



CTCs/CTMs Automated detection after size-based selection from blood of patients with melanoma

Jessica Groult¹, Charles Homsy², Ekaterina Tatarinova², Laetitia Da Meda³, Naoual Benali-Furet¹, Fei Ye¹, Alexandre Papine², Georges Uzan⁴, Celeste Lebbé³, <u>Janine Wechsler¹</u> and <u>Françoise Soussaline²</u>

(1) ScreenCell, Roger Pérez Centre, Sarcelles FRANCE; (2) IMSTAR, Paris FRANCE; (3) Onco-Dermatology AP-HP Saint-Louis Hospital, Paris F; (4) Inserm U1197, Paul Brousse Hospital, Villejuif - FRANCE

Introduction: Circulating Tumor Cells (CTCs) and Clusters (CTM) in the blood play a critical role in cancer staging and treatment management. Counting of CTCs/CTMs during cancer evolution was shown to be helpful for early detection of cancer relapse, but is limited by the current methodology constraints. Methods based on CTCs recognition by immunological technics could give rise to false negative results because the expression level of markers may differ in CTCs as compared to tumor tissues. And methods based on Cyto-Pathologist observation of CTCs/CTMs are more accurate, but time consuming and observer-dependent, thus cannot be applied in clinical use.

In this study, we analyzed 166 blood samples from a cohort of 131 patients with stage IV melanoma, from the onco-dermatology department in Saint-Louis Hospital, Paris.



ScreenCell technology

- Standardized IVD assays Platform
- Filter of fixed or live cells: 3 min
- Provide analysis of residual fluid
- Not depending on EpCAM

Method. Phase 1: CTCs & CTMs were extracted from 9 ml of patient peripheral blood, using ScreenCell[©] "Cyto" device, isolated by size-based technology on a porous membrane, the Isolation Support (IS), Each patient was analyzed 3 times (M0, M1 and M3). All ISs were MGG stained, read by visual scoring under microscope; then, scanned by Pathfinder™ reader-analyzer Capture Software yielding to generate a Digitized Filter on IS: D-IS.

Comparison of conventional visual scoring @40x with digitized D-IS on-screen scoring, with annotation of CTCs and CTMs, shown that the quality of the 3Ddepth corrected Image is adequate for (distant) scoring by the cytologist, with increased reliability and comfort.

Morphology criteria (Fig 2a, 2b)

CTC: Nucleus size >18mm, dark & irregular, high nucleocytoplasmic ratio CTM: Cluster > 2 CTCs











holder of 3 x IS / sample to improve membrane flatness

Digitized Filter

or (D-IS)



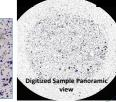
IMSTAR Pathfinder™ technology for blood-based test

- Tumor markers' morphology/phenotype
- Automated whole sample scoring: CTCs detection, counting/characterization
- Total gain of time: 2-8fold
- Accurate, operator-independent

Adaptation of Pathfinder capture Software: An Extended-Depth Focus (150 μm) algorithm provides 4Mpix images in perfect focus. Scanning time: 5-10 min for 2-3 cm² area (3cc)







Melanoma CTC modeling



Sets of Software Algorithms for Melanoma CTCs & CTMs detection:

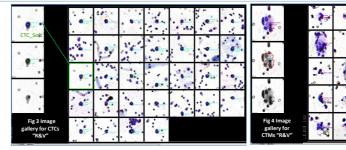
Cytologist's expertise was incorporated as "set of rules" on which are based recognition software algorithms, rules then used for "training" of software modules (proprietary Image Analysis Software Library). Two sets of trained algorithms for melanoma CTCs' & CTMs' scoring were finely tuned and integrated in Pathfinder™ Specific User Interface procedure for "Review & Validation" to assist in final interpretation.

AUTOMATED DETECTION & VALIDATION

Method Phase 2: In parallel, all Digitized Filters are analyzed by Pathfinder™ CTC-CTM BF software module and the cytologist counts CTCs and CTMs by visual scoring, and set these labels on each of D-IS

Cells automatically detected as suspicious are displayed in an Image gallery for fast review & validation on Pathfinder™ CTC – CTM "R&V screen", for final scoring and classification of CTCs as well as CTMs (Fig 3, 4).

All Images and Data are recorded in the Pathfinder software database.



RESULTS & DISCUSSION

CTCs: n= 166 digitized filters. Cytologist's on-screen CTCs counting on D-IS was compared to CTCs automated counting using the specific analysis/detection software (CTC Soft).

The cytologist' on-screen counting yields to a total of 2382 CTCs, while automated counting yields to 2375 CTCs. For 32 D-ISs, cytologist' on-screen CTCs number is inferior, and for 24 D-ISs, the cytologist on-screen CTCs counting is superior to automated counting. The 166 D-IS set were split in 2 groups: A = 85 IS with <8 CTCs and B = 85 IS with >8 CTCs. For group A, automated scoring is equal or superior to visual scoring in over 98% cases. For group B: Visual

scoring is equal or superior to the automated one in 90% cases CTMs: n= 70 digitized filters. Cytologist's on-screen CTMs counting and automated counting using specific analysis/detection software yields to 418 CTMs and 410 CTMs_Soft, respectively.

Both methods are in concordance in terms of CTCs' and CTMs' counting, thus in Digitized filters' (D-IS) classification success rate.

Total time/sample: 5 - 6 min.

CONCLUSION

The Pathfinder™ CTC software module was developed and partly funded in the frame of a French consortium project: ExpeVivo-CTC, for highly specific automated detection and counting of GMM stained melanoma CTCs/CTMs on IS Cyto® device. The accuracy and robustness validation of CTCs & CTMs scoring on Digitized – Filter (IS) was performed on stage IV melanoma patients from Saint-Louis Paris Hospital Onco-Dermatology Department.

The essential benefits of automated CTCs/CTMs detection are:

- High CTCs & CTMs counting and characterization accuracy Sensitivity 96.6% Specificity 100%.
- 2-fold gain of time reducing to 2 3 min the Expert time per D-IS (2 -3 cm² spread; 3ml blood).
- Operator independency Facilitate Junior Cytotech' training in a shorter time

We demonstrated that the combination of IMSTAR Pathfinder™ technology for assisted accurate scoring of CTCs /CTMs, adapted to ScreenCell® Cyto devices simple-to-use, inexpensive technology, could substantially facilitate melanoma patient's treatment follow-up, in clinical trials and ultimately for clinical application.











