Novel Monoclonal Antibodies to CTLA-4

Using Abeome's novel transgenic mouse antibody discovery platform, we have rapidly obtained functional, high affinity antibodies against human CTLA-4. Transgenic mouse B-cells expressing affinity-matured anti-CTLA-4 surface antibody were directly selected, and recombinant chimeric antibodies were immediately cloned and screened for CTLA-4 binding. Novel antibodies were discovered which block the CTLA-4-CD80 interaction, Further in vitro and in vivo evaluation of these lead molecules should support a valid clinical development path.

For Licensing Information, Please Contact:

Rick Shimkets
rick.shimkets@abeomecorp.com

Kirby Alton
kirby.alton@abeomecorp.com

Laboratories
150 Ben Burton Rd
Bogart, GA 30622
T- (706)850-2200

Contents:

I. CTLA-4

CTLA4 or CTLA-4, also known as CD152, is a protein receptor that functions as an immune checkpoint and downregulates immune responses. CTLA-4 is constitutively expressed in regulatory T cells but only upregulated in conventional T cells after activation – a phenomenon which is particularly notable in cancers.

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.

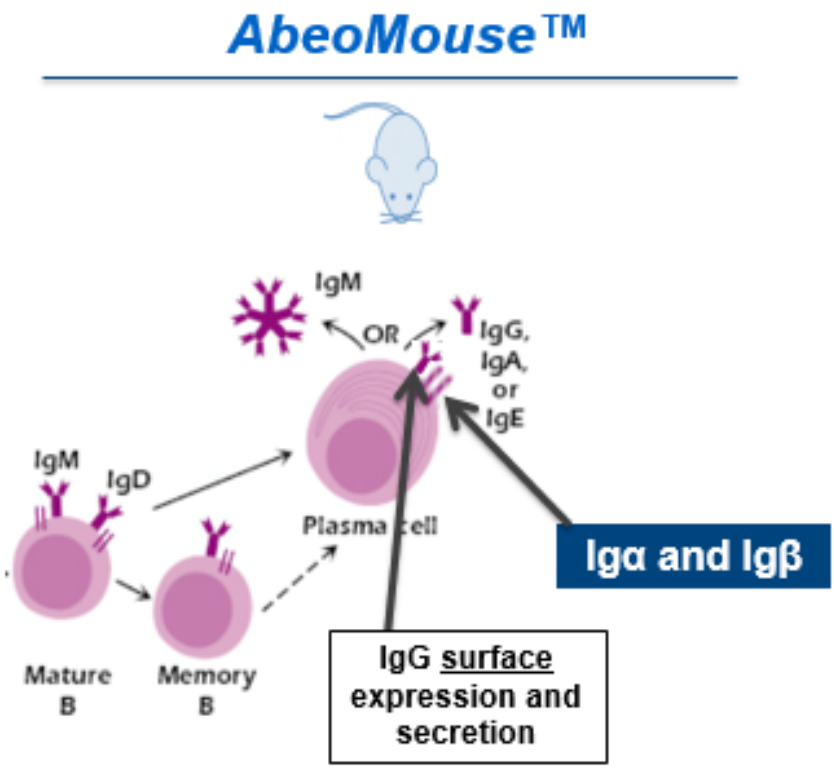

FIGURE 1. The transgenic AbeoMouse™ platform.
A novel antibody discovery platform that generates mature B cells with high surface IgG expression, allowing for the direct selection and cloning of antigen-specific B cells

III. Immunization With Human CTLA-4 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 CTLA-4 with a C-terminal Fc tag 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).

