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ORIGINAL REPORT

Intravesical rAd–IFNα/Syn3 for Patients With High-Grade, Bacillus Calmette-Guerin–Refractory or Relapsed Non–Muscle-Invasive Bladder Cancer: A Phase II Randomized Study

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ABSTRACT

Purpose

Many patients with high-risk non–muscle-invasive bladder cancer (NMIBC) are either refractory to bacillus Calmette-Guerin (BCG) treatment or may experience disease relapse. We assessed the efficacy and safety of recombinant adenovirus interferon alfa with Syn3 (rAd–IFNα/Syn3), a replication-deficient recombinant adenovirus gene transfer vector, for patients with high-grade (HG) BCG-refractory or relapsed NMIBC.

Methods

In this open-label, multicenter (n = 13), parallel-arm, phase II study (ClinicalTrials.gov identifier: NCT01687244), 43 patients with HG BCG-refractory or relapsed NMIBC received intravesical rAd–IFNα/Syn3 (randomly assigned 1:1 to 1×10^{11} viral particles (vp)/mL or 3×10^{11} vp/mL). Patients who responded at months 3, 6, and 9 were retreated at months 4, 7, and 10. The primary end point was 12-month HG recurrence-free survival (RFS). All patients who received at least one dose were included in efficacy and safety analyses.

Results

Forty patients received rAd–IFNα/Syn3 (1×10^{11} vp/mL, n = 21; 3×10^{11} vp/mL, n = 19) between November 5, 2012, and April 8, 2015. Fourteen patients (35.0%; 90% CI, 22.6% to 49.2%) remained free of HG recurrence 12 months after initial treatment. Comparable 12-month HG RFS was noted for both doses. Of these 14 patients, two experienced recurrence at 21 and 28 months, respectively, after treatment initiation, and one died as a result of an upper tract tumor at 17 months without a recurrence. rAd–IFNα/Syn3 was well tolerated; no grade four or five adverse events (AEs) occurred, and no patient discontinued treatment because of an adverse event. The most frequently reported drug-related AEs were micturition urgency (n = 16; 40%), dysuria (n = 16; 40%), fatigue (n = 13; 32.5%), pollakiuria (n = 11; 28%), and hematuria and nocturia (n = 10 each; 25%).

Conclusion

rAd—IFNα/Syn3 was well tolerated. It demonstrated promising efficacy for patients with HG NMIBC after BCG therapy who were unable or unwilling to undergo radical cystectomy.

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INTRODUCTION

Non–muscle-invasive bladder cancer (NMIBC) represents the most common disease state for patients with newly diagnosed bladder cancer.[1] Those with high-grade (HG) tumors are at significant risk for both recurrence and progression.[2,3] Bacillus Calmette-Guerin (BCG) represents the current preferred management.[4-6] Nonetheless, approximately 30% of patients will not respond to BCG; among those who demonstrate an initial response, more than 50% will experience recurrence and progression during long-term follow-up.[7]

The optimal management of patients with persistent or recurrent tumor after BCG remains controversial.[8] Although radical cystectomy provides cancer eradication,[9] many patients are elderly, have significant comorbidities with an attendant diminished performance status, and often are unwilling to undergo radical extirpative

ASSOCIATED CONTENT

Appendix
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Data Supplements
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surgery. Nonextirpative treatment options are available, but studies to date have included relatively small patient numbers and used varied definitions of treatment success[8,10-16] Indeed, the US Food and Drug Administration (FDA) and genitourinary oncology community agree that scant progress has been made in the management of this disease since the initial approval of BCG.[17-19] Thus an effective alternative to radical cystectomy for patients with disease recurrence after BCG treatment remains an important unmet clinical need.[17]

Recombinant intravesical interferon alfa-2b protein (IFNα-2b; Intron A; Merck, Kenilworth, NJ) demonstrated promising initial clinical results in NMIBC.[20,21] Intravesical IFNα-2b gene delivery offers a novel approach and increases the duration of exposure to IFNα-2b. Recombinant adenovirus (rAd)–IFNα-2b is a replication-deficient adenovirus-based gene transfer vector that encodes the human IFNα-2b gene.[22-24] Syn3, a polyamide surfactant, is incorporated into the drug formulation (rAd–IFNα/Syn3; Instiladrin, FKD Therapies Oy, Kuopio, Finland)[25] to enhance adenoviral transduction of the bladder lining. Dramatic enrichment of rAd–IFNα gene transfer and expression has been shown with Syn3 in both normal urothelium and human urothelial carcinoma that grows in mice.[22-25] rAd–IFNα-2b gene therapy mimics the physiologic events associated with viral infection, which results in local rather than systemic IFNα-2b production and subsequent tumor regression.[22]

A phase I dose-ascending study of rAd–IFNα/Syn3 was performed for patients with BCG-refractory and relapsing NMIBC.[26] Dose-dependent adenoviral gene transfer and urine concentrations of IFNα-2b were confirmed. Of 14 patients treated with dose levels of rAd–IFNα/Syn3 that resulted in measurable urine IFNα, six (43%) were free from recurrence at 3 months and had no dose-limiting toxicity, and two patients remained disease free at 29 and 39 months.[26] These provocative findings, predominantly at the two highest doses, prompted this phase II study, designed to evaluate the efficacy and safety of intravesical rAd–IFNα/Syn3 for patients with HG NMIBC refractory to, or with relapse after, BCG.

