Differential drug responses of circulating tumor cells within patient blood

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Abstract

Personalized medicine holds great promise for cancer treatment, with the potential to address challenges associated with drug sensitivity and interpatient variability. Circulating tumor cells (CTC) can be useful for screening cancer drugs as they may reflect the severity and heterogeneity of primary tumors. Here we present a platform for rapidly evaluating individualized drug susceptibility. Treatment efficacy is evaluated directly in blood, employing a relevant environment for drug administration, and assessed by comparison of CTC counts in treated and control samples. Multiple drugs at varying concentrations are evaluated simultaneously to predict an appropriate therapy for individual patients.

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1. Introduction

It is increasingly apparent that the most effective treatment for a cancer patient is a personalized approach based on predictive criteria for that individual. Traditional practice to achieve this goal has been to identify predictors of sensitivity or resistance in malignant cells. For example, it has been shown that panitumumab can be an effective therapy for colorectal cancer patients, but only in patients without KRAS mutation, which renders the treatment ineffective [1]. Thus, patients are screened for mutated KRAS prior to panitumumab treatment. Patients with non-small-cell lung cancer are evaluated for specifically mutated EGFR prior to being placed on gefitinib [2]. However, for more general chemotherapeutics, such as taxanes, no single mutation or marker has been identified that will serve as a reliable predictor of patient response. Chemotherapeutic resistance, both intrinsic and acquired, is a significant problem and is believed to result in failure in more than 90% of patients with metastatic disease [3]. In an attempt to determine patient-specific sensitivity to cytotoxic and cytostatic agents, studies have been conducted wherein tumor cells are biopsied and treated ex vivo. Unfortunately no significant benefit has been found in these types of assays because sensitivity ex vivo does not necessarily translate to a similar response in vivo [4]. This is likely due in part to spatial heterogeneity within tumors and the fact that biopsies only sample a small section of a tumor [5,6], and in part a consequence of the environment in which the cells are treated [7].

In recent years much interest has been focused on circulating tumor cells (CTC) [8]. Many studies have found that CTC appear early in the disease, and their prevalence in blood correlates with disease severity [9–12]. Clinicians are beginning to view CTC isolated from blood draws as a ‘fluid biopsy’, something of a snapshot of the current state of a dynamic tumor, and CTC are believed to reflect in some way the breadth of tumor heterogeneity [13]. Indeed, the case has been made that CTC are the relevant cancer cell subpopulation to target for therapy based on the fact that 90% of cancer deaths are due to metastasis [14]. In addition, the circulatory system, within which cancer cells are termed CTC, is the primary route of metastasis [15]. As such, CTC are being investigated on a patient-to-patient level for characterization purposes, such as epithelial-to-mesenchymal (EMT) state [16] and detection of surface markers that correlate with specific drug response [17].

We recently reported a technique for the isolation of CTC from patient blood in a relatively simple device using off-the-shelf components [18,19]. The device is modeled on an inflamed post-capillary venule and is functionalized with recombinant human E-selectin to rapidly bind flowing cells and anti-EpCAM antibodies to firmly adhere cancer cells. It has been suggested that E-selectin
plays a role in metastasis, specifically in the extravasation of metastatic cells [20–22]. In this paper we present a technique to rapidly screen patient samples for sensitivity to multiple chemotherapy drugs, and then isolated. This process is depicted schematically in Fig. 1. Peripheral blood was drawn from healthy volunteers after informed consent and transferred to 8 mL polypropylene round-bottomed tubes (BD Biosciences) in which the interior lumen had been blocked with 3% BSA for 1 h at room temperature. 50,000 breast cancer (BT20) or prostate cancer (PC3) cells were added to 5 mL of blood. The spiked blood was then perfused through an in vitro circulation microdevice (dimethylsulfoxide, DMSO) or one of three drug dosages based on published pharmacokinetic data (20% of peak plasma concentration (PPC), 100% PPC, and 300% PPC). Breast cancer spiked blood was treated with docetaxel (1 μg/mL, 5 μg/mL, 15 μg/mL) or doxorubicin (0.2 μg/mL, 1 μg/mL, 3 μg/mL); prostate cancer spiked blood was treated with docetaxel or mitoxantrone (1 μg/mL, 0.5 μg/mL, 2 μg/mL). Peak plasma concentrations were based on previous pharmacokinetic studies [34–36]. Samples were incubated for 24 h at 37 °C on a BioRad UltraRocker rocking platform (Hercules, CA).

