A First in Human Phase 1 Study of CG0070, a GM-CSF Expressing Oncolytic Adenovirus, for the Treatment of Nonmuscle Invasive Bladder Cancer

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Purpose: We assessed the safety, pharmacokinetics and anticancer activity of intravesical CG0070, a cancer selective, replication competent adenovirus, for the treatment of nonmuscle invasive bladder cancer.

Materials and Methods: A total of 35 patients received single or multiple (every 28 days × 3 or weekly × 6) intravesical infusions of CG0070 at 1 of 4 dose levels (1×10^{12}, 3×10^{12}, 1×10^{13} or 3×10^{13} viral particles). Response to treatment was based on cystoscopic assessment and biopsy or urine cytology. Urine and plasma CG0070, and granulocyte-monocyte colony-stimulating factor were measured in all patients. A subset of 18 patients was assessed for retinoblastoma phosphorylation status.

Results: Grade 1–2 bladder toxicities were the most common adverse events observed. A maximum tolerated dose was not reached. High levels of granulocyte-monocyte colony-stimulating factor were detected in urine after administration in all patients. Virus replication was suggested based on an increase in urine CG0070 genomes between days 2 and 5 in 58.3% of tested patients (7 of 12). The complete response rate and median duration of the complete response across cohorts was 48.6% and 10.4 months, respectively. In the multidose cohorts the complete response rate for the combined groups (every 28 days and weekly × 6) was 63.6% (14 of 22 patients). In an exploratory, retrospective assessment patients with borderline or high retinoblastoma phosphorylation who received the multidose schedules had an 81.8% complete response rate (9 of 11).

Conclusions: Intravesical CG0070 was associated with a tolerable safety profile and antibladder cancer activity. Granulocyte-monocyte colony-stimulating factor transgene expression and CG0070 replication were also suggested.

Key Words: urinary bladder; urinary bladder neoplasms; Adenoviridae; dose-response relationship, immunologic; toxicity

Abbreviations and Acronyms

AE = adverse event
BCG = bacillus Calmette-Guérin
CIS = carcinoma in situ
CR = complete response
DLT = dose limiting toxicity
E2F-1 = E2F-1 transcription factor
GM-CSF = granulocyte-monocyte colony-stimulating factor
IHC = immunohistochemistry
IVE = intravesical
NIBC = nonmuscle invasive bladder cancer
pRB = RB phosphorylation
RB = retinoblastoma
vp = viral particles

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In 2011 NIBC (stages Ta, T1 or CIS) accounted for an estimated 70% to 80% of the approximately 69,000 cases of bladder cancer.[1] The natural history of NIBC is marked by a high rate of recurrence and/or progression to higher tumor stage depending on tumor T stage and grade at diagnosis.[2] Especially concerning is CIS. Although CIS is limited to the urothelium, it is actually a multifocal, high grade disease with a significant risk

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of recurrence and progression after transurethral resection or ablation.[3,4] Stage Ta, which is often low grade, has a low rate of progression but a high rate of recurrence requiring repeat IVE treatment.[5]

IVE chemotherapy and BCG are commonly used as an adjunct to transurethral resection to decrease the recurrence risk or treat in situ or residual disease. BCG is the most effective first line treatment in the NIBC setting.[6–8] For patients in whom primary therapy fails, repeat BCG, chemotherapy and cystectomy are viable options depending on stage and grade at recurrence.[9] As reported in early phase studies, for CIS repeat treatment with BCG alone or combined with interferon yields a 20% to 50% CR, although with a limited response duration and systemic toxicity, including mycobacterial infection, cystitis, pain and rarely mortality.[10,11] The only agent formally approved by the Food and Drug Administration for CIS after BCG failure is valrubicin, which demonstrated a 21% CR and an 18-month time to treatment failure based on pooled data from several studies in a total of 90 patients.[12]

CG0070, a replication selective serotype-5 oncolytic adenovirus, is engineered to preferentially replicate in and destroy RB pathway defective cells using the E2F-1 promoter element to control virus replication and expression of the immunodulatory transgene GM-CSF.[13] Aside from insertion of the GM-CSF gene in place of the E3-19kb gene, which functions to down-regulate MHC I expression in infected cells, CG0070 encodes for an intact E3 region (fig. 1). Based on the frequency of RB pathway dysregulation in bladder cancer, it was hypothesized that permissive replication and transgene expression of CG0070 would yield targeted antitumor activity.[14,15]

