A BCG success story: From prevention of tuberculosis to optimal bladder cancer treatment

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ABSTRACT

BCG remains the most important vaccine for tuberculosis 100 years after its first use, and over the past 4 decades it has become the most widely accepted, effective drug used in the treatment of aggressive localized bladder cancer. This review chronicles the narrow path that led to approval and world-wide acceptance of BCG immunotherapy for bladder cancer while immunotherapy trials in other malignancies were abandoned. Six intravesical instillations of 5x10^8 CFU of BCG weekly after bladder tumor resection, first reported in 1976, is superior to resection alone and resection plus intravesical chemotherapy. Maintenance of effective immune stimulation is surprisingly difficult, but 3 weekly treatments 3, 6, and 12, 18, 24, 30 and 36 months after induction produces further significant reduction in tumor recurrence. This 3 week BCG maintenance schedule alone has reduced disease progression and mortality in multicenter randomized clinical trials. In the new age of immuno-oncology patients with many types of cancer now benefit from immunotherapy, but currently these modern agents are prohibitively expensive for most of the world. In contrast, the low cost and therefore low profitability of BCG has resulted in recurrent shortages that threaten both bladder cancer patients and children at risk for tuberculosis and other serious infections. Humanity has greatly benefited from early 20th century science that developed BCG and the benevolence of doctors Calmette and Guerin who put people over profit and widely shared cultures of the vaccine. The 21st century is bringing new immunotherapies and greatly expanding the types of malignancies that can be treated. Recombinant technology is expected to improve both the efficacy and production of BCG, hopefully expanding the availability of BCG and relieving the recurring supply shortage for both vaccination and cancer therapy.

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1. Introduction

Over eons the human immune system evolved to overcome multiple potentially-lethal bacterial and viral infections, and over generations, physicians and scientists discovered ways to use the human immune system to battle another potentially-lethal foe: cancer. BCG vaccination has prevented the death millions of children from tuberculosis and now has become a most effective treatment for bladder cancer. With the advent of immune checkpoint blockers, immunotherapy is currently the most exciting new cancer treatment modality, and can be expected to become the most effective treatment of many cancers. This review will describe how a vaccine first used in 1921 to prevent tuberculosis became a globally-accepted, highly-effective treatment of bladder cancer.

2. BCG, immunity and cancer

Before the identification of bacteria, pus was known to be associated with regression of tumors. In 2600 BCE Imhotep induced infection with incision and poultice to treat tumors. In the 18th and 19th centuries physicians incised tumors to induce infection and in 1894 the New York surgeon William Coley published responses to treatment with “Coley’s Toxin” [1]. This combination of Streptococcus pyogenes and Serratia marcescens was produced and marketed by Park-Davis and was commercially available until 1962. While responses clearly occurred, bacterial treatment remained controversial and the mixed results in clinical trials resulted in failure to achieve regulatory acceptance. BCG immunotherapy very nearly suffered the same fate.

Perhaps the first inkling that immunity induced by tuberculosis might protect from cancer came in 1929 when Pearl found that autopsies of those who had evidence of tuberculosis had a lower incidence of cancer than controls [2]. Mycobacterium tuberculosis...
was first shown to increase immune response in 1924 when Lewis and Loomis [3] found heightened antibody production following intraperitoneal injection of dead bovine tubercle bacillus in guinea pigs. BCG was subsequently manufactured in the 1940s as the Freund’s complete adjuvant to stimulate immune response. With the subsequent increased interest in the role of immunity in cancer and the advent of syngeneic tumor models, BCG and many other bacterial preparations were studied in animal models and then clinical trials in the mid 20th century. Enthusiasm for BCG in cancer treatment peaked in the early 1970’s when multiple animal models and clinical trials showed efficacy, most notably in melanoma where clinical responses were as high as 90% [4]. Neonatal vaccination in retrospective studies published in 1971 by Davignon [5] and in 1972 by Rosenthal [6] showed that BCG appeared to prevent childhood leukemia. In 1969 Mathe [7] reported a trial in 30 patients with acute lymphoblastic leukemia treated with combination chemotheraphy and then randomized to observation, BCG, or BCG plus irradiated allogeneic lymphoblasts, and found at 130 days that none of the control patients were recurrence-free compared with 45% of the 20 who were treated with BCG. Enthusiasm quickly faded however when large randomized controlled trials failed to confirm the benefit of BCG in the treatment of leukemia [8], lymphoma [9], colon cancer [10], ovarian cancer [11], lung cancer [12] and melanoma [13].

