CLAIMS

~~What is claimed is:~~

1. A purified composition of hyper-proliferative cancer stem cells comprising a sub-population of cells from a human cancer cell population, the cancer cell population expressing a range of fluorescent signals in response to a fluorescent adenosine triphosphate (ATP) imaging probe, and the sub-population of cells expressing an upper portion of the range of ATP-based fluorescent signals and separated from the human cancer cell population.

2. The composition of claim 1, wherein the upper portion comprises the top 10% of ATP-based fluorescent signals.

3. The composition of claim 1, wherein the upper portion comprises the top 5% of ATP-based fluorescent signals.

4. The composition of claim 1, wherein the composition is positive for one of a CD44 marker and an ALDH marker.

5. The composition of claim 1, wherein the sub-population of cells is stained with ATP-Red 1.

6. The composition of claim 1, wherein the composition is frozen.

7. A purified cell composition comprising a cancer stem cell sub-population stained with a fluorescent adenosine triphosphate (ATP) imaging probe and expressing a target portion of an ATP-based fluorescent signal range of a cancer cell population, the sub-population separated from the cancer cell population.

8. The composition of claim 7, wherein the cancer cell population expresses a range of ATP-based fluorescent signals, and the target portion of the ATP-based fluorescent signal range is one of an upper portion of the ATP-based fluorescent signals and a lower portion of the ATP-based fluorescent signals.

9. The composition of claim 8, wherein the target portion is one of the top 10% of ATP-based fluorescent signals and the top 5% of ATP-based fluorescent signals.

10. The composition of claim 8, wherein the target portion is one of the bottom 10% of ATP-based fluorescent signals and the bottom 5% of ATP-based fluorescent signals.

11. The composition of claim 9, wherein the composition is positive for one of a CD44 marker and an ALDH marker.

12. The composition of claim 1, wherein the sub-population of cells is stained with a fluorescent ATP imaging dye.

13. A purified composition of cells obtained by staining a human cancer cell population with a fluorescent adenosine triphosphate (ATP) imaging probe, separating a fraction of the human cancer cell population having a target portion of ATP-based fluorescent signals, and purifying the separated cells.

14. The composition of claim 13, wherein the target portion comprises one of the top 10% of ATP-based fluorescent signals, the top 5% of ATP-based fluorescent signals, the bottom 10% of ATP-based fluorescent signals, and the bottom 5% of ATP-based fluorescent signals.

15. The composition of claim 13, wherein the target portion comprises one of the top 10% of ATP-based fluorescent signals, the top 5% of ATP-based fluorescent signals, and the separated cells are positive for one of a CD44 marker and an ALDH marker.

16. The composition of claim 13, wherein the fluorescent imaging probe comprises ATP-Red 1.

17. A method of ATP-based cell fractionation, the method comprising:

staining cells in a cell population with a fluorescent adenosine triphosphate (ATP) imaging probe that fluoresces when bound to ATP;

measuring the ATP-based fluorescent signals of the stained cells in the cell population; and

separating the stained cells based on a target portion of ATP-based fluorescent signals.

18. The method of claim 17, wherein the target portion comprises one of the top 10% of ATP-based fluorescent signals, the top 5% of ATP-based fluorescent signals, the bottom 10% of ATP-based fluorescent signals, and the bottom 5% of ATP-based fluorescent signals.

19. The method of claim 17, wherein separating the stained cells based on target portion of ATP-based fluorescent signals comprises fluorescence-activated cell sorting (FACS) gating of the target portion of ATP-based fluorescent signals.

20. The method of claim 19, wherein the gates are set to collect at least one of (i) the stained cells having the top 10% of measured ATP-based fluorescent signals, and (ii) the stained cells having the bottom 10% of measured ATP-based fluorescent signals.

21. The method of claim 17, wherein the fluorescent ATP imaging probe comprises ATP-Red 1.

22. The method of claim 17, wherein the cell population is derived from one of blood, urine, saliva, tumor tissue, non-cancerous tissue, and a metastatic lesion.

