

WHAT IS CLAIMED IS:

1. ~~—A method for preparing a therapeutic population of tumor infiltrating lymphocytes (TIL) comprising:~~
 - (a) ~~—aseptically disaggregating a tumor resected from a subject thereby producing a disaggregated tumor product, wherein the tumor is sufficiently disaggregated so that the disaggregated tumor product can be cryopreserved;~~
 - (b) ~~—cooling the disaggregated tumor product to a suitable cryopreservation temperature;~~
 - (c) ~~—performing a first expansion by culturing the cryopreserved disaggregated tumor product in a cell culture medium comprising IL-2 to produce a first population of TILs;~~
 - (d) ~~—performing a second expansion by culturing the first population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a second population of TILs; and~~
 - (e) ~~—harvesting and/or cryopreserving the second population of TILs;~~
~~wherein the disaggregation comprises enzymatic disaggregation and/or physical disaggregation, wherein the physical disaggregation comprises repeated physical pressure applied to the resected tumor;~~
~~wherein the steps (a) to (e) are performed in a closed system.~~
2. ~~—The method of claim 1, wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm²~~
3. ~~—The method of any one of claims 1 or 2, wherein the disaggregated tumor product comprises a single cell suspension.~~
4. ~~—The method of any one of claims 1-3, wherein the resected tumor is not fragmented prior to disaggregation.~~
5. ~~—The method of any one of claims 1-4, wherein step (a) is performed at a temperature suitable for enzymatic digestion.~~

6.——The method of any one of claims 1-5, wherein step (b) comprises cooling the disaggregated tumor product directly to the cryopreservation temperature.

7.——The method of any one of claims 1-6, wherein the disaggregation period is 90 min. or less, or 75 min. or less, or 60 min. or less, or 50 min. or less.

8.——The method of any one of claims 1-7, wherein the disaggregation is continuous or proceeds in periods of at least one minute. period

9.——The method of any one of claims 1-8, wherein the tumor is not macerated.

10.——The method of any one of claims 1-9 which comprises cooling the disaggregated tumor product in a controlled temperature device programmed to reduce temperature at a constant rate.

11.——The method of claim 10, wherein the cryopreservation temperature is $80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{min}$ or $1.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$.

12.——The method of any one of claims 1-11, wherein the TILs comprise UTILs or wherein the TILs comprise MTILs.

13.——The method of any one of claims 1-12, wherein the first population of TILs is about 1-20 million TILs.

14.——The method of any one of claims 1-13, wherein step (c) includes growing TILs to produce the first population and the second expansion of step (d) comprises a rapid expansion.

15.——The method of any one of claims 1-14, wherein step (c) is performed for about two weeks and step (d) is performed for about two weeks.

16.——The method of any one of claims 1-15, wherein culturing in step (c) and/or step (d) includes adding IL-7, IL-12, IL-15, IL-18, IL-21, or a combination thereof.

~~17. — The method of any one of claim 1-16, which comprises placing resected tumor tissue into a flexible container with a disaggregation fluid, sealing the container, subjecting the tumor tissue to physical and/or enzymatic disaggregation, and cryopreserving the disaggregated tumor tissue.~~

~~18. — A therapeutic population of cryopreserved tumor infiltrating lymphocytes (TIL) obtained by the method of any one of claims 1-17.~~

~~19. — The therapeutic population of claim 18 wherein the population comprises about 5×10^9 to 5×10^{10} of T cells.~~

~~20. — A cryopreserved bag of the therapeutic population of claim 18 or 19.~~

~~21. — The cryopreserved bag of claim 20 for use in intravenous infusion.~~

221. A method for preparing a therapeutic population of tumor infiltrating lymphocytes (TIL) comprising:

(a) aseptically disaggregating a tumor resected from a subject thereby producing a disaggregated tumor product, wherein the tumor is sufficiently disaggregated so that the disaggregated tumor product can be cryopreserved;

(b) performing a first expansion by culturing the cryopreserved disaggregated tumor product in a cell culture medium comprising IL-2 to produce a first population of TILs;

(c) performing a second expansion by culturing the first population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a second population of TILs; and

(d) harvesting and/or cryopreserving the second population of TILs;
wherein the disaggregation comprises enzymatic disaggregation and/or physical disaggregation, wherein the physical disaggregation comprises repeated physical pressure applied to the resected tumor; and

wherein the steps (a) to (d) are performed in a closed system.

