Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Bacillus Calmette-Guerin

Evidence Summary

BCG promotes innate immunity system training in a manner that may protect against infections, and other immune-related conditions, but effects wane with time, and the impact of boosters is unclear.

Neuroprotective Benefit: Late life treatment with BCG in cancer patients is associated with lower risk for dementia. BCG may retrain the immune system into a neuroprotective state, but it is unclear whether it can benefit those with dementia.

Aging and related health concerns: Non-specific immune training by BCG may protect against and slow progression of some forms of cancer, autoimmune disease, and infectious respiratory diseases.

Safety: BCG vaccination is safe and well-tolerated for most people. Mild fever and injection site reactions are the most common side effects. Because it is a live vaccine, BCG can result in active infection in immunocompromised individuals.

Availability: Through national immunization programs in select countries, or by Rx for cancer treatment	**Dose**: BCG vaccination is typically given during the first year of life. Bladder cancer treatment involves induction and maintenance courses of BCG intravesical instillations.
Half-life: Immunity lasts approximately 20 years	**BBB**: N/A
Clinical trials: Aside from its use in tuberculosis prevention, BCG has been tested in small pilot clinical trials for type 1 diabetes, multiple sclerosis, Alzheimer's disease, and several cancers. BCG has been tested in larger Phase 2 and 3 RCTs for bladder cancer and Covid-19.	**Observational studies**: Neonatal BCG vaccination is associated with reduced childhood mortality in countries with a high pathogen burden. BCG treatment for bladder cancer has been associated with reduced risk for dementia in retrospective studies. Epidemiological studies are mixed regarding BCG's impact on the prevention of diabetes, atopic disease, and cancer.

What is it?

Bacillus Calmette-Guerin (BCG) is a vaccine designed for protection against the bacteria that causes tuberculosis, *Mycobacterium tuberculosis*, though it appears to offer a degree of protection against some other microbial pathogens as well [1]. The BCG vaccine, named for its creators, Albert Calmette and Camille Guérin, is a weakened live vaccine derived from *Mycobacterium bovis*, which is the main cause of tuberculosis in cattle, though it does also have the capacity to infect humans. The original virus was weakened through serial passage over 230 times in the course of ten years, and then distributed globally in 1924. Since that time, several different sub-strains of the BCG have emerged in different parts of the world, which can result in different immunological responses. The most common strains used worldwide are BCG Denmark, BCG Russia, and BCG Japan. The only strain that is currently manufactured in the US is the BCG-TICE strain, distributed by Merck. BCG is primarily administered to infants under the age of one in countries with a high burden of tuberculosis. It appears to be most effective during the early childhood years, though it offers only limited protection against pulmonary tuberculosis. BCG is also approved, in the form of intravesical instillation, in patients with non-muscle invasive bladder cancer [2]. Due to the ability of the BCG vaccine to exert non-specific (i.e. non-tuberculosis related) effects on immune system responses, it has been proposed as a therapeutic for other conditions with

immune dysfunction, including the autoimmune diseases type 1 diabetes, multiple sclerosis, and Alzheimer's disease.

Neuroprotective Benefit: Late life treatment with BCG in cancer patients is associated with lower risk for dementia. BCG may retrain the immune system into a neuroprotective state, but it is unclear whether it can benefit those with dementia.

Types of evidence:

- 4 retrospective reviews assessing AD risk following BCG treatment in bladder cancer
- 1 pilot clinical trial assessing biomarkers after late life BCG vaccination in an AD-risk population
- 1 small clinical study on late life BCG vaccination to enhance immunity in dementia patients
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

There is evidence from observational studies to suggest that late-life exposure to BCG is associated with a reduced risk for dementia.
A retrospective review of 1,371 bladder cancer patients (83% male) in Israel compared dementia rates between those who received adjuvant post-operative intra-vesical treatment with BCG with those who did not receive BCG with a median follow-up period of eight years [3]. Fewer people in the BCG-treated group (21/878 or 2.4%), developed Alzheimer's disease (AD) than those who did not receive BCG (44/ 493 or 8.9%). This translates to a four-fold higher risk in the non-BCG-treated group (Hazard ratio [HR]: 4.778, 95% Confidence Interval [CI] 2.837 to 8.046). BCG treatment did not significantly impact Parkinson's disease (PD) risk in this cohort, with rates of 1.9% and 1.6%, respectively. A second retrospective review by this group examined dementia rates in 12,185 patients (>80% male), in three cohorts from Israel and the United States, with non-muscle invasive bladder cancer who underwent at least one BCG instillation following tumor resection, relative to those who did not receive any BCG treatment, with a mean follow-up of 3.5 to 7 years [4]. In the largest cohort, 4.8% (75/1587) of BCG-treated patients developed AD, compared to 6.5% (336/5147) of those who did not receive BCG. Using the adjusted Cox proportional hazards regression model resulted in a hazard ratio of 0.787 (95% CI 0.612 to 1.012), which falls shy of statistical significance (p=0.062). Similar trends were seen in the other two smaller cohorts, with one showing rates of 3.2% (13/408) vs 5.8% (17/292), and the other showing rates of 0% (0/315) vs 2.9% (132/4,445), for the BCG-treated and non-BCG-treated groups, respectively. When

stratified by age, the risk reduction, up to 30.6%, was most prominent in those ≥75 years old. The effect appeared strongest in females, although this should be interpreted with caution, as the demographics of the population limited statistical power. In the largest cohort, BCG treatment was also associated with a reduced risk for PD (HR: 0.682, 95% CI 0.468 to 0.99), with incidence rates of 1.98% (33/1,669) and 3.0% (153/5,097) for BCG and non-BCG treatment, respectively. In contrast, BCG-treatment had no significant effect on incidence rates of stroke (HR: 1.048, 95% CI 0.846 to 1.229) or type 2 diabetes (HR: 0.710, 95% CI 0.429 to 1.490) in this cohort.

A separate group examined dementia risk in a racially and ethnically diverse cohort (16.8% non-Hispanic black, and 17.6% Hispanic) of 1,290 patients with non-muscle-invasive bladder cancer [5]. The overall dementia incidence rates were 3.1% (10/319) in the BCG-treated and 9.2% (89/971) in the non-BCG-treated groups (adjusted HR: 0.41, 95% CI 0.21 to 0.80).