METHODS

Study Design

This randomized, open-label, parallel-arm study was conducted across 13 centers in the United States between November 5, 2012, and April 8, 2015. The protocol, administrative oversight, and accrual timelines were designed and conducted by the Society of Urologic Oncology Clinical Trials Consortium. The study protocol and informed consent form were reviewed and approved by the respective responsible site institutional review boards and biosafety committees.

Patients

The trial was designed to enroll 40 patients unable or unwilling to undergo radical cystectomy, and there were two dosage groups of 20 patients each. Eligible patients were 18 years or older and had HG BCG-refractory or relapsed NMIBC, including papillary NMIBC alone (Ta or T1), carcinoma in situ (CIS) alone, or a combination of CIS and papillary disease. BCG-refractory disease was defined as the inability to achieve a disease-free state at 6 months after adequate induction BCG therapy with either maintenance or reinduction at 3 months. Adequate induction was defined as a minimum of five of six treatments, and adequate maintenance was defined as a minimum of two of three treatments. BCG relapse was defined as recurrence within 1 year after a complete response to adequate BCG treatment (at least five and two instillations). Patients were required to have undergone visually complete resection of papillary lesions by transurethral resection of bladder tumors. Patients could not have received intravesical therapy within 3 months before beginning study treatment, with the exception of cytotoxic agents when administered as a single instillation immediately after a transurethral resection. All participants who entered the study provided written or oral informed consent.

Random Assignment and Masking

Patients were assigned by computer-generated random assignment, with a constrained 1:1 sequence, to receive either low-dose (1×10^{11} viral particles [vp]/mL) or high-dose (3×10^{11} vp/mL) rAd–IFNα/Syn3. These doses were the most promising observed in the phase I study. The total doses administered were 7.5×10^{12} vp in the low-dose group and 2.25×10^{13} vp in the high-dose group. Treatment allocation was performed centrally with a block size of two for all patients who had successfully completed screening, with the constraint that the first four patients at each site were balanced between cohorts.

Procedures

rAd–IFNα/Syn3 in 75 mL was administered intravesically through a urethral catheter, with a planned retention time of 1 hour; an anticholinergic treatment was allowed to relieve urinary urgency and permit adequate retention. Patients without recurrence of HG disease at months 3, 6, and 9, as evaluated by cytology, cystoscopy, and biopsy (if clinically indicated) were then retreated at months 4, 7, and 10. At 12 months, a final efficacy evaluation was performed. This evaluation included a protocol-mandated biopsy from the site of the index tumor and at least five random biopsies, including the bladder dome, trigone, right and left lateral wall, posterior wall, and prostatic urethra in men with positive cytology or prior disease in this region.

During the study, patients were contacted weekly by phone for the first month after each treatment on days 7, 14 (of months 7 and 10 only), 21, and 28 (± 1 day) to provide information about adverse events (AEs) and concomitant medication use. Assessments for treatment failure were made between 14 and 7 days before retreatment. Patients who were withdrawn from treatment before study completion underwent a safety assessment at least 30 days after last administration of the study drug. All patients are being monitored in a 3-year long-term follow-up period to (1) determine recurrence of HG disease in those patients with a complete response and (2) to assess the long-term impact of treatment with rAd–IFNα/Syn3.

End Points

The primary end point was freedom from HG disease recurrence at 12 months, defined by a negative for cause or end of study biopsy. Secondary end points included response to treatment, defined as no evidence of recurrence of HG disease at 3, 6, and 9 months; incidence and time to cystectomy; and concentration of IFNα-2b in the urine. Safety assessments included physical examination, monitoring of vital signs, ECG, and standard clinical chemistry, hematology, and urinalysis assessments (performed by local laboratories). Safety end points include type, incidence, relatedness, and severity of AEs and severe (≥ grade 3) AEs (SAEs), as assessed by National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03).

Statistical Analyses

We determined that a cohort of 20 patients would be sufficient to give an 80% probability of rejection of a HG recurrence-free survival (RFS) rate of 10% with an exact 5% one-sided test when the true HG RFS rate was 35%. The operating characteristics for this Fleming design were calculated exactly with the binomial distribution described by A'Hern.[27] The hypothesis—that the response rate was equal to or less than the reference rate—was rejected if five or more of the 20 patients achieved HG RFS at 12 months. The proportion of patients who achieved HG RFS at 3, 6, 9, and 12 months was reported for each dose group, together with an exact 90% CI for the proportion. The time to HG recurrence or death was summarized with the Kaplan-Meier method. Analyses were performed with SAS (version 9 or later; SAS Institute, Cary, NC). Both the safety and efficacy (modified

intention-to-treat) analysis sets included all patients who received at least one dose of rAd–IFNα/Syn3. A data monitoring committee oversaw the study according to the data monitoring plan.

