Novel Antagonistic and Agonistic Monoclonal Antibodies to CD6

Using Abeome's novel transgenic mouse antibody discovery platform, we have rapidly obtained functional, high affinity antibodies against human CD6. Transgenic mouse B-cells expressing affinity-matured anti-CD6 surface antibody were directly selected, and recombinant chimeric antibodies were immediately cloned and screened for CD6 binding. Novel antibodies were discovered which block the CD6-ALCAM interaction, the CD6-CD318 interaction, both, or neither. Many antibodies were able to decrease IFN-gamma and IL-10 secretion on PBMCs, whereas two antibodies appear to stimulate IFN-gamma and IL10- secretion. The further in vitro and in vivo evaluation of these lead molecules should support a valid clinical development path.

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Contents:

I. CD6

CD6 is a type I transmembrane glycoprotein found on the surface of mature T lymphocytes (1). Similar to CD28, CD6 stimulation enhances anti-CD3 induced T cell proliferation in vitro (2,3). The extracellular region of CD6 contains three domains that show structural homology to proteins of the scavenger receptor cysteine-rich (SRCR) superfamily (1). The membrane proximal Domain 3 includes the binding site for activated leucocyte cell adhesion molecule (ALCAM/CD166), a natural ligand of CD6 and part of the Ig superfamily (4). More recently, an additional CD6 ligand, CD318, has been discovered, which binds to the membrane distal Domain 1 (5,6).

Several genetic studies have linked CD6 gene polymorphisms to multiple sclerosis (7,8). In addition, CD6 may play a key role in psoriasis, rheumatoid arthritis, and Sjogren's syndrome (9-12). Thus, CD6 represents a potentially attractive cell surface target for many T cell driven autoimmune diseases.

II. Abeome Antibody Discovery Platform: AbeoMouse™

We have developed a novel transgenic mouse system (AbeoMouse™) allowing for the direct selection of antigen-specific B-cells, paired with single-cell antibody gene cloning and screening. The AbeoMouse™ produces a 45-fold increase in surface immunoglobulin (Ig) positive antibody secreting cells and an accelerated immune response. Abeome's screening platform allows 1,000 times more affinity matured monoclonal antibodies to be isolated from a single AbeoMouse™ than by conventional technology. In contrast to other current antibody technologies, this platform allows for the enrichment and rapid cloning of specific, high-affinity chimeric antibodies against a target of interest. With this modular system, cloned variable regions (V-regions) may be swapped between multiple human Ig isotypes for empirical comparison of stability, affinity and functional potency, or to suit the specific therapeutic modality or effector function.

Specifically, the transgenic AbeoMouse™ has been engineered to constitutively express multiple genes, including the Igα/Igβ B-cell receptor proteins, resulting in a hyper immune response and surface antibody expression during all stages of B cell differentiation (Fig.1). This enables the selection and sorting of antigen specific B-cells producing the most affinity matured antibodies, and this technology platform has been applied to obtain antibodies against a diverse set of antigens, including but not limited to whole cells, peptides, glycoproteins, viral envelope proteins and mouse proteins, typically producing chimeric leads with low picomolar dissociation constants.

