Use of Fluorescence In Situ Hybridization to Predict Response to Bacillus Calmette-Guérin Therapy for Bladder Cancer: Results of a Prospective Trial

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Abbreviations and Acronyms

BCG = bacillus Calmette-Guérin
CIS = carcinoma in situ
FISH = fluorescence in situ hybridization
NMIBC = nonmuscle invasive bladder cancer
TUR = transurethral resection

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Purpose: No reliable methods currently exist to predict patient response to intravesical immunotherapy with bacillus Calmette-Guérin given after transurethral resection for high risk nonmuscle invasive bladder cancer. We initiated a prospective clinical trial to determine whether fluorescence in situ hybridization results during bacillus Calmette-Guérin immunotherapy can predict therapy failure.

Materials and Methods: Candidates for standard of care bacillus Calmette-Guérin were offered participation in a clinical trial. Fluorescence in situ hybridization was performed before bacillus Calmette-Guérin, and at 6 weeks, 3 months and 6 months during bacillus Calmette-Guérin therapy with maintenance. Cox proportional hazards regression was used to assess the relationship between fluorescence in situ hybridization results and tumor recurrence or progression. The Kaplan-Meier product limit method was used to estimate recurrence-free and progression-free survival.

Results: A total of 126 patients participated in the study. At a median followup of 24 months 31% of patients had recurrent tumors and 14% experienced disease progression. Patients who had positive fluorescence in situ hybridization results during bacillus Calmette-Guérin therapy were 3 to 5 times more likely than those who had negative fluorescence in situ hybridization results to experience recurrent tumors and 5 to 13 times more likely to have disease progression ($p < 0.01$). The timing of positive fluorescence in situ hybridization results also affected outcomes. For example, patients with a negative fluorescence in situ hybridization result at baseline, 6 weeks and 3 months demonstrated an 8.3% recurrence rate compared to 48.1% for those with a positive result at all 3 points.

Conclusions: Fluorescence in situ hybridization results can identify patients at risk for tumor recurrence and progression during bacillus Calmette-Guérin immunotherapy. This information may be used to counsel patients about alternative treatment strategies.

Key Words: urinary bladder neoplasms; mycobacterium bovis; in situ hybridization, fluorescence; treatment outcome; prognosis

More than 70,000 new cases of bladder cancer are diagnosed yearly, with the majority presenting as NMIBC.[1] Of nonmuscle invasive tumors 60% to 70% recur and 10% to 20% progress to muscle invasive disease.[2] Immunotherapy with BCG is the most effective intravesical treatment used in

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patients with high risk NMIBC.[3] However, treatment fails in a significant number of patients, who then require more aggressive interventions such as radical cystectomy and/or chemotherapy. If patients do not respond to intravesical BCG, radical cystectomy within the first 24 months after diagnosis is believed to improve survival by at least at 20%.[4] Thus, early identification of patients in whom BCG will fail would allow those patients to undergo earlier curative radical cystectomy and improve chances of survival.

Currently the gold standard for post-TUR surveillance is cystoscopy and urine cytology at regular intervals. These diagnostics rely on the detection of actual tumor recurrence and are poor predictors of therapy failure.[5,6] FISH analysis (UroVysion®) of exfoliated urothelial cells is highly sensitive and specific for urothelial cancer, regardless of whether the patient is being treated with intravesical immunotherapy.[7] Although FISH has been studied as a surveillance tool, there are limited data assessing the ability of FISH to predict bladder cancer recurrence or progression after the initiation of intravesical BCG.[8–11] In this prospective clinical study we determined whether the molecular recurrence of NMIBC, as defined by the presence of cytogenetically abnormal cells on FISH analysis, during intravesical immunotherapy could predict the clinical outcomes of tumor recurrence and progression to muscle invasion.

MATERIALS AND METHODS

Patients

All patients who were scheduled to receive intravesical BCG immunotherapy at our center since July 2005 have been offered participation in this prospective, institutional review board approved clinical trial (National Clinical Trial #01007058). Patients were eligible if they had pathologically confirmed primary or recurrent NMIBC documented within 6 weeks of enrollment and normal upper urinary tract imaging. Pathological inclusion criteria were similar to the European Organization for Research and Treatment of Cancer intermediate risk/high risk categories.[12] Patients were excluded from study if they had a history of prior pelvic radiation, had variant histological subtypes (squamous cell carcinoma, adenocarcinoma, micropapillary or small cell) or were immunocompromised. All patients with high grade tumors underwent re-resection between 4 and 6 weeks after the initial diagnosis to evaluate for occult muscle invasion. An immediate postoperative intravesical instillation of mitomycin C was administered when appropriate.

Intravesical Immunotherapy

Intravesical BCG was administered according to the protocol used in Southwest Oncology Group trial 8507.[13] All patients received an induction course of BCG consisting of 6 weekly treatments, then maintenance consisting of 3 weekly treatments at 3 and 6 months, and then every 6 months for a total of 36 months. Dose reductions were allowed at the discretion of the treating physician. As was reflective of our practice at the time of study initiation, augmentation of BCG with interferon alfa-2b was allowed at the discretion of the treating physician with the schedule of therapy similar to that outlined.[14]

FISH Assays

Urine samples were collected for analysis at baseline (after TUR and before initiation of intravesical BCG), at 6 weeks (right before the sixth instillation of BCG), at 3 months (at first maintenance immunotherapy and cystoscopic surveillance) and at 6 months (at second maintenance immunotherapy and cystoscopic surveillance) after the initiation of immunotherapy. FISH was performed according to instructions provided with the UroVysion Bladder Cancer Recurrence Kit. Freshly collected urine (volume 35 ml or greater) was immediately fixed with alcohol and cytospin preparations were prepared. After denaturation of DNA, fluorescent-labeled DNA probes comprising centromeric chromosome 3 (Spectrum Red), 7 (Spectrum Green), 17 (Spectrum Aqua) and locus specific identifier 9p21 (Yellow) were incubated with the specimen to hybridize with cDNA. Cells were counterstained with DAPI (4,6-diamidino-2-phenylindole). Interphase nuclei were analyzed using fluorescence microscopy to detect chromosomal copy number.[15] The 25 morphologically atypical nonoverlapping cells with distinct signals were scored. Atypical cytological features include patchy and lighter DAPI staining, nuclear enlargement and irregular nuclear contour. The assay was considered positive if at least 4 cells showed polysomy on at least 2 chromosomes (3, 7 or 17) and/or there were at least 12 cells with no signal (homozygous deletion) for 9p21.[16] On the rare occasion when a result was uninterpretable (collection or processing error), the assays were repeated as allowed by the protocol.

Patient Monitoring

Patients were monitored during BCG treatment according to normal practices at our institution using cystoscopy and cytology at 3-month intervals for 2 years and 3 to 6-month intervals thereafter. Repeat TUR and other treatments were performed as necessary. Patient management was not mandated based on results of the FISH assay but results were provided to the treating physician to be acted on if desired.

Statistical Analysis

Patient data were analyzed on an intent to treat basis. Descriptive statistics were used to summarize the study population characteristics. Recurrence was defined as any tumor found after the start of intravesical BCG regardless of grade or stage. Progression was defined as an increase in stage to muscle invasive disease. Logistic regression was used to assess relationships between patient and tumor characteristics and tumor recurrence or progression. Patient data were censored from time of recurrence, progression or date of the last documented cystoscopy if recurrence/progression was not observed. Univariate Cox proportional hazards regression was used to model the association between FISH results and the risk of recur-