SIRPαFc treatment targets human acute myeloid leukemia stem cells

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doi:10.3324/haematol.2019.245167

MATERIALS AND METHODS

AML patient samples

Peripheral blood was collected from AML patients at the Princess Margaret Cancer Centre after obtaining informed consent according to procedures approved by the Research Ethics Board of the University Health Network (UHN). Mononuclear cells were isolated by Ficoll density gradient separation and viably frozen in FCS with 10% DMSO. Patient samples were pre-screened for engraftment ability in NSG mice; only well engrafting ones were used for the study. LSC17 scores were measured on RNA extracted from bulk AML samples following a protocol modified from our previous study [1]. Samples were prepared and analyzed using an nCounter Analysis System Prep Station and Digital Analyzer (NanoString Technologies). Raw transcript counts were analyzed using nSolver analysis software (version 3.0.22) for quality control and normalization. The LSC17 score calculated for each patient was compared to the median of a reference AML cohort to determine if the patient had a high or low score. A manuscript is in preparation describing the assay methodology in detail.

Effects of SIRPαFc treatment in primary AML xenografts

Animal experiments were performed in accordance with institutional guidelines approved by the UHN Animal Care Committee. Transplantation of human cells into recipient mice was performed as previously described [2, 3]. NOD/Lt-*scid/IL2Rγ*^{null} (NSG) mice were sublethally irradiated with 225 cGy 24 hours before transplantation. Patient samples were depleted of T cells using the EasySep Human CD3 Positive Selection Kit (STEMCELL Technologies, Vancouver, Canada). AML samples were transplanted by intrafemoral (IF) injection at a dose of 1 to 5×10^6 cells per mouse. Starting 2 weeks post-transplantation, mice were treated with 5 mg/kg human

SIRPαFc (TTI-621, Trillium Therapeutics Inc., Mississauga, Canada) or 10 mg/kg control IgG 3×/week for 4 weeks. After 4 weeks of treatment, the mice were sacrificed and leukemic engraftment in the injected right femur (RF) and non-injected femur and tibias (BM) was evaluated by flow cytometry using human-specific monoclonal antibodies (mAbs). Cells reserved from RF and BM from treatment cohorts were pooled separately and frozen viably for secondary transplantation.

Flow cytometric analysis of AML engraftment

Leukemic engraftment was determined by the percentage of human CD45+/CD33+ cells in the murine bone marrow. The following human-specific mAbs were used for staining: anti-CD47-FITC clone B6H12, anti-CD45-APC, anti-CD38-PE-Cy7 (BD Biosciences), anti-CD47-FITC clone 2D3 (eBioscience, San Diego, CA, USA), anti-CD33-PE-Cy5 (Beckman Coulter, Brea, CA), anti-CD34-APC-Cy7 (Biolegend, San Diego, CA, USA). Data were acquired on a BD LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software v9.9.6 for Mac OS (Tree Star, Ashland, OR, USA).

Definition of response to SIRPαFc treatment

The definition of response was based on the relative reduction (RR) of leukemic engraftment in SIRPαFc-treated versus control-treated mice [4]. RR was calculated as [(mean%engraftment of control-treated mice) – (mean%engraftment of SIRPαFc-treated mice)] / (mean%engraftment of control-treated mice). Patient samples were classified as responders (R) if RR in the injected RF was >50%, partial responders (PR) if we observed 20 to 50% RR in the RF or >20% in the BM only, and non-responders (NR) if there was no statistically significant

difference in engraftment levels between control- and SIRPαFc-treated mice, or RR was <20% in both RF and BM.

Secondary transplantation assays

NSG mice were sublethally irradiated (225 cGy) 24 hours before transplantation. Bone marrow cells were harvested and pooled from cohorts of primary engrafted mice and depleted of contaminating mouse cells using Mouse Cell Depletion Kit (Miltenyi Biotech, Auburn, CA, USA). Human AML cells were injected IF at varying doses into cohorts of mice for limiting dilution analysis of LSC frequency. After 12 weeks, mice were sacrificed and the level of leukemic engraftment was evaluated by flow cytometry. Mice were scored as positive if there was a definite CD45+CD33+ human graft of greater than 0.1%. The LSC frequency and 95% confidence intervals were calculated using L-Calc software (STEMCELL Technologies, https://www.stemcell.com/l-calc-software.html).

Statistical analysis

Thirty independent patient samples were used in the study to capture the diversity of AML. Comparison of engraftment between drug- and control-treated mice was performed using two-tailed *t* tests (NS, $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$). Data were analyzed using Prism v.6 for Mac OS X (GraphPad Software, San Diego, USA).

References

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Supplementary Table 1. Clinical characteristics of AML samples at diagnosis.

