A Novel Anti-BCMA ADC for the Development of an Effective Multiple Myeloma Therapy

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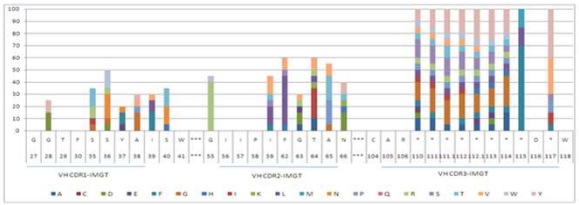
Abstract

B-cell maturation antigen (BCMA) expression is highly restricted to specific stages of B-cell development and it is not expressed on naïve or long-term memory B-cells. It is upregulated in multiple myeloma (MM) and has been shown to be a promising target for MM. Here we describe a novel anti-BCMA antibody, AVG-A11, isolated from AvantGen's yeast-display human antibody libraries, that specifically recognizes human and cyno-BCMA and does not cross-react with the related protein receptors TACI or BAFFR. It is 4 to 6-fold more potent in blocking the BCMA-BAFF interaction and inhibiting BAFF-induced NF-κB activation, respectively, in a head-to-head comparison with J6M0, the humanized anti-BCMA antibody that has shown promising results in clinical trials as the antibody-drug conjugate (ADC), GSK2857916.

In a surrogate ADC format using a MMAF conjugated anti-human Fc Fab mixed with either AVG-A11 or J6M0 IgG, AVG-A11 was 3 to 5-fold more potent against H929, MM.1S and RPMI8226 cells than J6M0 with EC_{50} values in the sub-nM range. Neither had a cytotoxic effect on BCMA-negative cells such as Raji (EC_{50}>1000 nM). Continuous live cell tracking revealed that AVG-A11 is able to trigger BCMA internalization more effectively than J6M0. In an ADC format, AVG-A11-mcMMAF showed potent cytotoxic activity on BCMA-expressing MM cell lines as well as human bone marrow mononuclear cells (BMMCs) from MM patients. Together these data indicate that we have successfully isolated a potent, fully human anti-BCMA antagonist directly from our yeast display human antibody platform that has been further developed into a potent lead therapeutic ADC candidate for effective MM therapy.

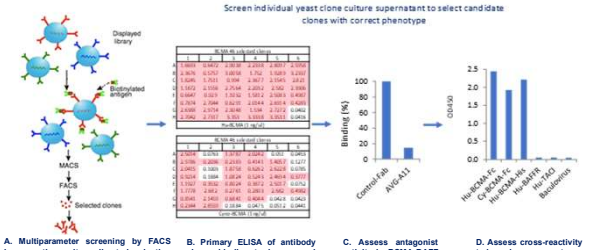
AvantGen's Yeast Display Human Antibody Libraries



- Constructed to mimic natural sequence variation in human antibodies to reduce immunogenicity and increase developability
- Informed by deep-sequencing human antibody clones from > 500 individuals; >350,000 antibody clones stratified by variable region sub-family, each has unique amino acid usage signature
- Largest known collection of yeast display libraries (>100 billion clones)
- Most potentially problematic sequence motifs have been removed from the library

Figure 1. AvantGen's Human Antibody Libraries. The above chart shows the amino acid frequencies observed in the 3 CDRs of antibody clones derived from the human heavy chain germline VH1-69 in our database generated by deep sequencing of human antibodies from >500 individuals. Each of the heavy and light chain libraries in AvantGen's antibody libraries is constructed to mimic the amino acid usage observed for that variable region sub-family.

Library Screening and Clone Selection



A. Multiparameter screening by FACS incorporating epitope-directed selection to enrich for clones that block BAFF binding to BCMA antigen

B. Primary ELISA of antibody clones binding to human and cyno BCMA-ECD

C. Assess antagonist activity in BCMA-BAFF competition assay

D. Assess cross-reactivity to homologous receptors

Figure 2. Isolating anti-BCMA clones with antagonistic activity. A. Cartoon of yeast library screening schema. Schema can incorporate cross species screening, in this case using differentially labelled human and cynomolgus BCMA-ECD-Fc, as well as epitope specific subtractive rounds of FACS to enrich for ligand-blocking antibody clones. **B.** Identifying BCMA-positive clones. The final enriched pool of yeast clones is plated then individual clones seeded into 96-well plates. After inducting antibody expression, the antibody-containing culture supernatants were used in primary ELISA assays to identify clones that bind both human and cyno BCMA recombinant antigen. **C.** Identifying clones that can inhibit BCMA binding to its ligand, BAFF by competitive ELISA. **D.** Identifying those clones that bind to BCMA but do not bind to homologous proteins, in this case TACI and BAFFR, or to baculovirus.

