



Intravesical bacillus Calmette-Guérin for bladder cancer: are all the strains equal?

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Abstract: Intravesical immunotherapy with bacillus Calmette-Guérin (BCG) is the standard of care for high-risk and intermediate-risk non-muscle-invasive bladder cancer (NMIBC). Several BCG strains are available. Despite originating all from subcultures of the same *Mycobacterium*, strains are genetically different which may lead to differences in treatment efficacy and adverse events. Identification of a more efficient strain and assessing its optimal administration schedule may improve oncological outcomes in NMIBC, specifically because of the worldwide shortage in BCG availability. This review focused on the antitumor effect of different BCG strains with a particular emphasis on the evidence underlying BCG dose and treatment schedules.

Keywords: Bladder cancer; bacillus Calmette-Guérin (BCG); strains; intravesical; immunotherapy

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Background

Trans-urethral resection of the bladder (TURB) followed by adjuvant intravesical instillation with bacillus Calmette-Guérin (BCG) is the most effective conservative therapy for the treatment of high-risk non-muscle-invasive bladder cancer (NMIBC) (1-3). It can significantly reduce the probability of both disease recurrence and progression, thereby potentially improving survival (4,5). However, treatment schedules vary widely in clinical practice and there is a significant lack of adherence to international guideline recommendations for its optimal use (6,7). While current guidelines do not make a clear recommendation regarding preferences for a BCG strain over the other, there is mounting data underlying clinically significant differences (1,8,9). Passaging and subculturing through different

distributors over the last decades may have, indeed, selected mycobacteria with differential biological activity profiles, virulences and reactogenicities (10,11). Various retrospective and prospective have aimed to compare the efficacy of different strains. Unfortunately, most with variable study designs, assertion bias, lead time bias, heterogeneity bias, lack of statistical power and study design issues such as maintenance cycles (12).

In this review, we sought to summarize the current evidence on the efficacy of different BCG strains in the treatment of NMIBC.

The BCG family tree

The first BCG strain was developed at the Pasteur institute in 1921 from an attenuated strain of *Mycobacterium bovis*.

