Editorial

Tailoring the dose of Moscow strain of intravesical bacillus Calmette-Guérin for Indian patients: A plea for urgent action

Intravesical bacillus Calmette-Guérin (BCG) has long been the mainstay of intravesical therapy for intermediate- and high-risk nonmuscle-invasive bladder cancer (NMIBC) to reduce disease recurrence and progression.[1] Although there is general consensus about the ideal instillation schedule of BCG, there seems to be a lack of consensus about the ideal dose of BCG in Indian patients. Single-center studies from India have reported that about half to two-thirds of our patients receiving BCG suffer from moderate to severe side effects.[2-4] This considerable toxicity calls for immediate measures to revisit the dose that we administer to our patients, in an attempt to reduce the adverse effects without compromising on the oncologic benefit of BCG.

HOW IT ALL BEGAN-INITIAL USE OF BCG IN NMIBC

In 1921, at the Pasteur Institute in Lille, France, Albert Calmette and Camille Guérin developed a vaccine against tuberculosis by 230 serial subcultures of *Mycobacterium bovis*. This attenuated nonvirulent substrain was called BCG. In 1976, Alvaro Morales from Queen's University, Canada, published his seminal paper on intracavitary instillation of BCG.[5] In 9 patients with recurrent NMIBC, the recurrence pattern improved remarkably from a total of 22 recurrences in 77 patient-months before BCG therapy, to a single recurrence in 41 patient-months after BCG instillation. Morales' initial work prompted randomized clinical trials, and in 1980, Donald Lamm first reported his results in 37 patients with a 22% recurrence rate in patients receiving BCG compared to 42% in the control arm.[6] Following further studies and increasing evidence, the US Food and Drug Administration approved BCG for intravesical use in NMIBC in 1990.

UNDERSTANDING THE BCG SUBSTRAINS

Mycobacteria contain certain gene groups known as regions of difference (RD). The wild-type *M. bovis* does not contain RD4 to RD11 which are present in *M. tuberculosis*. Through its 230 subcultures from 1908 to 1921, BCG also lost RD1 which is the fundamental difference between BCG and the wild-type *M. bovis*. All the BCG available today is derived from the wild-type *M. bovis*, and the lack of RD4-RD11 is also the reason why BCG is not susceptible to pyrazinamide. The BCG that was developed by Calmette and Guérin was distributed across various countries for clinical application. Daughter strains were extracted at different time periods and were named according to the place of origin or the manufacturer (Danish 1331, Connaught, Moscow, TICE, Tokyo, etc.).[7] Until 1960 when lyophilization (freeze-drying) was established, BCG cultures were maintained by continuous serial passage and this led to further genetic variations.[8] The BCG "family tree" depicted in Figure 1 shows the four main groups of BCG strains. The first group comprises the "early strains" while those in the other three groups are known as "late strains." BCG Danish 1331 (1331 signifies the number of serial passages that it underwent from the original BCG in 1921 till its extraction in 1954) was earlier used in India and belonged to Group 3, while the currently used Moscow (Russia) strain is an early strain (1924).

DOSE OF INTRAVESICAL BCG – COLONY-FORMING UNIT OVER MILLIGRAMS AND VIALS

BCG is a vaccine and like any other attenuated bacteria developed for medical use, its dosing measure is the colony-forming unit (CFU). CFU varies from one strain to another. Moreover, a vial of BCG of a particular strain may contain varying CFU depending upon the manufacturer, the lot number, among other factors.[9] This variation is the basis of different dose recommendations in milligrams for different strains.

