Supplementary Methods

Patients and samples

The study population consisted of 196 human subjects who underwent allogeneic HCT between 2008 and 2012 at Duke University Medical Center. Additionally, 45 patients who underwent lung transplantation (including 17 with biopsy-confirmed acute rejection and 28 without rejection), 38 patients who had sepsis with confirmed pathogens, and 10 healthy donors were enrolled as controls. Patients with sepsis were enrolled between 2005 and 2012, at the time of clinical presentation to Duke University Medical Center (Durham, NC), the Durham VA Medical Center (Durham, NC), or UNC Health Care (Chapel Hill, NC). They included 10 subjects with *Staphylococcus aureus* bacteremia, 10 subjects with *Escherichia coli* bacteremia, 10 subjects with *Clostridium difficile*-associated diarrhea, 4 subjects with *Streptococcus pneumoniae* pneumonia, and 4 subjects with acute cytomegalovirus infection. Clinical determination of sepsis was made by an expert in infectious diseases. Healthy control peripheral blood plasma samples were obtained from subjects participating in an influenza challenge study. All subjects were screened and determined not to have concurrent illness. All tested samples were baseline samples obtained prior to influenza virus inoculation. Enrollment took place in the United Kingdom during October 2009 with approved human study protocols.

qRT-PCR analysis of plasma miRNAs

Plasma miRNAs were quantified using SYBR Green-based quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). Mature miRNAs were

polyadenylated at the 3’ end with a Poly(A) Polymerase Tailing Kit (Epicentre Biotechnologies) and then converted into cDNA using the reverse transcriptase Superscript III (Life Technologies) and an oligo-dT primer with a universal tag. With this universal tag, qRT-PCR was performed with a miRNA-specific forward primer, a universal primer mix (UPM) containing two primers (long and short, molar ratio 1:5) to eliminate the non-specific priming, and Power SYBR® Green Master Mix (Life Technologies).

Table S1. Multivariate Cox regression analysis for the estimation of the correlation of miRNA signature in aGVHD patients with overall survival

	Hazard ratio	*P*
Age	0.970	0.265
Malignant (Yes/No)	0.000	0.987
Risk Score (low/high)	0.735	0.629
Donor type (Related/Unrelated)	2.697	0.107
Regimen Type (Non Myeloablative/ Myeloablative)	1.522	0.451
miRNA signature	2.110	0.010

Table S2 Spearman correlation analysis for miRNA panel by SPSS 16.0

		miR-423	miR-199a-3p	miR-93*	miR-377
miR-423	**Correlation Coefficient**	1.000	0.822	0.950	0.914
	Sig. (2-tailed)	\	< 0.001	< 0.001	< 0.001
miR-199a-3p	**Correlation Coefficient**	0.822	1.000	0.861	0.819
	Sig. (2-tailed)	< 0.001	\	< 0.001	< 0.001
miR-93*	**Correlation Coefficient**	0.950	0.861	1.000	0.932
	Sig. (2-tailed)	< 0.001	< 0.001	\	< 0.001
miR-377	**Correlation Coefficient**	0.914	0.819	0.932	1.000
	Sig. (2-tailed)	< 0.001	< 0.001	< 0.001	\

Table S3 Correlation analysis between miRNA panel and sIL-2Rα by SPSS 16.0

		miR-423	miR-199a-3p	miR-93*	miR-377
sIL-2Rα	**Correlation Coefficient**	-0.146	- 0.127	-0.135	-0.117
	Sig. (2-tailed)	0.059	0.101	0.081	0.132

Table S4 Comparison of AUC between single miRNA and miRNA panel for all samples by MedCalc

	AUC	*P* *
miR-423	0.718	0.048
miR-199a-3p	0.674	0.008
miR-93*	0.747	0.137
miR-377	0.769	0.387
miRNA panel	0.800	\

* *P* values were calculated between single miRNA (miR-423, miR-199a-3p, miR-93*, and miR-377) and miRNA panel using MedCalc statistical software.

