Radboud Repository

Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/22760

Please be advised that this information was generated on 2018-07-07 and may be subject to change.

Predictive Value of p53 Mutations Analyzed in Bladder Washings for Progression of High-Risk Superficial Bladder Cancer[1]

Jacqueline A. M. Vet,[2] J. Alfred Witjes, Salvatore A. E. Marras, Daphne Hessels, Henk G. van der Poel, Frans M. J. Debruyne, and Jack A. Schalken

Department of Urology, University Hospital Nijmegen, 6500 HB Nijmegen, the Netherlands

ABSTRACT

To assess the value of *p53* mutations in predicting the progression of superficial bladder cancer [transitional cell carcinoma (TCC)] and to define exactly when *p53* mutations occur in the process of tumor progression, 80 consecutive bladder washings from 26 high-risk (indicated by quantitative karyometric analysis) superficial TCC patients were examined by single-strand conformation polymorphism. Six of 13 patients who experienced clinical progression (progression to T_2 or higher) were found to have a *p53* mutation in one or more of their bladder washings. In the control group (no progression to invasive disease), only 1 of 13 patients had a *p53* mutation. For these high-risk superficial TCC patients, the occurrence of a *p53* mutation has a positive predictive value of 86% for the progression of disease. A negative predictive value of 63% was observed. Moreover, because *p53* mutations were found in samples prior to progression (mean, 8 months), they could identify patients who need changes in their treatment strategies to prevent progression to invasive disease. Despite these promising results, it is obvious that to increase not only the positive predictive value but especially the negative predictive value of this procedure to predict progression, additional prognostic markers are still needed.

INTRODUCTION

TCC[3] is the most common histological type of bladder cancer, presenting with a wide spectrum of clinical and pathological appearances. Superficial TCC accounts for approximately 80% of all newly diagnosed tumors in the bladder (1). Thirty to 90% of these tumors will recur, depending on stage, grade, multifocality, and treatment modality; however, only 10–25% become invasive or metastasize during the course of the disease (2, 3). Early identification of patients whose superficial bladder tumors will progress is very important, because changes in their treatment strategies can have a major impact on the outcome of disease. The morbidity of cystectomy, which at the moment is the only treatment to prevent progression to invasive disease, emphasizes the urgent need for markers that identify patients at risk for tumor progression.

The *p53* tumor suppressor gene, located on chromosome 17p13.1, is known to be involved in the development of bladder cancer. The p53 protein plays a role as a transcriptional regulator (4, 5) and is involved in the control of the cell cycle at the G_1 to S-phase transition (6, 7). In case of DNA damage, p53 can delay the progression through the cell cycle to permit DNA repair (5) or can initiate programmed cell death (apoptosis; Ref. 8). The loss of p53 regulatory functions can result in replication of incorrect DNA, leading to increased genetic instability (9). Allelic deletions of 17p were predominantly observed in invasive bladder tumors and, to a lesser extent, in high-grade superficial TCC but were absent in low-grade T_a tumors (10–12). Mutations in the *p53* tumor suppressor gene frequently occur in bladder cancer and appear to correlate with grade and stage (13–15). The relatively high frequency of *p53* mutations in T_2–T_4 tumors compared with that in T_1 tumors suggests their involvement in the progression of T_1 tumors to invasive disease (16). However, the exact point at which *p53* mutations occur during tumor progression from superficial (T_a) tumors to lamina propria invasive (T_1) and muscle invasive (T_2–T_4) tumors is still unclear.

From a large prospective study in which approximately 3000 bladder washings were investigated by quantitative karyometric analysis (17), we selected 26 patients who were indicated to be high risk (*i.e.*, suspected for recurrences) on basis of this analysis. We studied the value of *p53* mutations as an additional prognostic marker in this group of high-risk superficial TCC patients, using PCR-SSCP analysis. To define when *p53* mutations occur during the natural history of these tumors, we analyzed consecutive bladder washings from the same patients.

MATERIALS AND METHODS

Specimens. Eighty consecutive bladder-washing specimens from 13 patients with high-risk superficial TCC who underwent clinical progression to invasive disease (pT≥2) and 13 patients (age, sex, and follow-up matched) who did not experience progression were analyzed. The bladder washings were obtained from a large prospective study in which bladder washings of patients were collected for quantitative karyometric analysis (17). The bladder washings were collected just before the transurethral resections were done. If patients were diagnosed with superficial TCC, bladder washings were collected and analyzed with 3–12-month intervals. All patients were designated as having high-risk superficial TCC on the basis of quantitative karyometric analysis (17) and having intermediate

Received 12/7/95; revised 2/15/96; accepted 3/1/96.

[1] This work was supported by Dutch Cancer Society Grant NUKC9102.

[2] To whom requests for reprints should be addressed, at Public Health Research Institute, Department of Molecular Genetics, 455 First Avenue, New York, NY 10016. Phone: (212) 578-0871; Fax: (212) 578-0804.

[3] The abbreviations used are: TCC, transitional cell carcinoma; SSCP, single-strand conformation polymorphism; pT≥2, progression to T_2 or higher; CIS, carcinoma *in situ*.

