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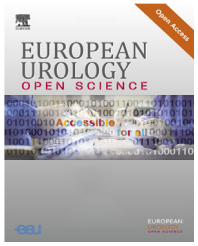

European Association of Urology

Brief Correspondence

Intravesical Ty21a Treatment of Non–muscle-invasive Bladder Cancer Shows a Good Safety Profile

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Abstract

Standard-of-care immunotherapy for non–muscle-invasive bladder cancer (NMIBC) with intravesical Bacillus Calmettte-Guérin (BCG) is associated with adverse events (AEs), disease recurrence/progression, and supply shortages. Preclinical data have shown that intravesical instillation of Ty21a/Vivotif, the oral vaccine against typhoid fever, may be an effective and safer alternative to BCG. We assessed the safety of intravesical Ty21a in NMIBC. For ethical reasons, patients with low- or intermediate-risk NMIBC not requiring BCG immunotherapy were enrolled. To determine the maximum tolerated dose, escalating doses of Ty21a/Vivotif were intravesically instilled in three patients once a week for 4 wk in phase 1a. In phase 1b, ten patients received the selected dose (1×10^8 CFU) once a week for 6 wk, as for standard BCG therapy. At this dose, all patients completed their treatment. Most patients experienced minor systemic AEs, while half reported mild local bladder AEs. AEs only occurred after one or two instillations for 40% of the patients. Ty21a bacteria were only recovered in three out of 72 urinary samples at 1 wk after instillation. Intravesical Ty21a might be well tolerated with no cumulative side effects, no fever >39 °C, and lower risk of bacterial persistence than with BCG. Ty21a treatment thus warrants clinical trials to explore its safety and antitumor efficacy in high-risk NMIBC. This trial is registered on ClinicalTrials.gov as NCT03421236.

Patient summary: We examined the safety of a new intra-bladder immunotherapy for non–muscle-invasive bladder cancer as an alternative to the standard BCG treatment. Our data show that the Ty21a vaccine might be well tolerated. Further studies are needed to determine the safety and antitumor efficacy of this treatment.

Some 70% of bladder cancers are non–muscle-invasive bladder cancer (NMIBC) at diagnosis [1]. The gold standard treatment for reducing the recurrence and progression of high-risk lesions is intravesical Bacillus Calmette-Guérin (BCG) immunotherapy. However, repeated BCG treatments are associated with significant side effects [2] and treatment

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failure (30–50%) [3], and there are currently manufacturing shortages [4], so there is a need for alternative or complementary treatments. The highly attenuated *Salmonella enterica* serovar Typhi strain Ty21a was obtained almost 50 yr ago via mutagenesis [5]. Ty21a was included in a commercial oral vaccine against typhoid fever (Vivotif) that has an excellent safety profile confirmed worldwide in more than 200 million vaccinees over the last 30 yr [6]. We provided preclinical evidence [7,8] of its safe intravesical use for induction of bladder tumor regression in an immunocompetent mouse model that closely mimics NMIBC [9]. Here we report on the safety of intravesical Ty21a in NMIBC patients. For ethical reasons, only patients with low- or intermediate-risk NMIBC not requiring BCG were enrolled. This trial is registered on ClinicalTrials.gov as NCT03421236.