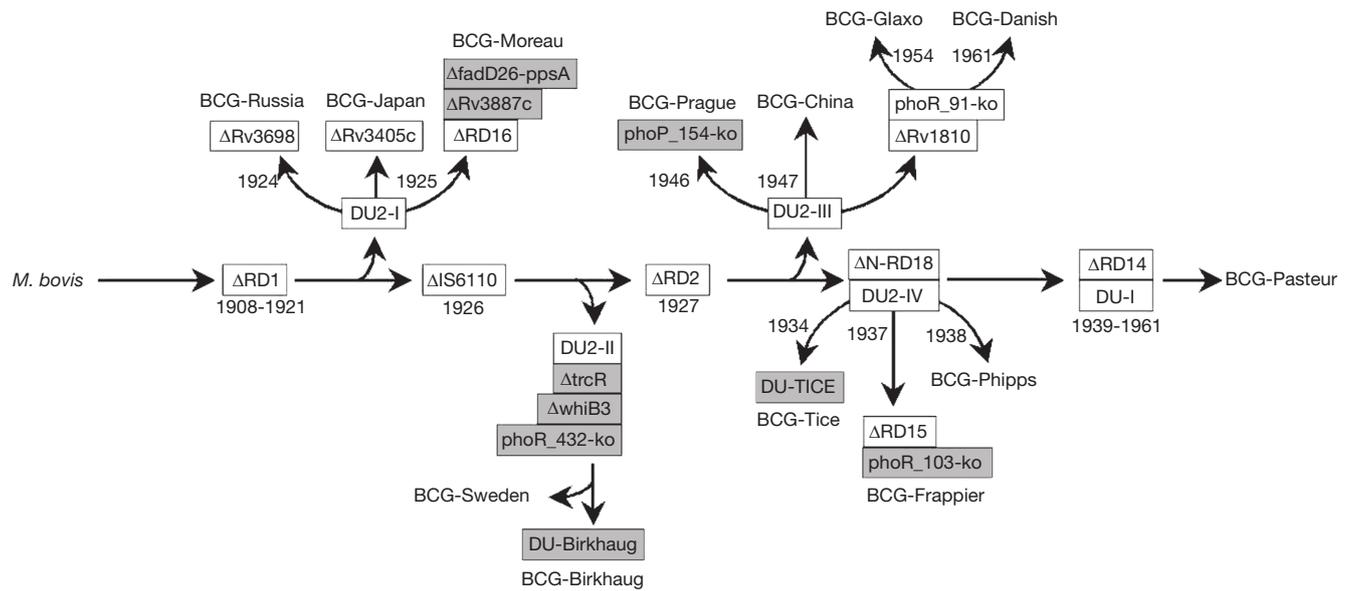


Figure 1 Phylogenetic tree of BCG highlighting genetic markers characterizing different strains proposed by Leung *et al.* (11). BCG, bacillus Calmette-Guérin.

Over the course of decades several laboratories developed their own daughter BCG strain and its primary use was in the prevention of tuberculosis. In 1976, Morales *et al.* used BCG for the first time in the treatment of bladder cancer. Using the vaccine manufactured by the Institute Armand Frappier (Montreal), these investigators demonstrated a favorable impact of the compound on bladder cancer recurrence rates (13). Subsequent studies confirmed the efficacy of BCG in reducing disease recurrence and, even, progression rates in appropriately selected patients with NMIBC. Federal Drug Administration approval for the treatment of high-grade NMIBC was finally obtained in 1990.

During decades the subculturing process of BCG, also known as passaging, caused a genetic evolution of the original strain (14,15). Therefore, it would be more appropriate referring to BCG strains in the plural, as they differ from a biologic, genetic point and probably clinical point of view. Studies on DNA sequences and their correlation with genetic and phenotypic differences revealed that BCG daughter strains have extensive differences in the level of expression of surface proteins and immunodominant protein antigens that influence virulence and reactogenicity (Figure 1) (10,11). Comparative genomics identified regions of difference (RD) such as deletions and insertions, which are used as genetic markers. These critical gene mutations

ultimately define the branches in the phylogenetic tree of BCG. The loss of RD1, for example, is the fundamental difference between *M. bovis* and BCG, and is present in all subsequent cultured strains (Figure 1).

Despite the long clinical experience with this compound, its mechanism of action is incompletely understood. The main antitumor effect relies on immune system stimulation with CD4+ and CD8+ lymphocytes, natural killer cells and granulocytes recruitment (16,17). To allow this process, attachment of BCG to the urothelium and internalization by cancer cells is required (18-20).

The ability of BCG to bind the urothelium is primarily mediated by fibronectin (21). The genetic evolution of BCG and the changes in protein expression could generate the hypothesis that there might be a clinical difference between strains. In an *in vitro* study, Hudson *et al.* could not corroborate this hypothesis. Authors found similar binding activities between three commercially available strains (BCG Tice, BCG Connaught, and BCG Armand Frappier) (22). The caveat being that these three strains are genetically closely related, which may have led to this lack of difference.

Ikeda *et al.* compared the characteristics of BCG Tokyo 172 with BCG Connaught in a mouse model. The mean percent of cancer cells with attached bacilli was 60.98% with BCG Tokyo-172 compared to only 13.13% with BCG Connaught (23). These findings suggest that BCG

substrains differ also in their biological activity as well as their interaction with the host. Whether this translates in different response rates and, consequently, clinical outcomes have still to be elucidated.

BCG concentrations

The guiding principle in drug administration is to maximize efficacy while minimizing toxicity. In the case of BCG, there has been no phase I trial, and the first administered dose of 120 mg [10^6 – 10^8 colony forming units (cfu)] by Morales *et al.* was partly arbitrary and partly based on animal studies (13). What the lowest dose of BCG is to maintain current efficacy is question of importance, especially in the current shortage of BCG availability. The Club Urológico Español de Tratamiento Oncológico (CUETO) 95011 trial compared 27 mg of BCG Connaught *vs.* 13.5 mg BCG Connaught *vs.* 30 mg Mitomycin. The authors conclude that 13.5 mg BCG Connaught has the same efficacy as 30 mg Mitomycin at the cost of higher toxicity and that 27 mg BCG Connaught seems to be the minimum effective dose for adjuvant treatment (24). Nevertheless, the short follow-up of 5 months in that study does not allow definitive conclusions.

