-ATG	NR
26	48	M	-55.5	h-ATG	PR
27	25	F	-97.9	r-ATG	CR
28	27	M	-15.4	h-ATG	PR
29	67	M	-22.0	h-ATG	PR
30	2	F	-85.4	r-ATG	NR
31	52	F	-62.0	h-ATG	PR
32	21	M	-57.0	r-ATG	NR
33	52	F	-44.9	r-ATG	NR
34	58	F	105.6	h-ATG	PR
35	7	F	-13.9	h-ATG	CR
36	23	M	2.1	h-ATG	PR
37	5	M	135.1	r-ATG	NR
38	17	M	-45.1	r-ATG	PR
39	50	F	NA	h-ATG	NR
40	75	F	-62.6	h-ATG	PR

IST= immunosuppressive therapy, h= horse, r=rabbit, ATG= Anti-thymocyte globulin,
CR = complete response, PR= partial response, NR = no response, NA = Not available

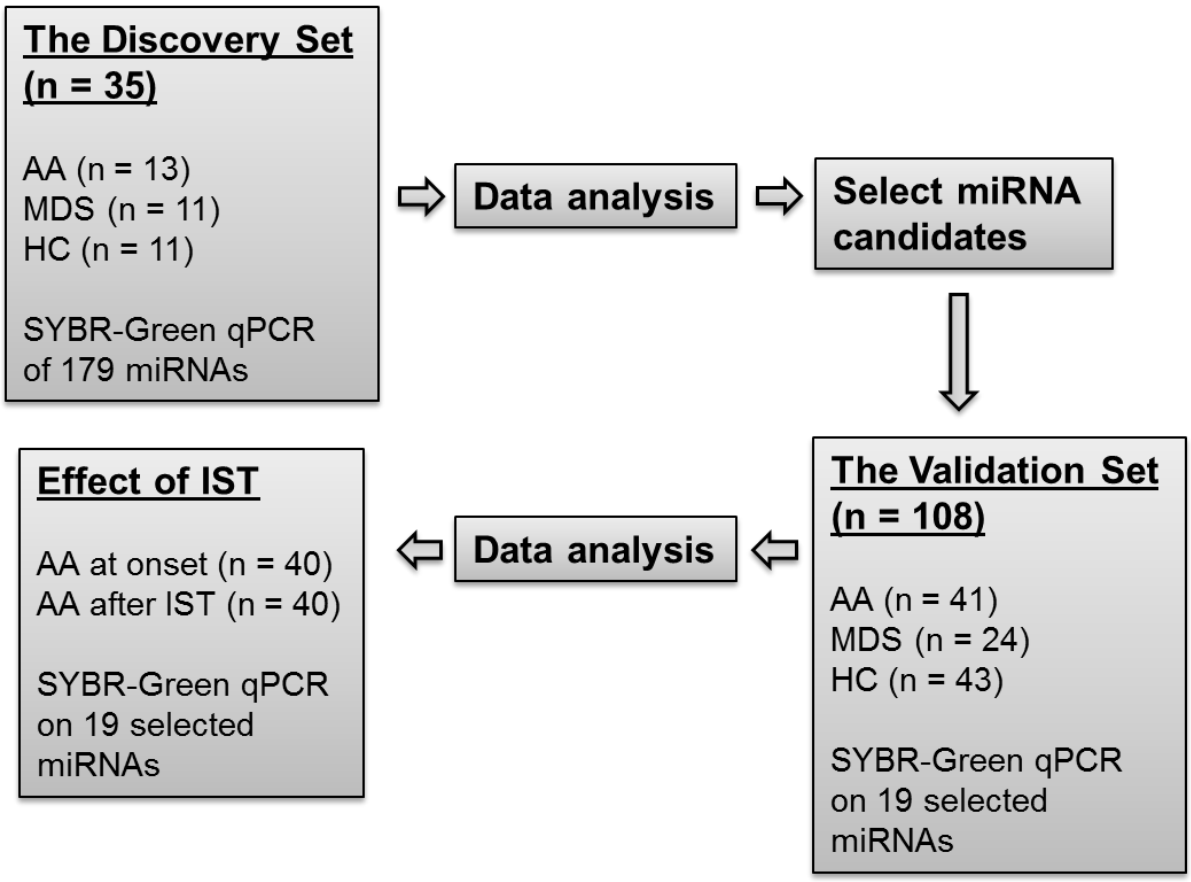

Figure S1. A summary of all analysis steps of the candidate miRNA.

