Project Title: Assessing mechanisms of small cell lung cancer drug resistance in circulating tumour cells

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Summary (250 words max single spaced):

SCLC is the most aggressive subtype of lung cancer and accounts for 13% of lung cancer diagnoses. Median survival, even with treatment, is under 10 months and 5-year survival is <5%. Most patients are treated with chemotherapy and radiation. The limited role of surgery means few biological specimens are available for research.

In the clinic, 70% of patient tumours initially respond to chemotherapy, but almost all will relapse within months with resistant disease. Understanding the mechanism leading to treatment resistance will be important to improving patient outcomes in SCLC.

A consequence of SCLC being an aggressive disease is that cancer cells disperse into the bloodstream of patients. Modern methods can detect these cells in peripheral blood. These “circulating tumor cells”, if successfully captured, can provide insight into the biology of this disease.

This study analyzes the CTCs from two pairs of cell lines, NCI-H69/H69AR and MAR/MARV6. Each pair consists of the parental cell line and its drug-resistant counterpart. We performed a lyoplate biomarker screening assay to determine the variation in biomarker expression between the cell lines. We then used flow cytometry to validate the findings of the lyoplate assay for CD9, CD49b, CD56, and CD99 which have been postulated to be associated with drug-resistance. Although the biomarkers tested did not correlate with drug-resistance across cell lines, we identified biomarkers which were consistent and provide future opportunities for research.

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Introduction

Lung cancer has the highest mortality of all common cancers, with small cell lung cancer (SCLC) having the worst prognosis due to both rapid disease progression and the early emergence of drug resistance. SCLC accounts for 16% of new lung cancer diagnoses in the United States and is strongly associated with heavy smokers. The progression of SCLC follows a typical pattern: 70% of patients respond to initial treatment with chemotherapy, followed by an aggressive relapse with drug-resistant disease within weeks to months. Even with treatment, the median survival is 6-9 months with a 5-year survival under 5 percent. In contrast, non-small cell lung cancer (NSCLC) has much higher rates of survival and prolonged response to treatment. This is in part due to many advances in drug treatment that have occurred over the last several decades. This disparity reflects differences in the biology of the two cancers as well as the difficulty of studying SCLC and advancing its treatment.

With respect to the biology of SCLC, current studies highlight the complexity of the disease. In general, lung cancers may originate from various cell types. Adenocarcinomas originate from alveolar type 2 cells while squamous cell carcinomas demonstrate a basal cell origin. SCLC appears to involve neuroendocrine cells, a rare type of sensory cell in the lung. In the vast majority of cases, multiple mutations have been identified in these cells. The most prominent of these mutations are in the tumor suppressor genes RB1 and TP53 which are almost universally present in patients with SCLC. TP53 is vital for many pathways involved in the process of DNA repair and Rb1 is a key regulator of progression through the cell cycle. It has been demonstrated that mice who have both genes deleted in the lung develop SCLC, highlighting the importance of these mutations in the disease process. Other mutations are also present, including those in the MYC family genes. The significance of other minor mutations remains unclear. A major focus of our current research is to identify biomarkers that can be used as surrogate markers for prognosis and the development of treatment resistant disease.

From a research perspective, SCLC is a difficult disease to study due to the nature of standard therapies used for treatment. SCLC is assumed to harbour micro-metastases at diagnosis, independent of clinical stage. Thus, chemotherapy remains the standard treatment for all cases with localized radiation therapy added in select cases. Surgical resection is rarely used for treatment, making it difficult to obtain tissue for research purposes. Instead, cell lines derived from small biopsies, pleural effusions, and bone marrow aspirates, done as part of the patient diagnostic work-up, remain the most commonly used research models. With the greater use of diagnostic imaging and smaller diagnostic biopsies, these tissue sources are becoming scarcer. The recent discovery of circulating tumor cells (CTCs) provides a new opportunity for molecular exploration of this disease. CTCs are individual or small clusters of tumor cells found in the peripheral blood of cancer patients. They have been postulated as being associated with tumor metastasis. CTCs have been detected in SCLC patients and research suggests an association between CTC number and prognosis, both at diagnosis and at disease progression.