Analytical Assays and Sample Testing

All analytical assays were developed and validated. Samples were tested according to good laboratory practices methods at Covance Laboratories Ltd (Harrogate, United Kingdom). Description of the assays and the results of sample testing are presented in the Appendix (online only).

RESULTS

Patient disposition is shown in Figure 1. Baseline characteristics are listed in Table 1.

Primary End Point: HG RFS

The12-month HG RFS rate was comparable between the two dose groups, with 33.3% of patients (7 of 21; 90% CI, 16.8 to 53.6) in the low-dose group and 36.8% (7 of 19; CI, 18.8 to 58.2) in the high-dose group alive and free of HG disease at 12 months. Overall, 35.0% of patients (14 of 40; 90% CI, 22.6% to 49.2%) remained free of HG recurrence at 12 months after the initiation of rAd–IFNα/Syn3 treatment (Table 2). Off-schedule disease assessments did not affect findings (Appendix, online only). The median time to HG recurrence or death was 6.5 months (90% CI, 3.52 to 12.78 months); the median time to HG recurrence was 3.52 months (90% CI, 3.02 to 12.78 months) for the low-dose group and was 11.73 months (90% CI, 5.88 months to not evaluable) for the high-dose group.

When patient subgroups and secondary end points were considered in exploratory analyses, the 12-month HG RFS rates were broadly similar for men and women, for younger and older patients, for refractory or relapsed NMIBC, for CIS only or papillary tumors and CIS, and for patients with Ta and T1 disease only (Table 2). Interestingly, of the 14 patients who were recurrence free at 12 months, 10 (71%) of the 14 had an antiadenovirus antibody response (defined as four times the predose titer), compared with 11 (24%) of 25 who experienced recurrence.

Significant levels of urine IFNα-2b were measurable in all patients in month 1 at days 2, 4, and 12 (Table 3). Of those patients who received a second dose, measurable IFNα-2b urine concentrations were noted in month 4 on days 2 and 4 after drug administration. Urine IFNα-2b concentrations did not appear to correlate with dose or clinical response.

In long-term follow-up, seven patients (18%) who withdrew from the study because of HG disease recurrence within the 12-month study period died at a median of 16 months (range, 2 to 26 months) after the withdrawal date. There is no indication that these deaths were treatment related. The cause of death was unknown in four patients, whereas two died as a result of progressive bladder cancer and one died as a result of liver failure unrelated to treatment 17 months after withdrawal from the study. The four patients for whom the cause of death is unknown were being observed locally after they completed their end-of-study evaluation. Fourteen patients (35%) who experienced an HG recurrence within the first year underwent a radical cystectomy at a median of 9 months (range, 4 to 28 months) from day 1 of month 1.

Patients are being monitored for 3 years to collect long-term follow-up data. Of the 14 patients who remained disease free at 12 months, additional follow-up data are being collected for 11; 3 withdrew from the study. Nine of these 11 patients are alive, and eight remained disease-free during a period of 15 to more than 36 months (Table 4). Two patients experienced HG recurrence at 21 and 28 months, respectively, from the start of treatment. One of these patients who experienced progression to muscle invasion underwent a radical cystectomy 31 months after the initiation of treatment and later died at 41 months. The other, who experienced recurrence at

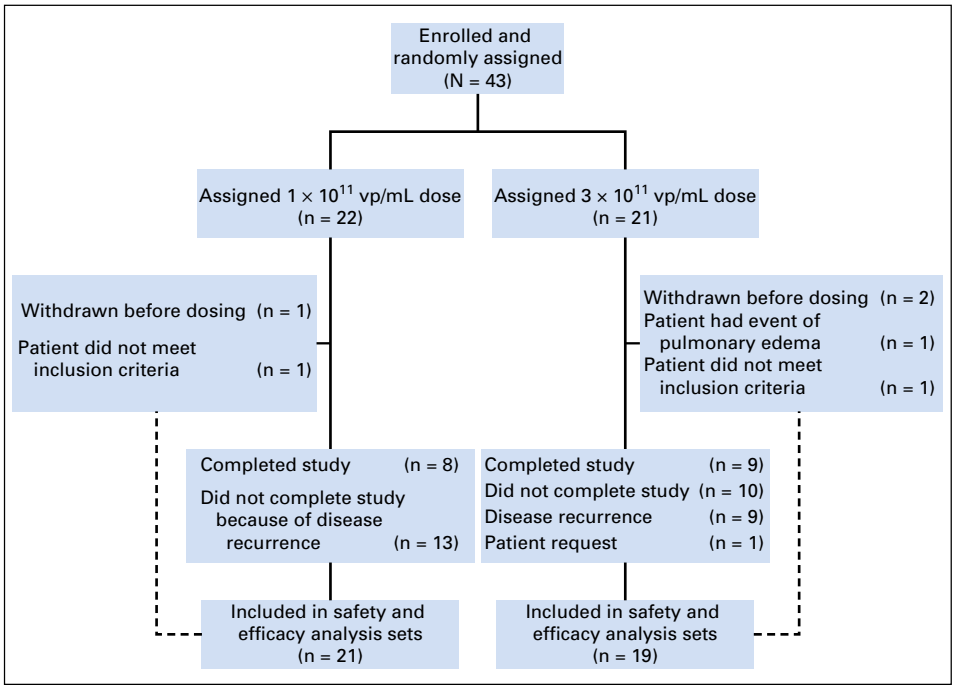

Fig 1. Patient dispositions. vp, viral particles.