When Morales first used BCG in 1976, he realized that he needed repeated administrations of the drug, rather than a single instillation, in order to induce a delayed hypersensitivity reaction in the bladder. Furthermore, the cutaneous response to intradermal BCG injection subsided in a week's time, and thus, a weekly instillation schedule was deemed suitable assuming that even bladder would recover from the BCG instillation in about a week. The Armand Frappier strain of BCG that was available to Morales in Canada was packed in boxes of six vials, and this led to the serendipitous decision of using a 6-weekly instillation protocol.[10] Each such vial contained 120 mg of the Armand Frappier strain with 7×10^6 CFU/g. This gave 8.4×10^8 CFU/instillation. Lamm *et al*. used the Pasteur strain in their RCT (randomized controlled trial) published in 1980.[6] The Pasteur and Armand Frappier strains are

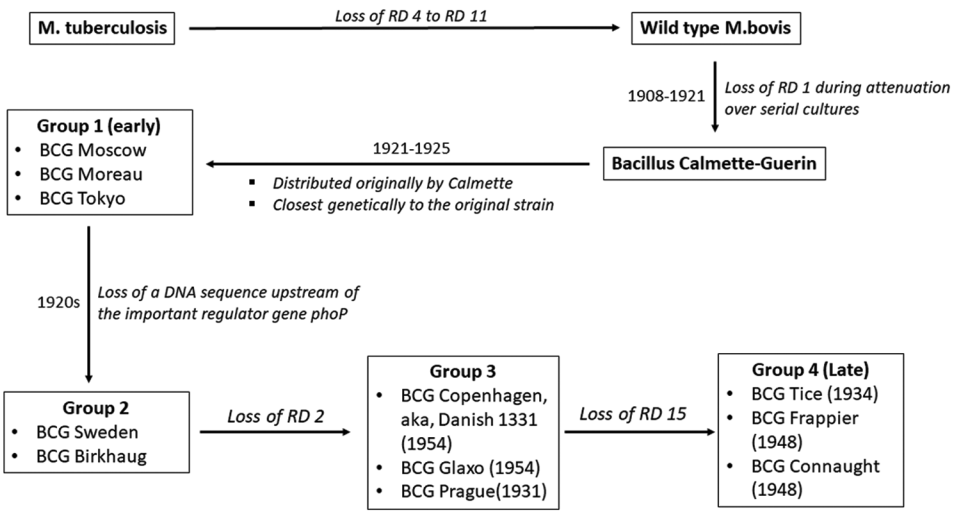

Figure 1: The bacillus Calmette-Guérin phylogeny tree

very closely related. In fact, it was Armand Frappier, a pioneer Canadian physician who studied the Pasteur strain in Paris and then took a flask of Pasteur BCG back with him to Canada, and subcultured another strain at his Institute of Microbiology and Hygiene in Montreal. The Pasteur strain in Lamm's study was also used at a dose of 120 mg. Here, each mg contained 1×10^7 CFU, resulting in 12×10^8 CFU (can also be written as 1.2×10^9)/instillation. Thus, the original BCG data suggested that a dose of 10^8–10^9 CFU for intravesical BCG is effective.[9]

After establishment of BCG as a potent intravesical agent for NMIBC in the 1980s, the Southwest Oncology Group initiated a trial to evaluate the role of maintenance BCG following the initial induction therapy. The results of this trial were published in 2000, and the recommended maintenance regimen is followed even today.[11] This trial used the Connaught strain of BCG at a dose of 81 mg/instillation, which contained $10.5 \pm 8.7 \times 10^8$ CFU (which can be written as 1.8×10^8 to 2.2×10^9 CFU). This dosage is again similar to that used in the earlier studies with the Armand Frappier and Pasteur strains. The Connaught strain was widely used in Europe, Japan, and America; however, its production was halted in 2017 resulting in a global BCG shortage.

