Characterization of JNJ-80948543 a novel CD79bxCD20xCD3 trispecific antibody for B-cell non-Hodgkin lymphoma

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[Supplemental Data]

Supplemental Table 1. CD79b and CD20 antigen density in hematologic tumor cell lines

Cell line	Cell line origin	CD79b ABC*	CD20 ABC*
CARNAVAL	DLBCL	220,000	250,000
OCI-Ly10	DLBCL	115,000	230,000
JeKo1	MCL	760,000	225,000
WILL2	DLBCL	7,200	1,200
HT	DLBCL	0	21,000
SUDHL1	ALCL	0	0
K562	CML	0	0

*ABC values are rounded and represent the results of 3 independent experiments.

ABC, antibody binding capacity; ALCL, anaplastic large cell lymphoma; CD, cluster of differentiation; CML, chronic myeloid leukemia; DLBCL, diffuse large B cell lymphoma; MCL, mantle cell lymphoma.

Supplemental Table 2. Cytotoxicity EC_{50} and maximum values observed for JNJ-80948543, CD79bxNullxCD3, NullxCD20xCD3 in T cell cytotoxicity assays conducted with tumor targets expressing CD79b and/or CD20 at 5:1 E:T ratios

OCI-Ly10 (72 hours)			
	JNJ-80948543	**CD79bxNullxCD3**	**NullxCD20xCD3**
EC_{50} (nM)	2.615	8.435	11.28
Max kill (%)	59.7%	37.0%	43.3%
CARNAVAL (72hours)			
EC_{50} (nM)	0.1973	1.972	0.9805
Max kill (%)	75.4%	74.0%	73.8%
WSU-DLCL2 (72hours)			
EC_{50} (nM)	0.0132	0.1285	0.7679
Max kill (%)	65.7%	71.2%	67.0%

EC_{50}, 50% effective concentration; E:T, effector to target; Max kill, maximum cytotoxicity;

T cell cytotoxicity assays were conducted using JNJ-80948543, CD79bxNullxCD3, NullxCD20xCD3, T cells derived from 3 healthy donors, and target cell lines expressing CD79b and/or CD20, for 72 hours. Data from 3 independent experiments were pooled and represented as means. EC_{50} was calculated using [inhibitor] vs. response – variable slope (four parameters) with GraphPad.

Supplemental Table 3. Cytotoxicity EC_{50} and maximum values observed for JNJ-80948543 in T cell cytotoxicity assays conducted with tumor targets expressing CD79b and/or CD20 at 5:1 and 1:1 E:T ratios

48 hours (means)					
	JeKo1	**CARNAVAL**	**OCILy10**	**HT**	**WILL2**
5:1					
EC_{50} (nM)	0.149	0.237	2.221	4.186	NE
Max kill (%)	90.8	78.4	31.0	59.1	NE
1:1					
EC_{50} (nM)	0.588	0.658	94.462[a]	NA	NA
Max kill (%)	65.8	35.0	62.8[a]	NA	NA
72 hours (means)					
	JeKo1	**CARNAVAL**	**OCILy10**	**HT**	**WILL2**
5:1					
EC_{50} (nM)	0.0092	0.092	27.042	2.016	31.971
Max kill (%)	99.0	98.5	81.8	86.8	95.5
1:1					
EC_{50} (nM)	0.155	0.536	16.543	NA	NA
Max kill (%)	85.9	77.8	49.3	NA	NA

EC_{50}, 50% effective concentration; E:T, effector to target; Max kill, maximum cytotoxicity; NA, not assessed; NE, not estimable.

T cell cytotoxicity assays were conducted using JNJ-80948543, T cells derived from 5 to 7 healthy donors, and target cell lines expressing CD79b and/or CD20, for 48 or 72 hours. Data from 6 independent experiments were pooled and represented as means.

[a]High variability across tested donors.

Supplemental Table 4: Cytokine Secretion EC_{50} (nM) and Maximum Values (pg/mL) Observed for JNJ-80948543 in T-cell Redirection Assays Conducted with CARNAVAL and OCI-Ly10 Cells at 5:1 and 1:1 E:T Ratios.

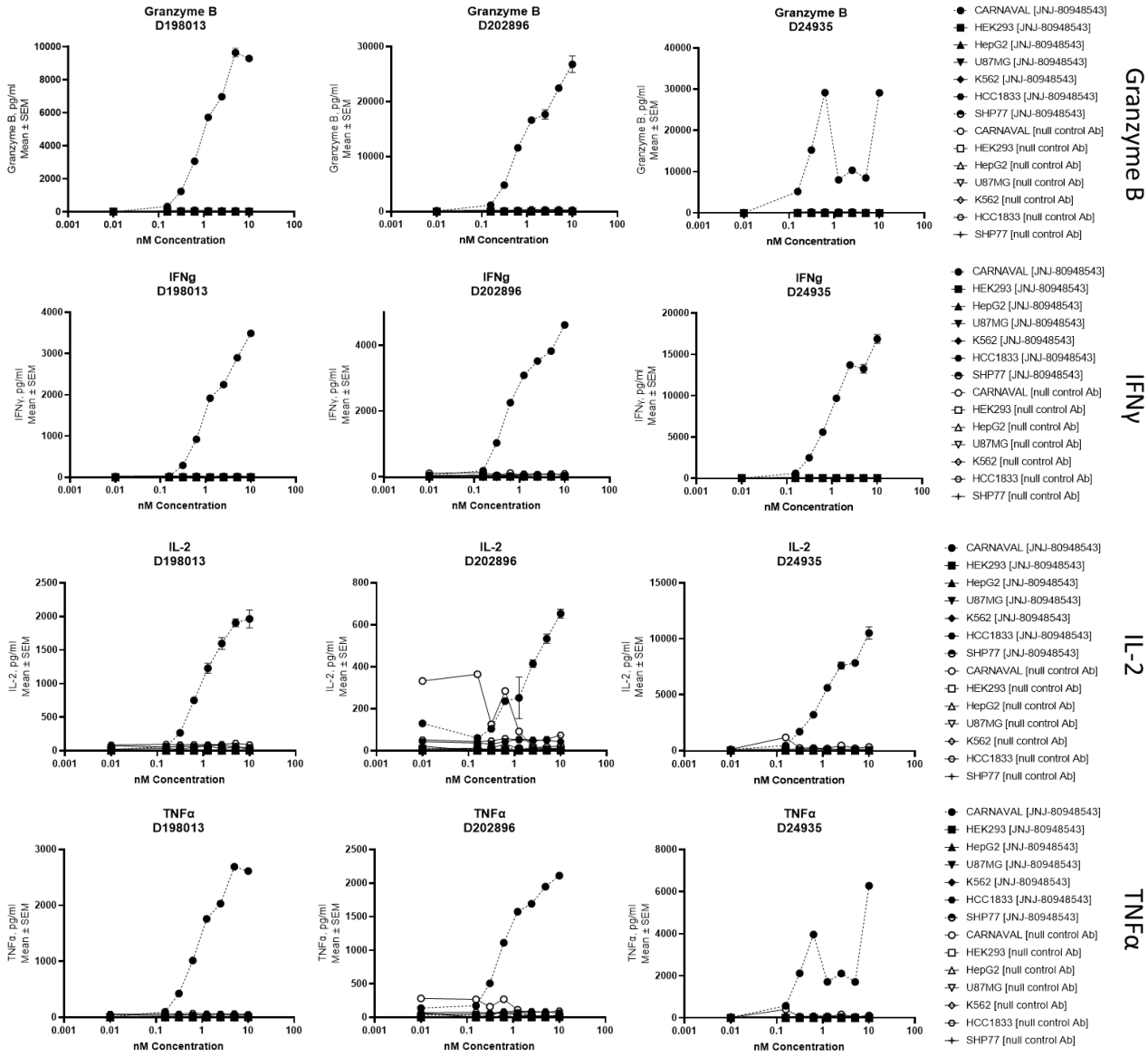
	48 hours				72 hours			
	CARNAVAL		OCI-Ly10		CARNAVAL		OCI-Ly10	
	E:T ratio							
	1:1	5:1	1:1	5:1	1:1	5:1	1:1	5:1
IFN-γ								
EC_{50}	2.097	NE	36.339	55.646	1.571	48.338	13.744	85.887
Max value	4,213.3	NE	5,630.8	16,564.2	12,185.5	30,242.4	16,383.0	59,934.1
IL-1β								
EC_{50}	NE	NE	ND	ND	NE	NE	ND	ND
Max value	NE	NE	ND	ND	NE	NE	ND	ND
IL-2								
EC_{50}	2.871	15.516	62.195	>100[a]	>100[a]	>100[a]	>100[a]	>100[a]
Max value	386.8	747.7	105.0	461.7	1,005.3	1,468.5	0.1	1,253.9
IL-4								
EC_{50}	1.894	7.946	NE	NE	14.044	37.367	NE	NE
Max value	2.1	11.1	NE	NE	3.0	11.1	NE	NE
IL-8								
EC_{50}	NE	NE	15.050	1.944	NE	NE	>100[a]	1.878
Max value	NE	NE	5.3	19.1	NE	NE	23.2	32.8
IL-6								
EC_{50}	NE	NE	1.251	NE	NE	NE	8.545	NE
Max value	NE	NE	5.5	NE	NE	NE	17.1	NE
IL-10								
EC_{50}	1.047	>100[a]	NE	NE	0.939	>100[a]	NE	NE
Max value	81.0	1,173.8	NE	NE	67.2	223,016.3	NE	NE
IL-13								
EC_{50}	0.429	3.392	NE	NE	0.202	17.497	NE	NE
Max value	0.2	42.5	NE	NE	42.9	77.3	NE	NE
TNF-α								
EC_{50}	2.158	60.226	>100[a]	>100[a]	10.573	>100[a]	33.971	>100[a]
Max value	282.0	980.4	234.6	1,310.3	397.0	2,382.7	344.8	3,143.2

CD, cluster of differentiation; EC_{50}, 50% effective concentration; E:T, effector to target; IFN, interferon; IL, interleukin; Max value, maximal cytokine release value; ND; not detectable; NE, not estimable; TNF, tumor necrosis factor.

T cells from 5 or 6 healthy donors were tested in T-cell redirection assays, incubated with JNJ-80948543 and $CD79b^{+}CD20^{+}$ CARNAVAL or OCI-Ly10 cells. The assay was conducted for 48 or 72 hours, with a single point per condition and donor. Supernatants were tested for the presence of cytokines. EC_{50} values could not be estimated for cytokines for which non-sigmoidal or decreasing response was observed and were listed in the table as NE. Data from 6 independent experiments are averaged and mean value reported.