AVG-A11 binds to native BCMA

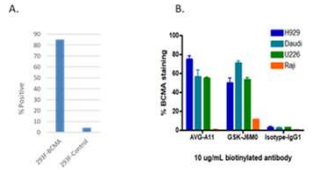


Figure 3. AVG-A11 as purified IgG recognizes native BCMA. A. Binding activity of AVG-A11 to transiently transfected HEK293 cells expressing BCMA compared to untransfected cells. **B.** Testing the binding activity to human H929, U266 MM and Burkitt lymphoma (Daudi) cells lines that express BCMA or Raji cells that do not express BCMA. Cells were incubated in the presence of 10 μg/mL (67 nM) biotinylated AVG-A11 or J6M0 for 30 minutes, washed and bound antibody detected with streptavidin-PE. Cell-bound fluorescence was measured using an iQue Screener Plus flow cytometer.

AVG-A11 binds with high affinity to BCMA but not to the related proteins, TACI and BAFFR

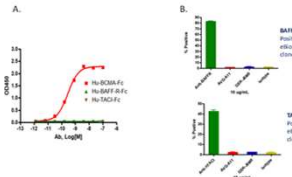
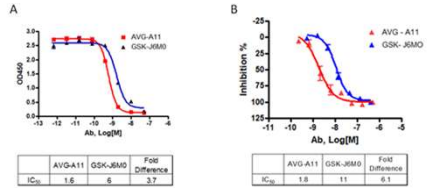


Figure 4. AVG-A11 binds with high affinity to BCMA but not to the related proteins, TACI and BAFFR. A. Determination of binding activity of purified AVG-A11 for BCMA by ELISA. Wells were coated with recombinant BCMA-ECD-Fc, TACI-ECD-Fc or BAFFR-ECD-Fc. Serial dilutions of purified AVG-A11 were added and bound IgG detected with goat anti-human Fc -HRP. Curve fitting using Prism software determined an apparent K_D value of 0.17 nM for BCMA-Fc. In contrast, no binding to either TACI or BAFFR was observed up to 100 nM. Octet analysis revealed a K_D value of 1.0 nM for human BCMA and 4.4 nM for cyno BCMA (data not shown) **B.** Degree of binding AVG-A11, GSK's J6M0 or isotype control IgG to HEK293F cells expressing human BAFFR (upper panel) or TACI (lower panel) at 10 μg/mL. Expression of BAFFR was confirmed using an anti-BAFFR antibody (clone 8A7,eBioscience Cat# 14-9117-82) and an anti-TACI antibody (clone 11H3,eBioscience, Cat# 13-9217-82). Both AVG-A11 and J6M0 showed very low background binding similar to the isotype negative control.

AVG-A11 blocks the BCMA-BAFF interaction and out-performs GSK-J6M0 in blocking BAFF-induced NF-κB activation



	AVG-A11	GSK-J6M0	Fold Difference
IC_{50}	1.6	6	3.7

	AVG-A11	GSK-J6M0	Fold Difference
IC_{50}	1.8	11	6.1

Figure 5. AVG-A11 is a potent antagonist of the BCMA-BAFF interaction. A. The ability of purified AVG-A11 compared to J6M0 to block BCMA binding to immobilized BAFF. Serial dilutions of AVG-A11 or J6M0 were mixed with 1.0 nM biotinylated BCMA and added to wells coated with BAFF. Bound biotinylated BCMA was detected with HRP-labeled streptavidin. Upper panel, inhibition curves; lower panel, IC_{50} values, which were calculated by curve fitting using Prism software. **B.** The ability of AVG-A11 compared to J6M0 to inhibit BAFF-induced NF-κB activation in HEK-Blue™ IL-1β reporter cells (InvivoGen, Cat# hkb-il1b) transiently transfected with plasmid encoding full-length human BCMA. Serial dilutions of the test antibody were added to the cells prior to the addition of 5 nM BAFF, and the cell mixtures incubated for 24 hrs. Secreted alkaline phosphatase stimulated by BAFF was then detected using QUANTI-Blue (Cat# rep-qbs). Inhibition versus antibody concentration curves are shown in the upper panel. IC_{50} values calculated by curve fitting using Prism software are shown in the lower panel.

AVG-A11 is more potent at triggering BCMA internalization than J6M0

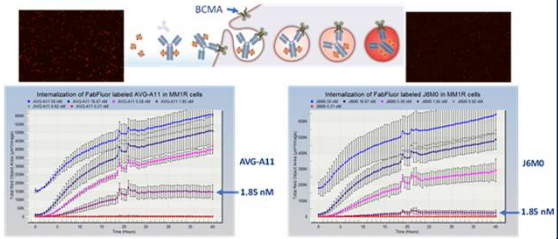
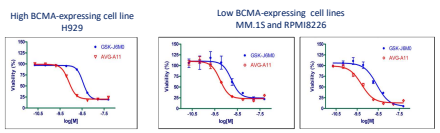


Figure 6. AVG-A11 induces more efficient BCMA internalization than J6M0 (experiments performed by ACEA). MM.1R cells were seeded in 96-well plates at 40,000 cells/well. AVG-A11 and J6M0 were each labeled with fluorescently labeled anti-Fab (Fab-Fluor) then the indicated concentration of Fab-Fluor AVG-A11 (left panels) or J6M0 antibody (right panels) was added to the wells. The cells were incubated in a xCELLigence RTCA real time cell analyzer and cultured at 37°C while intracellular fluorescence (uptake of the antibody-BCMA complex) was monitored every 30 minutes for 40 hours. The cellular uptake signal is clearly evident in the presence of 1.85nM AVG-A11 (see arrow and also the top left fluorescence image) but is at background levels for 1.85nM J6M0 (arrow and also top right fluorescence image). No uptake was seen in BCMA-negative cell lines under these conditions (data not shown).