In India, the Danish 1331 strain was used since the time BCG was introduced for intravesical use in NMIBC. The earliest report with this strain was presented by Kamat *et al*. in 1994 from the Tata Memorial Hospital in Mumbai.[12] They used BCG at a dose of 120 mg; however, it appears that even a lower dose of 40/80 mg would have provided enough CFU as per the reports of earlier studies with other strains. Later on, Danish 1331 was available in India as a 40-mg vial containing $1–8 \times 10^8$ CFU with three vials being used for each instillation ($3–24 \times 10^8$ CFU, which can be written as 3×10^8 to 2.4×10^9 CFU). A systematic review in 2017 identified only two more studies reporting the use of Danish 1331 strain and both these studies were from India.[13] The Danish 1331 strain became unavailable in India midway through the last decade. Currently, BCG Danish 1331 is manufactured in Copenhagen, Denmark, and is available as a 30-mg vial containing 2.5×10^8 CFU. Although this might seem as adequate dose, the manufacturer advises four vials (120 mg with 1×10^9 CFU) for every instillation.[14] This recommendation is not substantiated by any evidence and there is no data available on BCG toxicity from the local Danish population.

The use of different BCG strains across the world with their doses is depicted in Table 1. It can be seen that a single vial of all strains contains a comparable quantity of the vaccine in terms of CFU and only one single vial is used almost uniformly worldwide. Exceptions include the Moreau Rio strain where two vials are used and that is because a single vial contains less than adequate CFU. The other exceptions include the Danish 1331, Moscow, and Sofia SL222 strains where multiple vials are used per instillation in spite of seemingly adequate CFU in one vial. Of these, the Danish 1331 and Moscow strains have been used in India with two-third vials being used per instillation, without any definite evidence for it. Is it because the original dose used by Morales was 120 mg and, hence, we also used 120 mg? However, 120 mg of Danish 1331/Moscow strain contains three times the number of bacilli of *M. bovis* that are contained in 120 mg of the Armand Frappier strain used by Morales.

PRIOR ATTEMPTS IN INDIA FOR DOSE REDUCTION

As noted earlier, 120 mg has been the most commonly used "standard" dose of BCG in India. Vijjan *et al*. from Lucknow, India, conducted an RCT comparing standard dose (120 mg) to low-dose (40 or 80 mg) BCG of Danish 1331 strain.[2] They found that the median time to recurrence and the

Table 1: Comparison of the number of vials of various strains and doses and their global usage

Strain	Commercially available product	Countries where it is used	Dose (mg/vial)	CFU/vial	Number of vials per instillation
Armand Frappier	Not available now. Was used by Morales in his original report on intravesical BCG in 1976	NA	120	8.8×10^8	1
Pasteur	Not available now. Was used by Lamm *et al*. in the first RCT on intravesical BCG in 1980	NA	120	12×10^8	1
Connaught	Was available as TheraCys®/ Immucyst® (Sanofi Pasteur, France) till 2018 after which it was discontinued	Was widely used in Europe, USA and Japan till 2018	81	$10.5\pm8.7\times10^8$	1
Tice	OncoTICE® (Merck, USA)	USA, Canada, Australia, New Zealand, Europe, Pakistan	50	$2\text{-}8\times10^8$	1
RIVM	BCG-Medac® (Medac, Germany)	Europe (not widely used)	80	$2\times10^8\text{-}3\times10^9$	1
Moreau	Onko BCG 100® (Biomed Lublin, Poland)	Poland	100	3×10^8	1
Moreau Rio	Immuno BCG® (FAP, Brazil)	Brazil	40	8×10^7	2 (1.6×10^8 CFU)
Tokyo-172	Immunobladder® (Japan BCG Laboratory, Japan)	Japan, Hong Kong, Thailand	80	$0.4\text{-}0.5\times10^8$	1
Danish 1331	VesiCulture® (AJVaccines, Denmark)	Denmark, Argentine, Venezuela	30	2.5×10^8	4 (1×10^9 CFU)
Moscow	Onco-BCG® (Serum Institute of India, India)	India	40	$1\text{-}19.2\times10^8$	2 ($2\text{-}38.4\times10^8$ CFU) or 3 ($3\text{-}57.6\times10^8$ CFU)
		Lebanon	40		1
Sofia SL 222	Calgevax® (BB-NCIPD, Bulgaria)	Bulgaria	11.25	$1\text{-}3\times10^8$	3 ($3\text{-}9\times10^8$ CFU)

