

WHAT IS CLAIMED IS:

1. A continuous method for producing an antibody drug conjugate (ADC) composition comprising (i) conjugating an antibody or antigen-binding fragment thereof to a drug to form an ADC in a flow reactor, (ii) removing unconjugated drug, and (iii) exchanging the ADC into a stable buffer, wherein (i) to (iii) are performed continuously, and wherein single-pass tangential flow filtration (SPTFF), flow-through column-chromatography, and/or countercurrent diafiltration is used to remove the unconjugated drug ~~and/or exchange the ADC into the stable buffer~~.
2. The method of claim 1, wherein SPTFF and/or countercurrent diafiltration is used to remove the unconjugated drug and to exchange the ADC into the stable buffer.
3. The method of claim 1, wherein flow-through column chromatography is used to remove the unconjugated drug, and SPTFF and/or countercurrent diafiltration is used to exchange the ADC into the stable buffer.
4. The method of any one of claims 1-3, further comprising pre-processing the antibody or antigen-binding fragment thereof.
5. The method of claim 4, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed continuously with (i) to (iii).
6. The method of claim 4 or 5, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed using SPTFF and/or countercurrent diafiltration, optionally wherein only one SPTFF or countercurrent diafiltration step is used in the pre-processing.
7. The method of claim 4, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed in bulk prior to (i) to (iii).
8. The method of any one of claims 1-7, wherein the method further comprises pre-processing the drug for conjugation.
9. A continuous method for producing an antibody drug conjugate (ADC) composition comprising (i) pre-processing an antibody or antigen-binding fragment thereof, (ii)

conjugating the antibody or antigen-binding fragment thereof to a drug to form an ADC in a flow reactor, (iii) removing unconjugated drug, and (iv) exchanging the ADC into a stable buffer, wherein (i) to (iv) are performed continuously, and wherein single-pass tangential flow filtration (SPTFF), flow-through column-chromatography, and/or countercurrent diafiltration is used to remove the unconjugated drug ~~and/or exchange the ADC into the stable buffer~~.

10. The method of any one of claims 4-9, wherein the pre-processing of the antibody or antigen-binding fragment thereof comprises exchanging the antibody or antigen-binding fragment thereof into a buffer for conjugation.
11. The method of any one of claims 4-10, wherein the pre-processing of the antibody or antigen-binding fragment thereof comprises reducing the antibody or antigen-binding fragment thereof and/or oxidizing the antibody or antigen-binding fragment thereof, optionally wherein (a) the reducing and/or the oxidizing are achieved without using SPTFF or countercurrent diafiltration or (b) the reducing and/or the oxidizing are achieved using only one SPTFF or countercurrent diafiltration step.
12. A method for producing an antibody drug conjugate (ADC) in a continuous conjugation process, comprising (i) adding one or more conjugation reaction reagents to a conjugation reaction in a flow reactor while the conjugation reaction proceeds and after at least one ~~conjugation reaction product (ADC) has formed~~ and (ii) continuously removing unconjugated drug using (SPTFF), flow-through column-chromatography, and/or countercurrent diafiltration.
13. The method of claim 12, wherein the conjugation reaction reagent comprises an antibody ~~or antigen-binding fragment thereof~~.
14. The method of claim 12 or 13 wherein the conjugation reaction reagent comprises a drug attached to a linker.
- ~~15. The method of claim 12 or 13 wherein the conjugation reaction reagent comprises a linker.~~
- ~~16-15.~~ The method of claim 12 wherein the conjugation reaction reagent comprises an antibody or antigen-binding fragment thereof attached to a linker.

~~17. The method of claim 12, 13, 15, or 16 wherein the conjugation reaction reagent comprises a drug.~~

~~18. The method of any one of claims 12-17, wherein the conjugation reaction occurs in a flow reactor.~~

~~19.~~16. The method of any one of claims 12-~~18~~15, further comprising pre-processing an antibody or antigen-binding fragment thereof.

~~20.~~17. The method of claim ~~19~~16, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed continuously with the conjugation reaction.