2. The method of claim 1, further comprising cooling the disaggregated tumor product to a suitable cryopreservation temperature after step (a) and before step (b).

optionally wherein the cooling comprises cooling the disaggregated tumor product directly to the cryopreservation temperature.

233. The method of ~~claim 22~~claim 1 or 2, wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².

244. The method of any preceding claim~~any one of claims 22 or 23~~, wherein the disaggregated tumor product comprises a single cell suspension.

255. The method of any preceding claim~~any one of claims 22-24~~, wherein the resected tumor is not fragmented prior to disaggregation.

266. The method of any preceding claim~~any one of claims 22-25~~, wherein step (a) is performed at a temperature suitable for enzymatic digestion.

277. The method of any preceding claim~~any one of claims 22-27~~ **Error!**
Reference source not found., wherein the disaggregation period is 90 minutes~~minutes~~ or less, or 75 minutes~~minutes~~ or less, or 60 minutes~~minutes~~ or less, or 50 minutes~~minutes~~ or less.

288. The method of any preceding claim~~any one of claims 22-27~~, wherein the disaggregation is continuous or proceeds in periods of at least one minute.

299. The method of any preceding claim~~any one of claims 22-28~~, wherein the tumor is not macerated.

3010. The method of any preceding claim~~any one of claims 22-29~~, which comprises cooling the disaggregated tumor product in a controlled temperature device programmed to reduce temperature at a constant rate, optionally wherein the cryopreservation temperature is -80°C ± 10°C and the device is programmed to reduce temperature by 1°C/minute or 1.5°C/minute or 2°C/minute or 1°C/minute ± 0.5°C/minute or 1°C/minute ± 0.5°C/minute or 2°C/minute ± 0.5°C/minute.

~~31. The method of claim 30, wherein the cryopreservation temperature is $80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{min}$ or $1.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$.~~

~~3211.~~ The method of ~~any one of claims 22-31~~ any preceding claim, wherein the TILs comprise UTILs or wherein the TILs comprise MTILs.

~~3312.~~ The method of any preceding claim ~~any one of claims 22-32~~, wherein the first population of TILs is about 1-20 million TILs.

~~3413.~~ The method of any preceding claim ~~any one of claims 22-33~~, wherein step (b) includes growing TILs to produce the first population and the second expansion of step (c) comprises a rapid expansion.

~~3514.~~ The method of any preceding claim ~~any one of claims 22-34~~, wherein step (b) is performed for about two weeks and step (c) is performed for about two weeks.

~~3615.~~ The method of any preceding claim ~~any one of claims 22-35~~, wherein culturing in step (b) and/or step (c) includes adding IL-7, IL-12, IL-15, IL-18, IL-21, or a combination thereof.

~~3716.~~ The method of any preceding claim ~~any one of claim 22-36~~, which comprises placing resected tumor tissue into a flexible container with a disaggregation fluid, sealing the container, subjecting the tumor tissue to physical and/or enzymatic disaggregation, and cryopreserving the disaggregated tumor tissue.

~~3817.~~ A therapeutic population of cryopreserved ~~tumor infiltrating lymphocytes~~ (TILs) obtained by the method of any preceding claim ~~any one of claims 22-37~~, optionally wherein the population comprises about 5×10^9 to 5×10^{10} T cells.

~~39.~~ ~~The therapeutic population of claim 38 wherein the population comprises about 5×10^9 to 5×10^{10} of T cells.~~

~~4018.~~ A cryopreserved bag of the therapeutic population of claim ~~38-17~~ or 39, optionally for use in intravenous infusion.