CFU=Colony-forming unit, BCG=Bacillus Calmette-Guérin, NA=Not applicable, RCT = randomized controlled trial

rate of disease progression were comparable across the three arms. Furthermore, the incidence of side effects was significantly lower in the 40-mg arm compared to the other two arms. This led them to conclude that 40-mg BCG was as effective as a higher dose with significantly lesser toxicity. The fact that none of the patients were given maintenance therapy is a major limitation of this study. A year later, Agrawal *et al*. from Agra, India, reported their results of a similarly designed trial with the Danish 1331 strain.[3] Here, patients also received monthly maintenance instillations for 1 year following the induction therapy. Again, no significant difference was observed among the three arms with regard to disease recurrence and progression. Local and systemic toxicities were significantly lower in the 40-mg arm compared to the higher doses. These two papers represent the only studies reported with dose reduction using the Danish 1331 strain and both had similar conclusions. Seen from another perspective, both these studies actually did not compare a "low" dose with a "standard" dose. They actually compared the "normal recommended" dose of $1\text{–}8\times10^8$ CFU (40 mg) with higher doses of 80 and 120 mg which were being used without any supporting evidence, and hence, in hindsight, the results are not surprising.

Despite these results which were very meaningful for the Indian population, these studies did not change routine practice across the country because of the mentioned limitations. However, they definitely lay the foundations for better-designed and adequately powered randomized trials using the currently available Moscow strain in the Indian population.

The actual effect of using a lower than recommended dose was investigated by two large international studies – one by CUETO and the other by EORTC (30962).[15,16] Both studies compared the standard dose with a one-third dose; CUETO used the Connaught strain (81 mg vs. 27 mg) while the Tice strain (50 mg vs. 17 mg) was used in the EORTC study. While the CUETO study did find a significantly reduced incidence of adverse effects by using the one-third dose, no significant reduction was observed with the lower dose in the EORTC study. A point to note is that the CFU content of one vial of Connaught strain is variable with a large range, and the content is higher than that of one vial of Tice strain. The content of one Connaught vial also tends toward the upper limit of the recommended dose of $10^8\text{–}10^9$ CFU with its upper limit being 2.3×10^9 CFU. Thus, while reducing the number of instilled bacilli by using one-third vial of Connaught would lead to a decrease in toxicity, reducing the number of Tice bacilli would probably not have the same effect as its full dose anyways does not contain a high CFU.

Recently, a study conducted at a tertiary care center by researchers from the Serum Institute of India, Pune, which manufactures BCG in India, compared 80- and 120-mg doses of the Moscow strain in the Indian population.[17] Patients received induction as well as maintenance instillations for 3 years. At a follow-up of 36 months, the recurrence-free and progression-free survival rates were 86% and 94%, respectively, for 120 mg and 84% and 84%, respectively, with 80 mg. With regard to adverse effects, the incidence of fever, dysuria, and

burning micturition was significantly lower in patients receiving 80 mg. For hematuria ($P = 0.07$) and increased frequency ($P = 0.06$), the level of significance was tending toward 0.05. No differences were observed between the two arms for suprapubic pain and urinary tract infections. The authors reported that despite the difference in the incidence of adverse effects, these were mild and resolved within a few days with analgesics and antibiotics. This showed that 80 mg of Moscow strain was oncologically noninferior to 120 mg and was tolerated better. There is no clinical data available for comparison of 40 mg of Moscow strain with 80/120 mg.