Several large caveats of these studies need to be taken into consideration [6]. There is a strong potential for selection bias in the patient population who received the BCG-treatment, as they may have been less frail and better able to tolerate the treatment. Additionally, many studies suggest a link between cancer itself with dementia risk reduction [7], suggesting that differences in cancer profiles, which may have influenced treatment decisions, could differentially impact future dementia risk.

A fourth retrospective review attempted to get around some of these confounds by looking at the dose-response relationship. This study assessed the incidence of AD diagnosis in a population of 26,584 patients with high-risk non-muscle-invasive bladder cancer aged ≥ 66 years old in the US, using a Medicare database [8]. Overall, subsequent AD diagnosis rates were lower in those treated with BCG (964/13,496 or 7.1%) than for those without BCG exposure (1,228/13,008 or 9.4%) (HR: 0.73, 95% CI 0.67 to 0.79). Notably, the effect was dose-dependent, such that those who received the most instillations of BCG (13+) had the lowest dementia rates (HR: 0.55, 95% CI 0.47 to 0.64). A single induction session of 5 to 6 doses was associated with a 15% risk reduction, those receiving 7 to 13 doses had a 27% risk reduction, while those receiving over 13 doses had a 45% risk reduction, relative to those without BCG exposure. Similarly, in the racially and ethnically diverse cohort, the association was strongest in those who received both induction and maintenance BCG sessions (adjusted HR: 0.23, 95% CI, 0.0 to 0.96), and only borderline in those who received only the induction course (adjusted HR: 0.51, 95% CI 0.24 to 1.06) [5]. This suggests that the effect may be related to BCG, rather than the cancer or patient-specific factors. It is also in line with the results from studies in other chronic conditions, indicating that multiple doses of BCG are needed to elicit a protective effect.

Neonatal BCG vaccine treatment is not associated with altered dementia risk. An analysis of BCG regimens with AD incidence in European countries found a weak association in males, which did not

survive correction analysis [4]. The immunological effects of the BCG vaccine appear to last around 20 years, though there is some evidence to suggest that some features may last up to 40 years in some people [9]. As a result, the effects of the vaccine are tapering off prior to middle age, which is the time when dementia-related brain changes start to accumulate. This would suggest that BCG administration during mid to later life may more meaningfully impact neuro-immune trajectories that impact dementia risk. The potential impact of BCG instillation therapy in bladder cancer patients on dementia risk may be related to timing of the treatment, typically during this mid to late life window.

An open-label pilot clinical trial (NCT04449926) tested the impact of BCG vaccination during late life, with a mean age 64.3 for women and 65.7 for men, on AD biomarkers [10]. Forty-nine participants with a family history of dementia were stratified for AD risk using an Amyloid Probability Score (APS) based on the plasma Aβ42/40 ratio, age, and ApoE status. Participants were administered an initial dose of 0.1 mL (2 × 10^5 colony forming units [CFU]) BCG (Tice strain from Merck) via intradermal injection, followed by a booster one month later. Nine months following vaccination, Aβ42/40 levels were significantly higher for those under 65, but the effect was not significant in those over 65. The impact of vaccination was affected by a participant's immunological profile, including whether they had antibodies (IgG) to cytomegalovirus (CMV) as well as their CD4/CD8 T cell ratio. The persistent presence of CMV impacts the ability of the immune system, particularly T cells, to respond to other insults. The hypothesized mechanism by which BCG exerts its non-specific (i.e. non-tuberculosis-related) effects is through immune modulation. As such, the efficacy of BCG on chronic conditions may depend on the response capacity of a given person's immune system. In this cohort, biomarker AD risk was reduced most prominently following BCG in those without latent CMV and with a high CD4/CD8 T cell ratio, indicative of a more immunocompetent status. It is possible that a single course is sufficient in immunocompetent individuals, whereas multiple BCG courses may be needed in individuals with underlying immune system dysfunction, such as has been seen in the context of some autoimmune diseases.

Human research to suggest benefits to patients with dementia:

A study of 44 participants found that compared to age-matched controls, dementia patients were less likely to show a productive response on delayed hypersensitivity skin tests, which is indicative of the T cell and macrophage response to pathogens [11]. Following a course of four weekly intradermal injections of the BCG vaccine, 11 out of the 13 patients who initially had failed responses had at least a partial restoration, with seven rebounding to normal response levels. This suggests that multiple

treatments with BCG can modify immune responses in dementia patients, though does not address whether this can impact disease trajectory.

An open-label pilot study (NCT04507126) assessed the ability of the BCG vaccine to impact AD biomarkers in the blood and cerebrospinal fluid (CSF) as well as cognition in 20 older adults (≥ aged 55) with normal cognition or mild cognitive impairment (MCI). The study used two injections spaced four weeks apart of the Japan BCG strain at a dose of 1.8-3.9 x 10^6 CFU. The study was completed in mid-2022, but results have not yet been made available.

An open-label Phase 2 clinical trial (NCT05004688) is currently underway testing the effect of BCG immunization (two doses four weeks apart of the Japan BCG strain at 0.36-3.9 x 10^6 CFU per dose) on AD biomarkers and cognition in 15 patients with MCI or AD.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Immune modulation: The primary mechanism by which BCG is thought to exert its non-specific effects is via the modulation of immune system responses. BCG has been shown to be able to promote trained innate immunity, which involves the epigenetic and metabolic reprogramming of innate immune cells [12]. BCG can epigenetically influence the gene expression in monocytes/macrophages through chromatin remodeling, such as influencing methylation and acetylation patterns of histones, promoters, and enhancers. BCG appears to promote immune tolerance through the induction of genes that induce regulatory T cells, which promote self-tolerance and suppress immune responses. Innate immune training may influence the processing of amyloid precursor protein (APP) [13]. BCG has been shown to stimulate the production of IFN-γ. In the brain, IFN-γ promotes major histocompatibility complex (MHC) expression on astrocytes, which facilitates the processing of APP-derived fragments as self-antigens. In the absence of this pathway, there is preferential cleavage of APP to form Aβ42, which is not a suitable substrate for antigen presentation. As a result, there is a loss of tolerance, and subsequent activation of the immune system, which may result in neuronal damage. In addition to protecting against a deleterious immune response toward self-antigens, BCG-mediated immune training may facilitate productive immune responses against other pathogens, to protect against chronic latent infections which may otherwise drive an excessive low-grade inflammatory response [13]. For example, BCG has been reported to promote productive immune responses against herpes virus and gingivalis bacteria, pathogens which have been associated with AD risk. This suggests that by modulating the response of the immune system to self and microbial antigens, that BCG vaccination may prime the immune system toward a state that is less likely to drive AD pathology.

