

[4] The contribution of IgG2-mediated cytotoxicity in therapeutic efficacy is not well understood, nor are the quality attributes that influence IgG2-mediated cytotoxicity. The quality attributes that are impactful and predictive of IgG2-mediated cytotoxicity, and therefore suitable to monitor during IgG2 antibody manufacturing, are not well established. Therefore, there is a need to understand how certain quality attributes influence IgG2-mediated cytotoxicity, and modulate such quality attributes accordingly.

SUMMARY

[5] Based on the disclosure provided herein, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific aspects or examples of the invention described herein. Such equivalents are intended to be encompassed by the following examples (E).

E1. In one aspect, the disclosure provides a method of modulating Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing or decreasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or increasing or decreasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site.

E2. A method of increasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or increasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site.

E3. The method of E1 or E2, wherein an increase of about 1 percent of β -galactose increases Fc γ R-mediated cytotoxicity by about 0.55 percent to about 0.75 percent, such as about 0.55 percent, about 0.6 percent, about 0.65 percent, about 0.7 percent, or about 0.75 percent.

E4. A method of decreasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising decreasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or decreasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site.

E5. The method of E1 or E4, wherein a decrease of about 1 percent of β -galactose decreases Fc γ R-mediated cytotoxicity by about 0.55 percent to about 0.75 percent, such as about 0.55 percent, about 0.6 percent, about 0.65 percent, about 0.7 percent, or about 0.75 percent.

E6. The method of any one of E1-E5, wherein said Fc γ R is Fc γ RIIa.

E7. The method of any one of E1-E6, wherein said Fc γ R-mediated cytotoxicity is measured by an in vitro cytotoxicity assay, such as KILR™ Cytotoxicity Assay.

E8. The method of any one of E1-E7, wherein said Fc γ R-mediated cytotoxicity is Fc γ RIIa-mediated cellular cytotoxicity.

E9. In another aspect, the disclosure provides a method of matching the Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of a panitumumab sample to a reference value, comprising:

- (1) obtaining a reference value of Fc γ R-mediated cytotoxicity;
- (2) determining the Fc γ R-mediated cytotoxicity of said panitumumab sample; and
- (3) changing the Fc γ R-mediated cytotoxicity of said panitumumab sample by increasing or decreasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or increasing or decreasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site; such that the difference in Fc γ R-mediated cytotoxicity between the panitumumab sample and the reference value is about 35% or less.

E10. The method of E9, wherein the difference in Fc γ R-mediated cytotoxicity between the panitumumab sample and the reference value is about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, or about 5% or less.

E11. The method of E9 or E10, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is increased by increasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or increasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site.

E12. The method of any one of E9-E11, wherein an increase of about 1 percent of β -galactose increases Fc γ R-mediated cytotoxicity by about 0.55 percent to about 0.75 percent, such as about 0.55 percent, about 0.6 percent, about 0.65 percent, about 0.7 percent, or about 0.75 percent.

E13. The method of E9 or E10, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is decreased by decreasing the amount of terminal β -galactose at the N-297 glycosylation site of panitumumab, or decreasing the amount of panitumumab molecules that comprise G1, G1a, G1b, and/or G2 galactosylated glycan at the N-297 site.

E14. The method of any one of E9, E10, or E13, wherein a decrease of about 1 percent of β -galactose decreases Fc γ R-mediated cytotoxicity by about 0.55 percent to about 0.75 percent, such as about 0.55 percent, about 0.6 percent, about 0.65 percent, about 0.7 percent, or about 0.75 percent.

E15. The method of any one of E9-E14, wherein said Fc γ R is Fc γ RIIa.

E16. The method of any one of E9-E15, wherein said Fc γ R-mediated cytotoxicity is measured by an in vitro cytotoxicity assay, such as KILR™ Cytotoxicity Assay.

E17. The method of any one of E9-E16, wherein said Fc γ R-mediated cytotoxicity is Fc γ RIIa-mediated cellular cytotoxicity.

E18. In another aspect, the disclosure provides a method of modulating Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing or decreasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site, or increasing or decreasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E19. A method of modulating Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing or decreasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E20. A method of increasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site.

E21. A method of increasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising decreasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E22. The method of any one of E18-E21, wherein an increase of about 1 percent of fucosylated panitumumab molecules increases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E23. The method of any one of E18-E21, wherein a decrease of about 1 percent of afucosylated panitumumab molecules increases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E24. A method of decreasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising decreasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site.

E25. A method of decreasing Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E26. The method of any one of E18-E19 and E24-E25, wherein a decrease of about 1 percent of fucosylated panitumumab molecules decreases Fc γ R mediated cytotoxicity by about 2.70 percent to

about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E27. The method of any one of E18-E19 and E24-E25, wherein an increase of about 1 percent of afucosylated panitumumab molecules increases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E28. The method of any one of E18-E27, wherein said Fc γ R is Fc γ RIIa.

E29. The method of any one of E18-E28, wherein said Fc γ R mediated cytotoxicity is measured by an in vitro cytotoxicity assay, such as KILR™ Cytotoxicity Assay.

E30. The method of any one of E18-E29, wherein said Fc γ R mediated cytotoxicity is Fc γ RIIa-mediated cellular cytotoxicity.

