

# Morphological Characteristics of Malignancy in Circulating Tumor Cells (CTCs): A new approach to liquid biopsies utilizing standard Cytology Techniques and the FDA cleared Parsortix® PC1 System

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## 1 INTRODUCTION

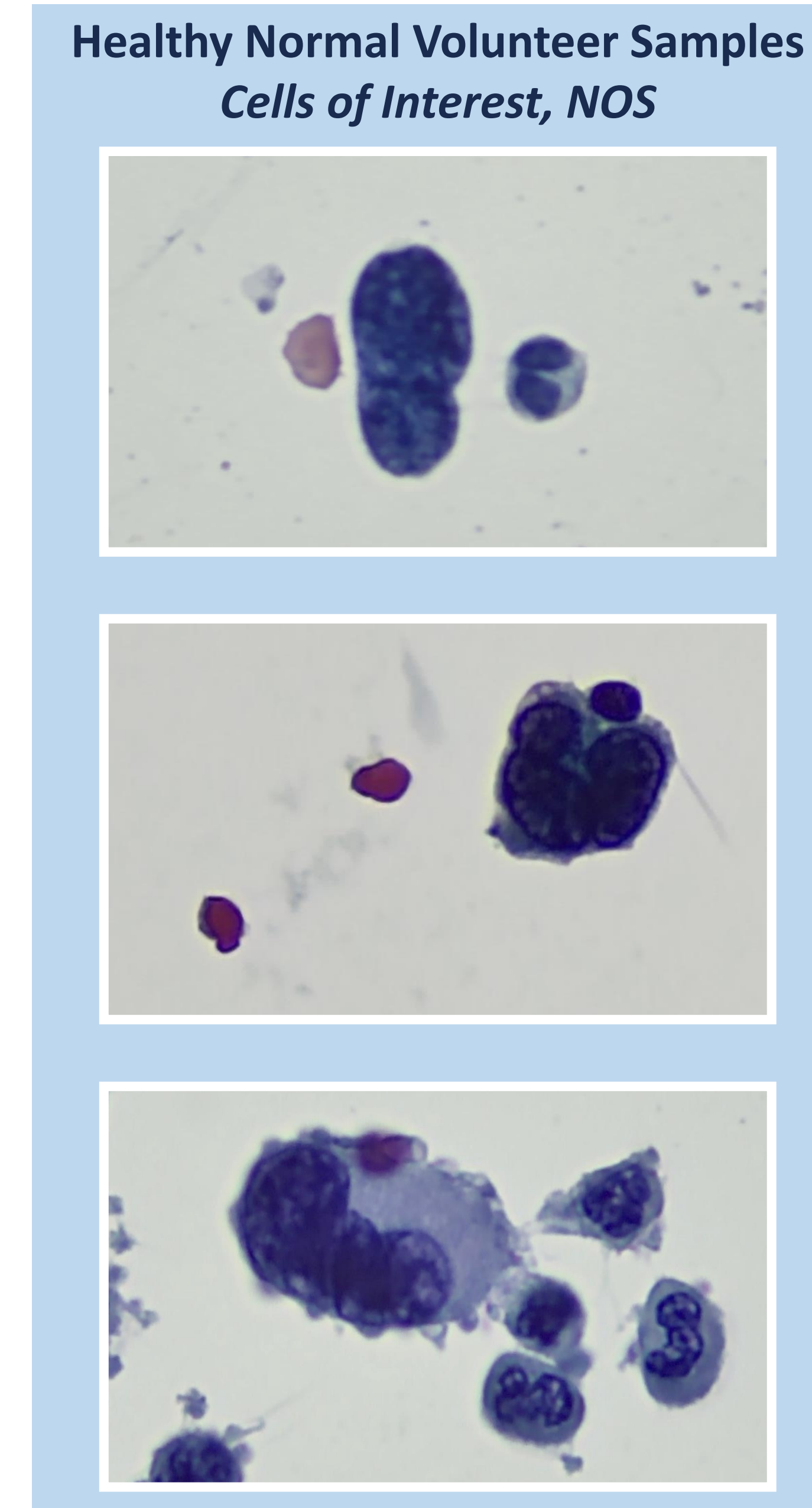
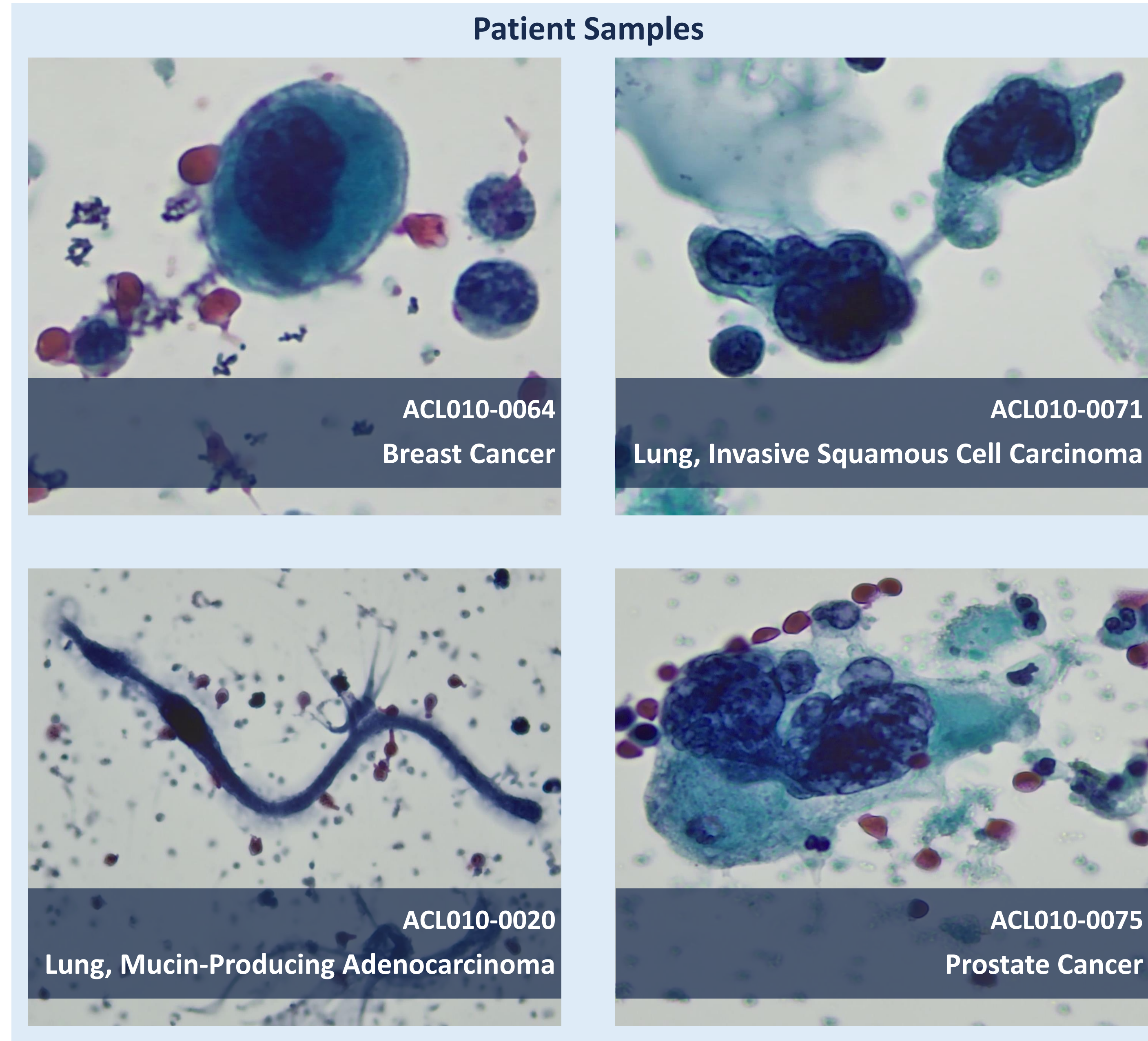
The process of metastasis is responsible for most cancer deaths. Circulating Tumor Cells (CTCs) are cells that have shed from the original tumor and have reached the circulation. Identification of CTCs has the potential to offer important information to patients and clinicians regarding disease status while allowing longitudinal monitoring in a non-invasive way. However, CTC tests currently in the market are expensive and are not covered by medical insurance, making them unaffordable for most cancer patients. Legacy CTC technologies also require the use of expensive immunofluorescence microscopes and reagents and are not easily implemented in standard clinical laboratory settings, thus limiting their use as standard of care.