We propose that CTCs can provide an opportunity to study markers of drug resistance in SCLC. There is evidence to suggest that surface proteins in drug-resistant cell lines are different than surface proteins in those that are drug-sensitive. In this study, these biomarkers are explored further for their potential in predicting drug resistance in a clinical setting.

Background

In order to study SCLC, cell lines were derived from body fluids of patients with SCLC. These cell lines were established by isolating cancer cells and growing these cells in media under ideal...
culture conditions. The cell lines NCI-H69, H69AR, MAR, and MARV6 are used in this project to study the relationship between drug resistance and expression of surface proteins on the cells.

Research conducted in Dr. S. Banerji’s lab is focused on identifying biomarkers on the surface of SCLC cell lines in vitro that may predict response and resistance to treatment in vivo. The lab specifically uses a high-throughput lyoplate antibody assay: a panel of cell surface antibodies that can be used to screen commonly expressed proteins on the surface of cells using flow cytometry. Using NCI-H69 and H69AR, it was found that specific biomarkers are only found in chemo-naive cells, some only in chemo-resistant cells, and some in both. The biomarker CD56 (commonly used for SCLC diagnosis) was identified as being associated with chemo-naive cells, while CD49b, CD9, and CD99 are associated with chemo-resistant cells. This current project works to confirm these findings using low-throughput assays and explore these surface markers in additional drug-sensitive and drug-resistant cell line pairs in vitro, as well as in primary cell cultures using CTCs from patients with SCLC.

Methods

Cell line culture

Cell lines NCI-H69 and H69AR were obtained from ATCC and grown in complete media (CM) consisting of RPMI-1640 with 10% fetal bovine serum (FBS) that was supplemented with penicillin/streptomycin and Corning Glutagrow Supplement. NCI-H69 was a cell line derived from a 56-year-old male with SCLC. H69AR was derived from this cell line after being exposed to increasing doses of doxorubicin over a period of time until drug resistance was developed. MAR and MARV6 were obtained thanks to the Dr. S.P. Cole lab at Queen’s University. The MAR cell line is the parent cell line from which MARV6 was derived. MARV6 was developed after exposing MAR to increasing doses of etoposide over several weeks. The MAR cell line was grown in CM and the MARV6 cell line was grown in CM supplemented with 0.2 µM of etoposide.

Drug Sensitivity Assays

For cell lines H69 and H69AR, doxorubicin was used to test drug sensitivity and, for the MAR lines, cisplatin and etoposide were used for this purpose. The drugs were prepared to produce final concentrations of 1000 µM, 100 µM, 10 µM, 1 µM, 0.1 µM, 0.01 µM, and 0.001 µM. The number of cells in each well varied depending on the cell type: for adherent cell lines, H69AR and MARV6, 1000 cells and for the suspension cell lines, H69 and MAR, 2000 cells. Each concentration of each drug was repeated three times and compared to a control group of cells without drug and a control group with DMSO. The cell plates were prepared and incubated for 72 hours. After 72 hours, the cells were left at room temperature for 30 mins to equilibrate. Cell Titre Glo ATP Assay solution was then added to each of the wells to lyse the cells and bind the fluorescent marker on the ATP molecules. To account for background fluorescence, the Cell Titer solution was also added to three wells containing only media. The plates were then read using a spectrophotometer and results were analyzed.

