# A comparison of CellCollector with CellSearch in patients with neuroendocrine tumours



## Dear Editor,

Circulating tumour cells (CTCs) have been hypothesised to be mediators of metastases (Fidler *et al*. 2003) but with numbers as low as $1/10^7$ white cells (Alunni-Fabbroni & Sandri 2010), their utility as biomarkers has been limited by low rates of detection and isolation. CTCs have been identified in patients with metastatic neuroendocrine tumours (NETs) using the FDA-cleared CellSearch (Janssen Diagnostics) technology, a semi-automated platform that uses immunomagnetic enrichment of CTCs based on expression of epithelial cell adhesion molecule (EpCAM) (Khan *et al*. 2011). Using this platform, CTCs were found in 36% of patients with pancreatic NETs and 53% of those with midgut NETs. The presence of CTCs is associated with a worse overall survival, and early changes in CTC number after treatment in NET patients are also prognostic (Khan *et al*. 2013, 2016). CTCs may also be considered as 'liquid biopsies', offering the opportunity to interrogate the molecular characteristics of the tumour. For such an approach to be broadly applicable, alternative technologies are required to increase number of CTCs isolated and the proportion of patients in whom they can be detected.

The CellCollector (GILUPI GmbH, Potsdam, Germany) is a novel medical device consisting of a 160 mm sterile steel wire of which the terminal 20 mm is coated with anti-EpCAM antibodies covalently coupled to a gold and hydrogel layer. The CellCollector is inserted into a peripheral vein enabling the circulating blood volume to be sampled. The wire is stained with fluorescently labelled antibodies and examined microscopically to identify CTCs. The clinical application of this device has been previously reported in patients with breast and lung cancer (Saucedo-Zeni *et al*. 2012).

In this study, we sought to compare the performance of the CellCollector and CellSearch in patients with metastatic NETs. Thirty-four patients provided written informed consent and were recruited into the study (Table 1). The protocol was approved by the central ethical review board (IRAS Project ID 105772). The CellCollector was inserted into the cubital vein via a 20G cannula and left *in situ* for 30 min after which it was removed, washed in phosphate buffered saline (PBS) and fixed in acetone. The cells were permeabilised (Triton X-100 in PBS, 0.1% concentration) at room temperature, washed in PBS and incubated with blocking buffer (bovine serum albumin (BSA)/PBS, 3% concentration). Immunostaining was performed with a solution containing FITC-conjugated antibodies against EpCAM (1:50; HEA125, Acris Antibodies, Herford, Germany), cytokeratin 19 conjugated with Alexa488 (1:50, A53-B/A2, Life Technologies), pan-cytokeratin-Alexa488 (1:50, C11, eBioscience, San Diego, CA, USA) and cytokeratin 7-FITC (1:50, LP5K Milipore). An Alexa-Fluor 647–conjugated anti-CD45 rabbit polyclonal antibody was added as negative marker to exclude white blood cells (1:25, MEM-28Exbio, Czech Republic). Finally, the wire was incubated in the nuclear stain, Hoesch 33342 (Sigma), (concentration 1 µg/mL). The wire was examined in a bespoke holder allowing inspection in four planes using an Axio Imager microscope with digital camera and AxioVision software.

CTCs were defined according to the following criteria: (1) intact cellular morphology, (2) cell diameter more than 4 µm, (3) positive for cytokeratin and nuclear stain, but negative for CD45 and (4) nuclear stain distinct from the cytokeratin or EpCAM staining. Examples of positively identified CTCs are shown in Fig. 1. The number of CTCs was enumerated by two independent operators who were blind to the patient's clinical information. Where there was disagreement between the two operators, a third operator arbitrated. A 7.5 mL peripheral blood sample was collected concurrently into a CellSave tube and analysed within 72 h by CellSearch as described previously (Khan *et al*. 2011).

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**Table 1** Clinicopathological details of study cohort.

| Primary | Midgut ($n=18$) | PNET ($n=14$) | Other ($n=3$) |
|---|---|---|---|
| Age: median | 59 | 58.6 | 50 |
| range | (40–74) | (36–66) | (40–56) |
| Sex: Female | 5 | 6 | 3 |
| Male | 13 | 8 | |
| Median duration | 64.5 | 32 | 62 |
| <25% Liver disease | 8 | 7 | 1 |
| >25% Liver disease | 10 | 7 | 2 |
| Primary resection | 11 | 2 | 2 |
| Grade 1 | 15 | 2 | 0 |
| Grade 2 | 3 | 10 | 2 |
| Grade 3 | 0 | 2 | 1 |
| Metastatic sites | | | |
| Lymph node | 16 | 12 | 2 |
| Bone | 4 | 3 | 2 |
| Lung | 1 | 1 | 1 |
| Peritoneal | 10 | 1 | 1 |
| Brain | 0 | 0 | 0 |
| Other | 2 | 1 | 0 |
| Previous therapy | | | |
| SST analogues | 13 | 5 | 2 |
| Chemotherapy | 1 | 5 | 1 |
| TAE | 1 | 0 | 0 |
| RFA | 1 | 0 | 1 |
| PRRT | 3 | 2 | 0 |
| Sunitinib | 0 | 0 | 0 |
| Everolimus | 0 | 0 | 0 |
| Interferon | 1 | 0 | 0 |

PRRT, peptide radiotargeted receptor therapy; RFA, radiofrequency ablation; SST, somatostatin; TAE, transarterial embolisation.

