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Immunotherapy for non-muscle invasive bladder cancer

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1 **Abstract**

2 Supplies of intravesical Bacillus Calmette-Guerin (BCG), the first-line treatment for most intermediate-

3 and high-risk non-muscle invasive bladder cancers (IR- and HR-NMIBC), have proven unreliable over

4 the last decade. This review considers the evolution of BCG immunotherapy for NMIBC: from the

5 discovery of the anti-tumour side-effects of Tuberculosis (TB) and subsequently the BCG vaccine, to

6 recent advances in novel immunotherapeutic agents. We summarise the evidence for alternative

7 options to standard intravesical BCG therapy regimens and describe the potential for immune

8 response manipulating drugs in the treatment of NMIBC. These new agents, including immune

9 checkpoint inhibitors, toll-like receptor agonists, and recombinant viral vectors, may provide better

10 options in the management of NMIBC in the future.

11

12 **Lay Abstract**

13 Many patients with non-invasive bladder cancers may need treatments into the bladder, including

14 one called Bacillus Calmette-Guerin (BCG). Unfortunately, the supplies of BCG have been interrupted

15 and somewhat unreliable since 2012. Because of this, we have been forced to look at other means of

16 treating our patients using drugs like BCG. This has made us think about how BCG treatment was first

17 developed more than forty years ago, and how it has evolved as a treatment for bladder cancer. In

18 this article, we review the current uses of BCG and other treatments for bladder cancer, and explore

19 what the future may hold for bladder cancer treatments.

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21 **(Tweetable abstract – optional):** This review explores the evolution of intra-vesical BCG therapy in IR-

22 and HR-NMIBC. We also describe new and emerging immunotherapeutics in the management of

23 NMIBC, which may become more important if the worldwide shortage of BCG continues.

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INTRODUCTION

Bladder cancer is the seventh most common cancer in Western society, with an annual global incidence of >430,000 cases(1). In the UK, we observe >10,000 new cases per year, with an estimated 70,000 patients living with bladder cancer at any one time. Greater than 90% are urothelial cell carcinomas (UCC) of the bladder (previously termed transitional cell carcinomas - TCC)(2).

Most patients in the UK (75-85%) present with non-muscle invasive bladder cancer (NMIBC: stages Ta/T1/Tis)(3); which represents a spectrum of disease from solitary small tumours with low-risks of recurrence or progression, to large multifocal disease with high-risks of early recurrence or progression to invasive or metastatic disease(4). Such progression to muscle-invasive bladder cancer (MIBC: stages ≥T2) occurs in up to 45% of NMIBC patients(4, 5), and is associated with worse outcomes than if the patient initially presented with MIBC, with a 5-year overall survival rate of only 27-50% despite radical therapies(6, 7). Intravesical Bacillus Calmette-Guérin (BCG) is central to the management of HR-NMIBC, and more recently IR-NMIBC, and has been used for over 40 years to reduce the risks of tumour recurrence and progression (rather than cure)(8). BCG thus represents one of the oldest immuno-oncology (IO) agents in current use. However, there has been a surge in the study and trial setting use of newer IO therapeutics for bladder cancers and other malignancies. In this review, we describe the history of BCG as an IO agent, the current use of BCG for bladder cancer, and the possible future of immunotherapy in bladder cancer.

BCG PAST: DISCOVERY, DEVELOPMENT & TREATMENT REGIMENS

Discovery & Development

The journey of discovery of the anti-tumour effects of tuberculosis (TB) to the development of intravesical BCG therapy is a fascinating series of linked events driving clusters of research, leading ultimately to the development of the BCG treatment regimes in use today.

In 1925, Dr Thomas Cherry noticed an inverse relationship between the incidence of cancers and tuberculosis (TB). Epidemiological data confirmed that as the incidence of TB declined, more people were dying from cancer(9). These findings were explored further by Dr Raymond Pearl in 1929, who conducted an autopsy series investigating TB lesions in two demographically matched patient groups. He concluded that active TB lesions were more than twice as likely to be present in the no malignancy 'control' group (16.3%) compared to the group with malignancies (6.6%)(10). From these findings stemmed the notion of the possible anti-tumour effects of TB.