~~41. The cryopreserved bag of claim 40 for use in intravenous infusion.~~

4219. A method for preparing a therapeutic population of tumor infiltrating lymphocytes (TIL) in a closed system, comprising:

- (a) (i) _____ cryopreserving a resected tumor and disaggregating the cryopreserved tumor, or
- (ii) _____ disaggregating a resected tumor and cryopreserving the disaggregated tumor, or
- (iii) cryopreserving a resected tumor and processing the tumor into multiple tumor fragments, or
- (iv) processing a resected tumor into multiple tumor fragments and cryopreserving the tumor fragments;
- (b) performing a first expansion by culturing the cryopreserved disaggregated tumor product in a cell culture medium comprising IL-2 to produce a first population of TILs;
- (c) performing a second expansion by culturing the first population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a second population of TILs; and
- (d) harvesting and/or cryopreserving the second population of TILs.

4320. The method of claim 4219, wherein the disaggregation comprises physical disaggregation, enzymatic disaggregation, or physical and enzymatic disaggregation.

4421. The method of ~~any one of claims 42 or 43~~ claim 19 or 20, wherein the disaggregation comprises repeated physical pressure applied to the resected tumor, optionally wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².

~~45. The method of any one of claims 42-44, wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².~~

4622. The method of any one of claims ~~42-45~~ 19-21, wherein the physical disaggregation comprises crushing and shearing.

~~47~~23. The method of any one of claims ~~42-46~~19-22, wherein the cryopreserved disaggregated tumor comprises a single cell suspension.

~~48~~24. The method of any one of claims ~~42-47~~19-23, wherein disaggregating is performed at a temperature suitable for enzymatic digestion.

~~49~~25. The method of any one of claims ~~42-48~~19-24, wherein cryopreserving comprises cooling the resected or disaggregated tumor directly to a set cryopreservation temperature.

~~50~~26. The method of any one of claims ~~42-49~~19-25, which comprises cooling the resected or disaggregated tumor in a controlled temperature device programmed to reduce temperature at a constant rate, optionally wherein the cryopreservation temperature is $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{minute}$ or $1.5^{\circ}\text{C}/\text{minute}$ or $2^{\circ}\text{C}/\text{minute}$ or $1^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$ or $1^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$ or $2^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$.

~~51.~~—~~The method of claim 50, wherein the cryopreservation temperature is $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{min}$ or $1.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$.~~

~~52~~27. A method for isolating a therapeutic population of TILs from tumor tissue resected from a subject, comprising:

(a) placing the resected tumor tissue in an automated device for semi-automated aseptic disaggregation of the tumor tissue comprising a programmable processor and a single use aseptic kit, wherein the aseptic kit comprises a closed system; wherein the aseptic kit comprises:

a disaggregation module for receipt and processing of material comprising tumor tissue;

an optional enrichment module for filtration of disaggregated solid tissue material and segregation of non-disaggregated tissue and filtrate; and

a stabilization module for optionally further processing and/or storing disaggregated product material,

wherein each of the modules comprises one or more flexible containers connected by one or more conduits adapted to enable flow of the tissue material there between; and

wherein each of the modules comprises one or more ports to permit aseptic input of media and/or reagents into the one or more flexible containers;

(b) aseptically disaggregating the resected tumor thereby producing a disaggregated tumor;

(c) performing a first expansion by culturing the disaggregated tumor in a cell culture medium comprising IL-2 to produce a first population of UTILs;

(d) performing a second expansion by culturing the first population of UTILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a second population of TILs; and

(e) harvesting and/or cryopreserving the second population of UTILs.

~~5328.~~ The method of ~~claims 32~~claim 27, wherein the disaggregation comprises repeated physical pressure applied to the resected tumor, optionally wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².

~~54.~~ ~~The method of any one of claims 52 or 53, wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².~~

~~5529.~~ The method of ~~any one of claims 52-54~~claim 27 or 28, wherein the resected tumor is not fragmented prior to disaggregation.

~~5630.~~ The method of any one of claims ~~52-55~~27-29, wherein the tumor tissue is not macerated.

~~5731.~~ The method of any one of claims ~~52-56~~27-30, wherein the disaggregation is performed for 90 ~~min.~~minutes or less, or 75 ~~min.~~minutes or less, or 60 ~~min.~~minutes or less, or 50 ~~min.~~minutes or less.

