Cell type-specific monoclonal antibody cross-reactivity screening in non-human primates and development of comparative immunophenotyping panels for CyTOF

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Monoclonal antibodies are a critical tool for immunologists, with applications including immunomicroscopy and cytometry, and non-human primates are essential models for drug development. Very few antibodies are raised against non-human primate antigens; instead, researchers typically use anti-human antibodies that are found to be cross-reactive with non-human primates. The NIH maintains a valuable database of cross-reactivity, but its coverage is not complete, especially for less common species. Furthermore, the database only indicates the presence or absence of staining and does not indicate if a different cell population is stained in the NHP species than in humans. We screened 332 antibodies in five immune cell populations in blood from cynomolgus macaques (*Macaca fascicularis*), rhesus macaques (*Macaca mulatta*), African green monkeys (*Chlorocebus aethiops*) and olive/yellow baboons (*Papio hamadryas anubis* x *Papio hamadryas cynocephalus*), thereby generating a comprehensive cross-reactivity catalog that includes cell type-specificity. We subsequently used this catalog to help create large CyTOF mass cytometry panels for three of those species and humans. The curated dataset containing the primary data for each antibody has been deposited as a browsable resource at <https://immuneatlas.org> and <https://flowrepository.org/id/FR-FCM-Z2Z7>.

Introduction

Non-human primates (NHPs) are critical components of drug development because of their similarity to humans. Many key immunology assays, such as flow cytometry, Western blots, immunohistochemistry and immunofluorescence microscopy, make use of antibodies to demarcate specific cell types and quantify signaling moieties. Very few antibodies are raised against non-human primate antigens; instead, researchers typically use anti-human antibodies that are cross-reactive with the non-human primate species that they are studying. To help researchers find antibodies for NHP research, the National Institutes of Health supports a highly valuable database of the cross-reactivity of commercially available antibodies with 13 NHP species (<http://www.nhpreeagents.org>). The database is derived from manufacturer and investigator reports, and typically provides a simple yes/no statement about whether a clone stains a species, with occasional comments about staining intensity or specificity. While an invaluable resource, the database is limited in its coverage. For example, prior to this study, only 28 CD markers had been evaluated in African green monkeys.

Additionally, with few exceptions, the database lacks information about the cell types bound by cross-reactive antibodies, and there are many known instances of antibody clones binding different cell types in different species. For example, granulocyte and monocyte marker expression is known to be substantially different in humans than in non-human primates. Anti-human CD33 clone

AC104.3E3 was reported in the NIH database and manufacturer's datasheet as cross-reactive with rhesus and cynomolgus macaques, but our lab and others determined that in those species, it prominently stains granulocytes (1, 2), while in humans it stains monocytes and classical dendritic cells. As another example, the Fcγ receptor CD16 is found on granulocytes in humans and sooty mangabeys, but not in macaques or baboons (3, 4), which will likely confound animal studies evaluating therapeutic antibodies, which may bind, transduce signals through and mediate internalization via this Fcγ receptor. Yet another example is CD56, which is expressed on monocytes in macaques (5), but is a canonical NK cell marker in humans. Thus, researchers must confirm that each clone they use is staining the cell population of interest through literature review or experimental verification.

Here we present an expansion of both the breadth and depth of primate cross-reactivity data. We screened 332 monoclonal antibodies in blood from two individuals of each of four NHP species: rhesus macaque (*Macaca mulatta*), cynomolgus macaque (*Macaca fascicularis*), African green monkey (*Chlorocebus aethiops*) and olive/yellow baboon (*Papio hamadryas anubis* x *Papio hamadryas cynocephalus* hybrid); and found more than 120 clones that stained one or more populations in each species. Furthermore, we included counter-stain antibodies that allowed us to determine staining specificity in at least five major immune cell populations. Data from the NHPs were compared to the same screen ran in our laboratory on human blood. Finally, we used the results from this screen to create the first

Figure 1. Five counterstains allow identification of at least five cell populations. Representative staining from a baboon shown. After gating by time to exclude artifacts, granulocytes were identified as CD66abce+/SSC-A+. Non-granulocytes were divided into B cells (CD20+/CD3-), T cells (CD3+/CD20-), NK cells (CD7+/CD3-/CD20-) and monocytes/dendritic cells (CD7-/CD3-/CD20-). *In species other than the African green monkey, dendritic cells could be separated on the basis of CD11b staining.

mass cytometry phenotyping panels for African green monkeys and rhesus and cynomolgus macaques. These panels allow for effective side-by-side comparisons between non-human primate and human blood. All data is publicly accessible at <https://immuneatlas.org> and <https://flowrepository.org/id/FR-FCM-Z227>.

