# Novel Monoclonal Antibodies to TROP-2

*Using Abeome's novel transgenic mouse antibody discovery platform, we have rapidly obtained antibodies of high affinity and neutralizing potency against human TROP-2 (TACSTD-2) B-cells expressing affinity-matured anti-TROP-2 surface antibody were directly selected, and recombinant chimeric antibodies were immediately cloned and screened for TROP-2 binding. The further in vivo evaluation of these lead molecules should support a valid clinical development path. For licensing information, please contact us:*

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## I. TROP-2 (TACSTD-2, tumor-associated calcium signal transducer 2)

Trophoblast cell surface antigen 2 (TROP-2, also known as TACSTD-2, 2i3, GA733-1, EGP, MR23, MR54, RS7, RS-7 and T16) is a transmembrane glycoprotein upregulated in nearly all cancer types independent of baseline levels of TROP-2 expression. There is a link between TROP-2 expression and enhanced tumor cell growth and proliferation. [1] Overexpression of TROP-2 has also been associated with drug resistance to therapeutics used for cancers, including Tamoxifen, which is a common drug used to combat breast cancer. [2] While its role in cancer progression is still being investigated, it has been clinically validated as a target for toxin-conjugate therapy by Sacituzumab govitecan, marketed as TRODELVY™ by Immunomedics. Accelerated approval was achieved in a phase 3 trial of triple negative breast cancer in a pretreated population. The drug was given FDA fast-track approval on April 22, 2020. Given the opportunity for improvement in both the targeting antibody and the toxin, new antibodies for TROP-2 were sought.

## 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.

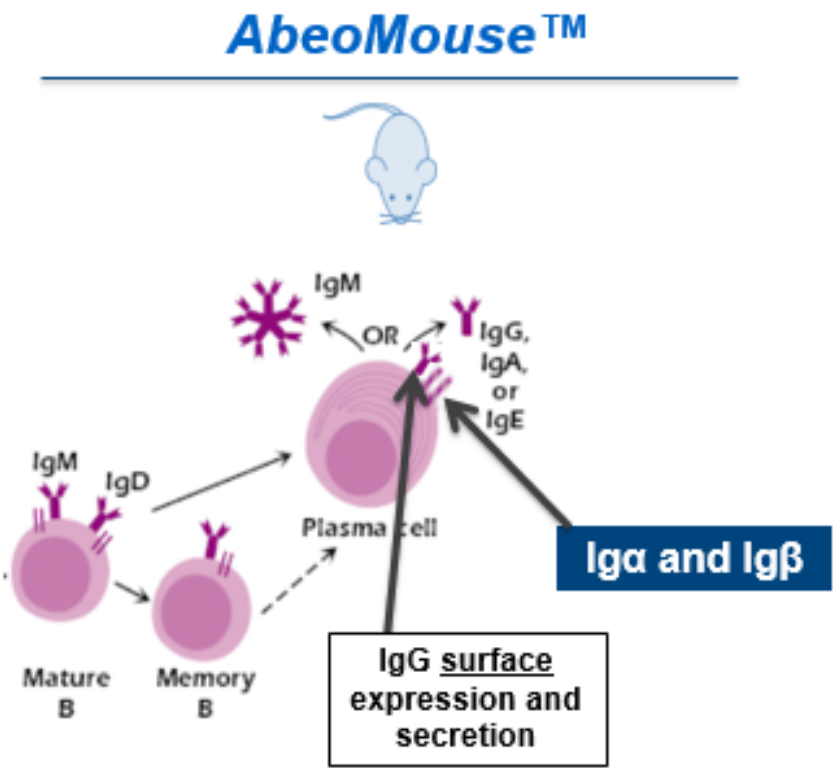



**FIGURE 1. The transgenic AbeoMouse™ platform.**
A novel antibody discovery platform that generates mature B cells with high surface IgG expression, allowing for the direct selection and cloning of antigen-specific B cells