Table 1. Baseline Patient Characteristics

Characteristic	No. (%) by rAd–IFNα/Syn3 Dose Group		No. (%) Overall (N = 40)
	1×10^{11} vp/mL (n = 21)	3×10^{11} vp/mL (n = 19)	
Median (IQR) age, years	70 (67-74)	73 (62-81)	70.5 (64.5-77.5)
Sex			
Male	19 (90)	14 (73.7)	33 (82.5)
Female	2 (9.5)	5 (26.3)	7 (17.5)
ECOG PS			
0	16 (76.2)	18 (94.7)	34 (85.0)
1	5 (23.8)	1 (5.3)	6 (15.0)
History of radiation therapy*	1 (4.8)	1 (5.3)	2 (5)
BCG failure classification			
Relapsed	10 (47.6)	9 (47.4)	19 (47.5)
Refractory	11 (52.4)	10 (52.6)	21 (52.5)
No. of previous BCG courses			
1	1	1	2
2	10	12	22
≥ 3†	10	6	16
Primary tumor classification at enrollment			
CIS	12 (57.1)	9 (47.4)	21 (52.5)
Ta	2 (9.5)	2 (10.5)	4 (10)
Ta and CIS	3 (14.3)	1 (5.3)	4 (10)
T1	2 (9.5)	4 (21.1)	6 (15)
T1 and CIS	2 (9.5)	3 (15.8)	5 (12.5)

Abbreviations: BCG, bacillus Calmette-Guerin; CIS, carcinoma in situ; ECOG PS, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; rAd–IFNα/Syn3, recombinant adenovirus interferon alfa protein/Syn3 (a nonreplicating recombinant adenovirus gene transfer vector for patients with high-grade BCG-refractory or relapsed non–muscle-invasive bladder cancer); Ta, papillary urothelial carcinoma confined to the mucosa; T1, micro-invasive urothelial carcinoma invasive into lamina propria but not muscularis propria; vp, viral particles.

*Radiotherapy was 10 or more years before screening in each of these three patients; as such, they were deemed eligible for study enrollment.

†Range of previous courses, 3 to 8.

21 months, remained alive and free from distant recurrence at 36 months. One patient free from bladder recurrence at 12 months died as a result of an upper tract tumor at 17 months.

Safety End Points

Overall, 39 patients (97.5%) experienced AEs during the study; 20 patients (95%) were in the low-dose arm, and 19 patients (100%) were in the high-dose arm (Data Supplement). In 34 of these patients (85%), at least one AE was considered to be drug related;: in 18 (87.5%) of 21 patients in the low-dose arm and in 16 (84.2%) of 19 patients in the high-dose arm. The most frequently reported drug-related AEs were micturition urgency in 16 patients (40%), dysuria in 16 patients (40%), fatigue in 13 patients (32.5%), pollakiuria in 11 patients (28%), hematuria and nocturia in 10 patients each (25% each). Notably, for the majority of patients (78%), the AEs were transient and classified as either grade 1 or 2. Nine patients (22%) reported a total of 19 grade 3 AEs: 12 in the low-dose arm (coronary artery occlusion, diarrhea, sepsis, arthralgia, renal neoplasm, transitional cell carcinoma, carotid artery occlusion, syncope, renal failure, nephroureterectomy, COPD, and hypotension) and seven in the high-dose arm (abdominal pain, back pain, fracture, syncope, dysuria [n = 2], and acute renal failure; Data Supplement). Although coded as A's according to convention, the renal neoplasm, transitional cell carcinoma, and nephroureterectomy reports in the low-dose arm reflect the diagnosis and treatment of a separate upper tract urothelial carcinoma in one patient. All grade 3 AEs occurred only once. There were no grade 4 or 5 events.

Overall, five patients exhibited a total of 10 SAEs: three patients had a total of eight SAEs in the low-dose group, and two patients had a total of two SAEs in the high-dose group. Of these, one episode of diarrhea (low-dose group; treated with 1,000 mL of 0.9% sodium chloride intravenously) and one episode of acute renal failure (high-dose group; urine culture, 59,000 to 99,000 colony-forming units/mL of *Klebsiella pneumoniae*; treated with antibiotics) were considered related to the study drug. Both resolved with medical therapy. There was no significant difference in the initial occurrence of AEs in those who received the low or high dose of rAd–IFNα/Syn3.

