A Non-invasive Diagnostics to Detect Circulating Leukemic Cells in Blood.

A microfluidic platform to monitor MRD in Leukemic patients

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BioFluidica

Abstract

Introduction : BioFluidica has developed a platform, The Liquid Scan™, to analyze circulating cancer cells in whole blood. The Liquid Scan™ is unique in its performance metrics compared to existing platforms by employing fully automated instrumentation in conjunction with a highly sensitive and specific cell detection microfluidic chip. This technology can be applied to a broad variety of solid tumors using tumor specific positive selection. Blood cancers such as Leukemias and Lymphomas generate cancerous cells that are differentiated from normal blood cells through the upregulated expression of cancer specific markers. The blood cancer cells can not be differentiated by size or other physical properties from normal blood cells. BioFluidica's microfluidic chip can be programed using capture compounds to target the cancer specific markers for specific cancer cell isolations. The capture and analysis of live circulating leukemic cells (CLCs) from whole blood, even when these cells appear undetectable by other methods.

The Liquid Scan™ has been applied to capture CLCs from patients suffering from acute myeloid, as well as acute lymphoblastic leukemia (AML and ALL, respectively). These leukemias are acute diseases of the blood and bone marrow, with mutational changes and clonal expansion of abnormal myeloid and lymphoblastic precursors respectively, claiming a great number of lives every year. Bone marrow biopsies/aspirations have been the standard methods for reliable diagnosis and monitoring of these diseases requiring large quantities of cancer cells for flow cytometry analysis. These procedures are extremely painful for patients, necessitate specialized centers and trained personnel, which increases the overall cost of treatment.

Methods: We have applied the Liquid Scan™ to capture and characterize CLCs, using specific antibodies to capture known clones of leukemic blasts. As a model, we use Kasumi-1, Kasumi 3, KG-1 and HL-60 cells for AML. For patient selection a panel of secondary (aberrant) marker was established (CD7, 56, 2, 5, 13, 19, 64. For ALL, SupB15 cells were selected to establish the CLC isolation using CD19 as an isolation target.

Results: We characterized the ability of the Liquid Scan™ to capture and release circulating leukemic cells from whole blood, by using cell line models spiked in whole healthy donor blood and applied to the microfluidic chip. Our results are directly applicable to patients in different stages of the diseases, both as an initial diagnostic tool, and as a way to monitor recovery and diagnose relapse.

Conclusions: Precision medicine (PM) is significantly changing the treatment of cancer and is bringing new hope for patients. Diagnostic testing for circulating tumor and leukemic cells are promising prognostic and predictive tools for selecting optimal therapies based on the patient's genetic content. BioFluidica's system is highly sensitive, with the capability of rapidly delivering purified and concentrated circulating cancer cells compatible with downstream molecular diagnostics.

Methodology

BioFluidica high through-put Rare Cell Isolation platform:

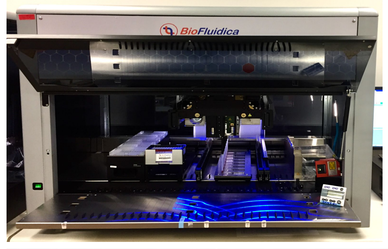


Figure 1. Biofluidica uses a fully automated robotic platform to load whole blood onto microfluidic chips surfaced with antibodies that target specific surface markers of circulating leukemic cells. The platform permits the use of whole blood with minimal pre-processing. Additionally, the programmable microfluidic chip design allows for extended contact between blood cells and the surface antibody, leading to specific capture of CTCs with desired surface markers.

Figure 2. Minimal manipulation of the blood sample is achieved by the use of high end instrumentation, with no tubing or valves that could increase shear stress and microclot formation. Whole blood is aspirated from the collection tube using non-bind pipette filter tips. The tips subsequently transfer the blood onto the microfluidic chip and push the blood through at an optimized flow rate. This maximizes the interaction of blood cells with the chip surface without introducing excessive shear stress. The flow rate is controlled by extremely precise robotic software, protecting the target cells from harsh or uncontrolled handling, leading to superior recovery.