Table 1 Clinical and pathological[a] data correlating with bladder washings of high-risk superficial TCC patients with and without progression of disease

	Progression	No progression
Stage		
T_a	6	5
T_a + CIS		1
T_1	3	4
T_1 + CIS	2	2
CIS	2	1
Grade		
1	1	
2	4	6
3	6	6
Age (mean, yr)	69.1	65
Sex ratio (male:female)	11:2	11:2
Follow-up		
Mean (mo)	50.5	53.9
Range (mo)	13–91	31–83

[a] Pathological data at the moment of first quanticyt sample; in case no pathological data are present, the previous diagnosis is taken into account.

or high-risk superficial TCC by calculating their prognostic index score based on tumor stage, multicentricity, recurrence rate, and number of bladder areas involved (18). All patients received optimal intravesical therapy (*e.g., Bacillus Calmette-Guérin* instillations) as soon as they were considered to have high-risk superficial bladder tumors. Clinical and pathological data for the high-risk patients are summarized in Table 1.

The bladder was washed with 50 ml saline that was immediately mixed with an equal volume of Carbowax (50% ethanol and 2% polyethylene glycol 1500) for fixation. After centrifugation, the supernatant was decanted, and the pellet was resuspended in 3 ml Carbowax and stored at −20°C.

For DNA isolation, 150 μl bladder-washing material were centrifuged for 5 min at 13000 rpm. After decanting the supernatant, the pellet was washed two times with a mixture of acetone:methanol (1:1). Subsequently, the cells were lysed in 50 μl lysis buffer [10 mM Tris-HCl (pH 8.8), 400 mM NaCl, 2 mM EDTA, 0.45% Tween 20, and 0.45% NP40] with 0.5 μg/μl proteinase K for 1 h at 60°C. Proteinase K was inactivated by boiling the sample for 5 min.

PCR-SSCP. PCR-SSCP analysis was performed to investigate *p53* mutations in exons 5–9 (19). Exons 8 and 9 were amplified together. The intron primers for amplification were: exon 5, sense, 5′-TCA CTT GTG CCC TGA CTT-3′; antisense, 5′-GAG GAA TCA GAG GCC TGG-3′; exon 6, sense, 5′-GAG ACG ACA GGG CTG GTT-3′; antisense, 5′-GAG ACC CCA GTT GCA AAC-3′; exon 7, sense, 5′-CCA AGG CGC ACT GGC CTC-3′; antisense, 5′-GCG GCA AGC AGA GGC TGG-3′; and exons 8 and 9, sense, 5′-CCT TAC TGC CTC TTG CTT C-3′; antisense, 5′-AAA CTT TCC ACT TGA TAA-3′.

A 2-μl aliquot of bladder-washing DNA solution was subjected to 40 cycles of PCR: 0.5 min at 95°C; 2 min at 55°C; and 1.3 min at 72°C.

Exons 5, 6, 8, and 9 were amplified in a 50-μl solution containing 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.75 mM $MgCl_2$, 250 μM deoxynucleotide triphosphates, 10 pmol of each primer, 3 μCi [α-^{32}P]dATP, and 1.5 units *Taq* polymerase (Perkin Elmer, Norwalk, CT). Exon 7 was amplified in the same buffer containing 1.4 mM magnesium chloride.

Five μl of the PCR product were diluted in 15 μl loading buffer (96% formamide, 20 mM EDTA, 0.05% bromophenol blue, and xylene cyanol), boiled for 3 min, and then quenched (10 min) on ice before loading (2 μl/lane). Each sample was applied to a 5% polyacrylamide (49:1)/Tris-borate EDTA (0.5×) gel with 10% (v/v) glycerol. Subsequently, electrophoresis was performed at room temperature for 16 h at 6 W (exons 8 and 9) and 5 W (exons 5–7).

Sequence Analysis. Sequencing of the double-stranded PCR products that showed a mobility shift on the SSCP gel was performed as described previously (20). Direct sequencing of the amplified product was performed if the intensity of the mutant DNA fragment was ≥50% of the wild-type DNA fragment. Whenever the intensity of the shifted DNA fragment was ≤50%, we excised the mutant fragment from the polyacrylamide gel, and the gel piece was immersed in 100 μl 20 mM Tris (pH 8.0)-1 mM EDTA solution and heated at 80°C for 15 min. The extract was briefly vortexed and incubated overnight at 4°C. After brief centrifugation, 10 μl of the supernatant were subjected to PCR reamplification for 45 cycles.

Before sequencing, the amplified PCR products were subjected to electrophoresis on a 2% low-melt agarose gel and further purified with the Magic Preps DNA purification system (Promega, Madison, WI). For sequencing in the AmpliCycle system (Perkin Elmer), we used internal primers: exon 5, sense, 5′-TGT CTC CTT CCT CTT CCT AC-3′; exon 6, sense, 5′-GGT CCC CAG GCC TCT GAT TC-3′; exon 7, sense, 5′-TCT TGG GCC TGT GTT GTC TC-3′; exon 8, sense, 5′-TTG CTT CTC TTT TCC TAT CC-3′; and exon 9, sense, 5′-CAC CTT TCC TTG CCT CTT TC-3′.

Electrophoresis was performed on a 6% polyacrylamide (19:1) gel containing 7 M urea.

