[0012] In certain aspects, the course of treatment is selected from the group consisting of selecting the anticancer drug, selecting the anticancer drug dose, and determining the length of treatment. In certain other aspects, the methods further include measuring expression and or activation levels of at least one other signal transduction molecule such as CRKL, AKT, STAT5 and SRC.

[0013] As such, the present invention provides: a method for selecting an anticancer drug in a subject having a hematological malignancy, the method comprising:

1) measuring the activation state level of BCR-ABL in an isolated cell from a sample from the subject;

2) incubating the isolated cell with at least one anticancer drug prior to initiation of treatment;

3) measuring the activation state level of BCR-ABL in the incubated cells; and selecting a course of treatment based upon the activation state level of BCR-ABL.

[0014] The present invention also provides a method for monitoring the efficacy of an anticancer drug in a subject, wherein the subject has a hematological malignancy, the method comprising:

a) measuring the activation state of BCR-ABL at T_0, prior to the first administration of the anticancer drug;

b) administering the anticancer drug to the subject, wherein the first administration of the anticancer drug is at time T_1;

c) measuring the activation state and or expression level of BCR-ABL at a time T_2 in a sample from the subject; and

d) determining a course of treatment based upon the activation state and or expression level of BCR-ABL.

[0015] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 illustrates a flow diagram of one embodiment of the present invention.

proximity-mediated immunoassay described herein. The methods of the present invention can be used to detect the levels of phospho-BCR-ABL in samples spiked with K562 cells. In particular, the measured levels of phosphorylated BCR-ABL relate to the number of K562 cells added to the blood samples. FIG. 9B shows total BCR-ABL recovery.

[0025] FIG. 10 tabulates patients analyzed in one embodiment of the invention. Patient 1 has active CML and has been receiving treatment since December 2006. Patient 2 who also has active CML has been receiving imatinib treatment since January.

[0026] FIG. 11A-B illustrates that Patient 1 (A) has a lower amount of phospho-BCR-ABL per ml (*e.g.*, 10,979 CU/ml ± 1,245 CU/ml) of blood as compared to Patient 2 (*e.g.*, 185,934 CU/ml ± 11,019 CU/ml) (B), suggesting that Patient 1 is responding to imatinib treatment. The values were determined without subtracting the blood background.

[0027] FIG. 12A-B show the detection of activated (phosphorylated) levels of BCR-ABL as determined by methods described herein. Cell lysate samples isolated from Patient 1 were diluted 1:5 and 1:20 according to the methods described in Example 6. The standard sample represents untreated K562 cell lysates with varying # of cells per 80 µl of lysate (e.g, 10000, 3000, 1000, 300, 100, 30, 10, or 0 cells / 80 µl). The top panel of FIG. 12A shows the images of the BCR-ABL CEER Assays.

[0028] FIG. 13A-B shows that *in vitro* treatment with imatinib of blood sample from Patient 1 dramatically decreased the amount of phosphorylated BCR-ABL, as compared to nilotinib treatment. FIG. 13A shows the images of the BCR-ABL CEER Assays.

[0029] FIG. 14 shows that activated BCR-ABL levels in Patient 1's blood sample change when treated with increasing amounts of BCR-ABL inhibitor (e.g., imatinib or nilotinib). Different ~~drug~~ concentrations of drug were incubated with Patient 1's blood sample for 1.5 hours at 37°C. The mean CU value after 1µM imatinib treatment was 26, and 110 after 0.1µM imatinib treatment. The top panel of FIG. 13 shows the images of the BCR-ABL CEER Assays.

[0030] FIG. 15A-B show that imatinib is more effective than nilotinib at reducing activated BCR-ABL protein in Patient 1's blood sample. The bar graphs show that 1µM imatinib treatment decreased activated BCR-ABL levels (A) as compared to the untreated sample. FIG. 15 B is after subtraction of blood background.