DISCUSSION

We report the results from a completed phase II trial of intravesical rAd–IFNα/Syn3 for patients with recurrent NMIBC after BCG. Several important findings emerge from the resultant data set, including a 12-month HG RFS of 35% by intention-to-treat analysis of all patients dosed. Notably, responses were durable: the majority remained disease-free for close to 24 months. We noted a 30% durable complete response for patients with any element of CIS and a 50% RFS for patients with papillary disease only at study entry. Likewise, the 12-month RFS in heavily pretreated patients was 31%. rAd–IFNα/Syn3 treatment was well tolerated; there were no grade 4 or 5 events, and no patients discontinued treatment because of drug-related AEs. Analytical assays indicated that IFNα-2b was measurable in the urine of all patients, which provided evidence for effective adenoviral-mediated gene transfer. Bioassays revealed no evidence for rAd–IFNα DNA in the blood, which provided additional reassurance for biosafety.

Several agents have been evaluated as second-line treatment after BCG; however, none (to date) have provided robust and durable responses. Valrubicin (Valstar; Endo Pharmaceuticals, Malvern, PA), the only agent currently approved by the FDA for the treatment of BCG-refractory CIS, provided a complete response rate of 18% at 6 months and a 1-year disease-free survival rate of approximately 10%.[16] Promising results from early-phase trials have been reported for intravesical taxane and gemcitabine.[10-14] Joudi et al[15] reported the final results from a national multicenter phase II trial of BCG plus IFNα-2b and noted that 45% of patients with BCG failure were free from recurrence at 2 years. However, only 44% were treated for an HG recurrence, and 61% received only one prior course of BCG.[15] A recent retrospective analysis of BCG and IFNα-2b reported a 38.6% RFS at 12 months. Again, many of these patients (20 of 44) received only one prior course of BCG, and 16 patients experienced relapse after 12 months.[27] Overall, the limited number of patients studied in previous trials, as well as the modest RFS with treatment despite a less stringently defined eligibility, illustrates the unmet need for effective and evidence-based second-line therapy for patients with BCG-unresponsive

Table 2. Incidence of HG RFS at 3, 6, 9, and 12 Months

Variable	rAd–IFNα/Syn3 Dose Group: 1×10^{11} vp/mL (n = 21) No. (%) of Patients	1×10^{11} vp/mL (n = 21) 90% CI (%)*	3×10^{11} vp/mL (n = 19) No. (%) of Patients	3×10^{11} vp/mL (n = 19) 90% CI (%)*	Overall (N = 40) No. (%) of Patients	Overall (N = 40) 90% CI (%)*
RFS at secondary end point analysis time						
3 months	10 (47.6)	28.6 to 67.2	13 (68.4)	47.0 to 85.3	23 (57.5)	43.3 to 70.8
6 months	8 (38.1)	20.6 to 58.3	9 (47.4)	27.4 to 68.0	17 (42.5)	29.2 to 56.7
9 months	8 (38.1)	20.6 to 58.3	9 (47.4)	27.4 to 68.0	17 (42.5)	29.2 to 56.7
12 months	7 (33.3)	16.8 to 53.8	7 (36.8)	18.8 to 58.2	14 (35.0)	22.6 to 49.2
HG recurrence-free subgroup at 12 months						
Refractory NMIBC (n = 31)					8 (38.1)	20.6 to 58.3
Relapsed NMIBC (n = 19)					6 (31.6)	14.7 to 53.0
CIS only (n = 21)					6 (28.6)	13.2 to 48.7
Papillary tumor (n = 9)					3 (33.3)	9.7 to 65.6
Ta + T1 disease only (n = 10)					5 (50.0)	22.2 to 77.8
Serum antiadenoviral antibody						
Positive (n = 22)					10 (45.5)	27.1 to 64.7
Negative (n = 17)					4 (23.5)	8.5 to 46.1

Abbreviations: CIS, carcinoma in situ; HG, high-grade; NIMBC, non–muscle-invasive bladder cancer; rAd–IFNα/Syn3, recombinant adenovirus interferon alpha protein/Syn3 (a nonreplicating recombinant adenovirus gene transfer vector for patients with high-grade bacillus Calmette-Guerin–refractory or relapsed NMIBC); RFS, relapse-free survival; Ta; papillary urothelial carcinoma confined to the mucosa; T1: micro invasive urothelial carcinoma invasive into lamina propria but not muscularis propria; vp, viral particles.
*CI is for the proportion of patients with HG RFS; 90% CIs are based on the exact binomial method.

disease that improves disease-specific patient outcomes and avoids cystectomy.