RESULTS

SSCP Analysis. PCR-SSCP analysis of exons 5–9 was performed on DNA isolated from bladder washings. Fig. 1*A*, *Lanes 5* (case 3) and *6* (case 6), shows, next to the wild type DNA fragments corresponding to exon 6, a clear mobility shift. The intensity of the wild-type DNA fragments compared with the mutated fragment could most likely be explained by the presence of normal cells (wild-type p53 phenotype) in the bladder washing. Fig. 1*B*, *Lane 6* (case 9), shows a clear mobility shift of the DNA fragment corresponding to exons 8 and 9 that were amplified together. The intensity of the mutated DNA fragment implies that in most cells in this bladder washing, the *p53* gene is mutated. Fig. 1*C* shows the sequence analysis result of the antisense strand of exon 6 (case 3); a transition from a T to a C is shown at codon 205, resulting in a change of a T to an L. Mobility shifts were observed in eight patients (Table 2). Mutations were found once in exon 5, two times in exon 6, and four times in exon 8. In one patient (case 1), two different mutations were detected in the consecutive bladder washings analyzed. A different mobility shift was observed by SSCP analysis, in concordance with the different nucleotide change. We found four transitions, G to A three times and A to G once, and three transversions, T to G once and G to C twice. In case 5, the known polymorphism in codon 213 of exon 6 (21) was

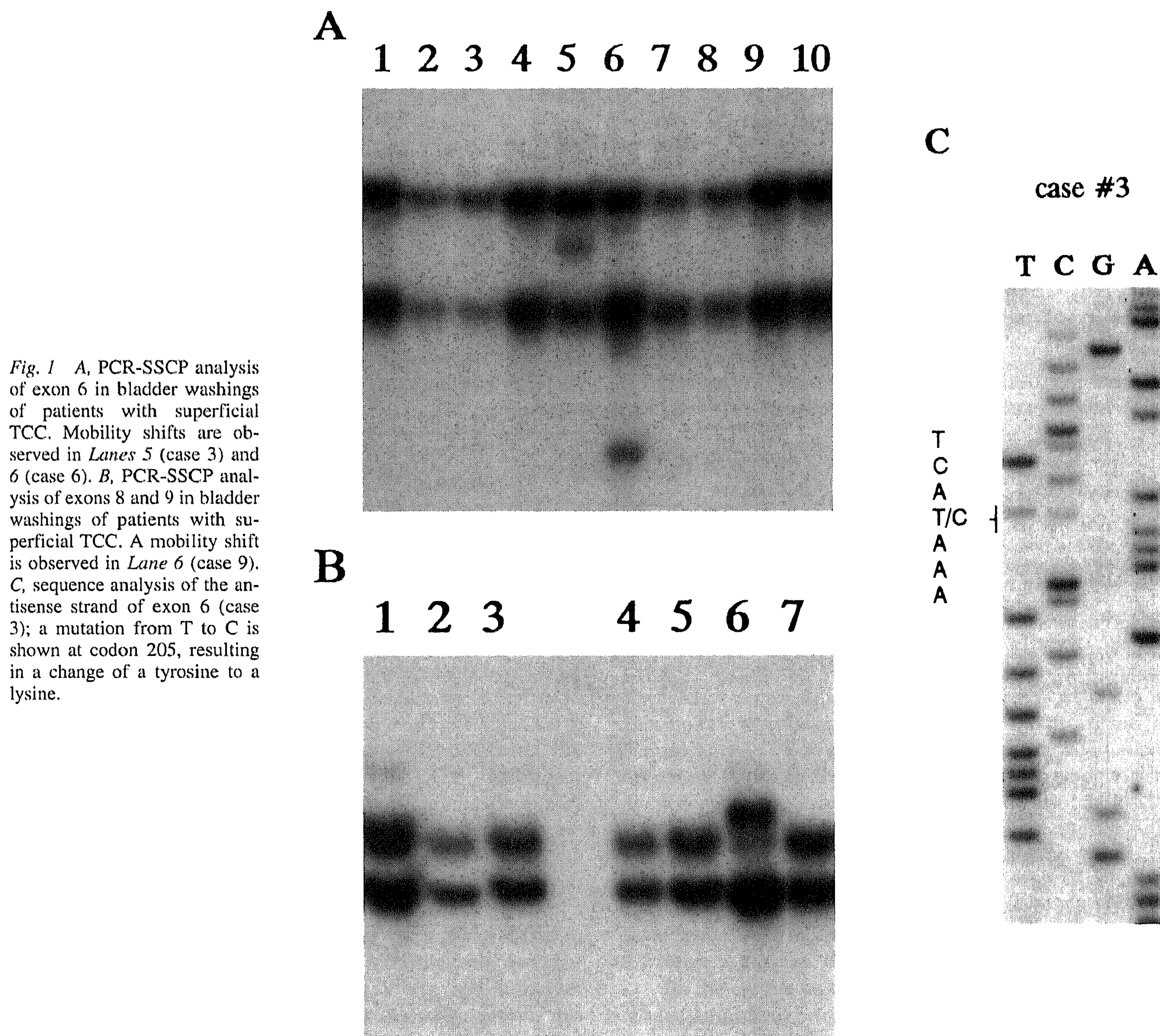

Fig. 1 *A*, PCR-SSCP analysis of exon 6 in bladder washings of patients with superficial TCC. Mobility shifts are observed in *Lanes 5* (case 3) and *6* (case 6). *B*, PCR-SSCP analysis of exons 8 and 9 in bladder washings of patients with superficial TCC. A mobility shift is observed in *Lane 6* (case 9). *C*, sequence analysis of the antisense strand of exon 6 (case 3); a mutation from T to C is shown at codon 205, resulting in a change of a tyrosine to a lysine.

detected. The identified point mutation, *i.e.*, an A to G transversion, did not result in an amino acid change (Table 2).