Lyoplate Assay

The BD Lyoplate Human Cell Surface Screening Panel (cat. # 560747) was used to determine the surface proteins expressed on the NCI-H69, H69AR, MAR, and MARV6 cell lines. Firstly, cells were dissociated using Accutase (cat. # AT104), then filtered using the Falcon 40 µm nylon filter to ensure a single cell suspension. Cells were diluted with BD Pharmingen Stain Buffer + EDTA (Cat # 554656) to a final concentration of 2x10⁵ cells/ml and then 100 µl was aliquoted into three
96 well plate. Following that, 10 µl of antibody from each well of the lyoplate was added to the corresponding wells on the 96 well plates. The plates were then incubated on ice. To perform the secondary antibody prep, 0.06 µg antibody/well (1:200 dilution) was used. For plates 1 and 2, 35 µl of goat anti-mouse antibody was diluted in 7 ml of PBS/FBS solution (2% heat-inactivated FBS in PBS). For plate 3, 20 µl of goat anti-mouse antibody was diluted in 4 ml of PBS/FBS and 15 µl of goat anti-rat antibody was diluted in 3 ml of PBS/FBS. After preparing the antibodies, 100 µl of antibody was added to the appropriate wells. This was then incubated for 10 min on ice, washed with 100 µl Stain buffer + EDTA, and centrifuged at 300xg for 5 minutes. After that, 150 µl were removed and the cells were washed again with 200 µl Stain buffer + EDTA and centrifuged. Afterwards, 200 µl of supernatant was removed and cells were ready to analyze using a Millipore flow cytometer. Markers that stained at least 20% of cells in a well were considered positive.

Flow cytometry

Flow cytometry was performed on cell lines NCI-H69, H69AR, MAR, and MARV6. For the adherent cell lines, Accutase (Cat # AT104) was used to remove H69AR cells from the flasks and, for MARV6, Trypsin-EDTA 0.25% (cat # 25200056) was used. Cells were then centrifuged, isolated, and incubated in 5 ml of cell dissociation solution consisting of 100 µl of 0.5M EDTA and 9.9 µl of PBS. The cells were passed through a Falcon 40 µm nylon filter, centrifuged, and re-suspended in BD Pharmingen staining buffer. Cells were counted and diluted such that there would be a total concentration of 2x10^6 cells/ml. In five test tubes, 100 µl were added to the unstained control tube and, in the rest of the tubes, 99 µl were aliquoted. The antibodies that were used were: FITC mouse anti-human CD9 monoclonal antibody (BD Biosciences; dilution: 1:100), PE mouse anti-human CD49b monoclonal antibody (BD Biosciences; dilution: 1:100), Alexa Fluor 488 mouse anti-human CD56 monoclonal antibody (BD Biosciences; dilution: 1:100), and PE mouse anti-human CD99 monoclonal antibody (BD Biosciences; dilution: 1:100). In each of the tubes, 1 µl of antibody was added from either CD9, CD49b, CD56, or CD99. The tubes were incubated and then rinsed with staining buffer, centrifuged, and re-suspended in 300 µl of staining buffer. The cells were then passed through a flow cytometer to obtain results.

Primary cell collection and culture

Patient samples were collected before patients began chemotherapy. After obtaining informed consent, 20 ml of peripheral blood is obtained from the patient and the buffy coat is isolated. The buffy coat is then suspended in CM. Currently there are nine cell lines: MB0500LU, MB0501LU, MB0502LU, MB0503LU, MB0504LU, MB0505LU, MB0506LU, MB0507LU, and MB0508LU. Some cell lines were also grown in NCI-H69 conditioned media. This media was extracted from the CM used to grow H69 cells for 4 days. The cells were filtered using Steriflip 0.45 µm vacuum filters (cat # SE1M003M00). Conditioned media was added to flasks in a 1:1 ratio with CM. New media is added to flasks every 72 hours for expansion.

Demographics of patients

The median age of the patients was 66 years, with a range from 54 to 87 years. Half of the patients were female and median pack years was 42, with a range from 15 to 80.