Approach

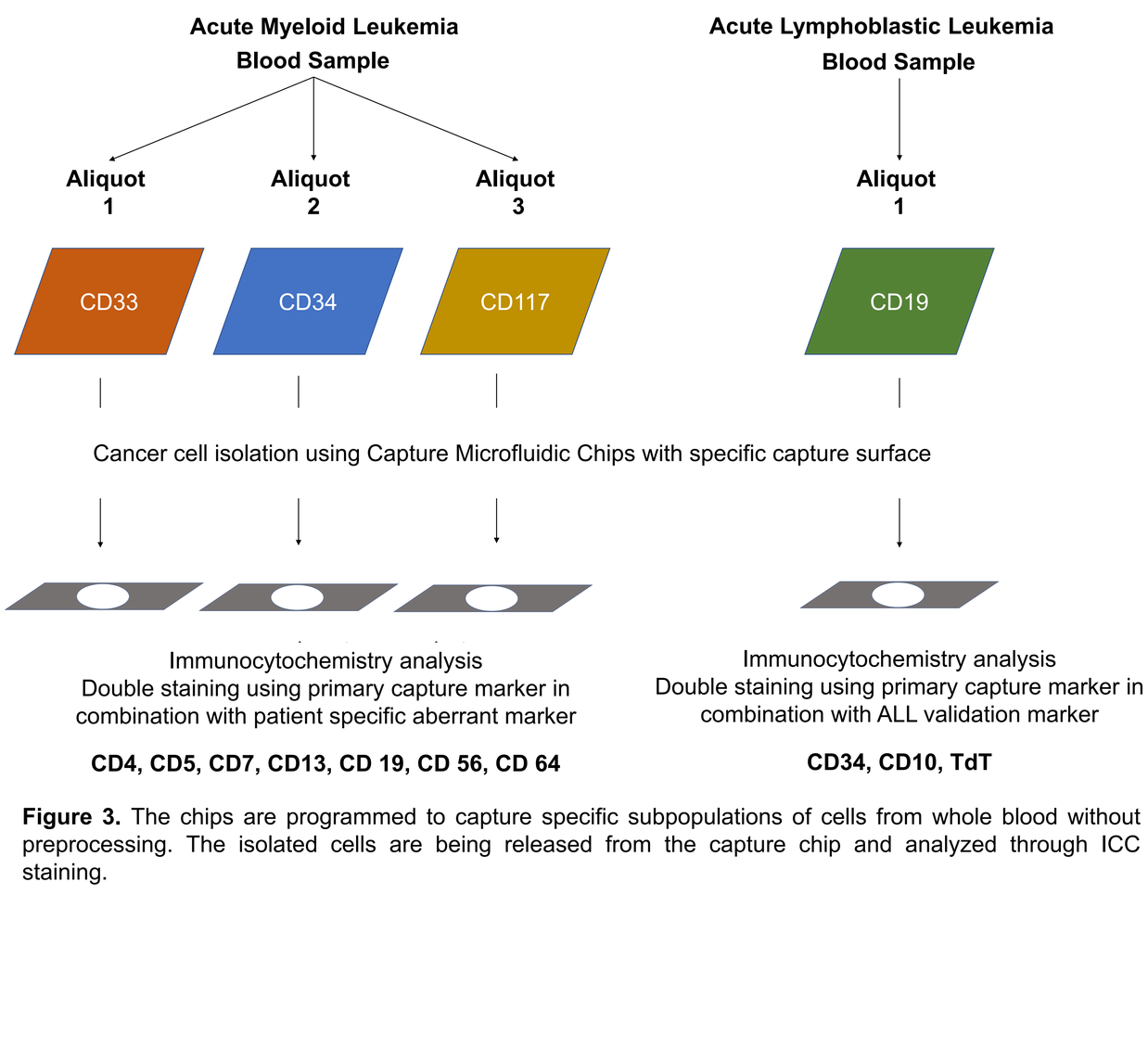


Figure 3. The chips are programmed to capture specific subpopulations of cells from whole blood without preprocessing. The isolated cells are being released from the capture chip and analyzed through ICC staining.

Results

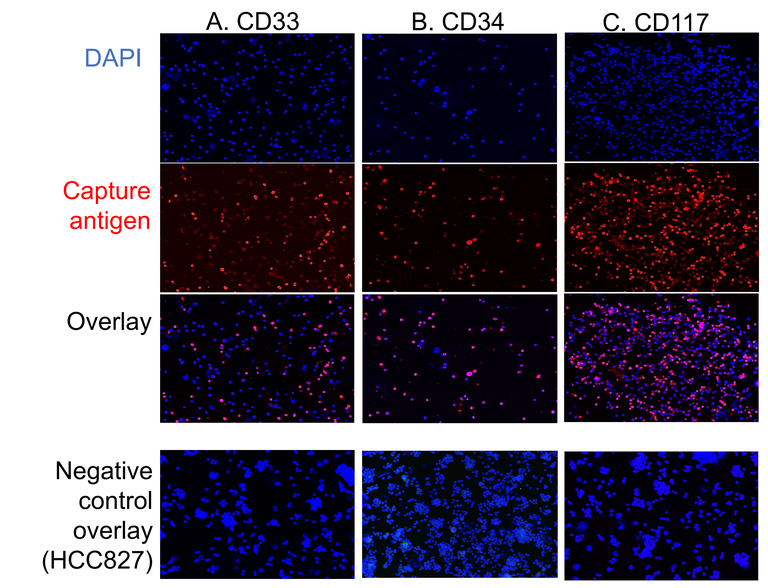
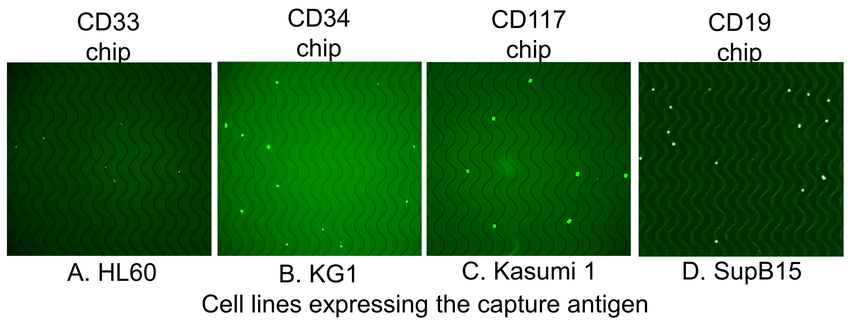