In this open-label phase 1 dose escalation study, 15 patients were recruited between May 2018 and May 2021 at our institution. Male and female patients with histological confirmation of low- or intermediate-risk NMIBC not requiring BCG treatment were included (Supplementary Table 1). A traditional 3 + 3 design for a dose escalation trial was adopted (Supplementary material) and adverse events (AEs) were graded using the Common Terminology Criteria for Adverse Events classification scheme. In phase 1a, eight patients were screened and five were enrolled (Fig. 1A). Among the first three patients (group A; Supplementary Table 1), who received the starting minimal dose of 1×10^8 colony-forming units (CFU) once weekly for 4 wk, two (67%) experienced mild AEs (grade 1; Table 1). A five-fold higher dose (5×10^8 CFU) was then instilled in a fourth patient (group B; Fig. 1A, Supplementary Table 1), who experienced a strong inflammatory syndrome (grade 2) 3 d later, with spontaneous remission within 48 h (Supplementary material). An intermediate dose was thus instilled in a fifth patient (2.5×10^8 CFU, group H1; Fig. 1A, Supplementary Table 1). At 1 wk after the first instillation, urinary culture revealed persistence of 1×10^6 CFU/ml of Ty21a bacteria and the patient reported mild (grade 1) local bladder and systemic AEs that all cleared after antibiotic treatment (Supplementary material). On the basis of these AEs, we considered 1×10^8 CFU of Ty21a as the maximum tolerated dose for phase 1b, which was instilled in ten patients once a week for 6 wk (group F; Fig. 1B, Supplementary Table 1). All patients completed the study. Most (n = 9, 90%) experienced minor systemic AEs (mainly general malaise and grade 1 AEs; Table 1) and half (n = 5, 50%) experienced mild (grade 1 or 2) local bladder AEs (mainly chemical cystitis and just one instance each of frequency and hematuria; Table 1). Four of the patients (40%) experienced AEs only after one or two instillations, while two patients (20%) reported AEs after five or six instillations. In addition, 30% of the patients reported AEs only after the fourth instillation.

Comparison of the frequency of instillations with systemic AEs (16.7% in group A and 41.7% in group F) and local bladder AEs (8.3% in group A and 18.3% in group F) shows that a majority of the Ty21a instillations were innocuous (Supplementary Table 2). Furthermore, no cumulative side effects were observed, as neither the number of patients reporting systemic or local bladder AEs nor the total number of systemic or local bladder AEs per instillation increased with successive intravesical Ty21a instillations