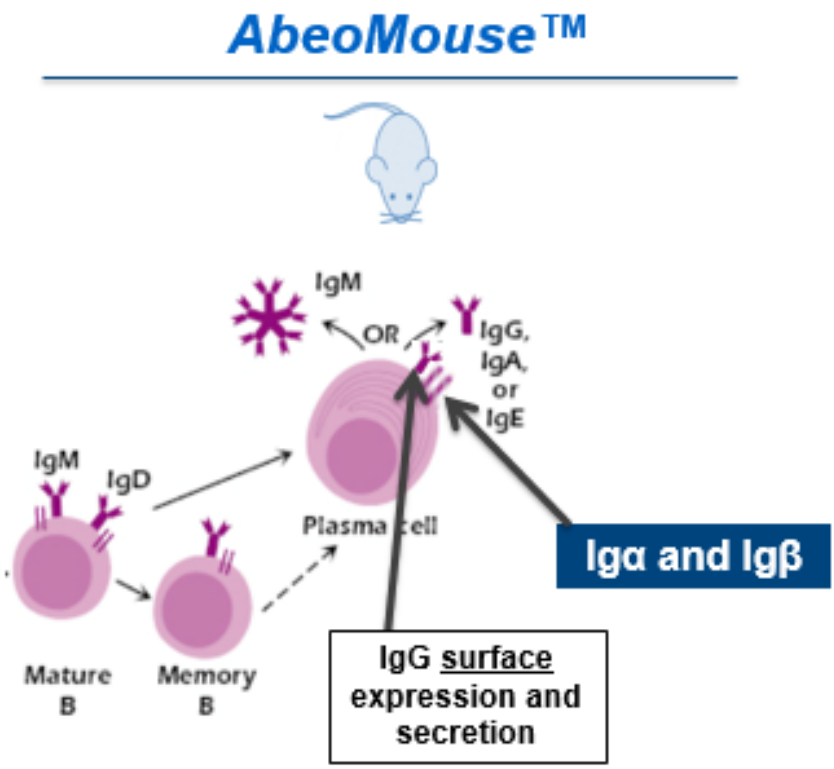


FIGURE 1. The transgenic AbeoMouse™ platform.
A novel antibody discovery platform that generates mature B cells with high surface IgG expression, allowing for the direct selection and cloning of antigen-specific B cells

III. Immunization With Human CD6 Extracellular Domain

Six AbeoMice™ 11-14 weeks of age were pre-bled to obtain baseline serum antibody levels and immunized subcutaneously or intraperitoneally (SQ or IP) with recombinant human CD6 with a C-terminal Fc tag (Met1-Glu398; Sino Biological) in a proprietary adjuvant. Booster injections were given every 14 days. Blood samples were taken and serum titers determined 7 days after each booster injection (Fig. 2, Table 1).

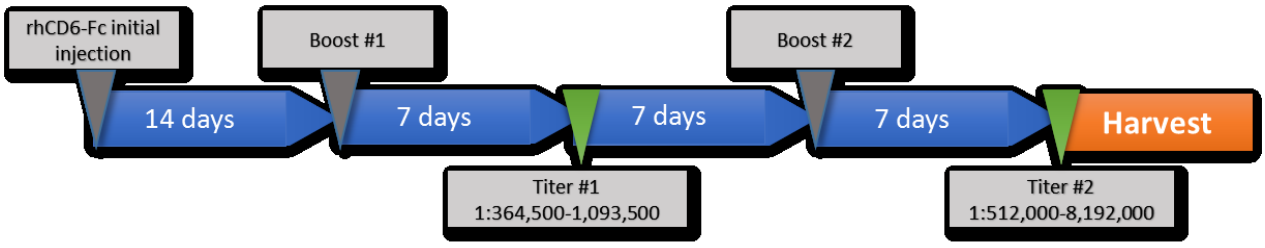


FIGURE 2. Immunization timeline for generating anti-CD6 antibodies

Table 1. Immunization and titer summary of mice immunized with CD6.

CD6 Immunization Summary											
					Injections				Titers		
Mouse	Protein	Sex	DOB	Age at Start	Route	Route	Route	Route			
					Initial	Boost	Boost	Boost 3	Titer 1	Titer 2	Titer 3
8571	CD6 - FC Tag	F	6/12/2018	14w	IP	IP	IP	NA	1,093,500	8,192,000	NA
4622	CD6 - FC Tag	M	7/3/2018	11w	IP	IP	IP	IP	1,093,500	2,048,000	NA
4065	CD6 - FC Tag	F	6/26/2018	12w	IP	IP	IP	IP	364,500	2,048,000	NA
4071	CD6 - FC Tag	M	6/26/2018	12w	SQ	SQ	SQ	NA	1,093,500	2,048,000	NA
4614	CD6 - FC Tag	F	7/3/2018	11w	SQ	SQ	SQ	SQ	364,500	512,000	512,000

IV. Harvest and Single Antigen-Positive Cell Sorting (Mouse #8571)

As a representative example of this campaign, mouse 8571 yielded 28 lymphoid tissue samples and bone marrow (Figure 3) which were harvested, processed, and pooled into a single suspension of lymphoid cells (8.9 x 10^8 cells). Erythrocytes were removed and resulting lymphocytes were depleted of cells expressing IgM antibodies by immuno-magnetic separation.