23. The method of claim 17, further comprising at least one of measuring ALDH activity of separated cells, measuring anchorage-independent growth of separated cells, measuring the mitochondrial mass of separated cells, measuring the glycolytic and oxidative mitochondrial metabolism of separated cells, measuring the cell cycle progression and proliferative rate of separated cells, and measuring the poly-ploidy of separated cells.

24. A method for separating and collecting metabolically hyper-proliferative cells from a cell population, the method comprising:

staining cells in a cell population with an ATP-labeling dye, wherein the ATP-labeling dye fluoresces when bound to ATP;

measuring the ATP-based fluorescent signals of the stained cells in the cell population;

separating the stained cells based on the measured ATP-based fluorescent signals; and

collecting at least a portion of the separated cells having a measured ATP-based fluorescent signal one of above a predetermined threshold and below a predetermined threshold.

25. The separating and collecting method of claim 24, wherein the ATP-labeling dye comprises ATP-Red 1.

26. The separating and collecting method of claim 24, wherein the predetermined threshold comprises a percentage of an upper portion of the measured ATP-based fluorescent signals.

27. The separating and collecting method of claim 26, wherein the predetermined threshold comprises one of the top 25%, the top 20%, the top 15%, the top 10%, the top 5%, the top 2%, and the top 1%.

28. The separating and collecting method of claim 24, wherein separating and collecting is performed using fluorescence-activated cell sorting (FACS).

29. The separating and collecting method of claim 24, wherein the separated cells are further separated based on a second marker.

30. The separating and collecting method of claim 29, wherein the second marker comprises one of CD44(+), CD133(+), ESA(+), ALDEFLOUR(+), MitoTracker-High, EpCAM(+), CD90(+), CD34(+), CD29(+), CD73(+), CD90(+), CD105(+), CD106(+), CD166(+), and Stro-1(+).

31. The separating and collecting method of claim 30, wherein separating cells based on a second marker occurs at least one of (i) prior to staining cells in the cell population with the ATP-labeling dye, and (ii) after staining cells in the cell population with the ATP-labeling dye.

32. The separating and collecting method of claim 31, wherein the second marker comprises an antibody coated on magnetic beads.

33. The separating and collecting method of claim 24, further comprising staining the cells in the cell population with a second marker, and wherein the measuring the ATP-based fluorescent signals of the stained cells in the cell population occurs after staining with the second marker and the ATP-labeling dye.

34. A method for identifying ~~and treating~~ cancer stem cells in a biologic sample, the method comprising:

~~obtaining a biologic sample from a patient;~~

staining cells in ~~the~~a biologic sample with an ATP-labeling dye, wherein the ATP-labeling dye fluoresces when bound to ATP;

measuring the ATP-based fluorescent signals of the stained cells in the cell population;

comparing the measured ATP-based fluorescent signals to a predetermined threshold indicating the presence of cancer stem cells~~; and~~

~~if the measured ATP-based fluorescent signals exceeds the predetermined threshold, administering to the patient at least one ATP-depletion therapeutic~~.

~~35. The method of claim 34, wherein the ATP-depletion therapeutic comprises one of Doxycycline, Tigecycline, Azithromycin, Pyrvinium pamoate, Atovaquone, Bedaquiline, Niclosamide, Irinotecan, Actinonin, CAPE, Berberine, Brutieridin, Melitidin, Oligomycin, AR-C155858, a Mitoriboscin, a Mitoketoscin, a Mitoflavoscin, a TPP-derivative, dodecyl-TPP, 2-Butene-1,4-bis-TPP, Doxycycline conjugated with a fatty acid, and a combination of Doxycycline, Azithromycin and Ascorbic acid.~~

~~36.~~35. A method of testing a candidate compound for anti-cancer activity, the method comprising:

staining a cancer cell population with an ATP-labeling dye, wherein the ATP-labeling dye fluoresces when bound to ATP;

measuring the ATP-based fluorescent signals of the stained cells;

separating the stained cells based on a target portion of ATP-based fluorescent signals to prepare a hyper-active cancer cell sub-population;

administering the candidate compound to the hyper-active cancer cell sub-population; and

measuring the effect of the candidate compound on the hyper-active cancer cell sub-population.