Correlation of *p53* Mutation and Progression of Disease. To assess the additional prognostic value of *p53* mutations for superficial TCC indicated as high risk on the basis of quantitative karyometric analysis, *p53* mutations were analyzed in bladder washings of 26 patients (Table 2). *p53* mutations were found in 6 (46%) of 13 patients with progressive superficial bladder cancer (Table 2A). One (7.7%) of 13 patients with no progression of disease (23-month follow-up; Table 2B) showed a *p53* mutation. The positive predictive value of *p53* mutations for progression of high-risk superficial TCC was 86% (6 of 7). The observed negative predictive value was 63% (12 of 19).

To assess when *p53* mutations occur during the process of tumor progression, consecutive bladder washings from the same patient were analyzed. The occurrence of a *p53* mutation during the course of the disease in individual patients ($n = 7$) is summarized in Fig. 2. Of these seven patients, six showed progression. It is noteworthy that the *p53* mutations were detected in bladder washings a mean of 8 (range, 4–14) months before progression to invasive disease. From two patients (cases 2 and 4), no bladder washings were available from the first period of disease (58 and 6 months, respectively). The first analyzed sample showed in both cases a mutation, followed by progression to invasive disease after 9 and 5 months, respectively. In two patients (cases 3 and 9), first the wild-type genotype was found. Within 12 and 9 months, respectively, a mutation was detected, followed by the occurrence of an invasive tumor 7 and 9 months later. In two patients (cases 1 and 6), mutated *p53* was detected at first diagnosis, followed by progression to invasive disease after 14 and 4 months, respectively. In only one patient (case 15)

Table 2 *p53* mutations analyzed (PCR-SSCP) in consecutive bladder washings of patients with superficial bladder cancer

Case	yr[a]	Stage/Grade[b]	*p53* mutation[c]	Amino acid change
A, patients with progression of disease (high risk)				
1	1990	A/1	Exon 8	Codon 285/GAG → AAG/glu → lys
	1992	2/3	Exon 8	Codon 281/GAC → CAC/asp → his
2	1992	1/3	Exon 5	nd
	1993	1/2B, CIS	Normal	
	1993	2/2	nd	
3	1990	A/2	Normal	
	1991	A/2	Normal	
	1991	1/2B	Normal	
	1991	1/3, CIS	Exon 6	Codon 205/TAT → TGT/tyr → lys
	1992	4/3	Exon 6	Codon 205/TAT → TGT/tyr → lys
4	1993	1/3, CIS	Exon 8	Codon 273/CGT → CAT/arg → his
	1994	>2/3	Exon 8	Codon 273/CGT → CAT/arg → his
5	1990	CIS	Exon 6[d]	Codon 213/CGA → CGG/arg → arg
	1991	NR	Exon 6	nd
	1992	atypia	Exon 6	Codon 213/CGA → CGG/arg → arg
	1992	4a/2–3	Exon 6	Codon 213/CGA → CGG/arg → arg
6	1991	CIS	Exon 6	Codon 194/CTT → CGT/leu → arg
	1991	2/3	Normal[e]	
7	1992	NR	Normal	
	1993	NR	Normal	
	1994	CIS	Normal	
	1994	1/3, CIS	Normal	
	1994	4a/3	nd	
8	1991	NR	Normal	
	1992	1/3	Normal	
	1992	2/3	Normal	
9	1993	1/3, CIS	Normal	
	1993	NR	Normal	
	1994	CIS?	Exon 8	nd
	1994	CIS?	Exon 8	Codon 280/AGA → AAA/arg → lys
	1994	NR	Normal	
	1994	2/3	Normal	
10	1992	NR	Normal	
	1994	NR	Normal	
	1994	≥2	Normal	
11	1991	A/2	Normal	
	1993	sq. metasplasia	nd	
	1993	(cystectomy; CIS + positive lymph nodes)		
12	1994	1/3	Normal	
	1995	4/3	Normal	
13	1992	A/2	Normal	
	1994	2/3	nd	
B, patients without progression of disease (high risk)				
14	1991	Recurrences	Normal	
	1991	Recurrences	Normal	
	1992	A/1	Normal	
	1994	Papillary multiple	Normal	
15	1991	1/3	Normal	
	1992	NR	Normal	
	1993	NR	Exon 8	Codon 280/AGA → ACA/arg → thr
	1994	A/3, CIS	Exon 8	Codon 280/AGA → ACA/arg → thr
	1994	NR	Normal	
16	1990	A/2B	Normal	
	1991	A/3, CIS	Normal	
	1992	1/3	Normal	
17	1990	A/2A	Normal	
	1991	1/3	Normal	
	1993	A/2B	Normal	
	1994	A/2A, CIS	Normal	
	1994	Papillary multiple	Normal	
18	1990	A/2B	Normal	
	1991	NR	Normal	
	1993	1/2, CIS	Normal	
19	1991	A/2A	Normal	
	1993	NR	Normal	
20	1990	1/2B	Normal	
	1991	1/2B	Normal	
21	1991	1/2B, CIS	Normal	
	1992	NR	Normal	
	1994	A/3	Normal	
22	1992	1/2, CIS	Normal	
	1992	1/2, CIS	Normal	
23	1992	A/3, CIS	Normal	
	1993	NR	Normal	
	1993	NR	Normal	
24	1992	NR	Normal	
	1992	Tumor	Normal	
	1993	NR	Normal	
	1994	NR	Normal	
25	1992	NR	Normal	
	1994	NR	Normal	
	1994	Papillary multiple	Normal	
26	1992	NR	Normal	
	1993	NR	Normal	
	1994	NR	Normal	
	1994	Papillary lesion	Normal	

