

Claims:

1. A method of preparing an antibody therapeutic comprising:
 - a. providing a dissociated cell sample from at least one solid tumor sample obtained from a patient;
 - b. loading the dissociated cell sample into a microfluidic device having at least one flow region and at least one isolation region fluidically connected to the flow region;
 - c. moving at least one B cell from the dissociated cell sample into at least one isolation region in the microfluidic device, thereby obtaining at least one isolated B cell; and
 - d. identifying at least one isolated B cell that produces antibodies capable of binding to a cancer cell-associated antigen.
2. The method of claim 1, wherein said isolation region comprises at least one conditioned surface that promotes B cell lymphocyte viability, said conditioned surface comprising covalently linked molecules, wherein said covalently linked molecules comprise alkylene ether moieties, amino acid moieties, saccharide moieties, or any combination thereof.
3. The method of claim 2, wherein said at least one conditioned surface comprises a layer of covalently linked hydrophilic molecules.
4. The method of claim 1, wherein said isolation region comprises at least one surface coated with a coating material that promotes B cell viability, wherein said coating material comprises a polymer comprising alkylene ether moieties, amino acid moieties, saccharide moieties, or any combination thereof.
5. The method of any one of claims 1-4, wherein the flow region of the microfluidic device comprises a microfluidic channel,
wherein each of the at least one isolation regions is part of a corresponding sequestration chamber, and wherein each sequestration chamber further comprises a

connection region fluidically connecting the corresponding isolation region to the microfluidic channel, and

wherein each of the at least one isolation region(s) forms a dead-end in the microfluidic device, and wherein, when the flow region is substantially filled with a flowing first fluidic medium and the isolation region(s) are substantially filled with a second fluidic medium:

- e. components of the second medium are able to diffuse into the first medium and components of the first medium are able to diffuse into the second medium; and
 - f. there is substantially no flow of the first medium from the flow region into the isolation region.
6. The method of claim 5, wherein the connection region has a width W_{con} of about 20 microns to about 100 microns.
7. The method of any one of claims 5-6, wherein each of the at least one isolation region(s) has a width W_{iso} that is greater than the width W_{con} of the corresponding connection region.
8. The method of claim 7, wherein each of the at least one isolation region(s) has a width W_{iso} that is about 50 microns to about 250 microns.
9. The method of any one of claims 5-8, wherein each of the sequestration chambers comprises a volume of about 0.5 nl to about 2.5 nl.
10. The method of any one of claims 1-9, wherein the microfluidic device comprises a substrate having a DEP configuration, and wherein moving the at least one B cell into the at least one isolation region comprises using DEP force to move the at least one B cell.
11. The method of any one of claims 1-10, wherein prior to loading the dissociated cell sample, the method further comprises labeling B cells in the dissociated cell sample with a detectable marker, and wherein moving the at least one B cell into the at least one isolation region comprises selecting at least one B cell for movement based on detection of the detectable marker.

12. The method of any one of claims 1-11, further comprising:
contacting the at least one B cell with a stimulating agent that stimulates B cell activation, wherein the stimulating agent comprises a CD40 agonist and/or one or more CD40L+ feeder cells, and wherein the at least one B cell is contacted with the stimulating agent for a period of one to ten days.
13. The method of claim 12, wherein the stimulating agent further comprises a toll-like receptor (TLR) agonist.
14. The method of claim 12, further comprising:
providing culture medium to the at least one B cell, wherein the culture medium comprises one or more growth-inducing agents that promote B cell expansion, and wherein the one or more growth-inducing agents include at least one agent selected from the group of IL-2, IL-4, IL-6, IL-10, IL-21, and BAFF.
15. The method of claim 14, wherein each B cell of the at least one B cell(s) is cultured in the isolation region of the microfluidic device to a cell count of about 8 to 20 cells.
16. The method of any one of claims 1-15, wherein identifying the at least one isolated B cell that produces antibodies capable of binding to the cancer cell-associated antigen comprises introducing a fluidic medium comprising the cancer cell-associated antigen into the microfluidic device, wherein the fluidic medium comprises soluble cancer cell-associated antigen or micro-objects that comprise the cancer cell-associated antigen.
17. The method of claim 16, wherein the micro-objects are beads, and the cancer cell-associated antigen is conjugated to the beads, or wherein the micro-objects are cells and the cancer cell-associated antigen is a membrane-associated antigen present on the cell surface of cancer cells present in the at least one tumor sample.
18. The method of claim 17, wherein the micro-objects are beads and the cancer cell-associated antigen is from a cell membrane preparation obtained from cancer cells isolated from the at least one tumor sample, or wherein the micro-objects are cancer cells isolated from the at least one tumor sample.