Shown are flow charts of miRNA analysis in the discovery and validation sets.

Supplemental Figure 2, Hosokawa et al.

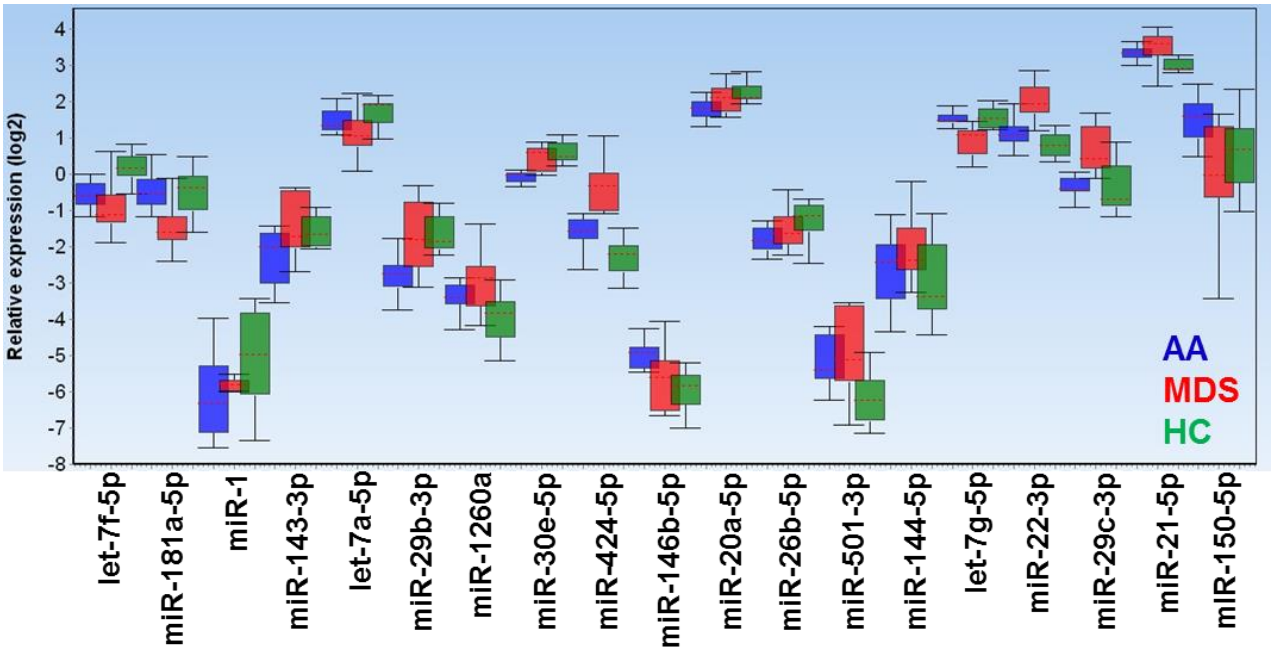

Figure S2. Box plots of the 19 miRNA expression levels in the discovery set.

Box plots show 19 miRNAs differentially expressed between AA, MDS, and HC in discovery set. The Y-axis indicates miRNA expression levels. Bars mean minimum and maximum values of miRNA expression. Blue bars = AA; red bars = MDS; green bars = HC.