PATIENTS AND METHODS

Patient Eligibility

Patients with NIBC based on cystoscopy and positive urine cytology or biopsy in whom at least 1 prior course of IVE BCG had failed were eligible for study inclusion. The Appendix (jurology.com) shows details of the main study entry criteria. This series received approval from an institutional review board at each site and was done in accordance with the 1964 Declaration of Helsinki[11] and International Conference on Harmonization/Good Clinical Practice guidelines. Written informed consent was obtained from each patient.

Study Design

All patients received CG0070 via IVE infusion after bladder pretreatment with 0.1% dodecyl maltoside and a saline bladder rinse.[16]

In the initial phase of the study, a single IVE infusion of CG0070 was administered at 1 of 4 dose levels (1×10^{12}, 3×10^{12}, 1×10^{13} or 3×10^{13} vp). Enrollment in multidose cohorts (1×10^{12}, 3×10^{12} and 1×10^{13} vp) of every 28 days $\times$ 3 or weekly $\times$ 6 was initiated thereafter in parallel. In the every 28-day cohorts patients with CR at the month 3 evaluation were eligible to receive 3 additional treatments every 28 days for a total of 6 instillations. Dose escalation was done using a standard 3 plus 3 phase 1 design until a maximum tolerated or maximum feasible dose was established.[17,18] CR and AE profiles tables were created. Progression-free survival was estimated using Kaplan-Meier curves.

Using National Cancer Institute Common Toxicity Criteria, Version 3 guidelines, all AEs were recorded from the day of the first CG0070 administration through 4 weeks after the last administration or until receipt of additional bladder cancer therapy other than CG0070. Routine hematology and biochemistry analyses were performed at a central laboratory. The Appendix (jurology.com) shows the exact DLT definition.

Pharmacokinetics

The concentration of CG0070 genomes and GM-CSF in urine and plasma was measured using quantitative polymerase chain reaction analysis and enzyme-linked immunosorbent assay, respectively. The lower limit of the quantitative polymerase chain reaction assay was determined to be accurate to 1,500 CG0070 copies per ml. Therefore, values less than 1,500 copies per ml were defined as below quantifiable limits. GM-CSF was determined using a Luminex® bead based assay, which allowed for semiquantitative assessment of serum GM-CSF levels.[19] The lower

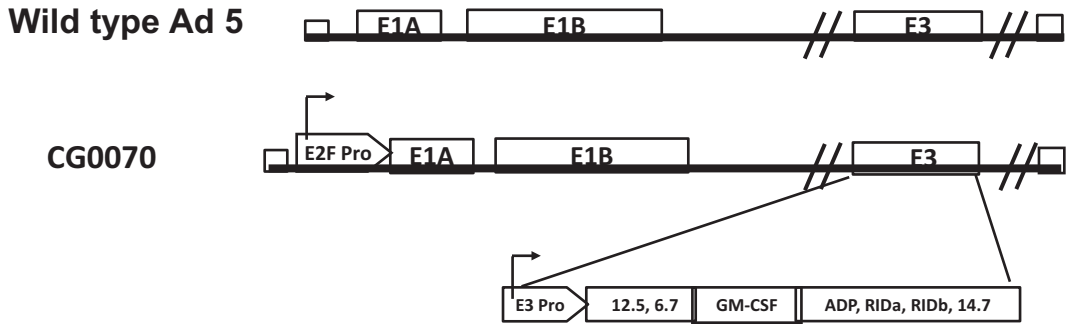

Figure 1. CG0070 design. E2F promoter (*Pro*) is active in RB pathway defective tumors. RB pathway is disrupted in about 80% of all cancers. GM-CSF has potential to stimulate systemic antitumor responses in situ.

limit of the assay was accurate to 15.6 pg GM-CSF/ml. Therefore, values less than 15.6 pg/ml were defined as below quantifiable limits.