Why has BCG immunotherapy become the widely-recognized and approved treatment of choice for high risk non muscle-invasive bladder cancer (NMIBC) when, despite clear evidence of efficacy, it is no longer used in other malignancies? Failure to confirm efficacy in other malignancies has been blamed on the use in advanced disease, often with concomitant chemotherapy, use of less-effective sub strains of BCG or non-viable preparations such as methanol extracts of BCG residue (MER), suboptimal immunization techniques and vaccination schedules, and comparison or combination of BCG with chemotherapy and other immunotherapies such as levamisole and C. Parvum. While we would like to believe that the success of BCG immunotherapy is related to the treatment techniques and schedules the authors developed, it may simply be that bladder cancer is the ideal condition for the application this immunotherapy.

The principles of BCG immunotherapy were developed in animal models, and were reviewed by Bast et al. in 1974 [14,15]. Studies showed that prior sensitization was needed to prevent growth of transplanted tumors, and once established, tumors were more difficult to treat. Juxtaposition of BCG and cancer cells was needed and remote injection of BCG could even stimulate rather than inhibit growth. The dose–response curve for antitumor response to BCG was shown to be bell-shaped: excess BCG could reduce response and even promote tumor growth [16]. Response to BCG was limited by tumor size, which may explain the quite consistent failure of BCG to improve the course of advanced disease. Immune competence, particularly the ability to respond to mycobacterial antigens, was correlated with tumor response and prior sensitization was needed when nonviable BCG preparations were used. Optimal success required a sufficient number of viable mycobacteria, as much as $10^8$ organisms in the guinea pig model, and close association of BCG and tumor cells. Effective BCG immunotherapy required an immunogenic tumor as well as an immune-competent host. All of these conditions can generally be met in patients with bladder cancer.

3. Bladder cancer and BCG

Non muscle-invasive bladder cancer (NMIBC) is particularly well-suited to benefit from BCG immunotherapy. NMIBC includes stages Ta, carcinoma limited to the epithelium, T1, invading the subepithelial connective tissue, and carcinoma in situ (CIS). CIS is an aggressive, often diffuse, flat malignancy confined to the urothelial layer that is difficult to visualize and therefore infrequently completely removed. Muscle-invasive bladder cancer (MIBC) is listed as a distinct, separate category because disease in the muscle cannot be reliably resected endoscopically and the risk for extravasational disease is high. Muscle invasive disease therefore requires much more aggressive treatment such as bladder removal, systemic chemotherapy, or radiation therapy. With the high risk of occult metastatic disease chemotherapy is generally combined with either cystectomy or radiation therapy. Clinical experience with bladder cancer echoes the lessons learned from the early animal models. Urothelial carcinoma, which comprises more than 90% of bladder tumors, typically presents with blood in the urine and is diagnosed by direct inspection of the bladder. Papillary tumors are resected cystoscopically and the tumor grade and stage determined by pathologic examination. Despite careful resection the risk for recurrence is very high, over 70%. Recurrence can be due to seeding of cells at surgery, incomplete resection, microscopic disease, or continued transformation and progression of urothelial cells to urothelial carcinoma. Instillation of BCG beginning about two weeks after surgery can provide direct contact of a sufficient number of mycobacteria to a maximally-reduced number of antigenic cells in generally immune-competent patients. These are ideal conditions for response to BCG. Unlike melanoma, where surgery is used to remove 100% of visible tumor and new tumors and metastases are generally remote, urothelial tumors of the bladder recur in the bladder, a site that is accessible by simple catheterization and where local immune stimulation can be given in the area most at risk for tumor recurrence. Urothelial carcinomas have a high number of mutations and therefore more potential antigenic targets. Poorly differentiated, high grade tumors are relatively more sensitive to BCG than well differentiated, low grade tumors. Bladder tumors are typically heterogenic, with mixtures of both high and low grade cells. Following BCG therapy, tumor recurrence is often low grade. Conversion of PPD to positive is associated with a better response to BCG. Those who remain PPD negative, and those who fail to develop antibodies to BCG have a higher recurrence rate [17]. Animal studies show that prior sensitization with BCG improves response [18,19]. An ongoing intergroup clinical trial S1602 is designed to determine whether or not pre-sensitization improves BCG response in bladder cancer [20].