[0031] FIG. 16A-D illustratesthe pathway profile of other phosphorlyated signaling transduction pathway components such as CRKL (A), AKT (B), STAT5 (C) and SRC (D). It

be analyzed for the level of expression and/or activation of one or more oncogenic fusion proteins such as BCR-ABL, substrates thereof, pathways thereof, or combinations thereof. Without being bound to any particular theory, eliminating the need for any wash steps after cell isolation is advantageous because cells of interest can be recovered from blood without changing the intracellular concentration of a therapeutic agent such as an anticancer drug (*e.g.*, a tyrosine kinase inhibitor). As set forth in the Examples below, cell isolation using the apparatuses described herein without any wash steps is contrary to the art-accepted practice of washing cells after isolation and provides cellular extracts from recovered cells without substantial dilution of a therapeutic agent such as an anticancer drug (*e.g.*, a tyrosine kinase inhibitor such as, *e.g.*, Gleevec®, Tasigna®, Sprycel®, *etc.*) inside the cells.

[0105] In particular embodiments, the apparatuses of the present invention are substantially similar or identical to the apparatus depicted herein. One skilled in the art will appreciate that the dimensions of one or more components of the apparatus described herein and illustrated can be varied, taking into account parameters such as, for example, the volume of sample to be loaded into the apparatus, the type of centrifuge to be used to spin the apparatus, the volume of lysis buffer to be added to the upper chamber of the apparatus, *etc.*

[0106] Turning now to FIG. 2A-G, as shown therein, there is a filtration device or apparatus for sample collection. FIG. 2A is the upper portion or chamber of the apparatus 201 which is a cylindrical tube with male helical ridges or threads 210. The upper portion with cap 215 is shown in FIG. 2B-C. This upper portion 201 can optionally have a cap 215 that snaps shut to prevent spilling of the sample. In certain embodiments, the snap-cap lid 215 is tethered via strap 217 to the upper portion and can be used to securely close the opening of the upper chamber after a sample and/or a reagent is added to the filtration device. The upper chamber 201 of the apparatus of the present invention preferably attaches to a lower ~~camber~~ portion or chamber 222 as shown in FIG. 2 D-E. The threads 210 of the upper portion fit securely into female grooves 221 of the lower portion or chamber 222. Preferably, the inner diameters of the upper and lower chambers are similar as to create a cylindrical tube which allows liquids to pass therethrough.

[0107] In certain aspects, the lower chamber 222 of the filtration device or apparatus is a cylindrical tube with an internal screw thread at one end 221 (FIG. 2E). In certain aspects, one or more (*e.g.*, a plurality such as two, three, four, five, six, seven, eight, nine, ten, or more) stacked filter membranes is placed between the screw threads of the upper chamber and lower chamber before the chambers are securely attached together. The filter(s) sits on pin wheel 225 of the lower chamber as shown in FIG. 2E.

4D depicts the collection tube 435 joined with the upper chamber 410 and lower chamber or portion 420. In FIG. 4D, view 405 is looking down on the device and view 461 is looking up at the device.

[0111] In some embodiments, in operation, a volume (e.g., 1 ml) of patient blood treated with a solution mixture comprising a protease and/or a phosphatase inhibitors is loaded into the upper chamber of an assembled cell isolation apparatus. The apparatus can be centrifuged in a tabletop or clinical centrifuge, such as an Allegra 6R centrifuge (Beckman), Sorvall Legend centrifuge (Thermo Scientific), or Heraeus Megafuge centrifuge (Kendro). In some aspects, the apparatus is centrifuged for 5-30 minutes at 600-2,000 rpms at 4°C. After centrifugation, the collection vessel containing the red blood cells (and plasma) is removed from the cell isolation apparatus, capped and set aside.

[0112] In further embodiments, a second collection vessel is attached to the filtration device. Non-limiting examples of a second collection tube for the cell lysate include 1.5 ml and 2 ml microcentrifuge tubes. Without a washing step, lysis buffer is added to the upper chamber of the filtration device. The upper chamber is capped and the filtration device and second collection tube are shaken vigorously. In certain instances, the cell isolation apparatus is incubated at 4°C for at least 1, 5, 10, 15, 20, 30, 60, or 120 minutes, preferably between about 15 to about 30 minutes. The apparatus can then be centrifuged (e.g., 3,000rpm for 5 min). The cell lysate can be transferred to another centrifuge vessel such as a microcentrifuge tube for storage at -70°C.