RB Protein Testing

IHC staining for pRB was performed on paraffin embedded tissue blocks from the initial diagnostic biopsy. Staining and scoring protocols for pRB (antiRB polyclonal antibody, RB-WL-1) were described previously.[20] pRB immunoreactivity was assigned to 1 of 3 categories of tumor cell nuclear staining, including low—less than 25%, borderline—25% to 50% or high—greater than 50%.

Efficacy Assessment

Responses were assessed per standard of care with cystoscopic evaluation in conjunction with biopsy of any suspicious areas of the urothelium or urine cytology at the discretion of the treating physician every 3 months until the patient went off study or disease recurred. Documentation of a pathological CR at cystectomy after CG0070 treatment (with no other interval therapy) was also considered CR.

RESULTS

Drug Exposure

A total of 35 patients with recurrent NIBC were enrolled in the study (table 1). Of the patients 13 were enrolled in the single dose study portion, including 3 each at dose level of 1×10^{12}, 3×10^{12} and 3×10^{13} vp, and 4 at the dose level 1×10^{13} vp due to administration error in 1 originally planned to receive dose level 3×10^{13} vp. A total of 22 patients were enrolled in the multidose portion of the study, including 9 in the weekly cohorts at dose levels 1×10^{12}, 3×10^{12} and 1×10^{13} vp, and 13 in the every 28-day cohorts at dose levels 1×10^{12} (7), 3×10^{12} (3) and 1×10^{13} vp (3). Seven patients were enrolled in the dose level 1×10^{12} vp, every 28-day schedule, including an additional 3 due to a DLT and another to replace a patient who withdrew early for nontoxicity related reasons following 2 treatments. The Appendix (jurology.com) shows patient demographics and dispositions.

Safety

Adverse events. The most common AE across dose cohorts and schedules was transient, local bladder toxicity (table 2). Most reported AEs were grade 1–2. None were considered clinically significant, as assessed by the treating physicians.

Three patients experienced a total of 6 grade 3 or greater AEs, including pollakiuria in 2, and lymphopenia, dysuria, urgency and nocturia in 1 each. A single DLT (grade 3 lymphopenia) was observed in conjunction with traumatic catheterization at treatment in the lowest dose cohort (1×10^{12} vp) on the every 28-day schedule. A single serious AE (grade 3 nausea and vomiting) unrelated to CG0070 was reported, which the investigator attributed to trimethoprim/sulfamethoxazole.

Three patients died while enrolled in the study, including none during the active treatment period. No death was considered related to CG0070. Generally, no trends in toxicity were associated with an increasing dose or administration schedule. The Appendix (jurology.com) shows the details of laboratory toxicity.

Urine GM-CSF. In the single and multidose cohorts, GM-CSF peaked in 94.3% of patients (33 of 35) on day 2 following the first treatment, while 1 had peak GM-CSF on day 3 and another had it on day 5 after the first treatment. Generally, mean day 2 GM-CSF levels were greater in the 3 highest dose cohorts after the first administration than in the lowest dose cohort. Mean day 2 GM-CSF levels for dose levels 1×10^{12}, 3×10^{12}, 1×10^{13} and 3×10^{13} vp were 5,267, 38,000, 36,432 and 62,907 pg/ml, respectively (fig. 2, *A*).

In the multidose groups, GM-CSF mean peak levels decreased with all subsequent treatments compared to the first infusion (figs. 3, *A* and 4, *A*). The higher dose cohorts were generally associated with higher GM-CSF mean peak levels on day 2 but with similar concentrations for subsequent dosing. The every 28-day cohorts were associated with the greatest decrease in GM-CSF after the initial dose.

Table 1. *Cases by stage and cohort*

Disease Stage	No. Single Dose (%)	No. Multidose (%)	Total No. (%)
Ta	3 (23.1)	12 (54.5)	15 (42.9)
T1	2 (15.4)	1 (4.5)	3 (8.6)
Ta + CIS	1 (7.7)	1 (4.5)	2 (5.7)
T1 + CIS	6 (46.2)	1 (4.5)	7 (20.0)
CIS only	1 (7.7)	7 (31.8)	8 (22.9)
Totals	13	22	35