The original trial of BCG in patients with recurrent bladder cancer used Armand Frappier BCG intravesically at a dose of 120 mg (8 x 10^8 CFU). BCG was also given percutaneously by applying BCG to the skin and puncturing the site with a Heaf gun, a 6 pronged spring-loaded device. Treatments were repeated weekly for 6 weeks [21]. The treatment schedule was arbitrary but it was known that at least a 3-week period of immunization was needed to mount a delayed hypersensitivity reaction. The percutaneous administration provided not only the possibility of an enhanced systemic recognition but a simple and readily-visible marker of immunocompetence. Frappier supplied the lyophilized BCG in packages of 6 vials. It was then decided to stop treatment at 6 weeks. Morales reported a 12-fold reduction in tumor recurrence in 9 patients post BCG, compared with pre BCG. This observation led the US National Institutes of Health to request contracts for a randomized controlled trial comparing standard surgical resection of bladder tumor with or without the addition of BCG. Two contracts were awarded and both found that BCG significantly reduced tumor recurrence compared with surgery alone [22,23], as illustrated in Fig. 1. Meanwhile, investigators around the world were doing Phase 2 trials using varying BCG preparations and routes of administration, including dermal scarification [24], intralesional injection [25] and even oral BCG [26]. The oral trial was followed by a 31- patient randomized comparison of bladder
tumor resection alone versus resection plus BCG. Only 1 patient treated with oral BCG had tumor recurrence compared with 7 treated with surgery alone [27]. Netto showed very high doses of oral Moreau BCG to have efficacy, but our comparison with intravesical versus oral Tice BCG failed to confirm efficacy of high dose oral BCG [28]. Subsequent comparison of intravesical BCG with high dose oral Moreau BCG by Netto's group also found intravesical administration to be superior [29]. Morales' original 6-week induction schedule [21] became the accepted standard, though percutaneous BCG was abandoned out of convenience and subsequent failure to demonstrate significant added benefit in randomized trials.

Direct antineoplastic activity of BCG against an existing cancer (rather than prophylaxis of recurrence) was first shown with an observational study of 7 patients with histologically documented carcinoma in situ (CIS) [30]. This report was remarkable not only because the complete response rate was 71% (7/9 patients) but also because the same response rate of CIS to BCG has been reported in multiple subsequent larger, controlled randomized studies [31].

BCG has been called “the vaccine that keeps on giving.” In addition to prevention of tuberculosis and reduction of childhood deaths, as illustrated elsewhere in this 100 year anniversary edition of the journal, vaccination with BCG has benefits that extend far beyond the initial intended use. A poignant example of this is an anecdotal twist of fate (see Fig. 2). Professor Guerin's daughter was treated successfully for bladder neoplasms with the vaccine co-developed by her father some 60 years earlier.

### 4. Maintenance BCG

The standard 6 week BCG induction regimen without prior BCG sensitization or concurrent percutaneous vaccination was so effective that it was very difficult to demonstrate that the addition of maintenance BCG significantly improved efficacy. Initial randomized comparisons of 6 week induction alone versus extended treatment using single instillations monthly [32] or quarterly [33], and even repeated 6 week instillations every 6 months [34] were relatively small but completely failed to show reduced recurrence. To date, the only randomized comparisons of BCG induction and maintenance to show significantly improved reduction in tumor recurrence have used the Southwest Oncology Group (SWOG) maintenance schedule of 3 weekly instillations at 3 and 6 months, then every 6 months to 3 years [35] (Fig. 3). The impressive benefit of the 3 week maintenance schedule is illustrated by the comparison of recurrence curves of the BCG arms of SWOG studies with the same entry criteria (Fig. 4). Less than 16% of patients completed the 27 BCG instillations as planned, suggesting that less treatment may be sufficient for some patients, perhaps particularly those with increased side effects. In this SWOG study 550 of 660 consenting high-risk Ta (non-invasive), T1 (lamina propria invasive) or CIS (carcinoma in situ) patients who were disease free after induction BCG were randomized to observation or 3 week maintenance BCG. Recurrence-free survival (RFS) increased from 36 to 77 months (P < 0.0001), clearly confirming that additional treatment after complete response was beneficial. The benefit of the 3 week schedule was confirmed by Hinotsu [36] in a 3 arm randomized trial of epirubicin chemotherapy, 6 week induction BCG and 18 months of 3 week maintenance in 115 evaluable patients. RFS was 28% with epirubicin, 65% with induction BCG and 85% with 3 week maintenance BCG (P = 0.019 versus induction BCG). Meta-analyses of studies that include varying maintenance BCG versus induction alone do confirm with statistical significance of <0.0001 that recurrence can be reduced with maintenance [37], but single instillations given monthly, quarterly, 3 year 3 monthly, as well as repeated 6 week instillations have not in individual studies achieved statistically significant reduction in recurrence compared with induction. Meta-analysis has also confirmed that maintenance BCG, but not induction BCG alone, is consistently superior to mitomycin chemotherapy [38]. In an individual patient data meta-analysis of 9 studies including 2820 patients, extended Mitomycin C (MMC) chemotherapy reduced tumor recurrence by 28% (P = 0.006) compared to induction BCG, and maintenance BCG reduced tumor recurrence by 32% (P = 0.0001) compared to MMC [39]. Further supporting the benefit of maintenance BCG, meta-analysis reveals that maintenance BCG significantly reduces disease progression compared with MMC (OR = 0.66, P = 0.02), but induction BCG alone does not (OR = 1.16, P = 0.6). With the worldwide BCG shortage investigators have tried to modify the 3 week maintenance schedule. In the multicenter NIMBUS trial [40] 412 patients were randomized to one year of the standard SWOG 3 week maintenance schedule (15 treatments) or induction at 1, 2 and 6 weeks with maintenance at 1 and 3 weeks at 3, 6 and 12 months (9 treatments). The study was closed when interim analysis found one year recurrence in the reduced-frequency BCG arm to be 27% compared with 12% in the standard frequency arm. The dramatic failure of reduced frequency BCG in the NIMBUS trial emphasizes the importance of six-weekly induction as originally performed by Morales and 3-weekly maintenance as developed by Lamm.