~~5832.~~ The method of any one of claims ~~52-57~~27-31, wherein after step (b), the method comprises (b') cryopreserving the disaggregated tumor in the stabilization module.

5933. The method of ~~any one of claim 5832~~, wherein step (b') comprises cooling the disaggregated tumor product directly to the cryopreservation temperature, optionally wherein the cryopreservation temperature is $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{minute}$ or $1.5^{\circ}\text{C}/\text{minute}$ or $2^{\circ}\text{C}/\text{minute}$ or $1^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$ or $1^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$ or $2^{\circ}\text{C}/\text{minute} \pm 0.5^{\circ}\text{C}/\text{minute}$.

~~60. The method of claim 59 wherein the cryopreservation temperature is $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by $1^{\circ}\text{C}/\text{min}$ or $1.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$.~~

6134. The method of any one of claims 52-6027-33, wherein the automated device further comprises one or more of, in any combination:

sensors capable of recognizing whether a disaggregation process has been completed in the disaggregation module prior to transfer of the disaggregated solid tissue to the optional enrichment module;

weight sensors to determine an amount of media required in the containers of one or more of the disaggregation module; the enrichment module; and/or the stabilization module and control the transfer of material between respective containers;

sensors to control temperature within the containers of the one or more of the disaggregation module; the enrichment module; and/or the stabilization module;

at least one bubble sensor to control transfer of media between the input and output ports of each container in the module;

at least one pump, optionally a peristaltic pump, to control transfer of media between the input and output ports;

pressure sensors to assess the pressure within the enrichment module;

one or more valves to control a tangential flow filtration process within the enrichment module; and/or

one or more clamps to control the transfer of media between the input and output ports of each module.

6235. The method of any one of claims 52-6127-34, wherein the programmable processor controls the disaggregation module to enable a physical and enzymatic breakdown of

the solid tissue material and/or wherein the programmable processor controls the stabilization module to cryopreserve the enriched disaggregated solid tissue in the container.

6336. The method of any one of claims ~~52-62~~27-35, wherein the disaggregation module repetitively crushes and shears the tumor tissue 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm², and the stabilization module cools the disaggregated tumor tissue to -80°C ± 10°C and the device is programmed to reduce temperature by 1°C/minute or 1.5°C/minute or 2°C/minute or 1°C/minute ± 0.5°C/minute or 1°C/minute ± 0.5°C/minute or 2°C/minute ± 0.5°C/minute.

6437. A flexible container suitable for use in a closed system for isolating a therapeutic population of TILs
for processing tissue comprising:
one or more layers made of a sealable polymer, wherein at least three edges of the flexible container are sealed during manufacturing;
an open edge on the flexible container through which tissue material is inserted during use; and
one or more connectors configured to couple the flexible container to at least one element through tubing;
wherein a section proximate the open edge is sealed after tissue material is positioned within the flexible container to form a seal.

6538. The flexible container of claim ~~64~~37, wherein the seal is formed using a heat sealer operating at a predetermined pressure, a predetermined temperature, and predetermined time frame.

6639. The flexible container of ~~any one of claims 64 or 65~~claim 37 or 38, wherein the flexible container is configured to be used with a device that mechanically crushes tissue material placed in the flexible container.

6740. The flexible container of any one of claims ~~64-66~~37-39, wherein the flexible container is suitable for repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm².

6841. A system for extraction of TILs from tumor tissue comprising:
a kit comprising:
a disaggregation flexible container;
a stabilization flexible container; and
at least one indicator tag positioned on at least one of the disaggregation flexible container or the stabilization flexible container capable of providing at least one of a source of tissue, a status of the tissue, or an identifier;
a disaggregation element capable of treating at least some tissue in a disaggregation flexible container to form a processed fluid;
an enrichment element capable of enriching at least some of the processed fluid to form the desired material;
a stabilization element capable of storing a portion of the desired material in the stabilization flexible container and optionally controlling freezing; and
at least one indicator tag reader positioned on at least one of the disaggregation element or the stabilization element capable of providing at least one of a source of tissue, or a status of the tissue at the stabilization element.

