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Phase 1b trial to evaluate tissue response to a second dose of intravesical recombinant adenoviral interferon α 2b formulated in Syn3 for BCG failures in non-muscle invasive bladder cancer

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Abstract

Purpose—A phase 1b trial was conducted to evaluate duration of production of IFN α following intravesical administration of recombinant adenovirus mediated interferon α 2b (Ad-IFN) formulated with the excipient Syn3. The primary aim was to determine if a second instillation three days after initial treatment produced prolonged urinary IFN production.

Patients and Methods—Seven patients with recurrent NMIBC after BCG were enrolled. Each treatment consisted of intravesical instillation of SCH721015 (Syn3) and Ad-IFN at a concentration of 3×10^{11} particles/mL to a total volume of 75mL given at day 1 and 4. Patients were followed for 12 weeks during which the magnitude and duration of gene transfer was determined by urine IFN α levels. Drug efficacy was determined by cystoscopy and biopsy and patients with a lack of recurrence at 12 weeks were eligible for second course of treatment.

Results—Seven patients were treated with an initial course (instillation on days 1 and 4), two had a complete response at 12 weeks and received a second course of treatment. One patient remained without evidence of recurrence after a second course (total 24 weeks). One patient suffered a non-treatment associated adverse event. Despite a transient rise in IFN α levels, sustained production was not demonstrated.

Conclusion—Ad-IFN α intravesical therapy has previously shown promising drug efficacy. A prior phase 1 trial with a single instillation compares similarly to the current study and suggests a second instillation is not necessary to achieve sufficient urinary IFN α levels.

Introduction

The American Cancer Society estimates 74,000 new bladder cancer cases and 16,000 bladder cancer deaths for 2015 in the United States. Therapy generally consists of frequent surveillance cystoscopy and resection for non-muscle invasive bladder cancer (NMIBC) with the use of intravesical therapy in patients with intermediate and high risk NMIBC. Radical

cystectomy, with or without the addition of systemic chemotherapy, is generally reserved for muscle invasive bladder cancer (MIBC) and also remains the treatment of choice for high risk NMIBC refractory to first line intravesical therapy. Intravesical Bacillus Calmette-Guerin (BCG) therapy has proven highly effective as primary therapy for high risk NMIBC with 5 year recurrence free survival rates of 60% achieved with maintenance therapy¹.

Unfortunately this leaves a substantial minority of patients with NMIBC who go on to fail BCG therapy in an era with only modestly effective second line intravesical therapies². In fact, NMIBC response rates are so poor after BCG failure that the most recent European Association of Urology guidelines state, "...treatments other than radical cystectomy must be considered oncologically inferior at the present time"³. Despite these recommendations, patients and clinicians are frequently challenged by patient factors including severe medical comorbidities and reluctance to undergo radical cystectomy, in no small measure due to the high complication rate⁴ in the perioperative setting. For this reason much interest has remained focused on the development of novel second line intravesical therapies for refractory NMIBC.

Among the most promising have been novel immunologic therapies directed at enhancing the native immune response in the bladder. Our previous phase 1 trial evaluated gene therapy via adenoviral mediated recombinant interferon alpha 2b (Ad-IFN α) formulated with the excipient SCH 209702 (Syn3), an agent which enhances gene transfer⁵. Through both direct and indirect cellular mechanisms.

Ad-IFN α has shown preclinical activity against malignant cells while sparing normal urothelium. The primary aim of the phase 1 trial was drug safety after a single instillation in patients with recurrent NMIBC after initial BCG plus maintenance therapy. Notably no dose limiting toxicity was encountered despite a dose escalation design. Additionally, clinical efficacy at three months demonstrated a complete response rate of 43%⁵ despite urinary IFN α levels returning to baseline levels in the majority of patients by 10 days following treatment⁵. In preparation for a phase 2 study we set forth to determine if additional intravesical instillations of Ad-IFN α could produce sustained IFN α production and possibly improve clinical efficacy.

In an orthotopic mouse model of human bladder cancer, a second instillation of intravesical Ad-IFN /Syn3 treatment produced a two-fold increase in urine IFN α levels (100,000 vs. 50,000 pg/mg) with associated prolonged IFN α elevations⁶. To aid in dose scheduling for a planned phase 2 trial we conducted a phase 1b trial of two instillations of Ad-IFN α /Syn3 in patients with recurrent NMIBC. We hypothesized that a second instillation would result in increased and prolonged IFN α production.