### 5. BCG compared to chemotherapy

BCG immunotherapy of bladder cancer was surprisingly slow to gain popularity among urologists despite clear evidence that it was superior to existing chemotherapies. BCG was approved in the US in 1990 based on the SWOG 8216 comparison of BCG and doxorubicin chemotherapy [31]. In 262 high risk bladder cancer patients followed for a median of 65 months and treated with intravesical and percutaneous BCG weekly for 6 weeks and then at 3, 6, 12 and 18 months versus 4 weekly doxorubicin induction and then monthly for a year, 5 year recurrence-free survival was 37% for BCG and 17% for doxorubicin (P = 0.015, Fig. 5). In patients with CIS complete response was 70% for BCG and 34% for doxorubicin (P < 0.001). Early BCG trials were associated with concern about side effects such as urinary frequency, dysuria, fever and even systemic BCG infection. Side effects were less with the popular and effective regimen of MMC chemotherapy. Comparisons of BCG and MMC that were done before optimal treatments protocols
were developed, such as 3 week maintenance BCG or 40 mg/20 cc dose of MMC, produced mixed results but generally favored BCG (Fig. 6). Meta-analysis confirmed that BCG is superior to intravesical chemotherapy, but randomized comparisons that do not use BCG maintenance, which is suboptimal, or include low risk patients, for whom BCG is relatively less effective, can produce conflicting results. In 9 comparisons of BCG and MMC, Bohle [38] found that progression was significantly reduced in 5 studies that used maintenance BCG (OR = 0.66, p = 0.02), but not in 4 studies using induction BCG (OR 1.16, P = 0.612). Similarly, in an independent patient data meta-analysis of 2,820 patients, maintenance BCG reduced tumor recurrence by 32% compared with MMC (P < 0.0001) but with BCG induction only (and often with additional MMC) recurrence was increased by 28% (P < 0.006) [39]. Meta-analyses often do not consider the dose and schedules of the treatments being compared. The multicenter SWOG comparison of 6 week induction BCG with MMC using the same 6 week induction and monthly maintenance schedule in both arms had to be terminated early by the ethical committee due to the significant increase in recurrence, particularly high grade disease, in the MMC arm [41] (Fig. 7).

From comparison studies we may conclude that low risk patients who have solitary noninvasive low-grade tumors are best treated with intravesical chemotherapy, often a single postoperative instillation. Intravesical chemotherapy significantly reduces recurrence, but not progression (Fig. 8). High risk patients, particularly those with CIS as well as those with high grade or T1 disease are clearly best treated with BCG. As discussed below, 3-week BCG maintenance is currently the only intravesical treatment demonstrated in randomized trials to improve survival. Intravesical chemotherapy has been used and widely studied for over half a century, but even when given with maintenance does not appear to improve survival [42].