Some 40 years earlier in the 1880s, Louis Pasteur successfully attenuated live strains of cholera and anthrax, and the rabies virus, creating non-virulent forms suitable for vaccination. In 1908, Albert Calmette and Camille Guérin adopted Pasteur's technique to create a successful vaccination against TB, known as Bacillus Calmette-Guérin or 'BCG'(11). Calmette and Guérin used isolated *Mycobacterium Bovis* from an infected cow; after 231 passages over 13 years, the bacterium was finally deemed innocuous, creating the live-attenuated BCG vaccine(12). The production of BCG vaccine provided a means for the therapeutic use of the anti-tumour effects of TB in a safer form.

Years later in 1959, Lloyd Old carried out the first study demonstrating the anti-tumour action of the BCG vaccine; mice with transplantable tumours given BCG vaccine demonstrated increased resistance to tumour growth compared to non-vaccinated control mice(13). These results supported Cherry's findings 30 years previously of the inverse relationship between the incidence of cancer and TB.

In 1966, Coe and Feldman investigated hypersensitivity responses in extracutaneous tissues of guinea pigs. They found that intra-vesical injection stimulated the greatest hypersensitivity response, and although not recognised at the time, this provided early evidence of the potential use for BCG in bladder cancer(14). Following this in 1971, a study by Zbar involving intralesional injections of BCG into hepatocarcinoma-induced guinea pigs, showed that animals injected with BCG did not develop metastases. For optimum effect, it was found that contact between the BCG and tumour cells was needed, and that tumours above a certain size were not effectively treated by BCG(15).

80   Results from these two studies formed the basis from which two concepts were derived:

81   1. The anti-tumour effect of BCG was better when restricted to one organ to maximise contact

82   2. The dose of BCG must be sufficient for the size of the tumour(s)

83   BCG was first used in the human bladder by deKernion *et al.* in 1975. A patient with an isolated

84   metastasis of a malignant melanoma in the bladder underwent cystoscopic intra-lesional injection of

85   BCG. Cystoscopy confirmed an active area of granulomatous inflammation, and subsequent excision

86   did not identify any residual cancer(16).

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88   **Treatment Regimens**

89   In 1976, the first intravesical instillation of BCG was performed by Morales. This was a small study of

90   nine patients with a previous history of 22 tumour recurrences in 77 patient-months. Patients received

91   120mg BCG in 50ml saline for each of six instillations administered weekly; following BCG instillation

92   only one recurrence was observed after 41 patient-months(17). Interestingly, this six-week regime

93   arose because the Frappier BCG strain used was packaged in six vials, and because adverse side-effects

94   of this strain diminished within one week, permitting weekly instillation(18). As identified previously

95   by Zbar, a 'sufficient' dose of BCG was required for anti-tumour effects, so taking into account the

96   added dilution from urine within the bladder, the 120mg dose was deemed suitable(18). Amid

97   increasing scientific and clinical interest in BCG therapy for NMIBC, larger studies were undertaken

98   that determined toxicity and efficacy. The US Food and Drug Administration (FDA) subsequently

99   approved the use of intravesical BCG instillation for the treatment of NMIBC in 1990.