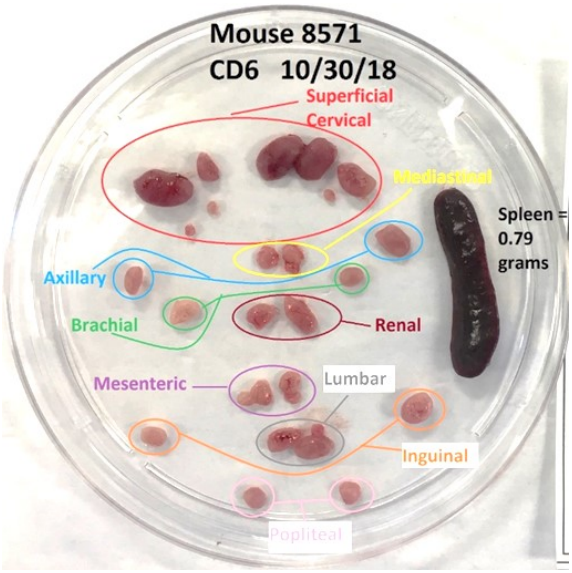


FIGURE 3. Lymphoid organs harvested from mouse 8571 and processed into a single cell suspension for FACS (fluorescent activated cell sorting). Abeome's transgenic mice have an enhanced immune response, greatly enlarged lymphoid organs (left), and typically an order of magnitude increase in the number of plasmacytes.

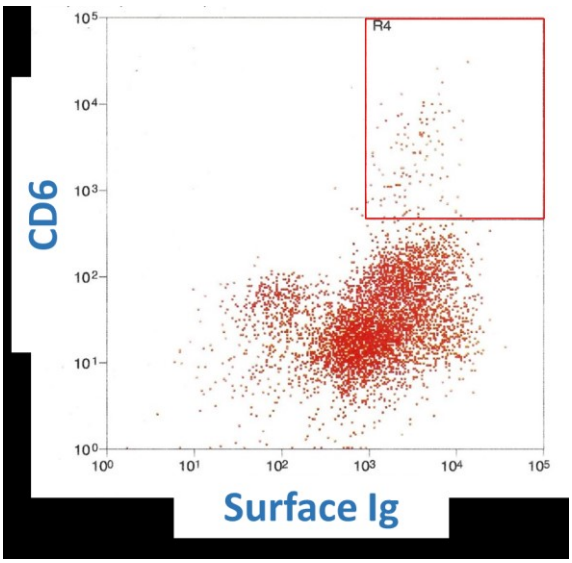


FIGURE 4. Single cell FACS sorting of lymphocytes expressing antibody to human CD6
The direct selection of cells surface-expressing anti-CD6 antibodies is accomplished by staining harvested B cells with fluor-labeled recombinant human CD6 and a polyclonal antibody recognizing mouse surface immunoglobulin. Double-positive cells (red box) are sorted at 1 cell/well into 96 well plates for RT-PCR and cloning.

V. High-Throughput Screening of CD6 Antibodies

After cell sorting, thirteen 96-well plates of single B cells, comprising over 1,000 antibodies, were subjected to nested RT-PCR using heavy and light chain variable region specific primer sets. Amplified V-regions were then fused with mammalian expression promoters and human Fc chains (IgG4 and kappa constant regions) by overlap PCR, generating transcriptionally-active PCR products. These individual paired heavy and light chain PCR products were transfected into HEK293 cells to generate supernatants containing secreted chimeric antibodies and which were subsequently screened for binding to CD6-Fc by ELISA. A representative set of screening data is shown (Fig. 5), which identified many antibodies positive for CD6 binding, but did not bind to a control human Fc protein. In total, we isolated and cloned nineteen chimeric monoclonal antibodies that bind strongly to human CD6. Binding of the chimeric monoclonal antibodies, designated ABM417-435, to human CD6 is shown in Figure 6.