[0113] FIG. 5A-D show an alternative embodiment of the filtration device or apparatus 500 for sample collection of the present invention. In this embodiment, there is an upper portion 501 and a bottom portion or chamber 530 with a sleeve or middle connector 522. The middle connector or sleeve 522 can optionally be fixed to either the upper 501 or bottom portion 530. As shown in FIG. 5B, in certain aspects, the upper portion has ridges or male ridges and can be fixed to the sleeve or bottom portion. In FIG. 5B, the apparatus is shown with cap 510. FIG. 5C shows that the bottom portion 530 can optionally have a cap 534 to keep this portion sealed. FIG. 5D depicts a lower chamber or portion 522 clearly showing female grooves. This portion can optionally be fixed to the bottom portion such that the sleeve ~~in~~is integral to the bottom portion of the device.

[0114] FIG. 6A-C show yet another aspect of the present invention. FIG. 6 A is a cross-sectional view of a device of the present invention, wherein the bottom portion 610 is joined with the upper portion 625 with sleeve 633 enabling joinder. Inset FIG. 6B is a close up of

and cell fragments. The supernatant of the cell lysate is transferred to another tube for storage at -70°C.

[0162] In yet another embodiment, the cell isolation apparatus comprises a tube filter unit and a collection vessel (FIG. ~~26~~2-6). The tube filter unit comprises a cylindrical tube affixed at one end with one or more (*e.g.*, a plurality of two, three, four, five, six, seven, eight, nine, ten, or more) filter membranes, wherein the membranes are able to retain healthy and malignant leukocytes and/or circulating tumor cells from whole blood samples. In particular instances, the filter membranes are (*e.g.*, 2, 3, or 4) layers of filters such as PALL filters (*e.g.*, Leukosorb Medium). In certain instances, each filter has a pore size of 8 μm, a thickness of 355.6-558.8 μm, and a leukocyte retention yield of 70-80%. In certain instances, the tube filter unit is attached to a collection vessel by way of a plastic adapter. The filter membrane of the tube filter unit is securely attached to the adapter which is positioned in the opening of a collection tube. Examples of collection vessels include, but are not limited to, 3 ml, 5 ml, 8 ml, 14 ml and 16 ml plastic culture tubes. In certain embodiments, 1 ml of patient whole blood treated with protease inhibitors and phosphatase inhibitors is loaded into the top of the tube filter unit of the cell isolation apparatus. The apparatus is centrifuged for 5-30 minutes at 600-2000 rpms (*e.g.*, 800 rpms) at 4°C in a tabletop centrifuge such as an Allegra 6R centrifuge (Beckman), Sorvall Legend centrifuge (Thermo Scientific), or Heraeus Megafuge centrifuge (Kendro). In certain instances, the collection tube containing the red blood cells is removed from the cell isolation apparatus and a new collection tube is attached. Without washing steps, 200 μl-1 ml of lysis buffer is added to the opening of the tube filter unit of the cell isolation apparatus. The apparatus is sealed or capped, and then shaken vigorously for 15-30 minutes at 4°C. The cell isolation apparatus is centrifuged and spun at about 3000 rpm for about 5 minutes in a tabletop centrifuge. The cell lysate can then be transferred from the collection vessel to another vessel for storage at -70°C.

[0163] In still yet another embodiment, the cell isolation apparatus of the present invention comprises a plurality of filtration devices or tube filter units and a multi-well or multi-tube collection vessel. Filtration devices and tube filter units described above can be attached to wells of a multi-format plate, such as a 12- well , 24- well, 48-well, or 96-well plate.

Example 2. Protocol for Tumor Cell Isolation From CML Patient Blood By Filtration Method Using Cell Isolation Apparatus.

[0164] This example details the protocol used to isolate and harvest CML tumor cells in individual patient whole blood using a method of the present invention. The whole blood

from patient can be *in vitro* treated or non- treated with BCR-ABL inhibitors prior to isolation.

[0165] Freshly drawn blood sample are best for isolation of tumor cells. If fresh samples cannot be obtained, blood samples can be processed within 1-3 days after being drawn. Samples collected in EDTA (Becton Dickenson cat. no. #366643, EDTA (K2) sterile tubes) are sent to for processing the day they are drawn. Samples must be kept at room temperature prior to processing. It is important to avoid refrigerating or freezing the samples at this point.

