Claims:

1. An analytical method comprising:

(i) conjugation of unoccupied cysteine sites of a cysteine-conjugated antibody drug conjugate (ADC) using an isotopically-labeled cytotoxin containing a carbonyl group and a reductant to produce an isotopically-labeled ADC sample; and

(ii) peptide mapping the sample.

2. The method of claim 1, wherein the cytotoxin is MMAF or MMAE.

3. The method of claim 1 or 2, wherein the ADC is first reduced by the reductant and then conjugated with the isotopically-labeled cytotoxin.

~~4. The method of any one of claims 1 to 3, wherein the reductant is dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP).~~

~~5. The method of any one of claims 1 to 4, wherein excess reductant is removed prior to the peptide mapping by eluting the sample through a size exclusion chromatography column.~~

~~6. The method of any one of claims 1 to 5, wherein conjugation occurs by reacting the ADC with the isotopically-labeled cytotoxin.~~

~~7~~4. The method of any one of claims 1 to ~~6~~3, wherein excess isotopically-labeled cytotoxin is removed prior to the peptide mapping by eluting the sample through a size exclusion chromatography column.

~~8~~5. The method of any one of claims 1 to ~~7~~4, wherein the peptide mapping comprises using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

~~9~~6. The method of any one of claims 1 to ~~8~~5, wherein the peptide mapping comprises denaturing the sample, reducing all remaining disulfide bonds, and alkylating resulting free sulfhydryls.

~~10~~7. The method of any one of claims 1 to ~~9~~6, wherein the peptide mapping comprises enzymatically digesting the sample to produce isotopically-labeled conjugated peptides and optionally quenching the enzymatic digestion by addition of a strong acid.

~~11~~8. The method of claim ~~10~~7, wherein the isotopically-labeled conjugated peptides are ionized and mass-to-charge ratios are detected, and the mass-to-charge ratios detected for the isotopically-labeled conjugated peptides are compared to non-isotopically-labeled conjugated peptides.

~~12. The method of claim 11, wherein the mass-to-charge ratios detected for the isotopically-labeled conjugated peptides are compared to non-isotopically-labeled conjugated peptides.~~

~~13~~9. The method of any one of claims 1 to ~~12~~8, wherein the method comprises reacting cytotoxin with isotopically-labeled water to produce the isotopically-labeled cytotoxin.

~~14. The method of claim 13, wherein the cytotoxin is reacted with isotopically-labeled water in acetonitrile.~~

~~15~~10. The method of any one of claims 1 to ~~14~~9, wherein the ADC is belantamab mafodotin.

~~16. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load at LC C214 is between about 56% to about 80%.~~

~~17. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2~~

~~comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load at HC C224 is between about 58% to about 81%.~~

~~18. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load of HC hinge DL2 at HC C230 and C233 is between about 15% to about 46%.~~

~~19. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load of HC hinge DL1 at HC C230 or HC C233 is between about 11% to about 15%.~~

~~20~~11. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load at LC C214 is between about 56% to about 80%, the percentage drug load at HC C224 is between about 58% to about 81%, the percentage drug load of HC hinge DL2 at HC C230 and C233 is between about 15% to about 46%, and/or the

percentage drug load of HC hinge DL1 at HC C230 or HC C233 is between about 11% to about 15%.

~~21. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load at LC C214 is between about 63% to about 76%, the percentage drug load at HC C224 is between about 65% to about 78%, the percentage drug load of HC hinge DL2 at HC C230 and C233 is between about 22% to about 40%, and/or the percentage drug load of HC hinge DL1 at HC C230 or HC C233 is between about 11% to about 16%.~~

~~22. A composition comprising an anti-BCMA antibody conjugated to a cytotoxic agent to form an antibody drug conjugate (ADC), wherein the antibody comprises a CDRH1 comprising the amino acid sequence according to SEQ ID NO:1; a CDRH2 comprising the amino acid sequence according to SEQ ID NO:2; a CDRH3 comprising the amino acid sequence according to SEQ ID NO:3; a CDRL1 comprising the amino acid sequence according to SEQ ID NO:4; a CDRL2 comprising the amino acid sequence according to SEQ ID NO:5; and a CDRL3 comprising the amino acid sequence according to SEQ ID NO:6; wherein the cytotoxic agent is MMAF or MMAE; and wherein the percentage drug load at LC C214 is between about 65% to about 71%, the percentage drug load at HC C224 is between about 68% to about 74%, the percentage drug load of HC hinge DL2 at HC C230 and C233 is between about 24% to about 30%, and/or the percentage drug load of HC hinge DL1 at HC C230 or HC C233 is between about 12% to about 18%.~~

~~23~~12. The composition of ~~any one of claims 16 to 22~~claim 11, wherein the anti-BCMA antibody is belantamab.

