stemming from physical and physiological damage the cells suffer in the process of cryopreservation. Finally, there is an ongoing need for a reducing cellular plasticity, necroptosis, or necrosis that results from physical and physiological damage cells incur in the course of cell manipulations *in vitro*, during liberation from tissue.

[8] The composition and methods thereof disclosed herein addresses these needs, wherein use of the composition may reduce the incidence of necroptosis or necrosis during cryopreservation, and may treat, prevent, inhibit, or reduce the incidence of cellular plasticity in a plurality of cells.

SUMMARY

[8a] In one aspect of the present disclosure, there is provided the use of a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound for cryopreservation.

[8b] In another aspect of the present disclosure, there is provided a method of cryopreservation, the method comprising the steps of:

(a) bringing one or more cells, tissues, or organs into ~~a plurality of cells in~~ contact with a cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound; and

(b) cooling the composition comprising one or more cells, tissues, or organs ~~the plurality of cells~~ of step (a).

in a plurality of cells.

[8c] In a further aspect of the present disclosure, there is provided a use of a composition comprising a necroptosis inhibitor compound comprising necrostatin-1 (Nec-1) for cryopreservation.

[8~~c~~d] In another aspect of the present disclosure, there is provided the use of a composition comprising at least one inhibitor of necrosis or necroptosis-inducing damage-associated molecular patterns (DAMPs) for preventing excessive necrosis and necroptotic cell death *in vitro* during cryopreservation, a freeze-thaw cycle, or a cooling cycle thereof, or a combination thereof, wherein said inhibitor comprises a cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound and wherein said inhibitor is a caspase-independent inhibitor.

[9] In one example, disclosed herein is a composition comprising a necroptosis inhibitor and a Bax channel inhibitor. In another example, the necroptosis inhibitor comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and the Bax channel inhibitor comprises a 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

[10] In a related example, a necroptosis inhibitor comprises a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one compound, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin compound, 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2-hydroxyethanone compound, (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide compound, methyl-thiohydantoin-tryptophan compound, (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide compound, 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl)phenyl)urea compound, or 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt of said necroptosis inhibitor compound.

[11] In another related example, the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.2nM – 2μM. In another related example, the concentration of said 3,6-Dibromo-α-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound, or

CLAIMS

What is claimed is:

1. Use of a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound for cryopreservation.

2. The use of claim 1, wherein the composition ~~further comprises a Bax channel inhibitor compound~~is caspase-independent.

3. The use of claim 1 or claim 2, wherein said necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an isomer thereof or a pharmaceutically acceptable salt thereof, and said Bax channel inhibitor compound comprises a 3,6-Dibromo-alpha-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride compound or an isomer thereof or a pharmaceutically acceptable salt thereof.

4. The use of claim 1 or claim 2, wherein said necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione, a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one compound, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin compound, 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2-hydroxyethanone compound, (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide compound, methyl-thiohydantoin-tryptophan compound, (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide compound, 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl) phenyl)urea compound, or 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one compound, or an isomer or pharmaceutically acceptable salt of said necroptosis inhibitor compound.

5. The use of any one of claims 1-4, wherein the necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an isomer thereof or a pharmaceutically acceptable salt thereof and the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said isomer thereof or said pharmaceutically acceptable salt thereof is 0.2nM – 2μM.

6. The use of any one of claims ~~3~~51-5, wherein the Bax channel inhibitor compound

comprises a 3,6-Dibromo-alpha-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound or an isomer thereof or a pharmaceutically acceptable salt thereof and the concentration of said 3,6-Dibromo-~~α~~alpha-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound or said isomer thereof or said pharmaceutically acceptable salt thereof is 0.5nM-50μM.

7. The use of any one of claims 1-6, wherein the composition further comprises a nicotinamide adenine dinucleotide (NAD), an adenosine triphosphate (ATP), a cyclosporin A, or a superoxide dismutase, or any combination thereof.

8. The use of claim 7, wherein said superoxide dismutase comprises a manganese superoxide dismutase or a zinc superoxide dismutase.

9. The use of any one of claims 7-8, wherein the concentration of said NAD is 5nM – 500μM.

10. The use of any one of claims 7-9, wherein the concentration of said ATP is 10nM – 1mM.

11. The use of any one of claims 7-10, wherein the concentration of said cyclosporin A is 1nM – 1mM.

12. The use of any one of claims 7-11, wherein the concentration of said superoxide dismutase is 0.001– 100 Kunitz Units (KU).

13. The use of any one of claims 7-12, comprising 20 nM 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione), 5nM 3,6-Dibromo-alpha-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride, 0.05 mM nicotinamide adenine dinucleotide (NAD), 0.01 μM adenosine triphosphate (ATP), 0.01μM cyclosporin A, and 0.1 Kunitz Unit manganese superoxide dismutase or zinc superoxide dismutase.

14. The use of any one of claims 1-13, wherein the composition further comprises a cryoprotective agent.

15. The use of claim 14, wherein said cryoprotective agent comprises DMSO, or serum, or any combination thereof.

16. The use of any one of claims 1-15, wherein the composition further comprises pharmaceutically acceptable excipients or carriers.