Results

Drug Sensitivity Curves

As demonstrated in Figure 1, NCI-H69 is drug-sensitive to doxorubicin while H69AR is drug-resistant. Similarly, the inhibitory concentration of 50% of cells (IC50) for the MAR and MARV6
for etoposide was determined to be 1.8 uM and 150 uM, respectively. This suggests that MAR is indeed the most sensitive of the cell lines to etoposide, whereas MARV6 is more drug-resistant.

**Lyoplate Biomarker Screen**

The lyoplate assay demonstrated the varied expression of biomarkers in the drug-sensitive and drug-resistant cell line pairs: NCI-H69 and H69AR; MAR and MARV6. There were some biomarkers expressed only in one cell line and some that were expressed in multiple cell lines.

**NCI-H69 and H69AR**

Figure 2 highlights the biomarkers that were positive in NCI-H69 and H69AR. Biomarkers highlighted yellow are those positive only in NCI-H69, those in red are positive only in H69AR, and those in orange were common to both NCI-H69 and H69AR. Of note, the biomarkers that were expressed exclusively on H69AR were: CD9, CD49b, CD49d, CD54, CD55, CD99, and CD99R. These biomarkers may provide a link between drug-resistance and biomarker expression.

**MAR and MARV6**

Comparing MAR and MARV6, Figure 3 highlights those exclusive to MAR in yellow, those exclusive to MARV6 in red, and those shared by both in orange. The surface proteins exclusive to the MARV6 cell line were: CD44, CD49c, CD51/61, CD56, CD58, CD61, CD63, CD146, and SSEA-3. Many biomarkers are shared between the MAR and MARV6 cell line which provides evidence for their shared lineage.

**Drug-sensitive cell lines**

Combining data from Figure 2 and Figure 3, Figure 4 highlights the biomarkers shared between NCI-H69 and MAR. Those biomarkers include: CD15, CD24, CD46, CD47, CD57, CD59, CD71, CD81, CD98, CD147, CD151, CD164, CD165, CD166, CD171, CD227, CD321 (F11 Rçptr), SSEA-1, and CD326.

**Drug-resistant cell lines**

Figure 5 highlights the biomarkers expressed on both H69AR and MARV6. Those were: CD44, CD46, CD47, CD49c, CD55, CD57, CD58, CD59, CD63, CD81, CD98, CD146, CD147, CD151, CD164, CD165, CD171, CD227, and CD321 (F11 Rçptr).

**Combined Analysis**

When comparing the drug-sensitive and drug-resistant cell lines with each other, and considering only the surface proteins that were positive for both cell lines in the drug-sensitive and drug-resistant pairs, Figure 6 highlights the surface proteins that were exclusive to the drug-sensitive cell lines and the surface proteins exclusive to the drug-resistant cell lines. The biomarkers common across drug-sensitive cell lines were: CD15, CD24, CD71, CD166, SSEA-1, and CD326. Exclusive to the drug-resistant cell lines were: CD44, CD49c, CD55, CD58, CD63, and CD146.

**Flow Cytometry**

**NCI-H69 and H69AR**

Flow cytometry confirmed the presence of CD9 (98.99%) and CD49b (95.19%) and the relative absence of CD56 (4.67%) in the H69AR cell line (Figure 7). The reverse is true with NCI-H69, with evidence supporting the presence of CD56 (13.49%) and absence of CD9 (0.78%) and CD49b (0.11%). With regards to CD99, it appears to be expressed in both cell lines, however at higher levels in H69AR (95.34%) than NCI-H69 (76.06%). This is consistent with the lyoplate data.
that CD56 is expressed in the drug-sensitive cell line, whereas CD9 and CD49b are expressed only in the drug-resistant cell line.