Table 2. *Most frequent AEs regardless of relationship to CG0070*

	% Frequency
Dysuria	71.4
Hematuria	42.9
Urinary frequency	42.9
Urgency	34.3
Urine abnormality	31.4
Bladder spasm	28.6
Nocturia	22.9
Fatigue	20.0
Arthralgia	17.1
Bladder discomfort	14.3
Abdominal pain	14.3
Myalgia	11.4
Influenza-like illness	11.4

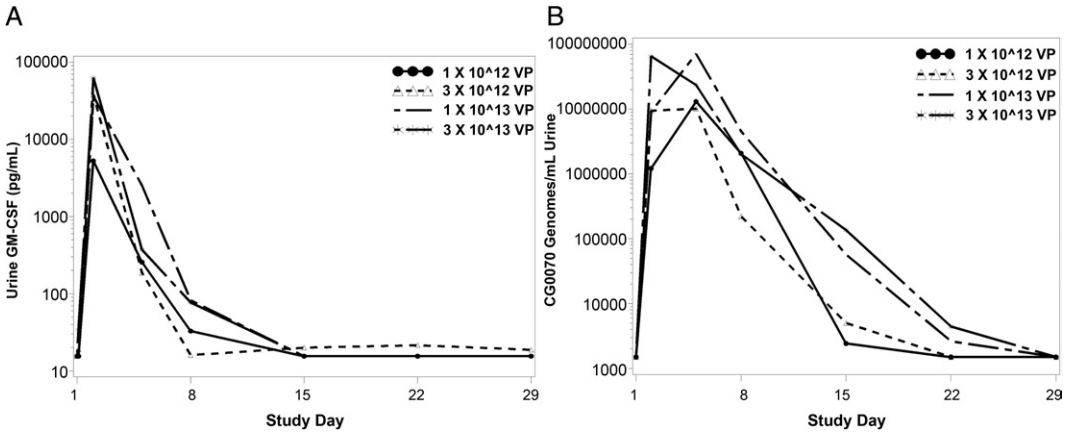

Figure 2. Geometric mean values in urine vs time in single dose cohorts for dose levels 1 to 4 (1×10^{12}, 3×10^{12}, 1×10^{13} and 3×10^{13} vp, respectively). *A*, GM-CSF on day 2 was 5,267, 38,000, 36,432 and 62,907 pg/ml, respectively. *B*, CG0070 genomes.

Plasma GM-CSF. In the single dose group, 84.6% of patients (11 of 13) had transiently detectable GM-CSF in plasma between study days 2 and 8. The peak GM-CSF level for any single dose patient was 313.33 pg/ml on day 2 at the dose level 2 cohort (3×10^{12} vp). Of the patients 33.3% (3 of 9) on the weekly × 6 schedule and 7.7% (1 of 13) on the every 28-day schedule had quantifiable GM-CSF levels in plasma.

Pharmacokinetics, Distribution and Clearance of CG0070 Genomes

Urine. In the single dose group, following the initial input and clearance of CG0070 on the day of treatment, the peak urine CG0070 genome level generally occurred on study day 5 with the mean genome concentrations decreasing after day 5 in all cohorts (fig. 2, *B*). Between days 2 and 5, 58.3% (7 of 12 patients assessed) had increases in urine CG0070 genomes and 25.0% (4 of 12 assessed) had a tenfold or greater increase. By day 29, 0% of patients (0 of 13) had quantifiable CG0070 genomes in urine, ie 1,500 copies per ml or greater.

For the weekly × 6 schedule, mean CG0070 genomes for the lowest dose cohort (1×10^{12} vp) was greater than for the 2 higher dose cohorts (3×10^{12} and 1×10^{13} vp, respectively) for most time points assessed (fig. 3, *B*). At 29 days after the last treatment, 11.1% of patients (1 of 9) had quantifiable CG0070 levels in urine.