2.4. Determination of false positive rate and capture efficiency

For the determination of false positive rates, 10 μL of whole blood was drawn from four healthy volunteers after informed consent and split into matched 5 μL samples. One sample of each matched pair was treated with 15 μg/mL docetaxel, and the other sample was treated with vehicle control. Samples were placed in BSA-blocked test tubes and placed on a rocker at 37 °C for 24 h. Buffo cells were isolated by Ficoll density centrifugation, washed, and suspended in calcium-saturated PBS. Samples were processed and stained in an identical manner to that used for CTC capture. For the determination of capture efficiency, 5 μL of whole blood was drawn from four healthy volunteers and the buffo cells isolated. The buffo cells were washed and suspended in calcium-saturated PBS. 1000 BT20 cells were added to each sample and immediately processed through the CTC capture device. Staining was carried out in an identical manner as that used for CTC capture, described below.

2.5. Spiking of cancer cell line cells into blood

Cancer cell line cells were spiked in blood, treated with chemotherapeutic drugs, and then isolated. This process is depicted schematically in Fig. 1. Peripheral blood was drawn from healthy volunteers after informed consent and transferred to 8 mL polypropylene round-bottomed tubes (BD Biosciences) in which the interior lumen had been blocked with 3% BSA for 1 h at room temperature. 50,000 breast cancer (BT20) or prostate cancer (PC3) cells were added to 5 mL of blood. The spiked blood was then perfused through an in vitro circulation microdevice (dimethylsulfoxide, DMSO) or one of three drug dosages based on published pharmacokinetic data (20% of peak plasma concentration (PPC), 100% PPC, and 300% PPC). Breast cancer spiked blood was treated with docetaxel (1 μg/mL, 5 μg/mL, 15 μg/mL) or doxorubicin (0.2 μg/mL, 1 μg/mL, 3 μg/mL); prostate cancer spiked blood was treated with docetaxel or mitoxantrone (1 μg/mL, 0.5 μg/mL, 2 μg/mL). Peak plasma concentrations were based on previous pharmacokinetic studies [34–36]. Samples were incubated for 24 h at 37 °C on a BioRad UltraRocker rocking platform (Hercules, CA).

2.6. Cell isolation and enumeration from spiked blood

Buffo cells were isolated from spiked blood using a Ficoll density centrifugation as previously described [18]. Briefly, buffo cells was washed with HBSS and any remaining red blood cells were lysed with erythrocyte lysis buffer for 1 min at room temperature (RT). Cells were washed with HBSS and re-suspended in 1 mL of flow buffer. Flow buffer was prepared by saturating PBS containing Ca2+ and Mg2+ with CaCO3, followed by sterile filtration through a 0.2 μm PTFE syringe filter (Millipore). Cells were perfused through the selectin-functionalized microdevice at a shear stress of 2 dyn/cm2. After flow, the microdevice tubes were washed with cell-free flow buffer. Cells were re-suspended from the tube by introducing trypsin for 10 min at RT. The recovered cells were plated onto glass bottom petri dishes (Grenier Bioone, Frickenhausen, Germany) and allowed to recover in media supplemented with 10% FBS for 4 h.

Cells were fixed in 4% paraformaldehyde for 45 min at RT. Plates were incubated with anti-EpCAM antibody conjugated to FITC diluted 1:100 in PBS for 1 h at RT followed by incubation with anti-CD45-APC antibody diluted 1:100 for 45 min at RT. DAPI was added and the plates were imaged using an Olympus IX81 fluorescence microscope (Center Valley, PA) or Zeiss LSM710 confocal microscope (Oberkochen, Germany) within the Life Science Core Facility at Cornell University. Cell counts were based on EpCAM and CD45 expression, nucleus size and shape, and cell size and morphology. A CTC was taken as any cell that met the following requirements: greater than 8 μm in size, nonsymmetrical nucleus, positive for EpCAM, negative for CD45. Fluorescent micrographs were taken at 20 randomly selected locations with in each well, and total cell counts estimated based on the total well area [18]. Processed cells that were not captured in the tube were collected, washed with PBS, and incubated with anti-EpCAM-FITC (clone 158202) for 1 h at RT. Stained cells were subsequently washed and stained with annexin-V and propidium iodide according to manufacturer instructions. Quantification was carried out using a Milipore Guava EasyCyte flow cytometer.