**MAR and MARV6**

Using the same flow protocol as above, cell lines MAR and MARV6 were also examined for expression of CD56, CD9, CD49b, and CD99 (Figure 8). MAR had the lowest abundance of CD56 with only 44.6% of cells expressing the protein while 83.63% of cells expressed the surface protein in MARV6. With regards to MAR, CD9, CD49b, and CD99 were expressed in 2.78%, 19.70%, and 1.05% of cells respectively and with MARV6 in 1.97%, 0.66%, and 0.57% of cells respectively. These findings suggest that, although there may be a connection between CD56, CD9, CD49b, and CD99 with drug resistance in H69AR cells, this relationship is less evident in the MAR cell lines. As can be noted, there is slightly higher expression of CD9 and CD49b in the drug-resistant MARV6 cell line, however this is only by a small amount. The relationship between drug-sensitive cell lines and CD56 is not consistent with these findings as the highest expression of CD56 was in the MARV6 cell line. We have not yet validated these findings with flow cytometry.

**Primary Patient Samples**

Due to the inconsistency of the cell surface marker expression between drug-sensitive and drug-resistant cells, when comparing the earlier NCI-H69/H69AR and recent MAR/MARV6 results, we have not yet explored marker expression on the surface of CTCs derived from patients. To date, CTCs were successfully isolated from the peripheral blood of 9 patients (Figure 9 and Table 1). Propagating these cells in culture media however has been difficult. Out of eight samples drawn from patients, only one sample appeared to grow in culture media. MB0500LU, MB0501LU, MB0502LU, MB0503LU, MB0504LU, MB0505LU, and MB0506LU failed to grow in either CM or conditioned media. Currently MB507LU appears to be growing well in CM supplemented with conditioned media.

**Culture appearance of cell lines**

In culture, cell lines MB0500LU, MB0501LU, MB0502LU, MB0503LU, MB0504LU, MB0505LU, and MB0507LU were adherent to the flask (Figure 10). MB0506LU grew as a suspension. The morphology of the cells also differed depending on the media in which they grew. In conditioned media, cells appeared to grow close together in thin strands. In CM, cells appeared circular in shape and dense in structure.

**Discussion**

We have attempted to explore whether biomarkers on the surface of cancer cells in SCLC patients have the potential of being used to predict clinically relevant disease states. Cell lines represent an accessible model in which to test the hypothesis. The NCI-H69/H69AR and MAR/MARV6 cell line pairs combined with high-throughput lyoplate screens provide a model for exploration.

With regards to the patient samples, establishing cell lines from primary culture proved to be difficult. The morphology of the cells and their adherent qualities did not allow for further experimentation with the cells. Although attempts were made to grow cells in a variety of media types, including CM and conditioned media, these attempts failed to produce cell lines that could be utilized for experiments. It is interesting to note that the majority of the patient samples grew as adherent cell lines. When compared to the NCI-H69/H69AR and MAR/MARV6 cell lines, where the drug-sensitive cell lines grew in suspension and the drug-resistant cell lines grew as adherent cells, it may be that cell morphology is related to drug-resistance. This is difficult to determine as experimentation with the patient samples is not possible without first improving their growing
conditions. Further studies are needed to determine the optimum media and conditions for these cell lines to flourish.

The lyoplate biomarker screens on the H69/H69AR and MAR/MARV6 cell lines point to the possibility of a link between disease states and biomarker expression by demonstrating the association between drug-resistance and specific biomarkers. Notably, the biomarkers which were found to be consistent across drug-sensitive and drug-resistant cell line pairs, and exclusive to those pairs, may provide opportunities for further research. For the drug-sensitive pairs, those biomarkers include CD24 and CD166. While CD24 has been associated with tumor aggressiveness and metastasis, studies have also shown its therapeutic potential.\textsuperscript{12} When targeted with monoclonal antibodies conjugated with doxorubicin, the combination proved to be more cytotoxic than with doxorubicin alone.\textsuperscript{12} These findings suggest that there may be an association between drug sensitivity and CD24. The surface protein CD166 has also been associated with poor prognosis in some studies.\textsuperscript{13} More research is needed to determine if these biomarkers are indeed associated with drug sensitivity.