For the every 28-day schedule, repeat administration was associated with the attenuation of urine CG0070 genomes (fig. 4, *B*). Mean CG0070 levels were generally higher for the highest dose level assessed (1×10^{13} vp). By day 29 after the last dose of CG0070 (or if not attained at this time, 3 months after the final treatment) 27.3% of the patients as-

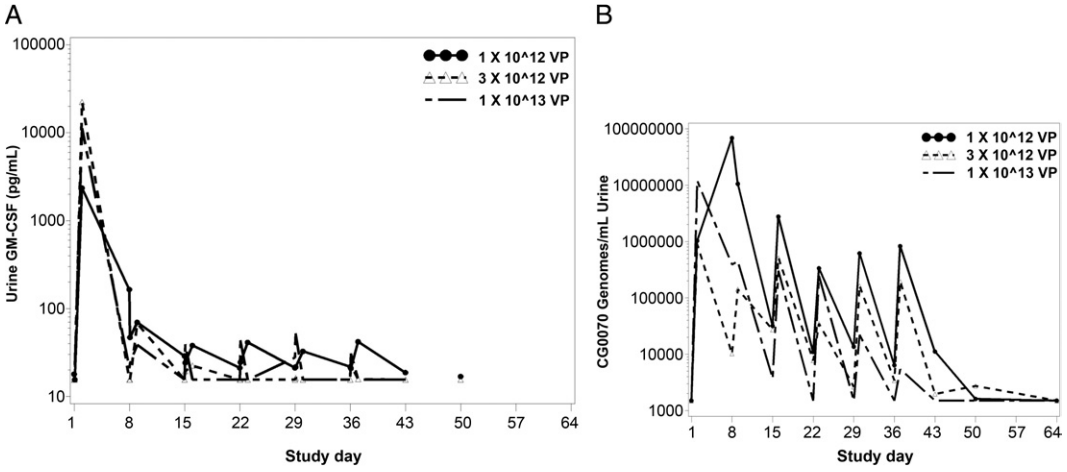

Figure 3. Geometric mean values in urine vs time in weekly dose cohorts for dose levels 1 to 3 (1×10^{12}, 3×10^{12} and 1×10^{13} vp, respectively). *A*, GM-CSF. *B*, CG0070 genomes.

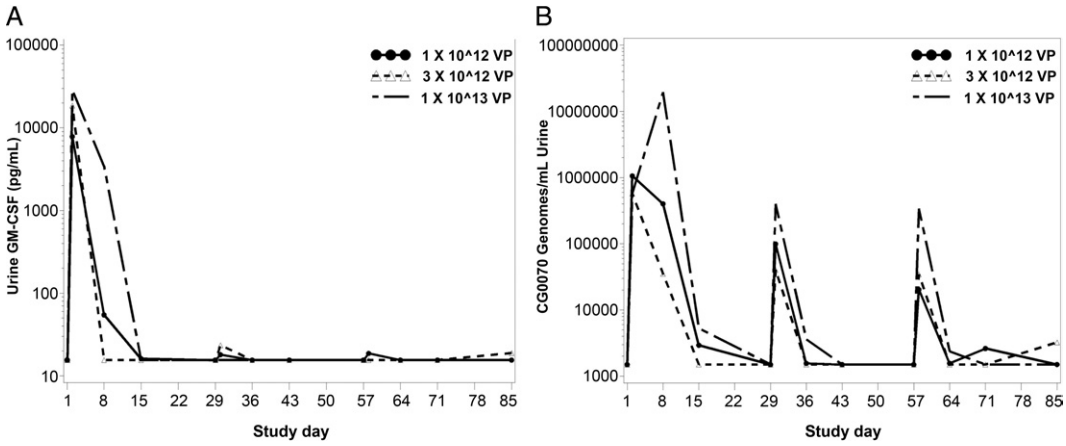

Figure 4. Geometric mean values in urine vs time in every 28-day dose cohorts for dose levels 1 to 3 (1×10^{12}, 3×10^{12} and 1×10^{13} vp, respectively). *A*, urine GM-CSF. *B*, urine CG0070 genomes.

sessed (3 of 11) had quantifiable CG0070 levels in urine.

Plasma CG0070 genomes. Overall, 7.7% (1 of 13), 11.1% (1 of 9) and 7.7% of patients (1 of 13) on the single, weekly × 6 and every 28-day schedules, respectively, had quantifiable CG0070 genomes in plasma at any time point, ie 1,500 copies per ml or greater. The highest plasma genome level for any patient was 9.15×10^5 vp/ml, which occurred 2 hours after the third administration in a patient with traumatic catheterization.