2.7. Patient sample isolation

Two tubes of peripheral whole blood (7.5 mL per tube) were collected from patients diagnosed with stage IV cancer by BioCytics Inc. at Carolina BioOncology Institute, PLLC, after informed consent. Patients were enrolled on Western IRB-approved Biocytics Protocol #0001 entitled “Pilot Study to Facilitate Development of an Ex Vivo Device Kit for Circulating Tumor Cell Harvesting, Banking, and Apoptosis-Viability Assay”. See ClinicalTrials.gov NCT# NCT00571389. Samples were analyzed from 3 breast cancer patients (Br1 through Br3), 2 prostate cancer patients (Pr1 and Pr2), 1 renal cancer patient (Re1), and 1 colon cancer patient (Co1). Samples were shipped overnight to Cornell University where they were split into 3 2.5 mL samples and treated with vehicle control, subclinical (20% PPC), or clinical dosages (100% PPC) of drug. Drugs were selected based on the cancer type. Prostate samples were treated with docetaxel and mitoxantrone; breast, colon, and renal samples were treated with docetaxel and doxorubicin. Samples were processed and enumerated in the precise manner as in cell spiking experiments as described above.

2.8. EpCAM and sialyl Lewis x expression following drug treatment

In order to determine whether the reduction in captured cells was from cell death or loss of adhesion ability, the expression of EpCAM and sialyl Lewis x, a selectin ligand moieties, was measured after drug treatment. BT20 cells were plated on 24 well plates in blood plasma isolated from healthy blood for 24h. Cells were then treated in plasma with the same dosages and drugs as in blood spiking experiments. The plates were incubated for 4 h at 37 °C. The cells were released from the plates with enzyme-free cell dissociation buffer (Life Technologies). Cells were stained with a 1:100 dilution of anti-EpCAM conjugated with FITC (clone HEA-125), or 10 μg/mL anti-sLeX for 30 min on ice. Cells stained with anti-sLeX were washed twice and counterstained with anti-mouse IgG conjugated to Alexa 488 (Life Technologies) for 30 min on ice. Cells were washed twice with buffer and analyzed using a flow cytometer.

2.9. Antibodies and reagents

Anti-EpCAM (clone 158210), anti-EpCAM-FITC (clone 158206) antibodies, mouse IgG2b-FITC isotype control, and recombinant human E-selectin were purchased from R&D Systems (Minneapolis, MN). Anti-CD45-APC (clone HI30) antibody, anti-sialyl Lewis x antibody (clone CSEXI1), mouse IgGl isotype control, and Annexin V-APC kit were obtained from BD Biosciences (San Jose, CA). Anti-EpCAM-FITC (clone HEA-125) was obtained from Miltenyi Biotec (Auburn, CA). Anti-mouse IgGl-Alexa488 secondary antibody was purchased from Life Technologies (Grand Island, NY). Halloysite nanotubes were a gift from NaturalNano (Rochester, NY). Ficoll Paque was purchased from GE Healthcare (Waukesha, WI). Erythrocyte lysis buffer was obtained from Qaigen (Germantown, MD). Docetaxel, mitoxantrone, and calcium carbonate were purchased from Sigma Aldrich (St. Louis, MO). Doxorubicin was purchased from Sellek-Pfizer (Houston, TX). ViaCount Viability Kit was purchased from Millipore (Billerica, MA). Hank’s balanced salt solution (HBSS), phosphate-buffered saline (PBS), PBS supplemented with calcium and magnesium, and trypsin were purchased from Life Technologies (Grand Island, NY). Parafaldohide was acquired from Electron Microscopy Sciences (Hatfield, PA). DAPI was obtained from Vector Laboratories (Burlingame, CA). Bovine serum albumin (BSA) was purchased from Sigma Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO) was obtained from Avantor Performance Materials Inc. (Center Valley, PA).
Fig. 1. Schematic of CTC analysis protocol.