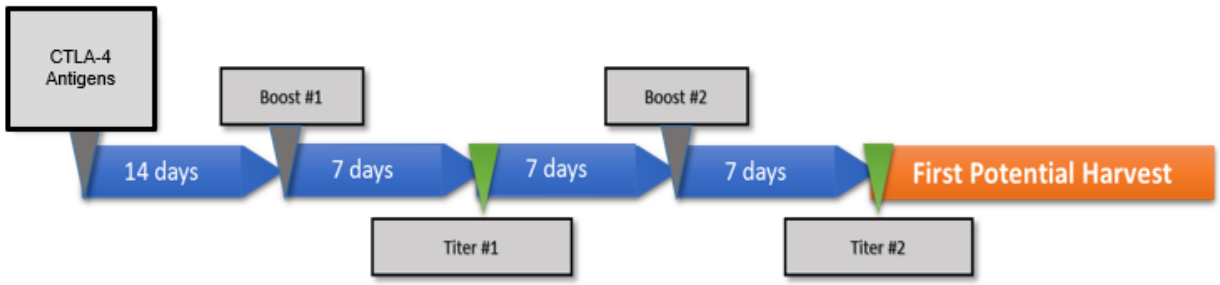

FIGURE 2. Immunization timeline for generating anti-CTLA-4 antibodies

Table 1. Immunization and titer summary of mice immunized with CTLA-4.

Six AbeoMice were immunized and the final titers of the 4 harvested were as follows:

4214 -- 1:128,000
5942 -- 1:128,000
4215 -- 1:512,000
5951 -- 1:128,000

IV. Harvest and Single Antigen-Positive Cell Sorting

AbeoMice yielded 20-35 lymphoid tissue samples each plus bone marrow (Figure 3) which were harvested, processed, and pooled into a single suspension of lymphoid cells. Erythrocytes were removed and resulting lymphocytes were depleted of cells expressing IgM antibodies by immuno-magnetic separation.

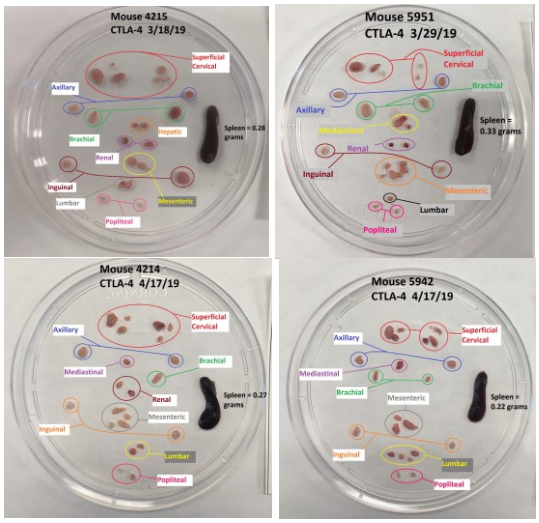

FIGURE 3. Lymphoid organs harvested from AbeomMice and processed into a single cell suspension for FACS (fluorescent activated cell sorting). Abeome's transgenic mice have an enhanced immune response, greatly enlarged lymphoid organs (left), and typically an order of magnitude increase in the number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

