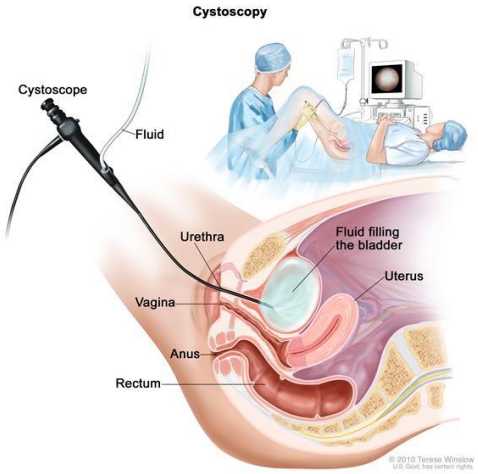
Urine as a liquid biopsy for highly sensitive detection of bladder cancer

HNPCC Annual Meeting, Hvidovre Hospital, 11th October 2018

Per Guldberg
Danish Cancer Society
Research Center

Gold standard for detection of bladder cancer

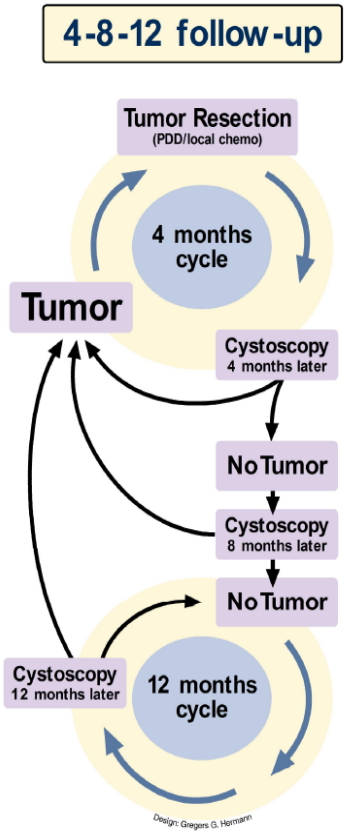


Flexible cystoscopy

Imperfect diagnostic test: False positives and negatives

Invasive and expensive

Long-term follow-up of patients with bladder cancer



Numbers from Denmark

- Population: 5.7 million
- New cases per year: 2,000
- 5,000 pts. with macr. hematuria (20% with tumor)
- 14,000 pts. with micr. hematuria (5% with tumor)
- 13,000 surveillance

Total: 32,000 flexible cystoscopies per year

DNA-based urine test

Identification of p53 Gene Mutations in Bladder Cancers and Urine Samples

DAVID SIDRANSKY, ANDREW VON ESCHENBACH, YVONNE C. TSAI, PETER JONES, IAN SUMMERHAYES, FRAY MARSHALL, MEERA PAUL, PEARL GREEN, STANLEY R. HAMILTON, PHILIP FROST, BERT VOGELSTEIN*

Although bladder cancers are very common, little is known about their molecular pathogenesis. In this study, invasive bladder cancers were evaluated for the presence of gene mutations in the p53 suppressor gene. Of 18 tumors evaluated, 11 (61 percent) were found to have genetic alterations of p53. The alterations included ten point mutations resulting in single amino acid substitutions, and one 24–base pair deletion. In all but one case, the mutations were associated with chromosome 17p allelic deletions, leaving the cells with only mutant forms of the p53 gene product. Through the use of the polymerase chain reaction and oligomer-specific hybridization, p53 mutations were identified in 1 to 7 percent of the cells within the urine sediment of each of three patients tested. The p53 mutations are the first genetic alterations demonstrated to occur in a high proportion of primary invasive bladder cancers. Detection of such mutations ex vivo has clinical implications for monitoring individuals whose tumor cells are shed extracorporeally.

