The extremely high cell freezing densities that we describe herein provide numerous advantages. One advantage is that sufficient ECs (e.g. HUVECs) for infusion into a patient can be provided in a single container - avoiding the need for combining the contents of multiple containers of ECs. Another major advantage is that the thawed EC-containing compositions can be diluted to directly yield a therapeutically useful EC composition – i.e. an EC composition having a suitable number and density of ECs for administration to a patient in a solution that is suitable for administration to a patient. This can be achieved because, at the level of dilution needed/used, the concentration of cryopreservatives in the diluted composition is safe for administration to a patient. This eliminates the need to remove the cryopreservatives by performing centrifugation and resuspension steps and also means that the need to transfer the ECs to different containers can be eliminated if desired. For example, if desired a suitable diluent composition can be added directly to the container in which the ECs were frozen and thawed to generate the final EC composition – which may then be transferred directly to a patient in a closed system.

Building on the discovery that ECs, such as HUVECs, can be frozen and thawed at the high densities described herein, we also sought to develop various other improvements to our compositions and methods for freezing and thawing ECs, and administering them to patients, including optimizing the level of human serum albumin (HSA) used.

The improved compositions and methods that we developed for cryopreservation and use of ECs are described further below and elsewhere throughout this patent disclosure.

In one aspect, provided herein is a composition comprising E4ORF1+ endothelial cells (ECs) at a density of from about 50 million cells per ml to about 150 million cells per ml in a freezing medium comprising an effective amount of a cryopreservative.

In another aspect, provided herein is a composition according to any of the preceding claims for use in therapy.

In another aspect, provided herein is a method of freezing endothelial cells, the method comprising: a. suspending endothelial cells (ECs) at a density of from about 50 million cells per ml to about 150 million cells per ml in a freezing medium, wherein the freezing medium comprises an effective amount of a cryopreservative, thereby creating a freezing composition, and b. subjecting the freezing composition to a gradual decrease in temperature to about -80°C to -90 °C.

Accordingly, in some ~~aspects~~ embodiments the present invention provides various compositions comprising endothelial cells (ECs) in freezing media. These compositions may exist at different temperatures and in different states – for example they may exist in a pre-freezing state, in a frozen / cryopreserved state, or in a thawed (post freezing / post cryopreservation) state.

For example, in one embodiment the present invention provides a composition comprising (a) endothelial cells (ECs) at a high density, and (b) a freezing medium comprising an effective amount of a cryopreservative. In some of such embodiments the ECs are at a density of from about 50 million cells per ml to about 150 million cells per ml. In some of such embodiments the ECs are at a density of from about 75 million cells per ml to about 125 million cells per ml. In some embodiments the ECs are at a density of about 100 million cells per ml.