advantageously the cells may be typically selected which have either identical HLA haplotypes (including one or preferably more HLA-A, HLA-B, HLA-C, HLA-D, HLA-DR, HLA-DP and HLA-DQ; preferably one or preferably all HLA-A, HLA-B and HLA-C) to the patient, or which have the most HLA antigen alleles common to the patient and none or the least of HLA antigens to which the patient contains pre-existing anti-HLA antibodies.

Tissue regeneration procedures by means of cell transplantation may be executed using a large variety of cell sources, and commonly using cells having proliferative capacity. For instance, in various human inborn metabolic diseases liver cell transplantation can restore at least some degree of metabolic control. In another example, intraportal transplantation of pancreatic islets offers improved glycaemic control and insulin independence in type 1 diabetes mellitus. For example, pluripotent stem cells capable of differentiating into a plethora of cell lineages, or progenitor cells committed to one or a few cell lineages (multipotent) and displaying varying degrees of differentiation may be used as a cell source for cell transplantation.

Despite some clinical success, current cell transplantation therapies are in need of further improvements. One concern among clinicians and health authorities are the potential consequences of procoagulant activity of certain transplanted cells on engraftment of the cells and other complications. For example, procoagulant activity of islet transplants has been reported to cause graft loss and intraportal thrombotic events (Beuneu et al. Diabetes, 2004, vol. 53, 1407-11; Moberg et al. Lancet, 2002, vol. 360, 2039-45). Procoagulant activity has been also observed in isolated primary hepatocytes (Stéphenne et al. Liver Transpl., 2007, vol. 13, 599-606).

Consequently, there persists an urgent need in the art to improve cell transplantation success and cell engraftment potential, and in particular to reduce prothrombotic complications associated with cell transplantation.

Furlani et al. Microvasc Res., 2009, vol. 77, 370-6 studied the kinetics of human mesenchymal stem cells after intravascular administration into SCID mouse cremaster vasculature by intra-vital microscopy. The authors proposed that intra-arterial mesenchymal stem cells infusion may lead to occlusion in the distal vasculature due to the cells' relatively large size.

Summary ~~of the invention~~

Having conducted extensive *in vitro* and clinical evaluations the inventors have learned that procoagulant activity previously reported for isolated primary cells such as

hepatocytes and islet cells is also observed for stem cells and progenitor cells such as mesenchymal stem cells. The procoagulant activity of stem and progenitor cells may be of concern for transplantation of these cells, in particular may cause undesired bloodstream modifications, loss of the transplanted cells, reduction of the cell engraftment potential and/or thrombotic events. Moreover, this procoagulant activity cannot be controlled by unfractionated heparin, the conventional anticoagulant for hepatocyte transplantation.

The inventors therefore investigated manners to counteract the procoagulant activity of transplanted cells and found that concomitant or associated administration of cells having procoagulant activity with an antithrombin activator and a thrombin inhibitor provides a particularly effective and safe combination for preventing deleterious procoagulant effects. Surprisingly, whereas concomitant administration of cells having procoagulant activity with either one of an antithrombin activator or a thrombin inhibitor alone does not adequately prevent thrombotic events at physiologically acceptable concentrations of respectively the antithrombin activator or thrombin inhibitor, the combination of the antithrombin activator together with the thrombin inhibitor can advantageously prevent cell therapy-induced thrombosis and thrombosis associated complications (e.g., local thrombosis and induction of local inflammation). Accordingly, the inventors realised a particularly advantageous and even synergistic combination therapy and clinical protocols useful for reducing procoagulant activity of transplanted cells, in particular stem and progenitor cells.

In a first aspect of the present disclosure, there is provided a combination comprising at least one antithrombin activator, at least one thrombin inhibitor, and cells selected from the group consisting of adult liver progenitor cells and liver myofibroblasts, wherein

- the antithrombin activator is selected from the group consisting of heparin, unfractionated heparin, low molecular weight heparin and fondaparinux; and
- the thrombin inhibitor is selected from the group consisting of bivalirudin and hirudin.

In a second aspect of the present disclosure, there is provided a pharmaceutical composition comprising the combination as defined above and one or more pharmaceutically acceptable excipients, wherein the pharmaceutical composition is liquid, semi-solid, or solid.

In a third aspect of the present disclosure, there is provided the use of the combination as defined above or the pharmaceutical composition as defined above in the manufacture of a medicament for the treatment of thrombosis or thrombotic complications caused by transplantation of said cells; or for inhibiting procoagulant activity of said cells *in vivo*.

In a fourth aspect of the present disclosure, there is provided a method for inhibiting *in vitro* the procoagulant activity of cells selected from the group consisting of adult liver progenitor cells and liver myofibroblasts, comprising providing a combination comprising said cells, at least one antithrombin activator, and at least one thrombin inhibitor, wherein:

- the antithrombin activator is selected from the group consisting of heparin, unfractionated heparin, low molecular weight heparin and fondaparinux; and

- the thrombin inhibitor is selected from the group consisting of bivalirudin and hirudin.