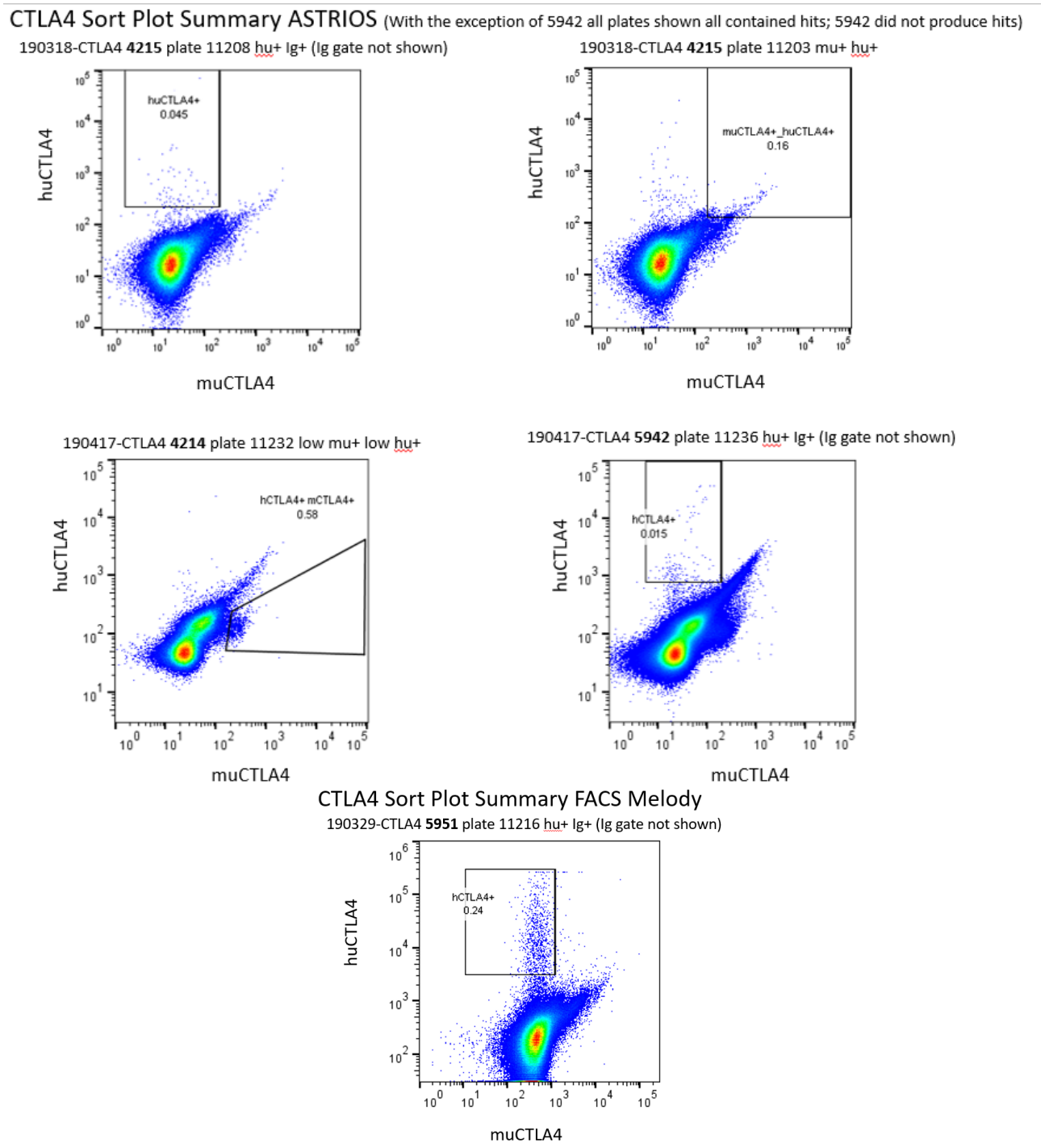

FIGURE 4. Single cell FACS sorting of lymphocytes expressing antibody to human CTLA-4
The direct selection of cells surface-expressing anti-CTLA-4 antibodies is accomplished by staining harvested B cells with fluor-labeled recombinant human CTLA-4 and a polyclonal antibody recognizing mouse surface immunoglobulin. Double-positive cells (black box) are sorted at 2 cells/well into 96 well plates for RT-PCR and cloning.

V. High-Throughput Screening of CTLA-4 Antibodies

After cell sorting, thirty 96-well plates of single B cells, comprising over 3,000 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 CTLA-4-HIS by ELISA. A representative set of screening data is shown (Fig. 5), which identified many antibodies positive for CTLA-4 binding, but did not bind to a control human Fc protein. In total, we isolated and cloned 26 monoclonal antibodies that bind strongly to human CTLA-4. Binding of the chimeric monoclonal antibodies, designated ABM467-493, to human CTLA-4 is shown in Figure 6. (ABM463 is an internal clinical control).