PROOF OF CONCEPT

Science 252:706-9 (1991)

Performance characteristics of a diagnostic test

! Sensitivity

Specificity

Positive predictive value

! Negative predictive value

Challenge #1: Coverage of DNA biomarkers

Driver mutations

Promoter hypermethylation

Copy number variations

Bladder cancer:

TERT 60-80%

FGFR3 30-40%

ONECUT2

TWIST1

BCL2

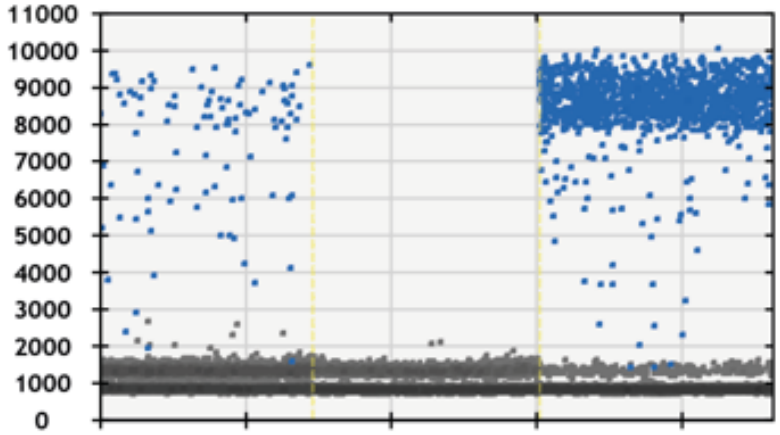
EOMES

VIM

SALL3

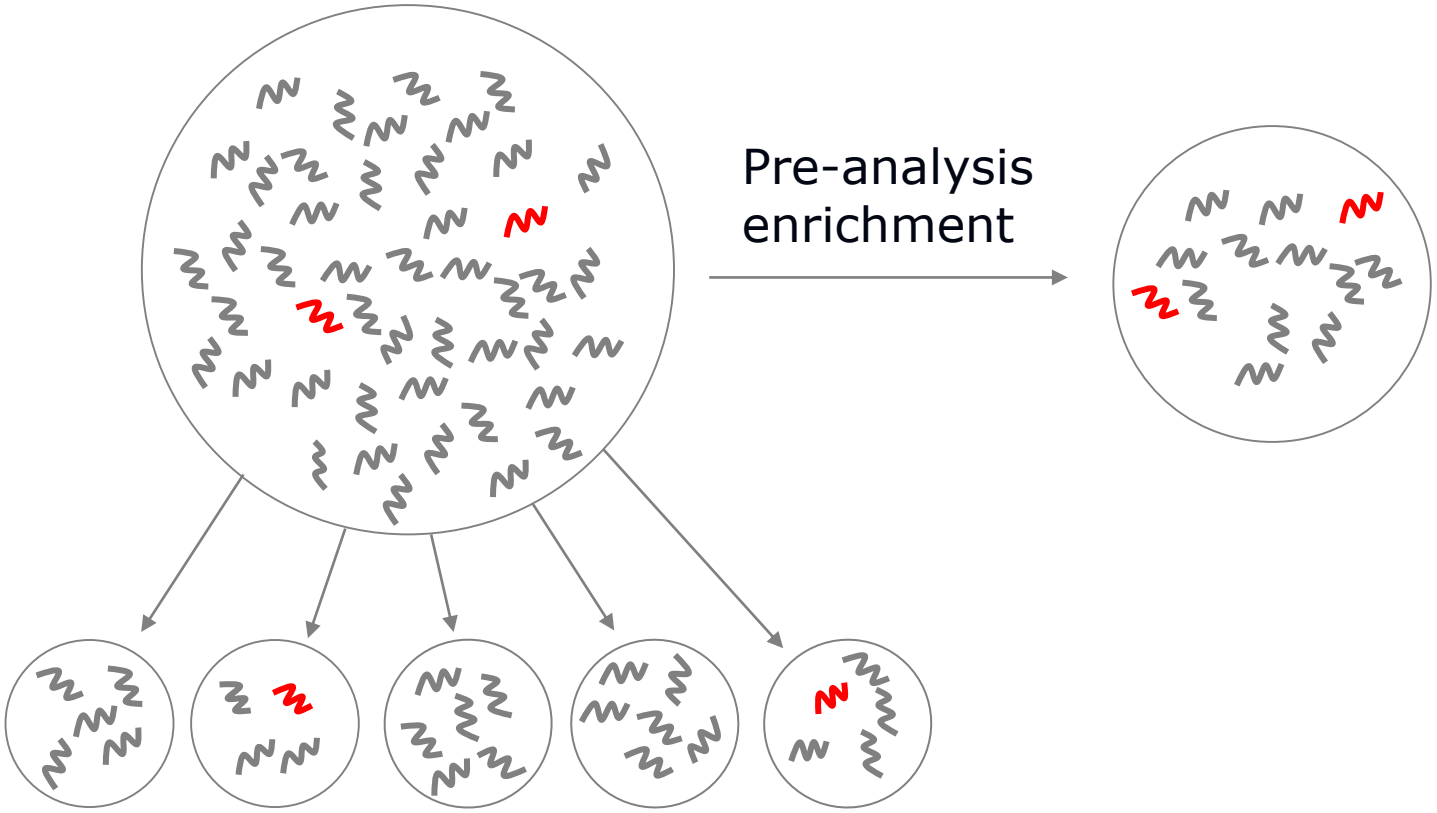
30-70% each

Challenge #2: Sensitive detection of rare DNA molecules

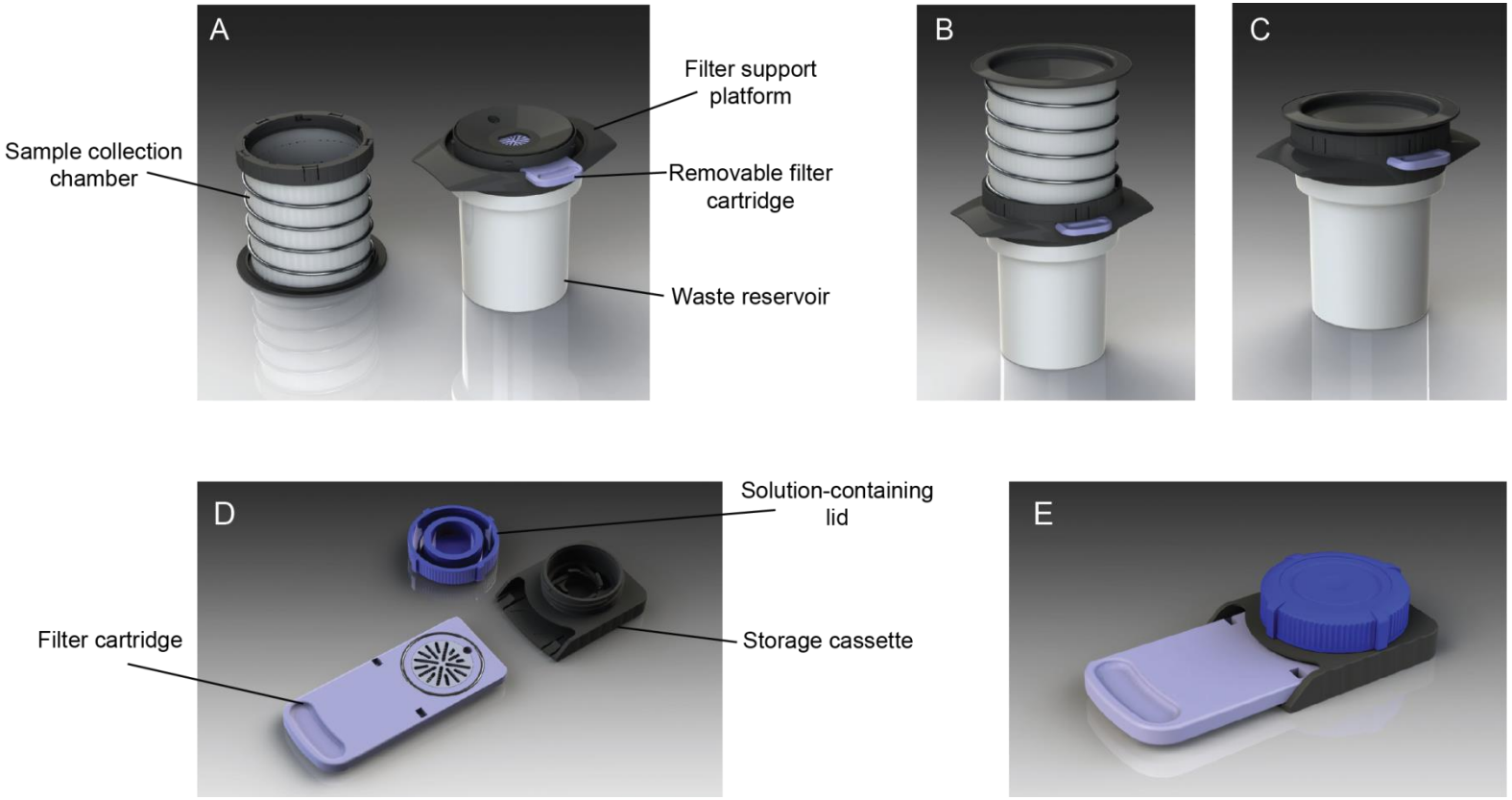


Droplet digital PCR (ddPCR) – down to 0.001% allelic fraction

Challenge #3: Rare molecules and sampling bias