Retrospective studies deliver controversial evidence. Single arm series evaluating dose reduction (i.e., 75 mg BCG Pasteur) showed similar outcomes compared to other series at the time (25–29).

In contrast, series directly comparing the administration of full with reduced dose of BCG showed that the full dose could achieve a better tumor eradication as well as longer recurrence-free survival. This was true independently of the investigated strain (30,31).

A small series evaluated the efficacy of 75 mg BCG Pasteur. During the study, the strain production was interrupted and had to be replaced by 27 mg BCG Connaught. Both compounds achieved comparable response rates, but numbers were small in both groups (n=32 and n=25) (32).

What these studies have in common, is the superior effect of full dose BCG to reduced dose in patients with multiple risk factors such as tumor multifocality and higher recurrence rates, suggesting a potential for a risk adapted dosage. This issue was addressed by the randomized EORTC 30962 study which compared a full-dose and one-third dose of intravesical BCG TICE, both with a 1- or 3-year maintenance course. Full-dose BCG was not superior to one-third dose BCG (5-year DFS: 62% *vs.*

59%; $P=0.092$). However, subgroup analyses revealed that in high-risk patients, 3 years of maintenance with full dose BCG significantly reduced recurrence rates compared to one third dose ($P=0.009$). There were no significant differences in side effects based on dose or duration of maintenance schedule given (33).

In a CUETO randomized trial including 500 patients showed no significant difference in disease-free survival, progression-free survival or overall survival between patients treated with a full dose of BCG (81 mg) compared to a one third reduced dose of BCG (27 mg) (34). Nevertheless, one should be aware of the lacking statistical power of the study to prove equivalence or non-inferiority beyond clinically acceptable margins. Therefore, results should be interpreted with caution.

Ikeda *et al.* performed DNA measurements on lyophilized preparations of BCG Tokyo127 and BCG Connaught and showed that BCG Tokyo172 contained $(48.8\pm 5.43)\times 10^8$ cfu per dose and BCG Connaught contained $(3.8\pm 1.4)\times 10^8$ cfu per dose but there were no differences in antitumor efficacy in a mice model (23). These results are corroborated by the findings of Rentsch *et al.* comparing BCG Connaught and BCG TICE. Differences in bacterial titer or formulations seem to have no influence on the ability to induce priming of BCG-specific T cells (35). The inferiority in efficacy of dose reduction seems to be independent of BCG strain. One retrospective single-arm study reported on the efficacy of 80 mg (6.5×10^6 cfu/mg) BCG Moreau (36). Patients were treated with maintenance cycles for up to 3 years. The reported 5 years RFS and PFS rates were 65% (95% CI, 52–74%) and 81% (95% CI, 65–90%), respectively. Heterogeneity in treatment schedules, analyses and definition of treatment failure do not allow a direct comparison between studies. Finally, the heterogeneity in distribution of colonies in the vials makes an aliquot distribution in the reduced dose impossible, so that the expected/calculated concentration of colonies forming units of BCG in the reduced dose is probably not accurate.

Treatment schedules

Despite general acceptance of BCG for the treatment of high-risk NMIBC, there are still some controversies regarding the optimal number of instillations and treatment duration.

Animal models showed that repeated instillations with BCG are required to stimulate a robust immune response (37). In the first series by Morales *et al.*, a 6-week protocol was

proposed. Six instillations were given because the strain came packaged in six separate vials and at least 3 weeks of treatment were thought to be necessary to achieve an adequate immune stimulation. Moreover, the weekly schedule was chosen to minimize side effects (13). In order to optimize treatment, maintenance instillations beyond the 6-week induction were adopted. First randomized trials on maintenance schedules failed to demonstrate a significant difference in treatment arms but these studies were underpowered (38,39).