Response

Rate. The response rate for all patients was 48.6% (17 of 35). Patients treated with the lowest dose (1×10^{12} vp) across all treatment schedules had the highest CR (61.5%) compared to the other dose levels, including 44.4%, 43.0% and 0.0% for 3×10^{12}, 1×10^{13} and 3×10^{13} vp, respectively. The median CR duration for responders was 10.4 months with responses ongoing at 17.0 months.

CR in the subset of patients with CIS only was 50.0% (4 of 8). In patients with CIS alone or in conjunction with Ta or T1 tumors CR was 41.2% (7 of 17) (table 3). CR in the every 28-day group was 53.9% (7 of 13 patients) and in the weekly × 6 group it was 77.7% (7 of 9) for a combined CR of 63.6% (14 of 22).

pRB status. A subset of 18 tissue blocks from the initial diagnosis was available for pRB IHC assessment, of which 5, 1 and 12 were low, borderline and high, respectively, for pRB staining. In general, there were too few patients per group to perform comparative analyses. However, the higher CRs were in the high pRB positive group (7 of 12 patients or 58.3%) and the borderline RB group (1 of 1 or 100%) with a rapid decrease in the response rate in the RB negative group (1 of 5 or 20%). This suggests the possibility of a pRB status response effect favoring patients with borderline or high pRB (table 3). Assessment of only patients with high or borderline pRB treated in the multidose subgroups (every 28 days and weekly × 6) revealed a CR of 81.8% (9 of 11). The best CR was for borderline or high pRB in patients treated with the weekly × 6 dose schedule since all 5 (100%) responded. These responses, which lasted from 3.5 to 18.1 months, were ongoing

Table 3. *CR and CR duration by dose, stage, grade and multidose subgroup plus pRB status*

Subgroup Examined	% CR (No. pts/total No.)	Median CR Duration (mos)
Overall	48.6 (17/35)	10.4
Dose level (vp):		
1×10^{12}	61.5 (8/13)	8.2
3×10^{12}	44.4 (4/9)	Not attained
1×10^{13}	50.0 (5/10)	Not attained
3×10^{13}	0.0 (0/3)	—
Stage:		
Ta	66.7 (10/15)	7.9
T1	0.0 (0/3)	—
Ta + CIS	50.0 (1/2)	Not attained
T1 + CIS	28.6 (2/7)	6.2
CIS only	50.0 (4/8)	8.4
Grade:		
I	83.3 (5/6)	8.2
II	50.0 (3/6)	11.8
III	60.0 (6/10)	Not attained
Multidose:		
Combined (every 28 days + wkly × 6)	63.6 (14/22)	Not attained
Every 28 days	53.9 (7/13)	15.3
Wkly × 6	77.8 (7/9)	Not attained
Multidose borderline + high pRB:		Not attained
Combined	81.8 (9/11)	
Wkly × 6	100.0 (5/5)	

at the last clinical assessments. Therefore, the median response duration for this subgroup had not been reached.

DISCUSSION

This study demonstrates the safety and activity of CG0070, a novel replication competent adenovirus designed for cancer targeted replication and GM-CSF transgene expression. Despite the inclusion of heavily pretreated and BCG refractory patients with a median of 4 previous resections (range 1 to 24), 2 cycles of BCG or BCG and interferon (range 1 to 9) and 1 of chemotherapy (range 1 to 4), IVE CG0070 was feasible and safe with the most common AE grade 1–2 bladder toxicities across all treatment groups. Furthermore, despite high dose treatment of up to 3×10^{13} vp, no treatment related serious AE and only a single DLT (transient grade 3 lymphopenia) was reported. Laboratory toxicity was similarly tolerable with no significant end organ dysfunction observed. No obvious trend toward increased toxicity was noted across doses, schedules or number of treatments.

To determine whether CG0070 achieved infection, replication and transgene expression in the urothelium, CG0070 genomes and GM-CSF levels were measured. All patients expressed high GM-CSF levels (up to 62,907 pg/ml) in urine, which generally peaked on day 2 (94.3% or 33 of 35) and generally increased with the dose level (fig. 2, *A*). The expression of endogenous GM-CSF due to intravesical adenovirus infection was possible. Unfortunately, previous reports of Ad-Ifn or Ad-p53 did not include information on urinary GM-CSF.[21,22]

Between days 2 and 5 after treatment 58.3% of patients (7 of 12) showed an increase in CG0070 genomes of any magnitude, while 25% (4 of 12) demonstrated at least a tenfold increase vs the nadir. This second wave of detectable CG0070 genomes suggests virus replication in the bladder. However, it is possible that this second wave represents the redistribution of sequestered CG0070 genomes from an unclear location in the bladder or another anatomical area.