### III. Immunization With Human TROP-2 Extracellular Domain

Five AbeoMice™ 8-11 weeks of age were pre-bled to obtain baseline serum antibody levels and then immunized subcutaneously or intraperitoneally with 20 μg of recombinant human TROP-2 extracellular domain (N-terminal segment His 27 - Thr 274 with a C-terminal human IgG1 FC tag; Acro Biosystems) in proprietary adjuvant. Booster injections with 10 μg of protein were given at days 14 and 28. Blood samples were taken and serum titers determined at days 21 and 35, resulting in extremely high titers, and mice were harvested at approximately 45 days after initial immunization (Fig. 2, Table 1).

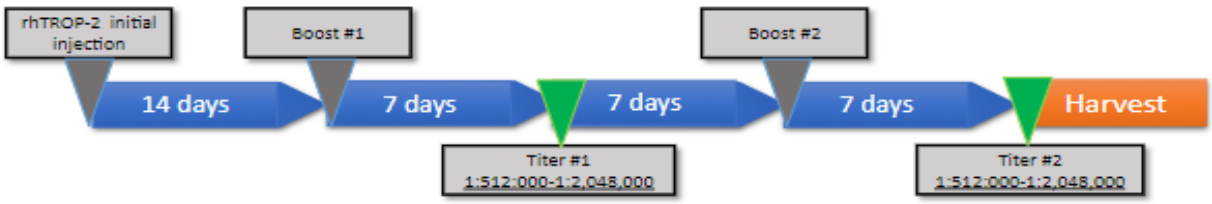



**FIGURE 2. Immunization timeline for generating anti-TROP-2 antibodies**

**Table 1. Immunization and titer summary of mice immunized with TROP-2.**

| Antigen | | | | | | | |
|---|---|---|---|---|---|---|---|
| hu TROP-2, FC tag | Proprietary adj-initial injection | Boost # 1 | Bleed # 1 | Titer # 1 | Boost # 2 | Bleed # 2 | Titer # 2 |
| Female ms # 559 | 5/14/2020 IP | 5/28/2020 | 6/4/2020 | 2,048K | 6/15/2020 | 6/22/2020 | 2,048K |
| Female ms # 231 | 5/14/2020 SQ | 5/28/2020 | 6/4/2020 | 512K | 6/15/2020 | 6/22/2020 | 512K |
| Male ms # 552 | 5/14/2020 SQ | 5/28/2020 | 6/4/2020 | 512K | 6/15/2020 | 6/22/2020 | 512K |
| Male ms # 597 | 5/14/2020 SQ | 5/28/2020 | 6/4/2020 | 512K | 6/15/2020 | 6/22/2020 | 512K |
| Male ms # 917 | 5/14/2020 IP | 5/28/2020 | 6/4/2020 | 512K | 6/15/2020 | 6/22/2020 | 2,048K |

## IV. Harvest and Single Antigen-Positive Cell Sorting (Mouse #597)

As a representative example of this campaign, mouse #597 yielded 26 lymphoid tissue samples and bone marrow (Figure 3) which were harvested, processed, and pooled into a single suspension of lymphoid cells (3.0 x $10^8$ cells). Erythrocytes were lysed using ammonium chloride and resulting lymphocytes were depleted of non-B cells and B cells expressing IgM antibodies by immuno-magnetic separation.