100   A meta-analysis conducted in 2002 concluded that BCG not only reduces recurrence of NMIBC but

101   also reduces progression to MIBC(19). With an initial six-week course of BCG deemed safe and

102   effective (what is now termed 'induction' BCG), attention then focused on prolonging the duration of

103   intravesical BCG treatment ('maintenance') in the assumption that further treatment could improve

104   oncological outcomes. Two early studies published in 1987 compared outcomes of patients receiving

105 maintenance therapy to induction alone. Interestingly, neither demonstrated significant differences

106 in rates of tumour recurrence or progression between groups, initially suggesting maintenance

107 therapy was not beneficial, yet increased patient side effects(20, 21). However, small sample sizes,

108 the presence of macroscopic tumour in many cases, and heterogeneity in both the tumour types

109 included and treatment schedules, may have confounded their results. Indeed, a subsequent larger

110 trial with 550 patients was published by Lamm in 2000(22). Here, a more homogenous patient cohort

111 with high-risk non-invasive papillary and carcinoma in situ, who were also macroscopically tumour

112 free, were randomised to receive either induction BCG only or induction plus maintenance BCG

113 (intravesical and percutaneous). The maintenance arm received BCG each week for three weeks at

114 intervals of 3, 6, 12, 18, 24, 30, and 36 months after induction. This study revealed that the median

115 recurrence-free survival was over twice as long for those receiving maintenance therapy (76.8

116 months) compared to those receiving induction only (35.7 months) (p<0.0001). Additionally, 5-year

117 survival was 83% in the maintenance arm compared to 78% in the induction only arm confirming the

118 patient benefits of maintenance BCG therapy. Furthermore, a 2003 meta-analysis concluded that

119 toxicity did not differ between patients receiving maintenance therapy and those who do not(23).

120 Therefore, despite increased healthcare costs and patient burden/morbidity, BCG maintenance

121 therapy became incorporated into numerous international guidelines for the treatment of both high-

122 risk, and more recently, intermediate-risk NMIBC(24).

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128 *[Figure 1 –The evolution of BCG therapy.]*

BCG PRESENT: SHORTAGES & TREATMENT EVOLUTION

Shortages

There have been several different strains of BCG available, including, but not limited to, Connaught, TICE, Pasteur, Danish, Moreau and Merck. Despite both a recent meta-analysis and review (25, 26) suggesting no superiority of one strain over another in the management of high-risk NMIBC, the Connaught strain appeared to be the most widely used. However, as of July 2012, there has been a worldwide shortage of BCG vaccine - the manufacturers of the BCG Connaught strain shut down temporarily following reports of US FDA regulatory breaches, and later stopped Connaught strain production indefinitely in 2016(27). There are several different strains of BCG available, but limited data comparing their relative efficacy(28). Many centres were forced to use Merck's TICE strain following the Connaught shortage, but a randomised controlled trial in 2014 suggested that TICE may be less effective than Connaught following induction. The Connaught strain was found to have 74% 5-year recurrence-free survival compared to 48% for TICE(28). An ongoing large-scale trial (the Southwest Oncology Group (SWOG) S1602), is further evaluating the efficacy of the Tokyo-172 BCG strain, after a smaller 2013 study suggested similar one-year recurrence-free survival to the Connaught strain, *i.e.* superior to TICE for both induction and maintenance(29). In addition, a 2016 retrospective study suggested that the Moreau strain had comparable efficacy to those more widely used, with 5-year recurrence-free survival of 65% and progression-free survival of 81%(30). Following this, a retrospective study in 2019 compared BCG TICE with BCG Moreau and found no difference between progression-free and recurrence-free survival, concluding BCG Moreau as an alternative effective substrain for the treatment of NMIBC in a time of shortage(31). SWOG S1602 is also evaluating potential benefit of priming with subcutaneous BCG prior to intravesical BCG to enhance T-cell entry into the bladder mucosa(32). It is clear that further studies are required to evaluate efficacy of alternative strains to help overcome the shortages of the currently-licensed and historically widely-used BCG strains. Notwithstanding, there is little incentive for companies to manufacture BCG - each dose is relatively cheap to purchase, but labour-intensive to produce (three

months of culturing and susceptibility to contamination)(33). Since one course of induction BCG

therapy (6 instillations) exceeds the dose required to meet the UK's annual BCG vaccination

requirements for one year, the shortages have caused major issues with continuing current BCG

regimes as a first-line treatment for most IR- and HR-NMIBC(34).