					Cytogenetics		Blood Parameters		Mutational Profiling		
Patient ID	**Age**	**Sex**	**AML type**	**Sample type**	**Normal vs. Abnormal**	**MRC Classification**	**WBC count ($\times 10^9$/L)**	**BM blasts (%)**	***NPM1c***	***FLT3*-ITD**	**LSC17 score**
AML3	84	M	*de novo*	Diagnosis	Normal	Intermediate	227	95	ND	negative	0.11
AML4	75	F	*de novo*	Diagnosis	Normal	Intermediate	227	80	ND	ND	1.12
AML5	53	F	*de novo*	Diagnosis	Normal	Intermediate	218	57	positive	positive	0.72
AML6	75	F	t-AML	Diagnosis	ND	ND	29	ND	ND	ND	0.27
AML7	26	F	*de novo*	Diagnosis	Normal	Intermediate	114	40	negative	positive	0.78
AML8	58	M	*de novo*	Diagnosis	Normal	Intermediate	102	70	positive	positive	0.90
AML9	72	M	s-AML	Diagnosis	Normal	Intermediate	43	ND	ND	ND	0.59
AML10	86	M	*de novo*	Diagnosis	ND	ND	218	ND	ND	ND	0.12
AML11	71	F	*de novo*	Relapse/Refractory	Abnormal	Intermediate	7	52	negative	positive	0.97
AML12	52	M	*de novo*	Diagnosis	Normal	Intermediate	163	90	positive	positive	0.87
AML13	79	F	*de novo*	Diagnosis	Abnormal	Adverse	75	50	ND	ND	0.84
AML14	62	M	*de novo*	Diagnosis	Normal	Intermediate	155	63	negative	negative	0.71
AML15	50	F	*de novo*	Diagnosis	Abnormal	Adverse	132	80	negative	negative	1.27
AML16	56	F	*de novo*	Relapse/Refractory	Normal	Intermediate	16	40	positive	positive	0.95
AML17	69	F	s-AML	Relapse/Refractory	Abnormal	Adverse	24	30	ND	ND	0.78
AML18	67	F	*de novo*	Diagnosis	Normal	Intermediate	100	94	positive	positive	0.94
AML19	74	F	*de novo*	Diagnosis	Normal	Intermediate	16	30	positive	positive	1.17
AML20	55	M	s-AML	Diagnosis	Abnormal	Adverse	29	62	ND	ND	1.33
AML21	54	F	*de novo*	Diagnosis	Normal	Intermediate	74	80	negative	positive	0.78
AML22	54	M	*de novo*	Relapse/Refractory	Abnormal	Adverse	47	80	ND	ND	1.22
AML23	55	M	t-AML	Diagnosis	Abnormal	Adverse	99	60	ND	ND	0.68
AML24	75	M	s-AML	Relapse/Refractory	Normal	Intermediate	4	23	ND	ND	1.09
AML25	55	M	s-AML	Relapse/Refractory	Normal	Intermediate	114	ND	negative	positive	0.81
AML26	33	M	*de novo*	Diagnosis	Normal	Intermediate	151	90	positive	positive	0.87
AML27	55	F	t-AML	Diagnosis	Abnormal	Intermediate	235	70	ND	ND	0.80
AML28	67	F	*de novo*	Diagnosis	Normal	Intermediate	40	92	negative	negative	0.54
AML29	69	M	t-AML	Diagnosis	Abnormal	Adverse	24	80	ND	ND	0.53
AML30	37	M	s-AML	Relapse/Refractory	Abnormal	Adverse	5	51	ND	ND	1.18
AML31	59	F	*de novo*	Diagnosis	Normal	Intermediate	43	90	positive	negative	0.17
AML32	48	F	*de novo*	Relapse/Refractory	Normal	Intermediate	107	85	positive	positive	0.60

t-AML, therapy-related AML; s-AML, secondary AML (post MDS/MPN); ND, not done. Median cutoff for high vs low LSC17 score is 0.51

Supplementary Table 2. SIRPαFc treatment reduces LSC frequency determined by secondary limiting dilution assays

	Primary mice			Secondary mice						
	In vivo	Cell			*n* mice	*n* mice	LSC frequency (1/n)		Fold	
Sample	response	source	Condition	Cell dose	engrafted	injected	Estimate	95% CI	change	*P* value
AML28	PR	RF	Control	1,000,000	6	7	353,479	158,428 – 788,670	↓ 5.4	0.003
				100,000	4	10				
			SIRPαFc	1,000,000	3	10	1,893,540	761,240 – 4,710,240		
				100,000	2	12				
AML30	PR	BM	Control	50,000	2	2	710	276 – 1,824	↓ 3.9	0.024
				5,000	10	10				
				500	4	8				
			SIRPαFc	50,000	2	2	2,791	1,379 – 5,648		
				5,000	9	10				
				500	0	8				
AML31	PR	RF	Control	100,000	7	10	111,175	53,072 – 232,888	↓ 10.3	0.006
				20,000	0	9				
			SIRPαFc	100,000	1	10	1,149,275	162,728 – 8,166,808		
				20,000	0	10				
AML32	NR	BM	Control	250,000	2	2	12,540	6,321 – 24,877	↓ 5.3	0.002
				50,000	10	10				
				20,000	6	8				
			SIRPαFc	250,000	2	2	66,965	33,128 – 135,363		
				50,000	6	10				
				20,000	1	8				

PR, partial responder; NR, non-responder; RF, injected right femur; BM, non-injected bone marrow.