Results

Counterstain panel and staining condition development

In order to identify the characteristics of these antigens across species, we first designed a panel of counterstain antibodies that delineate major circulating immune cell types in all five species (Figure 1). This panel readily identified granulocytes, B cells, T cells, NK cells and monocytes/dendritic cells. In all species except for African green monkey, we could additionally separate the monocytes and dendritic cells based on CD11b expression. In African green monkeys, CD11b (ICRF44) was non-reactive, and we chose not to include a substitute marker to maintain technical consistency across all species.

Additionally, we strategically selected the fluorophores to keep the PE channel, which was used for the screened antibody, free of bleed/compensation to avoid technical artifacts.

Fixation and red blood cell lysis conditions were selected after testing a large number of protocols on the basis of preserving antigen staining and effectively lysing non-human primate blood (data not shown). Fixation with approximately 0.3% PFA was low enough to avoid obvious loss of staining and sufficient to minimize morphological changes of cells as observed as forward and side scatter signals changing over the course of acquisition on the cytometer. Enzymatic lysis with VersaLyse was highly effective for both human and non-human primate blood, whereas other methods such as hypotonic lysis were inadequate or inconsistent for non-human primate blood.

Antibody reactivity screen

We established universal criteria for defining positive expression to determine antigen expression in an unbiased manner. An antibody clone was classified as reactive with a population if more than 10 percent of cells had a PE signal intensity greater than the 95th

percentile of the intensity of the corresponding isotype control for the same species and population (Table I). This threshold was found to accurately reflect the results of manual classification: We verified 500 of the 14,940 clone x species x population results, taking into consideration reported staining patterns (references included (2, 5, 7–13)) and the visual degree of separation from the isotype control, and calculated a false-positive rate of 7.4% and a false-negative rate of 1.6%, for an initial accuracy of 91% in our data. Then, we manually verified and corrected as necessary all discordant replicates and all clones that were classified as reactive in a non-human primate species but not in a human; thus, our final estimated accuracy exceeds 91%. All data from this project are publicly available, such that researchers can independently validate the expression of antigens of interest by accessing the primary data. In total, we identified 260, 153, 129, 161 and 147 clones that are reactive with one or more populations in human, cynomolgus macaque, rhesus macaque, African green monkey and baboon, respectively.

Researchers interested in evaluating the antibodies in this study are directed to examine the primary data at <https://immuneatlas.org> or <https://flowrepository.org/id/FR-FCM-Z227>, where one can compare the relative staining intensity and distribution of staining between species. Such review is critical when evaluating antibodies that stain rare populations—especially populations that comprise less than 10 percent of one of the populations that we delineated, or that show dim expression that might be excluded by the threshold for expression and staining that we applied. Additionally, we encourage diligence when interpreting markers such as CD41 and CD51/CD61, which are listed as reactive with all cell types, but in actuality are probably staining platelet fragments stuck to other cells, based on the known distribution of those markers in humans (14). It is also important to keep in mind that actual antibody-antigen specificity may vary by species due to differences in gene sequence, protein structure and post-translational modifications.

Notable examples of differences in expression patterns

As noted above, in macaques CD33 is found on granulocytes and CD16 is restricted to monocytes and dendritic cells. In this study, we found that African green monkeys share this same staining pat-

Figure 2. CD172g is expressed on monocytes and all granulocytes, but not on T cells, in examined NHP species. By comparison, CD172g is expressed on all T cells, a subset of granulocytes and a subset of B cells (data not shown) in humans. As discussed in the text, CD11b did not uniformly stain AGMs; thus, some of the CD11b-negative cells are monocytes. Blue: isotype control, orange: CD172g.

tern, albeit with weaker CD33 staining. Another notable difference (out of numerous idiosyncratic expression patterns observed) is that CD172g (signal regulatory protein (SIRP) γ , also known as SIRP β 2) is expressed on CD11b+ monocytes and granulocytes, but not on T cells, in all of the NHP species examined. In contrast, in humans this marker is expressed on T cells, some B cells and to some degree in granulocytes (Figure 2). Because it lacks a cytoplasmic signaling domain, CD172g is postulated to signal unidirectionally by activating the CD47-expressing cell and inducing T cell migration and prolifer-

ation in humans, mice and rats (15–17). Thus, our finding suggests a major difference in regulation of immune cell migration and adaptive response, which warrants further study.

Another notable example of the differences found is the presence of CD2 staining not only on T cells, but also on B cells in rhesus macaques, cynomolgus macaques, African green monkeys and to a slight extent baboons (Figure 3). CD2 is involved in adhesion, co-stimulation, antigen recognition and potentially differentiation (18, 19). In mice, virtually all circulating and bone marrow B cells express CD2

Figure 3. CD2 is expressed on B cells in NHPs. Blue: CD3+ T cells where expression is expected in humans; orange: CD20+ B cells.