For many years, the precise anti-tumour mechanism of BCG has remained unclear and is beyond the

scope of this article. However, various recent mechanistic studies have improved our

understanding(35, 36) which may prove helpful when investigating adjuncts to BCG use, that could

improve efficacy and/or reduce toxicity.

Treatment Evolution

BCG shortages continue to impact clinical practice(37). It has therefore been important to investigate

possible ways of reducing BCG use whilst maintaining clinical efficacy, specifically, the use of adjunct

therapies (in combination with BCG), and modifications to the current standard BCG treatment

regimes.

Combination Therapy

Mitomycin C (MMC) is a chemotherapeutic agent that was first found to reduce bladder cancer

recurrence following intravesical instillation in 1988(38). Mitomycin C binds and crosslinks DNA

through alkylation, preventing DNA synthesis(39). Electromotive drug administration (EMDA) is a

recent innovation that enhances drug efficacy by the processes of iontophoresis, electro-osmosis and

electroporation(40). When EMDA is utilised to deliver intravesical MMC it is thought that it can

penetrate 4-7 times deeper into the bladder mucosa(41), thereby increasing the concentrations of

MMC reaching cancer cells. Combinations of BCG and EMDA-MMC instillations have shown promise

as an alternative to the full course of both BCG induction and maintenance therapy(42).

180   Adapting Treatment Regimens

181   As described by Lamm, the dose-response curve for BCG is bell-shaped, indicating that excessively

182   high concentrations of BCG may worsen outcomes and may paradoxically enhance tumour

183   growth(43). If this curve peak can be determined however, treatment regimes could be optimised

184   further or tailored to each patient, with potentially improved outcomes. Unfortunately however,

185   uncertainty persists over the length and frequency of instillations during maintenance therapy, thus

186   the optimal schedule and length of the maintenance period remains an area of ongoing clinical

187   investigation.

188 *[Table 1: A summary of studies investigating different BCG regimens (D = dose, FD = full dose, AE = adverse events).]*

189   Oddens' 2013 RCT, summarised in **Table 1**, describes the potential to reduce the duration of BCG

190   maintenance after comparing three years to one year maintenance(44). For HR-NMIBC patients, three

191   years' full-dose BCG (maintenance) reduced tumour recurrences compared to one year full-dose, but

192   did not reduce disease progression or overall mortality. This trial also evaluated using 1/3 dose BCG

193   for both induction and maintenance; this appeared to have decreased efficacy but without reduced

194   side-effects/toxicity, however, patient drop-out was lower than with higher dose and longer

195   maintenance schedules. This suggests that current practice of three-year maintenance therapy with

196   full-dose BCG could be reduced to improve patient compliance with a small trade-off in reduced

197   efficacy. Furthermore, a reduced-dose regime would reduce financial costs and ensure BCG supplies

198   last longer.

199   Following Oddens' findings, a 2015 CUETO group RCT investigated the efficacy of three year

200   maintenance BCG (BCG instillation once every three months) versus BCG induction only (BCG once-

201   weekly for six weeks)(45). This maintenance schedule reduced the number of BCG instillations from

202   27 (the standard SWOG regimen) to 18. Interestingly, this study suggested that recurrence and

203   progression rates, and overall and cancer-specific survival, were no different between the induction

204   only and induction plus maintenance arms(45). Furthermore, there was lower attrition due to

205   toxicity/side-effects in the induction only arm compared to the maintenance arm (2.6% and 9.9%,

206   respectively). These data suggest that for maintenance therapy to be more effective than induction

207   alone, maintenance instillations must be more frequent than every three months.