~~37.~~36. The method of claim ~~36~~35, wherein the ATP-labeling dye comprises ATP-Red 1.

~~38.~~37. The method of claim ~~36~~35, wherein the target portion of ATP-based fluorescent signals comprises one of the top 25%, the top 20%, the top 15%, the top 10%, the top 5%, the top 2%, and the top 1%.

~~39.~~38. The method of claim ~~36~~35, wherein the hyper-active cancer cell sub-population is positive for one of a CD44 marker an ALDH marker.

~~40.~~39. The method of claim ~~36~~35, further comprising at least one of measuring ALDH activity of the hyper-active cancer cell sub-population, measuring anchorage-independent growth of the hyper-active cancer cell sub-population cells, measuring the mitochondrial mass of the hyper-active cancer cell sub-population, measuring the glycolytic and oxidative mitochondrial metabolism of the hyper-active cancer cell sub-population, measuring the cell cycle progression and proliferative rate of the hyper-active cancer cell sub-population, and measuring the poly-ploidy of the hyper-active cancer cell sub-population.

~~41.~~40. A method of diagnosing a risk of metastasis from a cancer sample, the method comprising:

~~obtaining a biologic cancer sample and a non-cancerous biologic sample;~~

determining ~~the~~ expression levels of ABCA2, ATP5F1C, COX20, NDUFA2, and UQCRB, in ~~the~~a biologic cancer sample; and

comparing the detected expression levels to baseline expression levels of ABCA2, ATP5F1C, COX20, NDUFA2, and UQCRB, in a non-cancerous biologic sample ~~from the patient; and~~

~~if the detected expression levels exceed the baseline expression levels, administering an ATP-depletion compound to the patient~~.

~~42. The method of claim 41, wherein the ATP-depletion compound comprises one of Doxycycline, Tigecycline, Azithromycin, Pyrvinium pamoate, Atovaquone, Bedaquiline,~~

~~Niclosamide, Irinotecan, Actinonin, CAPE, Berberine, Brutieridin, Melitidin, Oligomycin, AR-C155858, a Mitoriboscin, a Mitoketoscin, a Mitoflavoscin, a TPP-derivative, dodecyl-TPP, 2-Butene-1,4-bis-TPP, Doxycycline conjugated with a fatty acid, and a combination of Doxycycline, Azithromycin and Ascorbic acid.~~

~~43.~~41. A kit for identifying circulating tumor cells (CTCs) in a biologic sample, the kit comprising reagents for identifying an up-regulation of ABCA2, ATP5F1C, COX20, NDUFA2, and UQCRB in the biologic sample.

~~44.~~42. The kit of claim ~~43~~41, wherein the reagents comprise at least one antibody directed at one of ABCA2, ATP5F1C, COX20, NDUFA2, and UQCRB.

~~45.~~43. A method for detecting circulating tumor cells (CTCs) in a biologic sample, the method comprising:

determining the expression levels of ABCA2, ATP5F1C, COX20, NDUFA2, and UQCRB, in the biologic sample; and

indicating the presence of CTCs if the determined expression levels are upregulated relative to a control.

~~46.~~44. The method of claim ~~45~~43, wherein the biologic sample comprises one of blood, urine, saliva, tumor tissue, non-cancerous tissue, and a metastatic lesion.

~~47.~~45. The method of claim ~~45~~43, further comprising separating CTCs from the biologic sample by staining the sample with a fluorescent ATP-labeling dye, measuring the ATP-based fluorescent signals of the stained sample; separating the stained sample based on a target portion of ATP-based fluorescent signals; and collecting the cell sub-population having the target portion of AATP-based fluorescent signals.

~~48.~~46. The method of claim ~~47~~45, wherein the target portion of ATP-based fluorescent signals comprises one of the top 25%, the top 20%, the top 15%, the top 10%, the top 5%, the top 2%, and the top 1%.