(18). Kingma et al. previously reported that only a small subset (5.74 $\pm$ 2.46%) of normal human peripheral blood B cells express CD2, although approximately 25% of surveyed B cell neoplasms expressed CD2 (20). We observed no CD2+ B cells in human. B cell CD2 expression thus seems to have been lost evolutionarily, with the most abundant expression in the oldest species (mouse), moderate expression in macaques and African greens, less expression in baboons and essentially no expression in the youngest species (human). Interestingly, the ligand is not conserved between species: CD2 exclusively binds CD48 in mice and rats, while in humans it strongly binds CD58 and only weakly binds CD48, which is also co-expressed with CD58 (19, 21).

CytoF panel design

We used the results of the antibody screen, along with the results of several targeted follow-up experiments, to craft a set of parallel CyTOF mass cytometry panels for rhesus and cynomolgus macaque, African green monkey and human (Table II).

Wherever possible, we used the same antibody clones for all species. In the case of erythrocytes, we used the common marker CD235a for humans and CD233 for NHPs, instead of using the less common CD233 for all species (no anti-CD235 clone reactive with NHPs could be found). In the case of CD11c, clone 3.9 was only used in non-human primates because Bu15 was non-reactive, but 3.9 is reported to preferentially bind activated CD11c (22) and requires the addition of magnesium as a cofactor during staining (22, 23). We primarily use CD16 in lieu of CD11c during gating analysis for consistency. In the case of naïve and memory T cells, we observed very

Table II: CyTOF mass cytometry panels for orthologous phenotyping of humans and three species of NHPs. Canonical cell targets (rows) are listed along with mass channel, and antibody clones used for staining humans, macaques, and African green. Clones are listed in parentheses. * indicates weak staining.

Canonical Target	Channel	Human	Macaque	African green
Lymph/mono	115	CD45 (HI30)	CD45 (DO58-1283)	CD45 (DO58-1283)
Erythrocytes	113	CD235a (HIR2)	CD233 (BRIC6)	
Platelets	140	CD61 (VI-PL2)	CD61 (VI-PL2)	CD61 (VI-PL2)
Granulocytes	158	CD66abce (TET2)	CD66abce (TET2)	CD66abce (TET2)
Granulocytes	142		CD33 (AC104.3E3)	CD33 (AC104.3E3)*
B cells	173	CD20 (2H7)	CD20 (2H7)	CD20 (2H7)
B cells, class-switched	174	IgM (G20-127)	IgM (G20-127)	IgM (G20-127)
APCs (B, DC)	176	HLA-DR (Immu357)	HLA-DR (Immu357)	HLA-DR (Immu357)
DCs, B cells	162	CD1c (AD5-8E7)	CD1c (AD5-8E7)	CD1c (AD5-8E7)
cDCs	164	BDCA3 (1A4)	BDCA3 (1A4)	BDCA3 (1A4)
pDCs	148	CD123 (7G3)	CD123 (7G3)	CD123 (7G3)
T cells	157	CD3 (SP34.2)	CD3 (SP34.2)	CD3 (SP34.2)
CD4+ T cells	156	CD4 (OKT4)	CD4 (OKT4)	CD4 (L200)
CD8+ T cells	155	CD8a (RPA-T8)	CD8a (RPA-T8)	CD8a (RPA-T8)
Naïve T cells	166	CD45RA (HI100)	CD45RA (HI100)	
Myeloid cells	153	CD11b (ICRF44)	CD11b (ICRF44)	CD11b (ICRF44)
Myeloid, T subset	143	CD11c (Bu15)	CD11c (3.9)	CD11c (3.9)
Monocytes	151	CD14 (M5E2)	CD14 (M5E2)	CD14 (M5E2)
NK, mono, gran	159	CD16 (3G8)	CD16 (3G8)	CD16 (3G8)
Myeloid	142	CD33 (AC104.3E3)		
Monocytes	175		CD56 (NCAM16.2)	
NK cells	175	CD56 (NCAM16.2)		
NK cells	141	CD7 (M-T701)	CD7 (M-T701)	CD7 (M-T701)
NK cells	168	CD161 (HP-G310)	CD161 (HP-G310)	CD161 (HP-G310)

Figure 4: Parallel gating of blood from four species. CyTOF mass cytometry panels were developed to delineate at least 18 orthologous populations between species.

broad distribution of CD45RA staining, as previously reported (24, 25), with two clones (5H9 and HI100), which was difficult to gate (see online dataset). Staining of this marker was superior with the VersaLyse-based method than with the Triton-X100-based method (see methods) (data not shown).