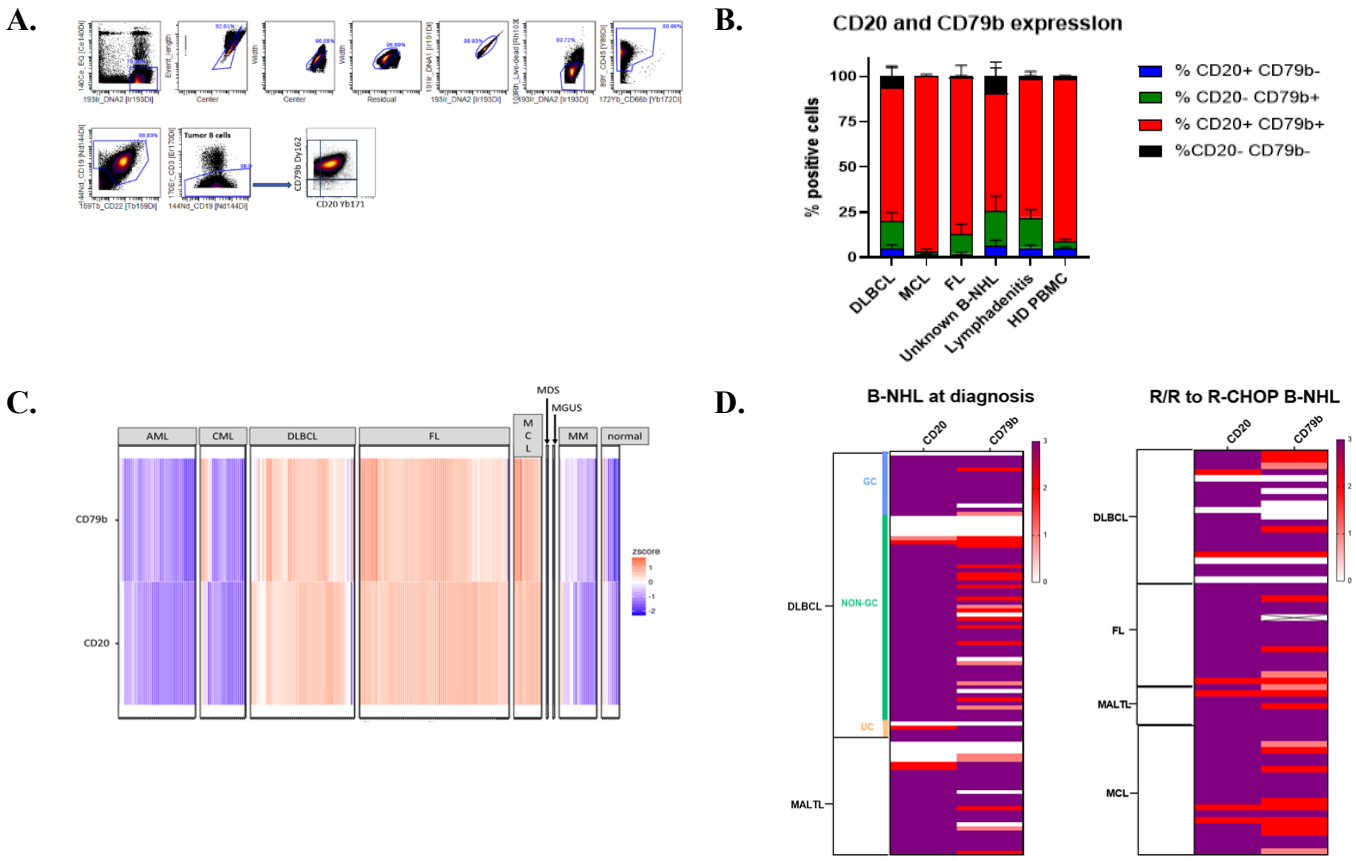
[a] EC_{50} value estimated to be higher than highest tested concentration.

Supplemental Figure 1. JNJ-80948543 Antigen Specific T-Cell Activation Dependent Cytokine Release



Primary T cells from 3 healthy donors (D198013, D202896, and D24935) were co-cultured with individual CD79b and CD20 antigen negative cell lines (HEK-293T, K562, HCC-1833, HepG2, U87MG and SHP-77) and an antigen positive control cell line (CARNAVAL, circle). Cells were treated with a titration of JNJ-80948543 (filled shapes) and null control Ab (open shapes). Supernatants were harvested at t=72hrs post-treatment and assessed for cytokines (Granzyme B, IFNγ, TNFα and IL-2) as a measure of antibody-induced T-cell activation. Technical replicates (n=3) were averaged and the mean ± SEM were plotted.

Supplemental Figure 2. CD79b and CD20 expression in B-NHL patient samples compared with patients with lymphadenitis or healthy donor B cells



(A) Exemplary hierarchical gating strategy for identification of tumor B cells in lymph node biopsies from patients with newly diagnosed B-NHL. Tumor B cells were defined as $CD45^{+}CD66b^{-}CD19^{+}CD22^{all}CD3^{-}$ (following the exclusion of debris, dead cells and doublets). $CD79b^{+}CD20^{+}$, $CD79b^{-}CD20^{+}$, $CD79b^{-}CD20^{-}$ and $CD79b^{+}CD20^{-}$ were identified within the tumor B cells.

(B) Dissociated lymph node tissues from treatment-naïve B-NHL patients (DLBC: n=6; FL: n=3; MCL: n=7; unknown B-NHL: n=5) and patients with lymphadenitis (n=7) or healthy donor peripheral blood mononuclear cells (n=9) were stained with Maxpar Direct Immune Profiling Assay antibody cocktail supplemented with anti-CD79b antibody to determine CD79b and CD20 expression in the B-cell lineage cell by CyTOF.

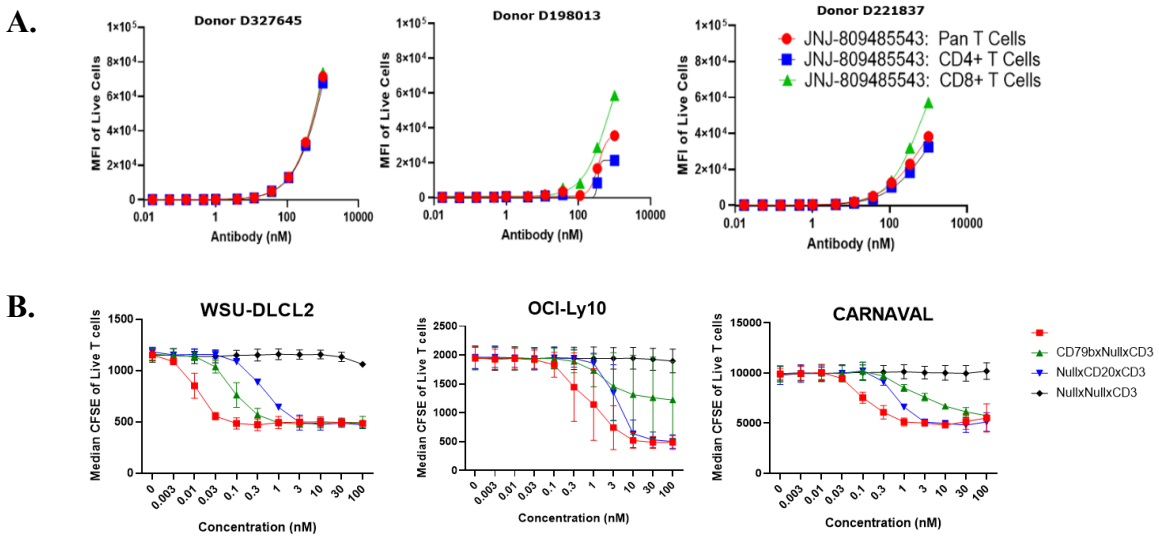
C) InforMe database was used to quantitate the relative mRNA levels of CD79b and CD20 across hematological malignancies using the GeneLogic Heme Plus 2.0 data set. mRNA levels are expressed as z scores, which describes the expression of a gene relative to the average across all of the samples. This analysis included n=226 samples obtained from tumor tissues: acute myeloid leukemia (AML; n=39), CML (n=23), DLBCL (n=53), FL (n=76), MCL (n=14), multiple myeloma (MM) (n=19), myelodysplastic syndrome (MDS; n=1), monoclonal gammopathy of undetermined significance (MGUS; n=1), and non-malignant non-lymphoid tissue control samples collected from patients (n=9; DLBCL [n=4], FL [n=3], MCL [n=2]).

(D) CD79b and CD20 IHC staining were performed on TMAs from B NHL patients at diagnosis or on FFPE samples from R/R to R-CHOP-treated B NHL patients. Staining intensity and utilizing scores (0, 1+, 2+, or 3+) are represented as a heat map and used for data visualization. In cases where range of intensity was provided, the more conservative (lower)

value was used for representation. Each row represents a single sample. A sample from the FL group could not be scored for CD79b due to tissue loss during IHC (crossed out). Samples at diagnosis consisted of n=28 MALTL samples, n=72 DLBCL samples. R/R lymphoma patient samples consisted of n=7 MALTL samples, n=21 DLBCL samples, n=16 FL samples, and n=20 MCL samples.

AML, acute myeloid leukemia; B-NHL, B cell non-Hodgkin lymphoma; CD, cluster of differentiation; CML, chronic myeloid leukemia; DLBCL, diffuse large B cell lymphoma; FFPE, formalin-fixed, paraffin-embedded; FL, follicular lymphoma; GC, germinal center; IHC, immunohistochemistry; MALTL, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; RCHOP, rituximab – cyclophosphamide – hydroxydaunorubicin – oncovin – prednisone/prednisolone; R/R, relapsed/refractory; TMA, tissue microarray; UC, uncharacterized for GC or NON-GC.