[a] Year of bladder wash sampling.
[b] Stage and grade of concomitant tumors. NR, no abnormalities seen by cytoscopy; sq, squamous; CIS?, suspicion for presence of CIS.
[c] nd, not done.
[d] Known polymorphism.
[e] Not a representative sample.

was a *p53* mutation not followed by progression to invasive disease; 2 years after diagnosis, the mutated *p53* was observed in the bladder washings, but until now (23 months later), no progression to invasive disease has been observed. The histological data of the tumors resected from the bladder at the moment of the first appearance of a *p53* mutation in the bladder washings are shown in Table 2.

DISCUSSION

Compared with superficial TCC, invasive disease constitutes a considerably greater threat to the patient's health. Because 10–25% of cases of superficial TCC progress to invasive disease (2, 3), prediction of tumor progression is an important issue in bladder cancer management. Multifocal occurrence of bladder tumors suggests a general disease of the urothelium

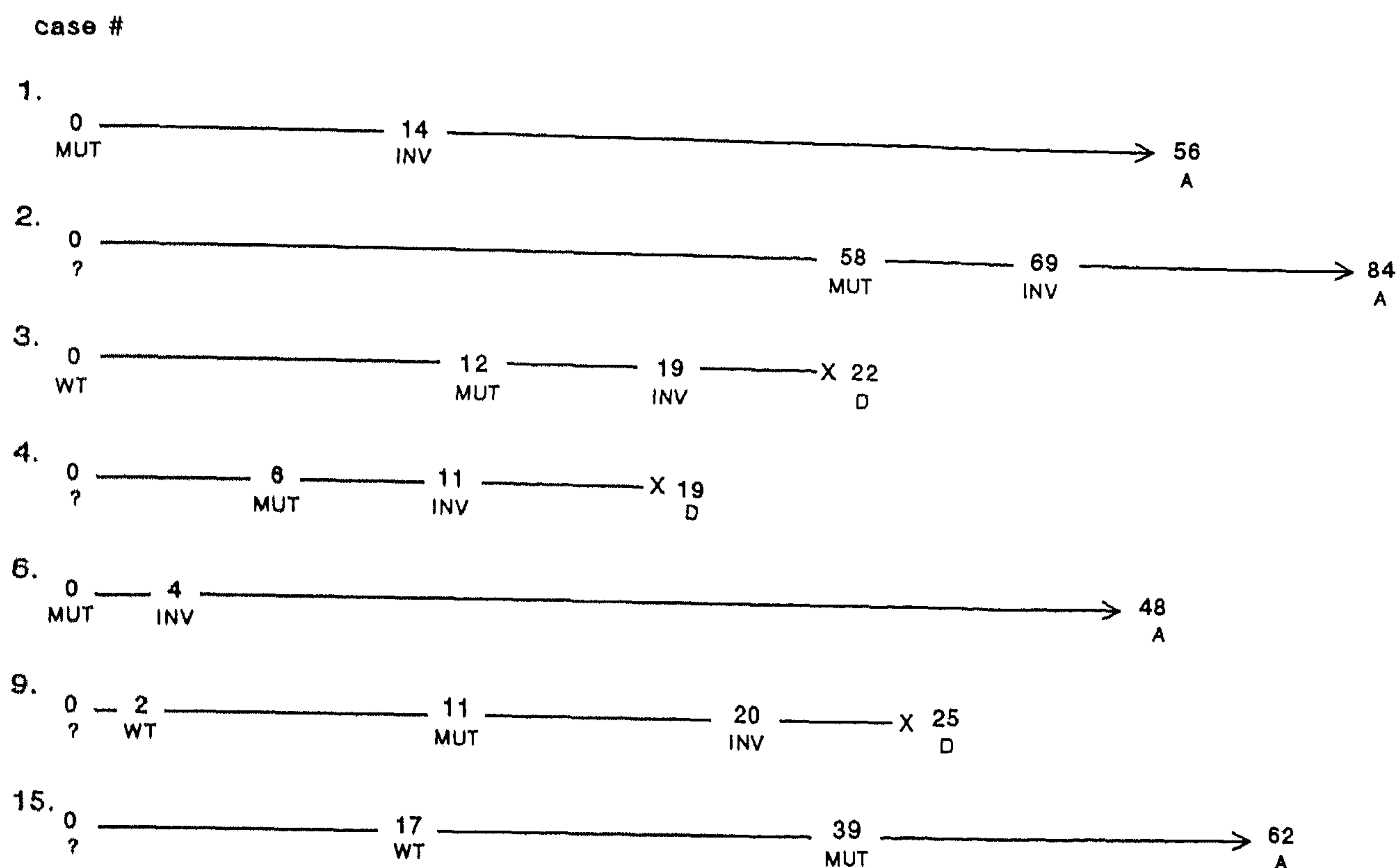

Fig. 2 Timing *p53* mutations in tumor progression cascade. *0,* first diagnosis; *?,* no bladder wash available; *WT,* wild-type p53; *MUT,* mutated *p53*; *INV,* occurrence of invasive tumor (pT≥2); *A,* alive; *D,* dead; time in months.