The experiments described here are especially valuable for the advancement of African green monkey (AGM) immunology because there is a dearth of literature discussing immunophenotyping and only 28 reactive antibodies listed in the NIH database. Nonetheless, this species is important in drug development—especially SIV research—and is the subject of an international effort to make it the most comprehensively characterized NHP by phenotype and genomics (26). Notably, the usage of AGMs is increasing due to a shortage of rhesus macaques for research (27). It was generally possible to use the same clones for African green monkeys as we were for macaques, with several exceptions (Table II). No reactive clones were found for CD11c; instead, we again rely on CD16 for gating monocyte subpopulations and CD123, CD1c and BDCA3 for gating dendritic cell subpopulations.

These CyTOF panels were exhaustively titrated to determine optimal staining concentrations for each antibody, then revalidated in at least two donors from each species. We subsequently contracted BioLyph LLC to lyophilize and package several thousand single-use pellets (“LyoSpheres”) of each of these panels for use in later studies. These pellets solve stability issues such as evaporation and precipitation that inhibit long-term studies and eliminate the technical variability and errors that arise from pipetting. As such, these panels allow for parallel evaluation of orthologous populations between species (Figure 4).

Discussion

We have substantially expanded the breadth and depth of available antibody cross-reactivity data between primate species, and have deposited the dataset containing the primary flow cytometry data online so that any user can easily compare staining patterns in these species, and even look at subpopulations of cells to check specificity—information not typically reported in the NIH database.

As a result of evaluating specific cell types, we have identified numerous antibodies that stain different populations in non-human primates than in humans. In light of this, researchers must take adequate steps to ensure they are evaluating the intended populations when using these reagents, and when evaluating immunotherapeutics targeting these proteins.

While we screened 332 different anti-human antibody clones, not all of the antigens targeted by those antibodies are expected to be present in the resting, peripheral blood cells that we tested. Indeed, only 78.3% (260/332) of the antibodies positively stained human blood. Thus, our screen did not evaluate markers that are found only in cells from bone marrow and other tissues, in progenitors or in activated populations. However, blood is the most easily accessed and likely the most common biological sample type used in research environments, and we thus expect this knowledge about blood to be of the greatest use to most researchers. We hope that the framework we have developed will be expanded upon with additional clones and/or additional species in future studies.

Finally, we present the first CyTOF mass cytometry panels for coordinated human and non-human primate research. We have continued to use these panels for large-scale comparisons of primate immune systems (in review).

Materials and Methods

Blood

Macaque blood was obtained from Valley Biosystems, Inc. (location withheld). African green monkey blood was obtained from Bioreclamation, LLC (Westbury, NY) and Worldwide Primates, Inc. (location withheld). Baboon blood was obtained from the Southwest National Primate Research Center, which is funded by the National Center for Research Resources (p51 RR013986) and supported by the Office of Research Infrastructure Programs/OD P51 OD011133. All animal blood was collected under an approved animal care and use protocol. Human blood was obtained from a healthy adult donor under an approved IRB protocol.

Antibody screen

Blood was collected in sodium heparin and processed within 24 hours of collection. Eight to 10 ml of blood was gently fixed and lysed by incubating with 792 μ l of 16% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA; final concentration approx. 0.3%) and 29.6 ml of VersaLyse (Beckman Coulter, Brea, CA) for 10 minutes at room temperature, then washed once with 0.01% BSA in PBS (“staining buffer”). Cells were resuspended in 6.5 ml of staining buffer, then incubated with 0.5 ml of human TruStain FcX Fc receptor blocking solution (Biolegend, San Diego, CA) for 10 minutes.

Five counterstain antibodies were then added: 500 μ l CD3 (SP34.2)-Brilliant Violet 421 (BD Biosciences, San Jose, CA), 500 μ l CD20 (2H7)-Brilliant Violet 605 (Biolegend), 1000 μ l CD66 (TET2)-APC-Vio770 (Miltenyi, San Diego, CA), 500 μ l CD11b (ICRF44)-PerCP/Cy5.5 (Biolegend) and 500 μ l CD7 (M-T701)-APC (BD Biosciences), for a final volume of 10 ml.

Subsequent steps were performed on an automation platform including an Agilent Bravo 96-channel pipetting robot, centrifuges, BioTek ELx405 96-channel aspirator/dispenser and Thermo Scientific MultiDrop dispenser. Twenty microliters of cells in the antibody cocktail were dispensed into every well of a 384-well plate using the MultiDrop. LegendScreen Human PE antibody screen plates (Biolegend) were rehydrated with the manufacturer-recommended 25 μ l of water. The screen consists of four 96-well plates; 5 μ l from each well were transferred to the single 384-well plate quadrant-wise. Staining reactions were incubated in the dark at room temperature for 30 minutes with 2.0 mm-radius orbital shaking, then washed three times with staining buffer and acquired on a BD LSR II with 405, 488 and 633 nm lasers using an HTS autosampler.