~~21.~~18. The method of claim ~~19-16~~ or ~~20~~17, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed using SPTFF and/or countercurrent diafiltration, optionally wherein only one SPTFF or countercurrent diafiltration step is used in the pre-processing.

~~22.~~19. The method of claim ~~19~~16, wherein the pre-processing of the antibody or antigen-binding fragment thereof is performed in bulk prior to the conjugation reaction.

~~23.~~20. The method of any one of claims ~~19~~16-22~~19~~, wherein the pre-processing of the antibody or antigen-binding fragment thereof comprises exchanging the antibody or antigen-binding fragment thereof into a buffer for conjugation.

~~24.~~21. The method of any one of claims ~~19~~16-23~~20~~, wherein the pre-processing of the antibody or antigen-binding fragment thereof comprises reducing the antibody or antigen-binding fragment thereof and/or oxidizing the antibody or antigen-binding fragment thereof.

~~25.~~22. The method of any one of claims 5-10 wherein the ADC is concentrated using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~26.~~23. The method of any one of claims 5-11 wherein the ADC is purified using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~27.~~24. The method of any one of claims 5-12, wherein the ADC is transferred to a formulation buffer using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~28.25.~~ The method of any one of claims 12-~~27~~24, wherein the ADC is concentrated, purified, and/or transferred to a formulation buffer using flow-through chromatography.

~~29.~~ A method for concentrating an antibody drug conjugate (ADC) comprising using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~30.~~ A method for purifying an antibody drug conjugate (ADC) comprising using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~31.~~ A method for transferring an antibody drug conjugate (ADC) to a formulation buffer comprising using single-pass tangential flow filtration and/or countercurrent diafiltration.

~~32.~~ The method of any one of claims 29-31, wherein the ADC was produced in a batch conjugation process.

~~33.~~ The method of any one of claims 29-31, wherein the ADC was produced in a continuous conjugation process.

~~34.26.~~ The method of ~~any one of claims 25, 29, 32, and 33~~claim 22, wherein the single-pass tangential flow filtration uses an ultrafiltration membrane.

~~35.27.~~ The method of ~~any one of claims 26, 27, 30, and 31~~ claim 23 or 24, wherein the single-pass tangential flow filtration uses a diafiltration membrane.

~~36.28.~~ The method of any one of claims 1-~~35~~27 wherein the method improves the consistency of the ADC production.

~~37.29.~~ The method of any one of claims 1-~~36~~28 wherein the wherein the method decreases the time for ADC production.

~~38.30.~~ The method of any one of claims 1-11, ~~24~~18, ~~23~~20-2724, and ~~29~~26-3729 wherein the single-pass tangential flow filtration and/or the or countercurrent diafiltration improves the consistency of the ADC production.

~~39.31.~~ The method of any one of claims 1-11, ~~24~~18, ~~23~~20-2724, and ~~29~~26-3830 wherein the single-pass tangential flow filtration and/or the or countercurrent diafiltration decreases the time for ADC concentration, purification, or transfer.

~~40.32.~~ The method of any one of claims 1-11, ~~2418~~, ~~2320-2724~~, and ~~2926-3931~~ wherein the single-pass tangential flow filtration and/or the or countercurrent diafiltration decreases the amount of buffer used.

~~41.33.~~ The method of any one of claims 1-~~4032~~, wherein the method further comprises in-line monitoring of an analyte.

~~42.~~ A method for producing an antibody drug conjugate (ADC) wherein in-line monitoring is used to measure the addition of a component to an ADC conjugation reaction.

~~43.~~ The method of claim 41 or 42 wherein the in-line monitoring measures the concentration of a component added to the ADC conjugation reaction.

~~44.~~ The method of any one of claims 41-43 wherein the in-line monitoring measures the flow rate of a component added to the ADC conjugation reaction.

~~45.~~ The method of any one of claims 42-44 wherein the component is an antibody or antigen-binding fragment thereof, a drug, a linker, drug attached to a linker, an antibody or antigen-binding fragment thereof attached to a linker, and/or a conjugation buffer.