Onc-ADaPT™ Clinical Laboratories, part of the ANGLE Group, have explored a method to identify CTCs using the FDA cleared Parsortix® System (ANGLE plc, Guildford, UK) followed by standard cytology processing, staining and analysis, significantly reducing the cost of operations and increasing the likelihood of being adopted into standard laboratory practices.



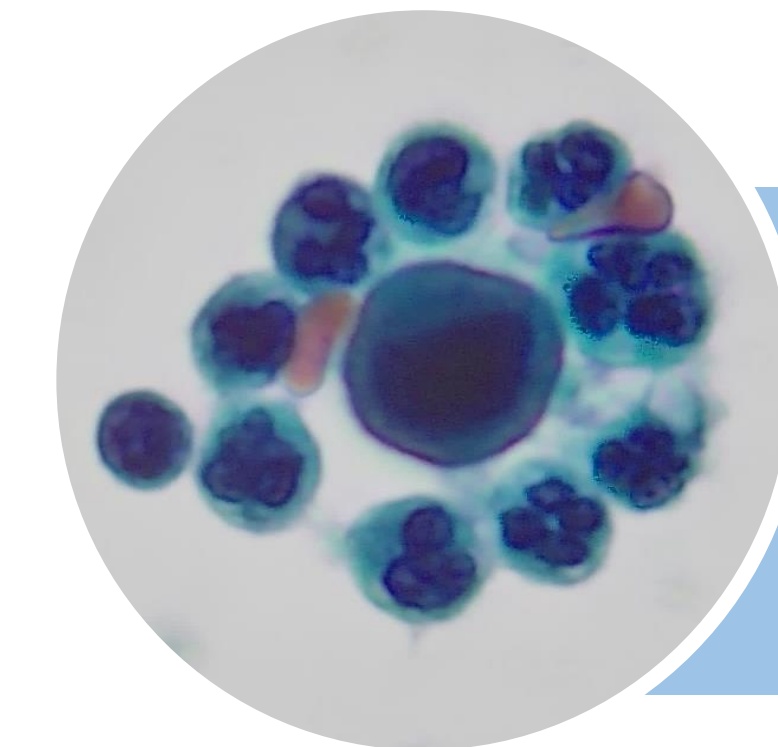
The Parsortix® PC1 system is an in vitro diagnostic device intended to enrich circulating tumor cells (CTCs) from peripheral blood collected in K2EDTA tubes from patients diagnosed with metastatic breast cancer. The system employs a microfluidic chamber (a Parsortix cell separation cassette) to capture cells of a certain size and deformability from the population of cells present in blood. The cells retained in the cassette are harvested by the Parsortix PC1 system for use in subsequent downstream assays. The end user is responsible for the validation of any downstream assay. The standalone device, as indicated, does not identify, enumerate or characterize CTCs and cannot be used to make any diagnostic/prognostic claims for CTCs, including monitoring indications or as an aid in any disease management and/or treatment decisions.

**HYPOTHESIS:** Parsortix® harvested cells can be processed by standard Pap Stain and brightfield microscopy to offer an inexpensive, morphology-based, test for CTCs.



## 3 RESULTS

During review of non-spiked, healthy normal volunteer (HNV) samples, cells of interest with abnormal-looking characteristics were identified. These cells were used to create a training set of normal and Not-Otherwise-Specified (NOS) images. Using the training set, CTs later analyzed the available patient samples. Patient samples presented various types of morphology, including bizarre shapes that require further evaluation and categorization.



*SkBr3 (breast cancer cell line) spiked into HNV blood surrounded by neutrophils and lymphocytes.*

## 4 CONCLUSIONS

- The morphology of metastatic malignant cells in whole blood can be utilized to identify CTCs using newly established background characteristics.
- CTC testing can be performed using the combination of the Parsortix® System, standard cytology staining and optimized analysis by qualified Cytologists.

## 2 MATERIALS & METHODS

The basic features of malignancy were listed by a Senior Cytologist (CT) and reviewed by another CT. Cancer cell lines were spiked into normal donor blood collected in Streck tubes. Non-spiked healthy volunteer blood was used to establish the background. The samples were then processed through the Parsortix® system producing 210µL suspension of enriched cells. The harvest was then processed using Cytospin, stained with standard Pap Stain and reviewed with brightfield microscopy by qualified CTs.