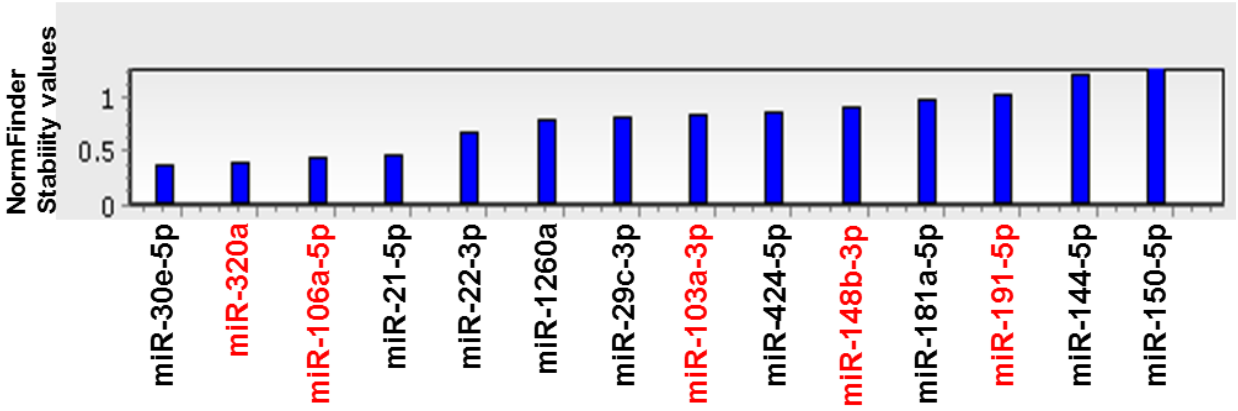

Figure S3. Normofinder represents the stably expressed miRNAs.

Five miRNAs (miR-103a-3p, miR-106a-5p, miR-148-3p, miR-191-5p, and miR-320a, shown in red color) with superior expression stability among all samples were included in the custom plate for data normalization in the validation set. Out of five reference genes defined by the discovery set, miR-106a-5p and miR-320a exhibited to have stable amplification in all of the individual samples with good expression in the validation cohort, whereby these two miRNAs were used to calculate ΔCT and further relative expression levels. The y-axis indicates the NormoFinder stability values.

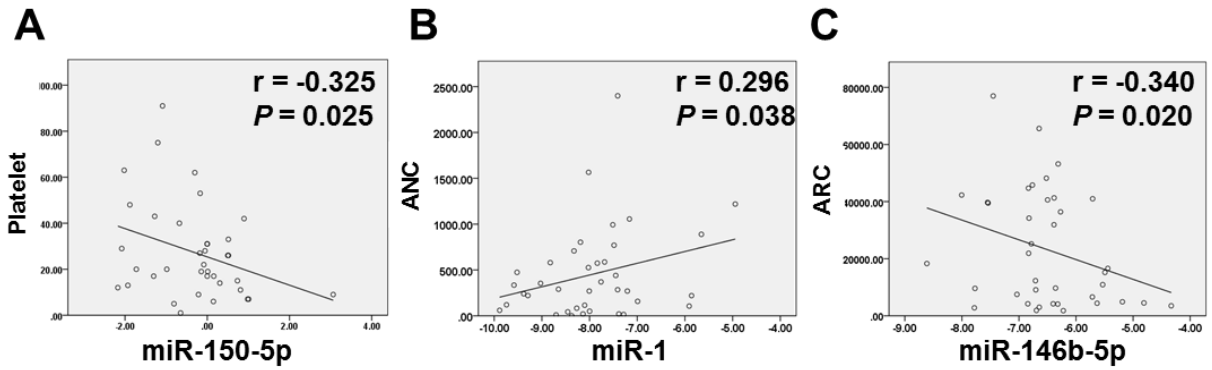

Figure S4. Correlations between miRNAs and clinical parameters in AA patients at diagnosis.

Pearson correlation coefficient was calculated between miRNAs and absolute neutrophil count (ANC), absolute reticulocyte count (ARC), or platelet count, respectively. (A) Negative correlation between the miR-150-5p expression levels and platelet count. (B) Positive correlation between the miR-1 expression levels and ANC. (C) Negative correlation between the miR-146b-5p expression levels and ARC. r, a correlation coefficient value.

Supplemental Figure 5, Hosokawa et al.