Figure S1

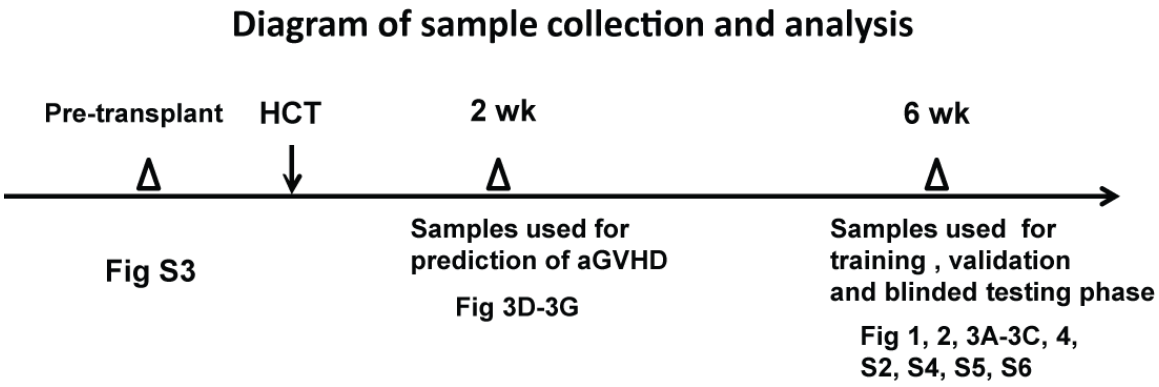

Figure S1. A schematic showing the time-points of sample collection and analysis methods used in different portions of the study.