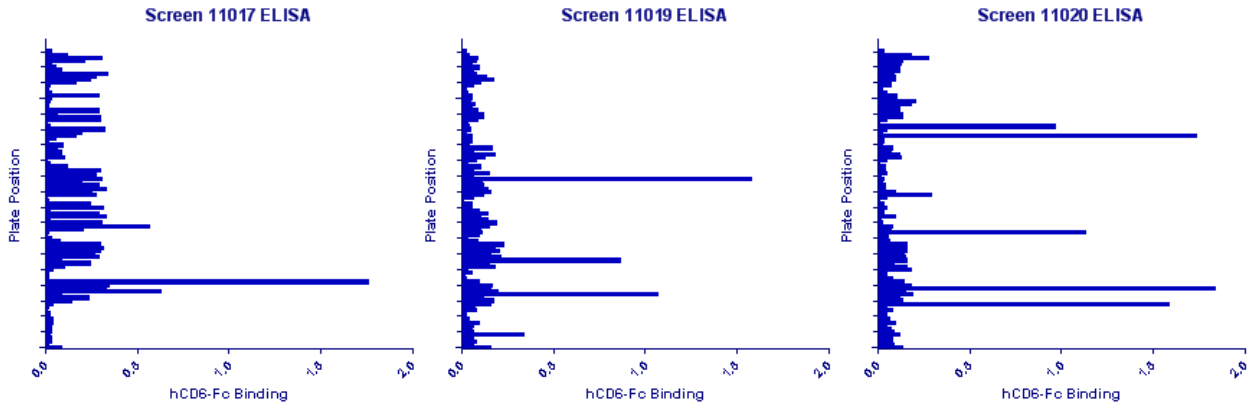


FIGURE 5. Representative ELISA screening data for anti-CD6 chimeric antibodies. Representative data from high-throughput screening of >1,000 novel antibodies revealed many candidates with strong binding to human CD6.

