

Supplementary methods

NK cell enrichment, expansion, and cryopreservation

PBMCs were collected from healthy donors via leukapheresis. CD3⁺ T cells were removed by VarioMACS (Miltenyi Biotec). Ten million CD3-depleted cells were seeded in 30 ml of CellGro SCGM medium (CellGenix with 1% autologous plasma, irradiated (2,000 rad) autologous PBMCs (5×10^7 cells), 500 IU/ml IL-2 (Proleukin), and 10 ng/ml anti-CD3 monoclonal antibody OKT3 (eBioscience) in an A-350N culture bag (NIPRO, Osaka, Japan). Cultured NK cells were restimulated on day 7 of the culture by adding 5×10^7 feeder cells per 1×10^7 NK cells in 60 ml of CellGro SCGM containing 1% plasma, OKT (10 ng/ml), IL-2 (500 IU/ml) and then transferred into an A-1000NL culture bag (NIPRO). Fresh medium with IL-2 (500 IU/ml) was added to the NK cell culture every 2 days to maintain a concentration of $0.5\text{--}2 \times 10^6$ cells/ml for 21 days. Harvested cells were suspended in freezing media (50% RPMI1640 (Gibco®) containing 20% albumin (GC pharma), 25% dextran-40 (DAI HAN PHARM), and 5% DMSO (Mylan Teoranta Limited) to make 1×10^8 cells/mL for cell cryopreservation. Frozen NK cells were preserved in liquid nitrogen until use. For infusion to patients, cells were thawed in 37°C water bath and immediately administered the thawed cells in the freezing medium (Min et al., 2018).

Pharmacokinetics of MG4101

Genomic DNA was extracted from PBMCs of recipients. Nested PCR was performed to detect the presence of allo-HLA-DRB1 genes of donor NK-cell origin. HLA-DRB1 exon 2 or the DRw52-group-specific part of DRB1 exon2 was amplified in the first PCR, and either the HLA-DRB1 allele-specific or the group-specific amplification was performed in the second PCR. The sensitivity of nested PCR was analyzed by target gene amplification from samples containing serially decreased amounts of donor-derived DNA mixed with a fixed amount of recipient-derived DNA: 10%, 1%, 0.1%, 0.01%, and 0.001% (vol/vol ratio). In addition, as an internal positive amplification control, amplification of a fragment of the human growth hormone gene (hGH) was included.

Cytokine measurements

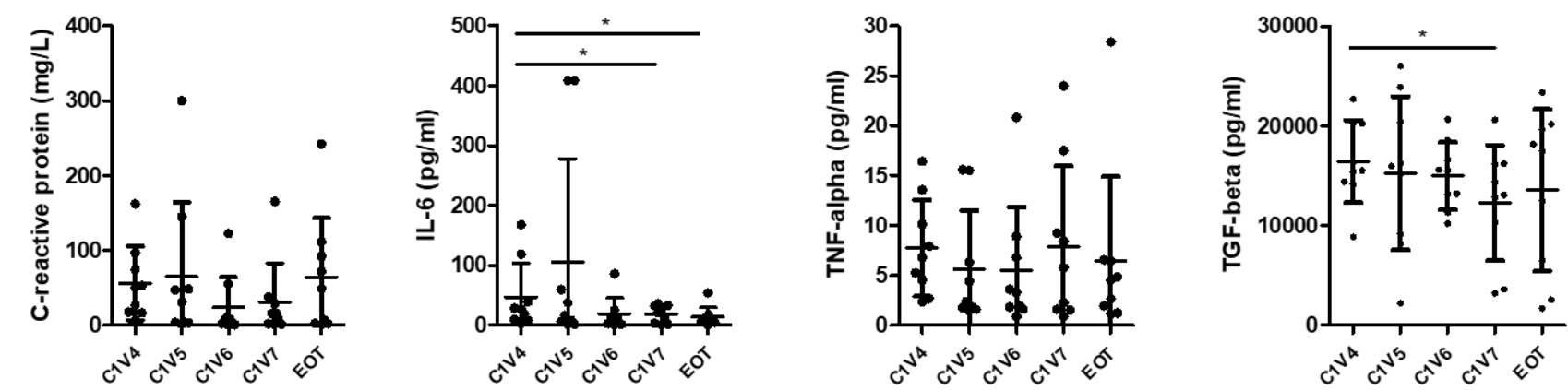
Blood samples for cytokine analysis were collected at specific time points. The serum samples of patients were immediately centrifuged at $800 \times g$ and 4°C for 10 min, divided into aliquots, and stored in a -80°C freezer for subsequent analysis. Serum IL-6, TNF- α , and TGF- β were measured with a sandwich enzyme-linked immunosorbent assay using Quantikine kits (R&D, Minneapolis, Minn). C-reactive protein (CRP) was measured with cardiac C-Reactive Protein (Latex) High Sensitive using Roche/Hitachi cobas c systems (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. The limit of detection for the cytokines was defined according to the manufacturer's instructions (IL-6, TNF- α : 0.156 pg/mL, TGF- β : 31.3 pg/mL and CRP: 0.15 mg/mL).