Figure 4. CD33 (A), CD34 (B) and CD117 (C) specific capture antigen staining of Kasumi 3 AML model cancer cell line. HCC827 lung cancer cell line was used as negative control.



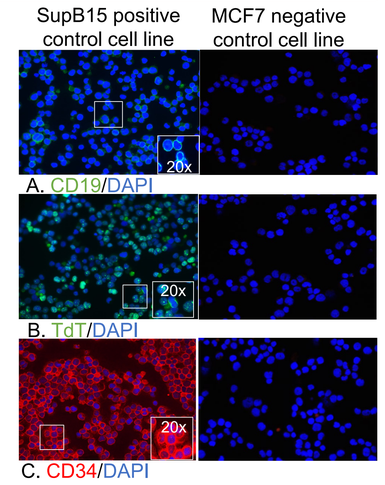
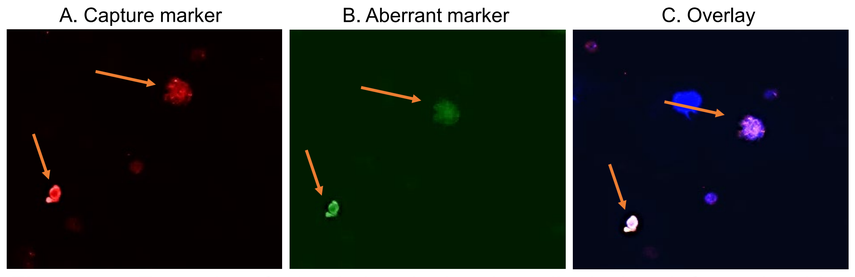


Figure 5. SupB15 cell line expressing CD19 specific capture antigen (A), and common ALL markers (B, C).

Figure 6. Targeted cell capture through programmed capture chips. Labeled target cells (green) spiked in healthy whole blood are specifically captured on the microfluidic chip coated with the capture antibody, serving as a model for AML (A-C) and ALL (D) patients.



AML patient: profile CD 34/CD13

Figure 7. Example of circulating leukemic cells isolated from the blood of an AML patient. Cells were characterized by ICC and show staining for the capture marker CD 34 (A), as well as the AML aberrant marker CD13 (B), confirming their identity as AML cells.

Conclusion

BioFluidica's cell-based microfluidic approach enables capturing whole, intact circulating leukemic cells from peripheral whole blood. The use of positive selection surface markers allows for the screening of leukemic patients for minimal residual disease and relapse, as well as for mutational changes and clonal expansion within the leukemic cell population. The high plasticity of the programmable chip allows for the targeting of multiple types of liquid and solid tumors. The models developed for both ALL and AML show reproducible capture and elution; a pilot clinical study following AML patients over time shows promising results. Our long term goal is the development of a fully integrated and automated microfluidic platform for the enrichment of circulating leukemic cells with high recovery and reproducibility. The platform will provide high specificity and throughput, and be cost effective as a commercially viable technology, while improving the diagnosis and follow-up of leukemic patients.

References

Jackson JM, Taylor JB, Witek MA, Hunsucker SA, Waugh JP, Fedoriw Y, Shea TC, Soper SA, Armistead PM. Microfluidics for the detection of minimal residual disease in acute myeloid leukemia patients using circulating leukemic cells selected from blood. *Analyst*. 2016;141(2):640-51.

Ravandi F, Walter RB, Freeman SD. Evaluating measurable residual disease in acute myeloid leukemia. *Blood Adv*. 2018;2(11):1356-1366.

Ofran Y, Rowe JM. Introducing minimal residual disease in acute myeloid leukemia. *Curr Opin Hematol.* 2015;22(2):139-45.

Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, Linda S, Martin PL, Pullen DJ, Viswanatha D. *et al.* Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood. 2018;*111:5477-5485.

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