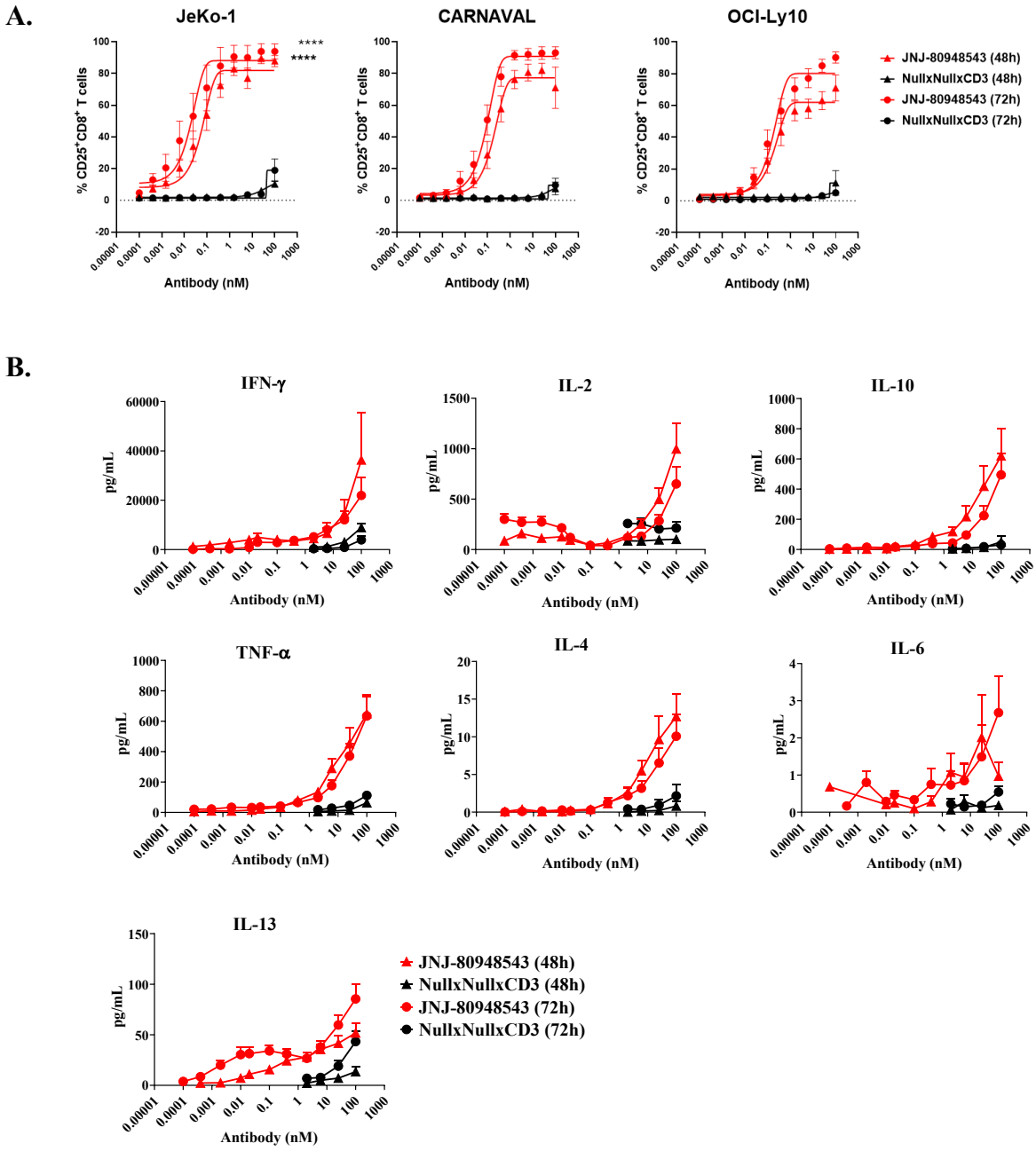
Supplemental Figure 3. Binding and in vitro functional characterization of JNJ-80948543



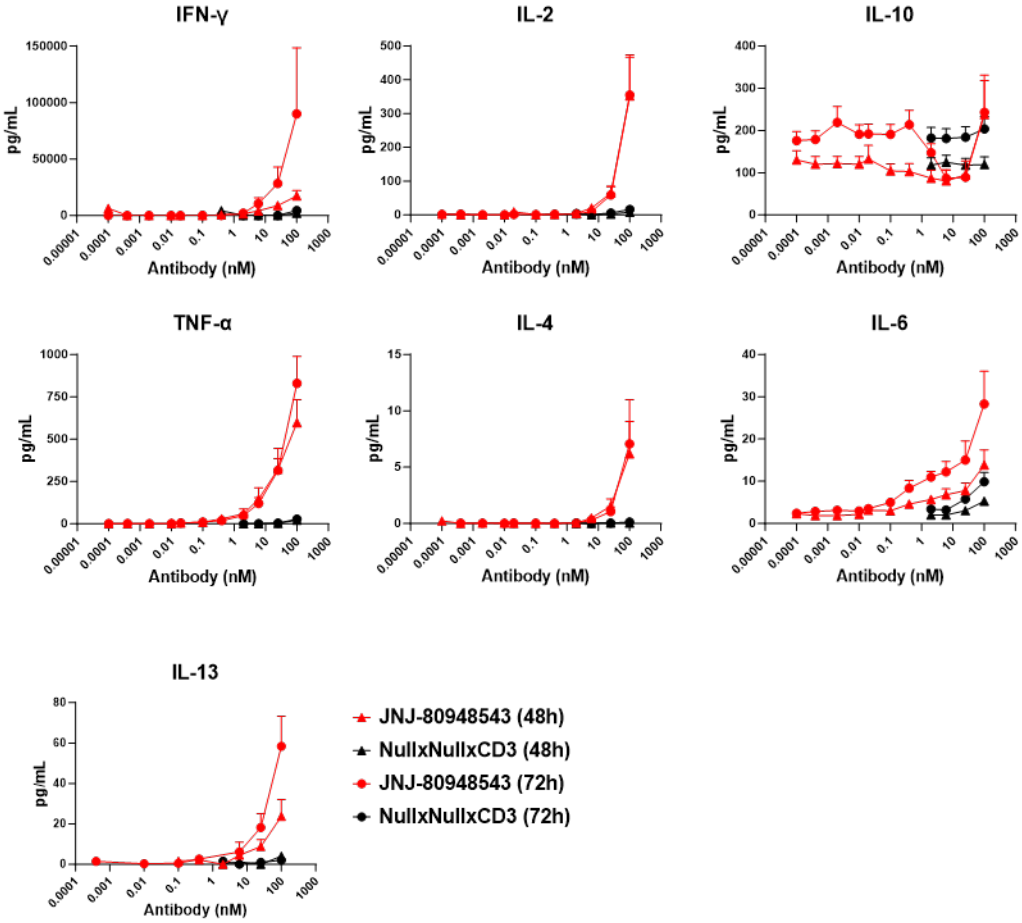
(A) Binding of JNJ-80948543 to primary T cell from 3 donors after 1 hour 37°C incubation. CD3, CD4 and CD8 T cell populations were assessed. Single data per point. Experiment was performed once with 3 T cell donors.

(D) T-cell proliferation was assessed in the presence of JNJ-80948543, CD79bxNullxCD3, NullxCD20xCD3, and NullxNullxCD3 antibodies and WSU-DLCL2, OCI-Ly10, CARNAVAL tumor cells and T cells from healthy donors at 5:1 E:T ratio. T-cell proliferation was assessed by flow cytometry using median CFSE decay as readout. Data from 3 independent experiments, 1 T cell donor per experiment are graphed as mean± SEM (n=3).

Supplemental Figure 4. JNJ-80948543 effect on T-cell activation in the presence of $CD79b^+$ $CD20^+$ B-NHL tumor cells in vitro



C.



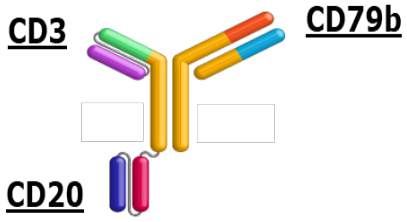
(A) The percentage of CD8 T-cell activation was determined by flow cytometry (Y axis) as percent of $CD25^+$ cells. Cancer cell lines were combined with $CD3^+$ pan T cells at a 1:1 effector-to-target ratio for either 48 or 72 hours with increasing concentrations (X axis) of JNJ-80948543 or NullxNullxCD3. Values are averages of 5 to 6 individual T cell donors. All cell lines are $CD79b^+/CD20^+$. Data from 6 independent experiments were pooled and represented as mean ± SEM (n=5-6).

(B) T cells from 5 or 6 healthy donors were tested in T cell redirection assays incubated with the indicated antibodies and $CD79b^+CD20^+$ CARNAVAL cells. The assay was conducted for 48 or 72 hours, at 5:1 E:T ratio. Supernatant was analyzed for inflammatory cytokines using MSD Proinflammatory kit (MSD K15049D). Data from 6 independent experiments are graphed as mean± SEM (n=5-6).

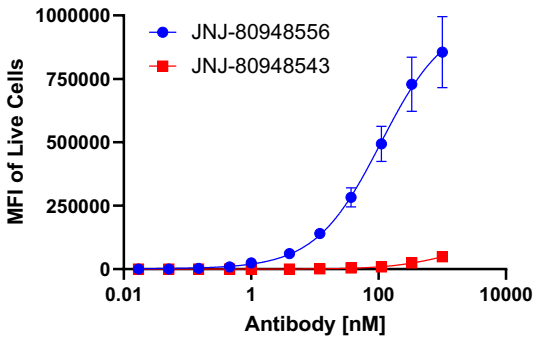
(C) T cells from 5 or 6 healthy donors were tested in T cell redirection assays incubated with the indicated antibodies and $CD79b^+CD20^+$ OCI-Ly10 cells. The assay was conducted for 48 or 72 hours, at 5:1 E:T ratio. Supernatant was analyzed for inflammatory cytokines using MSD Proinflammatory kit (MSD K15049D). Data are graphed as from 6 independent experiments mean± SEM (n=5-6).

