Supplemental information

Efficiently targeting neuroblastoma

with the combination of anti-ROR1 CAR NK cells

and N-803 *in vitro* and *in vivo* in NB xenografts

Yaya Chu, Gaurav Nayyar, Meijuan Tian, Dean A. Lee, Mehmet F. Ozkaynak, Jessica Ayala-Cuesta, Kayleigh Klose, Keira Foley, Alyssa S. Mendelowitz, Wen Luo, Yanling Liao, Janet Ayello, Gregory K. Behbehani, Stanley Riddell, Tim Cripe, and Mitchell S. Cairo

Table S1. Summary of current clinical trials utilizing anti-ROR1 targeted immunotherapies.

	Anti-ROR1 Agent	Patient Population	Phase	NCT Number	Status	Sponsor	Country
Anti-ROR1 Monoclonal Antibody	Cirmtuzumab	CLL	I/II	NCT02222688	Completed	Thomas Kipps	USA
	Cirmtuzumab	Metastatic Castration-resistant Prostate Cancer	I	NCT05156905	Recruiting	UCSD	USA
	Cirmtuzumab	CLL	I	NCT02860676	Completed	UCSD	USA
	Cirmtuzumab + Paclitaxel	Breast Neoplasms	I	NCT02776917	Active not_ recruiting	Barbara Parker, MD	USA
	Cirmtuzumab + Ibrutinib	CLL, SLL, MCL, MZL	I	NCT03088878	Active not_ recruiting	Oncternal Therapeutics, Inc	USA
	Cirmtuzumab + Venetoclax	CLL	I/II	NCT04501939	Active not_ recruiting	UCSD	USA
Anti-ROR1 Antibody Drug Conjugate	CS5001	Advanced Solid Tumor, Advanced Lymphoma	I	NCT05279300	Recruiting	CStone Pharmaceuticals	USA, Australia, China
	EMB07	Advanced Metastatic Solid Tumors, R/R Lymphoma	I	NCT05607498	Recruiting	EpimAb Biotherapeutics (Suzhou)Co., Ltd.	Australia,China
	NVG-111	CLL, SLL, MCL, FL, DLBCL, NSCLC, Malignant Melanoma	I	NCT04763083	Recruiting	NovalGen Ltd.	UK
	Zilovertamab vedotin	Triple-negative Breast Cancer, NS-NSCLC, NSCLC, Breast Cancer, Platinum-resistant Ovarian Cancer, Gastric Cancer, Pancreatic Cancer	II	NCT04504916	Completed	VelosBio Inc., a subsidiary of Merck & Co., Inc. (Rahway, New Jersey USA)	USA, Canada
	Zilovertamab vedotin	Urothelial Carcinoma	I/II	NCT05562830	Active not_ recruiting	Merck Sharp & Dohme LLC	USA, Australia, Chile, et al
	Zilovertamab vedotin	CLL, MCL, FL, MZL, DLBCL, RTL, BL, Lymphoplasmacytoid Lymphoma, T-cell NHL, ALL, AML, WM	I	NCT03833180	Active not_ recruiting	VelosBio Inc., a subsidiary of Merck & Co., Inc. (Rahway, New Jersey USA)	USA
	Zilovertamab vedotin	R/R DLBCL	II	NCT05144841	Recruiting	Merck Sharp & Dohme LLC	USA, Canada, China, et al
	Zilovertamab Vedotin+ R-CHOP	DLBCL	II	NCT05406401	Recruiting	Merck Sharp & Dohme LLC	Canada
	Zilovertamab vedotin +/- Nemtabrutinib	CLL, MCL, FL, RTL	II	NCT05458297	Recruiting	Merck Sharp & Dohme LLC	USA
	Zilovertamab vedotin + R-GemOx	DLBCL	II/III	NCT05139017	Recruiting	Merck Sharp & Dohme LLC	USA
Anti-ROR1 CAR T	LYL797	TNBC, NSCLC, Advanced Breast Cancer, Advanced Lung Carcinoma, Relapsed Cancer, Recurrent Breast Cancer, Recurrent NSCLC	I	NCT05274451	Recruiting	Lyell Immunopharma, Inc.	USA
	PRGN-3007	CLL, MCL, ALL, DLBCL, TNBC	I	NCT05694364	Recruiting	H. Lee Moffitt Cancer Center and Research Institute	USA
	RD14-01	Solid Tumor	I/II	NCT05748938	Recruiting	920th Hospital of Joint Logistics Support Force of People's Liberation Army of China	China
	RD14-01	Solid Tumor	I	NCT05638828	Not yet recruiting _	Shen Lin	China
	ONCT-808	R/R Aggressive B-Cell Malignancies	I/II	NCT05588440	Recruiting	Oncternal Therapeutics, Inc	USA
	Anti-ROR1CAR-TILs	Liver Cancer, Lung Cancer, Breast Cancer, Colo-rectal Cancer, Brain Tumor, Solid Tumor,	I	NCT04842812	Recruiting	Second Affiliated Hospital of Guangzhou Medical University	China