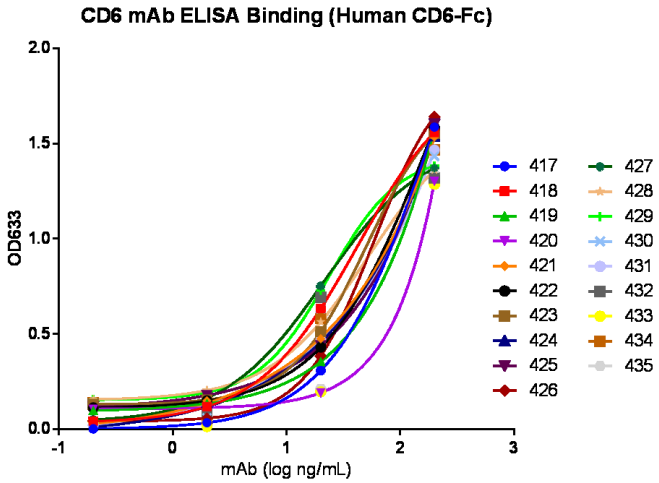


FIGURE 6. ELISA Binding of anti-CD6 Chimeric Monoclonal Antibodies

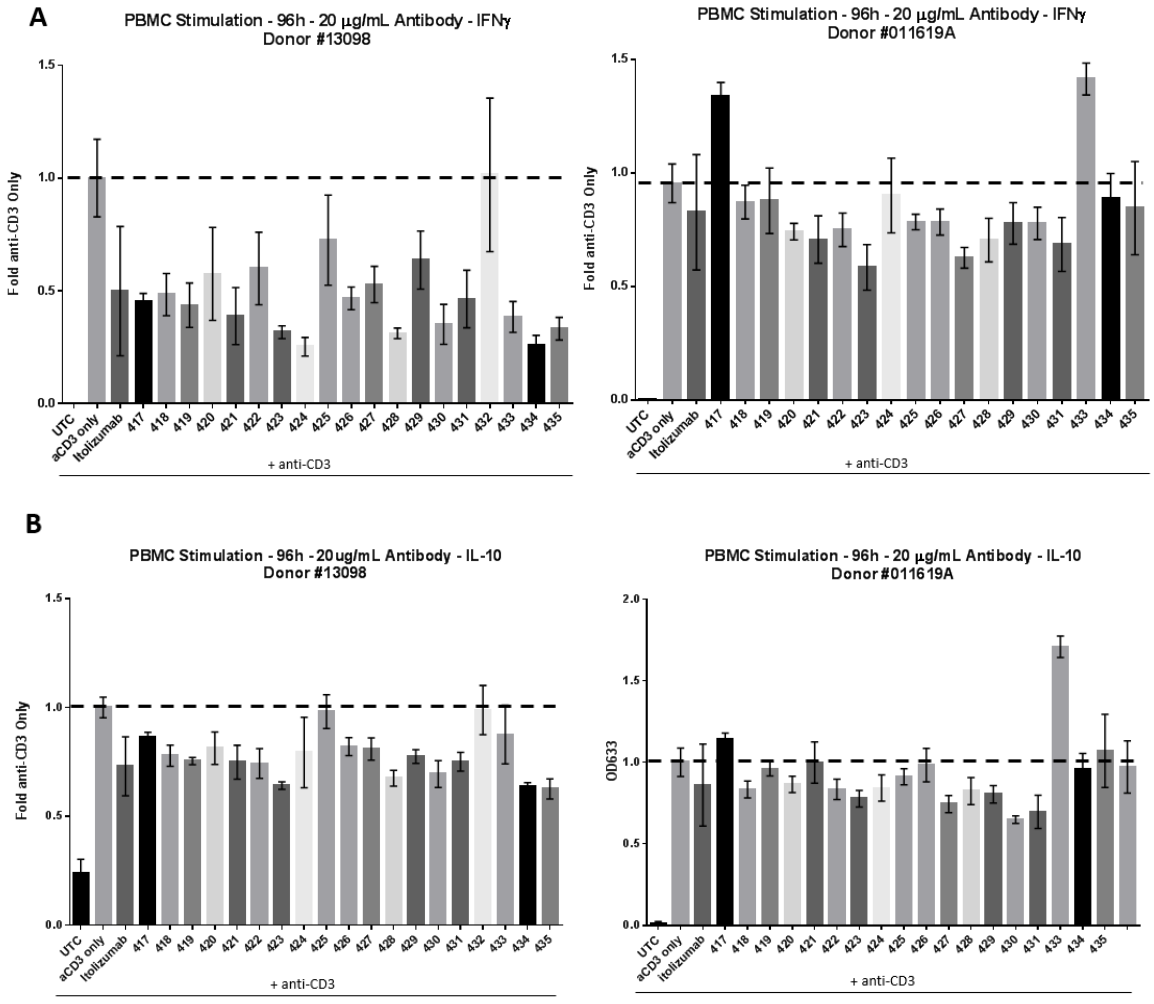
VI. Identification of anti-CD6 mAbs with Functional Properties

Human PBMC can be stimulated by immobilized anti-CD3 to produce IFNγ and IL-10 in the presence or absence of exogenous ALCAM co-stimulation (13). When treated with Itolizumab, an anti-CD6 humanized antibody, IFNγ and IL-10 levels from stimulated PBMC were reduced by 48% and 25%, respectively. In addition, in a PBMC stimulation model of Th17 polarization, treatment with Itolizumab reduced the surface expression of CD25 on both Th17 polarized and non-polarized, co-stimulated T cells (12). Based on these published studies, we hypothesized that agonistic or antagonistic anti-CD6 antibodies would modulate cytokine levels in PBMC cultures in the context of anti-CD3 activation.

To test this hypothesis, we stimulated PBMCs from two donors with plate-bound anti-CD3

(OKT3) in the presence of soluble or bead-bound anti-CD6 antibodies. At 96 hours, the culture supernatant was harvested and tested for IFNγ and IL-10 by ELISA. When anti-CD6 antibodies were present in soluble form (Fig. 7A, 7B), several of these showed antagonistic (e.g., ABM423, ABM424) activity on anti-CD3 activation of PBMCs. In contrast, two antibodies, ABM417 and ABM433, appeared to show agonistic or co-stimulatory activity with anti-CD3 with the second donor when treated in soluble form (Fig. 7A, right panel) or bead-bound presentation (Fig. 7C).