~~46.~~ The method of any one of claims 42-44 wherein the method further comprises in-line monitoring to measure the addition of a component to an in-situ reaction.

~~47.~~ The method of claim 46 wherein the component is a drug, a linker, and/or an in-situ reaction buffer.

~~48.~~ A method for producing an antibody drug conjugate (ADC) wherein in-line monitoring of an analyte is used to determine when (i) to stop adding conjugation buffer to an ADC conjugation reaction (ii) to stop recirculating conjugation buffer in an ADC conjugation reaction, and/or (iii) to start rinsing conjugation buffer from an ADC conjugation reaction.

~~49.~~ The method of claim 48 wherein the analyte is unconjugated drug or unconjugated drug attached to linker.

~~50.~~ A method for purifying an antibody drug conjugate (ADC) after an ADC conjugation process wherein the method comprises in-line monitoring of an analyte.

51. — ~~The method of claim 50 wherein the purification comprises filtration.~~
52. — ~~The method of claim 51 wherein the filtration is ultrafiltration or diafiltration, optionally wherein the diafiltration is countercurrent diafiltration.~~
53. — ~~The method of claim 51 wherein the filtration is tangential flow filtration.~~
54. — ~~The method of claim 53, wherein the tangential flow filtration is single-pass tangential flow filtration.~~
55. — ~~The method of any one of claims 42-54 wherein the analyte is in the retentate.~~
56. — ~~The method of claim 55 wherein the analyte is the concentration of unconjugated drug or unconjugated drug attached to linker.~~
57. — ~~The method of claim 55 or 56 wherein the analyte is the concentration of an ADC or an antibody or antigen-binding fragment thereof.~~
58. — ~~The method of any one of claims 55-57 wherein the analyte is the concentration of a component of a conjugation reaction buffer or a filtration buffer.~~
59. — ~~The method of any one of claims 55-57 wherein the analyte is the pH.~~
60. — ~~The method of any one of claims 50-59 wherein the analyte is in the permeate.~~
61. — ~~The method of claim 60 wherein the analyte is the concentration of an ADC or an antibody or antigen-binding fragment thereof.~~
62. — ~~The method of claim 60 or 61 wherein the analyte is the concentration of unconjugated drug or unconjugated drug attached to linker.~~
63. — ~~The method of any one of claims 50-62 wherein the purification comprises chromatography and the analyte is measured at the end of a chromatography column.~~
64. — ~~The method of claim 63 wherein the analyte is an ADC or an antibody or antigen-binding fragment thereof.~~

65. ~~The method of claim 63 or 65 wherein the analyte is unconjugated drug, unconjugated drug attached to linker, a component of a conjugation reaction buffer, or a component of a chromatography buffer.~~
66. ~~The method of any one of claims 42-65 wherein the conjugation reaction is a batch conjugation reaction.~~
67. ~~The method of any one of claims 42-65 wherein the conjugation reaction is a continuous conjugation reaction.~~
68. ~~The method of any one of claims 42-67, wherein the in-line monitoring is performed using variable path-length technology, a flow cell device, a UV sensor, Raman spectroscopy, or Fourier Transform Infrared spectroscopy (FTR).~~
69. ~~The method of any one of claims 42-67, wherein the in-line monitoring of the analyte is performed using a FlowVPE.~~
70. ~~The method of any one of claims 42-69, wherein the in-line monitoring improves ADC yield.~~
71. ~~The method of any one of claims 42-70, wherein the in-line monitoring improves ADC recovery.~~
72. ~~The method of any one of claims 42-71, wherein the in-line monitoring decreases time for ADC production.~~
73. ~~The method of any one of claims 42-72, wherein the in-line monitoring decreases the amount of buffer used.~~
74. ~~A method for producing an antibody drug conjugate (ADC) wherein in-line monitoring of an analyte is used to determine when to stop adding a conjugation reaction component to an ADC conjugation reaction.~~
75. ~~The method of claim 74, wherein the component is an antibody or antigen-binding fragment thereof, a drug, a linker, a drug attached to a linker, and/or a conjugation reaction buffer.~~
- 76.34. The method of any one of claims 1-~~75~~33, wherein the ADC comprises an antibody.

