A Novel Urine Cytology Stain for the Detection and Monitoring of Bladder Cancer

Noa Davis,*,† Yoram Mor, Pavel Idelevich, Dov Terkieltaub,† Vivi Ziv,‡ Adi Elkeles,† Sylvia Lew, Elimelech Okon,§ Menachem Laufer, Jacob Ramon, Daniel Kedar, Jack Baniel and Ofer Yossepowitch†

From Zetiq Technologies Ltd. (ND, PI, DT, VZ, AE), Tel Aviv, Patho-Lab Diagnostics Ltd. (SL) and LEM Pathology Laboratory (EO), Ness Ziona, Department of Urology, Chaim Sheba Medical Center (YM, ML, JR), Ramat-Gan and Institute of Urology, Rabin Medical Center (DK, JB, OY), Petach-Tikva, Israel

Abbreviations and Acronyms

UC = urothelial carcinoma

Accepted for publication June 24, 2014.

* Correspondence: Micromedic Technologies Ltd., Kiryat Atidim, Building 3, 5th Floor, 61581 Tel Aviv, Israel (telephone: 972-52-6100345; FAX: 972-73-2753443; e-mail: noa@m-medic.com).

† Financial interest and/or other relationship with Zetiq Technologies.

‡ Financial interest and/or other relationship with Micromedic.

§ Financial interest and/or other relationship with LEM Laboratories.

Purpose: CellDetect® is a unique platform technology comprising a proprietary plant extract and 3 dyes that enables color discrimination between malignant (red) and benign (green) cells based on specific metabolic alterations exclusive to the former. Preclinical studies and clinical trials demonstrated the applicability of the new technology in many cell culture lines and various cancers. We explored its performance characteristics in bladder cancer.

Materials and Methods: We performed an open label, 2-step study at tertiary medical centers. The study enrolled patients with newly diagnosed or a history of urothelial carcinoma. Step 1 involved staining archived biopsies. Slides were evaluated by 2 independent pathologists, who determined the concordance of the new staining technology with the hematoxylin and eosin based diagnosis. Step 2 included staining urine specimens with the new method and comparing findings to the patient final diagnosis and the results of standard urine cytology.

Results: A total of 58 archived biopsies were collected. The concordance of staining using the new platform technology with the hematoxylin and eosin based diagnosis was 100%. The new method applied to 44 urine smears showed 94% sensitivity and 89% specificity to detect urothelial carcinoma. Compared to standard urine cytology the new technology had overall superior sensitivity (94% vs 46%), particularly for low grade tumors (88% vs 17%, each $p < 0.005$). There was no significant difference in specificity between the 2 staining techniques.

Conclusions: Findings show the capability of CellDetect to accurately identify urothelial carcinoma. This indicates that the technology can be further developed to provide an alternative urine cytology test with diagnostic value that may have significant clinical benefits.

Key Words: urinary bladder, urothelium, carcinoma, diagnostic tests and procedures, staining and labeling

Bladder cancer is the fourth most common cancer in males and the eighth most common cause of cancer death with an estimated incidence of more than 74,690 new cases expected to have been diagnosed in 2014 in the United States and more than 15,000 deaths.[1] In patients diagnosed with nonmuscle invasive tumors there is up to an 80% chance of tumor recurrence,[2] rendering bladder cancer one of the most prevalent malignancies. Clinical guidelines recommend that patients with stage Ta, Tis or T1

0022-5347/14/1926-1628/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2014.06.079
Vol. 192, 1628-1632, December 2014
Printed in U.S.A.

bladder cancer should be followed with cystoscopy every 3 months for the first 2 years after tumor resection, semiannually during the subsequent 2 years and annually thereafter.[3] However, actual surveillance of these patients often deviates from standard protocols,[4] mostly due to the heavy work burden imposed on physicians, and the associated pain and discomfort that discourage patients.

In the search for alternative noninvasive diagnostic tools numerous urinary biomarkers to detect bladder cancer were developed and commercialized in the last 2 decades.[5,6] To date none of these tools has been implemented in routine clinical practice to supplant cystoscopy as the standard of care.

CellDetect technology is a novel cell staining method based on a proprietary plant extract that enables color discrimination between benign and malignant cells while preserving critical features of cell morphology.[7–10] The discriminative capacity of the stain is related to specific metabolic alterations and increased metabolic activity observed in neoplastic cells. Preclinical studies and clinical trials demonstrated the applicability of this technology in many cell culture lines and various cancers.[8,10] We explored the performance characteristics of CellDetect technology in bladder cancer.