[0166] The blood sample received in EDTA tubes (BD #366643) is treated with protease and phosphatase inhibitors. The tube of blood is mixed gently by slowly inverting 4-6 times, and then 0.05 mL of a protease and phosphatase inhibitor cocktail per 1mL blood is added. At this point in the protocol, tumor and white blood cells can be isolated from the blood sample by proceeding to the section of this example entitled "Isolation and lysis of tumor and white blood cells". Optionally, the blood sample can be treated with a drug prior to proceeding to that section.

Drug treatment of patient blood samples

[0167] The drug such as a BCR-ABL inhibitor are diluted in Hank's Balanced Saline Solution (HBSS) before it is added to the blood sample. 1.0 mL of the patient's blood sample is placed into a culture tube. The desired concentration of drug is added to each aliquot of the patient's blood. For instance, 10 µl, 1 µl or 0.1 µl that correspond to a drug concentration of 10 µM, 1 µM or 0.1 µM is added to a tube. One milliliter (1 ml) of untreated blood in a separate tube can be used as a control. The tubes are then incubated fro 4 hours at 37^{o}C in a CO_2 incubator. Afterwards, the drug treated sample can be further processed according to the procedure of the next section in order to isolate and lyse the tumor and white blood cells of the sample.

Isolation and lysis of tumor and white blood cells

[0168] Gently mix the blood sample to be added to the filtration device by slowly inverting the tube 4-6 times. Using a 1ml pipette, load 1mL of the blood sample to the upper chamber of the filtration device that is housed in a collection tube. After the sample has been loaded, snap close the filtration device. The cell isolation apparatus is ~~place~~placed in an Allegra 6R centrifuge and centrifuged for 15 minutes at 1,000 rpms at 4^{o}C. After centrifugation, the cell isolation apparatus is removed from the centrifuge. The first collection tube that contains the red blood cells is removed from the filtration unit and replaced with a second, clean

individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the ~~spirit or~~ scope of the appended claims.

WHAT IS CLAIMED IS:

1. An apparatus for isolating and separating leukocytes from red blood cells in a whole blood sample, said apparatus comprising:

a filtration device comprising an upper chamber, a lower chamber, and one or more stacked filter membranes between said upper and lower chambers, wherein said one or more stacked filter membranes are capable of retaining said leukocytes, wherein the inner diameters of the upper and lower chambers are similar as to create a cylindrical tube which allows liquids to pass therethrough; and

a collection tube for collecting red blood cells from said whole blood sample, wherein said filtration device is placed on top of said collection tube, and wherein said red blood cells are separated from said leukocytes and collected in said collection tube following centrifugation.

2. The apparatus of claim 1, wherein said whole blood sample is loaded into said upper chamber of said filtration device.

3. The apparatus of claim 1 or 2, wherein said filtration device comprises two, three, or four stacked filter membranes.

4. The apparatus of ~~any one of claims **1** to **3**~~claim 1 or 2, wherein said upper chamber further comprises a snap-cap lid attached thereto.

5. The apparatus of ~~any one of claims **1** to **4**~~claim 1 or 2, further comprising a second collection tube, wherein said collection tube containing said red blood cells is replaced with said second collection tube following centrifugation.

6. The apparatus of claim 5, wherein a lysate of said leukocytes is collected in said second collection tube following the addition of lysis buffer to said upper chamber and centrifugation.

7. The apparatus of claim 6, wherein said lysis buffer is added to said upper chamber without washing said one or more stacked filter membranes.

8. The apparatus of ~~any one of claims **1** to **4**~~claim 1 or 2, further comprising a second collection tube, wherein said one or more stacked filter membranes are removed from said filtration device following centrifugation and placed into said second collection tube.

9. The apparatus of claim 8, wherein said second collection tube contains lysis buffer.

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10. The apparatus of ~~any one of claims **1** to **9**~~claim 1 or 2, wherein said whole blood sample is obtained from a subject having or suspected of having a hematological malignancy.

11. The apparatus of claim 10, wherein said hematological malignancy is chronic myelogenous leukemia (CML).

12. The apparatus of claim 10 ~~or **11**~~, wherein said subject is either receiving or not receiving therapy with ~~a therapeutic agent~~an anticancer drug.