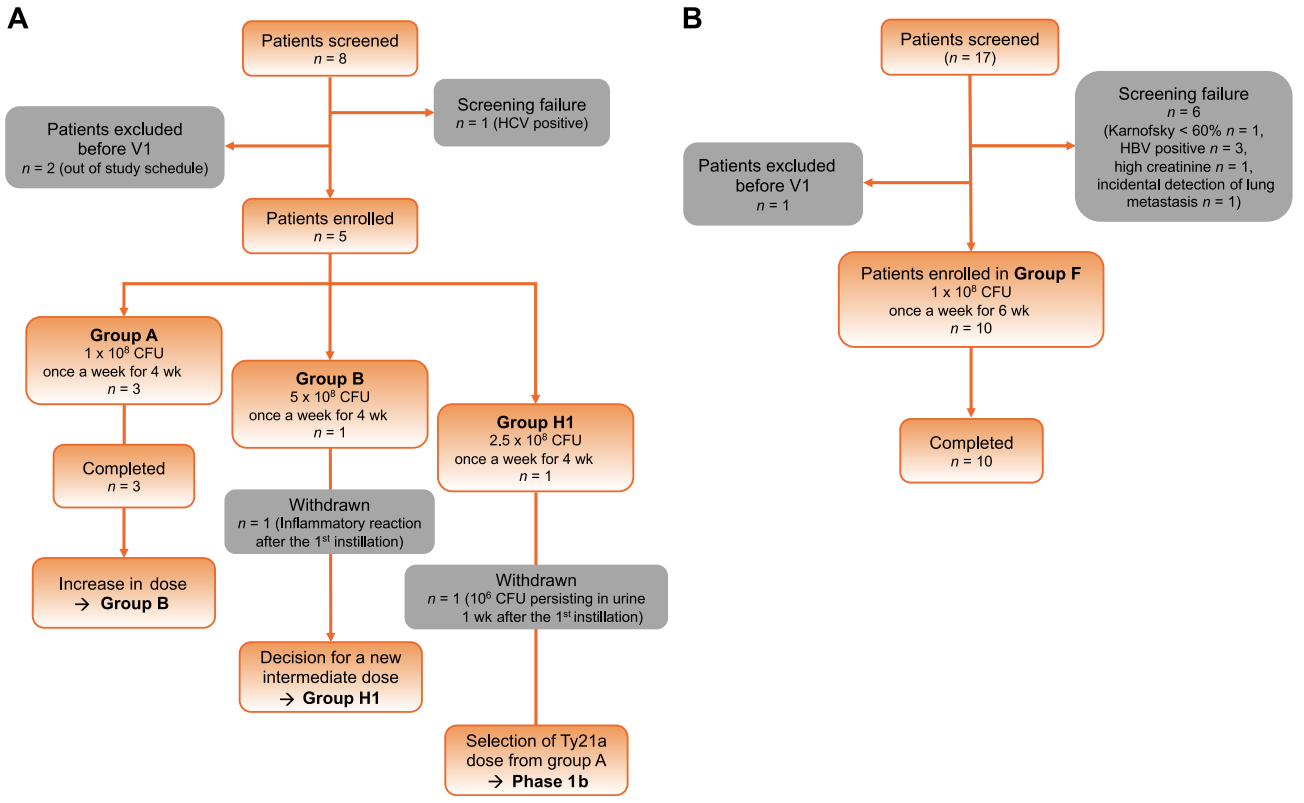

Fig. 1 – Study flow diagram. Patients screened and included in (A) phase 1a and (B) phase 1b. CFU = colony-forming units; HBV = hepatitis B virus; HCV = hepatitis C virus; V1 = first visit.

Table 1 – Systemic and local bladder AEs per patient[a]

	Patients, *n* (%)					
	Group A (*n* = 3)			Group F (*n* = 10)		
	Grade 1	Grade 2	Grades 1 + 2	Grade 1	Grade 2	Grades 1 + 2
Systemic AEs						
General malaise[b]	1 (33.3)	0	1 (33.3)	5 (50)	1 (10)	6 (60)
Fever >39 °C	0	0	0	0	0	0
Arthritis	0	0	0	0	0	0
Allergic reactions	0	0	0	0	0	0
Fever >38 °C [c]	0	0	0	3 (30)	0	3 (30)
Others[d]	1 (33.3)	0	1 (33.3)	3 (30)	0	3 (30)
Any systemic AE	2 (66.7)	0	2 (66.7)	8 (80)	1 (10)	9 (90)
Local bladder AEs						
Bacterial cystitis	1 (33.3)	0	1 (33.3)	1 (10)	0	1 (10)
Chemical cystitis[e]	0	0	0	2 (20)	1(10)	3 (30)
Frequency >1/h	0	0	0	0	1 (10)	1 (10)
Hematuria	0	0	0	1 (10)	0	1 (10)
Ty21a cystitis[f]	0	0	0	0	1 (10)	1 (10)
Other[g]	0	0	0	0	1 (10)	1 (10)
Any local bladder AE	1 (33.3)	0	1 (33.3)	3 (30)	2 (20)	5 (50)
Any systemic or local bladder AE	2 (66.7)	0	2 (66.7)	6 (60)	3 (30)	9 (90)
AEs after installations						
After 1–2 instillations	1 (33.3)	0	1 (33.3)	4 (40)	0	4 (40)
After 3–4 instillations	1 (33.3)	0	1 (33.3)	2 (20)	1(10)[i]	3 (30)
After 5 instillations	NA	NA	NA	0	2 (20)[j]	2 (20)
AEs only after the 4th dose	0	0	0	3 (30)	0	3 (30)
Asymptomatic urinary Ty21[h]	0			2 (20)		

AE = adverse event; NA = not applicable.

[a] AEs were graded using the Common Terminology Criteria for Adverse Events classification scheme.

[b] Flu-like symptoms, shaking chills, headache, discomfort, and/or fatigue.

[c] Three patients experienced 1-d fever (38.8 °C after the second instillation in patient 17F05, 38.9 °C after the first instillation in patient 22F07, and 38.5 °C after the second instillation in patient 23F08).

[d] Erythema, arthralgia, loss of appetite, and/or diarrhea.

[e] Urgency, urinary incontinence, urinary tract pain, and/or dysuria in the absence of urinary bacteria.

[f] Cystitis symptoms with concomitant urinary Ty21a detection (10^4 CFU/ml 1 wk after the second instillation in patient 19F06).

[g] Right hypochondrium pain.