Supplemental Figure 5. JNJ-80948556 profiling for binding to cancer cells and T cells and profiling for cytokine secretion

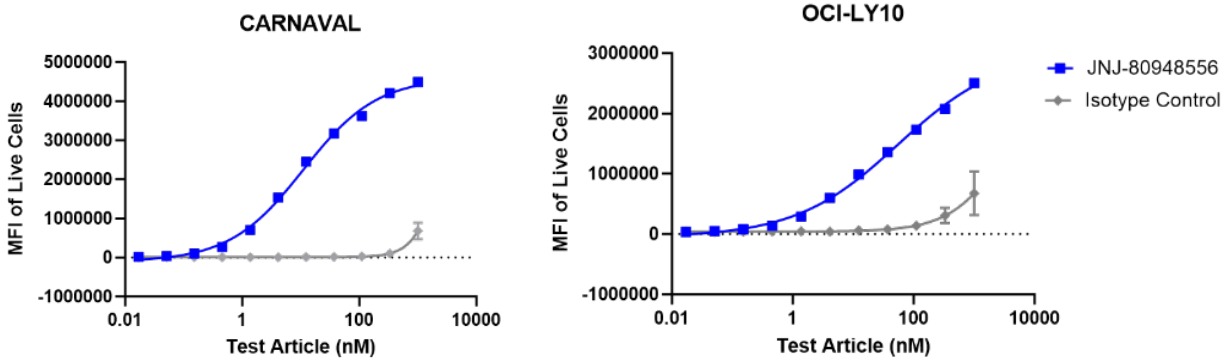
A. **JNJ-80948556** (higher affinity CD3)



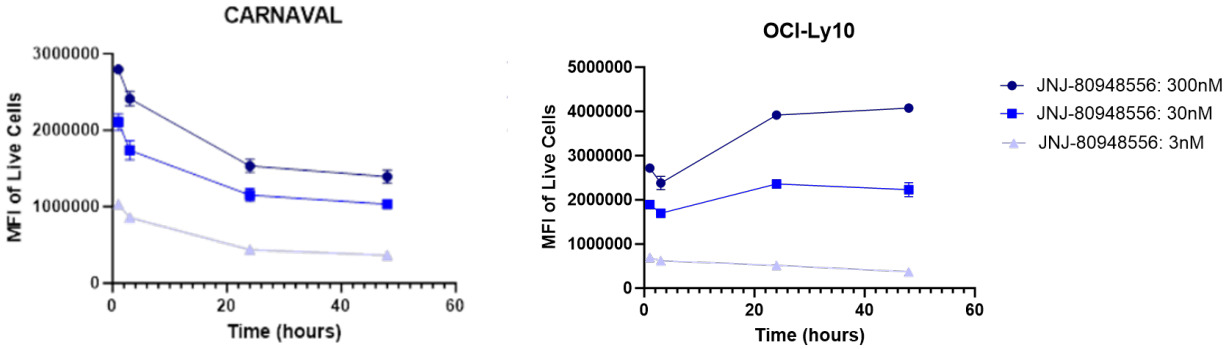
B.



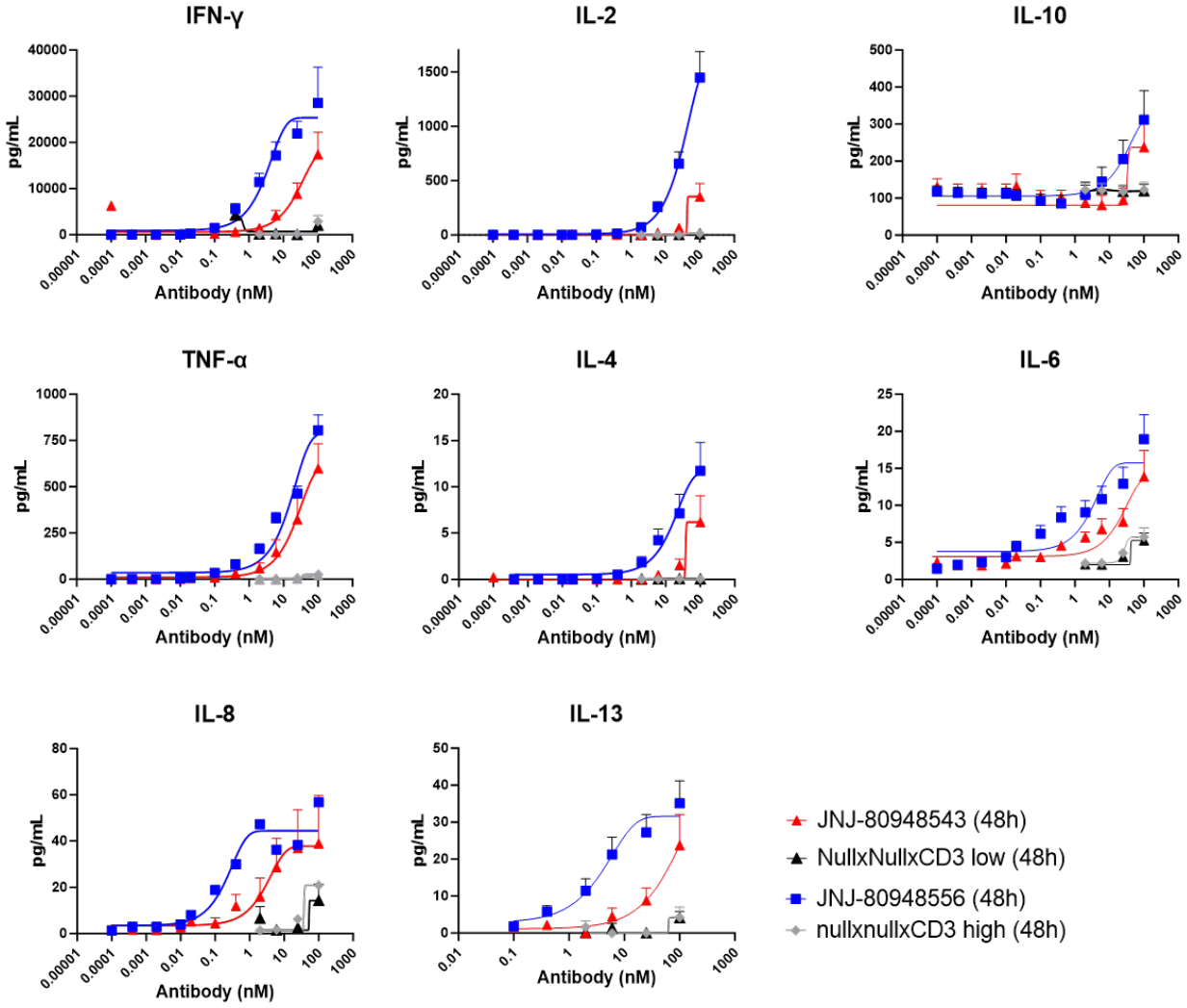
C.



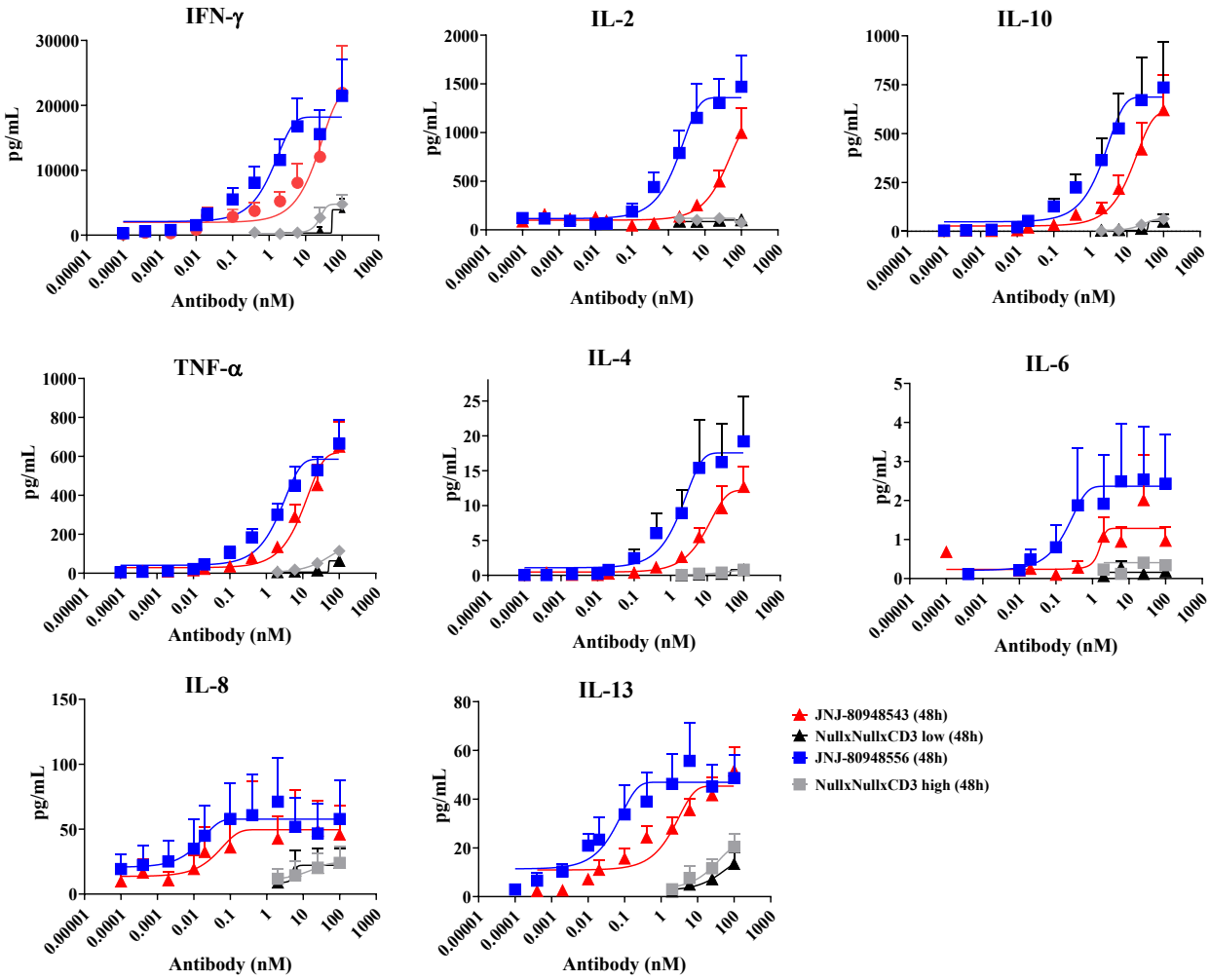
D.



E.


IFN-γ
IL-2
IL-10
TNF-α
IL-4
IL-6
IL-8
IL-13
pg/mL
Antibody (nM)
JNJ-80948543 (48h)
NullxNullxCD3 low (48h)
JNJ-80948556 (48h)
nullxnullxCD3 high (48h)

F.



(A) JNJ-80948556 trispecific antibody schematic.

(B) Binding of JNJ-80948543 and JNJ-80948556 to primary T cell from 3 donors after 1 hour 37°C incubation. Data from single experiment are graphed as mean± SEM (n= 3).

(C) Dose-dependent binding of JNJ-80948556 to CARNAVAL and OCI-Ly10 B-NHL cells at 1 hour. Data from two independent experiments are graphed as mean± SEM (n= 2 replicates).