17. A method of cryopreservation, the method comprising the steps of:

(a) bringing ~~a plurality of cells in~~one or more cells, tissues, or organs into contact with a cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound; and

(b) cooling the composition comprising ~~the plurality of cells~~one or more cells, tissues, or organs of step (a).

18. The method of claim 17, wherein the composition ~~further comprising a Bax channel inhibitor compound~~is a caspase-independent.

19. The method of claim 17 or claim 18, wherein said necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione, a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one compound, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin compound, 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2- hydroxyethanone compound, (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide compound, methyl-thiohydantoin-tryptophan compound, (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide compound, 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl) phenyl)urea compound, or 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one compound or an isomer or pharmaceutically acceptable salt of said necroptosis inhibitor compound.

20. The method of claim 17 or claim 18, wherein the necroptosis inhibitor compound comprises a 5-((7-cholor-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an isomer thereof or a pharmaceutically acceptable salt thereof.

21. The method of any one of claims 18-20, wherein the Bax channel inhibitor compound

comprises 3,6-dibromo-~~α~~alpha-(1-piperazinylmethyl)-9*H*-carbazole-ethanol dihydrochloride or an isomer thereof or a pharmaceutically acceptable salt thereof.

22. The method of any one of claims 20-21, wherein the necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an isomer thereof or a pharmaceutically acceptable salt thereof and the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said isomer thereof or said pharmaceutically acceptable salt thereof is 0.2nM – 2µM.

23. The method of any one of claims 21-22, wherein the Bax channel inhibitor compound comprises a 3,6-Dibromo-alpha-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound or an isomer thereof or a pharmaceutically acceptable salt thereof and the concentration of said 3,6-Dibromo-~~α~~alpha-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound or said isomer thereof or said pharmaceutically acceptable salt thereof is 0.5nM-50µM.

24. The method of any one of claims 17-23, wherein the composition further comprises a nicotinamide adenine dinucleotide (NAD), an adenosine triphosphate (ATP), a cyclosporin A, or a superoxide dismutase, or any combination thereof.

25. The method of claim 24, wherein said superoxide dismutase comprises a manganese superoxide dismutase or a zinc superoxide dismutase.

26. The method of claim 24 or claim 25, wherein the concentration of said NAD is 5nM – 500µM.

27. The method of any one of claims 24-26, wherein the concentration of said ATP is 10nM – 1mM.

28. The method of any one of claims 24-27, wherein the concentration of said cyclosporin A is 1nM – 1mM.

29. The method of any one of claims 24-28, wherein the concentration of said superoxide dismutase is 0.001– 100 Kunitz Units (KU).

30. The method of any one of claims 24-29, comprising 20 nM 5-((7-Chloro-1H-indol-3-

yl)methyl)-3-methyl-2,4-imidazolidinedione), 5nM 3,6-Dibromo-alpha-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride, 0.05 mM nicotinamide adenine dinucleotide (NAD), 0.01 µM adenosine triphosphate (ATP), 0.01µM cyclosporin A, and 0.1 Kunitz Unit manganese superoxide dismutase or zinc superoxide dismutase.

31. The method of any one of claims 17-30, wherein the composition further comprises a cryoprotective agent.

32. The method of claim 31, wherein said cryoprotective agent comprises DMSO, or serum, or any combination thereof.

33. The method of any one of claims 17-32, wherein the composition further comprises pharmaceutically acceptable excipients or carriers.

34. The method of any one of claims 17-33, wherein the one or more cells comprise one or more tissue culture cells, primary cells, egg cells, a tissue or a portion thereof, or an organ or a portion thereof, or any combination thereof.

35. The method of claim 34, wherein said one or more tissue culture cells or primary cells comprise stem cells, adult cells, transdifferentiated cells, dedifferentiated cells, or differentiated cells, or any combination thereof.

36. The method of any one of claims 17-35, wherein bringing in contact comprises perfusion of an organ or a portion thereof, a tissue or a portion thereof, or a cell.

37. The method of any one of claims 17-36, wherein the method further comprises preventing, inhibiting, or reducing the incidence of cellular plasticity, necroptosis, or necrosis, or inducing a state of metabolic suspension in the one or more cells, tissues, or organs, or inhibiting or reducing changes in the differentiation state of said one or more cells, by the step of: bringing said one or more cells, tissues, or organs in contact with said cryopreservation composition for preservation of one or more cells, tissues, or organs during cryopreservation, a freeze-thaw cycle, or a cooling cycle thereof, or a combination thereof.

38. Use of a composition comprising a necroptosis inhibitor compound comprising necrostatin-1 (Nec-1) for cryopreservation.

~~34~~39. Use of a composition comprising at least one inhibitor of necrosis or necroptosis-inducing damage-associated molecular patterns (DAMPs) for preventing excessive necrosis and necroptotic cell death *in vitro* during cryopreservation, a freeze-thaw cycle, or a cooling cycle thereof, or a combination thereof, wherein said inhibitor comprises a cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound and wherein said inhibitor is a caspase-independent inhibitor.