rather than a localized process. In this respect, both voided urine and bladder washings have the advantage over histological biopsies in that they reflect the general status of the bladder mucosa, because cells from the entire bladder are sampled. In 1945, Papanicolaou and Marshall had already shown that exfoliation of tumor cells in the urine is a powerful diagnostic tool for detection and grading of patients with TCC of the bladder (22). However, the sensitivity for detecting low-grade tumors and the reproducibility of this cytological grading system are disturbingly low (23). Because bladder washings enable the harvest of more and better-preserved material compared with voided urine (24, 25), van der Poel *et al.* applied quantitative karyometric analysis (2c deviation index and nuclear shape) to bladder washings to increase both the reproducibility and sensitivity of the cytological analysis (17). The sensitivity for detecting low-grade tumors increased substantially by using this analysis, and additionally, 86% of all tumor recurrences were preceded by cytoscopically negative samples with positive karyometric scores.[4] Notwithstanding this improvement, we attempted to enhance the clinical usefulness of this screening procedure by adding an additional marker. Although mutations in the *p53* tumor suppressor gene frequently occur in bladder cancer and correlate with grade and stage (13–16), until now, it has remained unclear exactly when during the tumor progression process *p53* mutations occur. We chose to analyze mutations in the *p53* gene directly because of the more objective nature of this analysis compared with immunohistochemical studies. Despite the fact that p53 accumulation and mutation of the *p53* gene are well correlated, in 20–30% of the cases, they appear to be separate events (26–28); therefore, there is no obligate causal relationship between mutation and protein overexpression.

Last year, Hruban et al. (29) showed that in the case report of Hubert H. Humphrey, a *p53* mutation was present in a urine cytology specimen 9 years before an infiltrating carcinoma of the bladder occurred. It should be noted that in this study, the mutation was first analyzed in the histological tissue, and then retrospectively, the known mutation was detected in the urine specimen. Although the method used is very sensitive, it has limited clinical relevance. In our study, we used PCR-SSCP analysis and applied it directly to bladder washings to define when *p53* mutations take place during the tumor progression process and, moreover, to assess the additional prognostic value of *p53* mutation analysis for patients who were indicated to be at high risk for recurrences on the basis of their quantitative karyometric analyses (17).

In the present study, we found *p53* mutations in the samples of all patients prior to progression, with a mean of approximately 8 (range 4–14) months. In two patients (cases 2 and 4), however, in the earlier courses of their disease, no bladder washings were done. Case 4 was diagnosed 6 months before the first mutation was found; therefore, the period between the first *p53* mutation and progression could have been, at most, 11 months. Case 2 could have had the mutation much longer, because we have no bladder-washing samples from the first 58 months of the disease. Unfortunately, because in both cases no histological specimens were available of this period of the disease, we were not able to check the *p53* status of the original tumors. In only one patient (case 15) has the appearance of a *p53* mutation not yet been followed by progression to invasive disease (23-month follow-up).

A drawback of analyzing *p53* mutations in bladder washings is illustrated by the absence or low number of mutated p53 cells in the bladder-washing specimens of cases 6 and 9 in the presence of concomitant invasive tumors. The existence of the same *p53* mutation, as detected in the previously analyzed bladder washings, was in both cases confirmed in the invasive tumors by analyzing the histological material (data not shown).

[4] H. G. van der Poel, unpublished data.

The invasive growth of the tumor most likely prevented the release of mutated p53 cells into the bladder washing.

We observed identical *p53* mutations in the consecutive bladder washings of four patients, which is in favor of the theory of the clonal origin of bladder tumors (30). However, there was one exception (case 1) in which we found different *p53* mutations in the individual bladder washings. These results are in agreement with the observed different *p53* gene mutations in CIS or dysplasia and consecutive invasive tumors in two patients in a previous study (10). Although different *p53* mutations do not absolutely prove that the clones are independent, they could indicate tumors that resulted from two independent transforming events. Six of 13 patients that clinically progressed (pT≥2) showed *p53* mutations in one or more of their bladder washings. In the control group (no progression), only 1 of 13 patients showed a *p53* mutation. In the group of superficial TCC patients at risk for recurrence, the occurrence of a *p53* mutation has a positive predictive value for progression to invasive disease of 86%. We observed a negative predictive value of 63%. This low negative predictive value implies that, although a *p53* mutation indicates a malignant potential for the tumor, the absence of a *p53* mutation does not guarantee a good prognosis, which we have already demonstrated for invasive bladder tumors (15). Other mechanisms as well can lead to tumor progression, and these may override or bypass the function of p53, *e.g.*, the epidermal growth factor receptor (31–33), E-cadherin (34, 35), or MDM2 expression (36).