- ~~77.35.~~ The method of any one of claims 1-~~7533~~, wherein the ADC comprises an antigen-binding fragment of an antibody.
- ~~78.36.~~ The method of any one of claims 1-~~7733~~, wherein the ADC comprises an antibody or antigen-binding fragment that specifically binds to CD37, CD33, FOLR1, CD123, CD19, cMET, ADAM9, or HER2.
- ~~79.37.~~ The method of claim ~~7836~~, wherein the antibody or antigen-binding fragment thereof comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of huMov19, Z4681A, or G4732A.
- ~~80.38.~~ The method of claim ~~7937~~, wherein the CDRs are the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDRs.
- ~~81.39.~~ The method of claim ~~7836~~, wherein the antibody or antigen-binding fragment thereof comprises VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 sequences comprising the sequences of SEQ ID NOs:1-6, respectively, SEQ ID NOs:7-12, respectively, SEQ ID NOs:13-18, respectively, SEQ ID NOs:19-24, respectively, SEQ ID NOs:25-30, or respectively, SEQ ID NOs:31-36, respectively.
- ~~82.40.~~ The method of claim ~~7836~~, wherein the antibody or antigen-binding fragment thereof comprises VH and VL sequences comprising the sequence of SEQ ID NOs:37 and 38, respectively, SEQ ID NOs:37 and 39, respectively, SEQ ID NOs:40 and 41, respectively, SEQ ID NOs:42 and 43, respectively, SEQ ID NOs:44 and 45, respectively, SEQ ID NOs: 46 and 47, respectively, or SEQ ID NOs:48 and 49, respectively.
- ~~83.41.~~ The method of claim ~~7836~~, wherein the antibody comprises heavy and light chain sequences comprising the sequences of SEQ ID NOs:50 and 51, respectively, SEQ ID NOs:50 and 52, respectively, SEQ ID NOs:53 and 54, respectively, or SEQ ID NOs:55 and 56, respectively.
- ~~84.42.~~ The method of any one of claims 1-~~8341~~, wherein the ADC comprises a linker selected from the group consisting of SMCC, sSPDB, and a peptide linker.
- ~~85.43.~~ The method of any one of claims 1-~~8442~~, wherein the ADC comprises a maytansinoid or an indolino-benzodiazepine.

~~86.44.~~ The method of claim ~~8543~~, wherein the maytansinoid is DM1 or DM4.

~~87.45.~~ The method of claim ~~8543~~, wherein the indolino-benzodiazepine is DGN462 or DGN549.

~~88.46.~~ The method of any one of claims 1-~~8745~~ wherein the ADC is IMGN529, IMGN779, IMGN853, IMGN632, or Kadcyla.

~~89.47.~~ A method for producing IMGN853 comprising (i) mixing huMov19 antibody and DM4-linked to sulfo-SPDB in a continuous conjugation process to form IMGN853 in a flow reactor, ~~optionally wherein in-line monitoring is used to measure the concentration of huMov19 antibody added to the conjugation reaction,~~ (ii) ~~optionally concentrating IMGN853 using single-pass tangential flow filtration and/or countercurrent diafiltration, optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate,~~ (iii) purifying IMGN853 using single-pass tangential flow filtration and/or countercurrent diafiltration, ~~optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate,~~ and (iv) exchanging the IMGN853 into a formulation buffer using single-pass tangential flow filtration and/or countercurrent diafiltration, ~~optionally wherein in-line monitoring is used to measure the concentration of impurities in the retentate.~~