In a fifth aspect of the present disclosure, there is provided a kit of parts or an article of manufacture comprising the combination as defined above and optionally comprising one or more pharmaceutically acceptable excipients.

In an sixth aspect of the present disclosure, there is provided the use of a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients, in the manufacture of a medicament for the treatment of thrombosis or thrombotic complications caused by procoagulant activity of cells selected from the group consisting of adult liver progenitor cells and liver myofibroblasts upon transplantation thereof; or for inhibiting procoagulant activity of said cells *in vivo*, wherein:

- the antithrombin activator is selected from the group consisting of heparin, unfractionated heparin, low molecular weight heparin and fondaparinux; and

- the thrombin inhibitor is selected from the group consisting of bivalirudin and hirudin.

In a a seventh aspect of the present disclosure, there is provided a method for inhibiting *in vitro* the procoagulant activity of cells selected from the group consisting of adult liver progenitor cells and liver myofibroblasts comprising contacting said cells with a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, wherein:

- the antithrombin activator is selected from the group consisting of heparin, unfractionated heparin, low molecular weight heparin and fondaparinux; and

- the thrombin inhibitor is selected from the group consisting of bivalirudin and hirudin.

Accordingly, the present disclosure~~an aspect~~ relates to a combination comprising cells having procoagulant activity, at least one antithrombin activator, and at least one thrombin inhibitor.

The present disclosure also~~Another aspect~~ relates to a combination comprising at least one antithrombin activator, at least one thrombin inhibitor, and cells selected from the group comprising or consisting of adult liver progenitor cells, mesenchymal stem cells (preferably bone marrow mesenchymal stem cells), skin fibroblasts, and liver myofibroblasts, more preferably selected from adult liver progenitor cells and liver myofibroblasts.

Where applicable, the combination may be configured for separate, simultaneous or sequential in any order administration of the cells, at least one antithrombin activator, and at least one thrombin inhibitor. Moreover, the cells, at least one antithrombin activator, and/or at least one thrombin inhibitor in said combination may be admixed or may be separate. Also disclosed is a method for producing said combination comprising combining the cells, at least one antithrombin activator, and at least one thrombin inhibitor.

at least one thrombin inhibitor. The cells, at least one antithrombin activator, and/or at least one thrombin inhibitor in said pharmaceutical composition may be admixed or may be separate. Also disclosed is a method for producing said pharmaceutical composition comprising admixing the cells, at least one antithrombin activator, and at least one thrombin inhibitor, each separately or in an admixture, with the one or more pharmaceutically acceptable excipients.

As well provided is a kit of parts or an article of manufacture comprising a combination comprising cells having procoagulant activity, at least one antithrombin activator, and at least one thrombin inhibitor, and optionally further comprising one or more pharmaceutically acceptable excipients.

As well provided is a kit of parts or an article of manufacture comprising a combination comprising at least one antithrombin activator, at least one thrombin inhibitor, and cells selected from the group comprising or consisting of adult liver progenitor cells, mesenchymal stem cells (preferably bone marrow mesenchymal stem cells), skin fibroblasts, and liver myofibroblasts, more preferably selected from adult liver progenitor cells and liver myofibroblasts, and optionally further comprising one or more pharmaceutically acceptable excipients.

The kit of parts or article of manufacture may be configured for separate, simultaneous or sequential in any order administration of the cells, at least one antithrombin activator, and at least one thrombin inhibitor. The cells, at least one antithrombin activator, and/or at least one thrombin inhibitor in said kit of parts or article of manufacture may be admixed or may be separate, particularly may be separate such as for example contained in separate containers. Also disclosed is a method for producing said kit of parts or article of manufacture comprising including the cells, at least one antithrombin activator, and at least one thrombin inhibitor, and optionally one or more pharmaceutically acceptable excipients, in a kit of parts or an article of manufacture. Also provided is the kit of parts or article of manufacture for use in any one and each of the herein-described indications.