METHODS

We performed an open label 2-step study in accordance with the Good Clinical Practice, Declaration of Helsinki 2008, and the Ministry of Health requirements and regulations in Israel. Step 1 involved staining archived bladder tumor specimens using the new CellDetect technology. Step 2 included staining voided urine specimens with the new technology. Specimens were obtained from patients with an intact bladder undergoing routine cystoscopic followup after resection of nonmuscle invasive bladder cancer who were deemed free of disease 12 months or longer before study participation. These patients served as controls. Specimens were also obtained from patients diagnosed with bladder cancer by cystoscopy who were scheduled for transurethral resection of bladder tumor.

Urine samples were obtained before cystoscopy or tumor/bladder removal. The minimal volume of urine required for analysis was 50 ml. All samples were analyzed by microscopy using a Neubauer hemocytometer. Samples that contained high levels of obscuring elements, eg erythrocytes or leukocytes, and those that were oligocellular were considered technically inadequate and excluded from analysis.

Serial sections from archived biopsies were deparaffinized and rehydrated. One section per biopsy was stained with hematoxylin and eosin according to standard protocol. Adjacent tumor sections were stained by CellDetect according to manufacturer instructions. Briefly, the CellDetect kit contains 4 principal components, including a proprietary plant extract and 3 dyes. The staining protocol involves fixation with 10% trichloroacetic acid followed by nuclear staining with hematoxylin and serial incubations in kit proprietary components with intermittent washes. All sections stained by this new method were analyzed by 2 independent pathologists using white light microscopy. The diagnosis was based on cell color and morphology. These readings were compared to the diagnosis based on hematoxylin and eosin staining, and the concordance between the 2 methods was assessed.

Urine samples were collected in clinic during the morning hours but not as first morning urine. They were processed to cytospin smears and fixed with 96% ethanol. Smears (1 slide per patient) were stained by the new method and analyzed by an expert cytopathologist under 20× magnification. Cell morphology was determined according to standard cytological criteria, namely an increased nucleus-to-cytoplasm ratio, nuclear irregularity, nuclear polymorphism and nucleoli. Cytoplasm and nucleus color was also documented. Cytology readings using the new method were compared to the patient final diagnosis and standard urine cytology readings based on hospital records when available.

RESULTS

A total of 58 eligible archived biopsy specimens were retrieved, including 22 (38%) with normal mucosa, 17 (29%) with stages Ta, T1 and Tis bladder cancer, and 19 (33%) with muscle invasive tumor (stage T2 or greater). Figure 1 shows representative images of CellDetect staining technology applied to tissue specimens. Cells comprising normal transitional

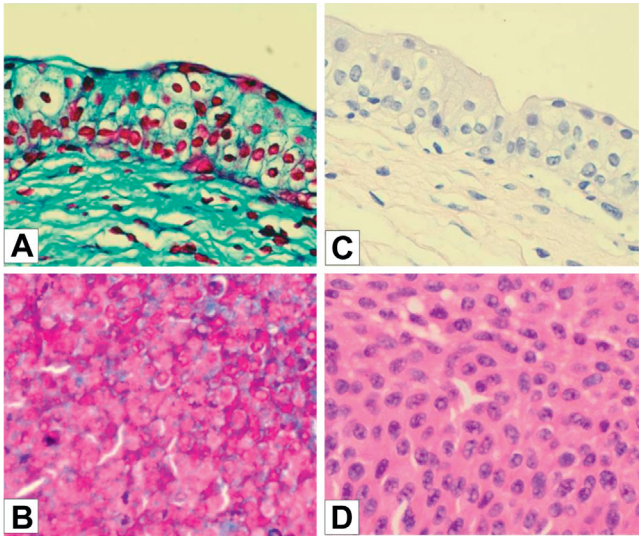

Figure 1. Photomicrographs show bladder transitional epithelium biopsy histological sections of normal epithelium (*A* and *C*) and UC (*B* and *D*). Cytoplasmic green/blue staining is characteristic of nonneoplastic states while neoplasm consists of cells with pink-magenta stained cytoplasm. CellDetect staining (*A* and *B*) and H&E (*C* and *D*), reduced from ×40.