The interobserver variation for CellSearch has been previously reported (Allard *et al.* 2004), and here we demonstrated good correlation between observers enumerating CTCs using the CellCollector achieving Spearman's correlation of 0.92 (95% CI: 0.85, 0.96) ($P<0.0001$) (Fig. 2A). The median number of CTCs enumerated with CellCollector was 6 (range 2–49), compared with a median of 0 (range 0–57) with CellSearch ($P<0.0001$ (Mann–Whitney $U$ test)). In 33 of 34 patients, there was ≥1 CTC found compared with only 16 of 34 patients with CellSearch (Table 2). Therefore, CTCs were detected in greater numbers and a greater proportion of patients with the CellCollector (Fig. 2B). The CellCollector identified CTCs in all midgut NETs and 12 of 13 PNETS.

We explored the prognostic relevance of CTC count according to CellCollector. With a median follow-up period of 13 months, overall survival data were insufficiently mature so we examined progression-free survival (PFS) as a surrogate. Overall, 14 patients had progression by RECIST criteria, and applying a cutoff of 7 CTCs, there was a significant difference in PFS (Cox Hazard Ratio 3.4, $P<0.05$). Using the same threshold in the Kaplan–Meier survival analyses (Fig. 2C), median PFS was 11 months for patients with ≥7 CTCs but not reached for those with <7 (log–rank, $P<0.05$).

Here, we have demonstrated for the first time that the CellCollector is able to detect CTCs in more NET patients and in greater numbers than CellSearch. However, the CellSearch has been extensively validated and remains a robust method for prognostication, whereas the CellCollector offers the potential to make molecular analysis of CTCs more widely applicable. Indeed, a recent study in lung cancer demonstrated both *KRAS* and *EGFR* mutations known to be present in the primary tumour, in CTCs derived from the CellCollector using chip-based digital PCR (Gorges *et al.* 2016). Other strategies to increase the volume of blood sampled for CTCs include the use of leukapheresis (Fischer *et al.* 2013). However, the leukapheresis product has a very high rate

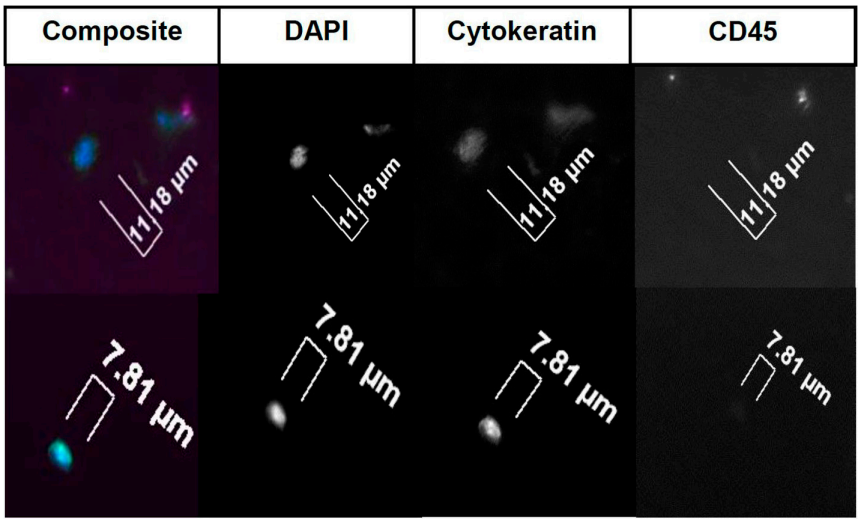


**Figure 1**
Examples of CTCs identified using immunofluorescent microscope, with signal for each channel demonstrated alongside composite image.



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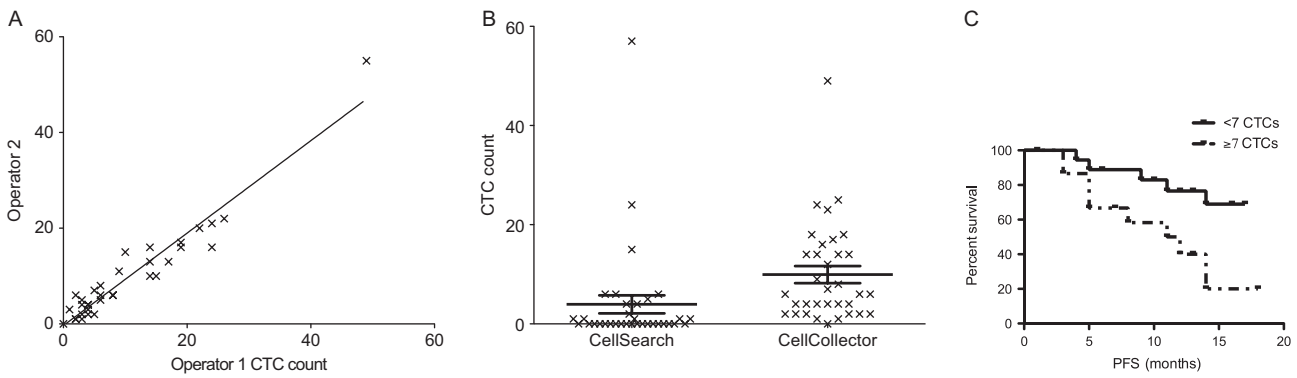


