Analysis of Circulating Tumor Cells in Early Stage Non-Small Cell Lung Cancer Patients Treated with Stereotactic Ablative Radiotherapy

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Introduction

Analysis of circulating tumor cells (CTCs) has shown significant promise as a prognostic marker in patients with advanced malignancies. Comparatively little data have been published on CTC analysis in early stage malignancies. We hypothesized that since dissemination of tumor cells via the circulation is required for the establishment of metastases, CTC analysis could be a useful biomarker for identification of early stage patients likely to develop distant recurrence. To begin to test this hypothesis, we set out to examine the feasibility of CTC analysis in non-small cell lung cancer (NSCLC) patients treated with stereotactic ablative radiotherapy (SABR).

Methods & Materials

• Population: 11 stage I and 9 more advanced (stages II-IV) NSCLC patients treated with radiotherapy.

• Samples: 20 peripheral blood samples were collected from 20 NSCLC patients.

• Control: 6 samples were also collected from 4 healthy volunteers.

• All samples were analyzed using the IsoFlux system from Fluxion Biosciences, which allows enrichment of viable CTCs using anti-EpCAM-coated magnetic beads in a microfluidic device.

• Resulting cell populations were stained with anti-cytokeratins (CK) and anti-CD45 antibodies, Hoechst Nuclear stain (DNA) and enumerated.

• Nucleated CK+/CD45- cells were considered to be CTCs.

• Parallel blood samples were processed and stored for molecular analyses.

• Statistical significance was assessed via Student’s t-test.

Results

Figure 2 CTC count by stage. Bars indicate sample means.

- Cell count differences were statistically significant between control versus stage I (p=0.01) (Figure 2).
- Only one stage I patient had ≥100 CK+/CD45- cells.
- No controls had ≥5 CK+/CD45- cells while 73% of stage I patients did (p=0.01).
- Gross tumor volume (GTV) correlated positively with CTC count (R²=0.4, p=0.04) (Figure 3).
- Median follow-up time for patients with follow-up samples is 6.5 months.

Figure 3 CTC count correlates positively with gross tumor volume (GTV) (R²=0.4, p=0.04).

Conclusions

• This exploratory study demonstrates the feasibility of isolation and enumeration of CTCs in stage I NSCLC patients treated with SABR.

• Question: Are early stage patients with the highest number of CTCs at highest risk for distant metastasis?
  - No recurrences identified to date but follow-up is short

• Plans:
  - Molecular analysis of the enriched CTCs collected from parallel blood samples is pending
  - Patients will be followed for outcome
  - Additional patients are being recruited

Figure 1. CTC isolation method. (A) Step 1 • Collect Blood; Step 2 • Label cells with anti-EPCAM magnetic beads; Step 3 • Load IsoFlux. (B) A permanent magnet attracts labeled CTCs while the flow forces rid the sample of background blood cells.