Files were gated and populations exported using a web-based, high-throughput cytometry analysis platform developed in our laboratory. Every well was manually gated by time to exclude anomalies caused by air bubbles or debris: temporal regions where the signal in the PE channel over time were inconsistent were excluded. Cell populations were then gated as described in the results section. Population gates were tailored to each species; tailoring to individual donors within a species was not necessary. The remaining analysis (statistics calculations, discordant replicate resolution, verification and reporting) was performed using Mathematica (Wolfram Research, Champaign, IL).

CyTOF mass cytometry

Purified antibodies (BioLegend, BD, Beckman Coulter) were conjugated to stable metal isotopes using commercially available polymeric chelators (“X8,” Fluidigm, Markham, Ontario, Canada).

Whole blood was lysed and prepared using the method of Chow and Hedley (6), which is better suited to high-throughput, plate-based assays than the Versalyse method described earlier. Briefly, we mixed 330 μ l of blood with 55 μ l of 16% PFA, incubated for 10 minutes, then added 1,615 μ l of 0.127% Triton-X100 (Fisher Scientific, Waltham, MA) in PBS (final concentration of 0.1% Triton) and incubated for 30 minutes. We then washed, stained with the metal-tagged antibodies for 30 minutes and washed again. Cells were subjected to a second fixation with 1.5% paraformaldehyde and DNA staining with iridium intercalator (Fluidigm), then acquired on a CyTOF 2 mass cytometer (Fluidigm).

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Declaration of interests: Z.B.B.H. is involved in the commercial development of the platform used to host the data presented here, although the data presented here will always be freely accessible.

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Table I: Summary of cell type-specific cross-reactivity. Clones were considered reactive if at least 10 percent of cells had a signal greater than the signal of the 95th percentile of corresponding isotype control or if they were manually classified as positive. Presence of letter in column indicates reactive staining detected in that cell population, absence of letter indicates clone is unreactive in that cell population. B: B cells, T: T cells, N: CD7+ NK cells, M: CD7- monocytes and dendritic cells, G: granulocytes, ?: inconclusive, insufficient number of cells acquired for a given population to make clear assessment. Two of each non-human primate species and one human were assayed.

Antibody	Clone	Human	Cyno	Rhesus	Baboon	AGM
CD1a	HI149	T G				
CD1b	SN13 (K5-1B8)	T G	M			T G
CD1c	L161	B M	B M	B M	B M	B
CD1d	51.1	B M				
CD2	RPA-2.10	TN	BTN	BTN	BTNM	BTN
CD3	HIT3a	T				
CD4	RPA-T4	T M	T			
CD5	UCHT2	BT G				
CD6	BL-CD6	BTN G				
CD7	CD7-6B7	TN				
CD8a	HIT8a	TN				
CD9	HI9a	?T?MG	BTNMG	BTNMG	BTNMG	BTNMG
CD10	HI10a	BTN G	MG	G	MG	G
CD11a	HI111	BTNMG	M			
CD11b	CBRM1/5	BT G				
CD11b	ICRF44	BTNMG	B NMG	BTNMG	B NMG	BTNMG
CD11c	3.9	B MG	B M	B	B M	
CD13	WM15	T MG				T MG
CD14	M5E2	BT MG	MG	MG		G
CD15	W6D3	MG				
CD16	3G8	BTNMG	TNM	TNM	TNM	N
CD18	TS1/18	BTNMG				
CD19	HIB19	B		B		
CD20	2H7	B	B	B	B N	B
CD21	Bu32	BT	B		B	B
CD22	HIB22	B				
CD23	EBVCS-5	?????				
CD24	ML5	BTNMG				
CD25	BC96	BTN			T	
CD26	BA5b	TN				
CD27	O323	BTN	BTN	BTN	BTNM	BTN
CD28	CD28.2	TN	T	T	T	T
CD29	TS2/16	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD30	BY88					
CD31	WM59	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD32	FUN-2	BTNMG	BT MG	BTNMG	BTNMG	B MG
CD33	WM53	NMG				
CD34	581					
CD35	E11	?????	BT MG	BT MG	BT MG	B G
CD36	5-271	BTNMG				
CD38	HIT2	BTNMG				
CD39	A1	BTNMG	BTNMG	MG	BTNMG	B MG
CD40	HB14	B M	B M	B	B	B M
CD41	HIP8	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD42b	HIP1	BTNMG				
CD43	CD43-10G7	BT?MG	T G		T	T MG
CD44	BJ18	BTNMG	BTNMG	BTNMG	BTNMG	BTN G
CD45	HI30	BT?MG				
CD45RA	HI100	BT?M				
CD45RB	MEM-55	BTNM	BTNMG	BTNM	BTNM	BTN
CD45RO	UCHL1	?????				
CD46	TRA-2-10	BTNMG				
CD47	CC2C6	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD48	BJ40	BTNMG	B M	B MG	B MG	BTNMG
CD49a	TS2/7	TNM	TN	T	T G	TN
CD49c	ASC-1	BTN				
CD49d	9F10	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD49e	NKI-SAM-1	TNMG	TNMG	TNMG	BTNMG	BTNMG
CD49f	GoH3	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD50	CBR-IC3/1	BTNMG	TNM		TNMG	
CD51	NKI-M9					
CD51,CD61	23C6	B M	T			M
CD52	HI186	BTNMG	M	M		
CD53	HI29	BTNMG				
CD54	HA58	BTNMG				
CD55	JS11	BTNMG	G		G	G
CD56	HCD56	N	NM	NM	NM	N