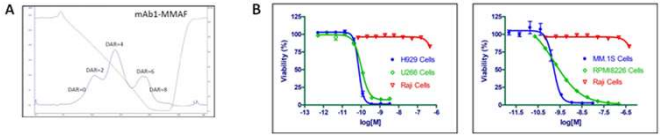
AVG-A11 out-performs GSK-J6M0 in killing MM cells in an ADC format



Antibody clone	H929		MM.1S		RPMI8226	
	EC_{50} (nM)	Fold improvement	EC_{50} (nM)	Fold improvement	EC_{50} (nM)	Fold improvement
AVG-A11	0.79	4.4	0.55	3.1	0.51	5.3
GSK-J6M0	3.5		1.7		2.7	

Figure 7. AVG-A11 shows higher potency than J6M0 in an ADC format using unmodified antibody complexed to anti-human Fc Fab-CL-MMAF. Cytotoxic activity of AVG-A11 or J6M0 against the high BCMA expressing MM cell line, H929, or the lower BCMA-expressing cell lines, MM.1S or RPMI8226, was determined by incubating cells in the presence of the indicated concentrations of primary antibodies, AVG-A11 or GSK-J6M0, followed with anti-human-Fc-CL-MMAF (Moradec, Cat# AH-202-AF) for 3 days (H929) or 6 days (MM.1S or RPMI8226). Cell viability was determined using CellTiter-Glo reagent (Promega, Cat# G7571/2/3). EC_{50} curves (upper panel) and EC_{50} values (lower panel) were determined by curve fitting using Prism software. Note: J6M0 was generated in-house using the published sequence for the variable domains using the same IgG1 Fc-expression vector used for expression of AVG-A11 in ExpiCHO cells. Therefore, the anti-Fab-CL-MMAF has identical affinity to both antibody clones

AVG-A11-mcMMAF is cytotoxic to MM cells but not to BCMA negative cells



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	H929	U266	MM.1S	RPMI8226	Raji
EC_{50} (nM)	0.074	0.1	0.16	0.18	>1,000

Figure 8. Cytotoxic activity of the AVG-A11-mcMMAF drug conjugate. A. Phenyl 650 hydrophobic interaction chromatography (HIC) profile of AVG-A11-mcMMAF. The average drug-antibody ratio (DAR) was 4.0. **B.** Cytotoxic activity of AVG-A11-mcMMAF against the BCMA-positive MM cell lines, H929, U266, RPMI8226 and MM.1S as wells as BCMA negative Raji cells. **C.** EC_{50} values that were determined by curve fitting using Prism software.

AVG-A11-MMAF exhibits cytotoxicity to BMMCs isolated from MM patients, but not BMMCs from a healthy individual

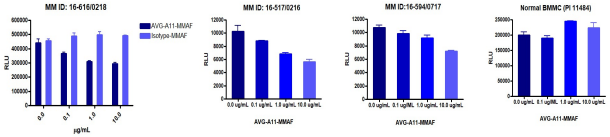

Figure 9. AVG-A11-mcMMAF shows dose-dependent cytotoxicity to bone marrow mononuclear cells (BBMCs) from multiple myeloma patients. About 200 K per well of BMMCs from 3 patients and normal BMMCs from one healthy individual were cultured in the presence of the indicated amount of AVG-A11-MMAF for 3 days. The amount of ATP was quantified with CellTiter-Glo.

Note, the conjugate selectively targets MM cells and not the other mononuclear cells from other hematopoietic lineages present in the BMMC population.

Summary

- A highly specific fully human antibody clone against human BCMA, AVG-A11, was isolated from AvantGen's yeast display libraries.
- AVG-A11 has high affinity for human BCMA (K_D of 1.0 nM) and cynomolgus BCMA (K_D of 4.4 nM), blocks the interaction of BCMA with its ligand BAFF with a Ki of 1.6 nM, and inhibits BAFF-induced NF-κB activation with IC_{50} of 1.8 nM, 4 and 6 fold more potent than J6M0, respectively.
- AVG-A11 does not cross react with the homologous proteins, TACI and BAFFR
- AVG-A11 appears to be more effective in triggering BCMA internalization than J6M0
- In an ADC format using a MMAF conjugated anti-human Fc Fab antibody, AVG-A11 is 3-5 times more potent than J6M0 at killing BCMA-expressing MM cell lines.
- AVG-11-mcMMAF can effectively kill MM cells present in BMMCs isolated from MM patients, without any effect on healthy BMMCs.