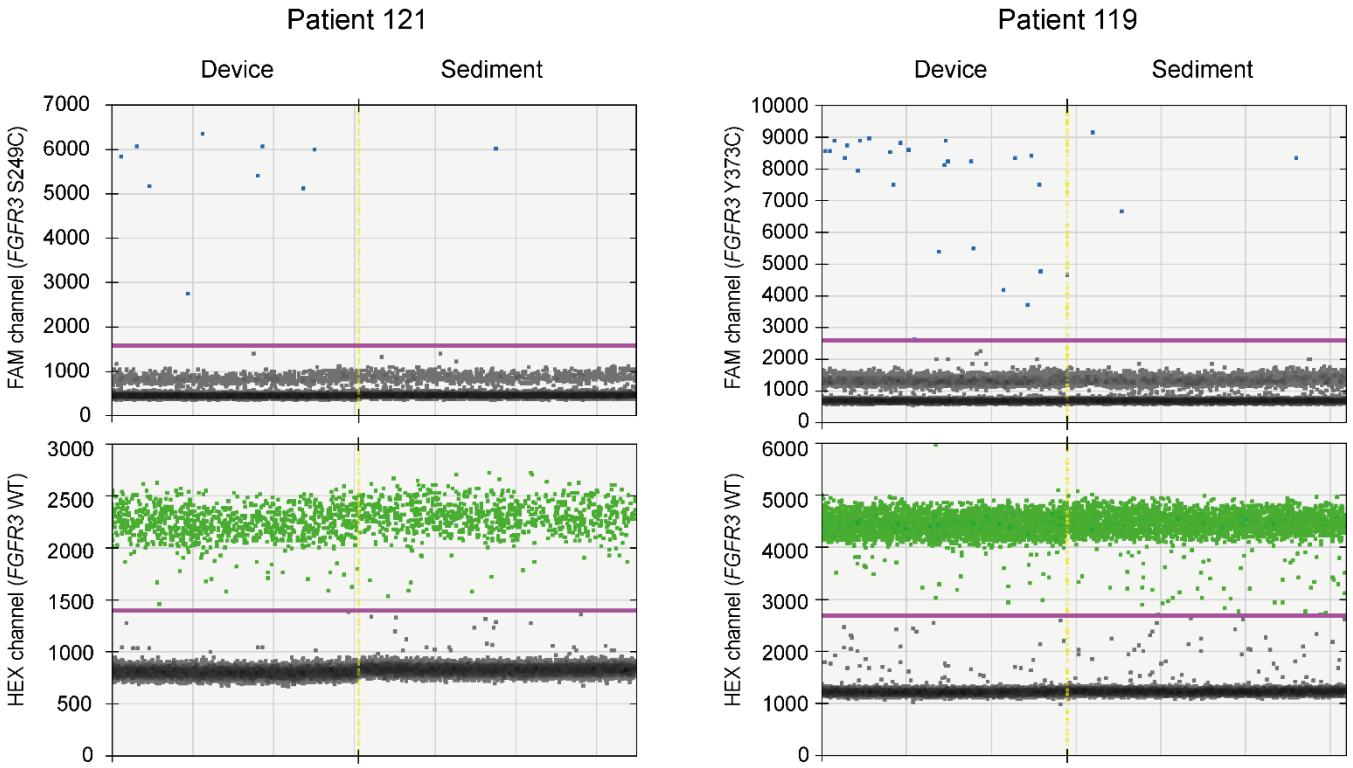


Filtration device for collection of cells from urine



Andersson et al. (2015) *PLoS One* 10:e0131889

Enrichment of tumor cells by filtration



Andersson et al. (2015) *PLoS One* 10:e0131889

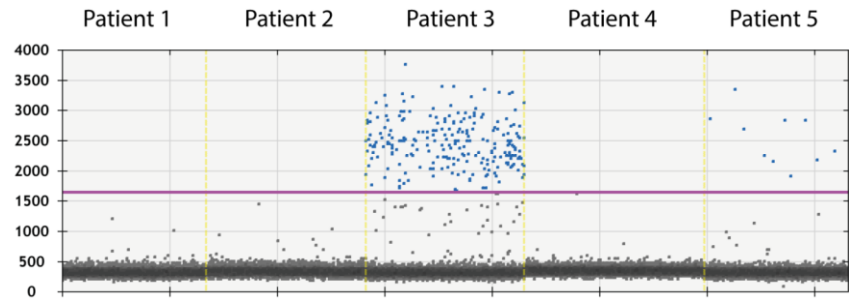
Urine-DNA test for bladder cancer

High diagnostic coverage of DNA biomarkers

High-sensitivity detection of biomarkers

Enrichment of tumor cells in sample

- *TERT*
- *FGFR3*
- *ONECUT2*
- *TWIST1*
- *BCL2*
- *EOMES*
- *VIM*
- *SALL3*



High sensitivity

Prospective blinded evaluation of urine-DNA testing in patients with macroscopic hematuria (N=475)