Figure S2

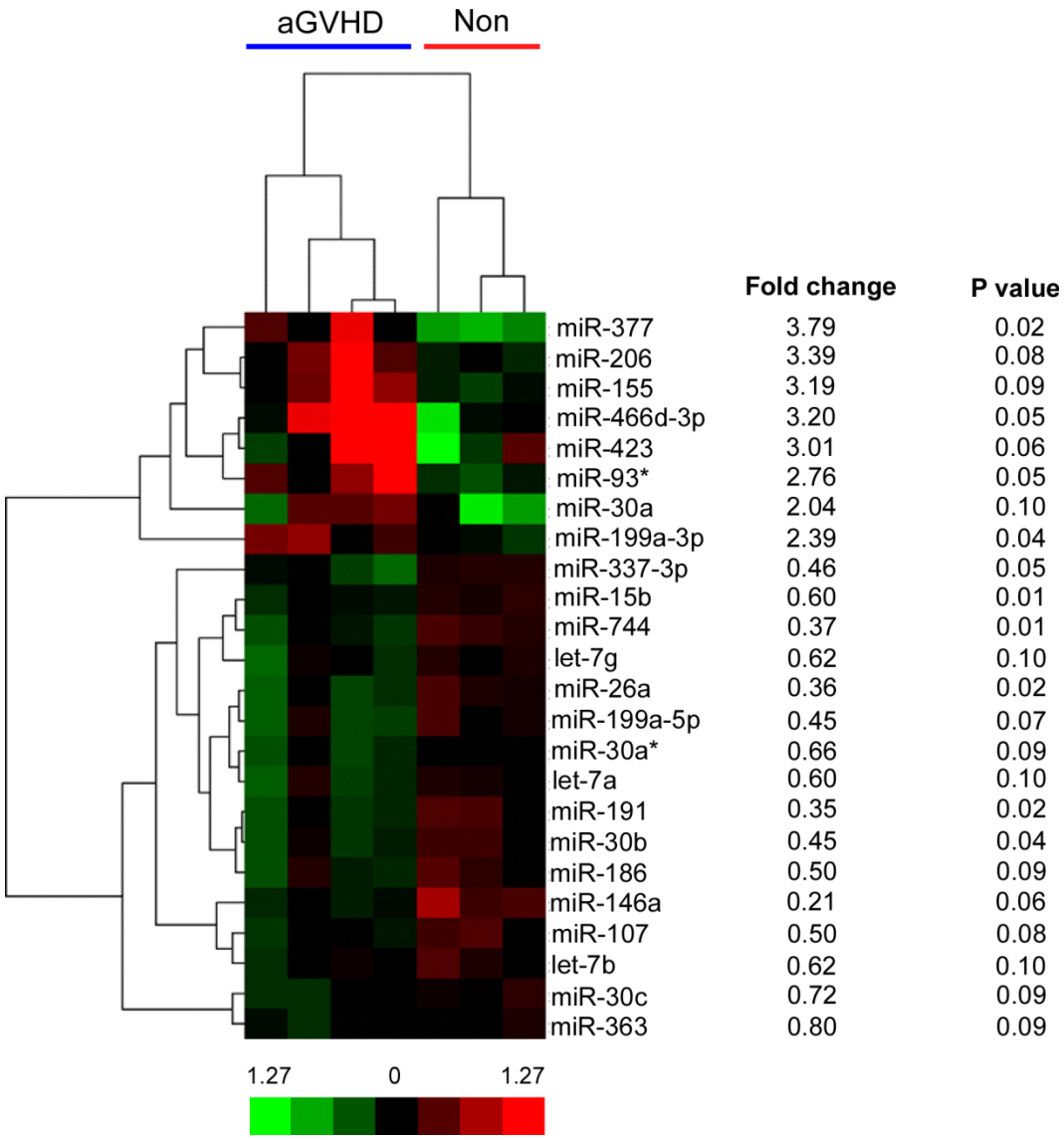

Figure S2. Hierarchical clustering analysis of differentially expressed miRNAs in plasma of aGVHD patients. miRNA profiling in plasma from 4 aGVHD patients and 3 non-GVHD patients 6 weeks after transplantation was performed by using a real-time PCR based high throughput miRNA array.

