

# HLDA10 Workshop – PART A

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HLDA10 is the tenth Human Leucocyte Differentiation Antigen (HLDA) Workshop. The HLDA Workshops provide a mechanism to allocate CD nomenclature through engaging in inter-laboratory studies. As the host laboratory, we invited researchers from national and international academic and commercial institutions to submit mAbs to human leucocyte surface membrane molecules, particularly those that recognised molecules to human myeloid cell populations and dendritic cells (DC). These mAbs were tested initially for activity and then distributed to 15 international laboratories to test the panel on different leucocyte populations. These populations included: blood dendritic cells, tonsil leucocytes, monocyte derived DC, CD34 derived DC, macrophage populations and diagnostic lymphoma and acute myeloid leukaemia samples. Each laboratory was provided with enough mAb to perform 5 repeat experiments. Submitted mAbs to some of the molecules were further validated to collate data required to designate a formal CD number. This collaborative process provides the broader scientific community with an invaluable data set validating mAbs to leucocyte surface molecules.

## THE PANEL

Code	Clone	Molecule	Label	Isotype	Provider
10-01	GE2	Clec7A/Dectin-1/BGR	purified	mouse IgG1	G. Brown
10-02	8F9	DNGR-1	purified	mouse IgG2aK	G. Brown
10-03	010e	CD1a	ascities	mouse IgG1	A. Bensussan
10-04	CL3	LPAP	purified	mouse IgG1	Filatov
10-05	7C5	unknown	S/N	mouse IgG1	H-J Buhring
10-06	FAB3480P	Clec2D/OCIL	PE labelled	mouse IgG1	R&D Systems
10-07	FAB17291P	Trem-2	PE labelled	rat IgG2b	R&D Systems
10-08	FMU-FAT-6	FAT1 cadherin	purified	mouse IgG1	B. Jin
10-09	9A11	Clec9A/DNGR	purified	mouse IgG1	C Reis e Sousa
10-10	0619	CD1a	ascities	mouse IgG2a	Bensussan
10-11	CL4	LPAP	purified	mouse IgG1	Filatov
10-12	58B1A2,	CD276 (NIH-3T3)	S/N	mouse IgG1	H-J Buhring
10-13	FAB1748P	Clec4A/DCIR	PE labelled	mouse IgG1	R&D Systems
10-14	FAB1750P	TIM-1	PE labelled	mouse IgG2b	R&D Systems
10-15	FAB812P	Fit-3/Flk-2	PE labelled	mouse IgG1	R&D Systems
10-16	FMU-FAT1-7	FAT1 cadherin	purified	mouse IgG1	B. Jin
10-17	HB3	CLEC12A/MICL	purified	mouse IgG1	R&D Systems
10-18	O249	CD1b	ascities	mouse IgG1	Bensussan
10-19	CL7	LPAP	purified	mouse IgG2a	Filatov
10-20	59A3B3,	unknown	S/N	mouse IgG1	H-J Buhring
10-21	FAB2806P	Clec4D/SF8	PE labelled	mouse IgG2b	R&D Systems
10-23	FMU-CRT-2	Calreticulin	purified	mouse IgG1	B. Jin
10-24	FAB2365P	TIM-3	PE labelled	rat IgG2a	R&D Systems
10-25	W4A5B3,	unknown	S/N	mouse IgG1	H-J Buhring
10-26	L161	CD1c	ascities	mouse IgG1	A. Bensussan
10-27	56A1C2	unknown	S/N	mouse IgG1	H-J Buhring
10-28	FAB238P	Clec5A/MDL-1	PE labelled	mouse IgG2b	R&D Systems
10-29	FMU-CRT-8	Calreticulin	purified	mouse IgG1	B. Jin
10-30	FMU-IL-13RA2-7	IL-13 Ra2	purified	mouse IgG1	B. Jin
10-31	FAB1900P	Clec5C/NKp80/KLRF1	PE labelled	rat IgG2a	R&D Systems
10-32	W5C4C5,	unknown	S/N	mouse IgG1	H-J Buhring
10-33	66E2D11,	unknown	S/N	mouse IgG1	H-J Buhring