Biomarkers consistent with the drug-resistant cell lines included CD44, CD55, and CD58. CD44 has been studied previously with conflicting findings. CD44 is a transmembrane glycoprotein involved in cell-to-cell adhesion. It is associated with many cancers and plays a role in metastasis.\textsuperscript{14} Some studies have associated the loss of CD44 with a worse prognosis.\textsuperscript{15} Some have also found that increased expression of CD44 was associated with increased resistance to radiation therapy and greater proliferation.\textsuperscript{16} Others did not find an association between CD44 expression and prognosis.\textsuperscript{17} Due to the conflicting nature of the studies, more information is needed to determine the feasibility of CD44 as a prognostic indicator. CD55 and CD58 have also been studied previously. CD55, also called decay-accelerating factor, is a protein which accelerates the decay of enzymes on the surface of cells responsible for activating complement and promoting cell death.\textsuperscript{18} CD58 is a surface protein associated with the regulation and effect of T lymphocytes on the cells. There have been studies showing the upregulation of CD58 in states of inflammation and downregulation of CD58 in tumor cells.\textsuperscript{19} Both CD55 and CD58 have not been shown to be associated with drug sensitivity.\textsuperscript{20,21}

The significance of CD15, CD49c, CD63, CD71, CD146, CD326, and SSEA-1 is not yet clear. These biomarkers provide future opportunities for research and exploration.

Because of the association between SCLC and the biomarkers CD9 and CD56, this study especially sought to study those biomarkers more closely. In a study by Kohmo et al, CD9 was found to be expressed in drug-resistant cell lines while not expressed at all in the parental cell lines.\textsuperscript{10} CD9 was also demonstrated to temporarily upregulate on the surface of H69 cells that were exposed to drug. CD9 disappeared after the drug was removed.\textsuperscript{10} Our study has independently confirmed this relationship between CD9 and drug-resistance using the lyoplate assay. A number of studies also aimed to establish the relationship between drug-sensitivity and CD56, although a clear relationship has yet to be described.\textsuperscript{22} CD56 is also used regularly to diagnose SCLC so prognostic potential in this biomarker is highly desired.

The lyoplate screen pointed to a strong relationship between CD9 and drug-resistance in the NCI-H69/H69AR cell line, as well as a relationship between CD56 and drug-sensitivity. However, this relationship was not observed in the MAR/MARV6 cell line. Using flow cytometry to validate the results from the lyoplate screen, it became clear that the distribution of biomarkers on the MAR/MARV6 cell lines did not point to as strong of an association between drug-resistance and CD56 and CD9 as they did on the H69/H69AR cell lines. In the NCI-H69 cell line, CD56 was consistently expressed while CD9 was minimally expressed. In the MAR cell line, CD56 was
expressed to a lesser extent than in MARV6, thus demonstrating the opposite trend. Although CD9 expression followed the same pattern in the MAR/MARV6 cell line as it did in the NCI-H69/H69AR cell line, the association was much smaller. Surprisingly, data from the lyoplate assays did not confirm the presence of CD9 in the MAR/MARV6 cell lines which contradicts findings of the flow cytometry data. This may be due to the low sensitivity of the lyoplate assay which did not account for the presence of CD9 in low percentages in the MAR/MARV6 cell lines.

The other biomarkers that were studied using flow cytometry also failed to show a trend. The CD49b biomarker did not show a strong correlation with drug resistance in the MAR/MARV6 cell line the same way it did for the NCI-H69/H69AR cell line and, although a trend was observed with CD99 and drug-resistance, the association was much smaller in the MAR/MARV6 cell lines than with the H69/H69AR cell lines.

Since our study only looked at two cell line pairs, it is possible these findings can be explained by the biological heterogeneity of SCLC. Previous research has shown that SCLC cell lines can be divided into classic SCLC and variant SCLC. Classic SCLC cell lines, which are the majority of cell lines, were found to express biomarkers that the variant SCLC cell lines did not. These biomarkers include BLI and DCC which are expressed in the classic cell line and not expressed in the variant cell line. It may be that H69/H69AR and MAR/MARV6 are different types of SCLC and therefore differ in biomarker expression. These proteins were not included in the lyoplate assay and so this has not yet been validated.