Further disclosed are ~~any one and each of the following aspects~~:

- a combination comprising cells having procoagulant activity, at least one antithrombin activator, and at least one thrombin inhibitor for use as a medicament;

- a combination comprising at least one antithrombin activator, at least one thrombin inhibitor, and cells selected from the group comprising or consisting of adult liver progenitor cells, mesenchymal stem cells (preferably bone marrow mesenchymal stem

- a method for inhibiting *in vitro* the procoagulant activity of any cells as described herein such as particularly cells having procoagulant activity comprising providing a combination comprising said cells, at least one antithrombin activator, and at least one thrombin inhibitor;

- a method for transplantation of any cells as described herein such as particularly cells having procoagulant activity to a subject in need of such transplantation comprising administering to said subject a therapeutically or prophylactically effective amount of a combination comprising said cells, at least one antithrombin activator, and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients;

- a method for treating thrombosis or thrombotic complications, particularly thrombosis or thrombotic complications caused by transplantation of any cells as described herein such as particularly cells having procoagulant activity, in a subject in need of such treatment, comprising administering to said subject a therapeutically or prophylactically effective amount of a combination comprising said cells, at least one antithrombin activator, and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients;

- a method for inhibiting procoagulant activity of cells, such as of any cells as described herein, *in vivo* in a subject in need of such inhibition, comprising administering to said subject a therapeutically or prophylactically effective amount of a combination comprising said cells, at least one antithrombin activator, and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients.

As well provided are uses of a combination comprising any cells as described herein such as particularly cells having procoagulant activity, at least one antithrombin activator and at least one thrombin inhibitor, in any one and each of the above-described indications.

The present disclosure further~~A further aspect~~ relates to a combination comprising at least one antithrombin activator and at least one thrombin inhibitor. Where applicable, the combination may be configured for separate, simultaneous or sequential in any order administration of the at least one antithrombin activator and at least one thrombin inhibitor. Moreover, the at least one antithrombin activator and at least one thrombin inhibitor in said combination may be admixed or may be separate. Also disclosed is a method for producing said combination comprising combining the at least one antithrombin activator and at least one thrombin inhibitor.

Further disclosed is a pharmaceutical composition comprising a combination comprising at least one antithrombin activator, at least one thrombin inhibitor and one or more pharmaceutically acceptable excipients. The pharmaceutical composition may be configured for separate, simultaneous or sequential in any order administration of the at least one antithrombin activator and at least one thrombin inhibitor. The at least one antithrombin activator and at least one thrombin inhibitor in said pharmaceutical composition may be admixed or may be separate. Also disclosed is a method for producing said pharmaceutical composition comprising admixing the at least one antithrombin activator and at least one thrombin inhibitor, each separately or in an admixture, with the one or more pharmaceutically acceptable excipients.

As well provided is a kit of parts or an article of manufacture comprising a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, and optionally further comprising one or more pharmaceutically acceptable excipients. The kit of parts or article of manufacture may be configured for separate, simultaneous or sequential in any order administration of the at least one antithrombin activator and at least one thrombin inhibitor. The at least one antithrombin activator and at least one thrombin inhibitor in said kit of parts or article of manufacture may be admixed or may be separate, particularly may be separate such as for example contained in separate containers. Also disclosed is a method for producing said kit of parts or article of manufacture comprising including the at least one antithrombin activator and at least one thrombin inhibitor, and optionally one or more pharmaceutically acceptable excipients, in a kit of parts or an article of manufacture. Also provided is the kit of parts or article of manufacture for use in any one and each of the herein-described indications.

Also disclosed are ~~any one and each of the ensuing aspects~~:

- a combination comprising at least one antithrombin activator and at least one thrombin inhibitor for use as a medicament;

- a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients, for use in transplantation of cells having procoagulant activity (i.e., in conjunction with cell transplantation);

- a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients, for use in transplantation of cell selected from the group comprising or consisting of adult liver progenitor cells, mesenchymal stem cells

as particularly cells having procoagulant activity, in a subject in need of such treatment, comprising administering to said subject a therapeutically or prophylactically effective amount of a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients;

- a method for inhibiting procoagulant activity of cells *in vivo* in a subject in need of such inhibition, comprising administering to said subject a therapeutically or prophylactically effective amount of a combination comprising at least one antithrombin activator and at least one thrombin inhibitor, or a pharmaceutical composition comprising said combination and one or more pharmaceutically acceptable excipients.

Preferably, any of the above methods may comprise the steps of: (a) preparing a composition comprising a cell suspension of the cells as described herein such as particularly cells having procoagulant activity in an aqueous solution containing the at least one antithrombin activator; (b) preparing an aqueous solution containing the at least one thrombin inhibitor (i.e., distinct from or separate from composition (a)); and (c) administering the composition as defined in (a) and the solution as defined in (b) simultaneously, separately or sequentially to the subject. Hence, preferably in the above ~~aspects~~embodiments, (a) a composition comprising a cell suspension of the cells as described herein such as particularly cells having procoagulant activity in an aqueous solution containing the at least one antithrombin activator is to be prepared; (b) an aqueous solution containing the at least one thrombin inhibitor is to be prepared (i.e., distinct from or separate from composition (a)); and (c) the composition as defined in (a) and the solution as defined in (b) is to be administered simultaneously, separately or sequentially to a subject.

As well provided are uses of a combination comprising at least one antithrombin activator and at least one thrombin inhibitor in any one and each of the above-described indications.