~~90.48.~~ A method for producing IMGN779 comprising (i) mixing Z4681A antibody and DGN462-linked to sulfo-SPDB in a continuous conjugation process to form IMGN779 in a flow reactor, ~~optionally wherein in-line monitoring is used to measure the concentration of Z4681A antibody added to the conjugation reaction,~~ (ii) ~~optionally concentrating IMGN779 using single-pass tangential flow filtration and/or countercurrent diafiltration, optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate,~~ (iii) purifying IMGN779 using single-pass tangential flow filtration and/or countercurrent diafiltration, ~~optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate,~~ and (iv) exchanging the IMGN779 into a formulation buffer using single-pass tangential flow filtration and/or countercurrent diafiltration, ~~optionally wherein in-line monitoring is used to measure the concentration of impurities in the retentate.~~

~~91.49.~~ A method for producing IMGN632 comprising (i) mixing G4732A antibody and sulfonated DNG549C in a continuous conjugation process to form IMGN632 in a flow

~~reactor, (ii) optionally concentrating IMG N632 using single-pass tangential flow filtration and/or countercurrent diafiltration, optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate, (iii) purifying IMG N632 using single-pass tangential flow filtration and/or countercurrent diafiltration, optionally wherein in-line monitoring is used to measure the concentration of unconjugated drug in the retentate, and (iv) exchanging the IMG N632 into a formulation buffer using single-pass tangential flow filtration and/or countercurrent diafiltration, optionally wherein in-line monitoring is used to measure the concentration of impurities in the retentate, optionally wherein the G4732A antibody is reduced and oxidized in only one SPTFF or countercurrent diafiltration step prior the conjugation process.~~

92.50. The method of any one of claims 1-~~28 or 34~~ 9149, wherein the method comprises increasing a first temperature of a conjugation process by at least 5°C to an elevated temperature that is no more than 55°C, wherein the elevated temperature is maintained for no more than 20 minutes.

~~93. A method of producing an ADC comprising increasing a first temperature of a conjugation process by at least 5°C to an elevated temperature, wherein the elevated temperature is maintained for no more than 20 minutes.~~

~~94. The method of claim 92 or 93 wherein the elevated temperature is no more than 55°C.~~

~~95. The method of claim 94, wherein the first temperature is increased by at least 10°C.~~

~~96. The method of claim 94, wherein the first temperature is increased by at least 15°C.~~

~~97. The method of claim 94, wherein the first temperature is increased by at least 20°C.~~

~~98. The method of claim 94, wherein the first temperature is increased by at least 30°C.~~

~~99. The method of any one of claims 92-98, wherein the elevated temperature is 35°C to 55°C.~~

~~100. The method of claim 99, wherein the elevated temperature is 40°C to 50°C.~~

- ~~101.—The method of any one of claims 92-100, wherein increasing the first temperature to the elevated temperature does not take longer than 2 minutes.~~
- ~~102.—The method of claim 101, wherein increasing the first temperature to the elevated temperature does not take longer than 1 minute.~~
- ~~103.—The method of any one of claims 92-100, further comprising decreasing the elevated temperature, optionally to the first temperature.~~
- ~~104.—The method of claim 103, wherein decreasing the elevated temperature, optionally to the first temperature, does not take longer than 2 minutes.~~
- ~~105.—The method of claim 104, wherein decreasing the elevated temperature, optionally to the first temperature, does not take longer than 1 minute.~~
- ~~106.—The method of any one of claims 103-105, wherein the steps of increasing the first temperature to the elevated temperature and then decreasing the elevated temperature are repeated at least twice or at least three times.~~
- ~~107.—The method of any one of claims 103-105, wherein the steps of increasing the first temperature to the elevated temperature and then decreasing the elevated temperature are repeated 2-20 times or 5-10 times.~~
- ~~108.~~51. The method of any one of claims 1-28 or 34-9149, wherein the method comprises decreasing a first temperature of a conjugation process by at least 5°C and no more than 30 °C to a reduced temperature, wherein the reduced temperature is maintained for no more than 20 minutes.
- ~~109.—A method of producing an ADC comprising decreasing a first temperature of a conjugation process by at least 5°C to a reduced temperature, wherein the reduced temperature is maintained for no more than 20 minutes.~~
- ~~110.—The method of claim 108 or 109, wherein the first temperature is decreased by no more than 30°C.~~
- ~~111.—The method of any one of claims 108-110, wherein the temperature is decreased by at least 10°C.~~