We recognize that this study is limited by its relatively small sample size and the lack of a comparative treatment arm. However, the trial was designed to determine optimal dosing and to provide preliminary efficacy to develop a definitive single-arm registration study.[12] Few agents have actually gone beyond phase I and II development, so it is readily apparent that the traditional pathway for drug registration does not work for NMIBC. This concern was addressed through deliberations among the Society of Urologic Oncology, American Urological Association, and FDA, with the consensus that a single-arm trial with a mixed population of papillary disease and CIS was appropriate for the BCG-unresponsive population, given that a minimal threshold of patients who had some component of CIS was met.[17]

Although the clinical impact of rAd–IFNα is encouraging, the mechanisms that mediate its antitumor activity remain undefined. In preclinical studies, IFNα and rAd–IFN beta inhibited angiogenesis,[28,29] and IFNα directly induced apoptosis in human bladder cancer cells by inducing autocrine tumor necrosis factor–related apoptosis-inducing ligand production.[30] Furthermore, rAd–IFNα overcame resistance to the IFNα protein in vitro and in animal models.[22] It is now well established that IFNα controls dendritic cell maturation and antigen presentation and promotes tumor recognition by T cells and natural killer cells, and that these effects likely play more important roles in tumor growth inhibition than the direct effects of IFNα on tumor cells.[31-33] Like IFN gamma, IFNα induces programmed death ligand 1 expression,[34] which may limit tumor immune recognition and almost certainly inhibits T-cell activation; this may

Table 3. Urinary IFNα-2b Concentrations After Treatment With rAd–IFNα/Syn3

Visit	Patients With Measurable Urinary IFNα-2b Concentrations: No. of Patients	% in Dose Group 1 (n = 40)	% in Dose Group 2 (n = 23)	Range of Urinary IFNα-2b Concentrations (IU/mL) for Patients With Measurable IFNα-2b*: In Dose Group 1 (n = 40)	In Dose Group 2 (n = 23)
M1D1	0	0		0	
M1D2	40	100		247-68,255	
M1D4	34	85		118-91,441	
M1D12	7	18		34-922	
M4D1	0		0		0
M4D2	17		74		54-11,587
M4D4	8		35		34-1,329
M4D12	0		0		0

Abbreviations: IFNα-2b, interferon alfa-2b protein; MID1, month 1 day 1; M1D2, month 1 day 2; M1D4, month 1 day 4; M1D12, month 1 day 12; M4D1, month 4 day 1; M4D2, month 4 day 2; M4D4, month 4 day 4; M4D12, month 4 day 12; rAd–IFNα/Syn3, recombinant adenovirus interferon alfa protein/Syn3 (a nonreplicating recombinant adenovirus gene transfer vector for patients with high-grade bacillus Calmette-Guerin–refractory or relapsed non–muscle-invasive bladder cancer).
*Urinary IFNα-2b concentrations were measured by ELISA. Concentrations were measured over 2 dosing cycles and the data are presented as both the number of patients with measurable IFNα-2b concentrations in each dosing cycle and the range of measurable protein concentrations in IU/mL.

Table 4. Durability of HG RFS Since Start of Treatment With rAd–IFNα/Syn3

Stage at Entry	Dose Group	Duration of Bladder HG RFS Since Day 1 (months)	Time of Last follow-Up from Day 1 (months)	Status at Last Follow-Up
Ta/CIS	High	21	47	Recurrence of HGD Died at 38 months
Ta	Low	28	41	Recurrence at 28 months Cystectomy at 31 months Died at 41 months
CIS	Low	15	15	CR Withdrew
Ta/CIS	Low	30	36	Recurrence of HGD
Ta	High	16	16	CR Withdrew
T1/CIS	Low	35	37	CR
T1	Low	30	50	CR
T1	High	36	36	CR
CIS	High	38	39	CR
CIS	High	34	37	CR
CIS	High	27	27	CR
CIS	Low	34	37	CR
T1/CIS	Low	17	17	Died of upper tract recurrence
Ta	High	13	13	CR Withdrew

NOTE. Duration of HG RFS represent the number of months from day 1 that a complete response within the bladder has been documented based on yearly reports. Three patients withdrew from the study shortly after the 1- month end-of-study evaluation. Two patients had recurrence of HGD at 21 and 28 months from day 1. One of these patients underwent a cystectomy but later died. One patient died of an upper tract tumor without a bladder recurrence.

Abbreviations: CIS, carcinoma in situ; CR, complete response; HG, high-grade; HGD, high-grade disease; rAd–IFNα/Syn3, recombinant adenovirus interferon alfa protein/Syn3 (a nonreplicating recombinant adenovirus gene transfer vector for patients with HG bacillus Calmette-Guerin–refractory or relapsed non–muscle-invasive bladder cancer); RFS, relapse-free survival; Ta, papillary urothelial carcinoma confined to the mucosa; T1, micro-invasive urothelial carcinoma invasive into lamina propria but not muscularis propria.

explain the resistance to rAd–IFNα by some of the bladder cancers treated in this study. Combination therapy with IFNα and an anti–programmed death 1 inhibitor was more efficacious in preclinical studies than either agent alone at inhibition of melanoma tumor growth, and combination trials in NMIBC are under consideration.[34] Finally, studies have demonstrated that local delivery of IFNα is better than systemic delivery to enhance tumor immune recognition, and viral transduction itself provides an important signal for kickstarting the immune system. Thus, in addition to serving as a bioreactor for sustained IFNα production (in contrast to the transient levels measured after intravesical instillation of the IFNα protein),[24] IFNα gene therapy should produce unique, desirable effects on antitumor immunity through local (as opposed to systemic) IFNα production and viral activation of intracellular pattern receptors. Thus, there are multiple reasons to explain the enhanced efficacy of rAd–IFNα compared with IFNα-2b in the treatment of refractory NMIBC.[35]