**Figure 2**
(A) Correlation between CTC identified by each operator for each wire enumerated. (B) Scattergram CTCs identified by CellCollector compared with CellSearch. (C) Kaplan–Meier survival for PFS when using 7 CTCs as threshold.

**Table 2** Demonstrates CTC count from both CellCollector and CellSearch for all 34 patients who underwent successful enumeration with each isolation method.

| Pt No. | Age | Primary | Grade | CTC by CellSearch | CTC by CellCollector | >25% Liver metastases | ≥3 sites of metastases |
|---|---|---|---|---|---|---|---|
| CD02 | 74 | Midgut | 1 | 4 | 14 | Yes | Yes |
| CD03 | 59 | Midgut | 1 | 1 | 2 | Yes | Yes |
| CD04 | 65 | PNET | 2 | 0 | 2 | No | Yes |
| CD05 | 72 | PNET | 1 | 1 | 1 | Yes | Yes |
| CD06 | 56 | Hindgut | 2 | 1 | 1 | Yes | Yes |
| CD07 | 71 | Midgut | 1 | 6 | 2 | No | Yes |
| CD08 | 64 | PNET | 2 | 0 | 4 | Yes | Yes |
| CD09 | 59 | Midgut | 1 | 1 | 2 | No | No |
| CD10 | 64 | PNET | 2 | 0 | 4 | Yes | No |
| CD11 | 66 | PNET | 2 | 0 | 4 | Yes | No |
| CD12 | 52 | PNET | 3 | 0 | 2 | No | No |
| CD13 | 61 | Midgut | 1 | 1 | 4 | No | No |
| CD14 | 65 | PNET | 3 | 6 | 9 | Yes | Yes |
| CD15 | 50 | Bronchial | 2 | 0 | 2 | No | Yes |
| CD16 | 54 | Midgut | 1 | 57 | 49 | Yes | No |
| CD17 | 54 | PNET | 1 | 0 | 8 | No | No |
| CD18 | 36 | PNET | 2 | 0 | 0 | No | No |
| CD19 | 40 | Midgut | 2 | 0 | 4 | Yes | Yes |
| CD20 | 67 | Midgut | 1 | 0 | 17 | Yes | No |
| CD21 | 54 | Midgut | 1 | 0 | 6 | No | No |
| CD22 | 66 | PNET | 2 | 0 | 24 | No | Yes |
| CD23 | 57 | Midgut | 1 | 0 | 14 | Yes | No |
| CD24 | 68 | Midgut | 1 | 0 | 14 | Yes | Yes |
| CD25 | 54 | Midgut | 1 | 0 | 6 | No | Yes |
| CD26 | 44 | Unknown | 3 | 24 | 25 | Yes | Yes |
| CD27 | 65 | Midgut | 1 | 0 | 16 | Yes | No |
| CD28 | 51 | PNET | 2 | 0 | 18 | Yes | Yes |
| CD29 | 44 | Midgut | 2 | 0 | 4 | No | No |
| CD30 | 66 | PNET | 2 | 5 | 14 | Yes | Yes |
| CD31 | 70 | Midgut | 1 | 0 | 6 | No | Yes |
| CD32 | 72 | Midgut | 1 | 4 | 18 | No | No |
| CD33 | 50 | Midgut | 2 | 6 | 7 | Yes | Yes |
| CD34 | 44 | Midgut | 1 | 15 | 23 | Yes | Yes |
| CD35 | 69 | PNET | 2 | 2 | 12 | No | Yes |



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of contaminating leucocytes and requires downstream enrichment methods to isolate CTCs. Compared with CellCollector, leukapheresis is also more time-consuming, expensive and onerous for patients (Stoecklein *et al*. 2016).

The CellCollector, like CellSearch, is limited by the dependence on EpCAM as a selection marker for CTCs, and a biologically important component of EpCAM-negative CTCs will not be sampled by either technology. Marker agnostic devices based on size exclusion or biophysical properties rather than antigen expression, offer an alternative method of CTC isolation but remain limited by the small volume of blood that can be sampled.

In summary, the CellCollector appears to be a promising innovation that may help enhance our understanding of CTC biology and the mechanism of metastasis.

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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