19. The method of claim 16, wherein introducing the cancer cell-associated antigen into the microfluidic device comprises:

flowing a fluidic medium that contains micro-objects that comprise the cancer cell-associated antigen through the flow region of the microfluidic device;

stopping the flow of the fluidic medium when at least some of the micro-objects in the medium are located in a portion of the flow region that is proximal to the at least one isolation region; and

moving at least one of the micro-objects into at least one of the isolation regions in the microfluidic device that contains at least one B cell, thereby producing at least one isolation region having at least one micro-object and at least one B cell.

20. The method of claim 16, wherein identifying the at least one isolated B cell that produces antibodies capable of binding to a cancer cell-associated antigen further comprises:

flowing a fluidic medium that contains a labeled antibody-binding agent through the flow region of the microfluidic device; and

stopping the flow of the fluidic medium when at least some of the labeled antibody-binding agent in the fluidic medium is located in a portion of the flow region that is proximal to the at least one isolation region; and

monitoring binding of the labeled antibody-binding agent to the cancer cell-associated antigen.

21. The method of claim 20, wherein the labeled antibody-binding agent is provided in a mixture with the cancer cell-associated antigen.

22. The method of any one of claims 1-21, wherein providing a dissociated cell sample comprises obtaining the at least one solid tumor sample, and dissociating single cells from the at least one solid tumor sample.

23. The method of any one of claims 1-22, wherein prior to loading the dissociated cell sample, the method further comprises:

fractionating the dissociated cell sample to isolate a B cell-enriched fraction that has a greater concentration of B cells than the original dissociated sample; and

loading the B cell-enriched fraction into the microfluidic device.

24. The method of claim 23, wherein the fractionating comprises selecting B cells from the dissociated cell sample using at least one marker chosen from CD19, CD20, IgM, IgD, CD38, CD27, CD138, PNA, and GL7.

25. The method of claim 23, wherein prior to loading the dissociated cell sample, the method further comprises:

fractionating the dissociated cell sample to isolate a cancer cell-enriched fraction that has a greater concentration of cancer cells than the original dissociated sample; and

loading the cancer cell-enriched fraction into the microfluidic device.

26. The method of claim 25, wherein the cancer cells are enriched using at least one marker specific for the cancer and/or using morphological differences.

27. The method of any one of claims 1-26, wherein the tumor that yielded the at least one tumor sample comprises a tertiary lymphoid structure.

28. The method of any one of claims 1-27, wherein the tumor that yielded the at least one tumor sample is a breast cancer, genitourinary cancer, renal cell carcinoma, testicular cancer, prostate cancer, ovarian cancer, uterine cancer, cervical cancer, a cancer of the nervous system, neuroblastoma, intestinal cancer, colorectal cancer, lung cancer, or melanoma.

29. The method of any one of claims 1-28, further comprising:

performing antibody sequencing on the at least one identified B cell or some or all of a clonal population of B cells derived therefrom.

30. The method of any one of claims 1-29, wherein the method further comprises generating an antibody therapeutic comprising at least heavy chain and light chain CDR sequences from the antibody of the at least one identified B cell.

31. The method of any one of claims 1-30, wherein the method further comprises using a microfluidic device to identify whether the antibodies produced by the B cell(s) are capable of binding to non-cancer cells.

32. The method of claim 31, wherein the non-cancer cells are from the patient that provided the at least one tumor sample.
33. An engineered T or NK cell comprising an antibody or fragment thereof displayed on its external surface, wherein the antibody or fragment thereof is identified by the method of any one of claims 1-32.
34. The engineered T or NK cell of claim 33, wherein the T or NK cell and the antibody or fragment thereof were obtained from the same patient.
35. Use of an antibody or fragment thereof produced by the method of any one of claims 1-32 in the preparation of a medicament for treating a patient having cancer.
36. The use of claim 35, wherein the tumor sample is from the same patient who is treated.