Neuroimmune mediated neuroplasticity: The immune system is known to play a role in brain development. Neonatal administration of BCG has been shown to influence neurodevelopment in rodents. Mice vaccinated with BCG D2-BP302 strain (10^5 CPU) at postnatal day zero (P0) showed evidence of increased hippocampal neurogenesis coupled with a neurotropic neuroimmune profile, including higher levels of IFN-γ, IL-4, TGF-β, BDNF, and IGF-1, as well as lower levels of TNFα and IL-1β, at two weeks of age [14]. This profile is consistent with a systemic Th1 immune bias. The vaccinated mice also showed better spatial cognition on the Morris water maze at four weeks of age, and were more resistant to impairments and anxiety stemming from an inflammatory stimulus (LPS) during adulthood. The latter may be related to a blunting of LPS-induced reduction in brain levels of serotonin (5-HT) [15]. The protective immune effects appear to stem from the IFN-γ-mediated recruitment of 1L-10 secreting T cells to the choroid plexus, which leads to the activation of macrophages in the meninges into an anti-inflammatory M2-like state [16]. Similarly, rats vaccinated with BCG D2-BP302 strain (10^5 CPU) at P0 showed increased levels of the neurotrophic factors BDNF and IGF-1, which correlated with increased dendritic spine length, complexity, and density in the hippocampus at two and four weeks of age [17]. The immune modulatory and neurotrophic effect was enhanced by the combined neonatal vaccination of rats with BCG and a seasonal influenza vaccine [18].

There is evidence from AD mouse models to suggest that BCG vaccination during adulthood can promote a similar type of neuroimmune remodeling with associated benefits on synaptic plasticity. In the APP/PS1 AD mouse model, BCG vaccination with the BP302 strain (four s.c. injections of 2 × 10^4 CFU at four weeks apart) at six to seven months of age induced an increase in circulating levels of the cytokine IFN-γ and enhanced the recruitment of M2-like inflammation resolving macrophages across the choroid plexus and perivascular spaces into the brain [19]. This shifted the cytokine profile in the brain to an anti-inflammatory state, increasing levels of IL-10, IL-4, and TGF-β, and reducing levels of the pro-inflammatory cytokines TNF-α and IL-1β. These were accompanied by increases in levels of the neurotrophic factors BDNF and IGF-1, resulting in a pattern that effectively mimics what is seen in the rodent brain following neonatal BCG vaccination. These mice also showed better cognition on spatial memory tests. Improvements were also seen in this model when the vaccination schedule was started later in the disease course, at nine months of age [20]. One study assessed the dose dependency, testing one, two, or three injections of live freeze-dried BCG D2-BP302 strain (1 × 10^6 CFU) starting at three months of age in the APP/PS1 model [21]. The group receiving three BCG vaccinations showed stronger immune modulation and a higher degree of synaptic plasticity in the hippocampus, relative to the other groups.

Together these studies suggest that BCG vaccination can enhance neuroplasticity via neuroimmune remodeling in animal models, and that in contrast to neonates where a single dose is sufficient, multiple doses are required in adults. The durability of the response is unclear. It will be important to know whether a onetime 'resetting' of the immune system is sufficient, or if additional measures are needed. With rodent neonate vaccination, the effects on neuroplasticity become less apparent by eight weeks of age. This suggest that periodic boosters may be needed in the adult animals, but the optimal interval is unclear, as is whether there is a ceiling on the number of doses that can be safely administered. Furthermore, there are the related questions of how long it takes for these neuroimmune changes to take place following vaccination and whether there is an optimal window for vaccination within the course of the disease.

Parkinson's disease: POTENTIAL BENEFIT (Preclinical)
BCG vaccination has been shown to protect dopaminergic neurons in the MPTP mouse model of PD. In one study, male mice were exposed to MPTP ten days after vaccination with live BCG (TheraCys® Connaught strain from Sanofi Pasteur, 2×10^7 CFU i.p.) [22]. BCG vaccinated mice had lower levels of microglial activation in the striatum in response to MPTP, resulting in more preservation of dopamine content. Another study assessed the impact of BCG vaccination using the same strain at 1 or 6 × 10^6 CFU 18 days prior to MPTP in male mice [23]. This dose range appeared to offer more protection relative to the higher dose used in the prior study. Levels of regulatory T cells (Tregs) were higher following vaccination, and this Treg response was correlated with the preservation of striatal dopamine. These studies suggest that BCG may prime the immune response in a manner where it is less likely to produce damage to the nigro-striatal dopaminergic system in response to an inflammatory agent. It is not clear whether there is a critical window of timing between vaccination and toxin exposure, and how this relates to the time course over which PD develops in humans.

APOE4 interactions: Not established.

Aging and related health concerns: Non-specific immune training by BCG may protect against and slow progression of some forms of cancer, autoimmune disease, and infectious respiratory diseases.