208   The 2020 phase III NIMBUS RCT investigated different induction and maintenance BCG instillation

209   frequencies(46). The reduced frequency group received three induction doses at weeks 1, 2 and 6,

210   compared to standard regime of once-weekly dosing for six weeks, and fewer maintenance cycles at

211   weeks one and three of months 3, 6 and 12, compared to the standard weekly dosing for three weeks

212   at months 3, 6 and 12, for one year. Overall, the reduced frequency group received a total of nine BCG

213   instillations, versus 15 for the standard/control group. Although the reduced frequency arm

214   experienced fewer adverse events, the trial was closed early due to a 'safety-relevant difference' in

215   recurrences between treatment arms: 27.1% recurrences after 12 months' median follow-up,

216   compared to 12.0% in the standard BCG arm.

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218   <u>Alternative Therapies</u>

219   Bladder cancer is said to be 'BCG-unresponsive' when there is persistent or recurrent CIS and/or

220   papillary tumour within 12 months, or recurrent high-grade Ta/T1 tumour within six months of

221   adequate BCG treatment(47). Adequate BCG therapy is defined by at least five out of six induction

222   doses with two out of three maintenance doses, or five induction doses plus two second course

223   induction doses(47).

224   For patients who may not tolerate BCG or are BCG-unresponsive, a novel rescue therapy was proposed

225   by Steinberg in 2015(48). This involved weekly intravesical instillations of gemcitabine immediately

226   followed by docetaxel (Gem/Doce) for six weeks(48). Gemcitabine (a deoxycytidine nucleoside

227   analogue) causes DNA synthesis inhibition, whilst docetaxel (an inhibitor of tubulin dis-assembly)

228   disrupts the cell cycle(48). This combination acts synergistically and leads to apoptosis and reduced

229   cell division, and ultimately tumour cell death.

230   Subsequently, a small clinical trial in 2017, suggested that this combination chemotherapy was safe

231   and effective for patients with high-risk NMIBC who were BCG naïve(49). Steinberg *et al.* performed

232   a retrospective review of patient records at multiple institutions examining the efficacy of

233   Gem/Doce(50). Recurrence-free survival of high-risk disease was 65% at one year and 52% at 2 two

234   years with only 3.3% of patients unable to complete the treatment course due to side-effects(50). This

235   confirmed the potential for Gem/Doce as a rescue therapy following BCG failure, and highlighted the

236   need for further (prospective) studies evaluating this combination therapy.

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BCG FUTURE: BUILDING ON THE IMMUNOTHERAPY PARADIGM

The significant reduction in risk of recurrence and progression of NMIBC in patients receiving BCG, has highlighted the importance of immunotherapy for bladder cancer. As understanding of the molecular events and characteristics of various cancers improves(51), more targeted novel therapies are being developed.

Immune Checkpoint Inhibitors

There are a plethora of clinical trials evaluating the efficacy of new and more established immune checkpoint inhibitors (ICIs) across multiple haematological and solid organ malignancies. Our understanding of ICIs is still in relative infancy but many are already showing great promise. For example, in the Keynote-057 trial, 101 BCG-unresponsive high-risk NMIBC patients received at least one dose of pembrolizumab during 24 months, or until tumour recurrence, or until dropout due to toxicity/side-effects(52). Keynote-057 found that 41% patients achieved a complete response (absence of recurrent high-risk NMIBC or disease progression) at three months(52), and 46% of those responding remained recurrence and progression free at 12 months(52). Furthermore, pembrolizumab treatment led to a 12-month progression-free survival of 83%(52). Despite inferiority in oncological outcomes compared to radical cystectomy, the use of pembrolizumab was approved by the FDA in 2017 following these promising data, and it is currently used in clinical practice. However, more studies are required to determine optimum treatment regimes. More recently, and for BCG-naïve high-risk NMIBC patients, the POTOMAC study assessed the efficacy of durvalumab (a PD-L1 inhibitor) in combination with BCG, compared to standard BCG therapy alone, in BCG-naïve high-risk NMIBC patients(53). It is anticipated that results from these and other clinical trials will lead to more options and greater success in the management of NMIBC, as stand-alone novel ICI therapy or in combination with BCG.

265 **Toll-like Receptor Agonists**

266 BCG immunotherapy is thought to act, at least in part, through interactions with three toll-like

267 receptors (TLRs); TLR2, TLR4 and TLR9(54).