[h] Urinary 10^4 CFU/ml 1 wk after the third instillation in patient 12F03 and 10^3 CFU/ml 1 wk after the first instillation in patient 23F08.

[i] experienced a Grade 2 AE after three instillations.

[j] One patient experienced a grade 2 AE after one instillation and one patient experienced a grade 2 AE after four instillations.

(Supplementary Table 3), which differs from results observed after intravesical BCG instillations [10,11]. In comparison to historical BCG side effects [12,13], Ty21a appears to induce fewer local bladder AEs, but more systemic AEs, although no high fever (>39 °C) was reported and general malaise was the most common minor systemic AE. Quite unexpectedly in the context of the mild AEs observed with the selected Ty21a dose, first instillation of a fivefold or 2.5-fold higher dose resulted in AEs that dissuaded us from further testing these doses. Of note, the recovery of a relatively high number of live Ty21a bacteria in the urine of the patient in group H1 is in contrast to our preclinical study, in which Ty21a bacteria did not persist in bladder tissues [7]. Shedding of Ty21a bacteria in the stool of vaccinees is a rare event, only occurring within the first 24 h after oral vaccination [6]. Although ingestion of higher doses ($3–10 \times 10^{10}$ CFU, tenfold higher than the usual oral vaccine dose) results in excretion in the stools of approximately 30% of recipients after 1 d, no bacteria were recovered 3 d later, strongly suggesting the inability of Ty21a to proliferate in vivo, at least in the gastrointestinal tract [14]. It is also highly unlikely that Ty21a can replicate in the urinary tract given the harsh environment of urine [15], the metabolic impairments in Ty21a [14], and the rare recovery (mostly asymptomatic) of wild-type *S. enterica* serovar Typhi from urine, even in endemic areas of typhoid fever [16]. In our study, intravesical instillation of 1×10^8 CFU resulted in recovery of Ty21a bacteria ($10^3–10^4$ CFU/ml) in only three out of 72 urinary samples collected 7 d after instillation (Table 1), one from a symptomatic patient with cystitis and the other two from asymptomatic patients. This suggests that persistence of Ty21a in the urinary tract is also a rare event at the selected dose, which should not jeopardize safety. This is in contrast to BCG bacteria, which are found in approximately 30% of urine samples 7 d after intravesical instillation [17] and whose subsequent local or disseminated infection can be the cause of the most severe, albeit rare, AEs associated with BCG immunotherapy [2]. Overall, our data suggest that intravesical Ty21a might be well tolerated with no cumulative side effects, no fever >39 °C, and a lower risk of bacterial persistence than with BCG.

Despite renewed interest in microbial cancer immunotherapy, intravesical BCG is still the only bacterial cancer therapy approved for clinical use. Building on our promising preclinical data, although limited by the small population included, our study shows that another commercial bacterial vaccine, Ty21a, is promising when used for intravesical instillation in NMIBC, with few and mild AEs at the selected dose. Future clinical trials are warrant to evaluate the safety and efficacy of intravesical Ty21a in reducing NMIBC recurrence and progression in high-risk patients and to explore the mechanisms underlying its effects.

Author contributions: Denise Nardelli-Haefliger had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Derré, Jichlinski, Lucca, Nardelli-Haefliger.
Acquisition of data: Benmerzoug, Bohner, Cesson, Chevalier, Crettenand, Dartiguenave, Derré, Domingos-Pereira, Jichlinski, Masnada, Lucca, Nguyen, Polak, Rodrigues-Dias, Roth, Schneider, Texeira-Pereira.
Analysis and interpretation of data: Cesson, Derré, Lucca, Nardelli-Haefliger.
Drafting of the manuscript: Cesson, Derré, Lucca, Nardelli-Haefliger.
Critical revision of the manuscript for important intellectual content: Benmerzoug, Bohner, Cesson, Chevalier, Crettenand, Dartiguenave, Derré, Domingos-Pereira, Jichlinski, Masnada, Lucca, Nguyen, Polak, Rodrigues-Dias, Roth, Schneider, Texeira-Pereira.
Statistical analysis: Nardelli-Haefliger.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2022.09.004.

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