10-34	BB27	CD101	ascities	mouse IgG1	A. Bensussan
10-35	FAB1859P	Clec7A/Dectin-1	PE labelled	mouse IgG2b	R&D Systems
10-36	FAB3479P	FPRL1/FPRL2	PE labelled	mouse IgG2b	R&D Systems
10-37	FMU-IL-13RA2-8	IL-13 Ra2	purified	mouse IgG1	R&D Systems
10-38	BGA69	unknown	ascities	mouse IgG1	B. Jin
10-39	F9-3C2F1,	unknown	S/N	mouse IgM	H-J Buhring
10-40	FAB1798P	Clec8A/LOX-1	PE labelled	mouse IgG2b	R&D Systems
10-41	FMU-IL-13RA2-14	IL-13 Ra2	purified	mouse IgG1	B. Jin
10-42	FMU-CRT-17	Calreticulin	purified	mouse IgM	B. Jin
10-43	DY12	CD245	purified	mouse IgG1	A. Bensussan
10-44	HEK5-1B3A3,	CD276	S/N	mouse IgG1	H-J Buhring
10-45	FAB6049P	Clec9A/DNGR	PE labelled	mouse IgG1	R&D Systems
10-46	IPS-K-2B6A8,	CD276	S/N	mouse IgG1	H-J Buhring
10-47	FAB3744P	FPRL1	PE labelled	mouse IgG2a	R&D Systems
10-48	DY35	CD245	ascities	mouse IgG1	A. Bensussan
10-49	58A2B11,	unknown	S/N	mouse IgG1	H-J Buhring
10-50	FAB154P	Axl	PE labelled	mouse IgG1	R&D Systems
10-51	FAB2946P	CLEC12A/MICL/CCL-1	PE labelled	mouse IgG2b	R&D Systems
10-52	FAB1517P	ULBP-3	PE labelled	mouse IgG2a	R&D Systems
10-53	AY19	IL-1RAcP	purified	mouse IgG1	B. Jin
10-54	FAB637P	Clec13A/CD302	PE labelled	mouse IgG1	R&D Systems
10-55	SC5	Vimentin	ascities	mouse IgM	A. Bensussan
10-56	FAB3131P	Tie-2	PE labelled	mouse IgG1	R&D Systems
10-57	FAB7436P	Clec14A	PE labelled	mouse IgG2b	R&D Systems
10-58	T-1A5	CD276	S/N	mouse IgG1	H-J Buhring
10-59	MDR64	unknown	ascities	mouse IgG1	A. Bensussan
10-61	ANC8D12	CD273, B7-DC	purified	mouse IgG1, K	Ansell
10-62	ANC8C9	GARP	purified	mouse IgG2a, K	Ansell
10-63	ANC10G10	GARP	purified	mouse IgG1, K	Ansell
10-64	MIH43	B7-H4	PE labelled	mouse IgG1, K	R&D Systems
10-65	8F9	Clec9A (DNGR1)	PE labelled	mouse IgG2a, K	R&D Systems
10-66	17G10.2	CD85g (ILT7)	PE labelled	mouse IgG1, K	R&D Systems
10-67	1D12	TIM-1	PE labelled	mouse IgG1, K	R&D Systems
10-68	1B4	TSLP-R	PE labelled	mouse IgG1, K	R&D Systems
10-69	CMRF-56	unknown	purified	mouse IgG1	D Hart
10-70	L4	P2X7	purified	mouse IgG2b	R Slutyer
10-71	111F8.04	DCIR	Alexa 488	mouse IgG1	Dendritics
10-72	9E8	Clec4A, (DCIR)	PE labelled	mouse IgG1, K	R&D Systems
10-73	50C1	Clec12A,	PE labelled	mouse IgG2a K	R&D Systems
10-74	24	CD85h (ILT1)	PE labelled	mouse IgG2b K	R&D Systems
10-75	F38-2E2	Tim-3	PE labelled	mouse IgG1 K	R&D Systems
10-76	HEK1/85	CCR5	PE labelled	rat IgG2a, K	R&D Systems
10-77	104A10.01	DORA	Alexa 488	mouse IgG1	Dendritics
10-78	9B9	Clec4D (Dectin-3)	PE labelled	mouse IgG2b K	R&D Systems
10-79	15 E 2	Clec7A (Dectin 1)	PE labelled	mouse IgG2a K	R&D Systems
10-80	TX45	MAIR II	PE labelled	mouse IgG1 K	R&D Systems
10-81	9F4	Tim-4	PE labelled	mouse IgG1 K	R&D Systems
10-82	CMRF-44	unknown	purified	mouse IgM	D Hart
10-83	118A8.05	DC-SIGN like	Alexa 488	mouse IgG1	Dendritics
10-84	36H2	DFD3	Alexa 488	rat IgG2a	Dendritics
10-85	CMRF-81	Tetanus toxoid		mouse IgG1, K	D Hart
10-86	EX32	Allergin	purified	mouse IgG2a	A Shibuya
10-87	EX33	Allergin	purified	mouse IgG2b	A Shibuya
10-88	Tx93	CD300H	Purified	mouse IgG2a	A Shibuya

