

composition of recombinant IL-2 (including aldesleukin) consistent in a sterile, stable lyophilized formulation in which the recombinant IL-2 is admixed with a water soluble carrier such as mannitol that provides bulk, and sufficient amount of sodium dodecyl sulfate to ensure the solubility of the recombinant IL-2 in water. The formulation is suitable for reconstitution in aqueous injections for parenteral administration stable and well tolerated in human patients. On the other hand, a detailed description of the process by which the PROLEUKIN™ product is prepared has been more recently disclosed in the European patent EP 1,688,146 B.

SUMMARY

In one aspect, the present disclosure refers to a method of producing aldesleukin suitable for the preparation of a composition comprising aldesleukin in a pharmaceutical aqueous vehicle, the method comprising: fermenting a bacterial cell transfected with an expression vector encoding an aldesleukin gene; disrupting the bacterial cell; collecting inclusion bodies containing aldesleukin; subjecting the inclusion bodies to dissolution by a sodium dodecyl sulfate (SDS) detergent followed by oxidation and a first chromatography; diluting with an organic nitrile and subjecting to a second chromatography; subjecting the aldesleukin to diafiltration and sterilization.

In another aspect, the present disclosure refers to a pharmaceutical composition containing the aldesleukin obtained by the method of claim 1 in which the aldesleukin: a) remains stable in the pharmaceutical liquid composition for at least a year as measured by the in vitro biological activity assay, b) have a stable aldesleukin/SDS aggregate distribution in the liquid composition at least for a year in the range of 4 - 18 nm range with a peak at about 8 nm as measure by dynamic light scattering, c) have a stable therapeutic activity, at least for a year in the liquid composition, as measure in an animal model of human cancer and d) is adequate to amplify human regulatory T cells in a cell culture.

One example of the present disclosure is a method to prepare aldesleukin highly stable in liquid pharmaceutical compositions for parenteral administration to a patient in need of an IL-2 immunotherapy, comprising, in general, the following steps: a) Fermentation of an *E.coli* strain transfected with an expression vector engineered to highly express the aldesleukin gene, b) Bacterial disruption, c) Collection of Inclusion bodies containing aldesleukin aggregates, d) Dissolution of the aldesleukin aggregates using SDS detergent, e) Oxidation with an oxidizing compound e.g. cupric chloride, f) Ceramic hydroxyapatite chromatography, g) Dilution with an

What is claimed :

1. A method of producing aldesleukin suitable for the preparation of a composition comprising aldesleukin in a pharmaceutical aqueous vehicle, the method comprising:
 - fermenting a bacterial cell transfected with an expression vector encoding an aldesleukin gene;
 - disrupting the bacterial cell;
 - collecting inclusion bodies containing aldesleukin;
 - subjecting the inclusion bodies to dissolution by a sodium dodecyl sulfate (SDS) detergent followed by oxidation and a first chromatography;
 - diluting with an organic nitrile and subjecting to a second chromatography;
 - subjecting the aldesleukin to diafiltration and sterilization.
2. The method of claim 1, wherein the bacterial cell is an *Escherichia coli* bacterial cell.
- ~~3.~~ ~~The method of claim 1, wherein the detergent is sodium dodecyl sulfate (SDS).~~
- ~~4.3.~~ The method of claim 1, wherein the oxidation is conducted with an oxidizing compound.
- ~~5.4.~~ The method of claim 1, wherein the first chromatography is a ceramic hydroxyapatite chromatography.
- ~~6.5.~~ The method of claim 1, wherein the second chromatography is a high-performance liquid chromatography (HPLC).
- ~~7.6.~~ The method of claim ~~6.5~~, wherein the HPLC is C4 column HPLC chromatography.
- ~~8.7.~~ The method of claim 1, wherein the aldesleukin remains stable in the pharmaceutical liquid composition for at least a year as measured by an *in vitro* biological activity assay.
- ~~9.8.~~ The method of claim 1, wherein the aldesleukin comprises a stable aldesleukin/SDS aggregate distribution in the liquid composition at least for a year in a range of about 4 - 18 nm with a peak at about 8 nm as measured by dynamic light scattering.
- ~~10.9.~~ The method of claim 1, wherein the aldesleukin has a stable therapeutic activity, at least for a year in the liquid composition, as measured in an animal model of human cancer.
- ~~11.10.~~ The method of claim 1, wherein the aldesleukin induces proliferation of human regulatory T cells in cell culture.

~~12.11.~~ 11. A pharmaceutical composition containing the aldesleukin obtained by the method of claim 1 in which the aldesleukin: a) remains stable in the pharmaceutical liquid composition for at least a year as measured by the in vitro biological activity assay, b) have a stable aldesleukin/SDS aggregate distribution in the liquid composition at least for a year in the range of 4 - 18 nm range with a peak at about 8 nm as measure by dynamic light scattering, c) have a stable therapeutic activity, at least for a year in the liquid composition, as measure in an animal model of human cancer and d) is adequate to amplify human regulatory T cells in a cell culture.