268 Toll-like receptors are a large family of cell membrane-spanning proteins that mediate multiple

269 intracellular mechanisms and pathways vital to the innate immune system. Agonists of TLRs have been

270 shown to possess anti-tumour activity across multiple malignancies(55), including, for example, the

271 TLR7 agonist Imiquimod, already in clinical use for basal cell carcinoma and some penile cancers(56).

272 Importantly for bladder cancer, TLRs are present in urothelium, and although their expression may ~~be~~

273 decrease in bladder tumours, their activity has been shown to persist(54). Therefore, TLR agonists

274 appear an attractive target in bladder cancer, and as such, agonists of TLR2, TLR4, TLR7 and TLR9 have

275 been investigated for anti-tumour effects in in-vitro and in-vivo bladder models(54). A small first phase

276 clinical trial of intravesical Imiquimod determined it was safe and had low systemic absorption and

277 toxicity(57). However, further studies are required to establish the optimal TLR(s) to target for NMIBC,

278 and thenceforth desired effects on bladder tumour recurrence or progression to MIBC.

279 An alternate TLR-mediated immunotherapy is the use of BCG cell wall skeleton (CWS). BCG-CWS is a

280 component of the BCG cell wall that can be produced readily, and provides ligands for TLR2 and

281 TLR4(54). Preliminary investigations have shown growth retardation of bladder tumours when

282 administered intravesically in mice(56). The Morales group demonstrated that for patients who have

283 failed standard BCG therapy, intravesical instillations of such a mycobacterial cell wall-DNA complex

284 possessed anti-tumour activity without significant toxicity or patient side-effects(58). These studies

285 highlight the promise and huge potential of immunotherapy, targeting Toll-Like Receptors, for the

286 management of bladder cancer, whether alone or in combination with BCG.

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Other Promising Immunotherapeutic Approaches

Interferon alpha-2b, a recombinant protein immune modulator, has been shown to induce bladder tumour regression in animal studies(59, 60). A subsequent clinical trial of different dosing regimes of Interferon alpha-2b in patients with BCG failure or BCG-unresponsive NMIBC, demonstrated good tolerability and suggested potential benefit in recurrence-free survival(61). This interferon was delivered in the form of a recombinant replication-deficient adenovirus gene vector 'Nadofaragene Firadenovec' (rAd-INFα/Syn3) which stimulates local urothelial INFα-2b production, leading to tumour regression(61). Consequently, a phase three trial recruited patients with BCG-unresponsive NMIBC, who received single intravesical doses of Nadofaragene Firadenovec, repeated at 3, 6 and 9 months, if there was no high-grade recurrence(62). Results were promising, with 53.4% of participants having a complete response (macroscopic tumour resolution) at month three, and 45.5% of patients were recurrence free at 12 months(62). This trial also demonstrated 91.9% 24-month overall survival for those receiving at least one dose, whilst significant side effects were only seen in 2% of patients(62). Therefore, Nadofaragene Firadenovec, as a novel and first-of-its-kind gene therapy, could be considered as a viable alternative for patients who fail to respond to BCG therapy (as an alternative to, or if unfit for, radical cystectomy), or even as a potential first-line treatment, for which further data are imminent.

Another trial evaluating a recombinant fusion protein in BCG-unresponsive HR-NMIBC patients is VISTA. This phase III non-randomised trial uses Vicinium, a potent inhibitor of protein synthesis that ultimately induces irreversible cell death. Intravesical Vicinium is administered twice weekly for six weeks then weekly for six weeks (induction), then every two weeks for up to two years (maintenance). The three-month complete response was 42% in patients with CIS, and 68% in patients with HR-NMIBC without CIS. Given these promising early results, Vicinium is undergoing FDA licensing application as a potential alternative to radical cystectomy in BCG-unresponsive patients(63).