Figure S3

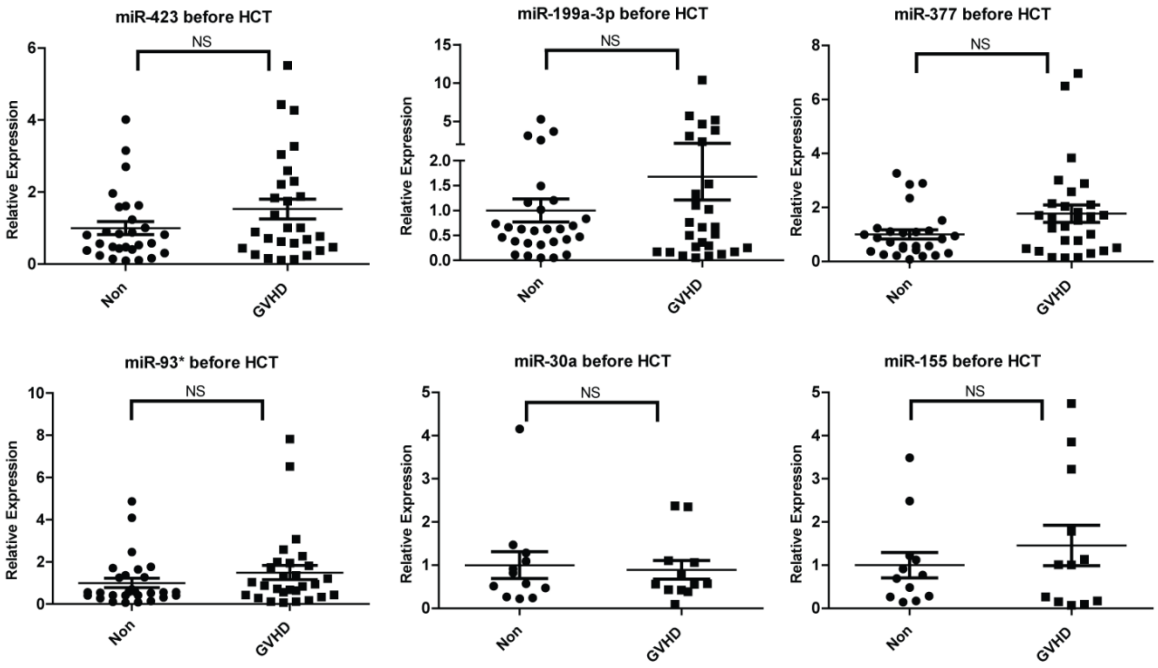

Figure S3. The expression of miRNA signature in plasma of aGVHD (n=28) and non-GVHD (n=28) patients before HCT.

Figure S4

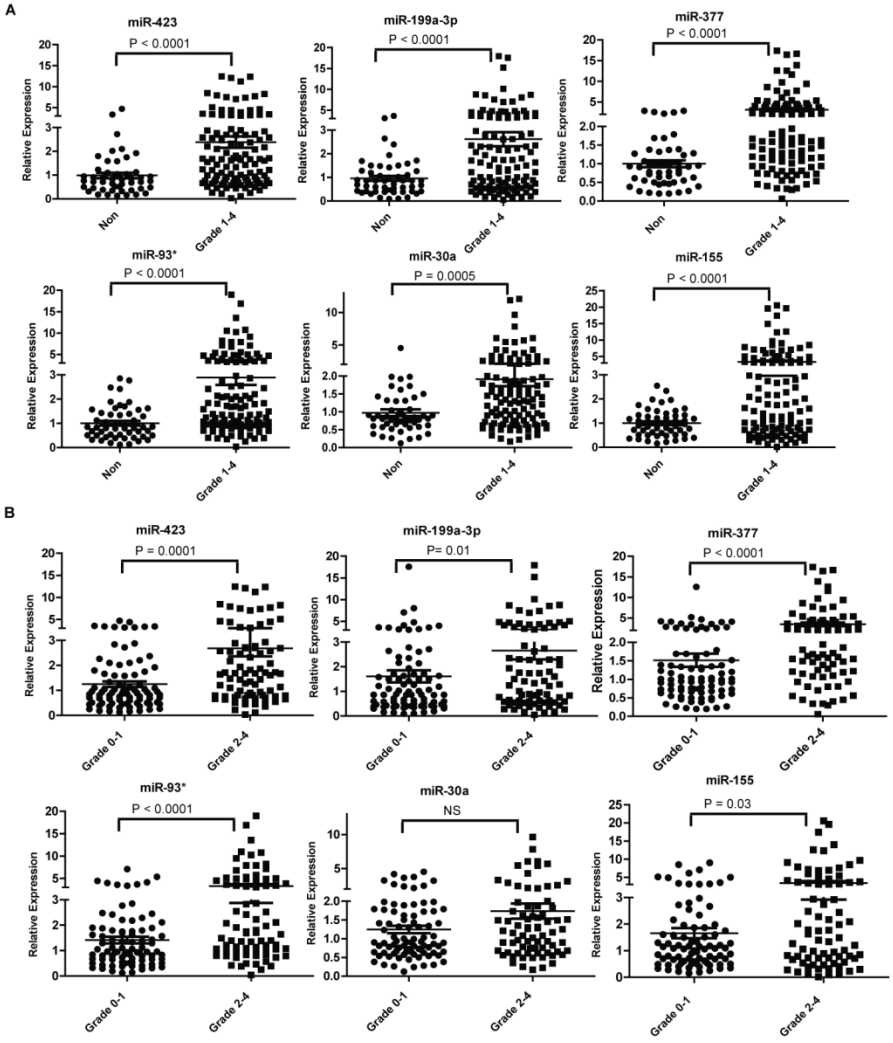

Figure S4. Comparison of miRNAs expression between different grades of aGVHD. (A) Expression levels of miR-423, miR-199a-3p, miR-93*, miR-377, miR-155, and miR-30a in all aGVHD patients (n=168, grade 1-4) and non-GVHD (grade 0). (B) Comparison of the levels of miR-423, miR-199a-3p, miR-93*, miR-377, miR-155, and miR-30a between patients with grade 0-1 and patients with grade 2-4.