Killer cell immunoglobulin-like receptor typing

The KIR alleles were determined by polymerase chain reaction using sequence-specific primers (PCR-SSP) methodology (J Korean Surg Soc. 2010 Jun;78(6):357-368.). Inhibitory KIR was typed for KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, and KIR3DL3 alleles, activating KIRs were typed for KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, and KIR3DS1

alleles, and pseudo-KIRs were typed for 2DP1 and 3DP1. Specific amplification of the KIR genes was performed using 50 forward and reverse primers.

Supplementary Figure S1. Cytokine (C-reactive protein, IL-6, TNF-alpha and TGF-beta) profiles after administration of MG4101 plus rituximab. * $p<0.05$



Supplementary Table S1. Baseline characteristics (n = 9)

Level	Subject No.	Age (y) [gender]	Histology	Stage	Prior treatment	Best response	Duration of response
1	8203101	64 [M]	MCL	IV	R-CHOP, R-ICED, auto-HSCT radiation therapy	PR	≥ 806 days
	8202101	64 [F]	DLBCL	III	R-CHOP, CHOP, IMEP, ICED, radiation therapy	PR	45 days
	8203102	38 [M]	DLBCL	IV	R-CHOP, ESHAP, Moseunetuzumab	PD	-
2	8203103	56 [M]	DLBCL	III	R-CHOP, ICED, Moseunetuzumab	PD	-
	8203104	69 [M]	DLBCL	III	R-CHOP + IT-MTX, ICED, radiation therapy	PD	-
	8203105	61 [F]	DLBCL	III	R-CHOP, ICED, auto-HSCT, GDP-8, mosunetuzumab	PR	≈28 days
3	8201101	80 [M]	MCL	IV	R-CHOP, Ibrutinib, Velcade/Cytarabine/Dexamethasone, Rituximab/ bendamustine, lenalidomide, UTX-TGF-2015 trial	PD	-
	8203106	57 [M]	MZL	I	R-CVP, BR/ibrutinib, ESHAP, radiation therapy, auto-HSCT	CR	≥ 359 days
	8203107	66 [M]	DLBCL	IV	R-CHOP, ICED, DHAP, GDP, NK cell treatment, lenalidomide	PR	≈28 days

Abbreviations: MCL, Mantle cell lymphoma; DLBCL, Diffuse large B-cell lymphoma; MZL, Marginal zone lymphoma; PR, Partial response; PD, Progressive disease; CR, Complete response

Supplementary Table S2. List of antibodies used in flow cytometry

Antibodies	Fluorophore(s)	Vendor	Catalog number
CD3	V500 and APC-H7	BD	561416
	APC-H7	BD	557832
CD4	APC-H7	BD	557871
	APC	BD	555349
	BV650	BD	563876
	BV786	BD	563877
CD8	APC-H7	BD	560179
	PE-Cy7	eBioscience	25-0088-42
CD14	PerCP-Cy5.5	BD	550787
CD19	PerCP-Cy5.5	eBioscience	45-0199-42
	FITC	BD	555412
	PE-TxR	BD	562294
CD56	FTIC	BioLegend	318304
	PE	BD	555516
CD45	BV421/V450	BD	560367
Foxp3	FITC	eBioscience	53-4776-42
CD25	PE	BD	555432
CD127	PE-Cy7	eBioscience	25-1278-42
CD45RA	Alexa Fluor® 700	BD	560673
CCR7	PerCP-Cy5.5	BioLegend	353220
PD-1	BV421/V450	BioLegend	329920
LAG-3	BV650	BioLegend	369315
LAG-3	BV650	BioLegend	369316
TIM-3	BV711	BD	565566
NKG2D	BV786	BD	743560
HLA-DR	BV711	BD	563696
41BB	PE	BioLegend	309804
CTLA-4	PE-Cy7	BioLegend	349914
TIGIT	APC	BioLegend	372706

Supplementary Table S3. PK of MG4101

Group	Recipient	1 st injection		
		1 hour	7 days	14 days
Level 1 (1×10^7 cells/kg)	8202101	+	+	-
	8203101	+	+	-
	8203102	+	-	-
Level 2 (3×10^7 cells/kg)	8203103	+	+	+
	8203104	+	-	-
	8203105	+	+	-
Level 3 (9×10^7 cells/kg)	8201101	+	+	+
	8203106	+	+	-
	8203107	+	+	-

Supplementary Table S4. KIR-HLA relationship

Group	Patient	Recipients		Donor				Donor KIR-L mismatch
		HLA status		KIR (genotype)				
		Bw	C	KIR2DL3	KIR2DL2	KIR2DL1	KIR3DL1	
Level 1 (1 × 10 ⁷ cells/kg)	8202101	Bw6	C1/2	P	N	P	N	None
	8203101	Bw6/4	C1	P	N	P	N	2DL1
	8203102	Bw6	C1	P	N	P	P	2DL1, 3DL1
Level 2 (3 × 10 ⁷ cells/kg)	8203103	Bw4/4	C1	P	N	P	P	2DL1
	8203104	Bw4/4	C1	P	N	P	P	2DL1
	8203105	Bw6	C1	P	N	P	P	2DL1, 3DL1
Level 3 (9 × 10 ⁷ cells/kg)	8201101	Bw6	C1	P	N	P	P	2DL1, 3DL1
	8203106	Bw6	C1	N	P	P	P	2DL1, 3DL1
	8203107	Bw6/4	C1	P	N	P	P	2DL1