Also provided is an arrangement comprising a surgical instrument or device for administration of a composition to a subject, such as for example systemically, topically, within an organ or tissue (e.g., portal vein of the liver, spleen, pancreas, liver, kidney capsule, peritoneum and omental pouch), and further comprising the combination or pharmaceutical composition comprising cells as described herein such as particularly procoagulant cells as taught herein, wherein the arrangement is adapted for administration of said combination or pharmaceutical composition for example

systemically, topically, within an organ or tissue. For example, a suitable surgical instrument may be capable of injecting a liquid composition comprising the combination or pharmaceutical composition taught herein, such as systemically, topically, within an organ or tissue.

Cells having procoagulant activity as intended throughout this specification encompass any cells which are capable of activating the coagulation cascade and to induce coagulation or clot formation. Procoagulant activity may be conveniently determined using any known coagulation test, such as without limitation thromboelastometry.

For example, cells may be denoted as having procoagulant activity in the sense of the present ~~invention~~disclosure when, in a standard thromboelastometry test, the cells display clotting time (CT) significantly shorter ($p<0.05$ applying a suitable test of statistical significance) than a negative control without addition of cells. Whereas thromboelastometry represents a standard laboratory technique, for reasons of further guidance suitable thromboelastometry for testing the procoagulant nature of the cells as intended herein may be as follows:

Measurements is performed on a ROTEM® delta analyser (Pentapharm, Munich, Germany). ROTEM® assesses the kinetics and quality of clot formation and clot lysis in real-time. The clotting time (CT) is defined as the period of time from the start of the analysis until the start of clot formation, until the 2 mm amplitude is reached. After a short rest period, 300 µl of whole blood is pipetted into a cup pre-warmed at 37°C. Suspended cells (5x10exp5) are subsequently added to whole blood (negative control: equal volume of suspension medium without any suspended cells). 20 µl of trigger reagent containing tissue factor (TF) at final dilution 1:17000/0.35 pM (such as Innovin, Siemens, Marburg, Germany) diluted in Owren buffer (such as obtainable from Clin-Tech Ltd, UK) is added to the cell-blood mixture followed by addition of 20 µl of 0.2 M CaCl2. After calcium addition, measurements starts automatically. If no coagulation is observed after 1800 sec, thromboelastometry is stopped.

As intended herein, cells as intended herein such as particularly cells having procoagulant activity may be of any origin and/or differentiation state. Preferably, the cells as intended herein such as cells having procoagulant activity are selected from the group consisting of stem cells and progenitor cells. More preferably, the cells as intended herein such as cells having procoagulant activity are mesenchymal stem cells. Also preferably, the cells as intended herein such as cells having procoagulant activity are adult liver-derived progenitor or stem cells.

measurable procoagulant activity of cells in factor VII deficient plasma may at least in part also be related to residual small amounts of factor VII.

The inventors have further realised that particularly preferred procoagulant cells as used herein may comprise a procoagulant activity component resistant to heparin. More specifically, such procoagulant cells will at least partly (e.g., only partly or wholly) retain their procoagulant activity as measured by thromboelastometry in the presence of heparin alone (i.e., in the absence of thrombin activator), such as particularly in the presence of unfractionated heparin, enoxaparin or fondaparinux, particularly at concentrations of 10 UI/ml, 1UI/ml and 0.34 mg/l, respectively. Hence, in further preferred embodiments, procoagulant cells as used herein may comprise a procoagulant activity component independent of the expression of tissue factor (TF) by the cells and may comprise a procoagulant activity component resistant to heparin.

An antithrombin activator as intended throughout this specification encompasses any agent capable of increasing the binding of antithrombin to any one or more of its targets.

In an embodiment, the antithrombin activator is selected from the group consisting of unfractionated heparin and low molecular weight heparin, preferably unfractionated heparin.

A thrombin inhibitor as intended throughout this specification is an agent capable of directly binding to thrombin and inhibiting or preventing thrombin-mediated fibrinogen activation.

In an embodiment, the thrombin inhibitor is selected from the group consisting of bivalirudin and hirudin, preferably bivalirudin. Advantageously, bivalirudin has a comparably short half live of about 35 to about 40 minutes, thereby allowing for a prompt return of a subject to a normal haemostasis status.

The above and additional aspects, preferred embodiments and features of the ~~invention~~present disclosure are described in the following sections and in the appended claims. Each aspect, embodiment or feature described herein may be combined with any other aspect(s), embodiment(s) or feature(s) unless clearly indicated to the contrary. In particular, any feature specified herein, and particularly any feature indicated as being preferred or advantageous, may be combined with any other feature(s) specified herein, and particularly with any other feature(s) indicated as being preferred or advantageous. The subject matter of appended claims is hereby specifically incorporated in this specification.