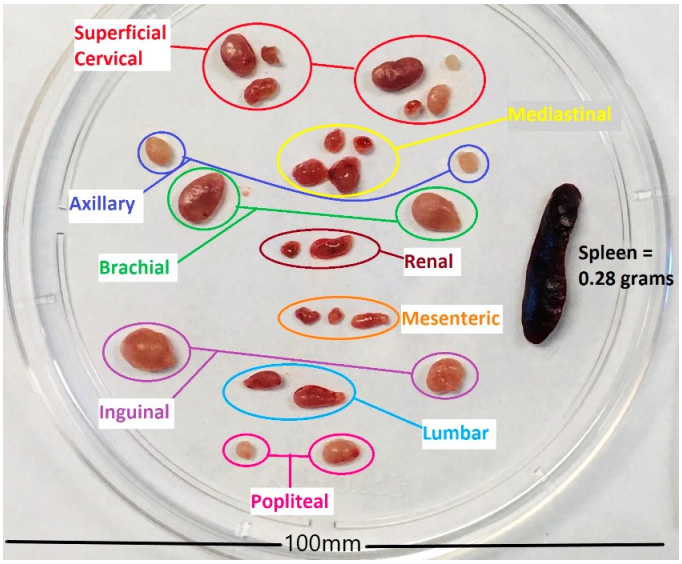



**FIGURE 3. Lymphoid organs harvested from mouse #597 and processed into a single cell suspension for FACS (fluorescent activated cell sorting).** Abeome's transgenic mice have an enhanced immune response, greatly enlarged lymphoid organs (left), and typically an order of magnitude increase in the number of antibody producing plasmacytes.

1.4 x $10^7$ IgM depleted B cells were labeled for FACS (fluorescent activated cell sorting). Specifically, cells were first incubated with purified rat anti-mouse CD16/CD32 (mouse Fc receptor block; BD Pharmingen) to block non-specific binding to mouse Fc receptors. Antigen specific antibody was detected with ifluor-633 labeled recombinant human TROP-2. Surface antibody was stained with dylight 488-GAMA-IgG. Antigen-positive cells were isolated as single cells by FACS as illustrated in Fig. 4 (red boxed cell population). Cells expressing surface antibody reactive to both human TROP-2 (ifluor-633) and GAMA-IgG (dylight 488) were singly deposited into wells of a 96 well plate. A total of 16,000 Ag+/Ig+ clones were sorted.

