Introduction

Monitoring CTCs from a blood draw could enable a minimally invasive, longitudinal observation of an evolving disease. Antibody drug conjugates (ADCs) are a promising approach to deliver highly potent chemotherapeutics to cells expressing target proteins. An ADC that targets ST4, an oncofetal antigen expressed on tumor initiating cells, is currently in clinical development. Patients with tumors or CTCs expressing ST4 may benefit from this therapeutic. We sought to develop a ST4 CTC test and to evaluate the incidence of ST4 expressing CTCs in breast, ovarian, and lung cancer patients.

Materials and Methods

Cell Lines: Three control cell lines with varying ST4 expression were used (NCI-H268: high, NCI-H1975: medium, and NCI-H460: low-negative) for assay development.

Patient Samples: Blood samples from breast (n=10), ovarian (n=9) and NSCLC (n=25) patients on active therapy were collected prospectively (ConversantBio Huntsville, AL) and shipped to ECS Sciences for analysis. All samples were appropriately consented. Each sample was accompanied by a pathology report giving details of indication.

Epic CTC Platform: (1) Nucleated cells from blood sample were placed onto slides and stored at -80°C. (2) Slides were stained with Cytokeratin (CK), CD45, DAPI and ST4 (utilizing a monoclonal antibody). (3) Slides were scanned in 4 channels (4) Pathology algorithm identified CTC candidates confirmed by human reader. (5) ST4 characterized on each CTC (Figure 1).

Results

Assay Development: A novel CTC assay utilizing a monoclonal antibody against ST4 was developed. Three cell lines with varying ST4 expression were spiked into normal whole blood to assess the linearity of CTC detection. These cell lines as well as ST4 high and ST4 low patient samples (previously assessed) were used to assess intra- and inter-assay reproducibility of % ST4(+) CTCs.

Patient Samples

Breast Cancer: 8 of 10 patients had traditional CTCs (CTC + CTC cluster) defined by CK positivity. 9 of 10 patients had CTCs when additional CTC subpopulations (CK-CTCs, and apoptotic CTCs) were considered. The mean CTC count for the 10 patients examined was 11 CTC/mL with all subpopulations (Figure 2). ST4 expression varied among the breast cancer patients (Figure 4), with the highest % positivity seen in patients with corresponding ER(+) primary tumors. The mean ST4 burden (calculated by adding the ST4 intensity value for each CTC within a sample and divided by the # of CTCs in that sample) was 26.3.

Ovarian Cancer: 2 of 9 patients had traditional CTCs. 3 of 9 patients had CTCs when additional subpopulations were considered. The mean CTC count for the 9 patients examined was 0.1 CTC/mL with all subpopulations (Figure 5). No ST4 positive CTCs were detected in these 9 ovarian cancer patients. The mean ST4 burden was 1.

Conclusions

ST4 expressing CTCs were detected in the blood of NSCLC and breast cancer patients who have received standard of care therapy. A higher percentage of breast cancer patients (80%) had CTCs than NSCLC (56%) or ovarian patients (22%). The number of traditional CTC/mL was greater in breast cancer patients (7.4 CTC/mL) than in NSCLC (2.8 CTC/mL) or ovarian cancer patients (0.1 CTC/mL). The ST4 expression was higher in NSCLC patients (mean ST4 burden of 39.1) than in breast cancer (mean ST4 burden of 26.3) or ovarian (mean ST4 burden of 1) cancer patients. This assay can be deployed in upcoming clinical trials to enumerate and characterize ST4 expressing CTCs as:

- Predicitive biomarkers
- PD/PK biomarkers

References

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