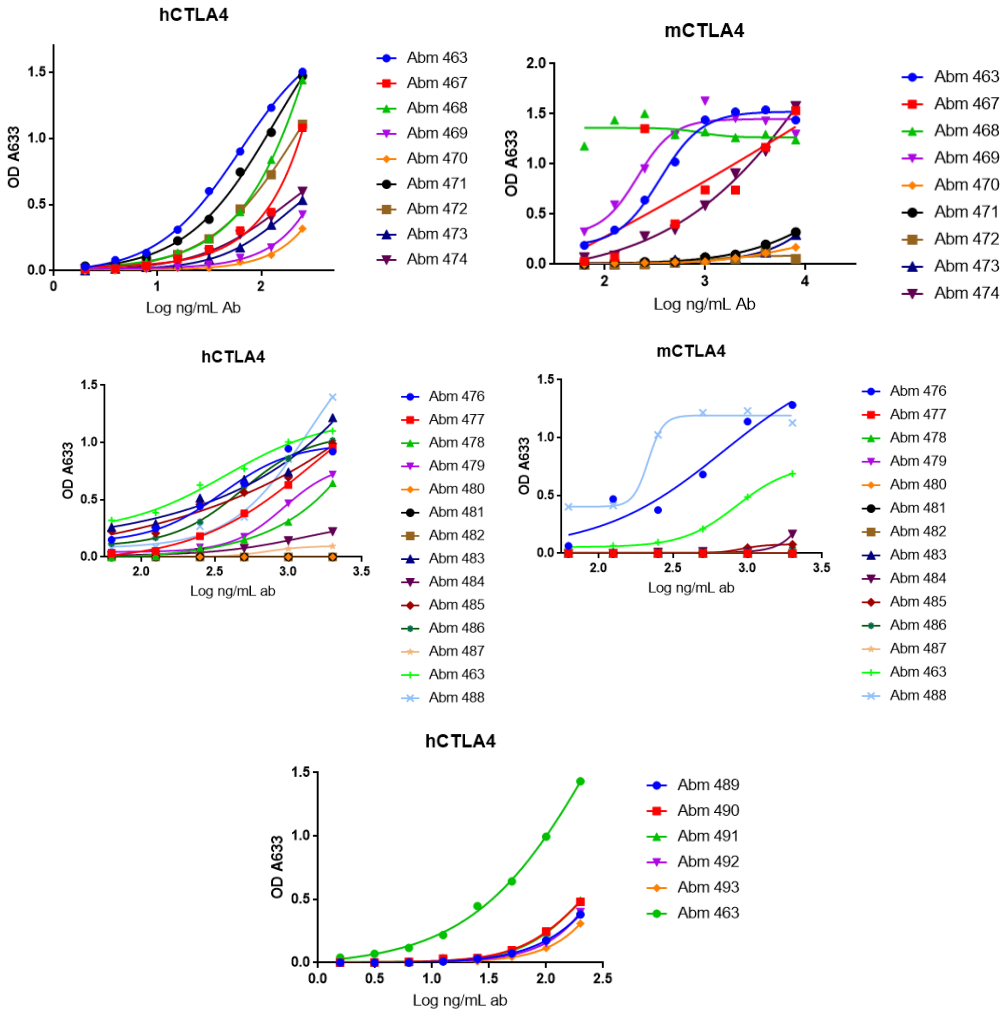

FIGURE 5. ELISA Binding of anti-CTLA-4 Chimeric Monoclonal Antibodies
Human CTLA-4 on left and Mouse on right.