~~24~~13. The composition of ~~any one of claims 16 to 23~~claim 11, wherein the ADC is belantamab mafodotin.

~~25. The composition of any one of claims 16 to 24, wherein percent DL2 is at least about 30%, about 15% to about 27%, or about 15% to about 32%; percent DL4a is at least about 30%, about 35% to about 38%, or about 30% to about 40%; percent DL4b is at least about 5%, about 7% to about 9%, or about 5% to about 10%; percent DL6 is at least about 10%, about 14% to about 20%, or about 10% to about 20%; and/or DL8 is at least about 1%, about 6.0% to about 12.0%, or about 4% to about 15%.~~

~~26. The composition of any one of claims 16 to 25, wherein percent DL0 is less than or equal to about 10% or about 5%.~~

~~27~~14. A pharmaceutical composition comprising the composition of ~~any one of claims 16 to 26~~claim 11 and at least one pharmaceutically acceptable excipient.

~~28. A method of treating cancer comprising administering to a subject in need thereof a therapeutically effective amount of the composition of any one of claims 16 to 26 or the pharmaceutical composition of claim 27.~~

~~29~~15. A composition of ~~any one of claims 16 to 26~~claim 11 or the pharmaceutical composition of claim ~~27~~ 14 for use in the treatment of cancer.

~~30~~16. Use of a composition of ~~any one of claims 16 to 26~~claim 11 in the manufacture of a medicament for use in the treatment of cancer.

~~31~~17. A method of determining conjugation levels of cysteine-conjugated antibody drug conjugates, the method comprising:

a) reducing the antibody drug conjugates to form reduced antibody drug conjugates;

b) conjugating the reduced antibody drug conjugates with an isotypically-labeled cytotoxin to form isotopically-labeled antibody drug conjugates;

c) producing isotopically-labeled conjugated peptides from the isotopically-labeled antibody drug conjugates and performing peptide mapping on the isotopically-labeled conjugated peptides;

d) detecting mass-to-charge ratios for the isotopically-labeled conjugated peptides; and

e) comparing the mass-to-charge ratios of the isotopically-labeled conjugated peptides to mass-to charge ratios for non-isotopically-labeled conjugated peptides to determine the conjugation levels of cysteine-conjugated antibody drug conjugates.

~~32~~18. The method of claim ~~31~~17, wherein the cytotoxin is MMAF or MMAE.

~~33~~19. The method of claim ~~31 or 32~~17 or 18, wherein the cysteine-conjugated antibody drug conjugates are first reduced by a reductant and then conjugated with the isotopically-labeled cytotoxin.

~~34. The method of claim 33, wherein the reductant is dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP).~~

~~35~~20. The method of claim ~~34~~19, wherein excess reductant is removed prior to the peptide mapping by eluting the sample through a size exclusion chromatography column.

~~36. The method of any one of claims 31 to 35, wherein conjugation occurs by reacting the cysteine-conjugated antibody drug conjugates with the isotopically-labeled cytotoxin.~~

~~37. The method of any one of claims 31 to 36, wherein excess isotopically-labeled cytotoxin is removed prior to the peptide mapping by eluting the sample through a size exclusion chromatography column.~~

~~38~~21. The method of any one of claims ~~31 to 37~~17 to 20, wherein the peptide mapping comprises using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

~~39~~22. The method of any one of claims ~~31 to 38~~17 to 21, wherein the peptide mapping comprises denaturing the sample, reducing remaining disulfide bonds, and alkylating resulting free sulfhydryls.

~~40~~23. The method of any one of claims ~~31 to 39~~17 to 22, wherein the peptide mapping comprises enzymatically digesting the sample to produce isotopically-labeled conjugated peptides and optionally quenching the enzymatic digestion by addition of a strong acid.

~~41~~24. The method of any one of claims ~~31 to 40~~17 to 23, wherein the method comprises reacting cytotoxin with isotopically-labeled water to produce the isotopically-labeled cytotoxin.

~~42. The method of claim 41, wherein the cytotoxin is reacted with isotopically-labeled water in acetonitrile.~~

~~43~~25. The method of any one of claims ~~31 to 42~~17 to 24, wherein the cysteine-conjugated antibody drug conjugates are belantamab mafodotin.