6. BCG and disease progression of bladder cancer

Some experts have said that BCG only delays the inevitable recurrence, progression and risk of death in bladder cancer, and...
recommend early cystectomy for high risk non-muscle invasive bladder cancer. Radical cystectomy has the advantage of removing not only the bladder, but also the lower ureters and prostate, frequent sites of bladder cancer extension that are not readily amenable to intravesical treatment. Cystectomy is major surgery, complications are common, and 90 day mortality is as high as 10 per cent. If recurrence and progression were inevitable it would be better to perform the procedure when the patient was younger and better able to tolerate major surgery. But are recurrence and progression inevitable?

Demonstration of significant reduction in progression is difficult in randomized clinical trials because it occurs late and in only a fraction of patients with tumor recurrence. With the exception of one study using hyperthermia, no intravesical chemotherapy has been demonstrated to reduce disease progression or mortality. While most BCG trials are not powered to demonstrate reduction in progression, meta-analysis and larger studies using 3 week maintenance BCG do confirm that immunotherapy with BCG reduces progression and as would be expected, mortality. In the SWOG 3 week maintenance versus induction BCG trial of 550

Randomized BCG vs. MMC Studies
Bohle A: J Urol. 169:90-5, 2003

| BCG | Rec. | MMC | ΔBCG | P value | Author/year |
|-----|------|-----|------|---------|-------------|
| 4%  | vs   | 34% | +30  | <.01*   | Pagano '87  |
| 28% | vs   | 62% | +24  | <.001†  | Fimblad '89 |
| 61% | vs   | 80% | +19  | NS      | Lee '92     |
| 47% | vs   | 42% | -5   | NS      | Witjes '94  |
| 64% | vs   | 42% | -21  | NA      | Vogt '95    |
| 46% | vs   | 43% | -3   | NS      | Vogt '95    |
| 43% | vs   | 56% | +9   | <.01*   | SWOG '96    |
| 51% | vs   | 66% | +15  | <.01*   | Malmstr. '96|
| 24% | vs   | 29% | +5   | NS      | Krege '96   |
| 38% | vs   | 62% | +24  | <.001†  | Ayed '98    |
| 32% | vs   | 54% | +22  | <.001†  | Milan '00   |
| 13% | vs   | 26% | +13  | <.01    | Nogueira '01|

Controlled comparisons generally favor BCG over MMC. All 6 BCG maintenance, but only 1 of 5 non BCG maintenance were significant.

Fig. 4. Randomized trials of single quarterly, monthly, and repeated 6 week BCG instillation every 6 months have failed to show significant benefit compared with induction alone. The entry criteria for SWOG studies comparing 3-week maintenance BCG with BCG induction, doxorubicin and MMC were identical. Recurrence curves support the observation that quarterly (SWOG 8216, bottom line) and monthly (SWOG 8795, second from top) BCG does not reduce recurrence compared with induction alone (solid line, 8507). The gold standard, 3- week maintenance (SWOG 8507, top line) is clearly superior to other BCG schedules studied (P < 0.001).

Fig. 5. The SWOG 8216 comparison of BCG and intravesical doxorubicin resulted in FDA approval of BCG. Recurrence curves are shown. The 19% 5 year reduction in recurrence with BCG versus intravesical doxorubicin can be compared with the 5 year reduction in recurrence with chemotherapy compared with surgery alone in the combined EORTC and MRC contemporary studies (See Fig. 8). Chemotherapy significantly reduced recurrence long term, but there was no reduction in disease progression or mortality. What about BCG?

Fig. 6. Response to intravesical treatment depends on dose, duration, concentration and treatment schedule. Mitomycin C (MMC), with a CR of 53% in CIS, was thought to be equal or superior to BCG. In low grade tumors the advantage of BCG over chemotherapy diminishes and when compared with BCG induction alone, MMC, especially if given with hyperthermia and optimal concentration and schedule, can be superior. Bohle et al have reviewed MMC BCG comparison studies and found evidence for overall superiority of BCG.

Lamm DL: N Engl J Med. 1991;325:1205
The 5 yr survival advantage was 4% at 5 years and 13% at 10 years (HR 0.87, 95% CI 0.83–0.92). Similarly, in Sweden a review of 4319 patients with T1 (lamina propria invasive) bladder cancer showed that those treated with BCG had a 21% reduction in mortality (HR 0.79, 0.66–0.96, p = 0.015) [45]. In both populations fewer than a fourth of the patients who were eligible for BCG received it, and that was before the worldwide BCG shortage.

