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# DETECTION OF JC/BK VIRUS IN PATIENTS WITH MULTIPLE SCLEROSIS TREATED WITH NATALIZUMAB

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## INTRODUCTION

Natalizumab was FDA-approved for the treatment of relapsing-remitting multiple sclerosis (MS) in November 2004 based on the results of two multi-center phase-III trials. However three months later, therapeutic use of natalizumab was suspended after progressive multifocal leuco-encephalopathy (PML) was detected in three patients who had participated in the phase III trials. PML is caused by JC virus infection and lysis of oligodendrocytes which leads to widespread white matter demyelination. After retrospective determination that the occurrence of PML was restricted to three out of the 3,417 trial patients, the FDA re-approved use of natalizumab as monotherapy for MS with a black-box warning about PML. In an attempt to closely detect any further cases of PML, natalizumab treatment is confined to infusion centers participating in the TOUCH™ program with mandatory monitoring and reporting requirements. To enhance the screening requirements of the TOUCH™ program we tested plasma and cerebrospinal fluid (CSF) of all patients for JCV/BKV (JC virus/BK virus) DNA prior to commencing and after every six months of treatment with natalizumab.

## METHODS

### Patient Selection

All patients receiving natalizumab were included in the analysis (n=200). Patients were considered for natalizumab if they had clinically definite MS and were unresponsive or intolerant to first line MS treatment. All patients were enrolled in the TOUCH™ Program.

### Clinical Design

This study is designed to enhance the surveillance of the TOUCH™ Program by introducing screening laboratory methods to detect viral replication before the development of PML. Thus all patients receiving natalizumab are screened for JCV/BKV DNA in plasma and CSF at baseline, and then after every 6 month treatment cycle. Natalizumab is discontinued in any patient who has a positive JCV/BKV test. All positive patients also undergo an immediate neurological evaluation and a brain MRI to detect clinical PML. All positive patients are kept treatment-free and have monthly CSF and plasma tests for JCV/BKV and clinical examinations until all tests return to normal.

Patients discontinuing natalizumab treatment at any point other than at a six month interval are also tested for plasma and CSF JCV/BKV DNA. Informed consent is obtained from all patients prior to obtaining blood and spinal fluid samples with an IRB-approved protocol.

### Laboratory Testing for JCV/BKV DNA

DNA extraction and qualitative real-time PCR (qPCR) for detection of JC and BK viruses from patient serum and CSF was done by Quest Diagnostics (Teterboro, NJ). DNA was extracted from 0.2 mL of clinical specimen using the automated MagNA Pure Total Nucleic Acid kit (Roche). Qualitative PCR reactions were done using TaqMan real-time chemistry (Roche). The primers and probes are proprietary but are directed to highly conserved regions of the large T antigen specific to each virus. There is no cross-reactivity of the primers and probes for one virus to the other, or with 20 viral and non-viral pathogens. Each reaction has an internal control to show that the PCR reaction has not been inhibited. The lower limit for detection of viral DNA of this assay is 500 copies/mL. In other studies on patients with PML it has been reported that there is a false-negative detection rate for JC virus of 15-25% with virtually no false-positive results. All positive tests are verified by repeat testing.

## RESULTS

### Post-treatment: 7 of 200 patients had positive Viral DNA

All 200 patients were JCV/BKV DNA free in CSF and plasma at baseline. However, seven of the 200 patients had detectable JCV or BKV DNA after six months of treatment. It is noteworthy that no differences could be discerned in age range, sex, past treatment record, or current medical history between patients who developed detectable JCV/BKV and those who did not (Table 1).

Five patients were positive for BKV DNA in the CSF, one patient was positive for JCV DNA in the CSF and one patient was positive for JCV DNA in the plasma (Table 2). After cessation of natalizumab treatment and the withholding of all immunomodulatory treatment, all seven patients converted to undetectable viral DNA within 6 months. It should be noted that all 7 patients with positive JCV/BKV tests have had no clinical or brain MRI features of PML at any point.

Table 1. Demographics of Positive JCV or BKV Patients  
RRMS- Relapsing-Remitting Multiple Sclerosis  
SPMS-R Secondary Progressive Multiple Sclerosis with Relapses

Patient ID	Age	Sex	Disease Duration	Disease Type	EDSS Score
1	32	F	12	SPMS-R	6.0
2	36	F	12	RRMS	3.5
3	65	F	28	SPMS-R	6.5
4	57	F	13	SPMS-R	5.0
5	38	F	30	RRMS	3.0
6	46	M	26	RRMS	3.5
7	54	F	12	RRMS	4.0

Patient ID	Pre-natalizumab treatment and drug-free washout period in months	Positive virus detection	Weeks until neg.
1	INF β-1a (weekly) and monthly I.V.I.G. 4	BKV in CSF	5
2	INF β-1b 24	JCV in plasma	8
3	MTX 2	JCV in CSF	4
4	MP 4	BKV in CSF	12
5	INF β-1a (weekly) 2	BKV in CSF	22
6	MTX 4	BKV in CSF	6
7	MTX 3	BKV in CSF	12

Table 2. Characteristics of Patients with Positive Viral Detection  
INF β-Interferon β; I.V.I.G.-Intravenous Immunoglobulin; MTX-Methotrexate; MP-Methylprednisolone

### JCV Positive Patients

Plasma JCV DNA appears to correlate with a state of immunosuppression whereas CSF JCV DNA is strongly associated with the development of PML. Among our 200 natalizumab-treated patients only 2 cases of JCV DNA, one each in plasma and CSF, were detected and after cessation of treatment both patients reverted to normal. Furthermore, the positive patients in our study were negative for JCV prior to natalizumab treatment and therefore the post-treatment conversion with detection of the virus could not be easily dismissed.

### BKV Positive Patients

We also tested for BKV, a closely related family virus to JCV. BKV is the causative agent of polyomavirus-associated nephropathy (PVN) of the kidney following renal transplant leading to a gradual loss of graft function. BKV is not causally related to PML but it is present with JCV in some brain samples of PML patients. Following natalizumab treatment for six months, 5 patients had detectable CSF BKV DNA. None of the 5 patients had any discernable clinical consequences of this CSF BKV conversion and with discontinuation of natalizumab treatment, all 5 patients have reverted to their baseline CSF status. Although BKV is not the causative agent of PML, the BKV conversion in natalizumab-treated patients is potentially worrisome due to its close relation to JCV.

## CONCLUSIONS

1. After 6 months of natalizumab therapy, the detection rate of JCV/BKV in plasma/CSF was 3.5%
2. Following cessation of natalizumab all 7 patients reverted to normal values within 6 months
3. No patient developed any clinical or radiological features of PML at any time during the study or in follow up (> than 6 months)
4. It may be prudent to test for plasma and CSF JCV/BKV DNA in all patients undergoing natalizumab treatment at 6 month intervals
5. This study is ongoing and the data after 18 months of treatment will allow for more robust conclusions