E31. In another aspect, the disclosure provides a method of matching the Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of a panitumumab sample to a reference value, comprising:

- (1) obtaining a reference value of Fc γ R-mediated cytotoxicity;
- (2) determining the Fc γ R-mediated cytotoxicity of said panitumumab sample; and
- (3) changing the Fc γ R-mediated cytotoxicity of said panitumumab sample by increasing or decreasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site; such that the difference in Fc γ R-mediated cytotoxicity between the panitumumab sample and the reference value is about 35% or less.

E32. A method of matching the Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of a panitumumab sample to a reference value, comprising:

- (1) obtaining a reference value of Fc γ R-mediated cytotoxicity;
- (2) determining the Fc γ R-mediated cytotoxicity of said panitumumab sample; and
- (3) changing the Fc γ R-mediated cytotoxicity of said panitumumab sample by increasing or decreasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site; such that the difference in Fc γ R-mediated cytotoxicity between the panitumumab sample and the reference value is about 35% or less.

E33. The method of E31 or E32, wherein the difference in Fc γ R-mediated cytotoxicity between the panitumumab sample and the reference value is about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, or about 5% or less.

E34. The method of any one of E31-E33, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is increased by increasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site.

E35. The method of any one of E31-E34, wherein an increase of about 1 percent of fucosylated panitumumab molecules increases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E36. The method of any one of E31-E33, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is increased by decreasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E37. The method of any one of E31-E34 and E36, wherein a decrease of about 1 percent of afucosylated panitumumab molecules increases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E38. The method of any one of E31-E33, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is decreased by decreasing the amount of panitumumab molecules that comprise fucosylated glycan at the N-297 site.

E39. The method of any one of E31-E33 and E38, wherein a decrease of about 1 percent of fucosylated panitumumab molecules decreases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E40. The method of any one of E31-E33, wherein the Fc γ R-mediated cytotoxicity of the panitumumab sample is decreased by increasing the amount of panitumumab molecules that comprise afucosylated glycan at the N-297 site.

E41. The method of any one of E31-E33 and E40, wherein an increase of about 1 percent of afucosylated panitumumab molecules decreases Fc γ R mediated cytotoxicity by about 2.70 percent to about 3 percent, such as about 3.0 percent, about 2.95 percent, about 2.90 percent, about 2.85 percent, or about 2.70 percent.

E42. The method of any one of E31-E41, wherein said Fc γ R is Fc γ R11a.

E43. The method of any one of E31-E42, wherein said Fc γ R-mediated cytotoxicity is measured by an in vitro cytotoxicity assay, such as KILR™ Cytotoxicity Assay.

E44. The method of any one of E31-E43, wherein said Fc γ R-mediated cytotoxicity is Fc γ R11a-mediated cellular cytotoxicity.

E45. In another aspect, the disclosure provides a method of modulating Fc gamma Receptor (Fc γ R)-mediated cytotoxicity of panitumumab, comprising increasing or decreasing the amount of panitumumab molecules that comprise a high-mannose glycan at the N-297 site.

E46. A method of increasing Fc gamma Receptor (FcγR)-mediated cytotoxicity of panitumumab, comprising decreasing the amount of panitumumab molecules that comprise a high-mannose glycan at the N-297 site.

E47. The method of E45 or E46, wherein a decrease of about 1 percent of high-mannose glycan increases FcγR-mediated cytotoxicity by about 1.20 percent to about 1.40 percent, such as about 1.2 percent, about 1.25 percent, about 1.3 percent, about 1.35 percent, or about 1.40 percent.

E48. A method of decreasing Fc gamma Receptor (FcγR)-mediated cytotoxicity of panitumumab, comprising increasing the amount of panitumumab molecules that comprise a high-mannose glycan at the N-297 site.

E49. The method of E45 or E48, wherein a decrease of about 1 percent of high-mannose glycan decreases FcγR-mediated cytotoxicity by about 1.20 percent to about 1.40 percent, such as about 1.2 percent, about 1.25 percent, about 1.3 percent, about 1.35 percent, or about 1.40 percent.

E50. The method of any one of E45-E49, wherein said high-mannose is Mannose-5 (Man-5).

E51. The method of any one of E45-E50, wherein said FcγR is FcγRIIIa.

E52. The method of any one of E45-E51, wherein said FcγR-mediated cytotoxicity is measured by an in vitro cytotoxicity assay, such as KILR™ Cytotoxicity Assay.

E53. The method of any one of E45-E52, wherein said FcγR-mediated cytotoxicity is FcγRIIIa-mediated cellular cytotoxicity.

E54. In another aspect, the disclosure provides a method of matching the Fc gamma Receptor (FcγR)-mediated cytotoxicity of a panitumumab sample to a reference value, comprising:

- (1) obtaining a reference value of FcγR-mediated cytotoxicity;
- (2) determining the FcγR-mediated cytotoxicity of said panitumumab sample; and
- (3) changing the FcγR-mediated cytotoxicity of said panitumumab sample by increasing or decreasing the amount of panitumumab molecules that comprise a high-mannose glycan at the N-297 site; such that the difference in FcγR-mediated cytotoxicity between the panitumumab sample and the reference value is about 35% or less.

E55. The method of E54, wherein the difference in FcγR-mediated cytotoxicity between the panitumumab sample and the reference value is about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, or about 5% or less.

E56. The method of E54 or E55, wherein the FcγR-mediated cytotoxicity of the panitumumab sample is increased by decreasing the amount of panitumumab molecules that comprise a high-mannose glycan at the N-297 site.