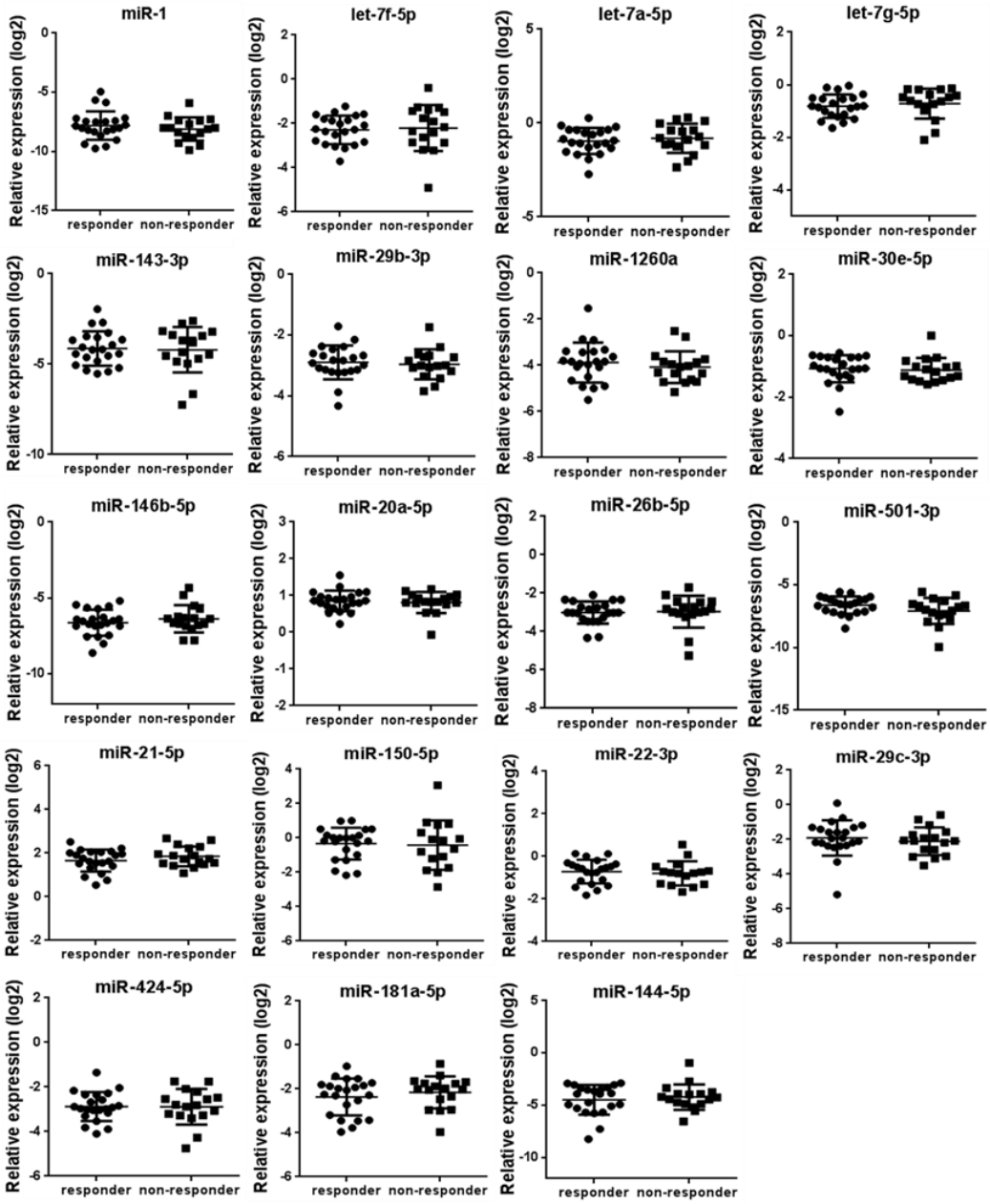

Figure S5. Comparison of 19 miRNA expression levels of plasma between responders and non-responders in AA patients at diagnosis.

Expression levels of 19 plasma miRNA at diagnosis were compared between two patient groups [responders (n = 23) vs non-responders (n=17)] in AA. None of the miRNAs was significantly different between the two groups.

Supplemental Experimental Methods

MiRNA profiling using the Plasma Focus microRNA PCR Panel

The panel assay includes wells of an interpolate calibrator (UniSp3) to account for run-to-run differences in amplification signal. Assays were carried out in a final reaction volume of 10 µl/wells, following the manufacturer's protocol, using 50x diluted cDNA and an equal volume of 2x SYBR Green master mix. qRT-PCR amplification condition was an initial hold at 95°C for 10 min, followed by 40 cycles of amplification (95°C for 10 sec and 60°C for 60 sec) and melting curve analysis. All qRT-PCR reactions were performed in 384-well plates using the ROX Reference Dye (Thermo Fisher Scientific, NY) and analyzed with the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Grand Island, NY).