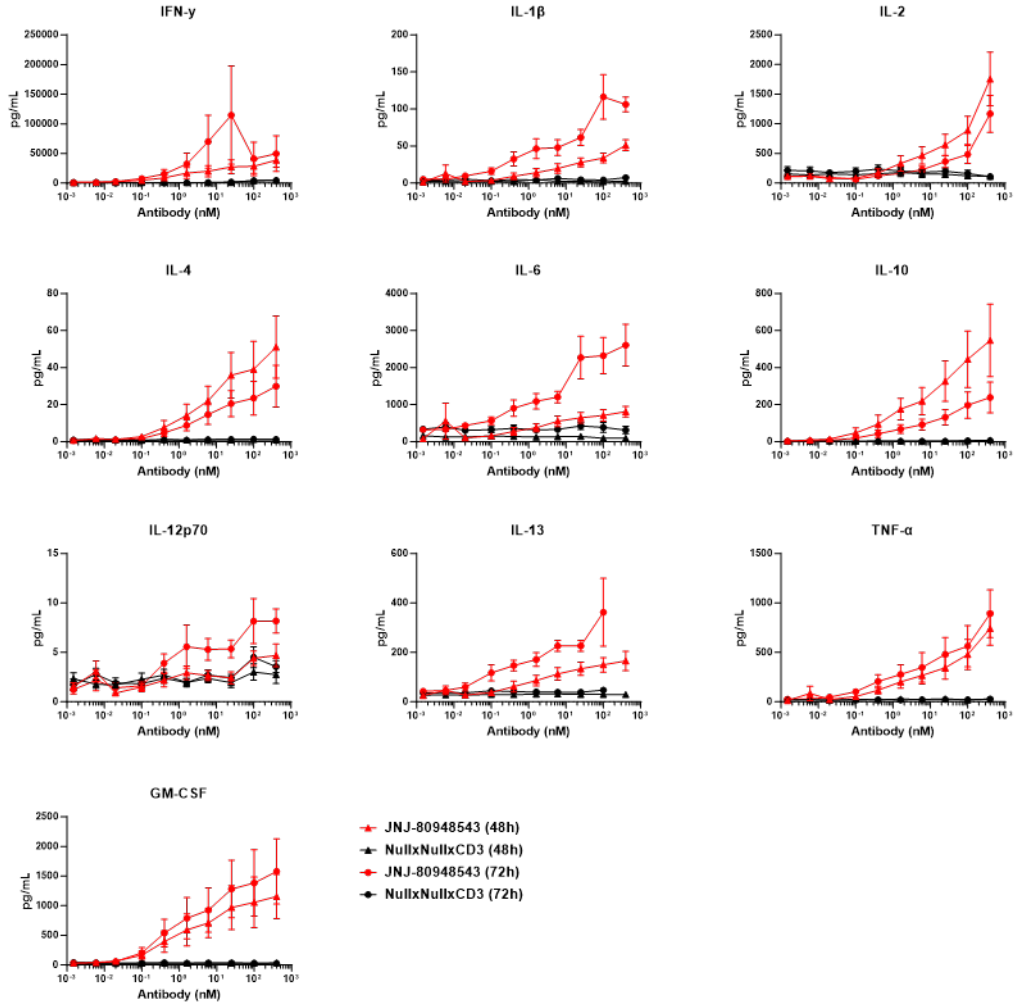
(D) Binding of JNJ-80948556 over time (up to 48h) to CARNAVAL and OCI-Ly10 B-NHL cells. Data from two independent experiments are graphed as mean± SEM (n= 2 replicates).

(E) T cells from 5 or 6 healthy donors were tested in T cell redirection assays incubated with the indicated antibodies and $CD79b^{+}CD20^{+}$ OCI-Ly10 cells. The assay was conducted for 48 hours, at 5:1 E:T ratio. Supernatant was analyzed for inflammatory cytokines using MSD Proinflammatory kit (MSD K15049D). Data from 6 independent experiments are graphed as mean± SEM (n=5-6).

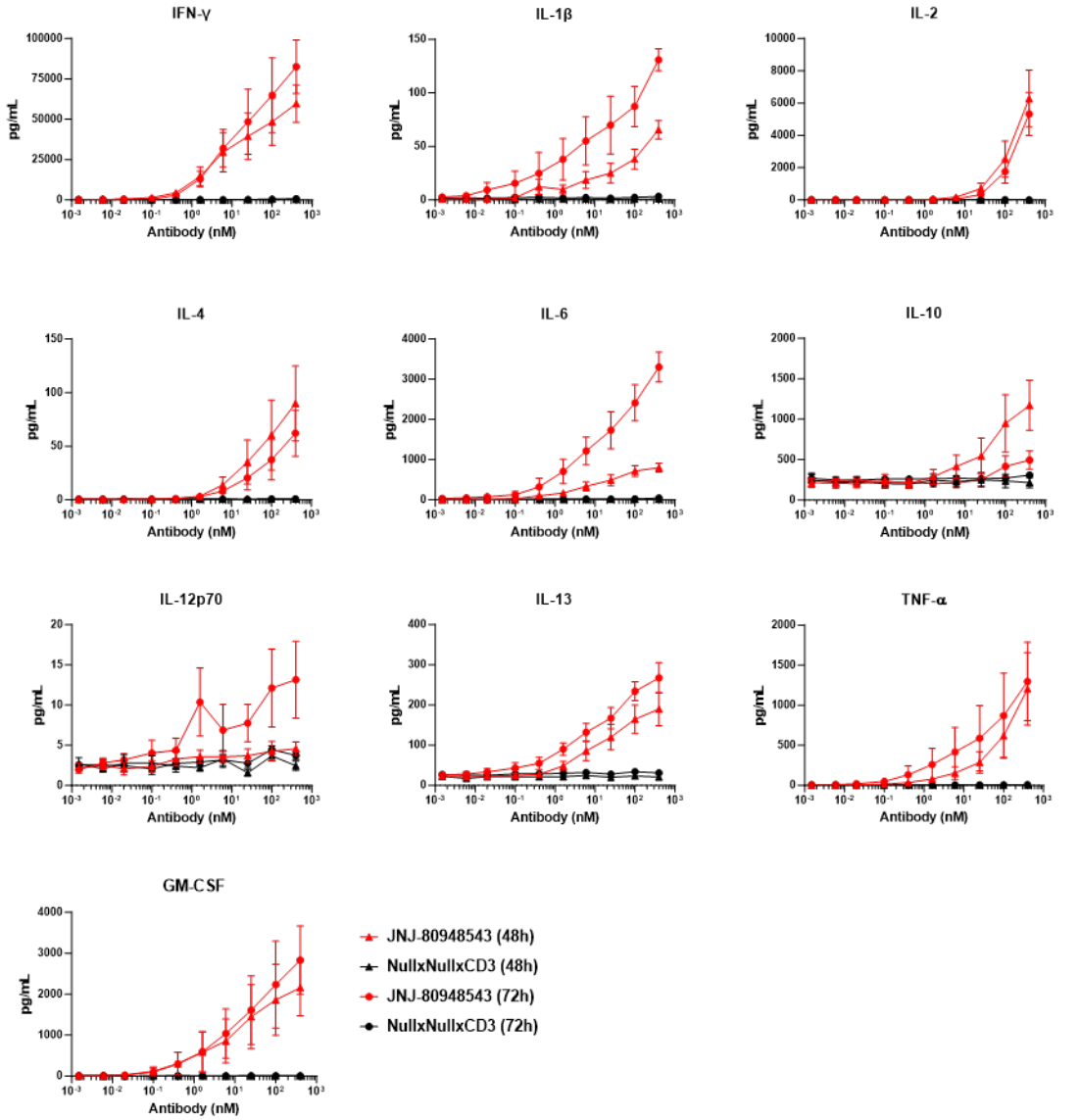
(F) T cells from 5 healthy donors were tested in T cell redirection assays incubated with the indicated antibodies and $CD79b^{+}CD20^{+}$ CARNAVAL cells. The assay was conducted for 48 or 72 hours, at 5:1 E:T ratio. Supernatant was analyzed for inflammatory cytokines using MSD Proinflammatory kit (MSD K15049D). Data from 5 independent experiments are graphed as mean± SEM (n=5).

Supplemental Figure 6. Effect of JNJ-80948543 on primary B-Cell cytotoxicity or Cytokine Induction in Whole-blood T-cell Redirection Assays, Conducted at 1:1 E:T Ratio in the Presence or absence of CARNAVAL or OCI-Ly10 Cells

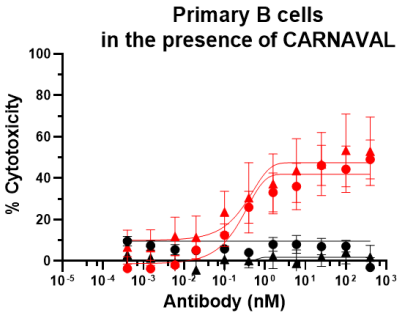
A.



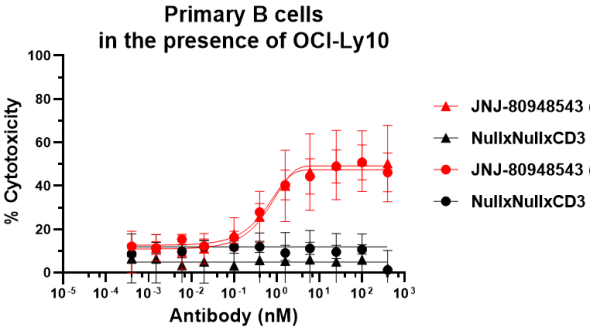
B.


IFN-γ
IL-1β
IL-2
IL-4
IL-6
IL-10
IL-12p70
IL-13
TNF-α
GM-CSF
pg/mL
Antibody (nM)
JNJ-80948543 (48h)
NullxNullxCD3 (48h)
JNJ-80948543 (72h)
NullxNullxCD3 (72h)