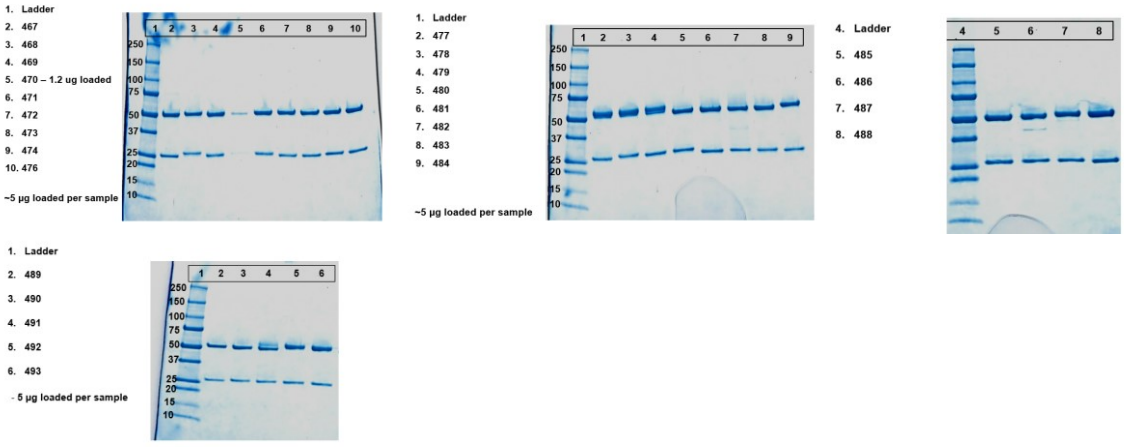

FIGURE 6. Denaturing Gel electrophoresis of chimeric antibodies

Antibodies were run on denaturing gels. ABM470 expressed poorly in 2 attempts. Extra banding in the heavy chains of ABM477, 478, 479, and 491 are noted. The heavy chains of these antibodies contain an N-glycosylation site in CDR2 as a result of somatic hypermutation changing a serine to an asparagine, and thus, the extra bands likely result from different glycosylation forms.

VI. Identification of anti-CTLA-4 mAbs with Functional Properties

A 2-cell assay system was employed to test whether any of the antibodies neutralized the interaction between CTLA-4 and CD80 (Promega J1631):

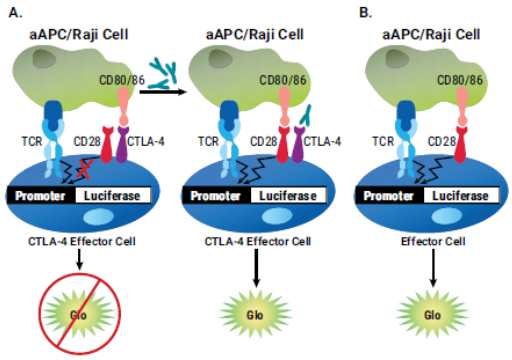

Figure 1. Representation of the CTLA-4 Blockade Bioassay. The bioassay consists of two genetically engineered cell lines, CTLA-4 Effector Cells and aAPC/Raji Cells. **Panel A.** When co-cultured, the CTLA-4/CD80 and CD86 interaction inhibits CD28 pathway activated luminescence. The addition of anti-CTLA-4 antibody blocks the CTLA-4/CD80 and CD86 interaction, thereby re-establishing CD28 pathway activated luminescence, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer. **Panel B.** When co-cultured with non-CTLA-4-expressing Effector Cells (Cat.# J1631), activation also induces luminescence by activation of the CD28 pathway but in a manner independent of anti-CTLA-4 antibody.

