bladder tumors. A sensitivity of 90% and specificity of 88% was achieved for bladder cancer detection by applying relative cut-off points to a quantitative TRAP assay [26]. Telomerase activity determination by the TRAP assay enables quantitative evaluations to be made, but is vulnerable to contamination by telomerase-positive, non-malignant cell types, such as proliferative stem cells and inflammatory elements [27,28]. Furthermore, because the TRAP assay detects enzyme activity and not simply the presence of the protein, valid findings require the presence of living cells. In native urine, suspended cells are exposed for various lengths of time to destructive substances and conditions including proteases, urea, and acidic pH values [10]. The alternative to the TRAP assay is detection of the structural or encoding RNA components of the telomerase enzyme, primarily the human telomerase RNA component (hTR) and hTERT mRNA [10,21,27,28]. Compared to other urine-based markers, quantitative detection of human telomere components by RT-PCR, particularly the RNA component (hTR), shows acceptable diagnostic accuracy with sensitivities of up to 77% (29). Clearly, telomerase is a promising marker for urothelial neoplasia, but these tests remain several steps removed from becoming a routine procedure which replaces cytology. In contrast, an *in situ* analysis would enable the morphological identification of hTERT-positive cells, and if used as an adjunct to cytology such analyses could be rapidly brought into practice. Good monoclonal antibodies that specifically recognize hTERT have been isolated only recently so few studies have focused on the immunohistochemical evaluation of hTERT in human tissues. In the current study, we demonstrated the feasibility of an hTERT immunoexpression assay on urine sediment samples, and investigated its utility as an adjunct to conventional urine cytology in the diagnosis of bladder carcinoma.

Our study has shown that through the application of a monoclonal antibody, hTERT can reliably be detected in paraffin-embedded solid tumor specimens and in urine sediments archived as cell blocks. In agreement with the few previous reports describing hTERT protein localization, we found hTERT to be localized to the nucleolus [13,30,31]. It is logical that hTERT is localized predominantly in the nucleolus, the site of nucleoprotein complex assembly, because hTERT is a subunit of the ribonucleoprotein telomerase enzyme [10]. In bladder tumor biopsy material we observed hTERT expression in cells adjacent to the tumor and expression was seen to diminish more distal to the primary lesion. This spatially associated hTERT positivity in apparently normal cells has been observed in other organs, including the colon [13], prostate [30] and melanocytic lesions [31]. The development of a reliable hTERT antibody has revealed this phenomenon and has the potential to better determine exactly where hTERT is expressed at the cellular level. The expression in normal cells could result from a number of possibilities, but the spatial relationship suggests that factors secreted from the tumor can induce hTERT transcription/translation in non-neoplastic cells.

Our goal was to evaluate the potential utility of hTERT immunodetection in urine sediments as an adjunct to cytology. Given the nature of the sample, which is often composed of scanty, dispersed cells, we did not attempt to estimate staining intensity or percentage of immunoreactive cells within the sample or block section. In this study, the presence (>3 cells observed) or absence of hTERT, specifically in transitional urothelial cells, was noted. The expression of hTERT was observed in 85% of the histologically confirmed bladder cancer cases, which represents a high rate of diagnostic sensitivity by examination of voided urine. Overall, the hTERT immunoassay demonstrated a positive predictive value of 77.8% and a negative predictive value of 75% in this study. The hTERT assay showed higher diagnostic sensitivity (84.8%) than published urine cytology data (~65%) for confirmed bladder carcinoma, however, the specificity of the hTERT immunoassay was 80%, lower than that obtained by cytologic evaluation (~90%). The specificity data do not support the use of hTERT immunodetection as a replacement for cytology, but the improved sensitivity of the assay suggests potential utility as an adjunct to cytology. It was notable that the majority of false-positive cases were found in the subset classified as cytologically atypical, but which have no evidence of disease to date. It will be interesting to see whether these patients will subsequently develop bladder malignancy in which case hTERT detection may have potential as an early detection or even predictive marker. However, specimens from patients with a history of bladder cancer are often deemed atypical cytologically, due to a 'field-effect', and in this study several cases were immunoreactive for hTERT. In agreement with telomerase activity assays in such patients [28], we did find hTERT-positive cells in patients who had no evidence of urothelial lesion at the time of sampling, but who did have a history of bladder cancer. Thus, it seems that hTERT detection may not be reliable in the monitoring of recurrent bladder cancer.

Our findings are in agreement with the previous telomerase-based studies in urological cancers, in that telomerase expression correlates with malignancy, but the use of immunohistochemistry adds specific advantages in the context of diagnosis. Although assays such as the TRAP assay or mRNA measurement can be designed to be semi- or fully quantitative, they do not provide information regarding the source of the protein. Immunohistochemical studies are beginning to reveal that normal cells can express hTERT under certain circumstances. In our study,