Fig. 2. Breast and prostate cancer cell lines are sensitive to docetaxel, doxorubicin, and mitoxantrone in vitro. Results are presented as the ratio of viable cells following 24 h of drug administration to the number of viable cells in the control sample. (A) BT20 cells treated with docetaxel. (B) BT20 cells treated with doxorubicin. (C) PC3 cells treated with docetaxel. (D) PC3 cells treated with mitoxantrone. Figures are representative of two independent experiments.
2.9. Cell viability

Cells were plated on 24 well plates and treated with drug at the same concentrations used in the isolation studies for 24 h at 37 °C. Cells were released from the plate with trypsin and washed with buffer. Cells were then diluted 1:10 in ViaCount viability reagent and incubated for 10 min at RT, according to the manufacturer instructions. The samples were then processed on a flow cytometer using built-in ViaCount software.

2.10. Statistics

All graphical error bars represent standard error of the mean. Significance was determined by performing an unpaired two-tailed t-test with α = 0.05 in GraphPad Prism.

3. Results

3.1. BT20 and PC3 cells showed dose dependent susceptibility to chemotherapeutic drugs in vitro

Chemotherapeutic drugs of interest (docetaxel, doxorubicin, mitoxantrone) were tested for their efficacy in vitro prior to testing the drugs in situ in whole blood (Fig. 2). Data are expressed as the number of viable cells relative to the untreated sample. BT20 showed dose-dependence, and this effect reached a plateau at ~50% viability with docetaxel. A similar effect occurred with doce-
taxel on PC3. Extended dose dependence was seen with doxorubicin and BT20 as well as with mitoxantrone and PC3, where viability was reduced to 3% and 1.5%, respectively.

3.2. Spiked BT20 cells are captured at high efficiency and identified with high specificity

1000 BT20 cells were spiked into buffy coat samples from four healthy volunteers and the mean recovery determined to be 82.0 ± 9.4% (mean ± SEM, Supplemental Fig. 1). Buffy coat samples were processed in an identical manner without spiked cells to determine false positive rates. The mean number of positively stained cells recovered from donor samples was 16.5 ± 9.4 cells per donor with no drug treatment, and 0 cells following treatment with 15 µg/mL docetaxel in matched samples.

3.3. BT20 and PC3 cells showed drug dependent susceptibility to chemotherapeutic drugs in whole blood

50,000 BT20 or PC3 cells were spiked into whole blood, treated with appropriate chemotherapeutic drug, and isolated as described above. The clinical dosage of each drug was taken to be the maximum plasma concentration determined by previous pharmacokinetic studies [34–36]. BT20 cells were treated with docetaxel (1 µg/mL, 5 µg/mL, 15 µg/mL) and doxorubicin (0.2 µg/mL, 1 µg/mL, 3 µg/mL). Cell counts of BT20 treated with docetaxel were reduced to 70.2 ± 5.4% (mean ± SEM), 43.9 ± 2.7%, and 47.7 ± 4.1% of the untreated sample. When treated with doxorubicin, cell counts decreased to 89.8 ± 9.2%, 70.9 ± 7.8%, and 68.8 ± 8.1%, with respect to the untreated control. PC3 cells were treated with docetaxel and mitoxantrone (0.1 µg/mL, 0.5 µg/mL, 1.5 µg/mL). Docetaxel treatment of PC3 cells reduced the cell count to 86.3 ± 7.1%, 41.7 ± 4.4%, and 60.3 ± 6.2% of control, while mitoxantrone treatment counts were 86.4 ± 0.8%, 54.7 ± 5.9%, and 54.5 ± 6.2% of the untreated control (Fig. 3, Table 1).

To confirm that the uncaptured cells were indeed rendered not viable rather than just non-adhesive, the cells from the syringe that did not stick to the tube were stained with annexin-V and propidium iodide. No significant number of viable EpCAM-positive cells were observed in any of the samples studied (data not shown).

3.4. Chemotherapeutic drug treatment did not cause loss of EpCAM or sialyl Lewisx expression

It was investigated whether the reduction of isolated cells as a result of drug treatment was due to drug efficacy or to simply loss of adhesion markers. To address this, the surface expression of EpCAM and sialyl Lewisx (sLe(x)) on BT20 cells was tested following drug treatment by flow cytometry (Fig. 4). No significant change in expression was seen post-treatment for any of the drug concentrations. This suggests that the reduced cell counts in drug treated samples were due to reduction in the number of viable cells rather than a loss of adhesion affinity per se.