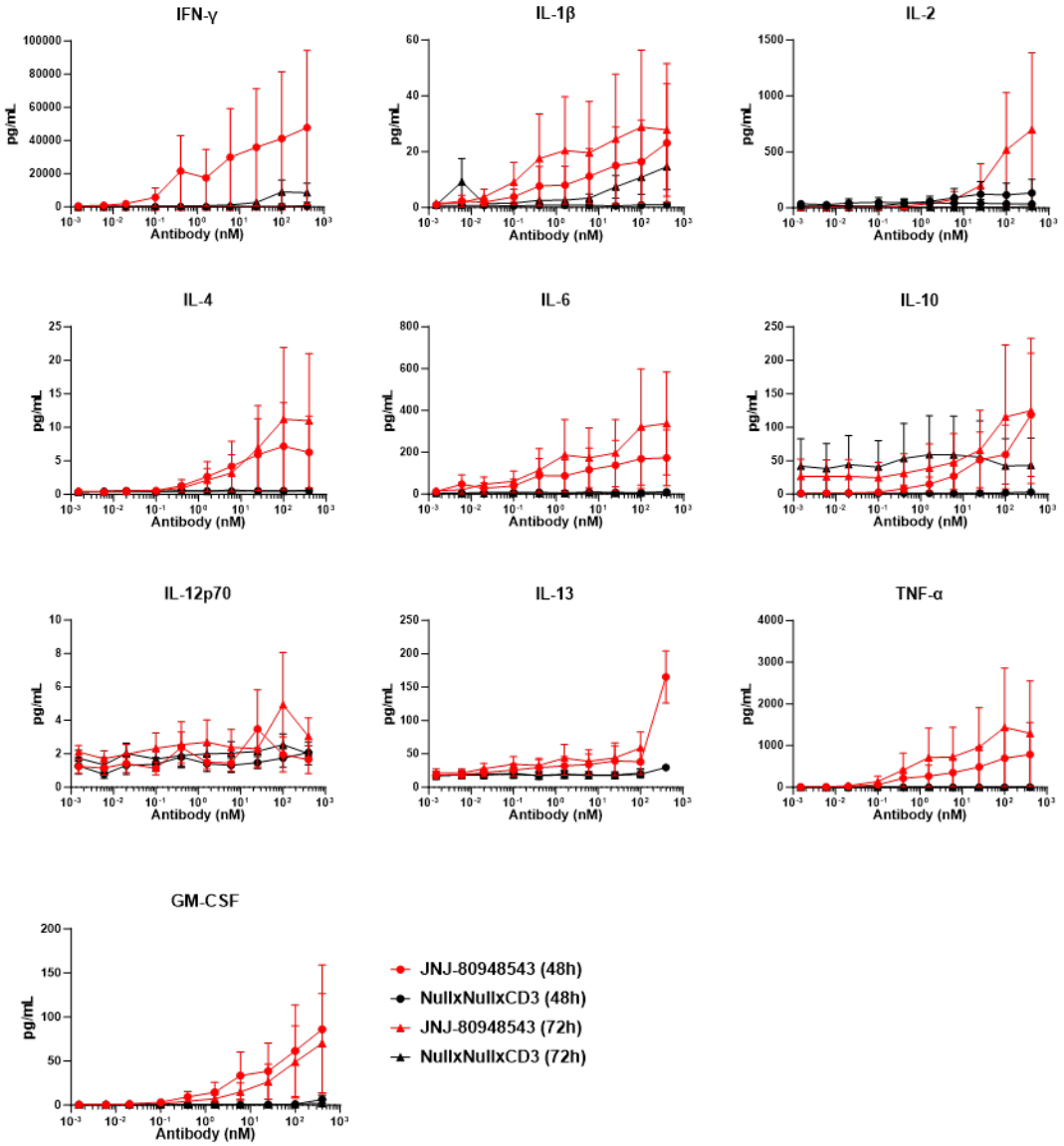
C.


Primary B cells
in the presence of CARNAVAL
% Cytotoxicity
Antibody (nM)

D.


Primary B cells
in the presence of OCI-Ly10
% Cytotoxicity
Antibody (nM)
JNJ-80948543
NullxNullxCD3
JNJ-80948543
NullxNullxCD3

E.



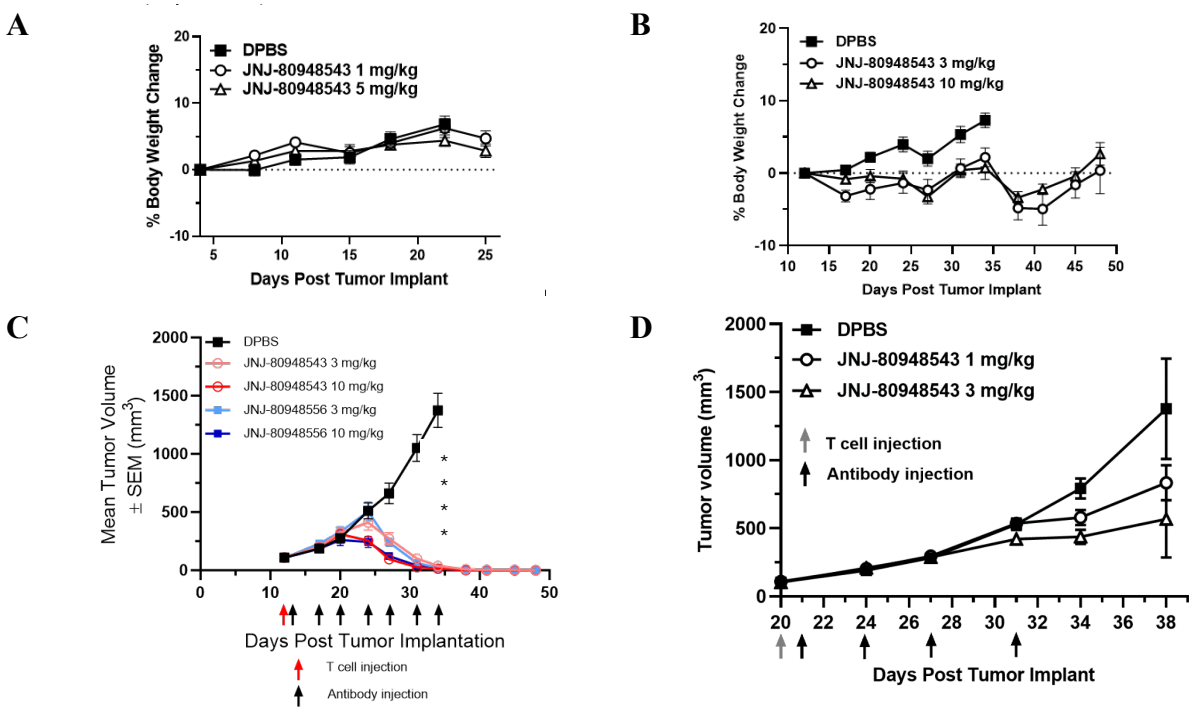
(A) Whole blood from 6 healthy donors was tested in T-cell redirection assays with the indicated antibodies and CD79b^{+}CD20^{+} CARNAVAL tumor cells. Cytokine levels were determined after 48 or 72 hours. The assay was conducted at an E:T ratio of 1:1; data were averaged and means ± SEM are graphed.

(B) Whole blood from 6 healthy donors was tested in T-cell redirection assays with the indicated antibodies and CD79b^{+}CD20^{+} OCI-Ly10 tumor cells. Cytokine levels were determined after 48 or 72 hours. The assay was conducted at an E:T ratio of 1:1; data were averaged and means ± SEM are graphed.

(C) and (D) Whole blood from 6 healthy donors was tested in whole-blood T-cell redirection assays with the indicated antibodies and CD79b^{+}CD20^{+} tumor cell lines, (C) CARNAVAL or (D) OCI-Ly10. The assay was conducted at an E:T ratio of 1:1 with respect to spiked-in tumor cells, but the E:T ratio for primary B cells ranged from 5:1 to 10:1. The percent cytotoxicity of primary B cells was determined after 48 or 72 hours. Data were averaged and means ± SEM are graphed.

(E) Whole blood from 6 healthy donors was incubated with the indicated antibodies (single point per condition per donor) for 48 or 72 hours. Cytokine production was measured; means ± SEM are graphed.

Supplemental Figure 7. Effect of JNJ-80948543 and JNJ-80948556 on OCI-Ly10 Tumor Volumes in T cell-humanized Mice



(A) Effect of JNJ 80948543 on Body Weight of T cell-humanized Mice Bearing SC CARNAVAL Tumors. Group body weights are graphed as the mean ± SEM (n=10/group). Tumor cells were implanted on Day 0, T cells were injected on Day 1, and mice were treated on Days 2, 5, 8, 11, 15, 18, 22, and 25. Data are displayed while at least 2/3 of animals remained in a group.

(B) Effect of JNJ-80948543 on Body Weight of T-cell-humanized Mice Bearing Established SC OCI-Ly10 Tumors. Group body weights are graphed as mean ± SEM (n=10/group). Tumor cells were implanted on Day 0, T cells were injected on Day 13, and dosing occurred on Days 14, 17, 20, 24, 27, 31, and 34. Data are displayed while at least 2/3 of animals remained in a group.

(C) T cell-humanized NSG mice injected SC with OCI-Ly10 tumors were dosed IP with JN48543 or JNJ-80948556 at 3 and 10 mg/kg for OCI-Ly10 (dosing time frame is denoted by bar below the X axis). Tumor volume was measured twice weekly and results presented as the mean tumor volume ± SEM for each group (n=10/group). Data are displayed while at least 2/3 of animals remained in a group. * Denotes significant difference ($p \leq 0.05$) compared with the DPBS control evaluated using a mixed model for repeated measures.

(D) OCI-Ly10 tumor cells were implanted on Day 0, T cells were injected on Day 20 when tumors reached a mean of 106 mm^3 (gray arrow), and dosing occurred on Days 21, 24, 27, and 31 (black arrows). Samples were collected for analysis 4, 24, 72, 96, and 168 hours post fourth dose. Tumor volumes are graphed as the mean ± SEM (n=15/group through Day 31; n=9/group on Day 34; n=3/group on Day 38).

Supplemental Methods

Null antibody generation

A null antibody for both the CD79b and CD20 arms (NullxNullxCD3) was generated as a negative control. In selected experiments, a NullxCD20xCD3 antibody was also generated and used. The fragment crystallizable (Fc) region of all the null antibodies was silent, as it contained the AAS mutations.

Cell lines

Human cell lines (CARNAVAL, JeKo-1, HT, SU-DHL-1, WILL-2, K562) were obtained from the American Type Culture Collection and Deutsche Sammlung von Mikroorganismen und Zellkulturen, or were licensed from the University of Toronto (OCI-Ly10). All cell lines were cultured at 37°C, 5% CO2 in complete culture medium (RPMI 1640 + GlutaMAX, 10% HI FBS) and tested negative for mycoplasma.

Parental CARNAVAL and OCI-Ly10 cells were transduced with Incucyte® NucLight Red lentiviral particles for expression of mKate2 red fluorescent protein. Flow cytometry-based sorting was performed to enrich the mKate2+ population, after which the pooled cells were kept under antibiotic selection.

Parental K562 cells were transduced with lentiviral particles containing a bicistronic plasmid encoding human CD79b-enhanced green fluorescent protein (eGFP) or CD20-mKate2. Antibiotic selection, with subsequent flow cytometry-based sorting, was performed to enrich CD79b+eGFP+ or CD20+mKate2+ expressing cells. To generate K562 cells expressing both CD79b-eGFP and CD20-mKate2, K562 CD79b-eGFP cells were transduced with lentiviral particles encoding CD20-mKate2. Flow cytometry-based sorting was performed to select for double-target-expressing cells, after which the cells were kept under antibiotic selection.

Pan T cells were collected from healthy donor leukopaks and isolated by negative selection. Frozen vials were stored in liquid nitrogen until use, then thawed and cells were transferred to fresh complete culture medium. Whole blood from healthy donors was collected in heparin tubes on the morning of the assay and stored at room temperature (RT) until plated.