ALL: Acute Lymphoid Leukemia; AML: Acute Myeloid Leukemia; BL: Burkitt Lymphoma; CLL: Chronic Lymphocytic Leukemia; DLBCL: Diffuse Large B-Cell Lymphoma; FL: Mantle Cell Lymphoma; MCL: Mantle Cell Lymphoma; MZL: Marginal Zone Lymphoma; NHL: Non-Hodgkin Lymphoma; NSCLC: Non-small Cell Lung Cancer; NS-NSCLC: Non-squamous Non-small-cell Lung Cancer; R-CHOP: Cyclophosphamide, Doxorubicin, Rituximab/Rituximab Biosimilar, Prednisone, Prednisolone; R-GemOx: Rituximab, Gemcitabine, Oxaliplatin; RTL: Richter Transformation Lymphoma; R/R: Relapsed/Refractory; SLL: Small Lymphocytic Lymphoma; TNBC: Triple-Negative Breast Cancer; UCSD: University of California, San Diego; WM: Waldenstrom Macroglobulinemia
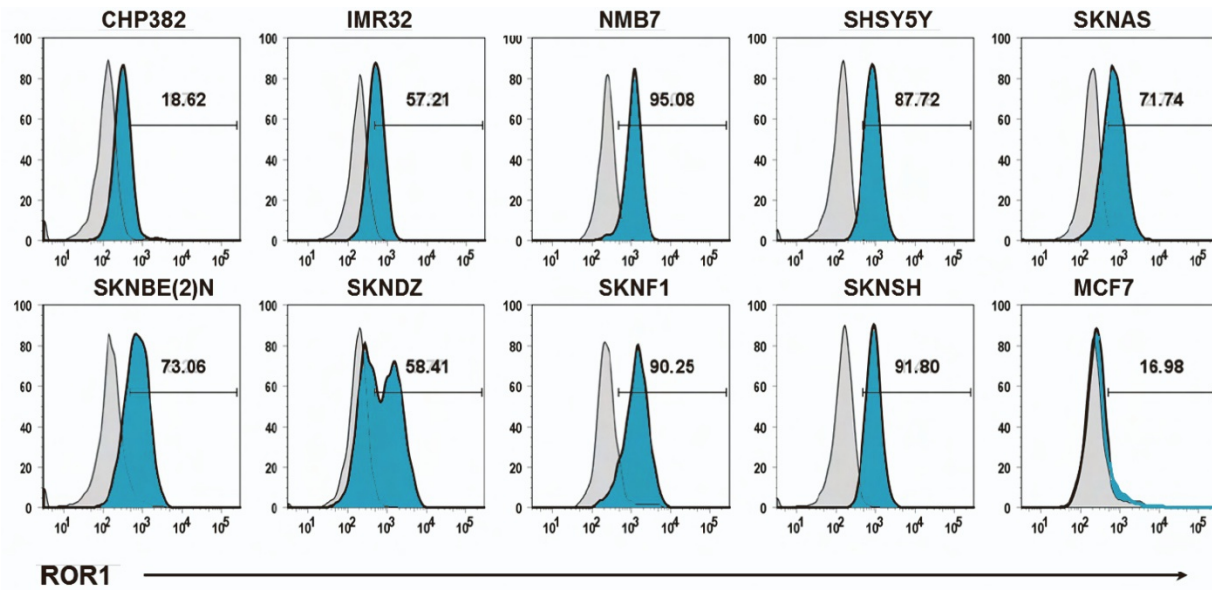
Fig. S1. Flow cytometry analysis of ROR1 expression on NB cell lines. ROR1 expression on NB cell lines was examined by flow cytometry analysis using anti-ROR1-APC antibody. ROR1 negative MCF7 cells were used as negative controls. Representative flow cytometric histogram data are shown.
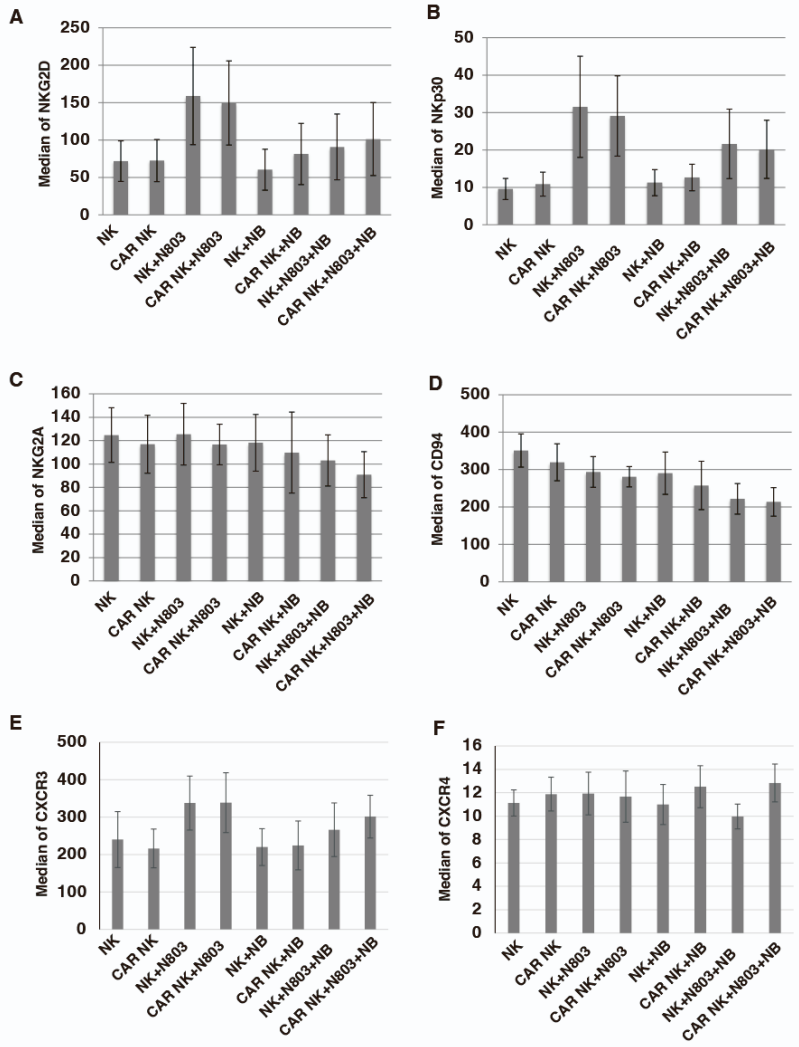
Fig. S2. High dimensional analysis of receptors on N-803 activated anti-ROR1 CAR NK cells with/without NB by mass cytometry. Anti-ROR1 CAR NK cells or NK cells were co-cultured with or without SKNFI and N-803 for two days. After the cells were stained and fixed, the samples were run on a CyTOF2. (A) The medians of NKG2D levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean$\pm$SEM. N=3. N-803 enhanced the expression of NKG2D on both NK and anti-ROR1 CAR NK cells. (B) The medians of NKp30 levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean$\pm$SEM. N=3. N-803 enhanced the expression of NKp30 on both NK and anti-ROR1

CAR NK cells. (C) The medians of NKG2A levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean±SEM. N=3. (D) The medians of CD94 levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean±SEM. N=3. (E) The medians of CXCR3 levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean±SEM. N=3. (F) The medians of CXCR4 levels by mass cytometry on anti-ROR1 CAR NK cells or NK cells under the indicated conditions were summarized as mean±SEM. N=3.