Types of evidence:

- 1 systematic review on childhood BCG vaccination and all-cause mortality
- 1 systematic review of BCG vaccination for atopic disease prevention
- 1 systematic review of epidemiological studies for BCG vaccination and Covid-19

- 1 meta-analysis of neonatal BCG vaccination on mortality in low-weight infants
- 1 meta-analysis on studies assessing BCG and pediatric leukemia
- 1 meta-analysis of clinical trials using BCG for bladder cancer
- 1 meta-analysis of RCTs for BCG vaccination in type 1 diabetes
- 3 reviews on BCG treatment for bladder cancer
- 2 reviews of BCG vaccination for autoimmune disease
- 2 clinical trials of BCG re-vaccination for Covid-19
- 2 clinical trials of BCG vaccination for Covid-19
- 2 clinical trials for BCG vaccination in multiple sclerosis
- 1 clinical trial of BCG vaccination for eczema prevention
- 1 clinical trial assessing adult BCG vaccination on *ex vivo* immune responses
- 1 clinical trial assessing the effect of age on BCG treatment for bladder cancer
- 1 epidemiological analysis of BCG vaccination and diabetes
- 1 observational study on BCG re-vaccination on mortality during adulthood
- 1 observational study on childhood BCG vaccination and cancer
- 1 retrospective cohort study of BCG vaccination and Covid-19
- 1 case-control study of BCG vaccination and Covid-19
- Numerous laboratory studies

Mortality: BENEFIT FOR INFANTS IN REGIONS WITH HIGH PATHOGEN BURDEN
BCG vaccination has been associated with decreased mortality in infants and children, particularly in regions with high pathogen burden [24], however, there is no clear evidence for a benefit in adulthood. A systematic review examined the association between childhood (< 5 years old) all-cause mortality and vaccination with BCG, diphtheria-tetanus-pertussis (DTP), and standard titre measles containing vaccines (MCV) in 34 birth cohorts [25]. BCG vaccination was associated with lower all-cause mortality in five clinical trials (n=3,508) with low to moderate risk bias (Relative Risk [RR]: 0.70, 95% CI 0.49 to 1.01) and nine observational studies (n=28,284) with high risk of bias (RR: 0.47 95% CI 0.32 to 0.69). BCG vaccination is recommended at six weeks of age, but the timing varied across the studies up to 4.8 months of age. The benefits appeared to decrease at later ages of vaccination. Similarly, an analysis of early BCG (prior to six weeks of age) in low-income countries in three RCTs (n= 6,583) found that vaccination reduced mortality within the first week by three-fold in low-birth-weight males, and by two-fold in females within two to four weeks of vaccination [26]. The protective effect in males was driven by a reduction in sepsis-related deaths. This suggests that the protective effects are rapid, related to effects on non-specific (not specific to tuberculosis infection) immunity, and that vaccination at birth

may be needed to protect the highest risk newborns. Since infants are most vulnerable to infectious diseases due to their immature immune systems, the protection afforded by BCG as an immune training agent would be expected to decline with age, as the immune system matures. Indeed, the results from a study of BCG re-vaccination on mortality beyond infancy failed to show a protective effect. The population based Karonga Prevention RCT included 46,889 individuals aged 3 months to 75 years in northern Malawi who received either BCG re-vaccination or placebo [27]. Of these, 3,709 were actively followed for up to 30 years. The analysis was divided into participants living in two different geographic regions of Malawi, and there were no significant differences in mortality rates with re-vaccination for either cohort (Northern cohort HR 0.94, 95% CI 0.74 to 1.20) (Southern cohort HR 1.06, 95% CI 0.88 to 1.27). The difference may be related to different primary causes of mortality in infants and adults. The non-specific effect on mortality in infants is driven by a reduction in deaths due to infectious diseases, whereas adults are more likely to die from non-communicable disease-related causes. Furthermore, the protection against infectious disease may be pathogen dependent, as a clinical study of 35 healthy adults in the UK found that BCG vaccination offered protection against Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumonia*, but not against the Gram-positive bacteria *Staphylococcus aureus and Streptococcus agalactiae*, in *ex vivo* assays [28].

Cancer Prevention: MODEST ASSOCIATION FOR REDUCED LUNG CANCER RISK

It was first reported in 1929 that the incidence of cancer was lower in patients with tuberculosis, suggesting that *Mycobacterium tuberculosis* pathogen and/or the immune response to it may promote the efficient clearance of nascent cancer cells [2]. There is evidence from some epidemiological studies to suggest that BCG vaccination may be associated with protection against some forms of cancer. A meta-analysis of 14 studies assessed the association between pediatric leukemia and early vaccination with the BCG vaccine, Triple vaccine, Hepatitis B vaccine (HBV), Polio, Measles, Rubella, Mumps (MMR), trivalent MMR vaccine or Haemophilus influenza type B (HiB) vaccine [29]. Twelve of the studies (n=3,764,593 participants) examined the relationship with BCG vaccination and found a non-significant trend toward reduced leukemia incidence (OR: 0.73, 95% CI 0.50 to 1.08). Vaccination of any type in the first year of life was associated with protection (OR: 0.58, 95% CI 0.36 to 0.91), but since BCG was the most common type of early vaccination, it is unclear whether BCG is mediating this effect. The lack of association with any of the other specific vaccination types with childhood leukemia suggests a specific role for BCG, but due to the low quality of evidence and high bias of the included studies, a clear determination cannot be made.

The strongest evidence to date comes from the 60-year follow-up of a clinical trial of 2,963 American Indian and Alaska Native participants who were vaccinated with BCG or placebo from ages 5 to 11

between December 1935 and December 1998 [30]. The overall cancer incidence rate did not differ between the groups (HR: 0.82; 95% CI 0.66 to 1.02), however, there was a lower incidence of lung cancer in the BCG vaccinated group (HR: 0.38, 95% CI 0.20 to 0.74). BCG vaccinated participants had a lung cancer rate of 18.2 cases per 100,000 person-years, while the rate was 45.4 cases per 100,000 person-years in the placebo group. The difference could not be attributed to differences in rates of tuberculosis, which is itself a risk factor for lung cancer. Since *Mycobacterium tuberculosis* is a respiratory pathogen, BCG may preferentially affect the immunoregulation of the lung, and because lung cancers tend to be highly influenced by their immune environment, BCG may foster an immune environment in the lung that is less conducive to cancer growth. It is possible that other immunologically active cancers could also be impacted by BCG, but that the numbers in this study were too small to detect a difference. The timing of vaccination may also have played a role, as cancers are most common in later life, during the time when immunity from childhood vaccination has substantially weakened. Vaccination during mid-childhood in this study, as opposed to infancy, may have extended the immunological effects into later years.