VII. CD6 Ligand Competition Studies



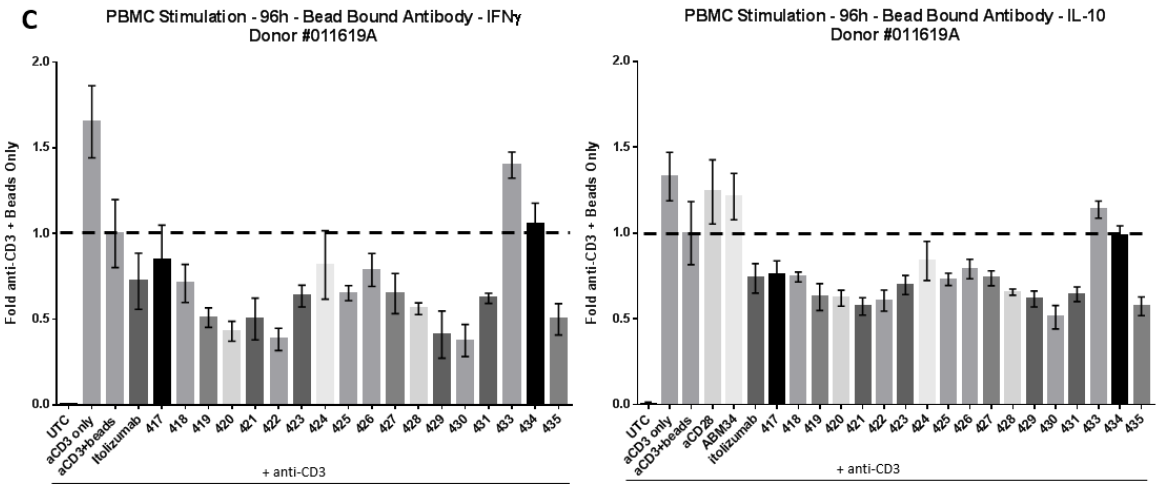


FIGURE 7. Modulation of PBMC activation by novel anti-CD6 antibodies

PBMCs from two donors were activated with plate-bound anti-CD3 for 96 hours, in presence or absence of anti-CD6 antibodies. At 96 hours, supernatants were assayed for IFNγ and IL-10 levels. A) IFNγ levels and B) IL-10 levels expressed as fold change from anti-CD3 only control in two different PBMC donors treated with soluble anti-CD6 antibodies. Panel C) IFNγ and IL-10 levels from one donor stimulated with anti-CD3 and treated with bead-bound anti-CD6 antibodies.

ABM423 most potently decreased IL10 and IFN-gamma secretion in PBMCs, and was humanized into ABM423.1. By ELISA ABM423.1 retained 100% of the binding activity of ABM423. Both ABM423 and ABM423.1 were retested for inhibition, while ABM433 was retested for activation using the same donor (Donor #011619A).