Subsequent RCTs have shown controversial results. Palou *et al.*, for example, randomized patients who were free of disease at six months to observation versus maintenance with 6-week instillations every 6 months for 2 years; BCG Connaught was used. No difference in recurrence could be observed ($P=0.07$) (40). Similarly, maintenance cycles using BCG Tokyo-172 did not improve RFS (41,42).

Interestingly, in the RCT by Rentsch *et al.*, patients treated with BCG Connaught had a significant longer 5-year RFS compared to patients treated with BCG TICE (74% *vs.* 48%; $P=0.001$). However, patients were not treated with maintenance cycles (35).

These findings were corroborated in large observational study by Witjes *et al.*, who showed that when no maintenance treatment was given, BCG Connaught was more effective than BCG TICE with regards to the time to first recurrence (HR 1.48; 95% CI, 1.20–1.82; $P<0.001$). However, when maintenance was given, BCG TICE was more effective than BCG Connaught regarding time to first recurrence (HR 0.66; 95% CI, 0.47–0.93; $P=0.019$) (43).

The Southwest Oncology Group (SWOG) 8507 trial delivers the most convincing evidence on the optimal instillation protocol (44). In this prospective trial patients, after a 6-week induction course of intravesical and percutaneous BCG Connaught, were randomized to a 36-month maintenance or observation. Patients allocated to the intervention arm had significant longer 5-year RFS (60%, 95% CI, 53–67% *vs.* 41%, 95% CI, 35–49%, $P<0.001$) and PFS (76%, 95% CI, 70–83%, *vs.* 70%, 95% CI, 63–76%, $P=0.04$) compared to those who underwent observation. This was true even if only 16% of the 243 maintenance cases received all 8 scheduled maintenance courses over the 3 years. Moreover, the proposed treatment schedule in the SWOG 8507 showed a significant improvement in RFS when retrospectively compared to a single maintenance, every 3 months for 2 years (SWOG 8216) (45) and a monthly maintenance for 11 months (SWOG 8795) (46).

Based on current evidence, we can conclude that, independently of the BCG strain used for induction, it is critical to adhere to treatment schedules with maintenance cycles in patients with high-risk disease, in order to improve treatment efficacy. Whether this is true in all patients with intermediate risk disease is still un-adjudicated. It appears that induction with BCG Connaught can achieve a stronger immune response, translating in better oncological outcomes and generating the hypothesis that the effect of specific BCG strains can be influenced by maintenance therapy. Discrepancy between study findings could be explained by small patient number and the selection bias of a more favorable disease. Indeed, in some studies the prevalence of low-grade disease reached 77% (41), other included patients who were free of disease at 6 months (40).

Differences in strains efficacy

Interestingly, despite decades of administration and widespread use, there are only few clinical trials directly comparing the efficacy of different strains. We could identify seven RCTs and one observational study (*Table 1*). Two studies showed a statistically significant difference in terms of RFS in favor of BCG Connaught when compared to BCG TICE, but only if no maintenance cycle was given (35,43). An explanation could be found in the mice model proposed by Rentsch *et al.* Indeed, authors found that intravesical BCG Connaught had a stronger immune system stimulation and greater cellular recruitment compared to BCG TICE ($P<0.002$) (35). The observation of Witjes *et al.* that maintenance cycles of BCG TICE can achieve a better RFS compared to maintenance with BCG Connaught (HR 0.66; 95% CI, 0.47–0.93; $P=0.019$) generates the hypothesis that the optimal efficacy of different strains may be dependent of different maintenance protocols (43).

Only in one of all RCTs, patients were treated with maintenance therapy (49). Since maintenance has shown to achieve better outcomes when compared to induction alone and is, therefore, the standard of care (33,44), results from trials in which maintenance was not administered should be interpreted with caution and are only hypothesis generating.

