

Eylea[®] (Regeneron, Tarrytown, NY) for the treatment of various ocular conditions, including wet type AMD, and is formulated for intravitreal administration. The fusion protein is also marketed as Zaltrap[®] (ziv-aflibercept) (Regeneron, Tarrytown, NY) for the treatment of certain types of cancer and is formulated for intravenous administration.

An attribute of a protein can have an important role in at least the quality of a protein product. Accordingly, provided herein are methods of characterizing and modifying the attributes of aflibercept and compositions comprising aflibercept with attributes, and related advantages.

SUMMARY

The present disclosure provides compositions of aflibercept, including compositions comprising a mixture of aflibercept species. Also provided herein are aflibercept species. In one aspect, the present disclosure provides a S1_Y92delY92L clipped species of aflibercept which lacks the initial 92 amino acids (SEQ ID NO: 5) of SEQ ID NO: 1 and which ~~comprising~~ comprises SEQ ID NO: 3. In another aspect, the present disclosure provides a mixture of aflibercept species including a S1_Y92del clipped species which lacks the initial 92 amino acids (SEQ ID NO: 5) of SEQ ID NO: 1, wherein the amount of the S1_Y92delY92L clipped species in the composition is less than 5.02%, as determined by reduced capillary electrophoresis sodium dodecyl sulfate (rCE-SDS). In yet another aspect, the present disclosure provides a method of producing a composition comprising a mixture of aflibercept species comprising: subjecting a cell culture fluid comprising a mixture of aflibercept species including a S1_Y92del clipped species which lacks the initial 92 amino acids (SEQ ID NO: 5) of SEQ ID NO: 1 to a purification process comprising an anion exchange chromatography (AEX) step followed by a hydrophobic interaction chromatography (HIC) step, wherein the purification process does not include a cation exchange chromatography (CEX) step; and wherein the amount of the S1_Y92del clipped species in the composition is less than 2%, less than 1% or less than 0.8% as determined by rCE-SDS. In a further aspect, the present disclosure provides a composition comprising a mixture of aflibercept species manufactured by the method as described herein.

In one example, a species of aflibercept has one or more different attributes than another species of aflibercept. In some examples, the difference is the presence or absence of an attribute. In another example, the difference is the level or amount of an attribute. Also, provided herein are methods of characterizing one or more attributes of aflibercept as well as methods of modifying one or more attributes of aflibercept, purifying aflibercept, and producing compositions of aflibercept.

One example of the present disclosure is an aflibercept species that is a Y92L clipped species of aflibercept. The Y92L clipped species can comprise SEQ ID NO: 3.

CLAIMS

What is claimed is:

1. A ~~Y92L-clipped S1_Y92del clipped~~ species of aflibercept ~~which lacks the initial 92 amino acids (SEQ ID ON: 5) of SEQ ID NO: 1 and which comprising-comprises~~ SEQ ID NO: 3.
2. A composition comprising a mixture of aflibercept species ~~including a S1_Y92del clipped species which lacks the initial 92 amino acids (SEQ ID ON: 5) of SEQ ID NO: 1~~, wherein the amount of ~~the Y92L-clipped S1_Y92del clipped~~ species in the composition is less than ~~25.0%~~, as determined by ~~reduced capillary electrophoresis sodium dodecyl sulfate (rCE-SDS)~~.
3. The composition of claim 2, wherein the amount of ~~the Y92L-clipped S1_Y92del clipped~~ species is less than ~~2-%, less than~~ 1 % or less than 0.8 % ~~as determined by rCE-SDS~~.
4. The composition of claim 2, wherein the amount of ~~the Y92L-clipped S1_Y92del clipped~~ species is about 1.1 % or about 0.4 % ~~as determined by rCE-SDS~~.
5. A method of producing a composition comprising a mixture of aflibercept species comprising:

subjecting a cell culture fluid comprising a mixture of aflibercept species ~~including a S1_Y92del clipped species which lacks the initial 92 amino acids (SEQ ID ON: 5) of SEQ ID NO: 1~~ to a purification process comprising an anion exchange chromatography (AEX) step followed by a hydrophobic interaction chromatography (HIC) step;
- ~~6. The method of claim 5~~, wherein the purification process does not include a ~~cation exchange chromatography (CEX) step; and wherein the amount of the S1_Y92del clipped species in the composition is less than 2 %, less than 1 % or less than 0.8 % as determined by rCE-SDS~~.
- ~~7.6.~~ The method of ~~any one of claims 5-6~~claim 5, comprising:
 - a) harvesting cells producing aflibercept;
 - b) subjecting the harvested cell culture fluid to a Protein A column; and
 - c) performing anion exchange chromatography (AEX) followed by hydrophobic interaction chromatography (HIC).
- ~~8. The method of any one of claims 5 and 7, wherein AEX is followed by CEX.~~

- ~~9.7.~~ The method of ~~any one of~~ claims 5-~~or 8~~6, wherein the cells are Chinese Hamster Ovary (CHO) cells.
- ~~10.8.~~ The method of any one of claims 5-~~9~~7, wherein the cells are grown by perfusion or fed-batch.
- ~~11.9.~~ The method of any one of claims 5-~~10~~8, wherein the cells are harvested by acid precipitation, centrifugation, depth filtration or any combination thereof.
- ~~12.10.~~ The method of any one of claims 5-~~11~~9, further comprising one or more viral inactivation and/or viral filtration step(s).
- ~~13.11.~~ The method of any one of claims 5-~~12~~11, further comprising a ultrafiltration/diafiltration (UF/DF) step.
- ~~14.12.~~ The method of any one of claims 5-~~13~~11, wherein the method does not comprise performing size exclusion chromatography (SEC).
- ~~15. The method of any one of claims 5, 7-14, wherein a lower amount of Y92L-clipped species is produced as compared to a purification process comprising an AEX step followed by a cation-exchange chromatography (CEX) step.~~
- ~~16. — The method of any one of claims 5-15, wherein the amount of Y92L-clipped species is less than 2 %, less than 1 % or less than 0.8 %.~~
- ~~17.13.~~ The method of any one of claims 5-~~16~~12, wherein the amount of ~~Y92L-clipped~~ the S1_Y92del clipped species is about 1.1 % or about 0.4 % as determined by rCE-SDS.
- ~~18.14.~~ A composition comprising a mixture of aflibercept species manufactured by the method of claim 5.