Material and Methods

Eligibility and Objectives

This was a single institution, single arm, phase 1b trial. Patients who had NMIBC refractory to BCG therapy and who refused radical cystectomy were considered eligible. BCG-refractory NMIBC was defined as recurrence or persistence of T1 bladder cancer after

treatment with a six-week induction course and at least one additional course of BCG (either a three-week maintenance course or a repeat six-week induction course). Additional study entry requirements remained unchanged from the phase 1 trial⁵. The study was approved by the University of Texas MD Anderson Cancer Center institutional review board.

The primary study endpoint was to determine if a second instillation of study drug at day four improved gene transfer efficacy as determined by the magnitude and duration of urinary INF α . Secondly, complete response, as defined by lack of tumor recurrence at three months following treatment was evaluated.

Treatment

As previously published, patients underwent intravesical treatment with Ad-IFN α constituted in the excipient Syn3⁵. The results of the prior trial determined the optimal concentration of Ad-IFN α to be 3×10^{11} particles per mL. A total volume of 75mL was constituted in a solution of Syn3 at a 1mg/mL concentration. Instillations were allowed to dwell for 1 hour and were administered at day one and four on an outpatient basis. Complete responders were allowed a second treatment cycle three months after the initial treatment.

Evaluation of Gene Transfer Efficacy

Urine samples were obtained pre-treatment and for 14 days following the initial treatment dose. Urine IFN α concentrations were determined on samples collected on ice and stabilized by the addition of buffer containing 10% bovine serum albumin and 50 mM (4-{2-hydroxyethyl}-1-piperazineethanesulfonic acid) HEPES (1 mL buffer to 10 mL of urine). IFN α levels were determined by ELISA from R&D Systems Inc. utilizing the manufacturer's protocol with the extended range standard curve as previously described⁵.

Results

Clinical Outcomes

A total of seven patients were enrolled and underwent protocol treatment. Salient clinical, pathologic, and demographic features are summarized in Table 1. All patients were male with a mean age of 77 years at onset of treatment (range 68 – 83 years). One patient (patient 1) withdrew 7 weeks after treatment due to worsening lower urinary tract symptoms and he represents the current study's only adverse event. Two of the seven patients (29%) had a complete response at three months (patients 4 and 7) and were eligible for a second treatment cycle. CRs following treatment cycles occurred in 2 out of 7 patients for an overall clinical efficacy of 29%.

One patient with T1 high grade disease at enrollment achieved a 3 month CR and remained disease free after the second treatment cycle (180 days after initial enrollment). After study completion all patients continued to receive care at our center and during prolonged follow up eventually all had disease recurrence and were recommended to undergo radical cystectomy. One patient refused and was lost to follow up (patient 6). Of the remaining six patients, 5 had organ confined, lymph node negative, disease at cystectomy and remain disease free. Patient 1 was found to have non-organ confined disease with multiple positive

lymph nodes at radical cystectomy. He succumbed to disease 20.1 months following study treatment and 16.7 months following radical cystectomy. Median follow up was 29.4 months (range 20.1 – 36.0 months) from day one of treatment to last confirmed contact with each patient.

Urinary IFN α Levels

Urinary IFN α levels over time are summarized in figure 1A for all instances, including the retreatments of patients 4 and 7. Three patients demonstrated transient secondary peaks in urinary IFN α after the second instillation and are highlighted in figure 1b. Despite these second peaks, sustained IFN α levels were not demonstrated and all patients returned to baseline levels by day 10.

Discussion

Bladder cancer is a common malignancy hallmarked by recurrences in NMIBC and frequently life threatening disease in MIBC. Although intravesical BCG remains the gold standard treatment for initial high risk NMIBC, recurrent/refractory NMIBC poses a clinical challenge. The high response rates seen in patient treated with BCG for primary CIS of close to 70%⁷ far over shadow those of second line therapies^{2,8–11}. For this reason, radical cystectomy remains the treatment of choice in eligible patients with recurrent NMIBC following optimized intravesical BCG therapy.

Patients however are frequently reluctant to undergo extirpative surgery and they are not without cause. Radical cystectomy is notable for an overall complication rate of 64% and a high grade complication rate of 13%¹². Additionally 30 day mortality rates range from 1.5 to 3%^{12,13} and add to this the impact of urinary diversion, which is the major source of complications following radical cystectomy⁴, it is not difficult to see why patients commonly seek second line therapies despite inferior oncologic outcomes.

To address this clinical need, novel second line agents for NMIBC are in desperate need. Numerous groups have primarily focused these efforts on cytotoxic intravesical chemotherapies. Unfortunately chemotherapeutics are well established to be inferior to BCG for the primary therapy of NMIBC¹⁴. Worse still are the results of intravesical chemotherapy as second line therapy, as demonstrated by the 1 year recurrence free survival rate of 21% seen in a phase 2 trial of Gemcitabine in patients who had recurrent NMIBC after BCG therapy¹¹. More promising results are achievable with the addition of heated chemotherapy as in the case of mitomycin-c thermo-chemotherapy, however recurrence free rates remain low at 56%¹⁵. One fact remains, the most effective intravesical therapy for NMIBC is BCG, an immune modulating therapy.