Antibody	Clone	Human	Cyno	Rhesus	Baboon	AGM
CD57	HCD57	N				
CD58	TS2/9	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD59	p282 (H19)	BTNMG	BTNMG	BTNMG	BTNMG	
CD61	VI-PL2	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD62E	HAE-1f					
CD62L	DREG-56	BTNMG				
CD62P	AK4					
CD63	H5C6	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD64	10.1	MG	MG	NMG	MG	MG
CD66a/b/c/e	TET2*	G	G	G	G	G
CD66a/c/e	ASL-32	G				
CD66b	G10F5	G				
CD69	FN50	TNM	TN	TN	G	B N
CD70	113-16	T				
CD71	CY1G4	BTNM				
CD73	AD2	BT G			TN	TN G
CD74	LN2	BT MG	B M	B MG	B M	BT MG
CD79b	CB3-1	BTNMG	T G	G	T G	T M
CD80	2D10	BTN				
CD81	5A6	BTNMG	T M	BT M	BT MG	BTNMG
CD82	ASL-24	BTNMG	T G		T G	T G
CD83	HB15e	T G		T	BT M	BT
CD84	CD84.1.21	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD85	17G10.2					
CD85	GHI/75	BTNMG				
CD85	MKT5.1	T MG	MG	MG	G	G
CD85d	42D1	T MG				
CD85h	24	MG		G		
CD85k	ZM4.1	M				
CD86	IT2.2	BT MG	B M	B MG	BT MG	BT MG
CD87	VIM5	T MG	MG	BTNMG	MG	NMG
CD88	S5/1	BTNMG			G	T
CD89	A59	NMG	NMG	NMG	MG	T G
CD90	5E10		BTNMG	BTNMG	BTNMG	BTNMG
CD93	VIMD2	M	MG	MG	TNMG	TNMG
CD94	DX22	TN	T G		T	T
CD95	DX2	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD96	NK92.39	TN G				
CD97	VIM3b	BTNMG		B		
CD99	HCD99	BT?MG	TN		T	T MG
CD100	A8	BTNMG	BTNM	BTNM	BTNMG	BTNM
CD101	BB27	BT MG	T MG	BTNMG	T MG	MG
CD102	CBR-IC2/2	BTNMG	BTNMG	BTNM	BTNM	BTNMG
CD103	Ber-ACT8					
CD104	58XB4					
CD105	43A3	NM				
CD106	STA					
CD107a	H4A3	BTNMG	T G	BTN	BT MG	BTNMG
CD108	MEM-150	B NM	B	B	B	B
CD109	W7C5	TNM	NM			TN
CD111	R1.302	N				
CD112	TX31	T MG				
CD114	LMM741	NMG	G			
CD115	9-4D2-1E4	G	G	TN G	M	T G
CD116	4H1	T MG				T
CD117	104D2	N				
CD119	GIR-208	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD122	TU27	TN	N	N	N	N
CD123	6H6	B NM				
CD124	G077F6	B				
CD126	UV4	TNMG	T M	T	T	T
CD127	A019D5	TN	T	T	TN	T
CD129	AH9R7	TN		TN		T G
CD131	1C1	MG	MG	MG	MG	G
CD132	TUGh4	BTNMG	MG	G		T MG
CD134	Ber-ACT35 (ACT35)	T				