Clinical evaluation: tumor N=99; no tumor N=376

	Cystoscopy	Urine-DNA test
Sensitivity	92%	97%
Specificity	57%	76%

AUC: 0.963

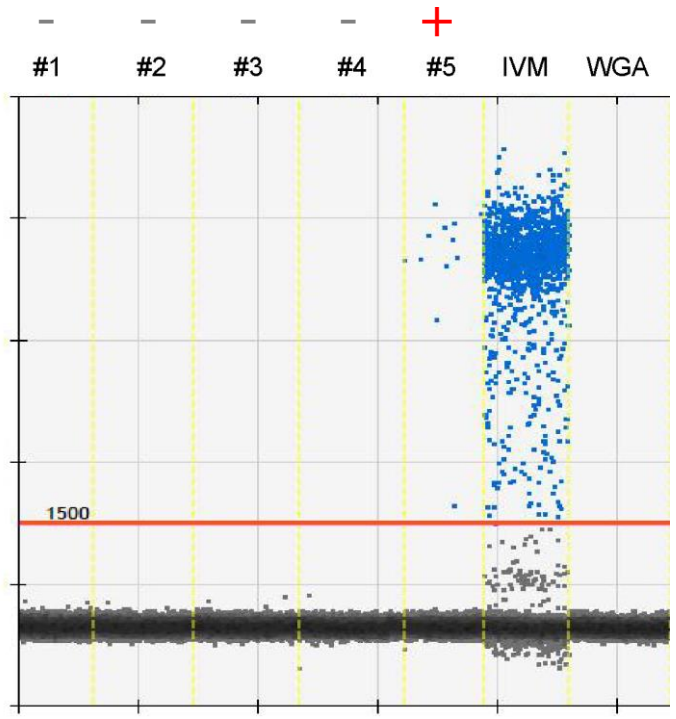
Negative predictive value (NPV): 99%

Dahmcke et al. (2016) Eur Urol, 70:916-919

Additional issue #1:
Release of DNA and cells from a tumor is random

- Prostate cancer
- GS 9
- 5 urine samples
- Biomarker: *GSTP1*

Repeated sampling may increase sensitivity



Larsen et al. (2018) J Urol

Additional issue #2:
Molecular disease may precede clinical disease

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Molecular disease may precede clinical disease

Urine samples collected from healthy individuals *prior* to clinical diagnosis of bladder cancer (N=606)

4.3 years before diagnosis

13.8 years before diagnosis

Are false-positives *true* false-positives?

	Cystoscopy	Urine-DNA test
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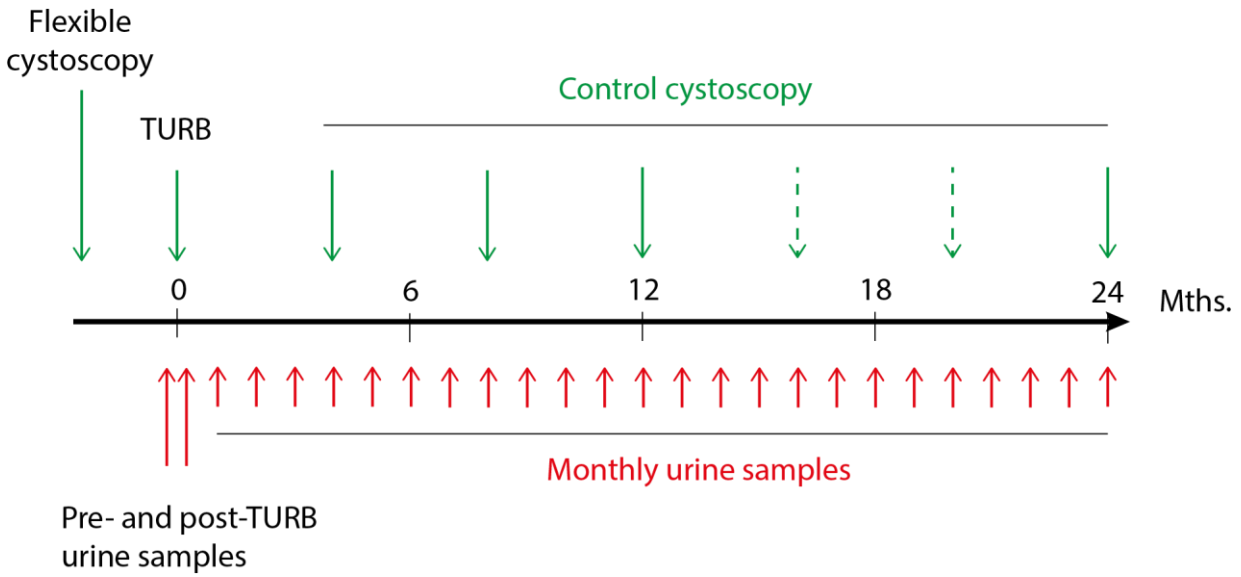
Surveillance trial



Danish Cancer Society

Christina Mackeprang Dahmcke

Christina Dahl

Louise K. Larsen

Anja Olsen

Anne Tjønneland

Herlev University Hospital

Kenneth E. Steven

Gitte Wrist Lam

Jørn Skibsted Jakobsen