Cancer Treatment: BENEFIT FOR BLADDER CANCER

BCG was originally tested in an adjuvant treatment in pediatric patients with acute lymphoblastic leukemia with mixed results [31; 32]. Subsequent clinical trials were conducted testing BCG in lung, prostate, colon, and kidney cancers with minimal to no success [2]. However, potential benefits could have been missed due to the advanced stages of the patients in these studies, who would not be expected to benefit based on defined prognostic factors. A set of four criteria was identified as predictors of optimal candidates for BCG therapy. These include the proximity of the administered BCG to the tumor cells, a small tumor burden, the immunological capacity of the patient, and the dose of *Mycobacterium* [31]. Based on these criteria, bladder cancer patients generally show higher potential for benefit relative to most other cancers, which is reflected in the clinical success for the use of BCG in this type of cancer [33]. Adjuvant intravesical instillation (administration into urinary bladder via catheter) of BCG following post-surgical tumor resection is currently approved by the FDA for use in non-muscle invasive bladder cancer [2]. Treatment involves an induction course followed by a maintenance course. While the maintenance courses have improved the durability of the response, 24 to 40% of patients still do not respond to BCG treatment [31]. Studies have been conducted to try to address whether particular factors related to the BCG treatment regimen could impact responses. A meta-analysis of 19 studies (n=4,345 patients) found that relapses were more common in those who received low-dose BCG rather than standard dose (Risk Ratio [RR]:1.17, 95% CI 1.06 to 1.30), and in those who received only the induction and not the maintenance dose (RR: 1.33, 95% CI, 1.17 to 1.50)

[34]. The BCG OncoTice strain (Merck) was associated with higher rates of relapse relative to the Connaught strain (Sanofi Pasteur), but strain differences did not meaningfully impact survival outcomes. While other analyses have indicated the advantage of maintenance courses on tumor recurrence rates, an optimal maintenance schedule has not yet been determined [35]. Since the non-specific immunological effects of BCG are expected to depend on the capacity of the immune system to respond, a clinical trial assessed the impact of age on the safety and efficacy of BCG [36]. The study found no significant differences in the safety or efficacy of intravesical BCG between patients under 75 and those over age 75.

The mechanism of benefit has not been fully elucidated, but is thought to involve the augmentation of innate immune responses and/or the induction of antigen-specific anti-tumor activity by the adaptive immune system [2]. The circulating profile of immunomodulatory cytokines and chemokines has been shown to be altered following intravesical BCG treatment. A meta-analysis of 15 studies (n=791 patients) concluded that the composition of tumor infiltrating immune cells has prognostic value with respect to the therapeutic response to BCG [37]. The anti-tumor response may be driven by the production of IFN-γ by T cells, leading to a shift in the immune response from Th2 to Th1 polarization [2]. Patients with a higher Th2 polarization at baseline tend to show a better response to BCG treatment.

Covid-19: POTENTIAL BENEFIT PRIOR TO DISTRIBUTION OF COVID VACCINES

The BCG vaccine is known to offer a degree of non-specific immunity toward other infectious diseases. In recent years there has been considerable interest in determining whether BCG offers protection against SARS-Cov2, the virus that causes Covid-19. In a retrospective cohort study including 6,201 healthcare workers in the US, SARS-Cov2 seroprevalence (3.8% vs. 5.2%, P=0.019) and Covid-19 symptoms (72.7% vs. 75.6%, P = 0.017) were lower in those with prior BCG vaccination, during the early phases of the pandemic in 2020 [38]. Early in the pandemic several studies found an inverse correlation between countries with high BCG vaccination rates and Covid-19 cases or deaths [24]. A major confounder of those studies was that the countries with higher BCG coverage tended to be poorer, with lower degrees of healthcare infrastructure, resulting in low testing capacity and an underreporting of cases [39]. A systematic review of 13 studies found that nine of the studies reported significant associations between BCG vaccination and Covid-19 outcomes, even after controlling for these confounding variables [40]. Prior BCG vaccination was associated with lower rates of seroconversion for SARS-Cov2. This effect was specific to BCG, as Covid-19 outcomes were not associated with vaccination rates for other infectious diseases, including pneumococcal, influenza, diphtheria-tetanus-pertussis, and measles. The results of this analysis suggest that BCG vaccination partially mediated the association between countries with higher BCG coverage and better Covid-19 outcomes. The effect was also

partially mediated by some of the confounding variables, particularly the median age of the population, and percentage over age 65, as countries with older populations tended to have worse Covid-19 outcomes. A high BCG vaccination rate amongst the young is thought to be a primary driver of the protection during the early phases of the pandemic via the establishment of herd immunity [41]. The BCG vaccine may have reduced the susceptibility of young people to Covid-19, which would have led to lower levels of transmission, thereby protecting the older members of the population, who would be more vulnerable to severe Covid-19, and for whom the protection of their childhood BCG vaccine would have already worn off. Men have generally been at higher risk for worse outcomes to Covid-19, likely related to the different immune responses in men and women. A case-control study including 147 participants from East Germany between the ages of 17 and 46 found that BCG vaccination during childhood significantly reduced the likelihood of Covid-19 infection for men, which effectively eliminated the sex difference in Covid-19 risk in this population [42].

In light of these findings, several clinical trials were conducted testing the efficacy of BCG against Covid-19. A Phase 3 RCT testing BCG vaccination in a high-risk population in India (n=495, aged 18-60) found that BCG vaccination did not significantly impact Covid-19 infection rates, as measured by PCR test (odds ratio [OR] 1.08, 95% CI 0.54 to 2.14), but did reduce symptomatic infections (OR 0.38, 95% CI 0.20 to 0.72) [43]. However, severe outcomes were low overall, likely due to the relatively younger age of the study population. The study used a freeze-dried live strain of BCG from the Serum Institute of India, with 0.1 mL intradermal injections containing between 0.2 and 0.8 x 10^6 CFU, and assessed outcomes after nine months. Following vaccination BCG antibody titers increased from 2.1 (0.01 to 19.0) IU to 7.8 (0.2 to 26.2) IU, over the course of the nine-month study. At the time of the study, delta and omicron, were the predominantly circulating strains. A caveat of this study is that due to high vaccination childhood BCG vaccination rates (~70%) in India, many of the participants likely had prior exposure to BCG, which may have impacted the time course and magnitude of the response to another BCG vaccination during adulthood.