In the multidose groups, the magnitude of peak CG0070 genome and GM-CSF concentrations in urine decreased with the second and subsequent treatments compared to peak levels following initial treatment (figs. 3 and 4). This suggests accelerated CG0070 clearance, perhaps due to the induction of anti-adenovirus immunity or a decreased number of replication permissive cells, eg E2F-1 expressing NIBC. Notably, all patients showed a high titer of anti-adenovirus neutralizing antibodies by 4 weeks after the initial CG0070 treatment with a significant increase at week 2, followed by a plateau in titers by week 4 (data not shown), supporting the possibility of enhanced clearance resulting from immune induction.

The CR and median duration of the CR across treatment groups were 48.6% and 10.4 months, respectively. Although small patient numbers, and the grade and stage heterogeneity of enrolled patients made comparison among subgroups problematic, some trends can be highlighted. Specifically, multidose cohorts in the aggregate and 10^{12} vp weekly $\times$ 6 groups appeared to be superior to single dose CG0070 with respect to antiNIBC activity (table 3). Patients with T1 disease failed to respond to therapy, while those with Ta and CIS regardless of grade appeared to benefit from treatment (table 3). Therefore, targeting CIS and Ta NIBC with 10^{12} vp per dose on a weekly $\times$ 6 schedule appears reasonable for future studies.

An exploratory, retrospective assessment of 18 patients with tissue available from the initial diagnosis suggested that those with high IHC pRB staining (greater than 50%) were more likely to respond to CG0070 (table 3), consistent with selective engineering of CG0070 to replicate preferentially in the presence of up regulated E2F-1. High pRB IHC staining, specifically in bladder cancer cells, is associated closely with p16/cyclin D1 and subsequent E2-1 up-regulation.[23,24] Interestingly, not all tested patients had increased IHC pRB but all showed robust GM-CSF expression and virus replication. This observation may have been due to E2F-1 up-regulation independent of increased pRB. Further studies are needed to better explore the association of RB pathway status and CG0070 activity.

Recently, it was reported that oncolytic virus therapies, in particular with GM-CSF expressing vectors, induce durable responses even in late stage solid tumors via direct oncolysis and the induction of anticancer immunity.[25,26] A major issue not addressed in this investigation is the relative contribution to overall CG0070 activity of direct viral oncolysis vs GM-CSF induced anticancer immunity. NIBC by definition lacks distant metastatic disease from which to gauge the possible effects of an immune or vaccine based mechanism of action, eg the response of uninfected tumors at sites distant from the bladder. Thus, it was impossible to clinically assess the potential impact of immune activation secondary to GM-CSF expression.

Unfortunately, tissue biopsies obtained in this study were not adequate to assess tumor infection, replication, local immune infiltration or tumor necrosis. Clinical testing of patient samples was not done to assess for the induction of antitumor immunity, eg cytotoxic T-lymphocyte activation or antibody induction. Investigation of the potential of CG0070 to induce anticancer immunity is critical to

the use of CG0070 beyond the limited confines of the bladder as a more broadly applicable tumor vaccine.

CONCLUSIONS

Although this study was primarily designed to assess the safety of CG0070 in the relatively limited compartment of the bladder, our investigation exceeded the outcomes normally associated with phase 1 dose escalation studies. A tolerable safety profile was established without attaining a maximum tolerated dose, and evidence of antitumor activity based on the complete response to treatment for CIS and residual Ta/T1 disease was noted. Furthermore, the pattern, magnitude and timing of measurable viral genomes and GM-CSF in urine support the possibility of virus replication and transgene expression after intravesical instillation. Based on the results presented, the frequent dysregulation of the RB pathway resulting in E2F-1 expression in cancer and the growing relevance of gene and immunotherapy for human malignancy, future studies of CG0070 are justified in NIBC as well as other neoplasms.

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