Antibody	Clone	Human	Cyno	Rhesus	Baboon	AGM
CD135	BV10A4H2					
CD137	4B4-1					
CD138	DL-101	TNMG	T G		T MG	T G
CD140a	16A1					
CD140b	18A2	G	G		T G	T G
CD141	M80	MG			M	MG
CD143	5-369	T G				
CD144	BV9					
CD146	SHM-57	BTNMG	MG		BTNMG	T MG
CD148	A3	BTNMG				
CD150	A12 (7D4)	BT	BT	BT	BT	BT
CD152	L3D10					
CD154	24-31	BTNMG				
CD155	SKII.4	NMG				
CD156c	SHM14	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD158a/h	HP-MA4	N	N		N G	
CD158b	DX27	N				
CD158d	mAb 33 (33)	TN G				
CD158e1	DX9	N				
CD158f	UP-R1	T MG				
CD161	HP-3G10	TNMG	TN	TNM	TNMG	N
CD162	KPL-1	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD163	GHI/61	M	MG	MG		G
CD164	67D2	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD165	SN2 (N6-D11)	BTNMG	BTNMG	B	BTNMG	BTNMG
CD166	3A6	BTNMG	BTNMG	BT MG	BT MG	BTNMG
CD167a	51D6					
CD169	7-239					
CD170	1A5	NMG				
CD172a	SE5A5	T MG	T MG	TNMG	MG	MG
CD172b	B4B6	MG				
CD172g	LSB2.20	T G	MG	TNMG	TNMG	MG
CD178	NOK-1	G			MG	
CD179a	HSL96					T
CD179b	HSL11					BT M
CD180	MHR73-11	B M	B M	B M	B M	B M
CD181	8F1/CXCR1	TNMG				
CD182	5E8/CXCR2	NMG				
CD183	G025H7	BTNMG	BTNMG	BTNM	BTNMG	BTNMG
CD184	12G5	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD193	5E8	T M				
CD195	T21/8	BTNMG	T MG	MG	T M	T MG
CD196	G034E3	BTNM	BTN G	BTN	BTN	BTNMG
CD197	G043H7	BT G	BT	BT	BTN	BT
CD200	OX-104	BT G			BT	B
CD200R	OX-108	BTNMG			BT MG	
CD201	RCR-401					
CD202b	33.1 (Ab33)					
CD203c	NP4D6					
CD205	HD30	G	BT MG	T MG	T MG	TNMG
CD206	15-2					
CD207	10E2					
CD209	9E9A8					
CD210	3F9	BTNMG	BTNM	M	BTN	BTNMG
CD213a2	SHM38	T G		T		
CD215	JM7A4	T G	T G	TN G	T G	T G
CD218a	H44	TNMG	N	TN	TN	TN G
CD220	B6.220	M	MG	M	M	MG
CD221	1H7/CD221	TNMG	T MG	T	BTN G	N G
CD226	11A8	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD229	HLy-9.1.25	BTN				
CD231	SN1a (M3-3D9)	T G	G		T	T
CD235ab	HIR2					B
CD243	UIC2	BTNMG			T	T
CD244	C1.7	TNM				
CD245	DY12	TNMG	M		TN	M
CD252	11C3.1	G				T G
CD253	RIK-2	TN G				N
CD254	MIH24	G				
CD255	CARL-1				T	T
CD257	T7-241	BT MG	T G	TN G	T MG	BT MG
CD258	T5-39			TN		
CD261	DJR1	G	T			T
CD262	DJR2-4 (7-8)	T MG				

Antibody	Clone	Human	Cyno	Rhesus	Baboon	AGM
CD263	DJR3	G				
CD266	ITEM-1					T
CD267	1A1	B	B	B	B	B
CD268	11C1	BT	BT M	B	BT MG	BT MG
CD271	ME20.4	N	TNM	TNM	BTNM	TNM
CD272	MIH26	BT				G
CD273	24F.10C12	T G	TNM	TNM	T M	M
CD274	29E.2A3	BTNMG	T G	N G	T MG	BTNMG
CD275	9F.8A4	B				
CD276	MIH42	TN G	N	N		TN
CD277	BT3.1	BTNMG	B	B	BTNM	BTN
CD278	C398.4A	TN	T	T	T	T
CD279	EH12.2H7	T	T	T	T	T
CD282	TL2.1	B MG				
CD284	HTA125	T M				
CD286	TLR6.127	MG				
CD290	3C10C5	B			B	
CD294	BM16	MG				
CD298	LNH-94	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
CD300e	UP-H2	M				
CD300F	UP-D2	M				
CD301	H037G3	BT MG	T MG		BT MG	BT MG
CD303	201A					
CD304	12C2					
CD307e	509f6	B				
CD314	1D11	TN G	TN	TN	TN	N
CD317	RS38E	BTNMG	BTNMG	BTNM	BTNMG	BTNMG
CD318	CUB1					
CD319	162.1	BTNM	G			
CD324	67A4					
CD325	8C11	G				
CD326	9C4					
CD328	6-434	NMG				
CD334	4FR6D3					
CD335	9E2	N				
CD336	P44-8					
CD337	P30-15	N	N	N	N	N
CD338	5D3					
CD340	24D2					
CD344	CH3A4A7	TNMG				TN G
CD351	TX61	N G				
CD352	NT-7	BTNMG	T MG		T G	T
CD354	TREM-26	TNMG	MG	T G	MG	G
CD355	Cr24.1					
CD357	621	TNMG	T G	TN G	T G	T MG
CD360	2G1-K12	BTNM	BTNM	BTNM	BTNM	B NM
4-1BB Ligand	5F4	TNMG	T G	TN	T G	TNMG
C3aR	hC3aRZ8	TNMG				
CSL2	1D9-M12	TNMG	BTNMG	BTNMG	BTNMG	BTNMG
CCR10	6588-5	BTNMG	B MG	BTNMG	BT MG	BT MG
CLEC12A	50C1	MG				
CLEC9A	8F9					
CX3CR1	2A9-1	TNMG	MG	G		M
CXCR7	8F11-M16					
Delta Opioid Receptor	DOR7D2A4					
DLL1	MHD1-314					
DLL4	MHD4-46					
DR3	JD3					
EGFR	AY13					
erbB3	1B4C3					
FcRL4	413D12					
FcRL6	2H3	N				B
FcεRIα	AER-37 (CRA-1)	M	M	M	M	
Galectin-9	9M1-3	T G	BT G		BT G	T
GARP	7B11					
HLA-A2	BB7.2					
HLA-A,B,C	W6/32	BTNMG	BTNMG	BTNMG	BTNMG	BTNMG
HLA-DQ	HLADQ1	B				
HLA-DR	L243	BTNM	B MG	BTNMG	BTNMG	BTNMG
HLA-E	3D12	BTNMG				
HLA-G	87G		MG	MG		M
HVEM	122	BTNMG	BTNMG	BTNM	BTNMG	BTNMG
IFN-γ R b chain	2HUB-159	T MG	G	T	T	T MG