Two studies failed to find a benefit for Covid-19 with BCG re-vaccination, however, both studies had major caveats. A Phase 2 RCT assessing the efficacy of re-vaccination with BCG in health care workers in Brazil (n=131) found that that there was no significant difference in the incidence rates of symptomatic Covid-19 with re-vaccination [44]. However, this study used the BCG Moscow strain, and re-vaccination did not induce NK cell activation at 15–20 days post-revaccination, as anticipated. As such, it is unclear whether re-vaccination generated a meaningful immunological response in this study. A Phase 3 RCT tested BCG re-vaccination in healthcare workers in South Africa (n=1,000), and re-vaccination also did not significantly improve Covid-19 outcomes in this study [45]. However, over half of the participants

had latent tuberculosis, which may have prevented immune training by BCG, as has been shown in animal models. This study used the BCG-Vaccin Statens Serum Institut, Danish strain 1331.
The efficacy of BCG vaccination during adulthood to protect against Covid-19 was tested as an extension of a clinical trial testing BCG in patients with type 1 diabetes, as this population is also considered to be at higher risk for Covid-19 (NCT02081326) [46]. This study included 144 participants in the US aged 18-50, 96 of whom received two doses (0.1 mL intradermally) of the Tokyo-172 BCG vaccine four weeks apart, followed by a booster one year later. This occurred over a period of time 2.5 to 3 years prior to the start of this study, which began at the start of the Covid-19 pandemic in January 2020 through April 2021. The efficacy rate was 92%, which is on par with the Covid-19 vaccines, with the caveat of a small sample size, and very delayed response rate. The cumulative incidence rate was 1% (1/96) for the BCG vaccinated relative to 12.5% (6/48) in the placebo group. The severity and duration of symptoms were also reduced in those receiving the BCG vaccine. The data from this study suggest, that similar to the other non-specific immune effects, it may take about two years from the time of vaccination for BCG to offer a sufficient level of protection against Covid-19, or potentially other future infectious diseases. Overall, these studies suggest that BCG vaccination offered a degree of protection against Covid-19, particularly in the early days of the pandemic, prior to the development of Covid-19 vaccines. Since Covid-19 vaccines offer more rapid and robust immunity against circulating strains, they are the best option for well-resourced countries. However, for countries with limited access to Covid-19 vaccines, BCG boosters to those with childhood vaccination could potentially provide benefit.

Autoimmune diseases

In autoimmune conditions, the immune system inappropriately attacks cells in the body as though they were foreign invaders. One potential solution is to re-train the immune system. Due to BCG's immune training capacity, it has been proposed as a potential therapeutic intervention for some autoimmune diseases [47]. Additionally, since childhood pathogen exposure plays an important role in shaping the immune system, a reduction in pathogen exposure during the early years of life due to improved hygiene is hypothesized to underlie the increase in rates of autoimmune and atopic diseases in developed countries [48]. Pathogen exposure is one aspect of the 'eposome' which represents the cumulative exposure to all environmental stimuli and potential antigens [49]. The innate immune system is trained through life exposures, particularly during early life. Historically, pathogen exposure was unavoidable, so the human immune system evolved in a context of early life pathogen exposure, such that this exposure may be needed for the proper training of the human innate immune system [48]. In the absence of this training, the immune system may become hyperresponsive to other environmental antigens. Consequently, in countries with low pathogen burden, early life vaccination

with a live vaccine, such as BCG, may promote innate immune system training that minimizes reactivity to harmless environmental antigens and autoantigens.

Type 1 diabetes: POTENTIAL BENEFIT WITH MULTIPLE VACCINATIONS

Various epidemiological studies have failed to show an association between the incidence of childhood type 1 diabetes and BCG vaccination. One epidemiological analysis including 396,118 participants from Quebec, Canada between the ages of 22 and 44, similarly found no association between childhood BCG vaccination (< 1 year) and childhood rates of type 1 diabetes [50]. However, the study did find an association for reduced risk of type 1 diabetes after age 30 (adjusted HR 0.65, 95% CI 0.44 to 0.95), as well as a reduced risk for type 2 diabetes (adjusted HR 0.85, 95% CI 0.79 to 0.92). Based on data from two prospective research registers in Sweden including 20,249 individuals with diabetes diagnosed between 1983 and 2007, type 1 diabetes rates progressively increased over time in younger individuals, whereas rates were stable or declined in those over 35 [51]. The shift toward higher rates corresponds well to a shift in BCG vaccination policy in Sweden [48], though other factors have also changed during that time period which could account for these findings, including rates of childhood obesity [51]. The ability of BCG to impact diabetes may be related to dosing. A study in Turkey found that receiving two or more BCG vaccination was associated with reduced risk of type 1 diabetes, while a single dose did not influence incidence rates [48].

This requirement for multiple doses has also been seen in clinical trials using BCG as a potential treatment for type 1 diabetes. A meta-analysis of four RCTs (n=198 participants) testing BCG vaccination in patients with type 1 diabetes found that there was no significant effect of BCG vaccination on lowering glycated hemoglobin (HbA1c) levels (mean difference [MD]: −0.12; 95% CI −0.53 to 0.30), though there was a tendency toward improvement [52]. The lack of efficacy in this analysis may have been driven by the inclusion of studies with inappropriately short time windows and use of a single dose. The study with the best outcomes included 282 participants for both clinical trials (n=52) and *in vitro* mechanistic studies (n=230), with monitoring up to eight years [53]. The participants in the clinical trial were vaccinated twice with the Connaught strain BCG intradermally, with four weeks between doses. The HbA1c levels in BCG vaccinated participants were reduced by over 10% after year three, by 18% after year four, and levels remained low over the next five years (from 7.36±0.44 at baseline to 6.65±0.36 at year eight). Notably, significant reduction in HbA1c levels were not seen prior to three years post vaccination, which is longer than the follow-up durations of the studies that failed to find benefit. This suggests that the benefits are derived from non-specific immune system training, which has been shown to occur around two years post-vaccination in adults. Mechanistically, BCG induced a metabolic switch in immune cells from oxidative phosphorylation towards aerobic glycolysis, thereby

enhancing glucose utilization, and lowering blood sugar levels. In combination with the change in metabolism, BCG led to a de-repression of signature Treg genes, thereby leading to enhanced expression and production. These studies suggest that the strain and dosing schedule of BCG may impact its potential therapeutic utility for type 1 diabetes, and likely other autoimmune diseases.