Despite the positive predictive value of 86%, the occurrence of a *p53* mutation is not an absolute sign for progression of disease. Whenever a high karyometric score is observed in a bladder washing of a superficial TCC patient, this is an indication for both a careful follow-up (at least every 3 months) and *p53* mutation analysis. As a control experiment, we also analyzed 35 bladder washings with low karyometric scores for the presence of *p53* mutations; in this group, no *p53* mutations were detected (data not shown). The occurrence of all *p53* mutations in the group of patients with a high 2c deviation index value (abnormal DNA content) suggests a link between p53 dysfunction and genomic instability (15, 37, 38). The combination of a high karyometric score and a *p53* mutation identifies patients with a high probability of progression to invasive disease within a relatively short time period (approximately 8 months). However, 54% of the high-risk patients without demonstrable *p53* mutations progress to invasive disease. Summarizing these data, it is obvious that more prognostic markers are needed to increase not only the positive predictive value but especially the negative predictive value of this screening procedure. Our choice to study *p53* mutations by SSCP does not exclude the usefulness of p53 immunohistochemistry on bladder washings, especially because it is more accessible as a diagnostic tool. We are currently studying the predictive value of p53 overexpression analyzed by immunohistochemistry in bladder washings for progression of superficial TCC.

For a group of superficial TCC patients indicated to be high risk based on karyometry (17), the occurrence of a *p53* mutation has positive predictive value for disease progression. Moreover, because *p53* mutations were found in samples prior to documented progression, their presence could identify patients who need changes in their treatment strategies to prevent progression to invasive disease. The clinical usefulness of *p53* mutations as markers with additional predictive value should now be assessed in a large prospective study.

REFERENCES

1. Soloway, M. S. Intravesical therapy for bladder cancer. Urol. Clin. North Am., *15:* 661–669, 1988.

2. Kiemeney, L. A., Witjes, J. A., Verbeek, A. L., Heijbroek, R. P., Debruyne, F. M., and the members of the Dutch South-East Cooperative Urological Group. The clinical epidemiology of superficial bladder cancer. Br. J. Cancer, *67:* 806–812, 1993.

3. Green, L. F., Mulcahy, J. J., and Warren, M. M. Benign papilloma or papillary carcinoma of the bladder. J. Urol., *110:* 205–207, 1973.

4. Fields, S., and Jang, S. K. Presence of a potent transcription activating sequence in the p53 protein. Science (Washington DC), *249:* 1046–1049, 1990.

5. Mack, D. H., Vartikar, J., Pipas, J. M., and Laimins, L. A. Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. Nature (Lond.), *363:* 281–283, 1993.

6. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.

7. Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T. D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell, *70:* 923–935, 1992.

8. Lowe, S. W., Ruley, H. E., Jacks, T., and Housman, D. E. p53-dependent apoptosis modulates the c cytotoxicity of anticancer agents. Cell, *74:* 957–967, 1993.

9. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C., and Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant *p53* alleles. Cell, *70:* 937–948, 1992.

10. Olumi, A. F., Tsai, Y. C., Nichols, P. W., Skinner, D. G., Cain, D. R., Bender, L. I., and Jones, P. A. Allelic loss of chromosome 17p distinguishes high grade from low grade transitional cell carcinomas of the bladder. Cancer Res., *50:* 7081–7083, 1990.

11. Habuchi, T., Ogawa, O., Kakehi, Y., Ogura, K., Koshiba, M., Hamazaki, S., Takahashi, R., Sugiyama, T., and Yoshida, O. Accumulated allelic losses in the development of invasive urothelial cancer. Int. J. Cancer, *53:* 579–584, 1993.

12. Dalbagni, G., Presti, J., Reuter, V., Fair, W. R., and Cordon Cardo, C. Genetic alterations in bladder cancer (see comments). Lancet, *342:* 469–471, 1993.

13. Sidransky, D., Von Eschenbach, A., Tsai, Y. C., Jones, P., Summerhayes, I., Marshall, F., Paul, M., Green, P., Hamilton, S. R., Frost, P., and Vogelstein, B. Identification of *p53* gene mutations in bladder cancers and urine samples. Science (Washington DC), *252:* 706–709, 1991.

14. Fujimoto, K., Yamada, Y., Okajima, E., Kakizoe, T., Sasaki, H., Sugimura, T., and Terada, M. Frequent association of *p53* gene mutation in invasive bladder cancer. Cancer Res., *52:* 1393–1398, 1992.

15. Vet, J. A., Bringuier, P. P., Poddighe, P. J., Karthaus, H. F., Debruyne, F. M., and Schalken, J. A. p53 mutations have no additional prognostic value over stage in bladder cancer. Br. J. Cancer, *70:* 496–500, 1994.

16. Spruck, C. H., Ohneseit, P. F., Gonzalez Zulueta, M., Esrig, D., Miyao, N., Tsai, Y. C., Lerner, S. P., Schmutte, C., Yang, A. S., Cote, R., Dubeau, L., Nichols, P. W., Hermann, G. G., Steven, K., Hoen, T., Skinner, D. G., and Jones, P. A. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res., *54:* 784–788, 1994.

17. van der Poel, H. G., Oosterhof, G. O., Schaafsma, H. E., Wiersma van Tilburg, A., Debruyne, F. M., and Schalken, J. A. Karyometry of bladder washings for the follow up of patients with transitional cell carcinoma of the bladder. Prog. Clin. Biol. Res., *378:* 9–18, 1992.