316   Cancer-specific vaccines have been trialled on a small-scale with intravesical immuno-stimulation by

317   BCG with encouraging results: a non-randomised study investigated 24 NMIBC patients in three

318   groups, receiving either vaccine injection (MAGE-A3) alone, or alongside one of two intravesical BCG

319   combinations(64). All groups seroconverted after the vaccination schedule, and half the participants

320   had detectable cancer-specific T-cells irrespective of group, yet only those receiving BCG had these

321   same T-cells detected in urine. This suggests there may be an enhanced vaccine response via immune-

322   stimulation which may help to localise cancer specific T-cells to the tumour.

323   Vγ9/Vδ2 T-cells are the main T-cell type in peripheral blood, and are involved in mediating immune

324   responses to a variety of diseases(65). Vγ9/Vδ2 T-cells can be activated by pyrophosphate-containing

325   phosphoantigens, and by specific prodrugs(65). Previously, pyrophosphate-containing

326   phosphoantigen use has been limited due to their instability in serum, however, prodrugs have been

327   synthesised that are much more stable in serum, and have also shown successful eradication of

328   bladder cancer cells *in vitro*(65). If these findings can be replicated *in vivo* and in clinical trials, and new

329   immunotherapy targets are identified, the management of NMIBC has a promising future.

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331   **CONCLUSIONS**

332   Intravesical BCG immunotherapy remains invaluable, and the standard of care for most patients with

333   intermediate- and high-risk NMIBC that do not need or want upfront radical cystectomy. However,

334   with increasing pressure on availability, and treatment failure rates of up to 40%(66), BCG is not the

335   best long-term solution for NMIBC. Fortunately, combination and novel immune- therapies are

336   becoming more refined, with improved efficacy, reduced toxicity, and fewer side-effects. Such

337   treatment options will be become ever more necessary due to the rising incidence of bladder cancer

338   with an ageing population and continued BCG shortages. Therefore, ongoing trials of

339   immunotherapeutic agents and novel means of delivering existing drugs (e.g. MMC) to improve

340 efficacy or decrease toxicity, are particularly important for the future of NMIBC management(42, 67,

341 68).

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345 **EXECUTIVE SUMMARY**

346 **History of BCG**

347 • The current standard BCG regime of six weeks induction followed by maintenance was

348   developed over the course of many years.

349 **BCG Shortage and different BCG Strains**

350 • There has been a worldwide shortage of BCG since 2012 (including crises in 2012 and 2014),

351   with differing efficacy between available BCG strains.

352 **Future directions of BCG treatment**

353 • There is the potential to reduce or shorten BCG treatment schedules to decrease BCG usage

354   in a time of continued shortage.

355 • BCG in combination with Mitomycin C, which may reduce BCG usage.

356 • Alternative therapies to BCG for those who are BCG-unresponsive.

357 **Future of Immunotherapy in NMIBC**

358 • Immune system targeting agents such as immune checkpoint inhibitors, toll-like receptor

359   agonists, and cancer-specific vaccines, may prove useful as an adjunct to BCG or in BCG-

360   unresponsive patients.

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Reference Annotations
9. *of interest: The inverse relationship between cancer and tuberculosis was recognised from epidemiological data. This was the basis for the anti-tumour function of TB.

562 **17. *of interest:** The first trial of intravesical instillation of BCG was performed. The findings from
563 this small trial were the beginning for clinical development of BCG as a therapeutic agent.
564
565 **22. **of considerable interest**: This trial evaluated the efficacy of the maintenance period compared
566 to induction therapy alone. The results showed that outcomes were greatly improved with
567 maintenance therapy.
568
569 **36. *of interest:** This trial proved the efficacy of intravesical MMC. This drug has since been used to
570 manage bladder cancer as an alternative to BCG for many years.
571
572 **54. *of interest:** This trial investigates the use a PD-1 inhibitor in combination with BCG or stand-
573 alone.
574
575 **59. *of interest:** The Morales group showed that using components of BCG intravesically can still
576 produce a response.