7. BCG shortage

The recurrent world-wide shortage of BCG began in 2012 with suspension of production of Connaught BCG in Canada due to a perceived risk of fungal contamination. A new facility was constructed to supply BCG, but that effort was abandoned due to a massive “100 year flood”. Subsequent shortages of purple potatoes and other supply problems reduced production of Tice BCG in the US. Strict regulatory hurdles, recurrent shortages of production supplies, increased acceptance and use of BCG for bladder cancer, and the limited profit in the production of BCG has led to long term, world-wide recurrent shortages. The shortage of BCG has resulted in the removal of bladders that could have been saved and increased tumor recurrence, morbidity, mortality and expense of bladder cancer [46]. The shortage of BCG is even more dangerous for infants. It is estimated that urgent measures to correct a projected 6.3% shortfall in infant BCG vaccination in 2015 saved 7433 (95% Uncertainty Range: 1074 to 65,278) childhood deaths from tuberculosis [47], and that does not even count the many more deaths from other diseases that appear to be prevented by BCG vaccination. Randomized trial [48] demonstrates that neonatal vaccination produces beneficial nonspecific effects and population studies suggest that childhood mortality may be reduced by as much as 50% [49].

The tragic shortage of BCG for childhood vaccination is worsened by the increased recognition of the unparalleled benefit and cost effectiveness of intravesical immunotherapy in bladder cancer. A single BCG instillation for bladder cancer requires ∼ 5 × 10⁸ CFU, potentially enough BCG to vaccinate as many as 1000 infants. As a cancer treatment BCG has long been criticized as a nonspecific immune stimulant whose mechanism is unclear. Recognition that this non-specificity is associated with protection from unrelated bacterial and viral pathogens, particularly respiratory virus infections possibly including COVID-19 [50] could increase the demand for BCG and risk further reductions in the supply. It is ironic that this daughter of the world’s most lethal human infection should provide so many benefits at such a low cost, and that the low profit due to the low cost should result in so much unnecessary suffering and death. Despite generous philanthropy to improve world health and well-meaning bureaucracy in so much unnecessary suffering and death. Despite generous philanthropy to improve world health and well-meaning bureaucracy to ensure the safety of drugs, the low profit of manufacturing BCG using current techniques provide no hope that the shortage will be relieved unless a new, more expensive and reliably-manufactured product such as recombinant BCG is approved.

8. Recombinant BCG

For more than 3 decades researchers have worked to develop recombinant BCG (rBCG) preparations in an effort to improve the safety and efficacy of BCG vaccination in the prevention of tuberculosis [51]. Genome sequencing of BCG strains has demonstrated deletion of epitopes that may account for decreased efficacy [52] and recombinant BCG (rBCG) have been constructed that heighten immune responses in vitro and in animal models [53]. It has been postulated that the genetic drifting inherent in the numerous passages of BCG has resulted in reduced capacity to activate important immunomodulatory factors such as “Simulator of Interferon Genes” [54].

Patients 10 year tumor recurrence was reduced from 52% to 25% (P < 0.0001), worsening-free survival increased from 52% to 60% (P < 0.04, one tail), and overall survival increased from 52% to 58% (P = 0.08, NS) [35] Fig. 9. Worsening-free survival is a surrogate for muscle invasive progression. To improve survival in patients at high risk for metastasis urologists often proceed with cystectomy, radiation or systemic chemotherapy when disease persists in the bladder. In the EORTC trial 30911, of 837 evaluable patients followed for 9.2 years after randomization to 3 week maintenance BCG or epirubicin chemotherapy on the same schedule, BCG significantly reduced recurrence (P < 0.0001), metastasis (from 9% to 5%, P = 0.046) and both disease specific and overall mortality (P = 0.023) [43] Fig. 10. The survival benefit of BCG immunotherapy is further supported by population studies. A retrospective US study of 23,932 patients showed that BCG reduced mortality in high-grade bladder cancer when given within 6 months of diagnosis (HR 0.78, 95% CI 0.72 to 0.85) [44]. The 5 yr survival advantage was further supported by population studies.
es” (STING), ligands/agonists that stimulate potent pro-inflammatory responses [54]. One promising rBCG that codes for STING has demonstrated superiority compared to standard BCG in the treatment of animal models of bladder cancer [55]. The Max Planck Institute in Berlin has developed a recombinant BCG (VPM2001) that has demonstrated safety and efficacy in vaccination trials and has been reported to have a 49% 60 month complete response in bladder cancer patients who have failed standard BCG [unpublished, Max Planck Institute, 2020]. Genetically-modified BCG designed to improve safety and more effectively stimulate and modulate the immune response, produced with modern technology that can more readily meet modern regulatory expectations has the potential to not only improve care but also alleviate the chronic supply shortage.