FIGURE 7. Human CTLA-4 Blockade Bioassay Mechanism

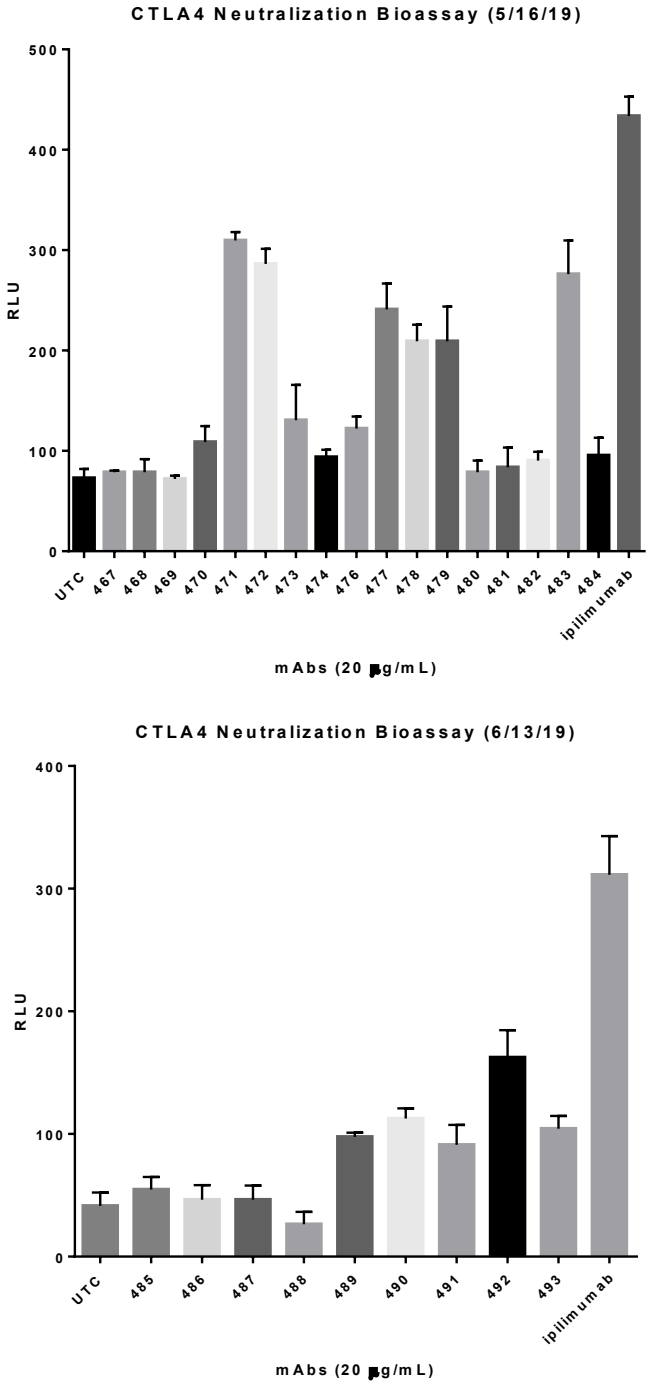

FIGURE 8. Testing functional blockade of human CTLA-4

Eleven Abeome antibodies tested demonstrated neutralization of CTLA-4 in the 2-cell assay at 20 μg/ml.

Table 2. Summary of characterized chimeric antibodies

Number	Human Binding	Mouse Binding	Functional neutralization
ABM467	Yes	Yes	No
ABM468	Yes	Yes	No
ABM469	Yes	Yes	No
ABM470	Yes	Weak	No
ABM471	Yes	Weak	Yes
ABM472	Yes	No	Yes
ABM473	Yes	Weak	No
ABM474	Yes	Yes	No
ABM476	Yes	Yes	No
ABM477	Yes	No	Yes
ABM478	Yes	Yes	Yes
ABM479	Yes	No	Yes
ABM480	Weak	No	No
ABM481	Yes	No	No
ABM482	Weak	No	No
ABM483	Yes	No	Yes
ABM484	Yes	Weak	No
ABM485	Yes	No	No
ABM486	Yes	No	No
ABM487	Yes	No	No
ABM488	Yes	Yes	No
ABM489	Yes	To be tested	Yes
ABM490	Yes	To be tested	Yes
ABM491	Yes	To be tested	Yes
ABM492	Yes	To be tested	Yes
ABM493	Yes	To be tested	Yes

Several of the Abeome-generated antibodies may be the basis for successful humanization and optimization campaigns to generate competitive CTLA-4 therapeutic antibodies.