3.5. Primary cancer blood samples show heterogeneous susceptibility to chemotherapeutic drugs

To investigate the relevance of this platform for clinical use, we tested primary blood samples from 7 cancer patients (3 breast, 2 prostate, 1 colon, 1 renal). Subclinical and clinical dosages were tested. Breast, colon, and renal blood samples were treated with docetaxel and doxorubicin, while prostate cancer blood was treated with docetaxel and mitoxantrone (Fig 5; Table 2). Overall, drug susceptibility for at least one of the drugs tested in 6 of 7 patients

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Experimental data detailing the number of cells captured from cell spiking in whole blood. PPC = 5 µg/mL docetaxel (DT), 1 µg/mL doxorubicin (DOX), and 0.5 µg/mL mitoxantrone (MTX).</td>
</tr>
<tr>
<td>Cell Line Treatment Donor Control 20% PPC 100% PPC 300% PPC</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>BT20 DT</td>
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<tr>
<td>BT20 DT</td>
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<tr>
<td>BT20 DT</td>
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<td>BT20 DOX</td>
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<tr>
<td>PC3 MTX</td>
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<td>PC3 MTX</td>
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</table>

Fig. 4. EpCAM and sialyl Lewisx (sLe(x)) expression of BT20 cells did not change following treatment with docetaxel or doxorubicin, as evaluated by flow cytometry. Data is presented in histograms wherein the black shaded region represents isotype control, the blue line is the control untreated sample, the red line is the 20% PPC, the orange line is 100% PPC, and the green line is 300% PPC. (A) EpCAM expression on cells treated with docetaxel (A) and doxorubicin (B) sLe(x) expression on cells treated with docetaxel (C) and doxorubicin (D). Figures are representative of three independent experiments.
was detected. The CTC from 3 patients were susceptible to only one of the drugs tested (Co1, Re1, Br3) while the CTC from another 3 patients were susceptible to both (Br1, Br2, Pr2). The remaining patient (Pr1) was not susceptible to either drug tested.

4. Discussion

In this paper a novel platform is presented for the prediction of cancer drug efficacy on a patient-to-patient basis, in a manner suitable for pre-screening prior to systemic administration. This platform was first characterized by spiking breast and prostate cancer cell lines at known quantities into healthy blood, creating model samples of blood containing cancer cells with well-defined susceptibilities. Based on studies of drug efficacy on these cell lines in media (Fig. 2), we were able to recapitulate the therapeutic effect in whole blood (Fig. 3). It is interesting to note that the effect of doxorubicin and mitoxantrone at their highest dosages was to eliminate nearly all cancer cells in media, however in whole blood...
there was no significant increase in cell elimination in response to the clinical dosage. The observed limit of efficacy to about 50% viability is likely due to various factors present in the milieu of whole blood. This underscores another advantage of our system, specifically that drug efficacy may be tested in the same environment in which it is actually administered. The dose dependence of treatment observed also demonstrates that it may be possible to identify patients that would respond to subclinical dosages at a level of efficiency equal to the maximum dosage, ameliorating detrimental side effects associated with chemotherapeutic toxicity.

A high degree of spiked cancer cell loss was observed following incubation of blood samples for 24 h on a rocker. The observed capture efficiency of 82% (Supplemental Fig. 1) strongly suggests that cell loss is due to cell death. Loss of cell viability is most likely due in part to the fact that the test tubes were thoroughly blocked with BSA and the motion of the blood from the rocker prohibited cell adhesion, contributing to cell death via anoikis [23,24]. Further cell death is likely the result of inhospitable factors within the whole blood collected from healthy volunteers, which would explain the relatively high degree of variability between donors (Table 1). Nonetheless, we were able to detect a therapeutic reduction in cell number, which is significant due to the fact that all comparable samples were matched. This is not expected to be the case for the clinical dosage. The observed limit of efficacy to about 50% viability is likely due to various factors present in the milieu of whole blood. This underscores another advantage of our system, specifically that drug efficacy may be tested in the same environment in which it is actually administered. The dose dependence of treatment observed also demonstrates that it may be possible to identify patients that would respond to subclinical dosages at a level of efficiency equal to the maximum dosage, ameliorating detrimental side effects associated with chemotherapeutic toxicity.