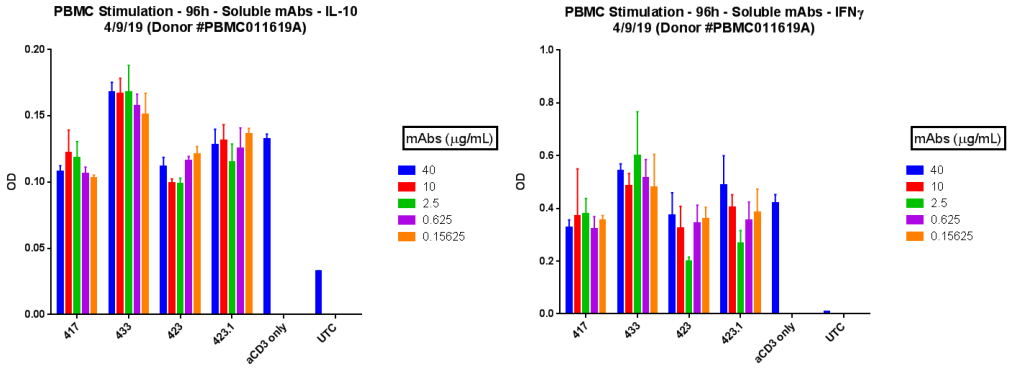


FIGURE 8. Modulation of PBMC activation by novel anti-CD6

In a replication experiment, PBMCs from donor 2 were activated with plate-bound anti-CD3 for 96 hours, in presence or absence of anti-CD6 antibodies. At 96 hours, supernatants were assayed for IFNγ and IL-10 levels. A) IL-10 levels and B) IFNγ levels expressed as fold change from anti-CD3 only control.

ABM433 continues to trend with an increase in cytokine secretion suggesting possible agonistic activity, while ABM423 and ABM423.1 show decreased cytokine secretion suggestive of antagonistic activity.

Antibody	Same Bin as ITZ?	Blocks ALCAM-CD6?	Blocks CD318-CD6?	Donor 1 PBMC IFNγ	Donor 1 PBMC IL-10	Donor 2 PBMC IFNg	Donor 2 PBMC IL-10	Donor 2 Bead-mAb PBMC IFNγ	Donor 2 Bead-mAb PBMC IL-10	KD-Human CD6
ABM417	YES	NO	YES	Decreased	Decreased	Increased	Increased	No Change	Decreased	107 pM
ABM418	YES	NO	YES	No Change	Decreased	No Change	No Change	No Change	Decreased	137 pM
ABM419	NO	NO	NO	No Change	Decreased	No Change	No Change	Decreased	Decreased	298 pM
ABM420	NO	NO	NO	No Change	Decreased	No Change	No Change	Decreased	Decreased	608 pM
ABM421	YES	NO	NO	No Change	Decreased	No Change	No Change	Decreased	Decreased	3.4 pM
ABM422	YES	NO	NO	No Change	Decreased	No Change	Decreased	Decreased	Decreased	1492 pM
ABM423	NO	YES	YES	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased	492 pM
ABM424	NO	YES	YES	Decreased	Decreased	No Change	No Change	Decreased	Decreased	<1 pM
ABM425	NO	NO	YES	No Change	No Change	Decreased	No Change	Decreased	Decreased	1159 pM
ABM426	NO	NO	YES	No Change	No Change	Decreased	No Change	Decreased	Decreased	20.9 pM
ABM427	NO	NO	YES	No Change	Decreased	Decreased	Decreased	Decreased	Decreased	492 pM
ABM428	NO	NO	YES	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased	658 pM
ABM429	NO	NO	YES	No Change	Decreased	Decreased	Decreased	Decreased	Decreased	813 pM
ABM430	NO	NO	YES	No Change	Decreased	Decreased	Decreased	Decreased	Decreased	1146 pM
ABM431	NO	NO	YES	No Change	Decreased	Decreased	Decreased	Decreased	Decreased	2016 pM
ABM432	NO	NO	YES	No Change	No Change	Not Tested	Not Tested	Not Tested	Not Tested	978 pM
ABM433	NO	YES	NO	Decreased	No Change	Increased	Increased	Increased	Increased	4921 pM
ABM434	NO	YES	NO	Decreased	Decreased	No Change	No Change	No Change	No Change	1597 pM
ABM435	NO	NO	NO	Decreased	Decreased	No Change	No Change	Decreased	Decreased	2130 pM
ITZ-G4	N/A	YES	YES	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased	2367 pM

SUMMARY TABLE

A total of 19 antibodies were tested for their binning with Itolizumab, blocking of the CD6-ALCAM interaction, blocking of the CD6-CD318 interaction, and of their ability to increase or decrease cytokine secretion when used to treat PBMCs. As summarized above, a variety of binding characteristics, affinities and activities on PBMCs were revealed.

