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Circulating tumor cells as a predictive biomarker in patients with small cell lung cancer undergoing chemotherapy☆

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A R T I C L E I N F O

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A B S T R A C T

Background: There are no biomarkers for assessment of disease burden or activity of therapy in SCLC.
Patients and methods: We conducted a prospective study enumerating serial CTCs in patients with newly diagnosed limited disease (LD) and extensive stage (ED) SCLC. CTCs demonstrating DNA damage and apoptosis based on γH2AX and M30 staining were also assessed. We correlated CTC number with disease stage, survival outcomes and tumor burden by RECIST.
Results: Between 03/2011-10/2013, 50 evaluable patients were enrolled (20 LD). Baseline CTC number was higher for ED (median CTC 71 vs. 1.5 for LD; *p 0.0004*). Patients with < 5 CTC had longer PFS but not OS (11 vs. 6.7 months, *p 0.0259 and* 15.5 vs. 12.9 months, *p 0.4357*). A higher cutoff (CTC < 50 or CTC ≥ 50) was significantly correlated with both OS (20.2 vs. 11.8 months, *p 0.0116*) and PFS (10 vs. 4.8 months, *p 0.0002*). Patients with < 5 CTC on day 1 of cycle 2 had longer PFS (10 vs. 3.17 months, $p < 0.001$) and OS (18 vs. 9 months, *p 0.0001*). Patients with an increase in γ2HAX-positive CTCs after chemotherapy had longer OS compared to patients without an increase (25.3 vs. 9 months, *p 0.15*).
Conclusions: This study demonstrates that CTCs at baseline and Cycle 2 of chemotherapy correlate with disease stage and survival in patients with SCLC, suggesting that CTCs may be used as a surrogate biomarker for clinical response. Confirmatory prospective clinical trials are needed before we can incorporate routine evaluation of CTCs into clinical practice.

1. Introduction

Small Cell Lung Cancer (SCLC) accounts for approximately 13% of all lung cancer cases [1,2] Most SCLC patients present with distant metastases and have a poor prognosis. All previous drug development strategies in SCLC have been typified by therapeutic empiricism without a sophisticated strategy for patient selection. Better biomarkers to identify patients destined to do well or poorly as early as possible in the treatment course could be very useful to accelerate the development of new agents. Given the difficulty in obtaining enough analyzable tissue from SCLC patients, there is an unmet need for a noninvasive biomarker that is prognostic and/or predictive of benefit with new therapeutic agents.

One simple user-friendly way to non-invasively obtain cancer cells for some cancer types is through analysis of circulating tumor cells (CTCs). CTCs can be detected by "CellSearch®", that enriches and enumerates CTCs utilizing an EpCAM-immunoferrofluid magnetic separation and differential staining of circulating white blood cells vs. cells of epithelial origin by selecting cells that are negative for CD45 (a pan leukocyte antibody) and positive with pan cytokeratin (CK) and nuclear material (DAPI) [3–8].

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1.1. CTCs in SCLC

Although it has been very difficult to identify CTCs in non-small cell lung cancer [9], this does not appear to be the case for SCLC. Hou et al. reported detection of CTCs in 85% of patients with SCLC using CellSearch®; CTC number fell following chemotherapy [10]. In another study, Naito et al. also detected ≥2 CTCs/7.5 ml in 68% of patients with SCLC, and found that patients with < 8 CTC lived longer than those with ≥8 CTC at baseline, after treatment, and at the time of relapse [11]. Normanno et al. and Hilermann et al., each individually reported detection of at least one CTC in 90% and 84% of patients with SCLC at baseline [12–14]. Change in CTC number following chemotherapy provided additional clinical information in the Normanno study (a significant decline in CTC count, after one cycle of chemotherapy was associated with a lower risk of death (HR 0.24, 95% CI 0.09–0.61)) [12]. Similarly, Hiltermann and colleagues showed that CTC count after one cycle of chemotherapy served as the strongest predictor of overall survival (HR 5.7; 95% CI 1.7–18.9; p = 0.004). Together these studies demonstrate that capture of CTCs by CellSearch® at baseline is feasible, reproducible, and predictive of response to chemotherapy. However, practical application of CTCs in SCLC remains limited as the previous studies have been restricted to certain subsets of SCLC (for example, the Normanno et al. study evaluated patients with extensive stage disease alone) and variable CTC cutoffs have been utilized for prognostic determination.

1.2. CTCs as biomarkers

In addition to being able to enumerate CTCs, CellSearch® also allows one to obtain additional pharmacodynamic information using immunohistochemical markers. The key criteria for such markers are that an antibody exists that can be used to stain cells on the CellSearch® platform and that the marker should be a validated measure of chemotherapy-induced DNA damage and/or cell death/apoptosis. Based on previous literature using, CellSearch®, two such makers that fit these criteria are the DNA damage marker γH2AX [15,16] and apoptosis marker M30 [17,18].