We have that shown that Ad-IFN α , acts through both direct and indirect mechanisms to produce cell death which implies that Ad-IFN α , can cause tumor cell death by other mechanisms including the production of bystander factors killing tumor cells beyond immunomodulation and the IFN α produced. We therefore developed the novel therapeutic, Ad-IFN α /Syn3, to effect gene transfer within the bladder and exploit these mechanisms of tumor cell kill against NMIBC. Our first human trial of using this gene therapy produced

promising results and a favorable toxicity profile. In this study a 43% CR at 3 months in a patient population enriched for resistant disease was found, namely those who have failed prior intravesical BCG plus maintenance therapy⁵. Additionally, despite dose escalation, no dose limiting toxicities were encountered⁵. With promising clinical efficacy and minimal side effects we are currently planning a larger multi-institutional phase 2 trial. To better inform on the optimal dosing strategy for this trial we sought to elucidate in the current study whether or not a second instillation of Ad-IFN α /Syn3 on day 4 of the treatment cycle provided an advantage to a single treatment. This was informed by preclinical animal studies where a second instillation did result in prolongation of IFN α production.

Our published pre-clinical data demonstrating prolonged, robust IFN α production with added instillations of Ad-IFN α /Syn3 in an orthotopic mouse model⁶. In that study a second instillation improved IFN α levels by two-fold (from 50,000 pg/mL to 100,000 pg/mL) and resulted in sustained production with IFN α levels for seven days, the full duration of the analysis. However, when designing the phase 1 trial, in an effort to limit the potential for toxicity a single instillation was used. Although we were encouraged by the clinical activity in that study, we hypothesized that treating with two instillations rather than one could increase the amount and duration of IFN α production. As a result, we conducted the current study to determine if IFN α levels analogous to our pre-clinical experience could be achieved. If so, this would serve as the basis for the treatment scheduled used in the planned phase 2 trial. Despite a small patient population the data would suggest no improvement across all participants and as such we concluded the study after enrollment and analysis of seven patients. Our study size falls in line with analogous small patient populations for other phase 1 studies (Table 2) with BCG refractory NMIBC. Nonetheless these data support the design and implementation of a future single instillation phase 2 study, and reinforce the potential for significant clinical activity after even transient gene transfer and expression.

Conclusion

Ad-IFN α /Syn3 is a novel gene therapy for recurrent BCG resistant NMIBC. Short term clinical activity is promising and two instillations versus one does not improve efficacy of significantly changed overall IFN α levels produced.

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Synopsis

A phase 1b trial to examine the clinical efficacy of a second instillation of adenoviral INF α 2b in patients with BCG refractory non-muscle invasive bladder cancer. This trial served as the basis for the dosing schedule of a phase 2 trial that is ongoing.

Figure 1. Urinary IFN α levels

A. Urine interferon- α (IFN α) levels are shown in all 7 patients at various times after intravesical treatment on day 1 and day 4. B. Urine IFN α levels are shown in individual patients who showed an increase in IFN α after the day 4 retreatment.

Table 1

Patient Demographics

Patient	Age	Disease at Enrollment	CR at 3months	Organ Confined*	Follow Up (Months)	Disease Status
1	68.3	Ta-High Grade + Tis	No	No	20.1	DOD
2	79.4	Tis	No	Yes	35.3	NED
3	82.6	Ta-High Grade + Tis	No	Yes	33.9	NED
4	76.0	Ta-High Grade	Yes (Retreated)	Yes	36.0	NED
5	68.9	Ta-High Grade + Tis	No	Yes	23.9	NED
6	76.2	Tis	No	Refused	26.4	AWD
7	81.0	T1-High Grade	Yes (Retreated)	Yes	29.4	NED

At radical cystectomy. DOD = dead of disease; NED = no evidence of disease; AWD = alive with disease

Table 2

Phase 1 Trials for Refractory/Recurrent NMIBC

Author	Treatment	Study Size (n)
Dinney et al ⁵	Intravesical Ad-INF α	17
McKiernan et al ¹⁶	Intravesical Docetaxal	18
Bassi et al ¹⁷	Intravesical Paclitaxel-Hyaluronic Acid	16
McKiernan et al ¹⁸	Intravesical Nanopartical Albumin Bound Paclitaxel	18