Antibody	Clone	Human	Cyno	Rhesus	Baboon	AGM
IgD	IA6-2	B				
IgG1, κ Isotype Ctrl	MOPC-21					
IgG1, κ Isotype Ctrl	RTK2071					
IgG2a, κ Isotype Ctrl	MOPC-173					
IgG2a, κ Isotype Ctrl	RTK2758					
IgG2b, κ Isotype Ctrl	MPC-11					
IgG2b, κ Isotype Ctrl	RTK4530					
IgG3, κ Isotype Ctrl	MG3-35					
IgG Isotype Ctrl	HTK888					
Ig light chain κ	MHK-49	B MG	G			
Ig light chain λ	MHL-38	B M	B MG	B MG	B MG	
IgM	MHM-88	B NMG	B MG	B NMG	BTNMG	BTNMG
IgM, κ Isotype Ctrl	MM-30					
IgM, κ Isotype Ctrl	RTK2118					
IL-28RA	MHLICR2a					
Integrin α9β1	Y9A2	MG	G	N	N	N G
integrin β5	AST-3T		G			
Integrin β7	FIB504	BTNMG	BTNM	BTNMG	BTNMG	BTNMG
Jagged 2	MHJ2-523	N				
LAP	TW4-6H10	M	MG		M	T M
Lymphotoxin β Receptor	31G4D8	M	M	M		BT MG
Mac-2	Gal397	T G	T G		T G	T G
MAIR-II	TX45	?????	T G	T	T	T G
MICA/MICB	6D4					
MSC	W7C6	T	T	T G	T M	T
MSC,NPC	W4A5	BT MG	T G	BTNMG	BT MG	BT MG
NKp80	5D12	N	TN	TNM	TNM	TN
Notch 1	MHN1-519	N				
Notch 2	MHN2-25	M				M
Notch3	MHN3-21					
Notch 4	MHN4-2					
NPC	57D2	?????				T
Podoplanin	NC-08					
Pre-BCR	HSL2	MG	M	M		
PSMA	LNI-17					
Siglec-10	5G6	B M				MG
Siglec-8	7C9	N				
Siglec-9	K8	NMG				
SSEA-1	MC-480	G				
SSEA-3	MC-631					B
SSEA-4	MC-813-70		MG	G	G	
SSEA-5	8E11	T				T
SUSD2	W3D5	B		TN		BT MG
SUSD2	W5C5	N	TNM		G	BTNMG
TCR gamma/delta	B1	T G	T	TN	TN	T
TCR Va24-Ja18	6B11					
TCR Va7.2	3C10	T				
TCR Vβ13.2	H132	?????				
TCR Vβ23	αHUT7					
TCR Vβ8	JR2 (JR.2)					
TCR Vβ9	MKB1					
TCR Vδ2	B6					
TCR α/β	IP26	T				
Tim-1	1D12	N G				
Tim-3	F38-2E2	N				
Tim-4	9F4					
TLT-2	MIH61	?????				
TNAP	W8B2	G	G		B MG	B G
TRA-1-60-R	TRA-1-60-R					
TRA-1-81	TRA-1-81	N				
TSLPR	1B4				M	
Vy9	B3					
β2-microglobulin	2M2	BT?MG	B N	B	B	B