Phosphorylated H2AX (γH2AX) is a marker of DNA damage and appears in the nucleus within minutes in a dose-dependent manner in cells treated with cytotoxic chemotherapy [15]. Wang et al. demonstrated that the percentage of γH2AX-positive cells increased in epithelial tumor cell lines [MCF7 (human breast adenocarcinoma), PC-3 (human prostate adenocarcinoma), HT-29 (human colorectal adenocarcinoma), and SKOV-3 (human ovarian adenocarcinoma)] treated with therapeutic concentrations of topotecan *ex vivo*. *In vivo*, the percent of γH2AX-positive CTCs increased post-treatment from a mean of 2% at baseline to 38% after a single day of chemotherapy in patients with a variety of advanced malignancies enrolled in phase I clinical trials [16].

M30 antibody recognizes a caspase-cleaved neoepitope of Cytokeratin 18 that is only revealed during apoptosis. M30 positive CTCs can be detected in patients with various malignancies. Rossi et al. sequentially assessed CTCs and M30-positive CTCs in breast cancer patients. Overall the number of total and M30-positive CTC decreased during treatment in six and increased in two of eight patients. They suggested that changes in the number of M30 positive CTCs may predict response to therapy [17,18].

We designed this study to prospectively assess the relationship of CTCs to disease stage, and survival, at baseline and prior to each chemotherapy cycle. In addition, our study sought to evaluate the applicability of CTCs as a potential, non-invasive, pharmacodynamic biomarker.

2. Patients and methods

2.1. Study design

We conducted a prospective trial at Abramson Cancer Center of the University of Pennsylvania, Philadelphia. Adult patients with a diagnosis of small cell lung cancer with measurable disease, receiving first line chemotherapy or chemoradiation, were included. All patients signed informed consent. The study was reviewed and approved by the Institutional Review Board at the University of Pennsylvania.

Peripheral blood was collected for CTC evaluation before the initiation of therapy (baseline) and Days 2 and 3 of chemotherapy for cycles 1, 2 and Day 1 of chemotherapy for Cycles 3 and 4. Another blood sample was drawn at the time of relapse, before initiation of salvage therapy. Blood samples were drawn into 10-mL evacuated tubes (CellSave, Janssen Diagnostics, LLC, Raritan, NJ). All CTC evaluations were performed on the CellSearch System (Janssen Diagnostics LLC, Raritan, NJ) without knowledge of patient clinical status. Computed tomography scans of the chest, abdomen and pelvis or positron emission technology (PET) scans were performed at baseline and every 6–12 weeks after initiating treatment, at the discretion of the treating clinician.

2.2. Detection of γ2HAX and M30

The samples were processed on the AUTOPREP using CellSearch® Epithelial Cell Kit (Janssen Diagnostics, Raritan, NJ) and then analyzed on the CellTracks® Analyzer II®. The CTCs were stained with the anti-γ2HAX-FITC antibody (Millipore (catalog # 16-202A, clone JBW301) at a final concentration of 1ug/ml. Monoclonal antibody M30 (Peviva, Stockholm, Sweden) was used at a final concentration of 0.05ug/ml to enumerate CTCs from patient samples and stained with anti M30-PE.

2.3. Radiographic assessments

Tumor measurements, and response assessments, all using RECIST 1.1, were performed by a dedicated thoracic radiologist (D.T.) on the baseline and follow-up scans. The radiologist was aware that these patients were undergoing CTC collection but this individual was blinded to the results.

2.4. Statistical analysis

The objective of this analysis was to assess the prognostic role of CTCs at baseline, prior to each chemotherapy cycle, with the additional exploratory goal to evaluate the role of CTCs as pharmacodynamic biomarkers. CTC numbers were characterized using mean, standard deviation (SD), median, and range. We correlated CTC number with disease stage and number of metastatic sites (mets), using non-parametric rank-based tests for association. CTC numbers were also correlated with response to therapy, progression-free survival (PFS) and overall survival (OS). PFS was calculated from the date of diagnosis until the earlier of date of progression or death. OS was calculated from the date of diagnosis until date of death or the date of last follow-up. Patients were censored at last follow-up if death had not occurred. Survival curves were compared using log-rank test. Cox proportional hazards regression was used to determine univariate and multivariate hazards ratios for OS. OS and PFS were analyzed for the overall population, and also stratified by stage alone, by dichotomized baseline CTC value alone (cutoffs set at 5, 10 and 50 CTCs respectively). These exploratory cutoffs were based on previously published results of CTC and analyses from various other disease groups including, but not limited to breast cancer, colorectal cancer, and small cell lung cancer [7,8,13,14].

Percentages of total CTCs expressing markers of γ2HAX or M30 were analyzed at baseline, and during consecutive chemotherapy days

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