Raw qRT-PCR amplification data were analyzed using GenEx6 software (Exiqon), according to the Exiqon's recommendations, which included the inter-plate calibration, approved quality controls (RNA-spike-in), and hemolysis test (miR-23a-3p –miR-451a). Plasma was analyzed for potential cellular miRNA contamination, due to hemolysis during the plasma samples preparation. A Δ CT value of established hemolysis markers [ΔCT (hemolysis) =CT (miR-23a-3p)-CT (miR-451a)] is recommended to use as the qualitative measurement of the hemolysis. Plasma samples with > 7 ΔCT values were not used for further analysis, due to a high risk of hemolysis. The discovery-set data were normalized using the global mean of all miRNAs with < 34 CT values.[1] Candidate reference miRNAs identified by variance analysis of the normalized dataset were further examined using the established algorithms, Normfinder.[2] Five miRNAs (miR-103a-3p, miR-106a-5p, miR-148-3p, miR-191-5p, and miR-320a) with superior expression stability among all samples were included in the custom plate for normalization in the subsequent validation set.

Validation of the miRNA profiling by custom PCR panel

Based on the results from the discovery set, we designed the custom microRNA PCR Panel for validation set. This panel covered the analysis of 19 human miRNAs for validation: five miRNAs (miR-103a-3p, miR-106a-5p, miR-148-3p, miR-191-5p, and miR-320a) as reference miRNAs, two miRNAs (miR-451a and miR-23a-3p) as hemolysis test, and six miRNAs as spike-in and other quality controls (Supplemental Table 3). This panel was used for profiling the validation set (108 of 41 AA patients, 24 MDS patients, and 43 HCs) and 40 AA patients with plasma samples collected after 6 months of IST.

Amplification conditions of qRT-PCR were an initial hold at 95°C for 10 min, followed by 40 cycles of amplification (95°C for 10 sec and 60°C for 60 sec) and melting curve analysis. All qRT-PCR reactions were carried out on 384-well plates with ROX Reference Dye (Thermo Fisher Scientific) and analyzed with the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems). qRT-PCR data from the custom PCR Panels were examined with GenEx6 software (Exiqon). In this process, the inter-plate calibration, approved quality controls (RNA-spike-in), and hemolysis test (miR-23a-3p –miR-451a) were included. Reference miRNAs, miR-106a-5p, and miR-320a were chosen by analyzing the suggested candidate genes in the applications "NormFinder" available in the GenEx6 software (Supplemental Figure 3). Normalization of individual miRNA levels was done to the above-mentioned reference genes. Quantification of relative miRNA expression on the validation set was performed with the comparative CT method using the formula 2^ ΔCT, where ΔCT = [(CT gene of interest- CT reference gene) sample A - (CT gene of interest - CT reference gene) sample B] by using miR-106a-5p and miR-320a as the reference miRNAs.[3]

Ingenuity® Pathway Analysis (IPA)

Pathway analysis was carried out to determine differentially regulated biological pathways by loading differentially expressed miRNAs with statistically significance into the Ingenuity® Pathway Analysis (IPA) software (www.ingenuity.com).

Statistics

Log conversion of the data in the discovery and validation sets was performed to obtain data more similar to a normal distribution for statistical testing. Unpaired two-tailed t-test for two-group comparison and one-way analysis of variance (ANOVA) for three-group comparison were performed. In the discovery set, candidate miRNAs were selected if significant differences ($P < .05$) among individual groups were observed by pair-wise group comparison (AA vs HC, MDS vs HC, and AA vs MDS). From candidate miRNAs, upregulated or downregulated 19 miRNAs found in each group were selected for the validation set analysis. A 1.5-fold-change (FC) threshold was chosen on the basis of its use in the literature.[1] Hierarchical clustering analysis was performed using Cluster 3 and results were displayed as heatmaps generated by TreeView.[4] In the validation set, association between group status and each of the selected miRNAs was assessed using logistic regression to control for the age and sex. Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were used to assess sensitivity and specificity of miRNA biomarkers for the diagnosis. Further, logistic regression was employed to develop a combined miRNA panel to predict the probability of developing AA, as previously described.[5, 6] Pearson correlation was performed to determine the correlation coefficient between miRNAs and clinical characteristics [age, absolute neutrophil count (ANC), absolute reticulocyte count (ARC), platelet count]. Paired two-tailed t-test was carried out based on within-patient

change before and after IST. *P* values from individual analysis were also adjusted for multiple comparisons using the approach of Benjamini and Hochberg to control the false discovery rate (FDR) at .05.

Supplemental References

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