~~**13**. The apparatus of claim **12**, wherein said therapeutic agent is an anticancer drug.~~

13~~**14**~~. The apparatus of claim ~~13~~12, wherein said anticancer drug is selected from the group consisting of a tyrosine kinase inhibitor, chemotherapeutic agent, hormonal therapeutic agent, radiotherapeutic agent, monoclonal antibody, vaccine, and combinations thereof.

14~~**15**~~. The apparatus of claim ~~14~~13, wherein said tyrosine kinase inhibitor is selected from the group consisting of imatinib mesylate~~, (Gleevec®),~~ nilotinib ~~(Tasigna®)~~, dasatinib ~~(Sprycel®)~~, bosutinib ~~(SKI-606)~~, gefitinib ~~(Iressa®)~~, sunitinib ~~(Sutent®)~~, erlotinib ~~(Tarceva®)~~, lapatinib ~~(Tykerb®)~~, canertinib ~~(CI 1033)~~, semaxinib ~~(SU5416)~~, vatalanib ~~(PTK787/ZK222584)~~, sorafenib ~~(BAY-43-9006),~~ leflunomide ~~(SU101)~~, vandetanib ~~(ZACTIMA™; ZD6474)~~, and combinations thereof.

15~~**16**~~. The apparatus of ~~any one of claims **12** to **15**~~claim 12, wherein said whole blood sample is incubated *in vitro* with said ~~therapeutic agent~~anticancer drug prior to isolation of said leukocytes.

16~~**17**~~. The apparatus of ~~any one of claims **1** to **16**~~claim 1 or 2, wherein said leukocytes are selected from the group consisting of normal leukocytes, malignant leukocytes, diseased leukocytes, and combinations thereof.

17~~**18**~~. The apparatus of ~~any one of claims **1** to **17**~~claim 1 or 2, wherein said apparatus is a plurality of filtration devices.

18~~**19**~~. A method for preparing a lysate of leukocytes from a whole blood sample without ~~substantial~~ dilution of a therapeutic agent, said method comprising:

(a) loading said whole blood sample into an apparatus of ~~any one of claims **1** to **18**~~claim 1 or 2;

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(b) centrifuging said apparatus to capture said leukocytes on said one or more stacked filter membranes and to separate said red blood cells into said collection tube; and

(c) lysing said leukocytes captured on said one or more stacked filter membranes with lysis buffer but without a wash step between steps (b) and (c) to thereby prepare a lysate of leukocytes.

19~~**20**~~. The method of claim 18~~19~~, further comprising replacing said collection tube with a second collection tube between steps (b) and (c).

20~~**21**~~. The method of claim 19~~20~~, further comprising centrifuging said apparatus containing said second collection tube after lysing said leukocytes in step (c) and collecting said lysate of leukocytes in said second collection tube.

21~~**22**~~. The method of ~~any one of claims **19** to **21**~~claim 18, wherein said whole blood sample is obtained from a subject receiving a therapeutic agent.

22~~**23**~~. The method of claim 21~~22~~, wherein said a therapeutic agent is an anticancer drug.

23~~**24**~~. The method of ~~any one of claims **19** to **21**~~claim 18, wherein said whole blood sample is incubated *in vitro* with a therapeutic agent prior to loading into said apparatus.

24~~**25**~~. The method of ~~any one of claims **19** to **24**~~claim 18, wherein the expression level, ~~and/or~~ activation level, or expression level and activation level of at least one oncogenic fusion protein ~~and/or,~~ signal transduction molecule, or oncogenic fusion protein and signal transduction molecule is measured in said lysate of leukocytes.

25~~**26**~~. The method of claim 24~~25~~, wherein the at least one oncogenic fusion protein comprises BCR-ABL.

26~~**27**~~. The method of ~~any one of claims **19** to **24**~~claim 18, ~~wherein said~~ wherein said apparatus is a plurality of filtration devices.

27. The apparatus of claim 1, wherein said upper chamber is a cylindrical tube with helical ridges or threads.

28. The apparatus of claim 4, wherein the snap-cap lid is tethered via a strap to the upper chamber.

29. The apparatus of claim 27, wherein the threads of the upper portion fit securely into the lower chamber.

30. The apparatus of claim 1, a filtration device wherein the upper chamber, and the lower chamber are screwed together.