References:

1. Aruffo A, Melnick MB, Linsley PS, Seed B. The lymphocyte glycoprotein CD6 contains a repeated domain structure characteristic of a new family of cell surface and secreted proteins. *J Exp Med*. 1991 Oct 1;174(4):949-52.
2. Zimmerman AW, Joosten B, Torensma R, Parnes JR, van Leeuwen FN, Figdor CG. Long-term engagement of CD6 and ALCAM is essential for T-cell proliferation induced by dendritic cells. *Blood*. 2006 Apr 15;107(8):3212-20.
3. Ibáñez A, Sarrias MR, Farnós M, Gimferrer I, Serra-Pagès C, Vives J, Lozano F. Mitogen-activated protein kinase pathway activation by the CD6 lymphocyte surface receptor. *J Immunol.* 2006 Jul 15;177(2):1152-9.
4. Whitney GS, Starling GC, Bowen MA, Modrell B, Siadak AW, Aruffo A. The membrane-proximal scavenger receptor cysteine-rich domain of CD6 contains the activated leukocyte cell adhesion molecule binding site. *J Biol Chem.* 1995 Aug 4;270(31):18187-90.
5. Saifullah MK, Fox DA, Sarkar S, Abidi SM, Endres J, Piktel J, Haqqi TM, Singer NG. Expression and characterization of a novel CD6 ligand in cells derived from joint and epithelial tissues. *J Immunol.* 2004 Nov 15;173(10):6125-33.
6. Enyindah-Asonye G, Li Y1, Ruth JH, Spassov DS, Hebron KE, Zijlstra A, Moasser MM, Wang B, Singer NG, Cui H, Ohara RA, Rasmussen SM, Fox DA, Lin F. CD318 is a ligand for CD6. *Proc Natl Acad Sci* U S A. 2017 Aug 15;114(33):E6912-E6921.
7. International Multiple Sclerosis Genetics Consortium. The genetic association of variants in CD6, TNFRSF1A and IRF8 to multiple sclerosis: a multicenter case-control study. *PLoS One*. 2011 Apr 28;6(4):e18813.
8. Swaminathan B, Matesanz F, Cavanillas ML, Alloza I, Otaegui D, Olascoaga J, Cénit MC, de las Heras V, Barcina MG, Arroyo R, Alcina A, Fernandez O, Antigüedad A, Urcelay E, Vandenbroeck K. Validation of the CD6 and TNFRSF1A loci as risk factors for multiple sclerosis in Spain. *J Neuroimmunol.* 2010 Jun;223(1-2):100-3.

9. Kofler DM, Farkas A, von Bergwelt-Baildon M, Hafler DA. The Link Between CD6 and Autoimmunity: Genetic and Cellular Associations. *Curr Drug Targets*. 2016;17(6):651-65.
10. Krintel SB, Essioux L, Wool A, Johansen JS, Schreiber E, Zekharya T, Akiva P, Ostergaard M, Hetland ML. CD6 and syntaxin binding protein 6 variants and response to tumor necrosis factor alpha inhibitors in Danish patients with rheumatoid arthritis. *PLoS One.* 2012;7(6):e38539.
11. Ramos-Casals M, Font J, García-Carrasco M, Calvo J, Places L, Padilla O, Cervera R, Bowen MA, Lozano F, Ingelmo M. High circulating levels of soluble scavenger receptors (sCD5 and sCD6) in patients with primary Sjögren's syndrome. *Rheumatology* (Oxford). 2001 Sep;40(9):1056-9.
12. Bughani U, Saha A, Kuriakose A, Nair R, Sadashivarao RB, Venkataraman R, Patel S, Deshchougule AT, S SK, Montero E, Pai HV, Palanivelu DV, Melarkode R, Nair P. T cell activation and differentiation is modulated by a CD6 domain 1 antibody Itolizumab. *PLoS One*. 2017 Jul 3;12(7):e0180088.
13. Nair P, Melarkode R, Rajkumar D, Montero E. CD6 synergistic co-stimulation promoting proinflammatory response is modulated without interfering with the activated leucocyte cell adhesion molecule interaction. *Clin Exp Immunol*. 2010 Oct;162(1):116-30.