9. Alternative immunotherapy for bladder cancer

BCG has demonstrated the potential of immunotherapy in bladder cancer, but other agents are needed to improve treatment options and manage those who are intolerant or resistant to BCG. Mycobacterium Cell wall Nucleic Acid (MCNA) is an immunomodulatory and antineoplastic agent derived from Mycobacterium phlei that is comprised of mycobacterial cell wall fragments com-
MCNA exerts its anti-cancer activity through a dual mode of action; an indirect immunotherapeutic effect (similar to BCG), stimulating anti-cancer cytokine production by immune effector cells and a direct chemotherapeutic effect (similar to cytotoxic agents). The immunomodulatory and anti-cancer activities of MCNA are mediated mostly through its DNA component, while the cell wall fragments function as a drug delivery system for the DNA. In early clinical studies the activity of the preparation against NMIBC was established [56]. Subsequent studies investigated the efficacy and safety of MCNA in the challenging population of patients who had failed BCG therapy. The results were encouraging and superior to other agents in management of this particularly challenging clinical situation [57,58], but like so many other treatments did not achieve registration with the US FDA. One efficient way to demonstrate efficacy of intravesical therapy is to show complete response in CIS as measured by negative biopsy and cytology. BCG, with a complete response rate of over 70%, is recognized as the treatment of choice for CIS (See Figs. 11 and 12). Many studies submitted for regulatory approval evaluate response in BCG unresponsive CIS. Previous immunotherapies have demonstrated some success, but entry criteria, duration of follow and number of patients evaluated prohibit direct comparison of efficacy. Acknowledging those major limitations, the CR rate in CIS has been reported to be 45 to 47% with interferon alfa 2b [59] and 50% with Keyhole Limpet Hemocyanin (KLH) [60]. While these response rates are similar to those seen with intravesical chemotherapy, the FDA requires strict adherence to defined categories of BCG failure. The complete response to BCG induction can be delayed: 24% of patients in the SWOG 8507 maintenance study who had persistent CIS at the initial 3 month cystoscopy after beginning induction went on to have complete response at 6 months without further treatment [35]. Patients who have not received an adequate number of BCG treatments or have responded and then had recurrence more than a year later are not necessarily BCG unresponsive. The FDA therefore defines specific criteria eligibility into trials of new agents. BCG refractory CIS is defined as failure to achieve complete response following adequate induction (at least 5 of 6 instillations) and at least 2 of a planned 3 week maintenance instillations or a second induction. BCG relapse is defined as recurrent malignancy within one year of adequate BCG. Without meeting these definitions regulatory approval would not be achieved in the US. Ongoing trials with encouraging intravesical therapy in BCG refractory and relapsing patients include agents such as Vicineum, an antibody/drug (Epcam/pseudomonas toxin) combination (39 to 57% CR at 3 months); ALT-803, an IL-15 stimulant (71% CR at any time), Nadofaragene Firadenovec, a reproductive deficient adenovirus plus the excipient Syn3 and interferon alfa gene (53% CR at 3 months) [61], and CG0070, also a reproductive deficient adenovirus, with an RB promoter and GMCSF (granulocyte–macrophage colony stimulating factor) gene (58% CR at 6 months) [62]. Another encouraging development, discussed in more detail below, is the advent of systemic immunotherapy. One such treatment, pembrolizumab, is now approved for NMIBC when intravesical treatments are unsuccessful. In CIS, 41% of 96 patients treated with pembrolizumab had CR at 3 months [63]. It is hoped that these agents and others, the product of modern science and production techniques, will be able to overcome the limitations of BCG and other intravesical therapies.
to meet and maintain the requirements for registration and provide an alternative to radical cystectomy when BCG and intravesical chemotherapy fail to prevent bladder tumor recurrence.