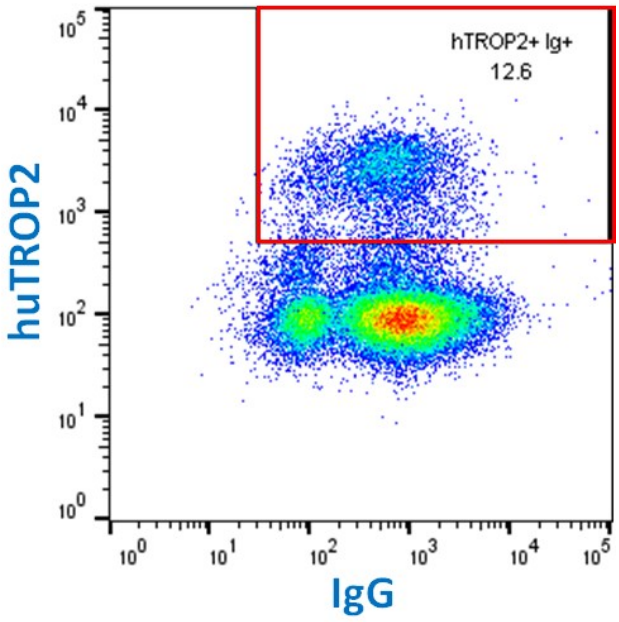


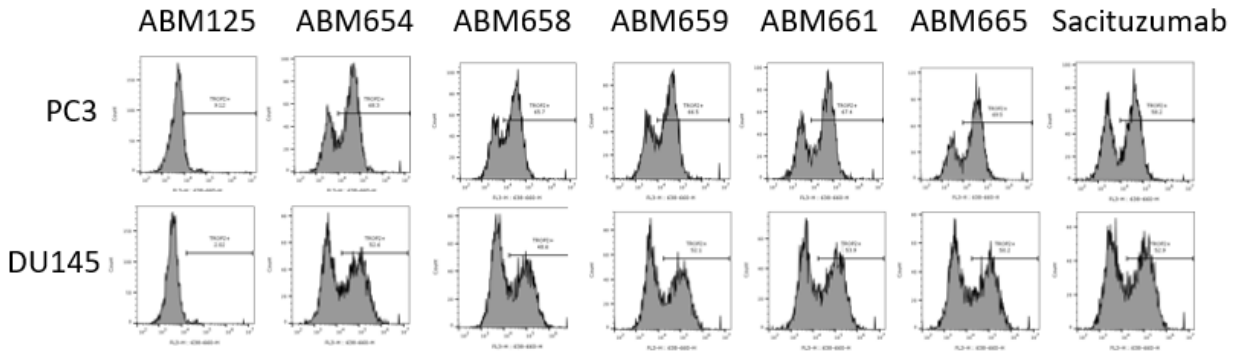

**FIGURE 4. Single cell FACS sorting of lymphocytes expressing antibody to human TROP-2**
The direct selection of cells surface-expressing anti-TROP-2 antibodies is accomplished by staining harvested B cells with fluor-labeled recombinant human TROP-2 and a polyclonal antibody recognizing mouse surface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

## V. High-Throughput Screening of TROP-2 Antibodies

After cell sorting, 15,936 B cells 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 of secreted chimeric antibodies and screened for binding to TROP-2 by ELISA. In total, we isolated and cloned more than 25 unique chimeric monoclonal antibodies (mAbs) that showed strong binding to human TROP-2. Antibodies were in either the same bin or different bins than Sacituzumab.

Antibodies were tested for their ability to bind native TROP-2 on the surface of cancer cell

lines PC3 and DU145. The majority of antibodies tested demonstrated binding to those cell lines with equivalent strength and frequency as Sacituzumab. Representative data are shown below:




## VI. Summary of Lead Discovery

Using our novel transgenic mouse antibody discovery platform, we have shown that we can rapidly obtain chimeric antibodies of high affinity against human TROP-2. B-cells expressing affinity-matured anti-TROP-2 surface antibody were directly selected, and recombinant chimeric antibodies were immediately cloned and screened for TROP-2 binding. The selection of a final lead antibody and isotype will be achievable upon pharmacokinetic and pharmacodynamic evaluation, which will further demonstrate their potential clinical utility.

## VII. Therapeutic Product Development

**Table 2. Summary of Current and Approved Anti-TROP-2 Antibody Agents**

**1) Immunomedics - Phase Ⅱ**
**2) Kelun Pharmaceutical - Phase II**
**3) Bio-Thera Solutions - Phase I**
**4) Junshi Biosciences/Hangzhou DAC - IND**
**5) Immunomedics - BLA Filing 6) Daiichi Sankyo - Phase I**

**1) Antibody TF2 - 111In-IMP-288; [111In]IMP-288 - NCT02300922**
**2) Antibody Drug Conjugate SKB264 - NCT04152499**
**3) Antibody BAT8003 - NCT03884517**
**4) rhAntibody Tub196 conjugate JS108 (DAC-002) - filed with NMPA**
**5) Sacituzumab govitecan - IMMU-132; TROP-2-SN-38,IMMU132; TROP2SN38 - NCT02161679 (Phase II), NCT04039230 (Phase I/II), NCT01631552 (Phase I/II), NCT03547973 (Phase II), NCT03901339 (Phase III), NCT04319198 (Phase III), NCT04320693 (Expanded Access), NCT03995706 (Phase I), NCT03964727 (Phase II), NCT03725761 (Phase II), NCT04230109 (Phase II), NCT04251416 (Phase II), NCT02574455 (P{hase III), NCT03992131 (Phase I/II)**
**6) Antibody DS-1062a - NCT03401385**

**References**:


1. Goldenberg et al. The emergence of trophoblast cell-surface antigen 2 (TROP-2) as a novel cancer target. *Oncotarget*, 2018, 9(48): 28989–29006.
2. Shvartsur and Bonavida. Trop2 and its overexpression in cancers: regulation and clinical/therapeutic implications. *Genes and Cancer*; 2015, 6(3-4): 84–105.