## PARTICIPANTS

Analysis	Participant CI	Site
Blood DC	DCBTG	Sydney
Blood DC	Anna Brooks	Auckland, NZ
Blood DC	Muzzafilla Haniffa	Newcastle, UK
pDCs	Daniel Benítez Ribas	Barcelona, Spain
B cells subsets (tonsil and spleen)	Pablo Engel Rocamora	Barcelona, Spain
monocyte macrophage subtypes	Richard Kroccek	Berlin, Germany
monocyte macrophage subtypes	Hannes Stockinger	Vienna, Austria
MoDC	Julia Almeida	Salamanca, Spain
MoDC	Hans-Joerg Buhring	Tuebingen, Germany
CD34 derived LC	James Young	New York, USA
AML	DCBTG	Sydney
Langerhans Cells	Andrew Harman	Sydney
Lymphomas	Kenneth Lee	Sydney
Blood DC	Unable to be completed from 1 lab	
CD34 derived cells	Unable to be completed	
Lymphomas, histiocytosis	Unable to be completed	

## ANALYSIS

Flow cytometry was used to test for binding to:

1. Myeloid, B cell and Hodgkins derived cell lines
2. Transient transfectants expressing cDNA for antigen
3. Fresh blood DC and monocyte populations
4. In vitro derived cell populations
5. Acute myeloid leukaemia samples
6. Immunohistochemistry of paraffin sections

Results were presented by participating laboratories in tabular formats with examples of results.

Data was collated for consistent results.

FCS files were clustered using cytobank SPADE.

## DC PANEL BACKBONE FLOW CYTOMETRY

Marker and Fluorochrome	Clone, Species and Isotype	Provided by
<b>CD3 V450</b>	UCHT1 Ms IgG1, κ	560365 BD Horizon™
<b>CD20 V450</b>	L27 Ms IgG1, κ	642274 BD Horizon™
<b>CD19 V450</b>	HIB19 Ms IgG1, κ	560353 BD Horizon™
<b>CD56 V450</b>	B159 Ms IgG1, κ	560360 BD Horizon™
<b>CD16 V500</b>	3G8 Ms IgG1, κ	561394 BD Horizon™
<b>CD14 PerCPCy™5.5</b>	M5E2 Ms IgG2a, κ	550787 BD Pharmingen™
<b>CD11c AF700™</b>	B-ly6 Ms IgG1, κ	561352 BD Pharmingen™
<b>HLA-DR APC-H7</b>	L243 Ms IgG2a, κ	641393 BD Horizon™
<b>CD304 APC</b>	7G3 Ms IgG2a, κ	Miltenyi -Biotec
<b>CD1c FITC</b>	L161 Ms IgG1	Miltenyi –Biotec
<b>CD141 PEVio770</b>	AD5-4H12 Ms IgG1	Miltenyi -Biotec
<b>CD34 PE-CF594</b>	581 Ms IgG1, κ	562383 BD Horizon™