112. ~~The method of any one of claims 108-110, wherein the temperature is decreased by at least 15°C.~~
113. ~~The method of any one of claims 108-110, wherein the temperature is decreased by at least 20°C.~~
114. ~~The method of any one of claims 108-113, wherein decreasing the first temperature to the reduced temperature does not take longer than 2 minutes.~~
115. ~~The method of claim 114, wherein decreasing the first temperature to the reduced temperature does not take longer than 1 minute.~~
116. ~~The method of any one of claims 108-115, further comprising increasing the reduced temperature, optionally to the first temperature.~~
117. ~~The method of claim 115, wherein increasing the reduced temperature, optionally to the first temperature, does not take longer than 2 minutes.~~
118. ~~The method of claim 116, wherein increasing the reduced temperature, optionally to the first temperature, does not take longer than 1 minute.~~
119. ~~The method of any one of claims 116-118, wherein the steps of decreasing the first temperature to the reduced temperature and then increasing the reduced temperature are repeated at least twice or at least three times.~~
120. ~~The method of any one of claims 116-118, wherein the steps of decreasing the first temperature to the reduced temperature and then increasing the reduced temperature are repeated 2-20 times or 5-10 times.~~
121. ~~The method of any one of claims 92-120, wherein the elevated temperature or reduced temperature is maintained for no more than 15 minutes.~~
122. ~~The method of any one of claims 92-121, wherein the elevated temperature or reduced temperature is maintained for 30 seconds to 15 minutes.~~
123. ~~The method of claim 122, wherein the elevated temperature or reduced temperature is maintained for 5 to 10 minutes or 10 to 15 minutes.~~

~~124. The method of claim 122, wherein the elevated temperature or reduced temperature is maintained for 1 to 5 minutes.~~

~~125.52.~~ The method of any one of claims 1-28, or ~~34-124~~51, wherein the method comprises changing a first pH of a conjugation process by at least 0.5 to an altered pH, wherein the altered pH does not exceed 9 and does not go below 4, and wherein the altered pH is maintained for no more than 20 minutes.

~~126. A method of producing an ADC comprising changing a first pH of a conjugation process by at least 0.5 to an altered pH, wherein the altered pH is maintained for no more than 20 minutes.~~

~~127. The method of claim 125 or 126, wherein the first pH is increased by at least 1, optionally wherein the altered pH does not exceed 9.~~

~~128. The method of claim 127, wherein the first pH is increased by at least 2.~~

~~129. The method of claim 127, wherein the first pH is increased by at least 3.~~

~~130. The method of claim 125 or 126, wherein the first pH is decreased by at least 1, optionally wherein the altered pH does not go below 4.~~

~~131. The method of claim 130, wherein the first pH is decreased by at least 2.~~

~~132. The method of claim 131, wherein the first pH is decreased by at least 3.~~

~~133. The method of any one of claims 125-132, further comprising changing the altered pH, optionally to the first pH.~~

~~134. The method of claim 133, wherein the steps of changing the first pH to the altered pH and then changing the altered pH are repeated at least twice or at least three times.~~

~~135. The method of claim 133, wherein the steps of changing the first pH to the altered pH and then changing the altered pH are repeated 2-20 times or 5-10 times.~~

~~136. The method of any one of claims 125-135, wherein the altered pH is maintained for no more than 15 minutes.~~

137. ~~The method of any one of claims 125-136, wherein the altered pH is maintained for 30 seconds to 15 minutes.~~
138. ~~The method of claim 137, wherein the altered pH is maintained for 5 to 10 minutes or 10 to 15 minutes.~~
139. ~~The method of claim 137, wherein the altered pH is maintained for 1 to 5 minutes.~~
140. ~~An ADC produced according to the method of any one of claims 1-139.~~