Multiple sclerosis: POTENTIAL BENEFIT IN EARLY STAGES

BCG was tested in a single crossover trial in 14 patients with relapsing-remitting multiple sclerosis (RRMS) who had not previously been treated with immunomodulatory therapies other than corticosteroids [54]. Freeze-dried BCG was administered as a single intracutaneous dose (1 mg/mL; Berna Institute, Basel). Patients received monthly gadolinium (Gd)-enhanced MRI brain scans during the six months prior and six months after vaccination. Among the 12 patients who completed the study, there were a total of nine relapses prior to vaccination and three after, coupled with a 57% reduction in brain lesions on MRI following BCG. Ten patients showed a decrease in MRI activity. This patient cohort was then followed out to 24 months, and the lesions that developed during the post vaccination period were found to be less likely to transition into a radiological correlate of sustained tissue damage, suggesting that tissue repair processes may have been enhanced following BCG vaccination [55].

BCG vaccination was also tested in a placebo controlled RCT in 73 patients with clinically isolated syndrome [56]. Clinically isolated syndrome represents a single CNS demyelinating event, which eventually converts into multiple sclerosis within two years in about half of all cases. Half of participants received a single intracutaneous dose of 0.1 mL freeze-dried BCG (1 mg/ML; Pasteur), and after six months all were transitioned into a preplanned 12-month study testing IM interferon-β-1a. At the end of six months, there was a significant reduction in gadolinium enhancing lesions (RR: 0.541, 95% CI 0.308 to 0.956), and a trend toward fewer relapses, which did not reach statistical significance. During the 60-month follow-up period, the cumulative probability of transitioning into multiple sclerosis was lower for those who received both BCG and IM interferon-β-1a (HR: 0.52, 95% CI 0.27 to 0.99), and vaccinated participants were more likely to remain relapse-free (57.6% vs 30%).

While these studies suggest that BCG vaccination may boost immune tolerance in a manner that mitigates immune-mediated damage to the CNS, the mechanism has not been elucidated, and it has not been established whether there is an optimal period in the disease course to start BCG vaccination, and the dosing frequency needed to maximize potential benefit. There is currently a clinical trial underway testing a single dose of BCG in patients with radiologically isolated syndrome, in which MRI lesions are detected without or prior to the onset of neurological symptoms to determine whether it can delay or prevent the transition to multiple sclerosis (NCT03888924).

Eczema: NO CLEAR BENEFIT FOR PREVENTION

The prevalence of atopic diseases, or those where an individual has an exaggerated immune response to a harmless environmental substance has been increasing, particularly in developed countries. This may be related to a shift in the pathogen exposure, which shapes immune system responses in children. Due to the role of BCG in immune training, many studies have suggested a connection between BCG vaccination and risk for childhood atopic diseases, such as eczema, also called atopic dermatitis [57]. A systematic review of 20 studies including 222,928 participants found that early BCG vaccination was associated with a reduced risk for atopic diseases (OR: 0.87, 95% CI 0.77 to 0.99), particularly in developed countries [57]. When separated into disease types, the effect was significant for childhood asthma (OR: 0.77, 95% CI 0.63 to 0.93), but not for eczema (OR: 0.94, 95% CI 0.76 to 1.16), however, the results could have been influenced by methodological limitations. An RCT analyzed the 12-month incidence of 1,272 infants with a family history of atopic disease vaccinated with the BCG-Denmark vaccine (Danish strain 1331) within 10 days of birth in Australia (NCT01906853) [58]. There was no significant difference in overall eczema incidence at 12 months (32.2% in the BCG group vs 36.6% in the control group). But there was a small decrease in the highest risk infants (adjusted risk difference −11.5%, 95% CI −21.9% to −1.2%). Altogether these studies do not provide evidence to support a role for BCG vaccination in the prevention of eczema.

Unlike autoimmune conditions such as diabetes, BCG is not considered a viable therapeutic option for eczema. There are case reports regarding the exacerbation of eczema in children following BCG vaccination [59], and BCG is not recommended in people with skin conditions such as eczema [60].

Atherosclerosis: UNCLEAR (Preclinical)

Active and latent tuberculosis infection is associated with increased risk for subclinical obstructive coronary artery disease [61]. It has not yet been studied whether BCG vaccination influences rates of atherosclerosis. Preclinical animal models have shown conflicting results regarding the atherogenic potential of BCG. In mice with a humanized lipoprotein profile (APOE*3-Leiden.CETP) fed a high-fat diet, vaccination with BCG (5 × 10^6 CFU i.v.) resulted in hepatic mycobacterial infection, which was accompanied by immune cell infiltration into the liver [62]. Hepatic uptake of plasma cholesterol was enhanced, resulting in reducing circulating levels and reduced atherosclerotic lesion formation. BCG (4-6 injections s.c. of extended freeze-dried Pasteur strain 1173P2) also reduced atherosclerotic lesions and pro-inflammatory cytokines in Apoe-/- and Ldlr-/- mouse models, which was associated with an increase in Tregs and IL-10 production [63]. In rabbits fed a high cholesterol diet for 10 weeks, BCG vaccination (4-13×10^6) at week two plus a booster, resulted in increased leukocyte activation, which aggravated atherosclerosis [64]. These studies have been criticized for the use of dosing ranges and schedules that

differ from what is used in humans. One study used a human equivalent dose of BCG (0.5 × 10^4 CFU/mL s.c.) in two-day old mice Apoe-/- mice, who were subsequently fed a high-fat diet [61]. In this study, BCG vaccination reduced atherosclerotic lesions at 16 weeks, which was driven by reduction in macrophage content, despite a higher lipid content. There was also a sex effect, as the anti-atherosclerotic effect was most prominent in females. The clinical relevance of this study is also questionable because in humans, atherosclerosis generally develops in mid to late life, when the effects of the vaccine have waned. From these studies, it remains unclear whether BCG vaccination during adulthood could mitigate or potentially exacerbate ongoing atherosclerosis.

Safety: BCG vaccination is safe and well-tolerated for most people. Mild fever and injection site reactions are the most common side effects. Because it is a live vaccine, BCG can result in active infection in immunocompromised individuals.

