Aim: Multiple myeloma (MM) is a neoplasm of plasma cells and is estimated to account for 1.4% of all new cancer cases in 2014. While originating in the bone marrow, MM cells have been detected in the peripheral circulation. Recent advent of CTC detection and capture techniques provides an opportunity for CTC-based pharmacodynamic assay development and single-cell molecular profiling. To this end, we developed an immunofluorescent cell-based assay to detect and characterize MM CTCs.

Methods: The MM CTC assay detects CD-138 as the MM CTC marker and was developed using MM.1S cells spiked into normal blood. Nucleated blood cells and MM.1S cells were plated onto glass slides, which were subjected to immunofluorescent staining followed by CTC analysis using the Pyxis™ Scanning Platform at Epic Sciences. The final MM CTC assay was tested for linearity, accuracy, reproducibility, specificity and sensitivity. Phosphorylated ribosomal protein S6 (pS6) expression was also quantitated on individual CTCs, identified as cells which are CD-138+, CD45-, and with intact DAPI nuclei. Clinical feasibility of the final 4-color assay detecting CD-138, CD45, pS6 and DAPI was assessed on MM patient samples.

Results: We developed a specific and sensitive assay against CD-138 for detecting MM CTCs. The assay did not detect any CD-138+ cells in healthy donor blood. Using a titration series of MM.1S cells spiked into normal blood, we determined the assay is linear (R^2 = 0.999) with 97.5% accuracy. Using a 4-color assay detecting CD-138, CD45, pS6 and DAPI, MM CTCs were identified on patient samples and pS6 expression levels were quantitated on each MM CTC. In addition to CD-138+/CD45- MM CTCs, a subpopulation of CD-138-/CD45- cells were also detected with distinct morphology from surrounding WBCs.

Conclusions: MM CTCs were detected in the blood of MM patients using the newly developed MM CTC assay. Biomarker expression levels can also be quantitated on each CTC and CTC subpopulation using a 4-color assay against the target of interest. This approach may prove useful clinically for pharmacodynamic testing in new therapeutics development and for monitoring and characterizing individual MM patient’s disease via liquid biopsies of the blood.

Disclosure: All authors have declared no conflicts of interest.