S.1.2 Structure

ABBV-399 is an antibody-drug conjugate (ADC) comprised of ABT-700 (anti-c-Met IgG1 antibody) conjugated to monomethylauristatin E (MMAE) via a valine-citrulline (vc) linker.

S.1.2.1 Antibody Structure of ABBV-399

ABT-700 is a humanized recombinant IgG1 kappa (κ) that is specific for a unique epitope of the human c-Met receptor. The antibody has further been engineered to (1) introduce a third disulfide linkage in the hinge-region critical to c-Met receptor antagonist activity and (2) remove C-terminal lysines from the heavy chain to eliminate lysine variants.

The antibody portion of the ADC has a general structure similar to normal human IgG1 kappa antibodies. The protein consists of two heavy chains paired with two light chains, and is expressed in Chinese hamster ovary (CHO) cells as a disulfide-linked tetramer.

Light Chain

The light chain of ABT-700 is humanized type kappa, consisting of 218 amino acids.

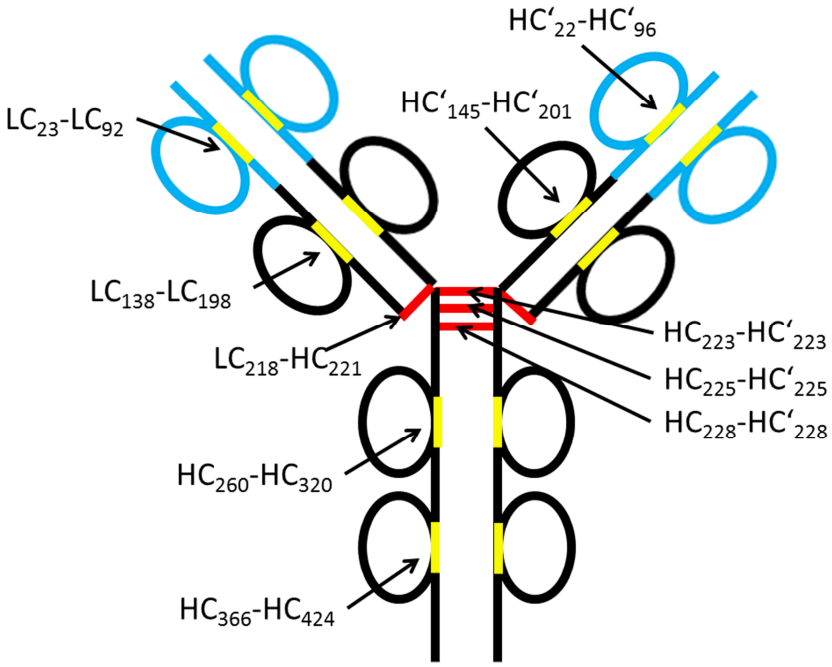
Heavy Chain

The heavy chain of ABT-700 is humanized isotype IgG1, consisting of 445 amino acids. As compared to the wild-type human IgG1 hinge sequence, the heavy chain contains a deletion of two amino acids and a threonine to cysteine mutation (D*KT*H*T*C vs. DCHC). In addition, the C-terminal lysine amino acid on the heavy chain was engineered out to eliminate heterogeneity at the C-terminus due to incomplete cleavage of the lysine. The heavy chain is post-translationally modified by addition of N-linked glycans to asparagine-296. The major glycans are fucosylated biantennary oligosaccharides containing zero, one, or two galactose residues. In addition, at the N-terminus of the heavy chain is a glutamine residue, which can undergo spontaneous cyclization to form a pyroglutamate residue.

ABBV-399 Disulfide Bond Structure

The monoclonal antibody portion of ABBV-399 consists of two IgG1 heavy chains paired with two kappa light chains. Each heavy chain contains 4 intrachain disulfide bridges between cysteines in positions 22 and 96, 145 and 201, 260 and 320, and in positions 366 and 424. Each light chain consists of 2 intrachain disulfide bridges; between cysteines in positions 23 and 92, and between cysteines in positions 138 and 198. In each antibody molecule, the two heavy chain molecules are linked by three interchain disulfide bridges, between the two cysteines 223, two cysteines 225 and two cysteines 228. Each heavy chain is paired with a light chain through a disulfide bridge between the cysteine in position 221 of the heavy chain and the carboxy terminal cysteine in position 218 of the light chain. A schematic representation of ABBV-399 with disulfide bond connectivity is shown in Figure 1. ABBV-399 is a conjugated derivate of ABT-700 where up to five interchain disulfide bonds are susceptible to reduction during the manufacturing process. For ABBV-399, intrachain disulfide bonds shown in yellow are present in all molecules. Interchain disulfide bonds shown in red are present in some molecules, depending on the number of conjugated species.