A recent meta-analysis addressed the efficacy of different BCG strains against other intravesical therapies. They found that BCG Tokyo-172 was associated with a nonsignificant better RFS compared to all other BCG strains, while BCG Connaught was associated with a nonsignificant increase in disease recurrence compared to BCG Pasteur, BCG TICE and BCG Tokyo-172. Authors concluded that BCG

Table 1 Clinical trials with heat-to-head BCG strain comparison

Study	Strains	Patients	Schedule	RFS	P	PFS	P	No. of AE (%)	P	Study type	Comment
Sengiku <i>et al.</i> [2013] (47)	Tokyo-172 Connaught	66 63	Induction	2-year 73.2%; 5-year 61.8% 2-year 68.8%; 5-year 56.0%	0.748	NA NA	NA NA	86 92	NS	RCT	Premature closure because of suspended BCG Connaught production in 2012 and therefore underpowered
Witjes <i>et al.</i> [2016] (43)	Connaught	957	Induction + maintenance (59%)	Without maintenance HR for TICE: 1.48; 95% CI: 1.20–1.82;	<0.001	HR: 1.08; 95% CI: 0.84–1.39	0.55	NA	NA	R	BCG Connaught reduces recurrence rates compared to BCG TICE when no maintenance is used, but the opposite is true when maintenance is given
	TICE	1,142	Induction + maintenance (18%)	with maintenance HR for TICE: 0.66; 95% CI: 0.47–0.93	0.019			NA			
Rentsch <i>et al.</i> [2014] (35)	Connaught	71	Induction	5-year 74%	0.01	5-year 94.1%	0.34	20 (28.0)	0.09	RCT	In experimental animal model, BCG Connaught induced a stronger immune response.
	TICE	60		5-year 48%		5-year 87.9%		25 (42.0)			
Vegt <i>et al.</i> [1995] (48)	TICE RIVM	140 149	Induction	5-year 36% 5-year 54%	NS	95% 94%	NS	NA NA	NS	RCT	Three arms: BCG TICE vs. BCG RIVM vs. Mitomycin-C
Mukherjee <i>et al.</i> [1992] (49)	Evans Pasteur	12 9	Induction followed by single dose every month	5-year 42% 5-year 44%	NS	NA NA	NA NA	NA NA	NA NA	RCT	
Inamoto <i>et al.</i> [2013] (50)	Tokyo low dose Connaught	18 20	Induction	1-year 72.2% 1-year 83.5%	0.698	NA NA	NA NA	14 (77.8) 14 (70.0)	0.77	RCT	
Fellows <i>et al.</i> [1994] (51)	Evans Pasteur	51 46	Induction	NA NA	NA	NA NA	0.14	26 (51.0) 22 (48.0)	NA	RCT	Marker lesion study assessing the ability of tumor eradication at 3 months
Witjes <i>et al.</i> [1996] (52)	TICE RIVM	140 149	Induction	64% 46%	NS	95% 94.6%	NS	NA NA	NS	RCT	Three arms: BCG TICE vs. BCG RIVM vs. Mitomycin-C; subgroup analyses based on stage and grade did not show a statistically significant difference

Induction defined as one instillation per week for 6 consecutive weeks. BCG, bacillus Calmette-Guérin; RFS, recurrence free survival; PFS, progression free survival; AE, adverse events; NA, not assessed; NS, not significant; RCT, randomized controlled trial; R, retrospective; HR, hazard ratio; CI, confidence interval.

Table 2 Randomized controlled trials comparing maintenance cycles versus induction only

