Neuroendocrine prostate cancer (NEPC) is an aggressive androgen independent variant of prostate cancer that most commonly arises in later stages of castration resistant prostate cancer (CRPC) as a mechanism of treatment resistance. Androgen receptor (AR) expression is typically low or absent in NEPC and the genes Aurora kinase A (AURKA) and N-Myc (MYCN) are frequently amplified (Beltran et al, 2011; Mosquera et al, 2013). There are no reliable serum markers to identify patients that are transforming to NEPC and incidence of CTCs in these patients is unknown. Detection of NEPC is challenging and clinical implications as these patients would not be expected to respond well to currently approved AR targeted therapies for CRPC. Circulating tumor cells (CTCs) are traditionally defined as EpCAM+cytokeratin positive (CK+) cells. CDA5 and morphologically distinct. However, recent evidence suggests that other populations of CTC candidates exist including cells that are EpCAM+cytokeratin negative (CK-), or smaller in size than traditional CTCs. CTC positive selection techniques that isolate CTCs based on size, density, or EpCAM positivity may miss CTC subpopulations. We aimed to molecularly characterize CTCs from patients with NEPC utilizing the Epic Sciences platform which performs no physical selection, and to correlate CTC results with patient matched clinical, molecular, and pathologic features.

**Methods**

Blood from 11 consecutive patients with metastatic prostate cancer and clinical or pathologic features suggestive of neuroendocrine prostate cancer (NEPC) were collected and shipped to Epic Sciences, where cells were identified utilizing Epic’s CTC collection and detection process (Figure 1). Traditional CTCs were identified as CK+CD45+ cells with intact DAPI nuclei and after pathologist review of their morphology, candidate CTC populations were identified as CK+CD45+ that were morphologically or cytologically malignant. Small nuclear size candidate CTCs were identified as CK+CD45+ cells with diameters similar to or smaller than that of a typical white blood cells (WBCs). Candidate CTCs were evaluated with prostate cancer relevant biomarkers, including androgen receptor (AR) expression by immunofluorescence (IF), a subset of CTCs were stained for EpCAM and Aurora kinase FISH. Exome and RNA sequencing were performed in patient matched metastatic biopsies obtained at same time of CTC analysis in 8 cases.

**Clinical Demographics**

Patients were classified as having pathologically confirmed NEPC arising de novo (n=2) or after treatment (n=4), or clinically diagnosed NEPC based on CRPC with highly aggressive visceral progression (n=5). NEPC patients were previously treated with abiraterone, docetaxel and/or cabazitaxel. Age range was 62-86 yrs. Sites of metastases included liver (7/11), bone (10/11), lymph nodes (8/11), pleura (1/11), lungs (2/11). Serum PSA, CellSearch® CTCs, and serum NE markers were variable including 5/11 patients with PSA<1 ng/ml (range 0.02-9.39) and 4/11 evaluated pts with CellSearch® CTC count of 0-3 (range 0-94). Metastatic tumor biopsies were evaluated in all cases including one rapid autopsy, with histology ranging from poorly differentiated carcinoma to small cell carcinoma. Neuroendocrine markers (NSE, chromogranin, synaptophysin) showed strong expression by immunohistochemistry in 6/11 cases. 6/8 evaluated tumors displayed amplification of Aurora kinase A by FISH. Patients were subsequently treated with platinum (4/11), AURKA inhibitor (4/11), hormonal therapy (2/11), or progressed to death within 1 day of CTC collection (rapid autopsy case).