Figure 1. Schematic Representation of ABT-700 /ABBV-399 with Disulfide Bond Connectivity



Structural Formulae

The predicted amino acid sequences for the light and heavy chains are presented in Figure 2 and Figure 3, respectively. Cys 218 of the light chain and Cys 221, Cys 223, Cys 225 and Cys 228 of the heavy chain undergo conjugation with vcMMAE in ABBV-399.

Figure 2. Amino Acid Sequence of the Light Chain of ABBV-399

1 DIVMTQSPDS LAVSLGERAT INCKSSESVD SYANSFLHWY QQKPGQPPKL
51 LIYRASTRES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQSKEDPL
101 TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
151 QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
201 THQGLSSPVT KSFNRGEC*

*conjugating cysteines

Figure 3. Amino Acid Sequence of the Heavy Chain of ABBV-399

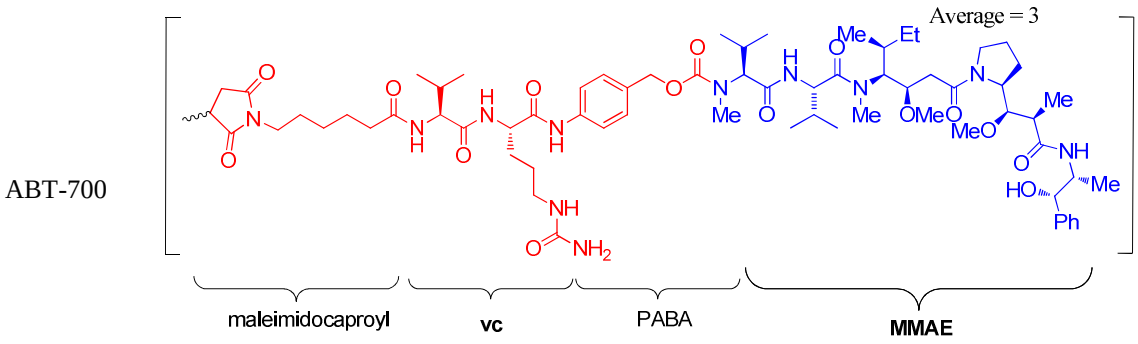
1 QVQLVQSGAE VKKPGASVKV SCKASGYIFT AYTMHWVRQA PGQGLEWMGW
51 IKPNNGLANY AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARSE
101 ITTEFDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
151 FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
201 CNVNHKPSNT KVDKRVEPKS C*DC*HC*PPC*PA PELLGGPSVF LFPPKPKDTL
251 MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
301 VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL
351 PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD
401 GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPG

*conjugating cysteines

S.1.2.2 Antibody-Drug Conjugate Structure of ABBV-399

ABBV-399 is derived from the conjugation of 2 to 10 vcMMAE molecules to partially reduced ABT-700. Only the interchain disulfides are reduced in the reduction step of the conjugation process. The structure of the ABBV-399 antibody-drug conjugate, highlighting a single drug-linker conjugated to a cysteine side chain is shown in Figure 4.

Figure 4. Structure of ABBV-399 Antibody-Drug Conjugate



Note: The maleimido caproyl valine-citrulline PABA linker is shown in red and the monomethylauristatin E (MMAE) drug is shown in blue. A total of n = 2, 4, 6, 8, or 10 molecules can be conjugated to a single antibody.

Twenty-three different isomers of antibody-drug conjugates are possible with drug-to-antibody ratios of 2, 4, 6, 8 and 10. A single isomer is possible for drug-to-antibody ratio of 10 while 4, 7, 7, and 4 isomers are possible for drug-to-antibody ratios of 2, 4, 6, and 8, respectively. The average drug-to-antibody ratio for final ABBV-399 after resin treatment is approximately 3.