Immunohistochemistry

To confirm specificity, the CD79 IHC antibody (clone D7V2F) was diluted at 1:100 and assessed by detection and absence of staining in $CD79b^+$ and $CD70^-$ cell lines. Tonsil tissue sections were included as positive controls for tissue staining of CD79b and CD20. The CD20 IHC antibody (clone L26) was ready-to-use purchased, validated by the vendor, and optimally diluted for use on the BOND system.

IHC staining, deparaffinization, and antigen retrieval process was performed on BOND RX automated stainer (Leica) using the BOND™ Polymer Refine Detection Kit. Epitope retrieval for CD79b was performed using BOND Epitope Retrieval Solution 2 at 100°C for 20 minutes, followed by 4 wash steps. For CD20, epitope retrieval was performed using BOND Epitope Retrieval Solution 1 at 100°C for 20 minutes, followed by 4 wash steps. Formalin-fixed paraffin-embedded (FFPE) tissues and tissue microarrays (TMA) were treated with 3% to 4% H_2O_2 for 5 minutes at RT to quench endogenous peroxidase activity, followed by a wash step (3 times with wash buffer). Anti-CD79b antibody and rabbit IgG (clone DA1E) isotype control were applied to the samples at 150 µL (0.15 µg/mL) per tissue and incubated for 30 minutes at RT. Anti-CD20 antibody was applied undiluted to the samples and incubated for 15 minutes at RT. After primary antibody incubation, tissues were washed 3 times with wash buffer, followed by adding a post-primary reagent rabbit anti-mouse IgG linker (to localize mouse antibodies) for 8 minutes at RT. The tissues were then treated with polymer anti-rabbit poly horseradish peroxidase – IgG for 10 minutes at RT, followed by 2 washes at 2 minutes each, and rinsed twice with distilled water. The refine reagent

3,3'-diaminobenzidine tetrahydrochloride hydrate was applied to tissues for 10 minutes at RT to visualize protein expression. Hematoxylin counterstain was applied and incubated for 5 minutes, followed by slide dehydration in a series of alcohols and xylene, then coverslipped.

All stained slides and TMAs were reviewed and scored by a Johnson & Johnson pathologist. All samples from relapsed/refractory lymphoma patients were scored based on percentage positive per neoplasm using staining intensity utilizing scores (0, 1+, 2+, or 3+). TMA was scored similarly. Scores were used for data representation.

Flow cytometry analysis of CD79b and CD20 expression on hematologic cancer cell lines

Quantum Simply Cellular microspheres were used according to manufacturer's instructions to correlate geometric mean fluorescence intensity with CD79b or CD20 antigen expression as molecules per cell on target positive and target negative cell lines. Values of isotype-stained cells were subtracted from values of cells stained for CD79b or CD20 to calculate receptor densities. Cells were considered negative for expression if the value was less than 2× the value of the isotype control.

CyTOF analysis of CD79b and CD20 expression on patient samples

Dissociated lymph node tissues from treatment-naïve B-NHL patients and patients with lymphadenitis (Fidelis or DLS) were stained with Rhodium-103 (Standard BioTools), followed by blocking of Fc receptors using human TruStain FcX (Biolegend). Cells were then stained with a Maxpar® Direct Immune Profiling Assay cocktail (MDIPA, Standard BioTools) supplemented with CD79b (clone CB3-1) and CD22 (clone HIB22). The cells were fixed in 1.6% formaldehyde solution and stained with Iridium-DNA intercalator (Standard Biotools). Prior to the acquisition, samples were resuspended in Maxpar® cell acquisition solution (Standard Biotools) containing 0.1x EQ™ four element calibration beads (Standard Biotools). Samples were acquired on the Helios mass cytometer (Standard BioTools).

Acquired FCS files were normalized using the processing module within the CyTOF® Software. All downstream gating and analyses were performed using the Cytobank software (Beckman Coulter).

CD79b and CD20 mRNA in silico analysis

Expressions of CD79b and CD20 (MS4A1) mRNA were extracted from extracted from an internal dataset from samples obtained from patients with different heme malignancies were profiled using the Affymetrix Human Genome U133 Plus2.0 microarray. These included 226 tissues samples which included acute myeloid leukemia (AML; n=39), CML (n=23), DLBCL (n=53), FL (n=76), MCL (n=14), multiple myeloma (MM) (n=19), myelodysplastic syndrome (MDS; n=1), monoclonal gammopathy of undetermined significance (MGUS; n=1), and non-malignant non-lymphoid tissue control samples collected from patients (n=9; DLBCL [n=4], FL [n=3], MCL [n=2]). The microarray probe sets were reannotated using the University of Michigan Brain Array custom CDF files (v20.0.0 – EntrezGene) and all samples were normalized in R 3.6.1 using the Robust Multi Average (RMA) normalization step present in the `affy` package (Bioconductor v3.2.0).

Off-Target Screening and Functional Specificity

The individual CD79b, CD20 and CD3 molecules were screened for binding specificity using the Retrogenix® Cell Microarray Technology screen (Charles River Laboratories). Briefly, test antibodies are screened against a library of 5,475 full-length human plasma membrane and cell-surface-tethered human secreted proteins and 371 heterodimers individually expressed on fixed HEK293 cells and detected with a AF647 anti-human secondary antibody. Specific interactions of the antigen binding domains are distinguished from antibody Fc or secondary antibody protein interactions.

For the functional specificity in vitro assay, tumor cell lines were selected from the Cancer Cell Line Encyclopedia (CCLE) transcriptomics data set. A panel of 6 cell lines were identified to be negative for CD79b, CD20 and CD3 (HEK-293T, K562, HCC-1833, HepG2, U87MG and SHP-77) that cumulatively express >50% of the known cell surface receptors using a cutoff of >5 TPM. Tumor cells and primary human T cells were plated at a 2:1 effector to target ratio and incubated with a titration of JNJ-80948543 or a NullxNullxCD3 control antibody. Supernatants were harvested 72 hours post treatment and assessed for cytokines (Granzyme B, IFNγ, IL-2 and TNFα) as a measure of antibody-induced T cell activation.

Incucyte® T-cell redirection assay using pan T cells and tumor targets

Tumor cell lines (CARNAVAL_mKate2, OCI-Ly10_mKate2, K562_CD79b_eGFP, K562_CD20_mKate2, K562_CD79b_eGFP_CD20_mKate2) were resuspended at 1×10^6 cells/mL in phenol-red-free complete medium.

JNJ-80948543 and NullxNullxCD3 antibodies were made at a 4× concentration of 400 nM in phenol-red-free complete medium and added to a 96-well U-bottom plate. The final antibody concentrations ranged from 100 nM to 0.1 pM.

Purified frozen pan T cells from 2 healthy donors were resuspended at 1×10^6 cells/mL in phenol-red-free complete medium. Pan T cells and tumor cells were combined at 1:1 and 5:1 effector-to-target (E:T) cell ratios and added to the serially diluted antibodies. Anti-CD25-Alexa 488 (1:1,000 dilution) was added to T cells cocultured with CARNAVAL_mKate2 or OCI-Ly10_mKate2 cells to measure T-cell activation over time. Analysis was performed on the Incucyte ZOOM live-content imaging system (Sartorius).

Images were automatically acquired in both phase and fluorescence channels every 6 hours for up to 6 days with a 4× objective lens (single image). Incucyte Zoom software (version

Incucyte 2019B Rev2) was used to detect target cells and T-cell activation based on mKate2 and CD25 expression, respectively, using optimized process definition parameters. For the analysis, the total red and green areas were quantified. For assessment of CD25 level, because CD25 signal in the first 48 hours showed high background fluorescence and provided a false positive signal, the scan values from the first 48 hours were excluded in further area under the curve (AUC) analysis. Beyond 48 hours, the background signal was sufficiently bleached and did not interfere with the readout. Data from different donors were graphed and AUC values were derived for each condition. After normalizing the data to the untreated control, antibody concentrations were plotted against the normalized values expressed as percent cytotoxicity as a concentration response

T-cell redirection assay using Pan T cells and tumor targets

CARNAVAL, OCI-Ly10, HT, WILL-2, JeKo-1, SU-DHL-1, and K562 cell lines were resuspended at 1×10^6 cells/mL in DPBS containing 0.1% bovine serum albumin (BSA) before incubation with carboxyfluorescein succinimidyl ester (CFSE; resuspended in 18 µL dimethyl sulfoxide and diluted 1:5,000) for 10 minutes at 37°C. Staining was quenched with 25 mL of complete medium, and cells were washed and resuspensed at 1×10^6 viable cells/mL in complete medium. JNJ-80948543 and NullxNullxCD3 antibodies were added to each well. For non-treated conditions, complete medium was added to the wells.

Purified frozen human pan T cells from 5 to 7 healthy donors were resuspended in complete medium at 3×10^6 cells/mL. Pan T cells and tumor cells were combined at 5:1 (all cell lines) and 1:1 (JeKo-1, CARNAVAL, OCI-Ly10) E:T ratios for each cell line, and the cell mixture was dispensed to the 96-well U-bottom plate containing the serially diluted antibodies. Plates were incubated at 37°C, 5% CO2 for 48 and 72 hours.

Supernatants were harvested for cytokine analysis. Cells were harvested to evaluate viability of tumor cells and T-cell activation. After cells were washed once in DPBS, viability was assessed by incubating the cells in Fixable Viability Dye eFluor™ 780 (diluted 1:1,000 in DPBS) for 30 minutes at 4°C. Viability staining was quenched by the addition of 100 µL/well of stain buffer, followed by centrifugation at 400× gravity (g) at RT for 5 minutes. Cells were resuspended in stain cocktail, incubated for 30 minutes at 4°C, and washed twice with 200 µL of stain buffer. Cell pellets were then resuspended in 100 µL of stain buffer and sample acquisition was performed on a FACSLyric flow cytometer (BD Biosciences). To assess cytotoxicity, the absolute count of the $CFSE^+$ cells was used to determine the number of viable cancer cells in each well. Viability of $CFSE^+$ cells was determined by using staining positivity of Fixable Viable cells in these assays were determined using Fixable Viability Dye eFluor™ 780.

The percentage of cytotoxicity was calculated using the formula:

$$\left(\frac{\text{Absolute \# of viable cancer cells}}{\text{Average of absolute \# of viable cancer cells in the untreated wells}}\right) \times 100$$

To measure the level of T-cell activation, the absolute percentage of CD69 and CD25 expression for either $CD4^+$ or $CD8^+$ T cells was used. It should be noted that SU-DHL-1 cells secrete IL-2 and IL-6 which will induce CD25 on T cells in the coculture system in the absence of antibodies. For the SU-DHL-1 coculture experiments only, the level of T-cell activation in the presence of antibodies was determined relative to the level of T-cell activation in the absence of antibodies.