In summary, rAd–IFNα/Syn3 was well tolerated and demonstrated promising efficacy for patients with HG NMIBC after BCG therapy. A phase III trial of high-dose rAd–IFNα/Syn3, which provided longer median HG RFS and equivalent biosafety, is ongoing.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Intravesical rAd–IFNα/Syn3 for Patients With High-Grade, Bacillus Calmette-Guerin–Refractory or Relapsed Non–Muscle-Invasive Bladder Cancer: A Phase II Randomized Study

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Appendix

Supplemental Methods

Role of the funding source. FKD Therapies Oy (Kuopio, Finland) provided funding to the investigators for study design, conduct, treatment administration, and data collection. The study database was held by the funder. All authors had unrestricted access to the raw and final study data and were responsible for data interpretation, the preparation of the report, and the decision to submit for publication.

Recombinant Adenovirus Interferon Alfa Protein/Syn3 production. First-generation replication-deficient serotype 5 adenovirus vector, which expressed human interferon alfa-2b (IFNα-2b) cDNA under a cytomegalovirus promoter, was produced under good manufacturing practice conditions in 293 cells, as previously described,[1] with slight modifications of the process. It was tested to be free of endotoxin, microbiologic contaminants, and other impurities. The structure of the vector was verified by sequencing. Production of recombinant IFNα-2b was verified from each production lot with immunologic methods. The excipient Syn3 is a polyamide surfactant that enhances adenoviral gene transfer to the bladder epithelium.[2,3]

Analytical Assays

Sample collections and assay methods. Whole blood and urine samples were collected on days 1 (predose), 2, 4, and 12 of months 1 and 4 for measurement of recombinant adenovirus IFNα-2b (rAd–IFNα-2b) DNA and IFNα-2b concentrations (urine only). Serum samples for IFNα-2b protein concentration measurements also were collected on days 1 (predose), 2, 4, and 12 of months 1 and 4. Serum samples for antibody assays were collected before dosing on day 1 of months 1, 4, 7, and 10, as well as at the month-13/withdrawal visit.

Urine samples for rAd–IFNα-2b DNA, IFNα-2b, and exploratory assays were collected into a sterile container and stabilized with the addition of buffer that contained 10% bovine serum albumin and 50 mM of HEPES (pH, 7.4). Two mL of buffer was added to each 20-mL sample of urine as soon as possible after collection of the urine sample. After addition of the stabilization buffer, aliquots were transferred into 2-mL cryotubes by using sterile pipette tips and were put on ice. Whole-blood samples for determination of rAd–IFN DNA by polymerase chain reaction (PCR) were collected into EDTA-containing tubes. Blood samples were collected at the required time points, were divided into sterile polypropylene cryotubes with sterile pipette tips, and were frozen at −70°C until shipment for analysis. Whole-blood samples for serum IFNα-2b measurements and for determination of anti-adenoviral and anti–IFNα-2b antibodies were drawn at the required time points. The samples were drawn into red top Vacutainer (Becton, Dickinson, and Co., Franklin Lakes, NJ) tubes and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 4°C, × 1,500 *g*, for 15 minutes, and the serum was separated into cryovials. All samples for all assays were frozen at −70°C within 5 hours of collection and were stored for shipment and analysis.

IFNα-2b protein concentration assay. Measurement of IFNα-2b concentrations in urine and serum samples was done by ELISA with a MesoScale discovery platform (Meso Scale Diagnostics, Bethesda, MD). Samples were incubated with a master mix to allow IFNα-2b to bind to biotinylated-anti–IFNα antibodies and sulfo-tagged (sTag)–anti-IFNα antibodies to form an antibody-bridge complex. After incubation, samples were added to the streptavidin-coated plate. The biotinylated–anti-IFNα antibodies bound to the streptavidin-coated plate, which allowed any unbound material to be washed away. Read buffer that contained tripolyamine was added. The sTag associated with anti-IFNα antibodies produced a chemiluminescent signal when an electrical voltage was applied. The concentration of IFNα-2b in samples was then back-calculated from a calibration curve. The method had a lower level of quantification of 31 IU/mL and an upper level of quantification of 2,000 IU/mL.

Analytical assays for rAd–IFNα DNA in blood and urine. To assess systemic exposure and urinary concentrations of rAd–IFNα vector DNA, a sensitive and specific quantitative PCR (qPCR) assay for the vector DNA was developed and validated. In both assay matrices, amplification was detected in all replicates of the standard curve (1×10^9 viral particles [vp]/225 μl to 1×10^3 vp/225 μl for all valid runs), and the correlation coefficient of the dilutions (R^2) was greater than or equal to 0.98 for all qPCRs performed. An assessment of the specificity of the qPCR assay was made with human and *Escherichia coli* DNA. No cross reactivity with either matrix was observed when 1-, 0.5-, and 0.1-μg templates were present in the qPCR assay. To determine if either human or *E. coli* DNA could interfere with the accuracy of the qPCR assay, a spike of 2×10^3 vp/2 μl of rAd–IFNα DNA (derived from the appropriate matrix matched standard) was spiked into a background of each concentration of genomic DNA.