WHY SHOULD WE BE CAUTIOUS WITH THE MOSCOW STRAIN IN USE CURRENTLY

The various BCG strains have distinct genetic differences and thus "X" CFU of one strain does not equal to "X" CFU of another and that is why a range of 10^8–10^9 CFU/instillation is recommended. These differences lead to variability in phenotypic characteristics, namely ability to bind to fibronectin and production of phenolic glycolipids and mycolic acids which play a role in virulence. They also differ in the production of interleukin (IL)-6, IL-8, tumor necrosis factor-α, IL-1β, and IL-12.[18] Furthermore, there are bound to be certain inherent differences in the way different populations respond to intravesical BCG with regard to both tolerability and oncological outcomes, and it is unclear whether there is any correlation between these two. Joshua *et al*. from Kochi, India, found that there was no difference in recurrence and progression rates in their patients who received induction therapy alone versus those who received maintenance therapy as well, suggesting that a preimmunized population like that of India has a higher immune response to intravesical BCG and may not require as high a dose or as long a regime as recommended by studies done on Western populations.[19]

As mentioned above, the Danish 1331 strain was used in India till about the middle of the last decade following which the Moscow (Russia) strain is being used now. A recently published report by Thyavihally *et al*. from Mumbai is the only study available that compares these two strains.[4] Over a period of 10 years, 68 patients received the Danish 1331 strain while 46 received Moscow BCG; both strains were used at a dose of 120 mg. A significantly higher proportion of patients in the Moscow strain group (67.4%) experienced moderate-to-severe side effects leading to BCG discontinuation, need of antitubercular therapy, or requirement of cystectomy, compared to the patients receiving the Danish 1331 strain (48.5%). The oncological outcomes with regard to recurrence and progression were comparable with the two strains.

Multiple studies have also shown that urinary levels of these cytokines are predictive of response to BCG therapy. Specifically, with the Danish 1331 strain, Kumar *et al*. randomized patients to receive either 40 or 120 mg of intravesical BCG and urinary IL-8 levels were measured immediately before and 2 and 4 h after BCG instillation.[20] The mean IL-8 level in BCG responders was significantly higher than compared to nonresponders. Furthermore, the mean IL-8 level in patients who received 40 mg was comparable to those who received 120 mg, proving that the quantitative response with 40 mg was not inferior to that with 120 mg.

In an attempt to compare the various strains, Secanella-Fandos *et al*. from Barcelona cultured three bladder tumor cell lines T24, J82, and RT4 (these represent histological tumor Grades 3, 3, and 1, respectively) with three early BCG strains (Japan, Moreau, and Russia) and five late strains (Connaught, Glaxo, Danish 1331, Phipps, and Tice).[21] This *in vitro* study revealed that the Russian and Connaught strains induced the highest inhibition of cell proliferation and also resulted in the highest production of IL-8. Tice was one of the least efficacious strains. For the Indian context, the Russian strain was significantly more efficacious than the Danish 1331 strain. These results further emphasize that the same dose in milligrams cannot be used for different strains as the strains differ in their immunogenicity and efficacy. Furthermore, the Moscow strain probably requires a lower dose than the Danish 1331 strain for the same level of efficacy, and this lower dose would lead to a decrease in the adverse effect profile.

The above reports suggest that the Indian population responds differently to intravesical BCG compared to the Western counterparts. Furthermore, there are no reports from outside of India about experience with the strains that have been used in India. Unlike the "global" shortage of BCG, we in India have the product readily available. It seems very consequent that we generate our own evidence regarding the oncological and adverse effect profile with the current strain and optimize the dose and probably also the instillation schedule for our patients. The optimal dose of the current Moscow strain for Indian patients cannot be extrapolated from previous studies performed with different strains on different populations. Considering the current high incidence of side effects, de-escalation of the BCG dose seems very logical and should be pursued earnestly and urgently to prevent further harm to our patients. *In vitro* studies with different doses could pave the way for clinical studies. Finally, if we do succeed in lowering the dose without compromising oncological efficacy, India could play a major role in providing BCG to other countries and help fight the global shortage.

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