Table 2

<table>
<thead>
<tr>
<th>Donor</th>
<th>Treatment</th>
<th>Control</th>
<th>20% PPC</th>
<th>100% PPC</th>
</tr>
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<tbody>
<tr>
<td>Br1</td>
<td>DT</td>
<td>7998 ± 905</td>
<td>2336 ± 255</td>
<td>1759 ± 423</td>
</tr>
<tr>
<td>Br2</td>
<td>DOX</td>
<td>4672 ± 609</td>
<td></td>
<td>715 ± 120</td>
</tr>
<tr>
<td>Br3</td>
<td>DOX</td>
<td>4288 ± 423</td>
<td>3188 ± 365</td>
<td>1429 ± 241</td>
</tr>
<tr>
<td>Br4</td>
<td>DOX</td>
<td>3188 ± 318</td>
<td>3106 ± 563</td>
<td>989 ± 167</td>
</tr>
<tr>
<td>Br5</td>
<td>DOX</td>
<td>9372 ± 550</td>
<td>5552 ± 550</td>
<td>7861 ± 879</td>
</tr>
<tr>
<td>Br6</td>
<td>DOX</td>
<td>8273 ± 533</td>
<td>4453 ± 449</td>
<td>3710 ± 473</td>
</tr>
<tr>
<td>Pt1</td>
<td>DT</td>
<td>7476 ± 524</td>
<td>9455 ± 1093</td>
<td>8786 ± 1070</td>
</tr>
<tr>
<td>Pt2</td>
<td>MTX</td>
<td>8300 ± 603</td>
<td>7146 ± 722</td>
<td>7531 ± 886</td>
</tr>
<tr>
<td>Pt3</td>
<td>DT</td>
<td>110765 ± 10336</td>
<td>52826 ± 5043</td>
<td>74952 ± 4918</td>
</tr>
<tr>
<td>Pt4</td>
<td>MTX</td>
<td>156087 ± 10276</td>
<td>87622 ± 9860</td>
<td>52606 ± 8115</td>
</tr>
<tr>
<td>Re1</td>
<td>DT</td>
<td>13,486 ± 576</td>
<td>11,076 ± 1011</td>
<td>12,423 ± 1345</td>
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<tr>
<td>Re2</td>
<td>DOX</td>
<td>11,269 ± 1034</td>
<td>6019 ± 486</td>
<td>5882 ± 843</td>
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<tr>
<td>Co1</td>
<td>DT</td>
<td>2749 ± 255</td>
<td>2529 ± 247</td>
<td>1072 ± 205</td>
</tr>
<tr>
<td>Co2</td>
<td>DOX</td>
<td>2466 ± 209</td>
<td>1292 ± 140</td>
<td>1677 ± 312</td>
</tr>
</tbody>
</table>

Clinical trials have been performed and more are in progress that monitor CTC count throughout the treatment of different cohorts of patients. It has been shown that CTC count is a reliable predictor of response and relapse [29,30]. The combination of these clinical trial findings with the suggestion that CTC may be the only deadly subpopulation of cancer cells (in that they propagate metastasis) makes CTC a particularly promising substrate for the development of personalized medicine determination in the clinic [31]. The assay developed here has the potential to be used in a number of ways. Patient cohorts could be selected based on drug sensitivity pre-screening. Alternatively, acquired resistance to chemotherapy can be monitored throughout the progress of clinical trials. Furthermore, as we have shown here for the administration of docetaxel and doxorubicin to renal and colon cancers, this platform allows for rapid screening of drugs approved for some cancers but remaining to be evaluated for others. This is particularly useful considering recent observations by the Cancer Genome Atlas Research Network that cancers from different tissues can have strikingly similar genetic signatures [32,33].

In conclusion, we have developed a novel platform for screening drug efficacies of chemotherapeutics using CTC enumeration as a diagnostic output and a predictor for drug susceptibility in individual patients. BT20 and PC3 cells were spiked into whole blood and treated with the purpose of validating this technique. The assay is carried out in a rapid procedure that, in a clinical setting, could predict a patient’s sensitivity in a single day. Two doses of two therapeutic agents were assayed simultaneously in this study; scale up to test more drugs is limited only by the quantity of blood that can be drawn from a patient and the required sample volume per test. Additionally, this technique is not limited to the isolation technique used in this paper: it can be adapted to any CTC detection or isolation method.

Conflict of Interest

John Powderly is Founder and CEO of BioCytics, and President of CBI. Bryan Greene PhD is CSO of BioCytics and Research Lab Director at CBI. Eric Keller is a Research Associate at BioCytics and is Operations Manager at CBI.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2013.08.026.