Study	Strains	Patients	Schedule	RFS	P	PFS	P
Lamm <i>et al.</i> [1991] (45)	Connaught	68 [‡] and 67 [§] ; 63 [‡] and 64 [§]	Doxorubicin; induction followed by single dose every 3 months for 2 years	5-year 17.2% [‡] and 18% [§] ; 5-year 36.9% [‡] and 44.7% [§]	<0.001	NR	NR
Lamm <i>et al.</i> [1995] (46)	TICE	186; 191	Mitomycin-C; induction followed by single dose every month for 1 year	45.7%; 59.7%	0.017	87.1%; 92.1%	0.48
Lamm <i>et al.</i> [2000] (44)	Connaught	192; 192	Induction Induction followed by 36 months maintenance	5-year 41; 5-year 60	<0.001	5-year 70; 5-year 76	0.04
Hinotsu <i>et al.</i> [2011] (54)	Connaught	41; 42; 32	Induction followed by 18 months maintenance; induction only; epirubicin	2-year 92.7%; 2-year 65.4%; 2-year 27.7%	<0.02	NR	NR
Badalament <i>et al.</i> [1987] (38)	Pasteur	46; 47	Induction; induction followed by single dose every month for 2 years	45%; 45%	NS	NR	NR
Hudson <i>et al.</i> [1987] (39)	Pasteur	21; 21	Induction; induction followed by single dose every 3 months for 2 years	71%; 67%	NS	NR	NR
Akaza <i>et al.</i> [1995] (41)	Tokio-172	55; 52	Induction; induction followed by single dose every month for 1 year	3-year 74.2%; 3-year 77.6%	NS	NR	NR
Palou <i>et al.</i> [2001] (40)	Connaught	61; 65	Induction; induction followed by 6 instillations every 6 months for 2 years	74%; 85%	0.07	NR	NR
Koga <i>et al.</i> [2010] (42)	Tokio-172	27; 24	Induction; induction followed by single dose at 3, 6 and 9 months	2-year 74.1%; 2-year 95.8%	0.078	96.3%; 100%	0.4
Sylvester <i>et al.</i> [2010] (55)	TICE	279; 281; 277	Epirubicin followed by 36 months maintenance; induction followed by 36 months maintenance; induction + INH followed by 36 months maintenance	47.3%; 63.3%; 60.3%	<0.001 [†]	NR	NR

Induction defined as one instillation per week for 6 consecutive weeks; 36 months maintenance include three weekly instillations of BCG at 3, 6, 12, 18, 24, 30 and 36 months. [‡], for patients without carcinoma *in situ*; [§], for patients with carcinoma *in situ*; [†], the two BCG treatment groups were pooled together. RFS, recurrence free survival; PFS, progression free survival; AE, adverse events; NR, not reported; NS, not significant; RCT, randomized controlled trial; R, retrospective; HR, Hazard ratio; CI, confidence interval; BCG, bacillus Calmette-Guérin.

is superior to chemotherapy in preventing recurrence, but no BCG strain was significantly superior when compared to the others (53). A direct comparison of BCG Tokyo-172 with BCG Connaught did not show a significant difference in RFS between the two arms (47). In this trial, complete response rates were 90.3% and 85.0% for patients treated with BCG Tokyo-172 and BCG Connaught, respectively (P=0.9) with 2-year RFS rates of 73.2% and 68.8%, respectively. This trial had to be closed prematurely due to suspended BCG Connaught production in 2012 and is therefore underpowered.

The absence of well-designed head-to-head trials, utilizing homogeneous contemporaneous cohorts and

therapeutic strategies in accordance with the standard of care, limits definitive conclusions on the superiority of one over the other BCG strains. Moreover, there is significant heterogeneity in oncologic outcomes between studies using the same BCG strain (Table 2) which makes comparisons even more challenging.

Conclusions and further perspectives

During decades BCG passaging caused genetic alterations of the original strain and generated a wide phylogenetic tree. However, due to study design and substantial differences in treatment schedules, it is still unclear whether

this evolution process translates in different potency of strains with differential induction of host response.

Further randomized trials are needed, taking into account the genetic profile for strain selection. Moreover, the horizon of intravesical therapy is expanding through studies involving novel immunomodulatory drugs. Nevertheless, the heterogeneity in BCG strains may make the results of the combination studies more heterogeneous, undermining therapeutic counselling when translated for patient counselling and differential clinical decision making relative to alternative therapeutic strategies. Combinations therapies are the most promising to improve upon the current treatment efficacy achieved by optimal BCG therapy. With the first immunotherapy, BCG, urologists have gained much experience and understanding of the immune system and its richness in helping prevent, treat and cure bladder cancer.

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Footnote

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