#5444

Detection of Circulating Cancer Cells from Lung Cancer Patient Blood and Correlation with Anti-cancer Therapy

Chi Wu[1], Longyun Li[1], Huajie Hao[2], Xiaoyun Zhou[1], Wenge Shi[2], Tony Reid[3], Mengjia Tang[2], Gioulnar I. Harvie[2], Helen Tao[2], Guoliang Tao[2], Ping Lin[2], Jia Xu[2]. *[1]Department of Respiratory Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China, [2]Aviva Biosciences Corp., San Diego, CA 92121; [3]Moore Cancer Center, University of California-San Diego, La Jolla, CA 92093*

Introduction

Despite the recent introduction of new agents to treat cancer, few patients are cured and the survival of patients with metastatic cancer that have failed first-line chemotherapy remains poor. The clinical development of new therapeutic technologies is impeded by the lack of efficient and quantitative methods to rapidly assess the response to therapeutic agents in clinical trials. Monitoring circulating cancer cells (CTC) has been shown to have clinical utility complementary with other cancer monitoring technologies, including imaging and assays for serum cancer biomarkers (Cristofanilli *et al.* 2005). Various technologies based on density separation, filtration, positive capturing or negative depletion, have been developed to monitor rare CTC from peripheral circulation of patients. Aviva proprietary rare cell enrichment technology based on negative immunomagnetic depletion does not rely on the expression of epithelial cell surface marker, whose levels vary greatly among circulating cancer cells dependent on disease stages and chemotherapy (Rao *et al.,* 2005; Thurm *et al.,* 2003, Pantel *et al.*, 2003). In addition, the physiological state of circulating cancer cells enriched by negative depletion are less disturbed by the enrichment process compared with positive selection, therefore they are more suitable for additional downstream analyses and applications.

Methodology

Validation of Aviva cancer cell enrichment technology: Various numbers of DAPI labeled MCF7 cells were spiked into healthy donor blood in a double blinded manner. After enrichment, the DAPI positive cells were counted using a fluorescent microscope system (Applied Imaging Corp.).

Blood Collection 7.5 ml venous blood sample was drawn into BD Vacutainer® ACD tubes after the initial 2 ml was discarded from 14 volunteers selected from PUMCH Department of clinical trials, 13 tuberculosis (TB) patients hospitalized in PUMCH, and 16 patients with stage III and IV lung cancers prior to chemotherapy in PUMCH. For CTC and CT scan correlation study, blood samples were drawn from 12 late stage lung cancer patients one day before and two weeks after cancer patients completed chemotherapy.

Sample preparation WBC were prepared and incubated with Apa 1 antibody coated magnetic beads (AVIVA Biosciences Corp.). After bead separation, enriched rare cell portion was centrifuged and the resulting cell pellet was suspended and spotted on coated slides, and fixed.

Cancer cell detection by immunofluorescence staining Slides were stained with anti-cytokeratin 8/18 Alexa 594, anti-cytokeratin 19 Alexa 488, and counterstained by DAPI and examined under a fluorescent microscope.

Cancer progression monitoring Clinical responses were monitored by CT scan two months after the completion of chemotherapy and judged according to the RECIST.

Summary of Key Findings

- Aviva proprietary rare cell enrichment technology based on negative depletion achieves good enrichment and recovery of spiked cancer cells (Fig. 1).
- We can detect cytokeratin positive cells from peripheral blood samples from a majority of late stage lung cancer patients (Fig. 2 and Fig. 3)
- The changes in the numbers of CK positive cells before and after chemotherapy correlate with chemotherapy and clinical responses based on CT scan results ($r = 0.553$, $P = 0.04$, Fig. 4), while Aviva cancer cell enrichment and detection technology potentially provides earlier indication of clinical responses .
- CK positive cells were also detected in a few peripheral blood samples from normal donors and TB patients. Since cytokeratin marker itself only suggests the positive cells are of epithelial origin, further enumeration in combination with tumor specific markers, such as EGFR, CD44v, CA19-9, could provide more complete information for clinical trial and potentially aid better clinical decision (Fig. 5).

References

- Cristofanilli, *et al.* J. Clin. Oncol. 2005;23: 1420-1430.
- Rao, *et al.* Intl. J. Oncol. 2005;27: 49-57.
- Thurm *et al.,* Clin. Cancer Res. 2003;9: 2598-2604.
- Pantel, *et al.,* Clin. Cancer Res. 2003;9: 6326-6334.

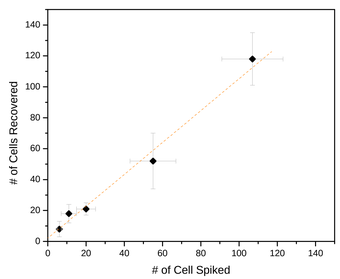

Fig 1. Recovery of spiked MCF7 prelabeled with DAPI using Aviva proprietary rare cell enrichment technology based on negative depletion (n = 3).

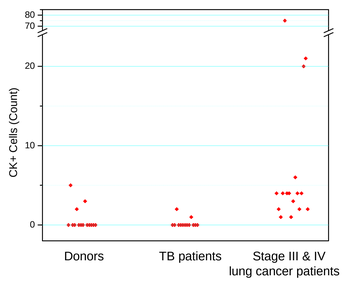

Fig 2. Recovery of CK 8/18 and 19 positive cells from patient populations using Aviva proprietary rare cell enrichment technology based on negative depletion.

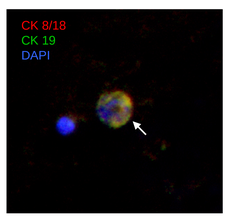

Fig 3. Representative image of CK8/18 and CK19 double positive cells recovered from late stage lung cancer patient population. The arrow indicates a CK positive cell.

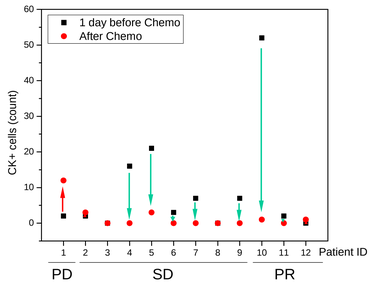

Fig 4. Changes in CTC numbers before and after chemotherapy correlates with clinical response assessed by CT scan. PD: progressive disease, SD: stable disease, PR: partial response

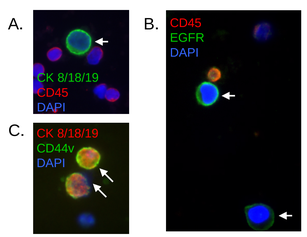

Fig 5. Developed additional markers for detecting circulating cancer cells. The arrows indicate isolated tumor cells.