Furthermore, there is considerable heterogeneity in SCLC clinically. As with many cancers, there are unlikely to be markers of drug resistance universal to all cases. SCLC is a complex disease with likely complex mechanisms contributing to drug resistance that need further exploration. To explore this concept further, a tissue microarray from sixty-five patients will be used to validate the findings of this study and explore the biomarkers associated with the drug-resistant and drug-sensitive cell lines (Table 2 and Figure 11). These cases represent SCLC patients with extremes of clinical outcomes: excellent response to treatment and long-term survival, poor responders to initial treatment (i.e. primary drug resistant), and early progressors, those who developed rapid secondary drug resistance. The tissue microarray will also be useful for comparing the expression of biomarkers on tissue samples with those in the CTCs of this study.

Patients are often left with uncertainty about their condition and whether or not they will benefit from chemotherapy. The goal of this research is to help clinicians use a minimally invasive test, a blood sample, to determine whether or not a patient will benefit from chemotherapy and for prognostication to greatly enhance patient care. These biomarkers associated with drug resistance may help avoid the ill effects of chemotherapy and instead focus more on palliative care and quality of life early in the treatment plan. Although the preliminary data appears inconsistent with regards to the specific biomarkers tested, further exploration is warranted.
Figures and Tables

Figure 1 Dose response curves for MAR/MARV6 and NCI-H69/H69AR with etoposide and doxorubicin, respectively. The IC50 of MAR and MARV6 were determined to be 1.8μM and 150μM. The IC50 of NCI-H69 and H69AR demonstrates the drug-resistance of H69AR.

Figure 2 Results of the lypoplate biomarker screen on the NCI-H69 and H69AR cell lines.

Figure 3 Results of the lypoplate biomarker screen on the MAR and MARV6 cell lines.
Figure 4 Comparison of results between the drug-sensitive cell lines, NCI-H69 and MAR.

Figure 5 Comparison of results between the drug-resistant cell lines, H69AR and MARV6.

Figure 6 Combining and comparing results of the drug-sensitive and drug-resistant cell lines.
Figure 7 Flow cytometry results for CD9, CD49b, CD99, and CD56 in NCI-H69 and H69AR. For NCI-H69, 0.78% and 0.11% of cells expressed the biomarkers CD9 and CD49b, respectively, whereas with H69AR 98.99% expressed CD9 and 95.19% expressed CD49b. 13.49% of NCI-H69 cells expressed the biomarker CD56 compared to only 4.67% of H69AR cells. With regards to CD99, 76.06% and 95.34% of cells expressed the biomarkers in NCI-H69 and H69AR, respectively.

Figure 8 Flow cytometry results for CD9, CD49b, CD99, and CD56 in MAR and MARV6. For CD9, 1.13% of MAR cells expressed the biomarker compared to 5.16% of MARV6 cells. 1.166% of MAR cells expressed CD49b compared to 1.34% of MARV6 cells. For CD99, 0.08% of MAR cells expressed the biomarker compared to 1.08% of MARV6 cells. 44.57% of MAR cells and 89.51% of MAR V6 cells expressed CD56.

Figure 9 Kaplan Meier curve representing the survival of the consented patients from time of diagnosis.

Survival proportions: Survival of Circulating Tumor Cells
Table 1 Demographics of patients consented in this study.

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<th>Cases</th>
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<th>Range</th>
<th>Pack Years (Median)</th>
<th>Years</th>
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Table 2 Demographics of patients consented for the tissue microarray

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<th>Range</th>
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Survival proportions: Survival of SCLC Response to Chemotherapy

Figure 10 High resolution images of NCI-H69, H69AR, and patient sample MB0507LU.

Figure 11 Survival curves for patients included in the tissue microarray.
References