Whole blood T-cell redirection assay

Fresh whole blood (50 µL) was added to Trucount tubes with 20 µL of BD Multitest staining mix. After incubation, the blood was lysed and fixed by adding 450 µL of 1× BD lysing solution. Lysed blood was acquired on a FACSLyric flow cytometer and the exact number of

T and B cells per µL of whole blood was calculated following the manufacturer's instructions for Trucount tube system.

JNJ-80948543 and NullxNullxCD3 were prepared at 4× concentration (1600 nM) and serially diluted at concentrations ranging from 400 nM to 0.38 pM for assay use. Two types of assays were performed, either with or without spiked-in, labeled cancer cells. In the spiked-in cancer cell setting, CARNAVAL and OCI-Ly10 cells cultured in complete medium were washed with DPBS containing 0.1% BSA and CFSE dye, and reconstituted and added to the cells as previously described, followed by incubation for 10 minutes at 37°C. Cold complete medium was added to quench the labeling and the cells were centrifuged at 400×g at RT for 4 minutes, washed, and counted. An E:T ratio of 1:1, with T cells from whole blood as effectors, and target cells comprising spiked-in labeled CARNAVAL or OCI-Ly10 tumor cells and primary B cells in the donor blood, were used. CFSE-labeled target cells were diluted in complete medium to the specific density required to achieve a 1:1 E:T ratio when adding 50 µL of the cell suspension per well to 100 µL of whole blood. In a 96-well U-bottom plate, 50 µL of the CFSE-labeled target cells were dispensed first for the spiked-in assay, while in the autologous assay, no CFSE-labeled target cells were added, but 50 µL of complete medium was added. Next, 50 µL of the 4× concentrated antibody dilution series were added to the wells, followed by 100 µL of whole blood. For each donor, single wells were tested per condition. Plates were incubated at 37°C, 5% CO2 for 48 or 72 hours.

After each timepoint, cells were harvested to evaluate viability of tumor cells and T-cell activation. Plates were centrifuged at 400×g for 3 minutes. From each well, serum supernatant was harvested for cytokine analysis. Blood and cell pellets were lysed with 200 µL/well of RBC lysis buffer, incubated at RT for 5 minutes, and centrifuged for 5 minutes at 400×g. The lysis process was repeated 4 more times, and cells were washed once in DPBS and resuspended in 100 µL of 1:1,000 diluted Fixable Viability Dye eFluor™ 780 in DPBS,

followed by incubation for 30 minutes at 4°C in the dark. Cells were centrifuged for 5 minutes at 400×g and after removing the supernatant, resuspended in 100 µL/well of Fc blocking reagent and incubated for 10 minutes at RT in the dark. Cells were then centrifuged for 5 minutes at 400×g and pellets were resuspended in 100 µL staining cocktail and incubated for 30 minutes in the dark at 4°C. Cells were acquired on a LSRFortessa flow cytometer (BD Biosciences).

Tumor cells or primary B cells were identified by gating on FSC and SSC to identify cell populations followed by $CFSE^{+}$ (tumor cells) or CFSE- followed by staining for $CD19^{+}$(primary B cells). LIVE/DEAD staining was performed to evaluate cell death.

T-cell activation were identified by gating on FSC and SSC to identify cell populations, then $CFSE^{-}$ to identify the blood population, followed by $CD3^{+}$ to identify T cells. LIVE/$DEAD^{-}$ staining was performed for live T cells. $CD4^{+}$ and $CD8^{+}$ subsets were identified and from each subset, CD69 and CD25 expression was determined.

Cytokine assay

Supernatants from the flow-cytometry based T-cell redirection assays were tested using the Human Pro-inflammatory Panel 1 kit (MSD). Frozen supernatants were diluted 1:10 in Diluent 2. A standard curve was prepared by reconstituting and serially diluting the provided calibrator in Diluent 2 as per the manufacturer's protocol. MSD Assay Plates (V Plex Proinflammation Panel 1 [human] kit) were prewashed per manufacturer's protocol and filled with the diluted supernatant or standards as per plate layout. Plates were incubated for 2 hours at RT followed by 2 hours of incubation with the antibody solution. Assay plates were read on a SECTOR imager (MSD). For serum cytokine samples from the whole-blood assays, an additional single-plex analysis from MSD for granulocyte-macrophage colony-stimulating factor (GM-CSF) was also performed according to the manufacturer's protocol. Extrapolation

of sample values from the standard curve for each cytokine was performed using the DISCOVERY WORKBENCH software (MSD, Version 4.0).

Antagonistic activity assay

OCI-Ly10 cells were harvested and resuspended at 9.52×10^5 cells/mL and added to U-bottom 96-well plate.

JNJ-80948543, CD79bxNullxCD3, NullxCD20xCD3, and NullxNullxCD3 antibodies were prepared at a 4× concentration of 4 μM in complete medium. A total of nine 3-fold serial dilutions were made and 35 μL of diluted antibody were added to each well of the 96-well plate. The final antibody concentrations tested in the assay ranged from 1 μM to 0.03 nM. As a control, complete culture medium containing 2.7% DPBS was prepared and added to the appropriate wells. The plates were incubated at 37°C, 5% CO2 for 24 hours.

After incubation, the plates were centrifuged at 800 rpm for 5 minutes and 50 μL of supernatant were transferred to a prewashed MSD Assay Plate (V Plex Proinflammation Panel 1 [human] kit). The MSD assay was performed as described per manufacturer's protocol, and the plates were read on a SECTOR imager (MSD). Cell viability was assessed in parallel after 24 hours incubation using CellTiter-Glo® Luminescent Cell Viability Assay glow according to the manufacturer's recommendations.

Xenograft mouse models

For all studies, female NSG mice (Charles River Laboratories) approximately 7 to 14 weeks of age and weighing approximately 25 g were used. All experiments were carried out in accordance with *The Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Johnson & Johnson, Beerse, Belgium.

CARNAVAL and OCI-Ly10 cells were cultured and harvested in their respective complete culture medium. On the day of tumor implantation, CARNAVAL cells were resuspended in

cold (4°C) serum-free medium at 5×10^6 cells/mL. OCI-Ly10 cells were resuspended in cold serum-free medium + Matrigel® (diluted 1:1) at 5×10^6 cells/mL.

Human pan-T cells were activated and expanded in vitro using the T-cell activation and expansion kit and grown in T-cell complete culture medium containing interleukin (IL)-2 at a concentration of 10 ng/mL, starting 3 days after thaw and activation. On the day of engraftment into mice, activation beads were removed from the T cells and cells were resuspended in serum-free medium at a concentration of 1×10^8 cells/mL, for an IP injection of 2×10^7 cells in 0.2 mL per mouse.

In all studies, T-cell-humanized mice were given fragment crystallizable (Fc) block at 0.2 mg/mouse IP and human immune globulin infusion at 10 mg/mouse IP at least 30 minutes prior to antibody dosing, to compensate for the low immunoglobulin environment in the NSG mouse strain.

In the prevention efficacy study, mice were randomized into groups of 10 and injected SC in the right flank with 1×10^6 CARNAVAL cells (0.2 mL). On Day 1, mice were humanized with T cells and starting on Day 2, mice were treated twice a week IP with 25 or 125 µg JNJ-80948543 (corresponding to 1 or 5 mg/kg, respectively) or DPBS, for a total of 8 doses.

In the established efficacy study, mice were injected SC in the right flank with 1×10^6 OCI-Ly10 cells (0.2 mL). On Day 12, mice were randomized into groups of 10 by tumor volume (mean tumor volume of 108 mm^3) and humanized with T cells on Day 13. On Day 14, mice were treated twice a week with IP dosing of 75 or 250 µg JNJ-80948543 or JNJ-80948556 (corresponding to 3 or 10 mg/kg, respectively) or DPBS, for a total of 7 doses.

To assess PD of JNJ-80948543 in established OCI-Ly10 tumors. Mice were injected SC in the right flank with 1×10^6 OCI-Ly10 cells in 0.2 mL. On Day 20, mice were randomized into groups of 15 by tumor volume (mean tumor volume of 106 mm3) and humanized with T

cells. On Day 21, treatment was initiated with IP dosing twice a week for a total of 4 doses of 25 or 75 µg of JNJ-80948543 (corresponding to 1 or 3 mg/kg, respectively) or DPBS. Tumors were collected from 3 animals per group and timepoint at 4, 24, 72, 96, and 168 hours post fourth dose to evaluate T cell infiltration by immunohistochemistry (IHC) analysis.

Tumor volume in SC models was calculated using the formula: tumor volume (mm^3) = ($D \times d^2/2$); where 'D' represents the larger diameter, and 'd' the smaller diameter of the tumor as determined by caliper measurements.

The percent TGI was defined as the difference between mean tumor volumes of the treated and control groups, calculated as % TGI = $((TV_c-TV_t)/TV_c)\times100$ where 'TV_c' is the mean tumor volume of the control group and 'TV_t' is the mean tumor volume of the treatment group. As defined by National Cancer Institute (NCI) criteria, ≥60% TGI is considered biologically significant.

The percent ΔTGI was defined as the difference between mean tumor volume of the treatment and control groups, calculated as % ΔTGI = $([(TV_c-TVc_0)-(TV_t-TVt_0)]/(TV_c-TV_{c0}))\times100$ where 'TV_c' is the mean tumor volume of a given control group, 'TV_{c0}' is the mean initial tumor volume of a given control group, 'TV_t' is the mean tumor volume of the treatment group, and 'TV_{t0}' is the mean initial tumor volume of the treatment group.

A CR for SC tumor models was defined as complete tumor regression, with no palpable tumor on the day of analysis.

Tumor volume was graphed using Prism. Statistical significance for most studies was evaluated for antibody-treated groups compared with controls on the last day of the study when at least two thirds of the control animals were still on study.

Animal Monitoring

Body weight and SC tumor volume were measured at least twice a week throughout each study. Animals were monitored daily for clinical signs related to graft versus host disease (GvHD; caused by human immune cells reacting to mouse tissue antigens) or tumor burden. When individual animals exhibited negative clinical signs, such as lethargy, ruffled and matted coat, hunched posture, cyanotic extremities, or dyspnea, or reached 20% body weight loss as compared with initial body weights, they were removed from the study and humanely euthanized. In the absence of negative clinical signs, animals were removed from SC tumor studies when a maximum tumor volume of $\geq$1,400 mm^3 was reached.