18. Kiemeney, L. A., Witjes, J. A., Heijbroek, R. P., Verbeek, A. L., and Debruyne, F. M. Predictability of recurrent and progressive disease

in individual patients with primary superficial bladder cancer. J. Urol., *150:* 60–64, 1993.

19. Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics, *5:* 874–879, 1989.

20. Kusukawa, N., Uemori, T., Asada, K., and Kato, I. Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. Biotechniques, *9:* 66–68,70,72, 1990.

21. Mazars, G. R., Jeanteur, P., Lynch, H. T., Lenoir, G., and Theillet, C. Nucleotide sequence polymorphism in a hotspot mutation region of the *p53* gene. Oncogene, *7:* 781–782, 1992.

22. Papanicolaou, G. N., and Marshall, V. F. Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. Science (Washington DC), *101:* 519–520, 1945.

23. Sherman, A. B., Koss, L. G., and Adams, S. E. Interobserver and intraobserver differences in the diagnosis of urothelial cells. Comparison with classification by computer. Anal. Quant. Cytol., *6:* 112–120, 1984.

24. Aamodt, R. L., Coo, J. S., Deitch, A., deVere White, R. W., Koss, L. G., Melamed, M. R., and Weinstein, R. S. Flow cytometric evaluation of bladder cancer: recommendations of the NCI flow cytometry network for bladder cancer. World J. Urol., *10:* 63–67, 1992.

25. Zein, T., Wajsman, Z., Englander, L. S., Gamarra, M., Lopez, C., Huben, R. P., and Pontes, J. E. Evaluation of bladder washings and urine cytology in the diagnosis of bladder cancer and its correlation with selected biopsies of the bladder mucosa. J. Urol., *132:* 670–671, 1984.

26. Esrig, D., Spruck, C. H., Nichols, P. W., Chaiwun, B., Steven, K., Groshen, S., Chen, S. C., Skinner, D. G., Jones, P. A., and Cote, R. J. p53 nuclear protein accumulation correlates with mutations in the *p53* gene, tumor grade, and stage in bladder cancer. Am. J. Pathol., *143:* 1389–1397, 1993.

27. Oyasu, R., Nan, L., Szumel, R. C., Kawamata, H., and Hirohashi, S. *p53* gene mutations in human urothelial carcinomas: analysis by immunohistochemistry and single-strand conformation polymorphism. Mod. Pathol., *8:* 170–176, 1995.

28. Vet, J. A. M., Bringuier, P. P., Schaafsma, H. E., Witjes, J. A., Debruyne, F. M. J., and Schalken, J. Comparison of p53 protein overexpression with *p53* mutation in bladder cancer: clinical and biological aspects. Lab. Invest., *73:* 837–843, 1995.

29. Hruban, R. H., van der Riet, P., Erozan, Y. S., and Sidransky, D. Brief report: molecular biology and the early detection of carcinoma of the bladder—the case of Hubert H. Humphrey. N. Engl. J. Med., *330:* 1276–1278, 1994.

30. Sidransky, D., Frost, P., Von Eschenbach, A., Oyasu, R., Preisinger, A. C., and Vogelstein, B. Clonal origin bladder cancer (see comments). N. Engl. J. Med., *326:* 737–740, 1992.

31. Neal, D. E., and Mellon, K. Epidermal growth factor receptor and bladder cancer: a review. Urol. Int., *48:* 365–371, 1992.

32. Neal, D. E., Sharples, L., Smith, K., Fennelly, J., Hall, R. R., and Harris, A. L. The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer (Phila.), *65:* 1619–1625, 1990.

33. Lipponen, P., and Eskelinen, M. Expression of epidermal growth factor receptor in bladder cancer as related to established prognostic factors, oncoprotein (c-erbB-2, p53) expression and long-term prognosis. Br. J. Cancer, *69:* 1120–1125, 1994.

34. Bringuier, P. P., Umbas, R., Schaafsma, H. E., Karthaus, H. F., Debruyne, F. M., and Schalken, J. A. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer Res., *53:* 3241–3245, 1993.

35. Otto, T., Birchmeier, W., Schmidt, U., Hinke, A., Schipper, J., Rubben, H., and Raz, A. Inverse relation of E-cadherin and autocrine motility factor receptor expression as a prognostic factor in patients with bladder carcinomas. Cancer Res., *54:* 3120–3123, 1994.

36. Lianes, P., Orlow, I., Zhang, Z. F., Oliva, M. R., Sarkis, A. S., Reuter, V. E., and Cordon Cardo, C. Altered patterns of MDM2 and TP53 expression in human bladder cancer (see comments). J Natl. Cancer Inst., *86:* 1325–1330, 1994.

37. Thomas, D. J., Robinson, M. C., Charlton, R., Wilkinson, S., Shenton, B. K., and Neal, D. E. p53 expression, ploidy and progression in pT1 transitional cell carcinoma of the bladder. Br. J. Urol., *73:* 533–537, 1994.

38. van Meyel, D. J., Ramsay, D. A., Casson, A. G., Keeney, M., Chambers, A. F., and Cairncross, J. G. *p53* mutation, expression, and DNA ploidy in evolving gliomas: evidence for two pathways of progression. J. Natl. Cancer Inst., *86:* 1011–1017, 1994.