6942. Use of a therapeutic population of TILs obtained by the method of any one of claims 1-36 in the manufacture of a medicament for treating cancer in a subject, wherein the therapeutic population of TILs is to be administered to the subject~~A method for treating cancer in a subject comprising:~~

- ~~(a) — aseptically disaggregating a tumor resected from the subject thereby producing a disaggregated tumor product, wherein the tumor is sufficiently disaggregated so that the disaggregated tumor product can be cryopreserved;~~
- ~~(b) — cooling the disaggregated tumor product to a suitable cryopreservation temperature;~~
- ~~(c) — performing a first expansion by culturing the cryopreserved disaggregated tumor product in a cell culture medium comprising IL-2 to produce a first population of TILs;~~
- ~~(d) — performing a second expansion by culturing the first population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a second population of TILs;~~
- ~~(e) — harvesting and/or cryopreserving the second population of TILs; and~~

(f) — administering the second population of TILs to the subject;
wherein the disaggregation comprises enzymatic disaggregation and/or physical disaggregation, wherein the physical disaggregation comprises repeated physical pressure applied to the resected tumor;

wherein the steps (a) to (e) are performed in a closed system.

70. — The method of claim 69, wherein the disaggregation comprises repeated physical pressure applied 120 to 360 times per minute at up to 6 N/cm², more preferably 3 N/cm²

71. — The method of any one of claims 69 or 70, wherein the disaggregated tumor product comprises a single cell suspension.

72. — The method of any one of claims 69–71, wherein the resected tumor is not fragmented prior to disaggregation.

73. — The method of any one of claims 69–72, wherein step (a) is performed at a temperature suitable for enzymatic digestion.

74. — The method of any one of claims 69–73, wherein step (b) comprises cooling the disaggregated tumor product directly to the cryopreservation temperature.

75. — The method of any one of claims 69–74, wherein the disaggregation period is 90 min. or less, or 75 min. or less, or 60 min. or less, or 50 min. or less.

76. — The method of any one of claims 69–75, wherein the disaggregation is continuous or proceeds in periods of at least one minute.

77. — The method of any one of claims 69–76, wherein the tumor is not macerated.

78. — The method of any one of claims 69–77, which comprises cooling the disaggregated tumor product in a controlled temperature device programmed to reduce temperature at a constant rate.

~~79. — The method of claim 78, wherein the cryopreservation temperature is — $80^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and the device is programmed to reduce temperature by by $1^{\circ}\text{C}/\text{min}$ or $1.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $1^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$ or $2^{\circ}\text{C}/\text{min} \pm 0.5^{\circ}\text{C}/\text{min}$.~~

~~80. — The method of any one of claims 69-79, wherein the TILs comprise UTHs or wherein the TILs comprise MTHs.~~

~~81. — The method of any one of claims 69-80, wherein the first population of TILs is about 1-20 million TILs.~~

~~82. — The method of any one of claims 69-81, wherein step (c) includes growing TILs to produce the first population and the second expansion of step (d) comprises a rapid expansion.~~

~~83. — The method of any one of claims 69-82, wherein step (c) is performed for about two weeks and step (d) is performed for about two weeks.~~

~~84. — The method of any one of claims 69-83, wherein culturing in step (c) and/or step (d) includes adding IL-7, IL-12, IL-15, IL-18, IL-21, or a combination thereof.~~

~~85. — The method of any one of claim 69-84, which comprises placing resected tumor tissue into a flexible container with a disaggregation fluid, sealing the container, subjecting the tumor tissue to physical and/or enzymatic disaggregation, and cryopreserving the disaggregated tumor tissue.~~

~~86. — The method of any one of claims 69-85, wherein the second population of TILs comprises about 5×10^9 to 5×10^{10} of T cells.~~

~~8743. The method of any one of claims 69-86~~use of claim 42, wherein the cancer is bladder cancer, breast cancer, cancer caused by human papilloma virus, cervical cancer, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC), lung cancer, melanoma, ovarian cancer, non-small-cell lung cancer (NSCLC), renal cancer or renal cell carcinoma.