Anti-IFN α antibody assay. Assessment of anti-IFNα antibody concentrations was done with a validated human anti-IFNα platinum ELISA from Affymetrix eBioscience (product code BMS217TEN; Thermo Fisher Scientific, Waltham, MA) with a mouse monoclonal anti-IFNα antibody as the positive control. The assay paradigm was a quasi-quantitative assay sequence that consisted of a screening assay to determine whether a positive signal existed, a competitive inhibition confirmation assay of the positive signal, and a titration assay that used serially diluted samples in buffer.

Antiadenovirus type 5 antibody assay. Antiadenovirus type 5 antibody concentrations were measured in serum from each patient with an ELISA-based assay. Serum samples from a predose dilution series were assessed for antiadenovirus antibodies to establish the baseline titer for each patient. Serum was diluted to the predose titer, and then 1:2 and 1:4 dilutions of the predose titer dilution were made for sample testing at each time point. Antibodies then were measured at the predose titer and in each dilution. In this quasi-quantitative assay, antibody titer results greater than twice the predose titer were considered significant.

Supplemental Results

Sensitivity analysis of primary end point. To assess the impact of off-schedule disease assessments on the primary efficacy end point, a sensitivity analysis was conducted in which 12 months was defined according to the assessment date as opposed to the nominal month-13 assessment. Results for the sensitivity analysis were identical to the primary efficacy end point: 14 of 40 patients (35%) overall showed high-grade recurrence free survival at 12 months and experienced comparable incidences for the dose groups (low-dose: n = 7 of 21 [33%]; high-dose: n = 7 of 19 [37%]).

IFNα-2b serum concentrations. Serum IFNα-2b concentrations were low. At day 2 of month 1, 31 of the 40 patients had concentrations less than 31 IU/mL (limit of assay quantification). Six patients had concentrations greater than 31 IU/mL but less than 50 IU/mL, and two patients had concentrations greater than 50 IU/mL but less than 160 IU/mL.

Blood and Urine rAd–IFNα DNA measurements. Median blood and urine concentrations of rAd DNA were measured with a qPCR assay that had a level of detection of 1×10^3 vp/225 μL. Importantly, no measurable rAd–IFNα DNA was detected in blood after the initial dosing. Of the 23 patients who received a second dose at month 4, only one patient (4.3%), randomly assigned to the 3×10^{11} vp/mL dose group, had a positive test result for a low level of virus detected at day 2 of month 1 (7.7×10^3 vp/225 μL), which was not measurable by day 4 of month 1.

As expected, all 40 patients had significant copies of rAd DNA in their urine at day 2 of month 1; the median value was 1.13×10^6 vp/225 μL. Thirty-nine patients had measurable concentrations of rAd DNA copies at day 4 of month 1. However, these were approximately three orders of magnitude lower; the median value was 8.08×10^4 vp/225 μL. Thirty-three patients (85%) had measurable concentrations at day 12 of month 1, and the median value was 2.3×10^4 vp/225 μL. In the 23 patients who received a second dose of rAd–IFN, 22 had measurable concentrations of rAd DNA at day 2 of month 4 and a median value of 5.13×10^5 vp/225 μL, and 20 patients had measurable concentrations of approximately eight times the level of detection at day 4 of month 4 and a median value of 8.45×10^3 vp/225 μL. By day 12 of month 4, only six patients (29%) had measurable copies of rAd–IFNα DNA in the urine. Results for the two dose cohorts were comparable.

Anti-IFNα antibody and antiadenovirus antibody concentrations. Anti–IFNα-2b antibody concentrations in serum were measured in serum from each patient. With the sole exception of one patient who had a weak 1:20 titer at day 12 of month 1, no other patient at any time point had measurable anti–IFNα-2b antibodies. Antiadenovirus type 5 antibody concentrations were measured in serum from each patient with a quasi-quantitative assay (see Covance Laboratories, Harrogate, UK for details).

Antibody data were collected at days 1 and 12 of month 1, day 1 of month 7, day 1 of month 10, and at the month-13/withdrawal assessment. The data demonstrated that 22 patients (55.0%) had a significant antiadenovirus antibody response (defined as four times the predose titer). Of the 14 patients who experienced a complete response, 10 (71%) had a significant antiadenovirus antibody response, and four (29%) did not demonstrate a significant response. These data suggest that a significant antiadenovirus vector antibody response does not appear to correlate with lack of efficacy. A definitive antibody titer for any of the positive patients was not determined.

Safety

A summary of all treatment-emergent adverse events is provided in the Data Supplement.