Antibodies Targeting HLA-G and PanCK for Capture and Identification of Circulating Choriocarcinoma Cells

Introduction
- Choriocarcinoma is a malignant, aggressive trophoblastic cancer occurring primarily in placenta characterized by extensive hemorrhage.
- Choriocarcinoma metastasizes via shedding and dissemination of tumor cells through the general circulation with early lung metastasis.
- Combination chemotherapy is an effective therapy for choriocarcinoma but the cancer may come back within a few months to 3 years after treatment.
- Detection of circulating choriocarcinoma cells for treatment and recurrence monitoring has not been previously reported in the literature.
- Pathologically, choriocarcinoma consists of multi-nucleated syncytiotrophoblasts and polyhedral, mononuclear cytrophoblasts, reminiscent of choriocarcinoma.
- In this Application Note, we applied CytoQuest™ CR system, HLA-G and PanCK monoclonal antibodies to capture and identify circulating choriocarcinoma cells.

Materials & Methods
- Peripheral blood of Choriocarcinoma patient was collected in Heparin Tube (02-689-6, BD).
- Two mL of blood were prepared for collecting the peripheral blood mononuclear cell (PBMC) by density gradient centrifugation using Leucosep® (163290P, Greiner Bio-One) and Histopaque®-1077 (10771, Sigma-Aldrich).
- PBMC fraction was harvested and resuspended in Wash Medium.
- Resuspended PBMC was loaded into the CytoQuest™ CR System and circulating choriocarcinoma cells were captured by HLA-G (KA4530, Abnova) immobilized CytoChipNano (U0095, Abnova).
- Immunofluorescence staining for detecting circulating choriocarcinoma cells was performed using PanCK, CD45, DAPI (KA4530, Abnova) as the instruction protocol.
- Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

Results
- Circulating Choriocarcinoma Cell Counts: In 2ml blood of Choriocarcinoma patient, 23 cells count as circulating choriocarcinoma cells (CK+, CD45-, DAPI+).

![Figure 1. Representative images of circulating choriocarcinoma cells (white arrow) and WBCs (yellow arrow) from choriocarcinoma patient. Circulating choriocarcinoma cells were detected by using immunofluorescence staining for PanCK (FITC, green), CD45 (PE, red) and Nucleus (DAPI, blue).](image)

Discussions
- HLA-G is a trophoblast-specific biomarker for circulating extravillous cytrophoblasts. HLA-G reactivity toward circulating choriocarcinoma cells is not known.
- PanCK biomarker is a hallmark cytoplasmic, cytoskeletal protein of epithelial cell origin, including benign adenomas and malignant carcinomas.
- A combination of HLA-G and PanCK monoclonal antibodies successfully captures and identifies mononuclear, circulating choriocarcinoma cells.
- These mononuclear cells most likely represent the cytrophoblast (instead of syncytiotrophoblast) population of the metastatic choriocarcinoma.
- Feasibility of a non-invasive diagnosis of metastatic choriocarcinoma has been demonstrated using Abnova’s CytoQuest™ microfluidic system and special antibody cocktail.
- Circulating choriocarcinoma cell detection offers beneficial therapeutic and recurrence monitoring of this highly metastatic yet treatable cancer.
References