Types of evidence:

- 1 systematic review and meta-analysis on the safety of infant BCG vaccination
- 1 systematic review on the safety of BCG re-vaccination
- 2 reviews of safety for intravesical BCG instillation in bladder cancer
- 2 RCTs for adult BCG re-vaccination for Covid-19
- 1 RCT on BCG strain comparative effectiveness for infant mortality
- Numerous laboratory studies

The BCG vaccine is one of the most commonly administered vaccines in the world, given to around 100 million children per year. It is primarily given to children under the age of one, as its efficacy to protect against tuberculosis declines considerably after age five [65]. The most common side effects are generally mild and include a local reaction at the injection site, fever, and headache [66].

A systematic review of 24 studies assessing the safety of BCG re-vaccination found that the adverse event profile was similar to original vaccination, primarily consisting of mild local or systemic reactions [67]. There was no evidence of serious adverse events in immunocompetent individuals. Similarly, BCG re-vaccination was found to be safe in clinical trials for Covid-19, with mild to moderate injection site reactions as the most common side effect [44; 45].

In patients with non-muscle invasive bladder cancer, treatment with intravesical BCG instillations results in flu-like symptoms and/or burning discomfort in the bladder in the majority of patients [68]. Side

effects resolve within 48 hours of instillation and are generally mild to moderate, which include urinary frequency, urinary urgency, nocturia, bladder pain, low-grade fever, chills, and hematuria [2]. Reported rare, but serious adverse events include Reiters syndrome, parotid (salivary) gland infection, femoral pseudoaneurysm, psoas abscess, iliac artery rupture, and diffuse arthritis.

Since BCG is a live vaccine, the primary concern and major serious adverse event is BCG infection with *Mycobacterium bovis*, requiring antituberculosis treatment [68]. This is primarily seen in immunocompromised individuals.

Drug interactions: BCG vaccination is contraindicated in immunocompromised individuals, due to the risk for active infection [69]. It is also contraindicated in individuals who are pregnant or breast-feeding. The vaccine may interact with antibiotics or immunosuppressant agents (WebMD).

Sources and dosing:

The BCG vaccine is typically given to infants during the first year of life in countries with a high burden of tuberculosis, and is less commonly administered in developed countries where levels of tuberculosis transmission are low [1]. There are various strains and formulations of the BCG vaccine, which differ in the immunological responses they produce, however, there is currently no consensus on whether there is an optimal version of the vaccine for tuberculosis, bladder cancer, or autoimmune disease [70]. Liquid versions of BCG had been used historically, but current vaccines primarily use a freeze-dried formulation [71]. Due to the serial passages and maintenance, the BCG strains have acquired a series of mutations that may affect both their ability to protect against tuberculosis and the nature of the non-specific innate immune system training, which influences protection against other pathogens/diseases [2]. Therefore, different strains may preferentially benefit different conditions, though no clear relationship has been established thus far. Since the distribution of BCG in low-income countries is largely done through UNICEF, global distribution of the vaccine depends on the strains produced by their suppliers [1]. The strains currently used for the UNICEF program are the BCG Denmark (Danish strain 1331, SSI), the BCG Russia (Russia BCG-I strain, Serum Institute of India), and the BCG Japan (Tokyo strain 172, Japan BCG Laboratory). An RCT comparing vaccination with these three strains in 12,057 infants in Guinea-Bissau found a trend toward fewer deaths with the BCG Japan vaccine, but overall, no significant difference in mortality at six weeks amongst the three strains [72]. There was, however, a difference in the immunogenicity of the strains, such that the BCG Russia strain was less immunogenic, based on the formation of purified protein derivative (PPD) responses and skin reactions, though it is debated how

well these surrogate responses reflect tuberculosis immunity. For bladder cancer, one study comparing the BCG Pasteur (Connaught strain) with the BCG-TICE strain found that the BCG Pasteur strain was associated with better survival, however, this study did not use maintenance dosing, so it is hard to interpret in the context of current dosing guidelines [2]. There is some evidence to suggest that the BCG Tokyo 172 strain may be the most efficacious, but head-to-head studies are needed to make a conclusive determination [2].
Since Sanofi-Pasteur discontinued production of the Connaught strain, there is currently only one US manufacturer for the BCG vaccine, which is the BCG-TICE strain produced by Merck [2].
The current dosing guidelines for intravesical instillation of BCG in non-muscle invasive bladder cancer are an induction course of six weekly instillations of BCG followed by a maintenance course of three weekly instillation treatments at 3, 6, 12, 18, 24, 30, and 36 months post tumor resection [2].

The BCG vaccine has not been approved for the treatment or prevention of other conditions, such as autoimmune diseases or Covid-19, so there are no official guidelines of safety or efficacy outside of tuberculosis and bladder cancer. The results from clinical trials suggest that multiple rounds of vaccination may be necessary for these non-specific effects and that the efficacy may not be apparent for at least two years after vaccination [46].
The timing of vaccination may also be critical for its effects outside of tuberculosis, depending on the typical age range in which a particular condition develops.

Research underway:

According to Clinicaltrials.gov, there are currently 148 active clinical trials using the BCG vaccine. These include trials for tuberculosis, bladder cancer, Covid-19, Alzheimer's disease, type 1 diabetes, radiologically isolated syndrome, melanoma, and other infectious diseases.

Due to the relatively low efficacy of BCG against tuberculosis, which may be related to the mutations that occurred during the serial passages and genetic drift over time, there are efforts to make recombinant versions of *Mycobacterium bovis*. VPM1002 is a genetically modified recombinant BCG vaccine derived from the BCG Danish, Prague strain. Clinical trials indicate that it is safe in infants with and without HIV exposure, and has fewer side effects than BCG, while eliciting a similar immune response [73]. Additional clinical trials testing VPM1002 are ongoing (Clinicaltrials.gov).

Search terms:

Pubmed, Google: BCG

- Alzheimer's disease, dementia, mortality, lifespan, cancer, cardiovascular, autoimmune disease, systematic reviews, meta-analysis, safety

Websites visited for BCG:

- Clinicaltrials.gov
- Drugs.com
- WebMD.com (Vaccine-percutatneous, Vaccine-intravescial)
- DrugBank.ca

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