10. BCG and the new immuno-oncology

Immune checkpoint inhibitors have begun a new era in cancer treatment: Immuno-Oncology (IO). Patients with advanced bladder cancer who have failed standard chemotherapy now often survive months or even years longer with systemic immunotherapy, therapy that is generally better tolerated than systemic chemotherapy. Immune checkpoint inhibitors of the PD-1/PD-L1 axis, which control programmed cell death, and CTLA4, which suppresses T cell activation, have been used in patients failing to respond to BCG. These and other approaches are actively being investigated for patients who fail BCG and for potential use as first line of treatment [64]. Urothelial carcinoma cells express lower levels of PD-1, but BCG can induce PD-L1 expression in Tregs and tumor tissue in BCG resistant patients, making consideration of combination therapy appealing. In patients with muscle invasive bladder cancer post radical cystectomy, Nivolumab in a randomized comparison with placebo extended disease free survival from 10.9 to 21.0 months (p = 0.0006) [65]. Despite impressive responses seen with PD-L1 inhibitors, the manufacturers of both durvalumab and atezolizumab have recently voluntarily withdrawn indications for use after failing to meet follow-up study end points subsequent to accelerated approval. Durvalumab withdrew the indication for first line treatment of metastatic disease and atezolizumab withdrew the indication for treatment of patients who failed platinum treatment. Clearly treatment with PD-L1, PD-1 and CTLA and the associated immune responses, like BCG, are complicated. Do some of the principles of BCG immunotherapy apply to modern immunotherapy? Do lessons learned with IO apply to BCG immunotherapy? The dose–response curve with BCG is bell-shaped, and excess BCG can reduce the response [16]. Might this apply to IO treatment? Is more IO always better? This is certainly not true of BCG. Maintenance BCG is reduced from 6 to 3 instillations after induction, the interval is increased from 3 to 6 months at 6 months, and dose is often reduced. Would similar reduction in dose and prolongation of treatment intervals apply to IO? Response to BCG can be delayed. In SWOG 8507 complete response in CIS increased from 58% at 3 months to 69% at 6 months without additional treatment. Three additional BCG instillations in the maintenance arm increased CR from 55% to 84% (P < 0.01) [35]. Patients treated with IO similarly appear to respond less quickly than those treated with chemotherapy, but even partial responses appear to be associated with prolonged survival. With BCG tumor recurrence after 6 months is considered to be treatment failure, and patients move on to other treatments, typically intravesical chemotherapy or cystectomy. Like BCG immunotherapy, IO maintenance after chemotherapy appears to prolong survival. The gut microbiome, and perhaps in the future the newly-identified urinary tract microbiome, is being extensively studied. The gut microbiome has been demonstrated to significantly influence the response to anti PD-1 immunotherapy. Alteration of the microbiome with diet, avoidance of antibiotics and even fecal transfer are being investigated and appear to reverse resistance to anti-PD-1 treatment [66]. Similar mechanisms would seem likely to apply to resistance to BCG immunotherapy. Measures of the immune response and tumor markers have been extensively studied but have not had a major impact on BCG immunotherapy of bladder cancer. The seismic shift to immuno-oncology will expand research into the mechanisms of tumor response and resistance. Resistance to BCG immunotherapy is now treated with removal of the bladder, in the future it could be managed by changing the gut flora. BCG vaccination to prevent tuberculosis might be similarly improved by better understanding of the microbiome.

11. Conclusions

Immunotherapy is an ancient treatment for cancer that was nearly abandoned following the advent of chemotherapy in the early 20th Century, but recognition of the important role of the immune system in cancer control sparked interest in the profound immune stimulation induced by BCG. Animal models demonstrated the effectiveness of BCG immunotherapy in many cancers, but in only one, superficial high-risk bladder cancer, was BCG to become the clear treatment of choice. Often treated as an erstwhile stepchild and spurned by surgeons as a treatment that would only postpone the inevitable curative extirpation of the bladder, it was said that BCG would only delay and not prevent tumor recurrence. Proponents of intravesical chemotherapy believed cytotoxic treatment was superior. Randomized clinical trials and real-world population studies have proved current BCG immunotherapy to superior to chemotherapy, and prevention of recurrence is long term in many patients. It was said that BCG does not prevent disease progression or mortality, and that maintenance of the immune stimulation was unnecessary, but again large randomized trials supported by population studies confirm the opposite. Immunotherapy has begun a new era with the advent of new techniques such as antibody based treatment and immune checkpoint inhibition. Immuno-oncology holds great promise for many malignancies. Bladder cancer and the Pasteur Institute’s century-old TB vaccine have been demonstrated to be uniquely suited for cancer immunotherapy research, which bodes well for current and future treatments that are building on the lessons of the past.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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