Cambridge University Hospitals NHS Foundation Trust

CYTOSYSTEMS Ltd Advanced Diagnostics For Cancer

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MP15-20: MCM-2 cell based assay is a sensitive and specific test for risk stratification of bladder cancer patients

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Introduction

- Cystoscopy remains the gold standard in the investigation of hematuria and follow up of patients diagnosed with urothelial carcinoma (UC) of the bladder.
- Many positive biomarkers of UC are proposed: most lacking the needed specificity and sensitivity. Very few studies seek to identify and confirm the disease-free state as the basis of a screening assay to avoid further, more invasive, testing.
- Minichromosome maintenance 2 protein (MCM2) is a marker of cell proliferation and ectopic expression is a characteristic feature of malignancy & pre-malignancy.
- These proteins, which are abundant throughout the cell cycle are down regulated following cell cycle exit by quiescence, differentiation or senescence

Objectives

- Evaluate accuracy of MCM2 positivity in diagnosis and surveillance of UC
- To stratify bladder cancer patients for *absence* of disease using a validated biomarker with the intention of safely reducing the need for, or frequency of, follow up cystoscopy.
- To achieve stratification by implementation of combined use of a biological biomarker, MCM2, with optimised sample collection for subsequent integration with an automated digital pathology platform.
- To conduct a prospective validation study to ascertain biomarker level cut offs and how many patients could have been predicted to have avoided cystoscopy

Methods

- A feasibility study was conducted on 176 patients and healthy volunteers from 3 centres in the UK from CS and GH clinics. The verification set comprised 149 volunteers.
- SurePath platform was used to process the urine cytology samples and slides stained using HRP-DAB or conventional Pap stains.
- Immunocytochemical analysis of MCM2 was performed as a marker for presence of UC.
- Feasibility data sets were used to determine MCM2 threshold for GH and CS counts using the optimised Youden's Index (J) and optimal sensitivity, establishing conditions such that there was a zero false negative rate (ZFN). Cut-off values were used to determine sensitivity, specificity, PPV and NPV.

Results

- Fig 1 Shows Pap (panel a) and anti-MCM 2/DAB stained (panel b) urothelial cells.
- Fig 2 Show feasibility-data ROCs for CS (panel a) and GH clinics (panel b). Area under curve values are 0.915 and 0.971 respectively.
- Table 1. Cut-offs and assay parameters determined from feasibility set, and parameters determined using these cut-offs with verification sets. The MCM assay is customizable allowing optimization for different patient cohorts, resulting in optimal sensitivity and specificty of GH (95.8/91.7) & CS (92.3/80.3) in the current assay format.

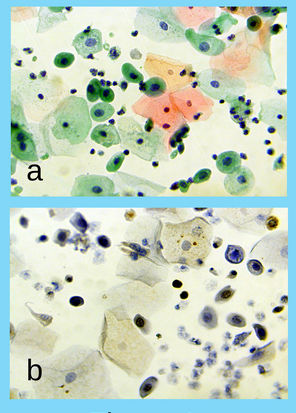

Figure 1

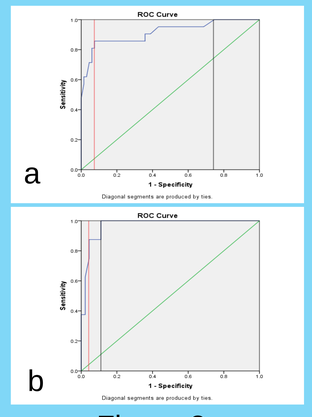

Figure 2

			Feasibility Set				Verification Set			
Clinical cohort	Cut-off type	Cut-off	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
CS clinic and healthy	J	81	81	94.1	73.9	96	92.3	80.3	75	94.2
	ZFN	13	100	29.7	22.8	100	100	45.9	54.2	100
GH clinic	J	130	87.5	95.6	77.8	97.7	70.8	95.8	94.4	76.7
	ZFN	44	100	88.9	61.5	100	95.8	91.7	92	95.7
Combined Clinics	J	122	82.8	94.5	75	96.5	84.1	90.6	86.9	88.5
	ZFN	13	100	54.1	29.5	100	100	50.6	60	100

Table 1

Conclusions

- MCM2 detection has the potential to be the basis for a reliable non-invasive test, for diagnosis of primary and recurrent UC which may be used to stratify cases where there is *no* likelihood of disease *i.e.* zero false negatives, thus avoiding invasive intervention and resulting in quantifiable health economic benefits.
- Clinically, false positives are acceptable, however from a health economic perspective a high sensitivity and specificity is required. Customizing the MCM assay for particular patient cohorts (CS, GH, high risk, low risk) allows both clinical and health economic requirements to be met.
- Together with patient history, the MCM stratification model may be adopted to identify a large subset of true negatives, resulting in potentially significant health economic benefits.
- To enhance clinical utility and accuracy, ongoing work includes incorporation of a cell collection device to be deployed in primary care (at point of sampling) to preserve analyte quality, plus development of an automated digital pathology platform and stratification algorithm.