Figure S5

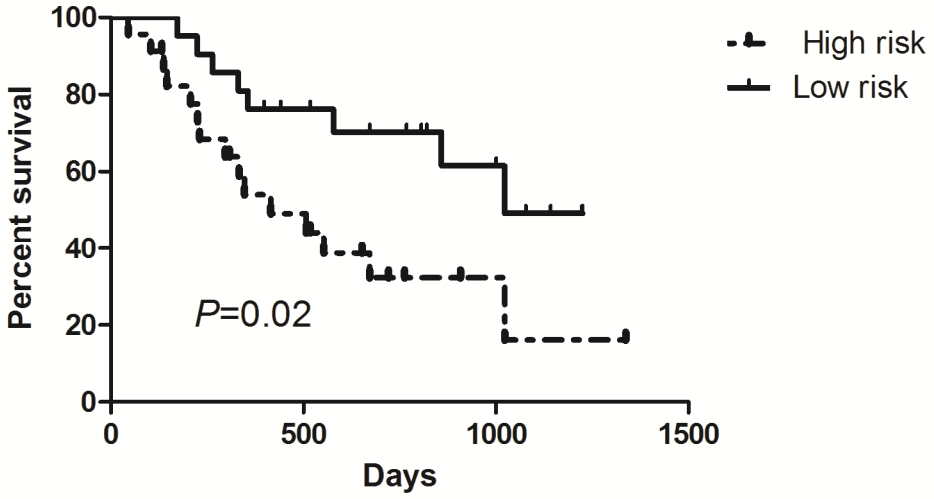

Figure S5. miRNA signature and overall survival of aGVHD patients. All aGVHD patients were divided into two groups (high-risk and low-risk) based on their predicted probabilities of developing aGVHD by detecting their expression of the miRNA panel. Overall survival was determined by Kapian-Meier Curve. *P* values were based on log-rank test.

Figure S6

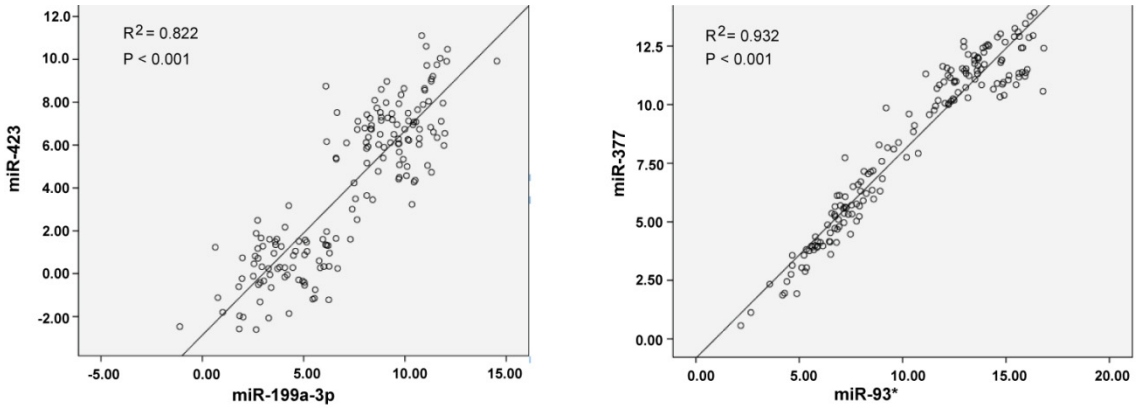

Figure S6. Spearman correlation analysis of miR-423, miR-199a-3p, miR-93*, and miR-377.